HUMAN RETROVIRUS INFECTION IN THE WEST OF SCOTLAND

by

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SUMMARY

Cases of AIDS have been reported in the U.S.A. since 1981 and its aetiological agent, human immunodeficiency virus type 1, HIV-1, has been spreading in the population for longer. A testing system was established at the Hepatitis Reference Laboratory, HRL, to identify those infected in the Glasgow and West of Scotland population. The performance of commercial screening tests was examined using sera locally available at the HRL. Confirmation of reactivity was confirmed by western blot analysis: other methods of confirmation were examined. The criteria of positivity on a western blot were established from observation of the antibody response in seroconversion and weakly reactive samples compared to false reactive or indeterminate samples. In this way genuine HIV-1 infection could be reliably diagnosed.

The prevalence of HIV-1 infection in the community was assessed and various risk groups identified and studied in detail. The groups at high risk of infection were the same as those initially reported from the United States, i.e. homosexuals (including bisexuals), intravenous drug abusers and haemophiliacs. A prevalence of less than 2% was found in drug abusers, in marked contrast to the situation in Edinburgh, with first infection detected in 1985. Infection in haemophiliacs pre-dated the discovery of HIV-1 with the earliest seropositive sample in Glasgow being 1981. Early infection of homosexuals was suspected to be around that time or before.

The progress of HIV-1 infection was followed in a group of patients attending the Infectious Disease clinics at Ruchill Hospital and in the HIV-1 infected haemophiliacs. Three marker changes associated with progression, i.e. loss of anti-p24, production of p24 antigen and raised β_2-microglobulin were examined along with clinical details to establish their usefulness in predicting onset of disease. An association with disease progression was observed with each marker. However no formula for progression based on marker changes could be established and individuality of response was the common theme. A association between age and disease progression was noted in the haemophiliacs.

ABBREVIATIONS

Ab	Antibody
Ag	Antigen
AIDS	Acquired immunodeficiency syndrome, also acquired immune deficiency syndrome
anti-gag(env)	Antibody to group specific antigen (envelope protein)
anti-HIV(1/2)	Antibody to human immunodeficiency virus (type1/type2)
anti-HTLV(I/II/III)	Antibody to human T cell lymphotropic virus (typeI/typeII/typeIII)
anti-p24 etc.	Antibody to the 24 kilodalton protein etc.
ARC	AIDS related complex
ATL	Adult T cell leukaemia
AZT	3'-azido-3'-deoxythymidine
Bi	Bisexual
β_2-mg	Beta 2 microglobulin
BTS	Blood Transfusion Service
^oC	degrees Celsius
CDC	Centre for Disease Control
cDNA	Complementary deoxyribonucleic acid
CDS	Communicable Diseases Scotland
Combi	Combination test
CP	Clinical progression
CSF	Cerebrospinal fluid
DA	Intravenous drug abuser
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EIA	Enzyme immuno assay
ELISA	Enzyme linked immunosorbent assay
env	Envelope
F	Female
FVIII	Factor VIII concentrate
FIX	Factor IX concentrate
FDA	Food and Drug Administration
FN	False negative
FP	False positive
gag	Group specific antigen
GP	General practitioner
gp	glycoprotein
GRI	Glasgow Royal Infirmary
GUM	Genito-urinary medicine
HBV	Hepatitis B virus
HCV	Hepatitis C virus
Het	Heterosexual
HIV-1(HIV-2)	Human immunodeficiency virus type 1 (type 2)

HLA	Human leukocyte antigen
Hm	Haemophiliac
HmA/HmB	Haemophilia A /Haemophilia B
Ho	Homosexual
HRL	Hepatitis Reference Laboratory
HTLV(I/-II/-III)	Human T cell lymphotropic virus (type I, II or III) also human T cell leukaemia virus (type I, II, III)
ID	Infectious Diseases
IF	Immunofluorescence
IgG	Immunoglobulin subclass G
IgM	Immunoglobulin subclass M
kb	kilobases
kd	kilodaltons
KS	Kaposi's sarcoma
LAV	Lymphadenopathy associated virus
LTR	Long terminal repeat
M	Male
min	minutes
NC	Nitocellulose
Neg	Negative
NF-KB	Nuclear factor Kappa B
OD	Optical density
OI	Opportunistic infection
OMP	Outer membrane protein
Orf	Open reading frame
Oth	Other
p17,p24 etc.	protein of molecular weight 17 kilodaltons (24 kilodaltons etc.)
PCP	Pneumocystis carinii pneumonia
PCR	Polymerase chain reaction
PGL	Persistent generalised lymphadenopathy
PHLS	Public Health Laboratory Service
pol	polymerase
Pos	Positive
QC	Quality control
RHC	Regional Haemophilia Centre
RHSC	Royal Hospital for Sick Children
RIA	Radioimmunoassay
RIBA	Recombinant immunoblot assay
RIPA	Radioimmunoprecipitation
RNA	Ribonucleic acid
RT	Reverse transcriptase
RTC	Regional Tranfusion Centre
RVL	Regional Virus Laboratory

s/co	Sample to cut-off ratio
SIV	Simian immunodeficiency virus
SNBTS	Scottish National Blood Transfusion Service
SV40	Simian virus 40
TMP	Transmembrane protein
Tx	Transfusion
UK	United Kingdom
US	United States
USA	United States of America
VP	Virological progression
WB	Western blot
WP	Weak positive

CHAPTER 1

INTRODUCTION

1. RETROVIRUSES

What is a Retrovirus?

The study of retroviruses has seen many advances in the past two decades, most notably since the detection and isolation of the first human retrovirus, Human T-cell Leukaemia Virus Type I (HTLV-I) by Poiesz et al.(1) in 1980-81 and subsequently Human Immunodeficiency Virus, HIV, (formerly Human T-cell Lymphotropic Virus Type III/Lymphadenopathy Associated Virus), the aetiological agent of Acquired Immune Deficiency Syndrome, AIDS (2, 3).

Retroviridae are enveloped, spherical viruses containing two copies of single-stranded, positive sense ribonucleic acid (RNA) and are distinguished by a viral-coded RNA-dependent DNA polymerase, a reverse transcriptase (RT). This reverse transcriptase catalyses the synthesis of double-stranded complementary deoxyribonucleic acid (cDNA) using RNA as a template. The cDNA circularises and becomes integrated into the host genome, thereby establishing latency which is an important feature of retroviral infection. Retroviruses were so named because they reverse what is the normal flow of genetic information as their RNA genome must be converted into DNA before viral expression.

Classification of Retroviruses (4,5)

The retroviridae are broadly classified by mechanism of transmission as either endogenous or exogenous retroviruses where the former are integrated into normal cellular DNA, probably as a remnant of an ancient infection and are passed vertically from generation to generation via the germ line, in a Mendelian fashion. Exogenous retroviruses can be split into two groups:

(i) those carrying onc genes derived from host DNA and directly coding for a protein involved in neoplastic transformation. Infection with such a virus leads to rapid development of malignancy and these are referred to as acute leukaemia viruses.

(ii) those which do not carry an onc gene, do not transform cells in vitro and are slow to produce disease in vivo. These are known as chronic leukaemia viruses.

There are 3 sub-families of retrovirus based on pathogenesis; (i) Oncovirinae: these viruses can induce tumour formation, e.g. feline leukaemia virus FeLV, bovine leukaemia virus BLV and human T-lymphotropic virus HTLV;

(ii) Lentivirinae: these are exogenous retroviruses which infect ungulate mammals especially sheep, goat and horses. Progression to disease is slow (over many years) and infection often results in degenerative diseases of the central nervous system, e.g. visna virus of sheep and equine infectious anaemia virus EIAV also feline immunodeficiency virus FIV, and human immunodeficiency virus HIV, etc. and

(iii) Spumavirinae, the foamy viruses which induce persistent infection but are thought to be non-pathogenic, e.g. simian virus 5 (SV5).

Retroviruses are also classified by morphology into type A, B, C or D particles as observed in electron microscopic studies. Type A produces an immature form and are only found intracellularly while the other three types produce particles which bud from the host cell membrane; type B have an eccentric core on budding, e.g. mouse mammary tumour virus MMTV, type C the most abundant sub-family, have a central, spherical core, e.g. FeLV, HTLV, BLV etc. and type D with a cylindrical central core, e.g. Mason-Pfizer and related primate retroviruses and HIV (6,7).

The Retroviral Genome, its Organisation and Gene Products

Some basic features of the genome organisation are common to all retroviruses (4 pp261-368, 5pp17-73).

The genome of a retrovirus contains 3 major structural genes,

gag which codes for the group-specific or core antigen, pol the polymerase gene and env the envelope gene which are flanked on either side in the proviral DNA by a series of nucleotides forming long terminal repeats (LTRs) (Fig.1.1). The LTR region contains the binding site for the RNA polymerase and important sequences involved in the control of replication of the virus, e.g. promoter and enhancer sequences. This region is also responsible for the integration of the DNA provirus into host cellular DNA. A number of other open reading frames are present between pol and env or env and LTR, encoding non-structural genes whose protein products may play a role in the regulation of viral expression and maturation.

The gag gene encodes a precursor protein which is cleaved to yield 3 or more internal structural proteins. The major core protein surrounds the viral RNA and associated nucleic acid-binding proteins. The pol gene encodes the enzymes required at various stages of the viral life-cycle; an integrase, reverse transcriptase and a protease. The env gene encodes a precursor envelope protein which may be glycosylated. This is cleaved to an outer membrane protein (OMP) and a smaller transmembrane protein(TMP), at least one of which is glycosylated. The TMP spans the lipid bilayer of the mature virus and is linked to the OMP by a disulphide bond. The OMP forms the spikes seen by electron microscopy and plays an important role in the binding of virus to its receptor and induction of neutralising antibody. The lipid bilayer surrounding the virus is derived from the host cell by budding of mature virus particles through the membrane of the host cell. The retroviral nucleic acid contains other open reading frames (orfs) varying in number and genome location in different viruses. These can be involved in control of gene expression, maturation and infectivity of the virus.

Retroviruses and their Association with Disease

The possible involvement of viruses in cancer was first postulated by Ellerman and Bang in 1908 (8), who showed that a filterable agent was responsible for the transmission of leukaemia in fowl. Peyton Rous (1910-1911) (9) also demonstrated that cell-free

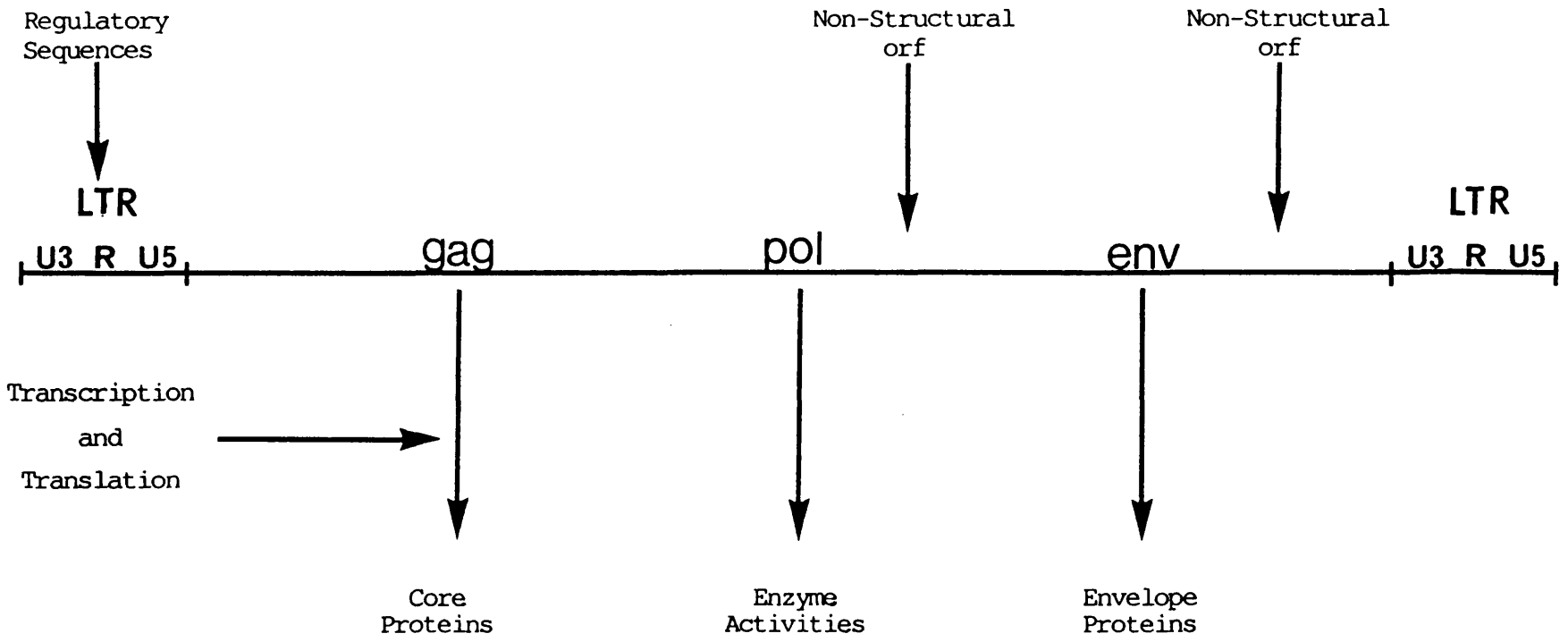


Figure 1.1 Basic proviral structure of the retrovirus genome.

filtrates were able to transmit tumours in chickens and he isolated the first such agent, Rous sarcoma virus (RSV). Many scientists did not believe that leukaemia was linked to cancer nor in the concept of infectivity in cancer so the subject lay dormant for several decades. In the 1950s, Gross et al.(10) isolated an RNA tumour virus from murine leukaemia cells. Further work followed on simian RNA tumour viruses. This fostered the idea that a virus can cause a silent or latent infection in its natural host but could be pathogenic in another host. E.g. SV40, a papova virus which is non-pathogenic in monkeys, its normal host has oncogenic properties when injected into baby hamsters.

As the work performed on mice involved using inbred laboratory strains there was a possibility that infections could be congenital and thus not an effect of the virus. Further progress was not seen until the 1960s when a retrovirus of cats, feline leukaemia virus FeLV (11) was discovered and it was observed that this virus could be transmitted horizontally amongst unrelated cats in household surroundings. Hence it was possible that a form of cancer could be transmitted and this work laid the foundations for the discovery of cancer-causing retroviruses in many species.

Retroviruses are the only known RNA viruses that can produce cancer and have been isolated from many species including sheep, chicken, cows, goats, horses etc. and now man.

HUMAN T-LYMPHOTROPIC VIRUSES TYPE I AND II (HTLV-I AND HTLV-II)

Isolation

The first human retrovirus was not detected until 1980-81 by isolation from cultured T cells. HTLV-I was isolated from two black US patients with T cell malignancies thought to be an aggressive variant of cutaneous T cell lymphoma (1,12).

Research had concentrated on the search for endogenous viruses and only those with high replicating activity could be detected with the methods available. The search for a human retrovirus was facilitated by two important findings in the 1970s. The first was

the independent and simultaneous discovery of the enzyme, reverse transcriptase (RT) by Temin and Mitzutani (13) and by Baltimore (14). A sensitive assay was developed to detect RT activity. The second finding was the discovery that a growth factor or its receptor were produced by T cells on stimulation by an antigen or various mitogens, e.g. phytohaemagglutinin (PHA), a plant lectin. Those T cells secreting this factor bind to other T cells bearing its specific receptor and in such a way promotes their continuous growth (15). This was called T cell growth factor (TCGF) now referred to as Interleukin-2 (IL-2). In vitro cultures of human lymphocytes could now be maintained by the addition of IL-2. In this way T cells from leukaemic patients and those with T cell malignancies were examined. Some cell lines were able to grow without in vitro activation, the membrane already contained IL-2 receptors in contrast to normal T cells. It was from these cells that HTLV-I was isolated. A second human retrovirus HTLV-II was isolated in 1982 from a patient, Mo, with a T cell variant of hairy cell leukaemia (16).

HTLV-I shares the qualities of both acute and chronic leukaemia viruses. It can transform primary cells in vitro as do the acute leukaemia viruses although it lacks an onc gene; it also produces clonally derived tumours in vivo and has a long incubation time to production of disease, similar to chronic leukaemia viruses.

Morphology and Size

The HTLV family derive their name from their tropism for T4 cells. HTLV-I and -II have the morphology of a "budding" type C retrovirus. The average size of HTLV-I is 100nm, but ranges from 90nm to 140nm in newly established cultures. One copy of proviral DNA is found in leukaemic cells and it is able to integrate anywhere in the host genome (17). This integration is conserved in the one patient but varies from patient to patient. HTLV-I shows greatest similarity to bovine leukaemia virus (BLV) (17) suggesting a distant evolutionary relationship.

The Genome, its Organisation and Gene Products (17,18)

The genetic organisation of HTLV-I and II is similar to other retroviruses, i.e. gag, pol and env genes but also includes regulatory genes rex, regulator of expression of virion proteins, and tax (transactivator) designated with a subscript number 1 or 2 pertaining to the relevant virus, e.g. rex_1 and tax_1 for HTLV-I etc. This tax gene is highly conserved in the HTLV family (HTLV-II, BLV and simian T lymphotropic virus type I, STLV-I). The protein product (p42 in HTLV-I, p38 in HTLV-II) operates in trans to activate viral gene expression and is involved in regulation of cellular genes (19). This unique tax sequence is capable of activating the gene for IL-2 and its receptor and therefore may play a role in the mechanism of oncogenesis of HTLV-I. The gene products of HTLV-I are described in Table 1.1. HTLV-II has 65% nucleotide sequence homology to HTLV-I and the encoded proteins are closely related (20).

HTLV and Disease

HTLV-I is associated with adult T cell leukaemia (ATL) (1,21) and more recently with a neurological disorder called tropical spastic paraparesis (TSP) or HTLV-I associated myelopathy (HAM) (22,23). ATL was first described as a new clinical syndrome in the 1970s in Japan (24) in particular among the residents of two southern Japanese islands, Shikoku and Kyushu. The link between HTLV-I and ATL was made when sera from Japanese ATL patients were tested and HTLV-I antibodies were present in almost all cases of ATL and in a high percentage of normal adults in endemic areas (25). Other geographical clusters, have been identified, in the Caribbean islands, areas of South America, Southern Italy and parts of Africa. The virus is also endemic in immigrant populations in Europe, e.g. West Indian population in England, Surinam population of Holland, etc. (18).

HTLV-II has not yet been definitely linked with any clinical disease state. Serological evidence of HTLV-II infection has been found in intravenous drug abusers in England (26) and New Orleans (27) and in an isolated, symptom-free population of Guayami Indians in Panama (28).

TABLE 1.1 HTLV-I genes, their protein products and related function

Gene	Precursor Protein(s)	Protein Products	Location in Virus and/or Function
gag	*p53 ---> p36,p32 p28,p26	p19	
		p24	Major internal core protein surrounding viral RNA
		p15	Nucleic-acid associated protein
pol		p14	Protease
		p99	Reverse transcriptase
		?p32	
env	gp61-gp68#	gp46	OMP
		gp21	TMP
rex_1		p27	Regulatory
tax_1		p24	Regulatory

gp - glycoprotein
OMP - Outer membrane protein
TMP - Transmembrane protein

* p53 represents a protein of molecular weight 53 kd.
# Molecular weight depends on the cell line used for isolation.

HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)

Isolation

The isolation of the third human retrovirus Human Immunodeficiency Virus, HIV, (formerly called HTLV-III/LAV) was first achieved by Luc Montagnier and colleagues at the Pasteur Institute in Paris, 1983 (2). They co-cultivated an HTLV-I-like retrovirus from the lymph nodes of a homosexual patient with lymphadenopathy and called it Lymphadenopathy Associated Virus, LAV. It was noted that although the core proteins were similar in size to HTLV-I they differed immunologically and no cross-reaction was detected. Sufficient quantities of virus could not be produced to allow full characterisation of this isolate due to the cytopathic effect of LAV, in vitro. So the full impact of this discovery was not realised until one year later when Popovic et al. (3) described the establishment of a permissive T-cell population, that could grow continuously and produce virus after infection, from a neoplastic, aneuploid T-cell line, HT, derived from a patient with lymphoid leukaemia. Using clones of these T-cell lines, specifically H9, Gallo et al. (29) were able to isolate a 'novel' retrovirus from patients with AIDS and those at risk for AIDS. Robert Gallo and co-workers from the National Cancer Institute (NCI) in Bethesda, Maryland, called their virus Human T-Cell Lymphotropic Virus Type III (HTLV-III) because they noted many similarities to the other human retroviruses notably T4 tropism, Mg^{2+} dependent reverse transcriptase and its cytopathic nature. Sarngadharan et al. (30) showed antibodies in patients at risk for AIDS and that 88% of patients with AIDS had antibody to this virus. Thus HIV appeared to have a role as the aetiological agent for AIDS. The pioneering work of Robert Gallo and co-workers at the NCI on HTLV-I allowed the rapid characterisation of HIV and development of immunological reagents for a screening assay (30,31). In August 1984, Levy et al. (6) isolated a third set of viruses, AIDS-associated retroviruses (ARV) from San Francisco AIDS

patients, which were antigenically and structurally related to LAV. The above isolates are all variants of the same virus now called Human Immunodeficiency Virus, HIV, the name proposed by the International Committee of Virus Taxonomy and Nomenclature in May 1986 (32).

HIV has been isolated from plasma (33), lymphocytes in the peripheral blood (6,29) as well as from cells in the bone marrow, lymph nodes, brain, semen, cervical secretions, CSF, tears, saliva, and breast-milk of infected persons (34-39). The major routes of transmission are through sexual contact; male to male, male to female and female to male, parenterally from blood and blood products and perinatally, pre-, post- or during delivery.

Morphology and Size

Negative staining electron microscopy revealed no information about virus morphology, but by thin sectioning the core of HIV-1 could be seen to have type D morphology. As it buds from the cell membrane, the core is crescent-shaped, this condenses and becomes an eccentric bar-shaped or conic core in the mature particle. Extracellular particles are 90-130nm in diameter. This core contains the capsid proteins and two copies of the positive sense single-stranded RNA genome and associated with this is the viral-encoded reverse transcriptase. The envelope of the virus consists of a lipid bilayer, contributed by the membrane of the host cell through which the envelope glycoproteins project (40). The outer envelope protein appears as projections or spikes on the outer surface of mature virions. A close evolutionary relationship exists between HIV-1 and the lentivirus family particularly visna virus of sheep (7). There are many similarities, e.g. in morphology, budding and formulation of electron dense core, variation in <u>env</u> region, nucleotide sequence homology in <u>gag</u> and <u>pol</u> regions, neurotropism, genome organisation and the large size of the envelope proteins. <u>In vivo</u> lentiviruses are cytopathic and cause debilitating diseases in contrast to type C human retroviruses HTLV-I and -II which cause lymphoproliferation of infected cells and T-cell malignancies.

The Genome, its Organisation and Gene Products (18,41,42)

The RNA genome of HIV is 9.2 kilobases, (kb), long and the integrated provirus 9.6 kb. The integrated genome is flanked on either side by LTR regions with the usual U3 R U5 motif which contain regulatory sequences recognised by various host transcription factors, e.g. NF-KB (43) and by viral regulatory gene products. In addition to the three classical retroviral genes, gag, pol and env, there are at least six further genes vif (virion infectivity factor), vpr (viral protein R), vpu (viral protein u),tat (transactivator), rev (regulator of expression of viral proteins), and nef (negative factor). The latter three have regulatory functions and the functions of at least two are unclear. This level of genomic complexity distinguishes the lentiviruses from other retroviruses. All the gene products of HIV-1 are immunogenic but this thesis is concerned with the detection of antibodies to the structural proteins of the virus and these will be discussed in greater detail. The products of the non-structural genes are involved in control of the replication and infection pathway of the virus. Their functions and their role in the complex regulatory mechanisms of HIV-1 expression and pathogenesis are described more fully in a recent review article by W.C. Greene (44).

Structural Genes (18,41)

The gag gene encodes a p55 precursor which is cleaved by the viral-coded protease into 3 major proteins. A phosphorylated p24 is the major core protein and this surrounds the nucleoprotein core. The amino-terminal protein p17 is associated with the inner surface of the phospholipid bilayer and probably stabilises the exterior and interior components of the virion. The carboxy-terminal p15 is cleaved into two proteins p7 and p9, which are nucleoproteins; the p7 binds directly to the viral RNA.

The pol gene encodes several important viral enzymes; an aspartyl protease, the reverse transcriptase, ribonuclease, and an integrase. These functions are so crucial to the virus that the amino-acid sequences are well conserved amongst all established

retroviral sequences to date. The amino-terminal protein is an aspartyl protease which cleaves the precursor proteins. The middle region of the pol gene encodes the reverse transcriptase which exists as a heterodimer, p66/p51 in its most active form (45). (The old numbering p64, p53 on the early version western blot has been used throughout this thesis). The ribonuclease function is also here and is used to digest the small RNA sequences used as template for DNA synthesis. The third pol gene product is a 31 kilodalton (kd) endonuclease/integrase which cleaves the closed circular DNA, made from the viral RNA and integrates it into the host cell DNA.

The envelope proteins of HIV-1 are larger in size than type C retroviruses and are more similar to lentiviruses. The glycosylated precursor, gp160, is cleaved by a cellular protease to an outer membrane protein (OMP), gp120 and a transmembrane protein (TMP), gp41; these are linked by disulphide bonds in the mature virion (46). There are 31 potential N-linked glycosylation sites on the env protein, as predicted from the nucleotide sequence, with greater than 20 sites on the gp120 and up to six sites on the gp41. The carbohydrate moiety, in the form of oligosaccharide chains is added and modified by host cell enzymes and contributes half the molecular mass of gp120; gp120 is reduced to p80 on deglycosylation (46). Gp120 occurs as spikes on the outside of the virus and a rapid loss of some of these gp120 molecules has been seen, by electron microscopy, to occur in vitro during maturation of HIV virions (40).

Gp120 mediates binding of the virus to its receptor. For HIV this is the T helper cell (T4) antigen, referred to as the CD4 antigen (47). The receptor binding site on the gp120 appears to be conserved in different isolates. It was the remarkably selective depletion of $CD4^+$ lymphocytes in AIDS patients that led investigators to show the tropism of HIV for $CD4^+$ cells, predominantly T4 cells. Although HIV-1 infects predominantly T4 helper/inducer cells, other cell types can be infected in vitro, e.g. EBV-transformed B cells, brain cells, muscle cells from a rhabdomyosarcoma cell line etc. (41,48) and most importantly monocytes and macrophages which are considered to be major reservoirs of the virus in the body as they are on the whole refractory to the cytopathic effects of HIV-1. There is evidence to

suggest that infection is not always mediated by the CD4 antigen (48). In addition another component on the cell surface is required to achieve virus entry. It is currently believed that binding of a fusogenic domain in the gp41 with another cell-membrane protein is the required secondary event (49). The oligosaccharide side chains may also be involved.

Nucleotide sequence data from many isolates of HIV-1 illustrate an extensive degree of variation. Not only does variation exist amongst geographically distinct isolates and between sequential isolates taken from the same individual but also that multiple variants co-exist in an individual at any one time (50-52). Variation occurs mainly through nucleotide substitutions but also through short sequence deletions and insertions. These are usually in multiples of three to preserve the reading frame. The consequences of such variation may affect the three-dimensional protein structure or conformation of an epitope. This diversity is greatest in the OMP, gp120, and these are localised to the "hot spots of mutation" or hypervariable regions which are interspersed with conserved regions. There are five such regions, V1-V5, and computer-assisted analysis of the predicted env protein gp120 sequences of seven HIV-1 isolates locates the majority of antigenic epitopes in these hypervariable regions (53).

The envelope proteins are the major target of neutralising antibodies (54) and cytotoxic T-lymphocytes (CTLs) (55). In addition a highly conserved epitope at the 3' end of gp120 is the target of the antibody-dependent cellular toxicity (ADCC) process (56). What role all these play in establishing an anti-viral or protective state in an infected individual is unclear and requires further evaluation. Neutralisation sites have been identified on both gp120 and gp41 and antibodies can cross-neutralise other strains of HIV-1, however neutralisation titres are low. The major neutralisation epitope is situated on the immunodominant loop in the V3 region of gp120 (57). This antigenic site has been found to vary in different isolates but a small GPGR amino acid sequence, i.e. glycine, proline, glycine, arginine at the top of the loop is generally conserved. Sequence changes in these regions may be an adaptive response by HIV to evade

the host immune system and maintain a persistent latent infection as proposed for other lentiviruses particularly equine infectious anaemia virus, EIAV, (58).

HIV-1 and Disease

It was noted in the late 1970s, early 1980s that young, highly promiscuous, homosexual men, in San Francisco and New York, began to suffer and die from infections normally considered harmless or causing only mild disease. There was also an increase in the occurrence of uncommon types of cancer particularly Kaposi's Sarcoma (KS). An endemic form of KS exists in Africa but otherwise it is rare in the population especially in young adults. Haematological studies revealed that certain cells of the immune system were present in the wrong proportions and most obvious was the decrease in numbers of T helper cells. So it appeared that an immune deficiency was allowing opportunistic infections of fungal, protozoal, viral and bacterial origins to be life-threatening in otherwise healthy, young men. This acquired immune deficiency syndrome or AIDS was first identified as a new disease in 1981 (59).

AIDS was originally defined by the CDC (60), the Centre for Disease Control, U.S.A., as occurring in a person:

(i) with reliably diagnosed disease that is moderately indicative of a defect in cell-mediated immunity, e.g. KS in a patient less than 60 years old or an opportunistic infection, OI; and

(ii) who has no known underlying cause of cellular immune deficiency or any other cause of reduced resistance reported to be associated with disease.

Early epidemiological evidence suggested AIDS was a new infectious disease that could be horizontally transmitted by intimate contact or blood products (61). The specific finding that haemophiliacs developed AIDS after transfusions of Factor VIII (FVIII) and that plasma is filtered in the preparation of FVIII suggested "a filterable agent" was responsible. A virus, specifically a retrovirus, was suggested because a virus of cats, feline leukaemia virus (FeLV) causes an immune deficiency similar to AIDS. The T cell

tropism and manner of transmission of the agent was reminiscent of HTLV-I and HTLV-II. These human retroviruses normally induce a lymphoproliferative response but can also have immunosuppressive activity in vitro. AIDS appeared to be spreading to the HTLV-I endemic areas of Africa, Europe, Haiti, etc. Gelmann et al. (62) looked for evidence of HTLV-I in AIDS; only 2 out of 33 AIDS patients contained HTLV-I proviral sequences and these were in black homosexuals who may have been predisposed to HTLV-I if born in an endemic area. Essex et al. (63) could only detect anti-HTLV-I in approximately 25% of AIDS and pre-AIDS patients. This was insufficient evidence to establish a causal relationship of HTLV-I to AIDS.

Other candidate viruses, e.g. cytomegalovirus, Epstein-Barr virus, etc. were also implicated in AIDS due to the high rates of infection with these agents in the population with AIDS. However these viruses were more opportunist than causal in association. A novel retrovirus type, HIV, was subsequently isolated from AIDS and pre-AIDS patients as described previously. Serological evidence (30) showed that 90% of AIDS patients had antibodies to the viral proteins of HIV and that this virus could be isolated from patients with AIDS (29).

AIDS has such a devastating effect on the human body due to the selective tropism of HIV-1 for the T4-helper cell, a key cell of the immune system. Other cell types, e.g. monocytes and macrophages can also be infected and act as reservoirs of HIV-1. Following invasion of a foreign body, the T4 cell is involved in the orchestration of events leading to an immune response and has been termed the 'leader of the immune orchestra'. The pathogenic effects of HIV-1 are mediated predominantly through gp120 attachment to the CD4 receptor on infected and uninfected T4 cells leading to syncytium formation and cell death. Direct or indirect effects on other cells in the immune system also contribute to the development of disease but discussion of this is beyond the scope of this thesis. However depletion of the T4 cell population through the cytopathic effect of HIV allows opportunist micro-organisms to spread unhindered and cause death.

HUMAN IMMUNODEFICIENCY VIRUS TYPE 2

Isolation

A second human immunodeficiency virus, HIV-2, was first isolated from two HIV-1 seronegative patients with an AIDS-like illness from Guinea-Bissau and Cape Verde, West Africa (64) and this virus was more closely related to a simian immunodeficiency virus, SIV_{mac}, than to HIV-1. This confirmed serological evidence of the emergence of a new human retrovirus (65).

Initially there was some confusion due to the apparent isolation by Kanki et al. (65) of HTLV-IV from asymptomatic Senegalese women. HTLV IV shared similarities and serological cross-reactivity with a monkey retrovirus STLV-III_{agm}, isolated by the same group, which was non-pathogenic in its host, African green monkeys (agm). However restriction mapping confirmed by sequencing revealed that HTLV-IV and STLV-III_{agm} were greater than ninety-nine percent similar and that both were identical to a particular isolate of SIV from a macaque, SIV_{mac251} which causes an AIDS-like illness in captive macaques (66). This was the isolate used in serological studies performed by Kanki and Essex and they subsequently readily admitted their mistake to the scientific community (67) - that their isolates of HTLV-IV and STLV-III_{agm} were a result of laboratory contamination of their cell stocks by SIV_{mac251}. Thus the French isolate of LAV-2 is the virus known to-day as HIV-2. It has been proposed that HIV-2 arose from horizontal infection of a human, 30-40 years ago, by a retrovirus of sooty mangabeys, SIV_{sm} (68). These monkeys are native to West Africa and SIV_{sm} causes an AIDS-type illness in captive macaques, although non-pathogenic in its host.

The Genome, its Organisation and Gene Products (69)

The genome and its organisation are similar to that of HIV-1 although it is genetically and antigenically more closely related to SIV sharing about 75% homology to SIV_{mac} and only about 45% homology to HIV-1. The *gag*, *pol*, *env* and similar regulatory genes, i.e. *tat*,

rev, nef, vif and vpr are present. The vpx region located between the pol and env genes is unique to HIV-2 and SIV but the function of its gene product(s) are unknown. Serological cross-reactivity exists between HIV-1 and HIV-2 and this can be seen on heterologous western blots (70). Anti-HIV 2 reacts readily with the core protein and to a lesser extent with the envelope proteins of HIV-1. In contrast anti-HIV 1 sera react mainly with the gag and pol gene products of HIV-2. In addition HIV-2 antibodies will neutralise HIV-1 but HIV-1 antisera do not neutralise the type 2 virus.

HIV-2 Seroprevalence

HIV-2 is prevalent in many West African countries where it is a more common cause of AIDS than HIV-1. There is evidence that HIV-2 has been present in West Africa as early as 1966 (71) though others dispute this (72) and are cautious, not wishing to increase the stigma regarding Africa as the progenitor since it already bears much of the blame for the emergence and spread of HIV-1. HIV-2 infection is rare outside Africa. A few cases have been described in Europe, Brazil, the USA and Canada (73); the majority of reported cases had West African connections being either natives of the area or through sexual contacts. In the United Kingdom (UK) to date there have been 12 cases of HIV-2 identified. Nine of the 12 had connections with Africa, predominantly West Africa and each had a risk factor for HIV-2 infection (74). Since June 1990, the UK Blood Transfusion Service has been screening for anti-HIV 2 in combination with anti-HIV 1 in all blood donations. In the first six months of testing one donor was found to be infected with HIV-2 (74).

Transmission of HIV-2 occurs in a similar way to HIV-1, i.e. sexually, parenterally via blood or blood products and perinatally. The predominant mode of spread is through sexual intercourse between men and women, as is the situation with HIV-1 transmission in Africa.

HIV-2 and Disease

HIV-2 causes an AIDS-type illness similar to that caused by

HIV-1. Some features of HIV-2 infection appear to be different to that of HIV-1, most notably that of a longer latency period to disease progression, up to sixteen years has been reported (75). Dual infection with HIV-1 and HIV-2 has been documented (76) but what influence this has on disease progression has yet to be fully evaluated. Care must be exercised in interpreting apparent dual infection based on serological studies due to the nature of antibody cross-reactions to both viruses.

2. SEROLOGICAL MARKERS OF HIV INFECTION AND THEIR DETECTION

SEROLOGICAL TIME COURSE OF INFECTION

Primary infection with HIV-1 may be symptomless but can result in an acute illness with a variety of presentations, e.g. fever, rash, nausea, diarrhoea, myalgia, sore throat, lymphadenopathy, etc. (77). This is reminiscent of other viral illnesses; influenza, rubella etc., but also includes some neurological involvement (78). This mononucleosis-type syndrome has been described for all risk groups (77-81). No anti-HIV 1 is detectable at this time although symptoms have been known to persist until seroconversion (79). An increase in the number of circulating T8 cells and therefore a change in the T4:T8 ratio is seen. The estimated time to development of symptoms is 3 to 6 weeks (80), however a British nurse developed symptoms 2 weeks after a needlestick injury (81). In contrast acute illness is rarely documented in haemophiliacs (82). Therefore the incubation time and severity of symptoms may vary according to the route and size of the virus inoculum.

The first serological marker detected is HIV-1 antigen (Ag) (83). This antigen, mainly core (p24) Ag is present in the free state and in immune complexes. Antigenaemia occurs before or at the time of primary symptoms (84). In most cases Ag is present early and transiently. Goudsmit et al. (85) detected HIV-1 Ag in 11 out of 35 homosexual men who seroconverted during the period of study, in 5 men this was prior to antibody seroconversion. Allain et al. (86) found a similar prevalence (35%) of antigen positivity in a group of

seroconverting haemophiliacs; antigen was present up to 9 months prior to seroconversion in 9 of the antigen positive patients. Timing of patient sampling probably precludes detection of HIV-Ag in many studies as it is produced transiently. It is also possible that antigen may not be produced at all in some cases or that the Ag test lacks sensitivity in the acute phase. However, HIV-1 can be isolated from plasma at the time of primary infection (87,88) and reflects a true viraemic phase prior to seroconversion. Persons at this stage are potentially highly infectious.

A primary anti-HIV 1 IgM response has been detected; it occurs one to two weeks after onset of antigenaemia, it may persist in the presence of IgG and may circulate for 8 to 41 weeks (83,89,90). The IgM antibody recognises only a limited group of antigens, mainly core p17 and p24 (89) proteins, on a western blot. HIV-1 IgM is not always detected prior to the IgG antibody response even by the most sensitive tests and therefore its role in blood donor and clinical screening is of very limited value.

Some 6 to 8 weeks elapses between infection with HIV-1 and production of IgG antibodies. Analysis of seroconversion samples using western blots showed a predominant anti-p24 reactivity as the first evidence of infection followed by other gag proteins then env and pol (79,91-93). In contrast radioimmunoprecipitation (RIPA) detected anti-env (anti-gp160) before anti-gag reactivity (80,91). This illustrated differences in detection ability of the two techniques at that time. Western blots now detect anti-env during seroconversion (94). Allain et al. (86) have also shown, using a competitive enzyme immunoassay (Abbott Diagnostics), that anti-env antibodies are produced earlier than anti-gag antibodies in HIV-1 infection.

From the information described above, a diagrammatic representation of the putative time course of serological events in HIV-1 infection can be constructed and is shown in Fig.1.2. In summary HIV-1 antigen is produced around the time of acute infection, 3-6 weeks after encountering the virus. This acute infection is manifested by symptoms of a viral-like illness. An IgM response may be detected around this time followed 2-4 weeks later by anti-HIV 1

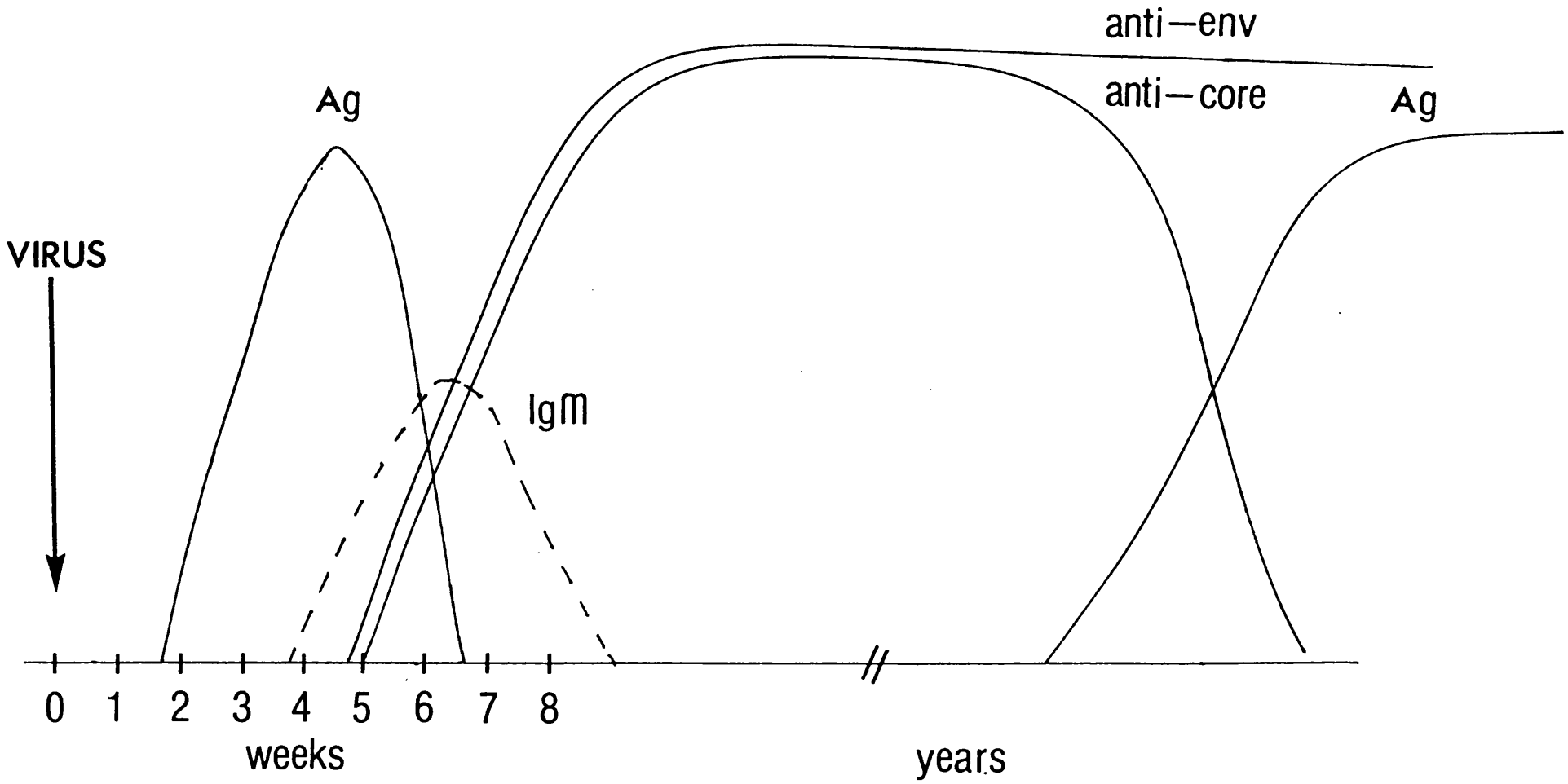


Figure 1.2 Serological time course of HIV-1 infection.

Ag : Antigen

IgG. It is important to note that not everyone has a documented illness and it may not always be possible to detect serum antigen during the acute stage. The best and most reliable marker is still the detection of anti-HIV 1 IgG. Thus antibody negative, virus culture positive/plasma viraemia presents a problem when screening. In the blood transfusion situation this puts potential recipients at risk. Transmission of HIV-1 by antibody negative blood donors has been documented (95,96) and occurred in the West of Scotland, where two recipients were infected from a single donor (97). Thus reducing this window period has been the goal of those involved in development and manufacture of screening tests for HIV-1.

Infection with HIV-1 is life-long because once integrated into the host genome chronic infection is established. Serum HIV-1 Ag is usually undetectable following seroconversion and during the ensuing chronic phase, probably due to the formation of immune complexes with anti-HIV 1. Persistence of serum antigen after seroconversion has been associated with rapid clinical progression (99). HIV-1 infection is not a truly latent infection as recent evidence has shown, by culturing HIV-1 from plasma, that a small amount of viral replication occurs throughout all stages of infection and thus such body fluids are potentially infectious (33,100).

The end stage of HIV-1 infection is AIDS. Development of clinical syndromes associated with AIDS is often, though not always, accompanied or preceded by serum antigenaemia. Goudsmit et al. (85) detected HIV-1 serum antigen in 86% of adult and paediatric cases of AIDS but less often, only 8%, in asymptomatic patients. In addition early serological studies using western blots had noted a decline or loss of core antibody in AIDS patients (92,101,102). A longitudinal study by Lange et al. (92) revealed that anti-core reactivity decreased before or at the onset of symptoms. In contrast, anti-env reactivity was retained without evidence of decline and no association of anti-env with other markers and clinical outcome could be established (103).

A link between the changes in these two markers, loss of anti-p24 and appearance of serum Ag with clinical progression was further established (104-107). Lange et al. (104) found that

persistent antigenaemia and decline of anti-p24 reactivity preceded the onset of AIDS. Complexing of excess Ag was implicated in the mechanism of reduction of anti-p24 reactivity. However Nishanian et al. (98) recently reported that the decline of anti-p24 was most likely a result of the reduced ability of the immune system (IS) to produce anti-p24 as they could show no excessive Ag production. Detection of such changes in antibody and antigen indicated increased expression of viral genes and thus a switch from latent to active HIV-1 infection. Persistent activation of viral genes would herald clinical progression.

A representation of this information is shown in the extended part of Fig.1.2. However it is important to note that this pattern is not standard in all AIDS patients and that deviations do occur, e.g. lack of detectable antigen during AIDS, simultaneous presence of both antibody and antigen etc. The time to development of AIDS is variable and only estimates can be made from following cohorts of seropositives. Measurement of serological markers may predict progression and could aid in assessing the prognosis of a patient. Loss of antibody and/or appearance of antigen may be associated with a poor prognosis and may allow earlier intervention of drug therapies.

ANTI-HIV DETECTION

Antibody Screening

Upon infection with a viral agent, the body's immune system, specifically the B-cells are stimulated to produce antibodies. Hence detection of antibodies to a specific virus in the blood of a patient indicates previous exposure to the virus. As the nature of the infectious agent causing AIDS, the methods by which it is transmitted and the population most at risk of being infected were elucidated, antibody screening tests were developed to identify those who had been infected. In this way an attempt could be made to control the spread of virus by education, information and counselling the seropositives to adopt a change in life-style towards low-risk behaviour and to identify infected blood donations and so prevent transfusion of HIV by

blood and blood products. Transfusion-acquired AIDS was a particular problem in the USA, early in the epidemic, because of the policy in the American blood banks of paying donors for their blood donation. Large numbers of the homosexual population made regular visits. So there was clearly a need for a reliable test to screen out infected blood and prevent it being used in transfusions or the manufacture of blood products.

The technology and reagents involved in identification and characterisation of the virus were used in the development of a screening test (30). The first assays were introduced in 1984, and early in 1985 the United States Food and Drug Administration (FDA) licensed the first commercial kit. Since then there has been a profusion of commercial kits available for the detection of anti-HIV and the majority of tests use enzyme-linked immunosorbent assay (ELISA) technology.

A good screening test requires high sensitivity and specificity to prevent false negatives and false positives, either of which would have disastrous consequences in any screening programme, particularly in the Blood Transfusion Service (BTS). Ideally the test would detect antibody at all stages of infection, especially new infections when a patient seroconverts and antibodies to only a few proteins are present and in the late stages of infection when antibody titres are decreasing. The test needs to be robust, user friendly, adaptable for screening either large or small numbers depending on requirement, with few steps to reduce the possibility of human error and have a short performance time to allow high throughput especially for centres such as the BTS who are testing large numbers of sera. The design of suitable tests can be divided into a few basic types as described by Mortimer et al. (108). These are described more fully in Appendix 1.

Screening has been performed at the Hepatitis Reference Laboratory (HRL) since April 1985. Prior to this time a small amount of testing was performed by immunofluorescence using LAV-infected and uninfected ready-prepared slides (Diagnostics Pasteur). Routine screening of all blood donations by the Blood Transfusion Service (BTS) began in the United Kingdom on the 14th October 1985. Before screening commenced an assessment of five commercial kits, using a

panel of 360 selected sera (including blood donors, high-risk groups and sera likely to cause false positive readings) was performed by the PHLS Virus Reference Laboratory at Colindale (108). This evaluation identified problems with certain kits especially the performance of heat-treated specimens which gave rise to false positive results. But, in general, the kits were found to be adequately sensitive with the Wellcome and Organon kits being particularly good due to a higher specificity. In an evaluation of six first generation ELISAs, Reesink et al. (109) also found high values for sensitivity with good specificity particularly in the Wellcome and Pasteur tests. The Wellcozyme anti-HTLV III EIA (Wellcome Diagnostics) was chosen for use in the Scottish BTS.

Frequent evaluations have been performed on the commercial kits as they have evolved and improved with appropriate use of seroconversion panels, rather than dilutions to assess test performance.

First Generation Tests

The first generation of tests used cell culture derived viral lysate to coat the solid phase and a number of problems were identified with this source of antigen. The first FDA licensed kit, the Abbott HTLV III Antibody EIA was found, in use, to lack specificity. False positives occurred in testing patients with antibodies to human leukocyte antigens (HLA), especially haemophiliacs and renal unit patients. T cell-associated HLAs become incorporated into the surface of the virus as newly synthesised virus particles are released by budding out from the T cell membrane. These are then present in the viral preparation used to coat the bead. Testing with anti-HLA sera by Kuhnl et al. (110) showed that 37% HLA-DR antisera gave positive results in this test; they suggested that DR4 is present on the H9 cell, the cell line clone used to grow HIV-1 (3) and is therefore present on the antigen-coated beads. The H9 cell line has been HLA-typed by Weiss et al. (111) and found to include DR4 and DQW3 amongst other HLA specificities. However evaluation of the HLA specificity of sera reactive in the HIV ELISA test systems is no

substitute for a confirmatory test to show an HIV-specific reaction since genuine positives may also have an anti-HLA serotype (112). Heat inactivation of the virus (56^{o}C for 30 minutes) in patients' sera also interfered with the test, increasing the optical density and giving false positive results but the mechanism for this is unknown (113).

Rapid Tests

A number of alternative assays were developed which were rapid to perform, required minimal equipment and no automation. These qualities are important in field studies or in developing countries where money and other resources are limited, and conditions for storage and testing are far from ideal. In such situations tests need to be robust, sensitive and easy to perform without necessitating the use of electrical equipment or other expensive hardware. These tests include the Dipstick and HIVCHEK (Du Pont) and Testpack (Abbott) and a particle aggulination test, Serodia-HIV (Fujirebio Inc.). This latter test was found to be highly sensitive and specific (114). However others were short-lived on the market, e.g. Dipstick (Du Pont) which lacked sensitivity. However more recently, five of these rapid, visually-read assays including Serodia-HIV (Fujirebio Inc.) and HIVCHEK (Du Pont) were evaluated on fresh clinical samples in Zaire. The sensitivity of these assays ranged from 84.6% to 99.1% and the specificity from 92.7% to 98.8% (115). The authors suggested these tests although less sensitive could be used in developing countries particularly for large-scale blood donor screening.

Second Generation Tests

As more information was gleaned about the virus and the immune response to it, tests were evaluated and improved in an effort to close the window of infection, i.e. that time between infection with the virus and production of antibodies. A second generation of screening assays were developed using recombinant proteins or synthetic peptides to avoid the problem of anti-HLA reactivity.

Sequences from env regions were the predominant epitopes coated onto the solid phase. First generation tests detected anti-p24 predominantly. It was thought to be the first antibody produced (92,93,101). However evidence from other serological studies showed that anti-env was the most reliable marker of infection as it was produced early in response to infection and also retained in the later stages of disease (86,91,94).

Second generation tests detected anti-HIV earlier than first generation assays. In a comparison of three first and three second generation tests (from the same manufacturers), second generation tests were far more sensitive than the first generation ELISAs both in earlier detection of anti-HIV 1 at seroconversion and in testing serial dilutions (116).

Combination Tests

The isolation of HIV-2 from West African AIDS patients and the subsequent emergence of HIV-2 infection in the USA and Europe prompted the development of anti-HIV 2 ELISAs (Diagnostics Pasteur). Some serological cross-reaction occurs between HIV-1 and HIV-2 and it was found that the anti-HIV 1 assay could detect HIV-2 seropositivity. The type II assay format for HIV-1 (competitive test) was the worst performer in detecting anti-HIV 2 (117); the affinity of the cross-reacting antibodies may not be sufficient to compete with the anti-HIV 1 conjugate. The prevalence of HIV-2 seropositives detected by indirect anti-HIV 1 ELISAs ranged from 70% to 93%, clearly demonstrating the serological cross-reactivity of HIV-1 and HIV-2 (117).

Screening assays for anti-HIV 1 have now incorporated recombinant protein or synthetic peptides from HIV-2 onto the solid phase and thus anti-HIV 2 reactivity can be assayed in the same sample well as anti-HIV 1. The second generation anti-HIV 1 assays had achieved high degrees of sensitivity and specificity up to this point and it was thought that the addition of a second virus might compromise this. However 9 out of 9 combination (combi) tests retained 100% sensitivity in detecting anti-HIV 1 in a PHLS/Department

of Health (DH) evaluation (118). Specificity ranged from 94.6% to 100% in the tests examined. In a supplement (119) to this evaluation, anti-HIV 2 performance was assessed in the same 9 kits. All assays examined were found to be adequately sensitive as screening tests for the detection of anti-HIV 2. Sensitivity was highest in the tests developed by Bio-Stat Diagnostics Ltd. and Wellcome Diagnostics. It is interesting to note that the Abbott Anti-HIV 1/2 Testpack a rapid test, despite low specificity, was as sensitive as the conventional ELISAs in detecting anti-HIV 1 and anti-HIV 2.

A lack of readily available HIV-2 antibody positive samples and seroconversion panels in the UK prevents a larger evaluation of anti-HIV 2 detection performance. However the priority in screening is the detection of anti-HIV 1 as HIV 2 infection is still rare in the UK. Further serological discriminatory tests and confirmatory tests have to be performed to establish the exact nature of the reactivity of any test specimens in these combination assays.

Confirmatory Tests for Detection of Antibody

The problem of false negatives (FN) can only be addressed as test sensitivities improve since this occurs usually in persons infected with the virus who present for testing in the initial incubation phase. However false positives (FP) can be tested further. A positive HIV test result is devastating and has far reaching consequences for the patient. Therefore a screening programme for testing members of high and low risk populations must attempt to minimise both the FN and the FP rate to give the best predictive value for the test result. All ELISA reactives were therefore further assayed by a test with a higher specificity which was usually of different technology and used an alternative source of antigen. Since a positive confirmatory test is a definitive diagnosis of HIV infection these tests must be subject to strict criteria of interpretation.

Four types of tests have been used in this capacity.

1. An indirect immunofluorescent (IF) test. Slides were coated with HIV-1 infected and uninfected cells. Antibody in the

test specimen bound to viral antigens on the slide was detected with a fluorescent conjugate.

2. A competitive enzyme immunoassay using a dual-bead system (Abbott Confirmatory EIA, now Abbott ENVACOR HIV-1 EIA) was developed by Abbott Laboratories. A recombinant protein from the core sequence and from the envelope sequence of the viral genome were coated onto individual beads and assayed independently.

Two further test systems examined the antibody response to individual viral proteins.

3. The western blot assay (WB): viral proteins from a cell culture derived lysate are electrophoretically separated in a polyacrylamide gel. The proteins, separated according to molecular weight are electrophoretically blotted onto a nitrocellulose (NC) membrane. The NC is cut vertically into strips and antibodies in the test sera are reacted with the viral proteins bonded to the NC. Incubation of the strip with an enzyme conjugate followed by substrate reveals an insoluble coloured product at the site where antibody in the clinical specimen has bound. The nine structural proteins of HIV-1 form a distinct separation pattern and it is therefore easy to examine the antibody response of an individual to a range of viral antigens.

4. In the radioimmunoprecipitation assay (RIPA) human serum is reacted with metabolically radiolabelled viral-infected cell cultures. The antibody-antigen immune complexes formed are removed and separated onto a gel. The result is visualised following autoradiography. Those viral antigens recognised by antibodies in the test serum form a distinct separation pattern on the gel.

Immunofluorescence

Initially immunofluorescence was favoured as a screening test in some laboratories. As a confirmatory test, it performed well in comparisons with other confirmatory tests. Carlson et al. (120) found 100% agreement with WB results.

In the hands of experienced persons, IF is a good reliable and rapid test. However the differentiation of specific from

non-specific fluorescence may be a problem for persons with little experience of the technique.

The Confirmatory EIA

The Confirmatory EIA (Abbott Diagnostics) has the advantage of being a ready-to-use ELISA requiring no extra specialised equipment for a diagnostic laboratory. In a small evaluation the Confirmatory EIA results were in complete agreement with IF or WB results in sera from high-risk donors, with only two samples, from low-risk blood donors, which were discrepant (121). However as the screening tests improved, the sensitivities of the second generation ELISAs bettered that of the Confirmatory EIA and its role changed to that of a supplementary test for the screening ELISAs rather than confirmatory.

Radioimmunoprecipitation

RIPA has been used primarily as a research tool for characterising viral proteins (46). Since the viral lysate is prepared under milder conditions than that for WB, this helps to preserve the conformational epitopes. The antibody-antigen complexing reaction occurs in a liquid phase allowing maximal exposure of epitopes, and thus it is a sensitive technique, particularly for the detection of envelope glycoproteins. This is an advantage in the diagnosis of early HIV-1 infection. Tersmette et al. (122) developed a glycoprotein-enriched assay (GRIPA) which they found to be at least as sensitive as the WB on seroconversion samples. In addition those samples considered to be false positive (FP) by WB were negative by GRIPA (and virus culture). RIPA results would therefore be useful in distinguishing between false positivity and seroconversion in an indeterminate WB where an isolated p24 reactivity by RIPA was claimed to represent a true antibody response in contrast to an isolated p24 reactivity on WB (122,123).

However to perform RIPA requires facilities to grow virus and use radioisotopes. This is often impracticable and beyond the scope of most diagnostic and some reference laboratories. RIPA is not

performed routinely as a confirmatory test for anti-HIV 1; the WB with its new enhanced envelope detection performs adequately.

Western Blotting

The technique of western blotting, was first described by Towbin et al. in 1979 (124). Its use and development as a tool in the confirmation of HIV infection was its first introduction into diagnostic virology. The setting up and running of electrophoretic gels and blots is a time-consuming process requiring specialised equipment and technical expertise. However commercial kits are available, only one of which has been licensed by the FDA (Western Blot Assay kit for the detection of IgG antibodies to HIV-1, Du Pont). In a comparison of 3 WB tests, an in-house test and two commercial kits, the Du Pont test had the highest sensitivity and the highest specificity (125).

Pre-blotted nitrocellulose is supplied and the western blot test becomes an ELISA, where the product of the enzyme-substrate reaction forms an insoluble precipitate at the site of the Ab-Ag complex formation on the NC.

The nature of the test requires interpretation of the result from a series of bands on the NC strip. Initial problems arose because the test, whether in-house or commercial, was not fully standardised. Individual laboratories detected different bands in the same collections of sera, thus making evaluation and the setting of criteria for positivity very difficult. At the start of screening and confirmation, the predominant reactivity observed was anti-p24. In 1985 the CDC guidelines on the minimum requirement for a positive blot was reactivity to p24 and/or gp41 (126). However caution in interpreting an isolated anti-p24 reaction was necessary following reports of false positive or indeterminate results. In 1986 Biberfield et al. (127) first reported the presence of false positive WB bands at p24 and p55 in 3 low risk blood donors. These donations were reactive in 3 screening ELISAs but negative by 2 or 3 other ELISAs and by IF. The same ELISA reactivity and anti-gag WB pattern were present in follow-up samples, 1-3 months later. Further reports

in the literature corroborated this finding and demonstrated that bands at p17, p24 and p55 alone or in combination was the motif most commonly seen in falsely reactive sera (128,129).

The cause of this FP reaction is unclear. Blomberg and Klasse (125) noted that 3 out of 12 commercial species anti-human IgG conjugates gave relatively strong bands at p24 and a rabbit anti-tubulin serum reacted to proteins indistinguishable from p24 and p55. In addition anti-HLA class I and II sera reacted with proteins migrating close to gp41 and p31 respectively. Clearly some cellular human proteins from the cell culture which co-migrate with the viral proteins are the cause of some FP reactions. The use of WB strips prepared from mock-infected cell culture lysates has had some success in confirming the cellular nature of FPs.

A variety of criteria have been established and one in particular has been recommended by the Centre for Disease Control, Atlanta (130), i.e. a positive result is determined by the presence of antibodies to any two of p24, gp41 and gp160/120 since this gave the highest percentage of positives and the lowest percentage of indeterminate results.

The western blot test is not the gold standard and various problems can be identified including the subjective nature of interpretation, lot-to-lot variability, length of time to perform, and the occurrence of FPs. Despite these shortcomings the western blot remains the test of choice in most reference laboratories. More information regarding antibody specificity can be instantly obtained from a WB result. Different patterns on the WBs of individual patients were noted and it was speculated that a WB may give extra information relating to the stage of infection. Both the banding pattern and the intensity of reaction can give such an indication, e.g. at seroconversion, high intensity to few bands and in terminal stages where lack of anti-p24 is a common feature.

Recombinant Immunoblot Assays (RIBA)

The newest development is that of recombinant immunoblot assays (RIBAs). These consist of nitrocellulose strips with lines of

recombinant proteins painted onto the surface. Obvious advantages include the exclusion of human cellular proteins, choosing immunogenic regions from which to make peptides and the addition of HIV-2 specific recombinant peptides to allow the simultaneous discrimination of HIV-1 and HIV-2. These tests, in general, contain a maximum of about 4 peptides for HIV-1 and only one peptide for HIV-2. Therefore a decision regarding positivity is based on a reaction to fewer bands and these represent linear rather than conformational epitopes.

3. EPIDEMIOLOGY OF AIDS AND HIV-1 INFECTION

AIDS: THE EPIDEMIC

The acquired immune deficiency syndrome, AIDS, was first described by Gottlieb et al. in 1981 (59) following their previous report of an increased incidence of Pneumocystis carinii pneumonia (PCP) in young homosexual males in the U.S.A. (131).

The first report (131) discussed the occurence of PCP in 5 previously healthy homosexual men in Los Angeles. Prior to this time, PCP had only been found in patients with an already impaired immune system or in persons undergoing immunosuppressive treatments. At the same time there was a report of a rare malignant tumour of blood vessel tissue in the skin and internal organs called Kaposi's sarcoma (KS), occurring in twenty-six previously healthy homosexual men in New York and California (132), some of whom also had Pneumocystis pneumonia. Increased reports after this time described patients suffering from overwhelming infections with opportunistic micro-organisms either protozoal, fungal, bacterial or viral in origin, e.g. cryptosporidium, candida, mycobacterium or cytomegalovirus (CMV) respectively. Something was happening in the gay population as increasing numbers fell victim to a devastating depression in immune function through an alteration in T cell subsets allowing opportunistic infections (OI) to ravage the immune system and kill otherwise healthy, active, young men. Various physicians, immunologists and epidemiologists at the time became aware of this but were puzzled; perhaps a new and particularly virulent strain of CMV

or a new infectious agent was the cause. These patients had acquired an underlying immune deficiency which allowed such infections to become established. The cause, however, remained unknown.

The United States Centre for Disease Control (CDC) began a national surveillance for these conditions, PCP and KS, in June 1981 and produced a guideline definition (60). These criteria were specified to help detection of AIDS in the population and to identify those at risk. A task force (Kaposi's sarcoma and opportunistic infection, KSOI, task force) was sent out from the CDC to identify what common factors, if any were present in those patients with symptoms (133). Various aspects of homosexual behaviour were examined; a bad batch of nitrate inhalants, a stimulant used frequently by homosexuals was suspected. However the argument for a sexually transmitted infectious agent was strengthened when the astonishing numbers of possible sexual contacts (up to 250 or more per year) were revealed and indeed it was this factor and the incidence of venereal disease associated with increased number of sexual contacts which distinguished the cases from the controls.

AIDS was originally thought to be a 'gay-person's' disease and the name Gay-Related Immune Deficiency or GRID was coined. The increased surveillance of clinical cases and their contacts implied an infectious aetiology. The causative agent was unknown but its spread in the gay population was reminiscent of hepatitis B, a blood-borne disease. Thus if it could be spread sexually, it could also be spread parenterally and thus additional at-risk populations were identified. Case reports of unusual opportunistic infections were noted in haemophiliacs (Hm), intravenous drug abusers (DA), Haitians, recipients of blood and blood products, sexual contacts of these groups and in babies particularly in those with a drug abusing mother and more frequently in ethnic populations, blacks, hispanics and Haitians (60,61).

Although the first case reports came from the U.S.A. in the late 1970s, early 1980s, it soon became clear that this was not just an American health problem. Reports of AIDS soon followed from Europe among the same risk groups; homosexuals, drug abusers and haemophiliacs. In addition, an AIDS-like illness was reported in

Negro patients, native to equatorial Africa, who were in hospital in Brussels and Paris. Further investigation revealed AIDS in Africa itself, particularly Zaire and Rwanda at this time and later in most Central and East African countries (134). But for those African patients in hospital in Europe, this disease syndrome may have gone unnoticed in this continent for longer as multiple infections especially of a respiratory nature, e.g. tuberculosis, are a routine medical problem in Africa. Various aspects of AIDS in Africa and the similarity to a monkey disease have led many to believe that the origin of AIDS and its causative agent come from Africa itself.

Thus the world was on the brink of a global epidemic of a killer disease with an infectious aetiology in the early 1980s. Until the causative agent was identified, a diagnosis of AIDS was based on clinical observation of opportunistic infections. In addition several prodromal stages or pre-AIDS conditions were noted, i.e. persistent generalised lymphadenopathy (PGL) and AIDS related complex (ARC) (135). Several staging systems have been developed to classify patients at the various stages leading to development of AIDS. The two most commonly used are: the CDC system (Stages I through IV) which is based primarily on clinical signs, symptoms and opportunistic infections; an alternative method, the Walter Reed (WR) staging system involves 6 stages, WR1 to WR6, defined by T4 cell counts, other immune indicators, e.g. skin tests, and clinical manifestations (136). The CDC definition has been updated and further signs and symptoms associated with AIDS have been added (137) including neurological involvement (e.g. encephalopathy) and that of a wasting syndrome or slim disease which was a major feature of disease presentation in Africa (138). Thus cases of AIDS were reported to the CDC allowing epidemiologists to examine the dynamics of the epidemic as it changed over time. When reporting started in the USA, in 1982, the doubling time, i.e. that time for the number of reported cases to double was 5 months, by 1985 this had increased to 11 months (139).

Since the start of the epidemic case reports and transmission dynamics have been analysed and extrapolated to make predictions about the numbers of cases expected over a specified time period. The reported cases are likely to represent the minimum number of cases at

any one time due to the many problems in epidemiological surveillance of such a disease. Under-reporting and delays in reporting make it more difficult to establish trends. The up-dating of the AIDS case-definition also affects the numbers and encourages retrospective reporting giving a false boost in the total numbers at any one time.

To the end of June 1990 a world total of 266,098 cases of AIDS, across 5 continents from 157 countries, had been reported to the World Health Organisation (WHO) (140). The WHO estimates that the true figure is closer to 700,000 and expects a cumulative total of one million cases worldwide by the early 1990s and 5-6 million cases by the year 2000. The majority of cases come from the Americas (61%) with 50% of the world total in the U.S.A. alone and 25% from the African continent with Europe contributing 13% of all cases. The numbers quoted from these countries represent absolute numbers and at such a high level are worriesome, however they are not rates per capita so the extent of the problem is not evident from the bare data. To the end of June 1990, the total number of cases of AIDS reported in the UK was 3,433 (141). This represents a rate of 59.8 per million population. The majority (80%) were homosexual or bisexual, only 3% were drug abusers, 6% were haemophiliacs with between 1-4% in the other risk groups. In the same time period, a total of 155 cases of AIDS (136 male and 19 female) had been recorded in Scotland; this is a rate of 30.3 per million population. A breakdown of the total by risk group reveals 51% were homosexuals (or bisexuals) and 28.4% were drug abusers. AIDS in Scotland has been recorded in all risk groups (Fig.1.3). The mortality rate has always been close to 50%. However as patients now live longer once diagnosed with AIDS the rate has dropped to around 43%.

In Glasgow the first case of AIDS was a homosexual diagnosed retrospectively in 1984. He had travelled to the U.S.A./Caribbean. A total of 53 cases (37 deceased) have now been reported in Glasgow and the West of Scotland (Mrs. G. Allardice, CDSU, personal communication) up to the end of June 1990. The majority (43%) of cases are homosexuals, however the numbers in the other risk groups are increasing. This is most probably due to the time of arrival of the virus in the different risk groups.

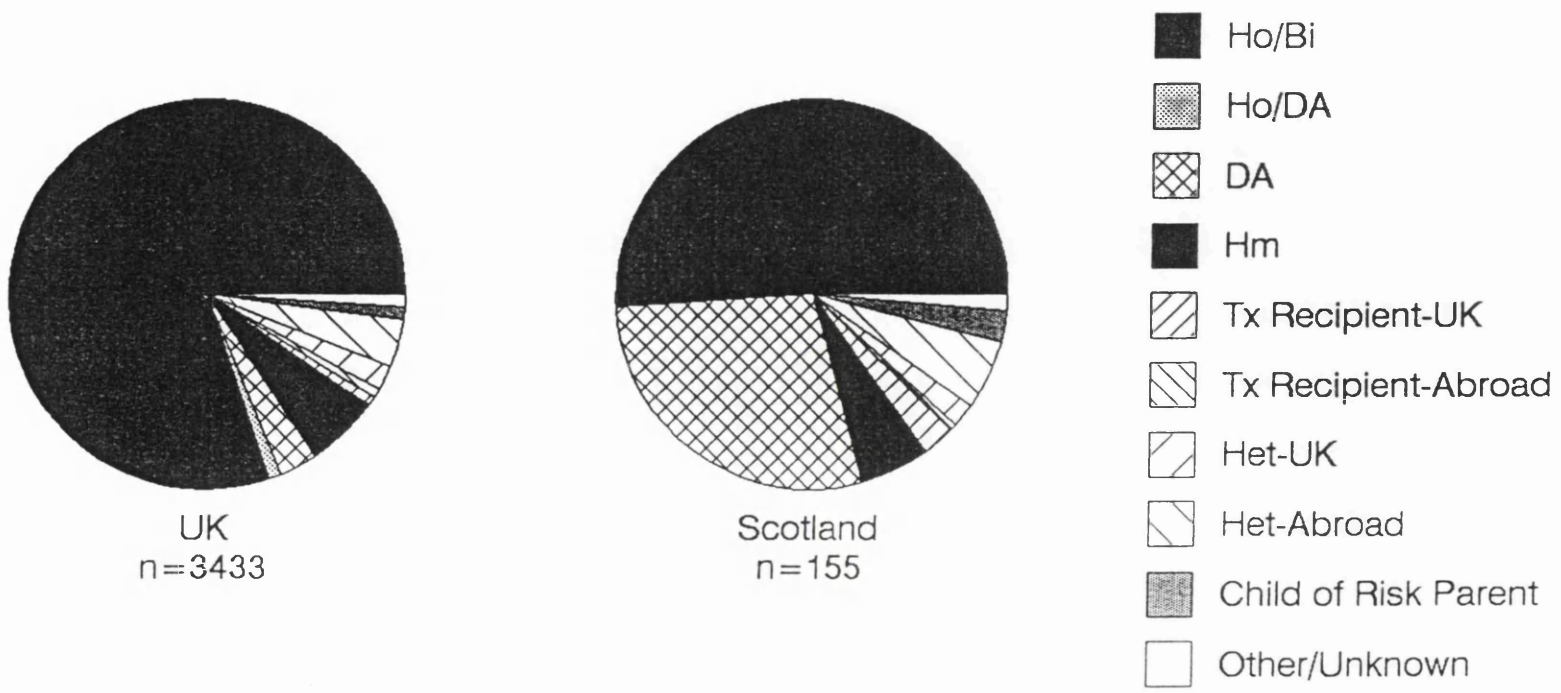


Figure 1.3 Cases of AIDS by risk group in the UK and Scotland to the end of June 1990

An average of 9 new cases of AIDS per quarter in Scotland and 186 new cases in the U.K. have been reported since September 1987. However this has increased to 14 per quarter between June 1989 to June 1990 in Scotland and 265 per quarter in the UK. (Figures extracted from the surveillance data published in the CDS weekly report, AIDS News Supplements). Again this is probably due to the later arrival of the virus in certain at-risk populations who are now contributing to the AIDS figures. In addition measures introduced to slow down the disease process, e.g. drug therapy, means a longer incubation period between infection and AIDS for those infected earlier.

HIV-1 INFECTION

However, reports of AIDS cases give no information about new infections, only about those already infected. In addition the numbers of persons with AIDS make up only a small fraction of the total who harbour the potential to develop AIDS. An analogy to this "tip of an iceberg" phenomenon has frequently been made. The discovery and characterisation of the infectious agent causing AIDS added a new dimension to the epidemiology. When a screening test was developed the numbers who had been exposed to the virus could be determined. This is the reservoir from which AIDS cases arise. Early serological studies showed a high prevalence of HIV-1 infection, determined by the presence of anti-HIV 1 in patients' sera in the population groups with AIDS. Identification of these at-risk groups and modes of spread could be helpful in limiting the spread of the epidemic through information, education and counselling aimed at encouraging behavioural change and therefore reducing viral transmission.

Modes of Transmission (142-144)

HIV-1 is transmitted primarily during sexual contact, through parenteral exposure to blood and blood products and perinatally from mother to child. Sexual contact between males is the most common route of spread reported to date. The epidemic in the U.S.A. began

in this group. In the U.K. up to the end of June 1990, 52% of reported seropositives are homosexual men. Although HIV-1 has been isolated from semen, additional homosexual practices causing trauma to the lining of the rectum facilitate entry of HIV-1. Thus anal intercourse transmits HIV efficiently. The risk of infection increases with the number of sexual partners, i.e. as the chances of coming into contact with an infected person increase. The receptive partner is at greater risk of acquiring HIV-1. A few cases involving orogenital contact have been reported; this is a rather less efficient mode of transmission. The frequency of female to female transmission is very low; in one case this was reported to have resulted from traumatic sex practices.

Male to female and female to male transmission can occur as HIV-1 has been isolated from both semen and cervical secretions. Heterosexual spread was noted to be on the increase in the U.S.A. during the late 1980s, this was originally through contact with high risk groups. However heterosexual spread of the virus outwith the high risk groups is the worry and concern of the 1990s. This is highlighted by the situation in Africa where the HIV-1 infected male to female ratio is approximately one. This is principally in the sexually-active (25-40 years old) age groups suggesting that heterosexual contact is the major means of transmission of the virus in this continent (134). The ratio of male to female HIV-1 seropositives in the U.K. is 8.35 up to the end of June 1990 (145) and heterosexual contact contributed less than 7% of the total seropositives, the majority of whom had known exposure abroad, predominantly in Africa (145). More recent reports suggest this is increasing, although the total numbers are still small. Current surveillance data indicate the greatest increase is in males or females with partners from abroad (146). Different rates of heterosexual transmission have been noted. There is also varied opinion on the role of co-factors and risk factors influencing transmission, e.g. numbers of sexual contacts, frequency of contact, infection with other sexually-transmitted diseases, disease stage of index patient, genetic factors (HLA type) etc. (147). These may operate more or less efficiently in different populations and in

different risk groups.

Parenteral exposure occurs in intravenous drug abusers, DAs, through the sharing of needles and syringes contaminated with infected blood. Injecting practices such as 'wash-out' coupled with the large groups who share together in shooting galleries promote the spread of the virus in the drug abusing community. It is important to note that the drug abusers are the major reservoir for perinatal transmission and spread into the heterosexual population through non-drug abusing sexual contacts.

Transfusion of infected blood and blood products is associated with a high rate of seroconversion. All components of blood, e.g. platelets, concentrated red cells, plasma, etc. can transmit infection. A more advanced disease stage in the donor is associated with a greater risk of seroconversion in the recipient and possibly an increased rate of progression to AIDS in the recipient. Transmission by this route can now largely be prevented both by appropriate donor deferral and blood donor screening. However cases have been reported of seroconversion in recipients after transfusion with screened blood. The donors, seronegative at the time of donation have subsequently developed anti-HIV 1. All tissue and organ donors are also screened for HIV-1 antibodies as transmission has been documented in transplant of tissues such as kidney, liver, heart, pancreas and bone. Clotting factor concentrates (Factor VIII and Factor IX) were responsible for infection in the haemophiliac population. A method of heat treating the factor concentrates to inactivate the virus was introduced in 1984 which resulted in a reduced incidence of infection. The risk is now virtually nil, perhaps in time with the current advances in genetic engineering, recombinant FVIII will be used routinely thereby removing the risk of infection with any blood-borne virus.

The main risk of parenteral exposure to HIV-1 in health-care workers (HCW) is through needle-stick injuries and has been associated with a 0.9% seroconversion rate. Three health-care workers seroconverted following non-needlestick exposures of infected blood probably via mucous membranes or non-intact skin. Two laboratory workers became infected while working with concentrated virus. A total of 28 cases of documented seroconversion in HCW has been

reported world-wide including nurses, surgeons, dentists etc. (148). Therefore rates of transmission of HIV-1 in HCW are low and can remain so if safety precautions are adhered to and enforced.

The true rate of perinatal transmission is unknown and requires close follow-up of children born to seropositive mothers due to the passive transfer of maternal antibodies which can persist up to 12-18 months. Most transmission is thought to occur in utero during pregnancy or at birth in the immediate post-partum period as HIV has been isolated from foetal tissues and cord-blood. In addition breast-feeding is contra-indicated in seropositive mothers as this may be another mode of transmitting the virus. Perinatal transmission has been a significant problem in other areas of the world especially the U.S.A. and Africa; at one time in New York AIDS was the leading cause of paediatric deaths. It was also found in the U.S.A. (New York City) that infection rate varied with race and that it was greater in negroes and hispanics than caucasians; why this is so is unclear (144). A multi-centre study of paediatric infection in Europe (149) has found that the rate of transmission (13%) is now much lower than the original estimates. Current surveillance data include all antibody positive children no matter whether this is passive antibody from the mother or genuine antibody synthesised in response to true infection. Therefore many of those quoted in this risk category may not be genuinely infected.

There is no current evidence to suggest that other or more unusual modes of transmission of the virus exist. No non-sexual, household contacts of HIV-1 infected individuals were shown to seroconvert (150). There is no indication that HIV can be insect-borne despite the high numbers of cases in Africa where arthropod vectors are a common means of disease transmission (134). Thus HIV has only been reported in the risk groups described. Continued epidemiological surveillance is required to monitor the appearance of new risk groups and new modes of viral transmission.

The U.K. surveillance, including Scotland, England, Wales and Northern Ireland, of HIV-1 antibody positive reports totalled 14,090 to the end of June 1990 (145). The largest contribution was from the homosexual (including the bisexual) population with 51.2% of the

total. Fourteen percent were drug abusers, 9% haemophiliacs, 16% other or undetermined and the remaining risk groups, i.e. homosexual drug abusers, blood transfusion recipients, heterosexual contact and children of infected or at risk parent(s) only between 1 and 3%.

On closer examination of the figures, there emerges an interesting geographical difference in the seroprevalence of certain risk groups throughout the U.K. Forty-three per cent of the total homosexual/bisexual seropositives are located within the North West Thames reporting region with a further 25% from two other Thames regions (North East and South East) (145). In contrast 49% of the total drug abusers reported are from Scotland. A breakdown of the Scottish figures reveals that one area in particular, Edinburgh, is the greatest source of seropositive drug abusers. Evidently different behavioural and biological factors promoting transmission vary in different areas and allowed a rapid spread of infection in a high proportion of risk group members in a relatively short space of time. This is particularly so when there is a large pool of at-risk persons. The pockets of high prevalence of infection remain, although there is evidence that all risk groups in all areas, to a greater or lesser extent, are infected.

NATURAL HISTORY OF INFECTION

Since the start of the epidemic it has become evident that development of AIDS in HIV-1 seropositives is not an uncommon outcome. The numbers of cases of AIDS will increase despite a stabilising or slowing down in the incidence of new infection. Provisions for health care have to be made available to meet the demands of a growing epidemic and therefore estimates of the expected number of new AIDS cases are required. In general, estimates represent the worst-case scenario. Most estimates made at the start of the epidemic were not fulfilled due to changes in many epidemiological and behavioural parameters.

The risk of developing AIDS increases with the duration of infection with HIV-1, but the rate at which AIDS develops is variable. The incubation period may depend on the route of infection, the size

of inoculum, immune response in host, etc. The best estimates can be obtained from cohort studies where the length of time of infection is known, this is particularly so in transfusion-related cases. Most estimates suggest that the incubation period to AIDS is long, in general, between 7 and 10 years (151,152). No difference in progression rate was found in a study on a small sample of haemophiliacs and homosexuals from two different areas of the U.S.A. (153) and in a comparison of individual studies of these risk groups by Moss and Bacchetti (154). Cohort studies on cumulative progression rate where date of seroconversion is either known or estimated find that 10-20% of seropositives will progress to AIDS in 5 years (154-157) and 30-40% will have AIDS at 8 years after seroconversion (158-160). Thus the studies suggest that the probability of progression increases with length of time of seropositivity and that in the absence of treatment most persons will progress to AIDS within 10 years. Medley et al. (151) noted an age-related difference in latency period. In children under 5 years of age with transfusion-associated infection rapid progression occurred, on average in 1.97 years compared with 8.23 years in those aged 5-59 years. Studies on haemophiliacs suggests an older age at seroconversion correlated with a more rapid disease progression (158).

An interplay of viral and host co-factors can influence the rate of progression. This is an important consideration for those infected as behavioural factors, e.g. continuation of high risk activities is with in the control of the individual, but other biological and genetic variables are not. Co-infection with other viruses, intercurrent acute infections, virulence of the infecting strain of HIV-1 and host genetic factors have all been thought to contribute to progression, however the extent of the contribution of such factors is difficult to ascertain. Viruses such as HTLV-1 and the herpesvirus family are thought to interact at the molecular level enhancing viral replication (161). Other microbial infections and T cell mitogens which can stimulate the immune system may trigger increased viral replication (162). Variation in the replication capacity and cytopathic effect of different strains of HIV-1 *in vitro* has been noted (163), what significance this has *in vivo* is

uncertain. Certain human leukocyte antigen (HLA) phenotypes have been associated with development of AIDS particularly HLA-DR3 and associated antigens (164). Any one or a combination of the above factors can contribute to the rate of developing AIDS or whether an individual develops AIDS at all, however these factors cannot be routinely measured in the laboratory to give any indication of onset of disease.

Several prognostic indicators of progression have been identified in a number of European cohort studies (99,104,105,107). An association between loss of antibody to the core protein p24, production of free viral antigen, and decline in T4 cell counts with development of HIV-related symptoms were the most common findings. Other markers reflecting the more general state of the immune system, e.g. β_2-microglobulin, neopterin, α - and γ- interferon, interleukin-2, immunoglobulin levels etc. have also been evaluated in predicting disease progression. These markers may indicate those infected persons who will progress more rapidly and would therefore benefit from earlier intervention and prophylactic drug therapies as it becomes increasingly more likely that AIDS is the end stage for all HIV-1 seropositives. Measurement of the viral and some immune markers is possible using the commercial test kits which are available.

OBJECTIVES OF STUDY

When this study was begun in October 1985 the first objectives were to establish a reliable screening and confirmatory test system for HIV-1 to cover Glasgow and the West of Scotland. Using this system the epidemiology of HIV infection in the community was studied; risk-groups were identified and the prevalence of infection in these groups was measured over time.

Public concern about HIV and AIDS led to major health service commitments including establishing an outpatient and counselling clinic at Ruchill Hospital. It was thus possible to follow patients with infection over many years. Virological progression of the disease in such patients was investigated to see if there was any relationship to clinical progression.

CHAPTER 2

DETECTION OF HIV-1 ANTIBODY: SCREENING AND CONFIRMATORY TESTING

A. SCREENING FOR ANTI-HIV 1

INTRODUCTION

Antibodies synthesised in response to infection with human immunodeficiency virus type 1 (HIV-1) are detected in the serum of individuals by enzyme-linked immunosorbent assay (ELISA).

The first HIV-1 antibody test kits were developed in 1984, following the isolation of the putative agent for AIDS and its characterisation in terms of size, morphology and the nature of its structural proteins. Test kits have been commercially available since 1985 and the choice has been extensive. The tests discussed here comprise two basic designs as described more fully in Appendix 1; the Type I or direct binding/antiglobulin assay and the Type II or competitive assay.

The kits are evaluated and the performance characteristics e.g. sensitivity and specificity, defined by the manufacturers before use in routine diagnostic testing (Table 2.1). Since there is no recognised gold standard for establishing the presence or absence of HIV-1 antibodies in human blood the assumptions in Table 2.1 are required, that is,

(a) assuming 100% prevalence of antibodies in AIDS patients

(b) assuming 0% prevalence in random blood donors.

Initial evaluation of the available kits was performed by the Public Health Laboratory Service (PHLS), Virus Reference Laboratory, Colindale, London (108) prior to the beginning of routine blood donor screening in October 1985.

With the development of new technologies and the advent of improved tests, regular evaluations have been carried out by the PHLS. The most recent of these evaluations has been that of the combination ELISAs for HIV-1 and HIV-2 (118,119).

A second, related human immunodeficiency virus, HIV-2 causing an

TABLE 2.1

Performance characteristics of HIV-1 antibody ELISAs, defined by the manufacturers

	Abbott HTLV III Antibody EIA	Abbott Recombinent HIV-1 EIA	Wellcozyme anti-HTLV III	Wellcozyme HIV Monoclonal EIA
Sensitivity# (%)	93.4	100	100	100
Specificity* (%)	99.8	99.84	100	99.98
Date:	March 1985	July 1987	January 1987	September 1987

Assuming 100% prevalence of antibodies in AIDS patients

* Assuming 0% prevalence in random donors

AIDS-like disease in West Africa was isolated from two male patients with AIDS in Guinea Bissau and Cape Verde in 1986 (64). This virus can be transmitted in the same way as HIV-1. Very few cases of HIV-2 infection have been identified in the UK; a total of 12 by the end of December 1990 (74), but one of these was in a blood donor detected in 1989. Therefore it was decided by the BTS to screen for previous exposure to this virus and so eliminate it from the blood bank supplies. HIV-2 has been incorporated into screening tests and the ELISAs now take the form of a combined assay for antibodies to HIV-1 and HIV-2. These combination assays have been available since the end of 1988. In the UK Blood Transfusion Services the combined screen was introduced on 1st June 1990.

Evaluation of screening assays at the HRL has depended on the testing of a number of samples from high risk individuals. These included -

(i) seroconversions, where specimens are available both pre- and post- infection;

(ii) weak antibody positives where no previous antibody negative sample is available but blood samples have been withdrawn shortly after seroconversion before the levels of antibody saturate the test system, and

(iii) dilutions of antibody positive sera, either serial dilution of a single sample or in the case of pooled sera where one or more samples in the pool may be positive.

The results presented here are not intended as an evaluation of the test kits, but simply a look at the improvement in performance characteristics (i.e. sensitivity and specificity) of the tests available over the first four years of anti-HIV 1 screening.

The following results demonstrate how the different ELISAs performed with the array of samples at the HRL.

METHODS

The test kits used throughout this study were:

Abbott HTLV III Antibody	EIA	Type I	First Generation
Wellcozyme anti-HTLV III	EIA	Type II	First Generation
Abbott Recombinant HIV-1	EIA	Type I	Second Generation
Wellcozyme HIV Monoclonal	EIA	Type II	Second Generation

An alternative ELISA, the Dipstick assay (DuPont), is discussed and an introduction to the combination anti-HIV 1 plus anti-HIV 2 assays is made. The tests employed have been more fully described in Appendix 2.

Prior to commercial kits being introduced a selection of samples were tested by immunofluorescence (IF) using ready-prepared slides coated with LAV infected and uninfected cells (Pasteur). After April 1985, enzyme-linked immunosorbent assays (ELISAs) were used to screen for anti-HIV 1.

RESULTS

1. DETECTION OF HIV-1 ANTIBODY BY FIRST GENERATION TESTS

The first commercial test used at the HRL in April 1985 was the Abbott HTLV III Antibody EIA (Abbott Laboratories). Samples found seropositive were confirmed by IF using the slides, described above, at this time.

The haemophiliacs form a discrete population and stored sera were available from the mid-70s. Thus, retrospective screening was performed using the Abbott ELISA. At this time 14 out of 111 adults and 16 out of 57 children were found seropositive in 1982-84 samples. The test was used in a semi-quantitative fashion, as an increase in optical density (OD) was observed in those with serial samples. A low OD reading implies low antibody load. This may be an indication of recent infection or a property of the antibody species itself where only high avidity antibodies have bound to the solid phase and have therefore been detected. Table 2.2 shows the results from testing serial samples from 7 haemophilia patients. A slow rise in HIV antibody titre occurs in these patients. The results demonstrated that the Abbott ELISA could detect seroconverting patients and that low OD values could represent genuine, specific HIV-1 antibody.

The second test which came into operation at the HRL in October 1985 was the Wellcozyme anti-HTLV III ELISA (Wellcome Diagnostics). During the period of overlap, when both tests were being used, a few available samples demonstrated the increased sensitivity and reduced number of false positives in the Wellcome test at this time (Table 2.3). Sample 3 was from a hepatitis B surface antigen positive blood donor in 1986 identified through retrospective testing of such donors by the BTS. The ELISA result, when tested by the Abbott and Wellcome first generation tests at the HRL was negative at this time. Further testing was performed for the BTS, since their screening result by the Wellcozyme anti-HTLV III EIA was initially reactive and repeated within a 10-20% cut-off range. The sample gave three bands (p17, p24, gp41) on a western blot and the ENV bead was positive on

TABLE 2.2

Abbott HTLV III Antibody EIA results
from serial samples of seven haemophiliacs

Patient	Date	ELISA Result OD	Cut-off
Ha	21. 5.82	0.045	
	2. 7.84	1.570	
	8. 8.84	1.830	
	1. 4.85	>2.000	
			c/o 0.134
Hb	24. 2.82	0.045	
	6. 4.83	1.236	
	29.10.84	>2.000	
			c/o 0.134
Hc	29. 1.82	0.059	
	6. 8.82	0.126	
	10. 3.83	1.349	
	26.10.83	1.909	
	2. 7.84	>2.000	
			c/o 0.134
Hd	18. 1.82	1.654	
	17. 9.82	1.905	
	5. 7.83	>2.000	
			c/o 0.133
He	11. 1.82	0.092	
	26. 5.82	0.488	
	18. 1.83	1.286	
	24. 6.83	1.376	
	3. 9.84	1.826	
	25. 2.85	>2.000	
			c/o 0.133
Hf	31. 8.82	0.093	
	4. 2.83	0.284	
	9. 6.84	1.580	
			c/o 0.135
Hg	29.11.82	0.830	
	22. 2.83	1.127	
	13. 8.84	1.607	
			c/o 0.135

TABLE 2.3

First generation ELISA results and confirmatory results on several interesting samples.

Patient Category	Abbott HTLV III Ab EIA	Wellcozyme anti-HTLV III EIA	Confirmed Result*
1. LP1#	N	P	Positive
2. Drug Abuser	N	Eq**	Positive
3. Blood Donor	N	N	Positive
4. Blood Donor	WP	N	Negative
5. Renal Unit	WP	N	Negative

N - Negative
P - Positive
* - Confirmed by western blot (DuPont) and the Abbott Confirmatory EIA
# - Low Positive Control 1 (Virus Reference Laboratory, PHLS, Colindale, England)
**Eq - A cut-off range (c/o ± 10%) was defined in the Wellcome test. Samples with ODs within this range were defined as equivocal until confirmed.

WP - Weakly Reactive by ELISA
Patient 4 - s/co 1.33
Patient 5 - s/co 2.32

the Abbott Confirmatory EIA. At this time the sample was considered a confirmed positive and has subsequently retested positive by the third generation screening ELISAs and by a GACELISA developed by Parry et al. (165)

Many lessons were learned from the use and performance of these tests in the field. The rapid developments occurring in this area and the need for improvement meant that second generation tests quickly became available for use.

2. DETECTION OF HIV-1 ANTIBODY BY SECOND GENERATION TESTS

Weakly reactive serum samples and those from patients who seroconverted, which were originally screened by the Wellcozyme anti-HTLV III EIA and confirmed by western blot, were tested by the second generation assays from Abbott and Wellcome Diagnostics.

In order to compare intra- and inter-assay results, the optical density (OD) readings were assigned a score on a scale of 1-20. The optical density distance, i.e. the difference between the maximum and the minimum readings was divided into 20 equal intervals, 10 parts on either side of the cut-off (166). The interval size was determined thus:

$$\frac{OD_{cut\text{-}off} - OD_{minimum}}{10}$$ for intervals 1-10 (i.e. Ab negative)

and

$$\frac{OD_{maximum} - OD_{cut\text{-}off}}{10}$$ for intervals 11-20 (i.e. Ab positive)

If the OD was greater than 2.00 (>2.00) in the Abbott test, a category, 21, was used. The test system is saturated at this point and the spectrophotometer is unable to give an OD greater than 2 units

at this wavelength. Since the Wellcome tests are competitive ELISAs these formulae were reversed, i.e. the lower interval 1-10 represents

$OD_{maximum}-OD_{cut-off}/10$ and the upper interval,

$OD_{cut-off}-OD_{minimum}/10$, to allow the test scores to be comparable to those for the Abbott ELISA.

Performance of Second Generation Antibody Tests:

Seroconversion Samples

The scores for a group of patients who seroconverted over the period of the study have been represented in a graphic form in Fig.2.1. The results of the two second generation tests are comparable and the scores are higher than those of the same sample in the first generation test in 14 of the 23 individuals. The best separation of negative and positive ODs around the cut-off is achieved using the HIV Monoclonal EIA (Wellcome). It is clear, with reference to certain marked specimens in Fig.2.1 that the second generation tests are more sensitive than the first generation test shown here.

Weak Positive Samples

Table 2.4 shows the results for the same 3 ELISAs on a group of patients, where the original ODs of the sera were judged to be weakly reactive in the screening ELISA and showed a pattern associated with recent infection on western blot. Certain results (patients 3, 4, 7, 8, 10) which had been borderline on previous testing were clearly positive by the second generation tests. Weak positives were now apparently strong positives. This can also be seen from the plots of the OD scores (Fig.2.2). Again the monoclonal test from Wellcome gives the clearest results with good separation of positives and negatives and there are fewer samples near the cut-off.

Serial Dilutions

A panel of dilutions from HIV antibody positive blood donations provided a good set of samples to examine the performance of the same

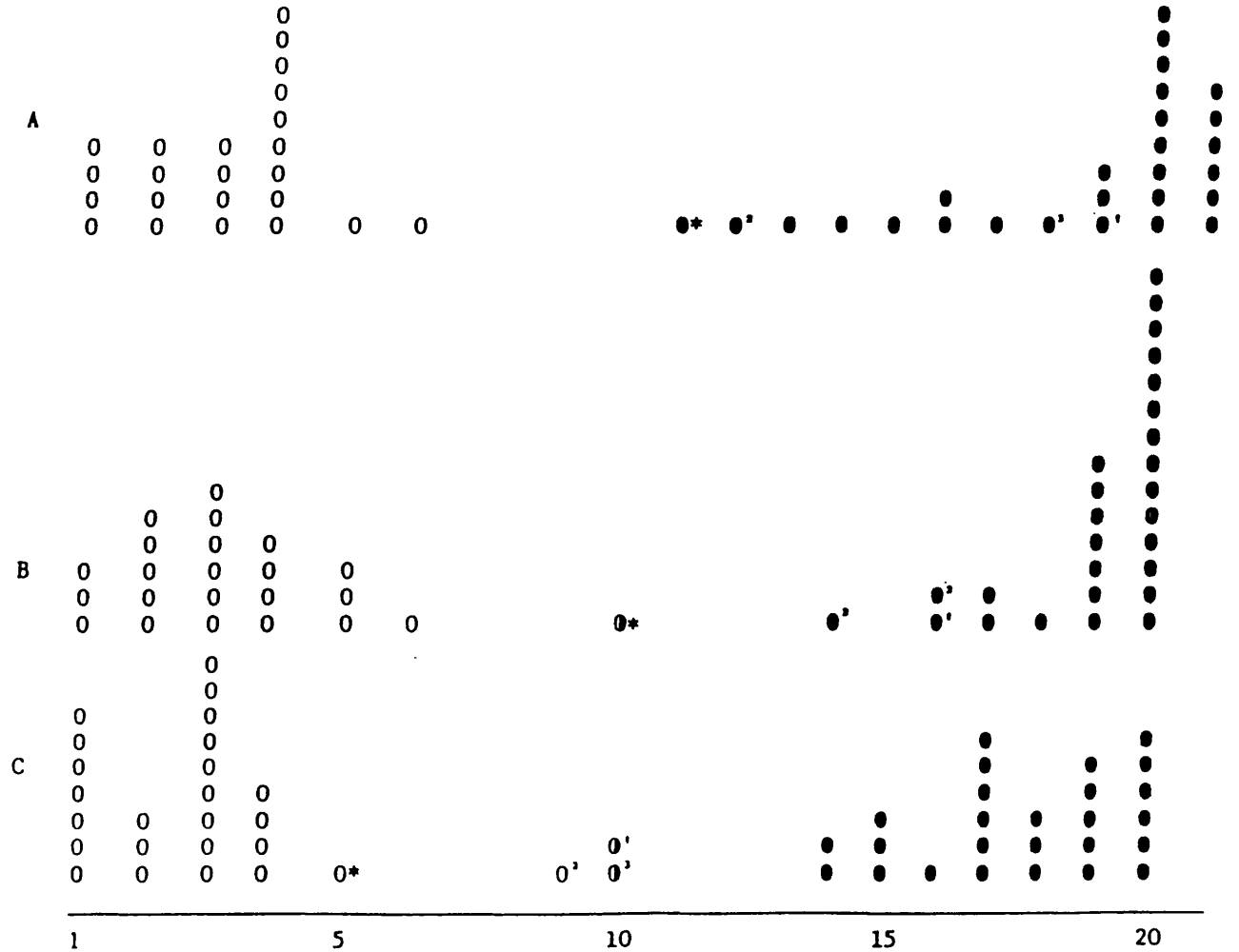


Figure 2.1 Graphic representation of ELISA optical density scores in a group of patients who seroconverted for HIV-1

A Abbott Recombinant HIV-1 EIA (2nd Generation)
B Wellcozyme HIV Monoclonal EIA (2nd Generation)
C Wellcozyme anti-HTLV III EIA (1st Generation)

* same sample represented in A,B and C; ' same sample represented in A,B and C;
' same sample represented in A,B and C; ' same sample represented in A,B and C

TABLE 2.4

Optical density scores in samples from patients with weakly reactive ODs on initial testing

Patient No.	TEST A	TEST B	TEST C
1.	19	18	17
2.	21	19	20
	20	20	20
	20	20	19
	21	20	19
3.	**11**	**12**	**10**
	NT	NT	13
	13	15	11
	15	17	14
	21	20	20
4.	**16**	**16**	**10**
	16	15	13
	20	18	17
5.	19	20	18
6.	17	18	19
	19	20	18
	21	20	20
7.	**15**	**17**	**9**
	21	20	20
8.	**12**	**18**	**11**
	12	17	11
	19	NT	16
9.	17	19	15
	21	19	19
	21	NT	20
10.	**14**	**16**	**9**
	17	19	13
	20	19	19
	20	20	20
11.	12	16	13
	17	19	14
	16	20	20
12.	19	17	15
	16	20	NT

A - Abbott Recombinant HIV-1 EIA (2nd Generation)
B - Wellcozyme HIV Monoclonal EIA (2nd Generation)
C - Wellcozyme anti-HTLV III EIA (1st Generation)

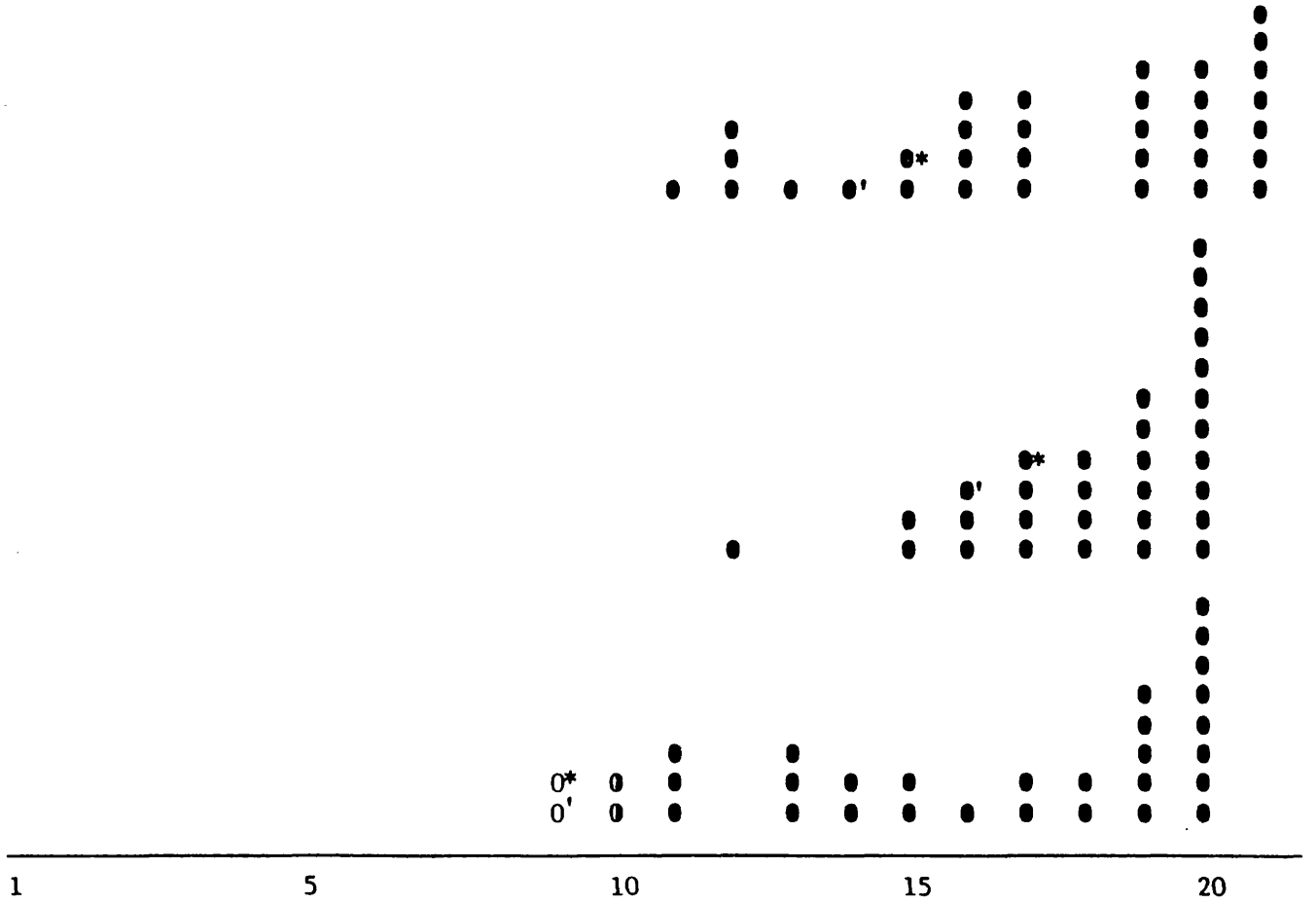


Figure 2.2 Graphic representation of ELISA optical density scores in a group of patients recently infected with HIV-1

A Abbott Recombinant HIV-1 EIA (2nd Generation)
B Wellcozyme HIV Monoclonal EIA (2nd Generation)
C Wellcozyme anti-HTLV III EIA (1st Generation)

* same sample represented in A,B and C; ' same sample represented in A,B and C

3 ELISA systems. Table 2.5 shows the dilutions and their test results. This also gives an indication of the sensitivity of the tests. However, there is a drawback: the ELISA only picks up that species of antibody which is present in the highest concentration in the serum and remains when the sample is diluted. This is not always the same species that would be present in a genuine early sample from a seroconversion. The result also depends on the viral antigens present on the solid phase.

A marked difference in the results between the first and second generation ELISAs can be seen in S3 and S4, Table 2.5. Overall, the Abbott Recombinant HIV-1 EIA is able to detect antibody at higher dilutions. Certain marked samples in Table 2.5 were sufficiently close to the cut-off, in the various tests, to have been noted and retested or confirmed if this were a clinical situation.

Pooled Sera

A number of biochemistry quality control (QC) sera were tested for HIV. This QC material was produced from large pools of human and bovine sera for use in the calibration of a variety of clinical biochemistry tests.

Human-derived material carried the risk of being anti-HIV positive prior to the implementation of blood product screening. Only one single donor was required to be infected with HIV to contaminate a whole batch.

This pooled material was screened for HIV in 1985 and 1986; one company in particular actively pursued this testing facility in order to ensure customers that their products were safe to handle and unlikely to contaminate other material or machinery.

The QC material was supplied lyophilised and was reconstituted in deionised water, except in those samples where a diluent was supplied. The reconstituted samples were screened in three ELISA systems and confirmed by western blot. Table 2.6 shows typical results obtained on this set of samples. It was noted that this material performed less well in the competitive ELISAs: however the second generation tests were better than the first generation test used. Although, in general, the pooled material showed low ELISA

TABLE 2.5

Serial dilutions of seropositives tested by 3 ELISA systems

		Abbott Recombinant HIV-1 EIA (2nd Generation)	Wellcozyme HIV Monoclonal EIA (2nd Generation)	Wellcozyme anti-HTLV III EIA (1st Generation)
S1.	Neat	+	+	+
	1:2	+	+	-*
	1:4	+	-*	-
	1:8	+	-	-
	1:16	+*	-	-
	1:32	-	-	-
S2.	1:30	+	+	+
	1:100	+	+	+
	1:300	+	+	-
	1:1000	-**	-	-
	1:3000	-	-	-
S3.	1:1000	+	+	-*
	1:2000	+	+	-
	1:4000	+	-	-
	1:8000	+	-	-
	1:16000	-	-	-
S4.	1:100	+	+	+
	1:300	+	+	-≠
	1:1000	+	+#	-
	1:3000	-	-	-
	1:10000	-	-	-
S5.	Neat	+	+	+
	1:10	+	+	+
	1:30	+	+	+
	1:100	+	+	+
	1:300	+	+	-*
	1:1000	+	-*	-
S6.	1:30	+	+	+
	1:100	+	+	-*
	1:300	+	+	-
	1:1000	-#	-	-
	1:3000	-	-	-

* - within c/o + 10%
≠ within c/o + 20%
** - within c/o - 20%
\# - within c/o - 10%

TABLE 2.6 Optical density readings from 3 ELISAs on a selection of quality control material.

	A	B	C	Western Blot Confirmation
QC 1	0.515	0.372	0.612	p17, 24, 31, gp41, p53, 64, gp160/120
QC 2	0.448	0.532	0.842	p24, 31, 53, 64, gp160/120
QC 3	0.786	0.421	0.454	p17, 24, 31, 53, 55, 64, gp160/120
QC 4	0.468	0.399	0.672	p17, 24, 31, gp41, p53, 55, 64, gp160/120
QC 5	0.436	0.539	0.772	p24, 53 (55), 64, gp160/120
Cut-off	0.178	0.779	0.531	
QC 6	0.771	0.251	0.559	p24, 31, gp41, 53, 55, 64, gp160/120
QC 7	0.832	0.235	0.431	do.
QC 8	0.524	0.495	0.772	do.
QC 9	0.543	0.552	0.820	do.
Cut-off	0.185	0.579	0.605	
QC 10	0.828	0.238	0.528	p24, 31, gp41, p53, 55, 64, gp160/120
QC 11	0.263	0.635	1.020	p24, 31, 55, 64, gp160/120
Cut-off	0.154	0.612	0.789	
QC 12	0.948	0.247	0.523	p17, 24, 31, gp41, p53, 55, 64, gp160/120
QC 13	0.840	0.348	0.667	p17, 24, 31, gp41, p53, 55, 64, gp160/120
QC 14	0.499	0.736	0.952	(p17), p24, 31, 53, 55, 64, gp160/120
Cut-off	0.246	0.612	0.509	

A - Abbott Recombinant HIV-1 EIA (2nd Generation): B - Wellcozyme HIV Monoclonal EIA (2nd Generation):
C - Wellcozyme anti-HTLV III EIA (1st Generation)

reactivities, multiple bands were present on WB described later in this Chapter (Fig.2.11).

All non-human-derived material that was tested was anti-HIV negative.

These results give no information regarding the infectivity of the product.

3. DETECTION OF HIV-1 ANTIGEN IN SEROCONVERSIONS AND WEAK POSITIVE SAMPLES

HIV-1 antigen (Ag) is the first serological marker to be produced on infection. When the first antigen test became available (January 1987) a number of samples from patients who were suspected of being recently infected were tested. No serum Ag was detected in 13 samples from 10 patients prior to seroconversion where the time interval from the last HIV-1 antibody negative sample to the first antibody positive sample ranged from 35 days to 196 days. In addition, Ag was detected and confirmed in only one out of six patients with equivocal antibody ELISA reactive (but WB confirmed) samples, i.e. at seroconversion (data not shown).

Table 2.7 shows the results of Ag testing in two patients in whom the time course of HIV-1 infection was followed by testing sequential samples. This gives a clearer indication of when antigenaemia occurs in acute infection. The Ab serology is discussed later in this Chapter along with western blot results (Table 2.14). In one case (Tx) the date of infection with the virus was known. HIV Ag was detectable in the first serum sample available for screening, after infection at day +38 and was present in a further two samples up to day +42. These results, however, were unconfirmed due to the low sample volumes in each case. No HIV-1 Ab was detected in these samples by ELISA or WB. Thus Ag was detected 19-23 days prior to the first specimen found to contain antibody. In contrast in patient Ho the date of infection with HIV was unknown (Table 2.7), but several samples around the time of antibody seroconversion were available for testing. The patient was found to be both antibody and antigen negative three months earlier (day -101). One sample, 6 days prior

TABLE 2.7

Antigen results in sequential samples from two patients.

Patient	Day	HIV-1 Antigen Result (Abbott)#	HIV-1 Antigen Result (DuPont)#
Tx	-35	N	
	0*	No sample	
	+3	N	
	+38	R (s/co 1.46)	
	+40	R (s/co 1.68)	
	+42	R (s/co 1.54)	
	+61	N	
	+80	N	
	+308	N	
Ho	-101	N	N
	-6	R (s/co 1.32)	R (s/co 10.51)
	-2	N (s/co 0.90)	R (s/co >17.09)
	0*	N (s/co 0.92)	R (s/co >17.09)
	+2	N (s/co 0.96)	R (s/co 11.75)
	+22	N	N

The sample to cut-off ratio of reactive and high negative results are indicated

* In patient Tx, day 0 represents the date of infection with HIV-1, i.e. the date of the platelet transfusion. In patient Ho, day 0 represents the first specimen with detectable anti-HIV-1 since the exact date of infection with the virus was unknown.

to the appearance of HIV-1 antibody, was reactive in the antigen test. Three further samples at day -2, day 0 and day +2, where day 0 was the first specimen with detectable, but unconfirmable anti-HIV 1, gave negative OD readings in the antigen test. However the ODs were close to the cut-off (c/o minus 10%) giving sample to cut-off (s/co) ratios between 0.90 and 0.96 and clearly stood out from the other negative samples on that test run. These were referred to as high negative results and it appeared that the antigen level was decreasing. A sample at day +22 was HIV-1 Ag negative. These results were interesting and the Du Pont p24 core antigen EIA was also performed. Discrepant results were found and this is indicated in Table 2.7. All the samples were reactive in this test apart from day +22 and in two cases there was sufficient antigen to saturate the test system. No neutralisation confirmatory tests were performed at this time. This difference in the results may be explained by the use of Triton X-100 in the DuPont assay. This detergent disrupts HIV-1 virions and releases Ag in the sample. The Triton X-100 treatment step was later introduced into the Abbott antigen test protocol. The day +2 sample then retested positive by the Abbott test and was confirmed by neutralisation.

4. RAPID ANTIBODY SCREENING TESTS

A number of sera from different risk groups, whose reactivity in other test systems was known, were tested using the Dipstick assay (Du Pont). A limited amount of testing was performed using this assay and the results are shown in Table 2.8. The negative and strong positive sera gave clear results and presented no problems, although one AIDS patient gave a weak one plus (1+) reading. The remaining three out of the seven weak ELISA reactives did give equivocal results, namely a tinge of colour. In addition five of the nine false positives gave equivocal results and clearly these sera would have to be tested further.

TABLE 2.8

HIV-1 antibody test results using the Dipstick Assay (DuPont)

Category of Sera	No. Sera Tested	Du Pont Dipstick Positive
HIV-1 Ab positive	10	10 (100%)
HIV-1 Ab negative	6	0 (100%)
Weak ELISA Ab positive	9	6 (66.7%)
False positive	9	2 (22.2%)

5. COMBINATION ANTI-HIV 1 AND ANTI-HIV 2 SCREENING TESTS

The first combination test in use at the HRL was the Abbott Recombinant HIV-1/HIV-2 EIA (Abbott Diagnostics) in March 1989 (Appendix 2). A number of sera were examined and the results compared to the test in use at that time (Abbott Recombinant HIV-1 EIA). The results are shown in Table 2.9.

Anti-HIV 1 Detection by Combination Tests

All strong HIV-1 positives were detected by the combination test; but three samples from patients considered to be at an early stage of infection were apparently missed. The reason for this is unknown. Some problems had previously been noted when testing older stored sera indicating that there could be loss or deterioration of antibody in such samples. Sera are stored in plastic vials at -20^{o}C; the effects on serum proteins of repeated freezing and thawing are unknown, however contamination, proteolytic degradation and some binding of proteins to the plastic vials has led to discrepant results after a period of storage.

A number of sera from low risk individuals which had reacted falsely by different ELISAs including the Abbott Recombinant HIV-1 EIA, were tested. A reduced number of false positives was found using the combination EIA. This was not unexpected as past experience in testing low risk sera by different ELISAs showed that the various tests flag a different set of samples as falsely reactive.

Four internal quality control sera and nine biochemistry quality controls were tested and compared to the results of the HIV-1 assay. Three results in the HIV-1/HIV-2 tests were discrepant; two quality control sera were negative by the test criteria, but the OD was within 10% of the cut-off, and one biochemistry serumwas negative.

The lack of sensitivity noted in the above results was also seen with respect to testing serial sample dilutions of HIV-1 seropositives (Table 2.10).

TABLE 2.9

Results of screening various groups of sera with the combination, HIV-1 plus HIV-2, assay versus the HIV-1 antibody assay.

Category of Specimen	Total Nos. Tested	Abbott Recombinant HIV-1 EIA Positive	Abbott Recombinant HIV-1/HIV-2 EIA Positive
HIV-1 Ab positive	9	9	9
HIV-1 Ab negative	4	0	0
WPs	11	11	8
FPs	19	11	2
QC	13#	13	10
HIV-2 Ab positive	10	6	9

- Including 9 biochemistry quality control sera described earlier.

WPs - Weak Positives
FPs - False Positives
QC - Quality Controls

TABLE 2.10

Performance of single Sample dilutions in the combination test versus the HIV-1 antibody assay.

Dilutions	Abbott Recombinant HIV-1 EIA	Abbott Recombinant HIV-1/HIV-2 EIA
S1. Neat	+	+
1:2	+	+
1:4	+	+
1:8	+	+
1:16	-*	-
1:32	-	-
S2. 1:30	+	+
1:100	+	+
1:300	+	+
1:1000	-	-
1:3000	-	-
S7. 1:1000	+	+
1:2000	-*	-
1:4000	-	-
1:8000	-	-
1:16000	-	-

* within c/o -20%

Anti-HIV 2 Detection by Combination Tests

Nine out of ten sera, brought from Portugal, and taken from patients with HIV-2 infection, were detected by the combination HIV-1 and -2 test (Table 2.9). All these samples are positive for HIV-2 antibody by ELISA (ELAVIA Ac-Ab-Ak II, Diagnostics Pasteur). Unfortunately, little is known about these samples and it may be that the negative sample was from a patient who was seroconverting or possibly even at a terminal stage. Six out of the 10 sera were detected by the HIV-1 screen, indicating the cross-reactive nature of proteins from related viruses.

Although no increased sensitivity or specificity was seen with this combination test, it did provide a means of testing for HIV-2 antibodies in the population being screened. There have been no persons found to be HIV-2 antibody positive in 18 months of routine screening with the combination HIV-1/-2 test.

6. HIV-2 ANTIBODY DETECTION

An HIV-2 Ab ELISA (Diagnostics Pasteur) was used prior to the introduction of the combination tests, for specific anti-HIV 2 requests. In addition, some testing was performed on a small number of HIV-1 Ab positive persons who came from Africa or were infected in Africa. Ten out of 15 HIV-1 Ab positives reacted in the HIV-2 ELISA. To establish the nature of this reactivity, whether simply cross-reaction with HIV-2 encoded proteins or more interestingly a genuine HIV-2 infection or dual infection, an HIV-2 Western Blot (Du-Pont) was performed. The results are shown in Table 2.11. The major HIV-2 structural proteins were identified as follows - (Instruction Manual, Western Blot IgG Assay Kit for the Detection of HIV-2 Antibodies, Du-Pont, March 1988):

gag proteins	:	p56 (gag precursor) p26, p16
pol proteins	:	p68 (reverse transcriptase) p36 (endonuclease)
env proteins	:	gp140, gp105 gp41 (transmembrane protein)*

*The molecular weight of the transmembrane protein of HIV-2 is usually quoted as gp36.

Comparison with the positive control indicated that the predominant reactivity was to the core proteins. Two samples (MUH and BV, Table 2.11) required further investigation because of the presence of a band in the high molecular weight regions, gp105 in BV and gp140 in MUH. The samples were sent to the Research and Diagnostics Unit, Diagnostics Pasteur, France, where a peptide test had been developed. Synthetic peptides (Genetic Systems, Seattle, USA) mimicking the gp41 HIV-1 and the gp36 HIV-2 transmembrane glycoprotein specific epitopes were coated onto strips of nitrocellulose and detected by an ELISA method thus discriminating between anti-HIV 1 and anti-HIV 2 reactivity. BV was positive on the HIV-1 peptide only but MUH reacted against both peptides and it was suggested that he was infected with an HIV-1 isolate more closely related to HIV-2 than the prototype HIV-1 (Diagnostics Pasteur, personal communication). This test is now available commercially as the Pepti-LAV 1/2 (Diagnostics Pasteur).

7. HTLV-I/-II ANTIBODY DETECTION

An ELISA (Abbott HTLV-I EIA) is only performed on those samples for which there is a specific request. Some screening has been performed on HIV-1 risk groups, predominantly DAs and this is discussed in Chapter 3. No evaluation of the commercially available ELISAs can be performed due to a lack of HTLV-I and HTLV-II antibody positive sera.

TABLE 2.11

HIV-2 western blot results on HIV-1 antibody positive sera reactive in an Anti-HIV 2 ELISA (Diagnostics Pasteur)

Patient	Reason	Dilutions	Western Blot
OC	ex-Malawi	1:100	p26, (gp41), p56, p68
MUH	ex-Africa	1:100	p26, p36, gp41, p56, p68, gp140
GR	Sexual contact with African	1:100	p26
SR	-	1:100	p26, p36
PN	ex-Uganda	1:100	p26, gp41, p56
BV	Contact in Zambia	1:20	p26, gp41, p56, p68, gp105
A3/86	BTS reference	1:100	p26, p36, (gp41)
A6/86	BTS reference	1:100	p26, gp41
A7/87	BTS reference	1:100	p26, p36

DISCUSSION

The presence of specific antibody to HIV means that an individual has been exposed to HIV at some time in the past. Antibody positive persons are potentially capable of transmitting the virus; this implies that an individual is continually or chronically infected with HIV. The ability to transmit HIV may not always be present, the potential is, however.

Although the result of a screening test does not give any measure of the infectivity nor any information on the prognosis of an individual a positive result is devastating for the patient. Therefore, the test has to achieve a high degree of sensitivity and specificity to ensure confidence in the result. The consequences of a wrong result to a patient, especially with such an emotive subject as HIV are disastrous.

There is no gold standard test for anti-HIV so the characteristics of an assay are assessed on certain populations. There have been no large scale evaluations performed at the HRL principally because a proper evaluation of specificity requires the testing of thousands of samples. However, several groups of interesting clinical samples have been examined over the period of the study and useful information produced on specificity and sensitivity.

A good screening test has to be robust, easy to install, easy to operate with minimal handling, i.e. user-friendly, give reproducible results, available at a low cost per test, and adaptable for either large scale automated use or limited numbers, in addition to the essential qualities of high sensitivity and specificity. The occurrence of false negatives is a problem because they pass through the system and are not retested or tested by another system. False positives can be eliminated by further tests and the numbers in this category were reduced when confirmatory testing was used, either immunofluorescence or western blotting.

Some ELISAs have traded one quality, e.g. high sensitivity at the expense of another, e.g. that of specificity and many tests had their teething problems in the beginning which have been ironed out with use. It is important that test performance is subject to

rigorous criteria of validity each run.

First Generation Tests

The first test, the Abbott HTLV III EIA, was subject to both false negatives (FN) and false positives (FP).

False positive reactions with human sera occurred through cross-reactions of antibodies to human leukocyte antigens (HLAs) specifically the DR locus of the Class II HLAs which are expressed on the surface of the HIV-infected H9 cell culture material used as an antigen source (110-112). This was seen in a few multiply-transfused renal unit patients tested at the HRL. It was also noted in the literature that heat inactivation of the virus (56^{o}C for 30 min) in patients' sera led to an increase in absorbance and in some cases to false positive reactivity (113). The mechanism for this is unknown. However this was not routine practice at the HRL and was therefore not a problem.

The Wellcozyme anti-HTLV III EIA also used human cell culture derived material as an antigen source. The cut-off value was based on the OD of a weak positive serum and a grey zone for retesting was identified as 10% either side of this value. This test was also subject to false negative (167) and false positive results (168). In addition an edge effect was noticed when the microtitre plate was incubated on a heating block. The ODs at the edge were higher than those in the centre of the plate and was a potential source of error (169). The edge effect was overcome by the use of a water bath or incubator. This test was highly favoured by the BTS and most centres used it for screening. The Glasgow and West of Scotland BTS achieved an increased sensitivity by an extra 30 min incubation of sera prior to addition of the enzyme-labelled HIV antigen conjugate (170). Any donor sera with an OD repeatedly outwith 3.2 standard deviations of the test negative mean, i.e. that of the samples under test and/or repeatedly reactive were sent for confirmation to the HRL.

The Wellcome test appeared to be able to detect recent infections with a higher degree of accuracy (Fig.2.1; Fig.2.2). A different epitope may be exposed on the solid phase in different tests

and therefore different antibodies are being detected. It was initially thought that antibodies to core proteins were the first species produced after infection as they are present in abundance. As time progressed it was established from seroconversion studies that antibodies to envelope glycoproteins were produced early in infection (86) and reliable detection of seroconversion required these epitopes to be present as capture antigen on the solid phase. Epitopes on the envelope proteins used in the development of the Wellcome test were perhaps exposed in such a way as to be better able to detect early infection.

Second Generation Tests

Lessons learned from the first generation assays helped to improve the tests, e.g. the false positives due to anti-HLA cross-reactions were reduced by using recombinant peptides on the solid phase (Abbott) instead of cell culture derived viral lysate. The use of recombinant protein(s) derived from sequences of HIV-1 proteins permitted a choice of which epitopes to include on the solid phase and this was the prerogative of the manufacturer. Thus the solid phase could be coated with a choice of antigens of different specificities and in different proportions. The second generation Wellcome test used the same first generation test antigen on the solid phase but it was bound to the microtitre well by monoclonal antibody. The tests evolved with the aim of detecting HIV-1 antibody at its earliest production in an infected person and therefore closing down the window of infection, i.e. that time between exposure to the virus and the first production of antibodies.

The sera used in studies at the HRL were chosen to assess how efficient the new assays were at picking up anti-HIV 1 when it is present in those patients recently infected. The results show that the new generation of tests were indeed an improvement; the test signals were amplified and ODs were more positive in the second generation tests. A sample previously considered a weak positive now gave a strong positive result and the tests showed a greater separation of negative and positive results, therefore permitting

greater faith in the result being genuine. Although there was the added danger of amplifying false positive reactions and introducing a further new set of specimens which reacted falsely in the second generation tests, confirmatory testing was available to screen out the problems.

It can be seen using these panels of pedigreed anti-HIV 1 positive samples that different tests do show variation in their ability to detect antibody to different parts of the virus and at different time points in infection; competitive assays appear better than the antiglobulin kits in detecting antibody at seroconversion whereas the latter have the edge in endpoint dilution studies. It is important to note that although dilution studies may give an indication of sensitivity of a test, where the antibody species present in highest concentration is measured, this will vary in different people and will depend also on disease stage. A test giving a high sensitivity in this situation does not indicate its use in the diagnostic laboratory where sensitivity depends on being able to detect genuine HIV antibody early in infection. Specimens from recent infections give a better, more true picture of test performance than dilutions since this is the situation in a genuine clinical setting.

A subtle difference existed between the serial dilutions, tested and presented in Table 2.5 and those of the pooled sera containing HIV shown in Table 2.6 The latter were originally thought to be ELISA false positives but multiple bands were shown by western blot. In contrast the western blots of single sample dilutions could eventually show a single band at higher dilutions (Fig.2.10). The pooled material is assumed to be a dilution of an HIV positive; it is not known exactly what is in these samples or what they are diluted in, if anything, prior to lyophilisation. It would appear that there was more than one infected donor in the pool.

Antigen Testing

The initial testing with the antigen assay(s) was performed to establish its usefulness in the diagnostic laboratory. HIV-1 Ag

has been detected in sera and CSF prior to antibody production (85,86) and has also been detected at the time of acute illness (84). In one of the two patients studied, Tx, antigen was present 3 weeks prior to the production of antibody. This fits with the putative time course of HIV-1 infection and is comparable with other studies of incubation times. Patient Ho was more unusual in that antigen was present at antibody seroconversion. The patient had a documented illness consistent with acute HIV-1 infection, i.e. lymphadenopathy, pyrexia etc. in the 3 to 4 weeks prior to seroconversion.

Such a test would be useful in detecting infection early and thus closing the window of infection. A programme of HIV-Ag screening of blood donors in Germany failed to detect additional HIV-infected individuals in over 150,000 donations (171). Thus its lack of sensitivity for this marker, even although it can detect picogramme quantities, and the transient nature of early antigenaemia meant that it could not be used routinely to detect infected persons prior to the production of antibody. However it may prove useful in situations such as needlestick injuries where a known exposure has occurred and regular samples are being withdrawn from the patient. The test was used for some time as part of a battery of tests on reference samples sent from the Regional Transfusion Centres (RTC) in Scotland and Northern Ireland. However, its major use at the HRL is in the follow-up of anti-HIV 1 seropositives. This is discussed further in Chapter 4.

Rapid Tests

A number of alternative antibody screening tests, including the DuPont Dipstick Assay, were developed to appeal to a variety of users. These were designed for use 'in the field' or 'at the patient's bedside' and particularly in developing countries where problems such as maintaining test kits at $4^{o}C$ are as much a challenge as performing and reading the results of the assay. The results indicate that the Dipstick test lacked sensitivity and therefore confirmation of a result was still required, especially in those samples giving only a tinge of colour in the final substrate solution. This test was

short-lived on the market. No further work has been performed on these rapid tests at the HRL.

Combination Tests

The arrival of the combination (combi) tests where both HIV-1 and HIV-2 proteins were present on the solid phase brought with it a new challenge. A specimen reactive in such a test would have to be tested further to distinguish between anti-HIV 1 and anti-HIV 2 or to confirm a dual infection. In addition, it was thought the sensitivity of anti-HIV 1 detection would be compromised by the presence of HIV-2 on the bead. Strong positives were flagged easily but in other clinically relevant cases (WPs, Table 2.9) it was suspected that the use of older, stored sera which had been frozen and thawed numerous times may have affected the test performance. In practice the test has proved sufficiently sensitive for the detection of HIV-1 Ab with respect to seroconversion samples. The combi test detected all but one of 10 serum samples from HIV-2 infected individuals from Portugal. No assessment of test performance with respect to HIV-2 Ab detection could be made due to the lack of HIV-2 Ab positive samples available locally.

HIV-1 and HIV-2 share a 45% homology both at the genomic level and in amino-acid sequence (69) and it is not surprising that antibody cross-reactions do occur. The simplest method of distinguishing between HIV-1 and HIV-2 reactivity would be by using individual ELISAs specific for anti-HIV 1 only or anti-HIV 2 only, i.e. ELISAs incorporating non-conserved epitopes.

HIV-2 Antibody Screening

The results with the HIV-2 antibody ELISA used at the HRL (Diagnostics Pasteur) indicates that it lacks specificity since 75% of the HIV-1 seropositive samples tested did react but were not confirmed as HIV-2 Ab positive. Again sensitivity could not be assessed due to a lack of HIV-2 seropositive samples. Therefore, a problem exists in finding a reliable method of determining the nature of the reactivity

in the combi test. The answer may lie in the use of recombinant immunoblots containing more than one epitope from each of HIV-1 and HIV-2. This is discussed further at the end of this Chapter.

CONCLUSION

The phenomenal pace of AIDS research has allowed constant and continued advancement and updating of diagnostic screening. The tests available are always improving as technology and test development keep up with current research.

Questions to be asked on developing an antibody assay for testing clinical samples and blood donors include which antibodies are produced during both early and late infection and are the corresponding antigens represented on the solid phase? This question has been effectively answered as the sensitivity and specificity improved in the second generation tests. The window period between infection and production of antibody has been narrowed.

The tests have now incorporated screening for a second retrovirus, HIV-2, and epitopes from the envelope protein are included on the solid phase. Although this virus was first isolated in regions of West Africa, cases have now been reported in Europe (74) and America (73). Inevitably the virus will spread either through direct transmission in one geographical area or as infected persons travel round the world and it is therefore necessary to be able to detect such persons and limit the spread. Future developments may include adding epitopes of the human T-cell leukaemia virus family into the primary screening stage. One of the major considerations is protecting the blood supply and prevent transmission of such viruses by this route.

Great variation exists within each retrovirus family. The envelope proteins of HIV-1 are highly variable; isolates obtained from a single patient from patients infected from the same source and from geographically diverse areas are different (50-52) so it is important that tests are continually evaluated especially on local samples to ensure that antibody to emerging strains of virus can be detected.

B. CONFIRMATORY SCREENING

INTRODUCTION

If screening tests were perfect, there would be no need for confirmatory tests. However, although there has been a great improvement, screening tests are not 100% sensitive and 100% specific, so confirmation of a result is required. The more important quality of a confirmatory assay is specificity, since samples which have reacted in the screening test are to be tested by this method. The sensitivity should preferably be much better than but must be at least as high as the screening test. An ideal confirmatory test would be able to detect antibody to a range of viral proteins, e.g. core and envelope and to detect antibody to a variety of strains of HIV.

Initially immunofluorescence was used as a screening test prior to the advent of a commercial ELISA in April 1985 and then as a confirmatory test for the ELISA results, for some months during 1985. This was rapidly superseded by western blotting at the end of 1985.

Two tests, in particular were used at the HRL; the Western Blot (Du Pont) and the Confirmatory EIA (Abbott Diagnostics).

RESULTS

1. CONFIRMATORY EIA

This dual-bead confirmatory ELISA was developed by Abbott Laboratories in 1986 for use with their antibody screening test (Appendix 2). The core bead is coated with a protein which contains part of p17, all the amino acids of p24 and part of p15 and thus detects anti-p24 and anti-p55. The env bead is coated with all the amino acids of gp41 and part of gp120 and detects anti-gp41 and anti-gp160 according to the manufacturer's instructions.

Initial evaluation of the test showed that the core bead lacked sensitivity. Table 2.12 shows that 14 of the 53 seropositives were negative with the core bead and positive on the env bead. These samples would be considered positive by the manufacturer's criteria,

TABLE 2.12

Performance of confirmed HIV-1 seropositive and HIV-1 seronegative samples on the Abbott Confirmatory EIA

	HIV-1 Seropositive	HIV-1 Seronegative
No. tested	53	52*
No. ENV bead Positive	53	0
No. CORE bead Positive	39	0

* Low risk blood donors sent to the HRL for confirmatory testing. These had been found to be reactive by ELISA in this or a previous specimen. All were found to be HIV-1 Ab negative.

TABLE 2.13

Comparison of core antibody reactivities on both the Abbott Core bead and Western Blot (Du Pont) on the HIV-1 seropositives in Table 2.12

		Western Blot p24 and/or p55		
		Positive	Negative	
Core Bead	Positive	38	1#	39
	Negative	11*	3	14
		49	4	

Seropositive haemophiliac

* Including 2 with p55 only and no p24 band on a western blot

i.e. repeatedly positive on one bead. These 14 core bead negatives consisted of:

(a) 3 who were symptomatic or at a late stage of infection (two of whom had no p24 or p55 on a western blot). (Table 2.13).

(b) 5 early infections, one with a follow-up sample which remained core negative, and

(c) 6 whose stage of infection was unknown, one of whom was p24/p55 negative on a WB. This also included two samples from one patient at different times both of which were core negative.

However, the env bead was sensitive in detecting Ab early in infection (Table 2.14) and because of this the ELISA was included as part of a battery of tests on samples sent for confirmation from the BTS for a number of years.

The test was subsequently called the Abbott ENVACOR HIV-1 EIA and was intended to supplement the screening result rather than being a confirmatory test since the second generation Abbott Recombinant HIV-1 EIA was equally, if not more, sensitive.

Serial specimens from a number of patients were tested and showed loss of core antibody as time progressed although an anti-core reaction was often still visible on a western blot. The negative anti-core result, in some cases, correlated with the production of p24 antigen in the serum and in some cases preceded the onset of symptoms and/or antigenaemia. The role of this core bead ELISA in association with other markers of progression, e.g. presence of p24 antigen will be discussed in Chapter 4.

2. WESTERN BLOTTING

Viral proteins are electrophoretically separated on a polyacrylamide gel and then blotted onto a nitrocellulose membrane. This is subsequently divided into strips for each individual sample. Antibodies to specific viral proteins are then detected by type I ELISA methodology using the nitrocellulose strip as the solid phase.

Although all the gene products of HIV are immunogenic <u>in vivo</u> it

TABLE 2.14

Development of markers of HIV-1 infection in a transfusion recipient (Tx) and a homosexual (Ho)

Patient	Day	ELISA	ENV	Core	Western Blot
Tx	0*	NS	NS	NS	NS
	+3	N	NT	NT	No bands visible
	+38	N	N	NT	No bands visible
	+40	N	N	NT	No bands visible
	+42	N	N	NT	No bands visible
	+61	P	P	NT	p24, p55, gp160/120
	+75	P	NT	NT	p24, p55, gp160/120
	+78	P	NT	NT	p24, p55, gp160/120
	+80	P	P	NT	p24, p55, gp160/120
	+103	P	P	N	p17, p24, p55, gp160/120
	+115	P	P	N	p17, p24, gp41, p55, gp160/120
	+308	P	P	P	p17, p24, p31, gp41, p53, p55, p64, gp160/120
Ho	-101	N	NT	NT	NT
	-6	N	N	N	No bands visible
	-2	NT	NT	NT	No bands visible
	0*	P	N	N	p24, gp160/120
	+2	P	P	N	p24, p55, gp160/120
	+22	P	P	Bd	p17, p24, p55, gp160/120
	+40	P	P	P	p17, p24, p31, gp41, p53, p55, p64, gp160/120

N = Negative; P = Positive; Bd = Borderline; NT = Not Tested

NS = No Sample

* = In patient Tx, day 0 represents the date of infection with HIV-1, i.e. the date of the platelet transfusion. In patient Ho, day 0 represents the first specimen with detectable but unconfirmable anti-HIV 1 because the exact date of infection with the virus was unknown.

is the structural proteins which are detected by western blot strips prepared from cell culture derived viral lysate. Nine proteins, seven structural and two precursor proteins are separated according to molecular weight from a viral lysate prepared from HIV-infected cells. The molecular weights of these proteins are p55, p24, p17 (gag), p64, p53, p31 (pol) and gp160, gp120, gp41 (env) (Fig.2.3). When arranged according to molecular weight on a strip they form a distinct and unique pattern. Hence the antibody specificity of an individual for HIV can be examined by western blot and a decision made as to the specificity of the reaction seen in the ELISA test.

Initially both an "in-house" western blot (WB) and a commercial Western Blot kit (Du Pont) were available. The high molecular weight env proteins were not present on the original WB kits from Du Pont in late 1985. However, by March 1986, gp160/120 were detected on these strips, often as a single smeared band, characteristic of envelope glycoprotein transfer to nitrocellulose. The unreliability of detecting high molecular weight envelope proteins on the in-house strips, and its lack of sensitivity, meant that the commercial kit was used routinely for confirmation of samples reactive by ELISA. These were designated as strong positive or weak positive by OD readings in the ELISA test. Weak positives implied low levels of antibody and were tested at a dilution of 1:20. Strong positives were tested at a dilution of 1:100.

Strong Positives

Those sera which reacted strongly by ELISA produced 8 or 9 bands and were therefore easy to confirm. Fig.2.4 shows western blot strips from different risk groups tested at 1:100, demonstrating an antibody reactivity to all the viral proteins. These strips were taken from the same batch and so the bands could be easily aligned. No difficulty was found in confirming samples which reacted in a similar way.

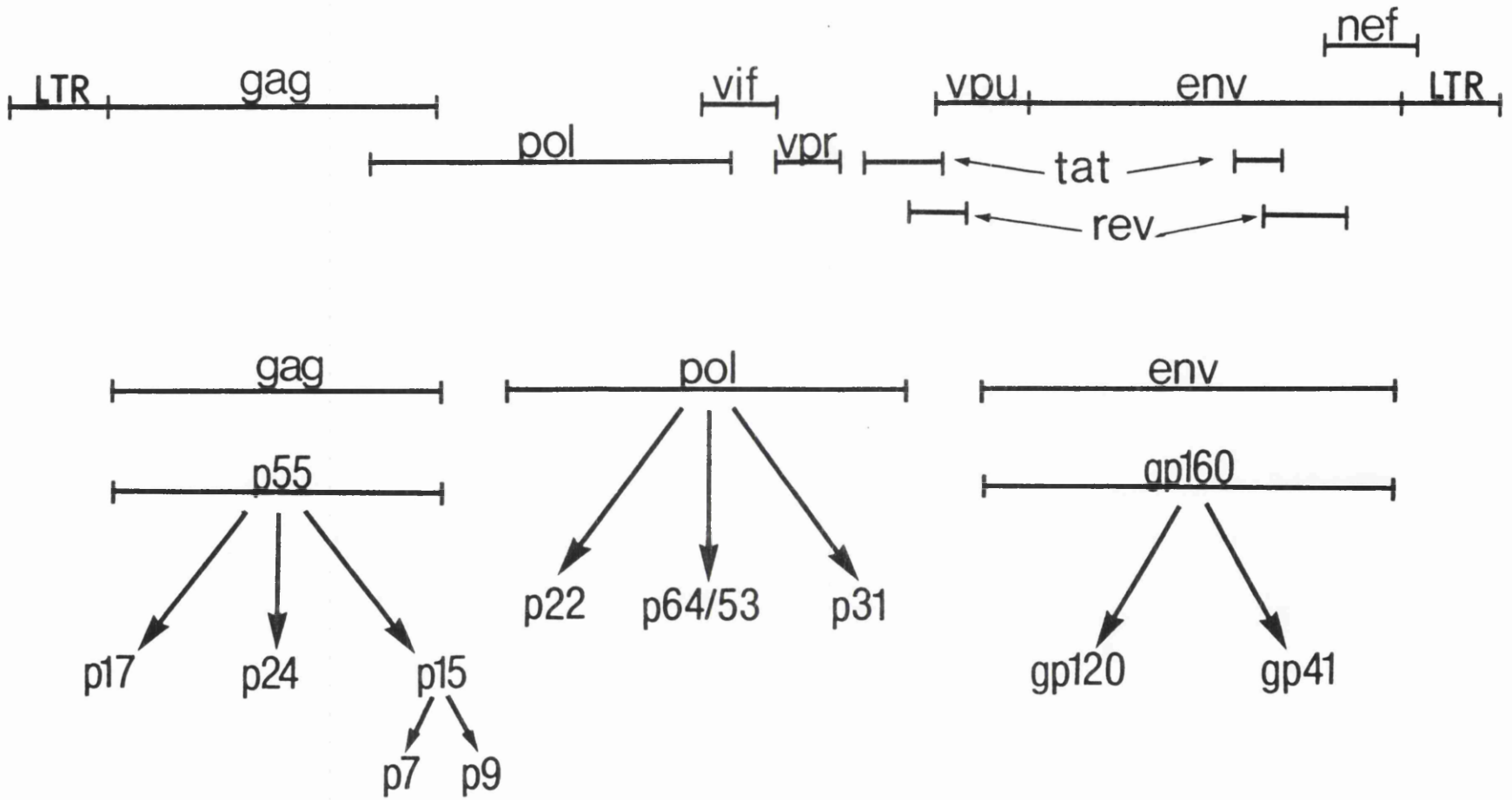


Figure 2.3 HIV-1 genome organisation and structural gene products.

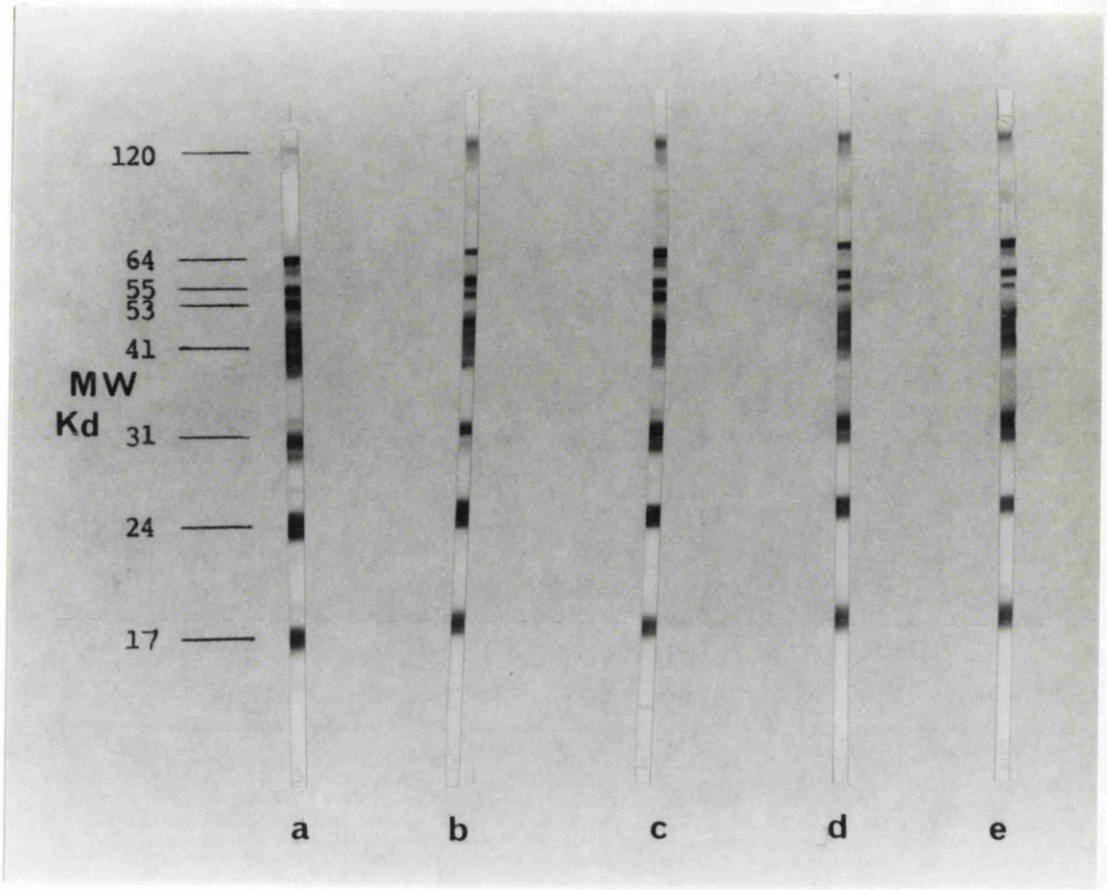


Figure 2.4. Strong ELISA reactives confirmed by western blot at 1:100 dilution

a Contact in Africa
b Homosexual
c Homosexual
d Drug Abuser
e Drug Abuser

Weak Positives

The decision was taken to test weak ELISA reactives at 1:20 dilution to ensure detection of antibody even at very low levels, because of the role of the HRL as an HIV Reference Laboratory. This dilution was chosen to maximise sensitivity without producing too high a background. Fig.2.5 shows 3 weak ELISA positives and the weak positive WB control (Du Pont) at 1:20 and 1:100 dilution. In general the strongest bands are present at both dilutions but the 1:20 blot gives more information.

Problems arose when weak ELISA reactives showed only a few bands, even when tested at 1:20, on a western blot. Therefore strict criteria about what constituted a positive WB had to be established.

Establishing the Criteria of Positivity

Initial criteria of positivity for this test system, in the USA, were the presence of antibodies to p24 and/or gp41 (126). However the occurrence of false positives producing bands at either p17, p24 or p55 alone or a combination of any two was noted in several reference samples in the HRL and noted in the literature (127-129). These criteria would mean that such samples would be wrongly reported as positive with all the consequences for the patient.

An important distinction could be made by examining those sera from expected low-risk individuals flagged by the strict criteria attached to screening at the BTS and those from high risk groups being screened in a clinical situation at the HRL. Initially, the criteria of positivity at HRL were established from the observation of seroconversions in the drug abusing population in Glasgow and the West of Scotland in 1986. Fig. 2.6 illustrates the pattern found in early infections in drug abusers; all samples were tested at 1:20 dilution except numbers 5, 8, 10 and 11.

The samples were tested at different times using different batches of strips. It can be seen that there is batch variation in the exact positions of the bands. They are not always at the same electrophoretic distance from the top of the gel but their relative

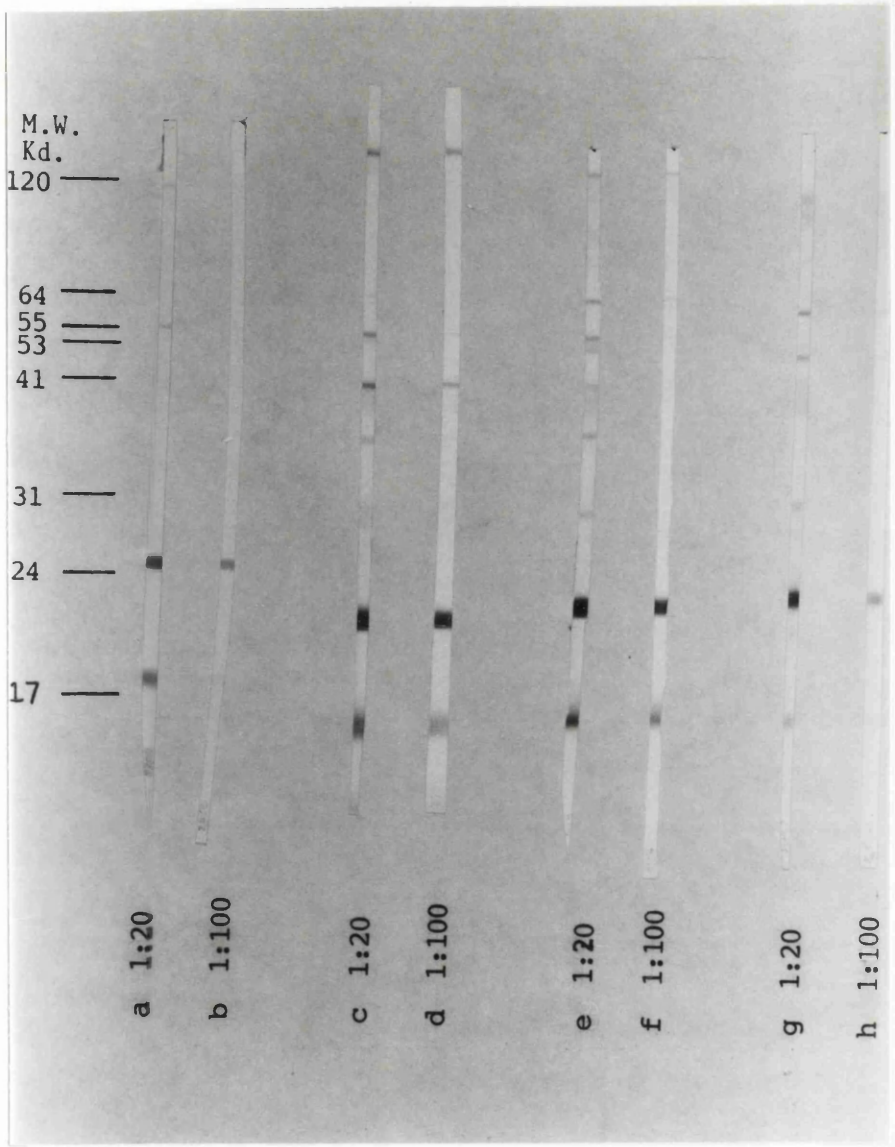


Figure 2.5. Weak ELISA reactives tested at 1:20 versus 1:100 dilution by western blot.

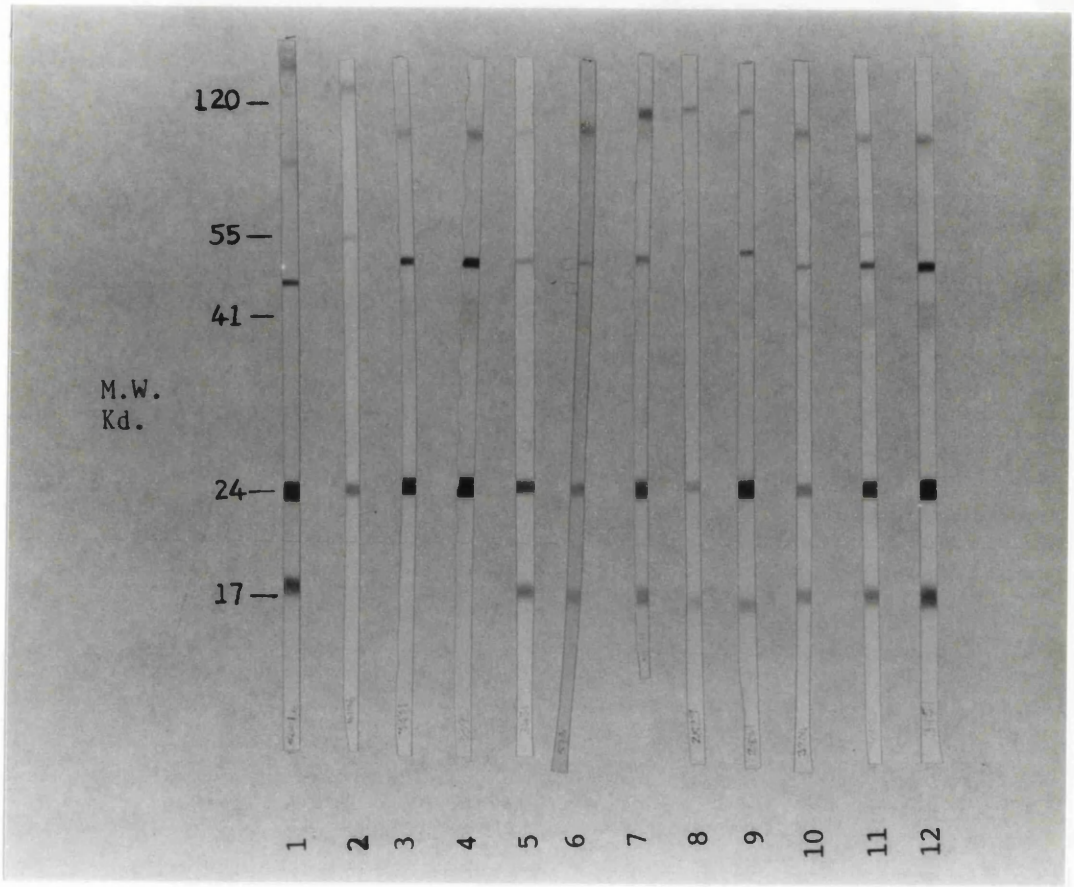


Figure 2.6. Early infection with HIV-1 in 12 DAs confirmed by western blot.

All samples tested at 1:20 dilution except nos. 5, 8, 10 and 11.
Strips aligned on the p24 band.

positions are the same. In Fig. 2.6 the p24 band has been aligned in all the samples and this has resulted in the other bands being slightly out of line in most cases.

All strips show gp120 with at least two other bands. Even on the weakest reacting strip (2) three bands are visible. No earlier samples from these patients were available which would have shown which band or bands were the first to be detected. It was therefore decided that the criteria of positivity would be 3 bands one of which had to be gp120. In early infections the pattern was most commonly p24, p55, gp120, a pattern reported by others (94,123). Antibodies to p17, p24, gp41, p55 and gp120 are the major bands represented on the WB strips in Fig. 2.6 and this is indicated on the left hand side of the diagram. Sample 11 also shows anti-p53 and anti-p64. (Symptomatic patients at advanced stages of HIV-related disease are also noted to have few bands on a western blot but the pattern is different and is described later (Western Blot and Disease Progression)).

Some time later serial samples became available from two further seroconversions which permitted confirmation and refinement of the above criteria. A set of samples was made available to us by Dr. Sheila Cameron from a leukaemic patient who received a transfusion of platelets and subsequently became infected with HIV-1. These platelets were from a donor whose donation contained no detectable antibody but who was in the seroconversion window, was infectious and infected two recipients. Sequential samples were available from the platelet recipient and the time course of HIV-1 infection, with respect to production of markers, could be determined.

The results are shown in Table 2.14 (patient Tx) and Fig.2.7. The first detectable antibody response was found in the sample on day 61, by two ELISAs (Abbott Recombinant HIV-1 and Abbott Confirmatory EIA) and WB (p24, p55 and gp160/120). Antibody to the envelope glycoprotein was detected by the confirmatory EIA but this test did not detect core, p24 and p55 antibodies, until after day 115 on the next available sample at day 308, although they were visible on a western blot on day 61. Antibodies to the other proteins developed slowly. This may be attributed to the immunosuppressed state of the patient.

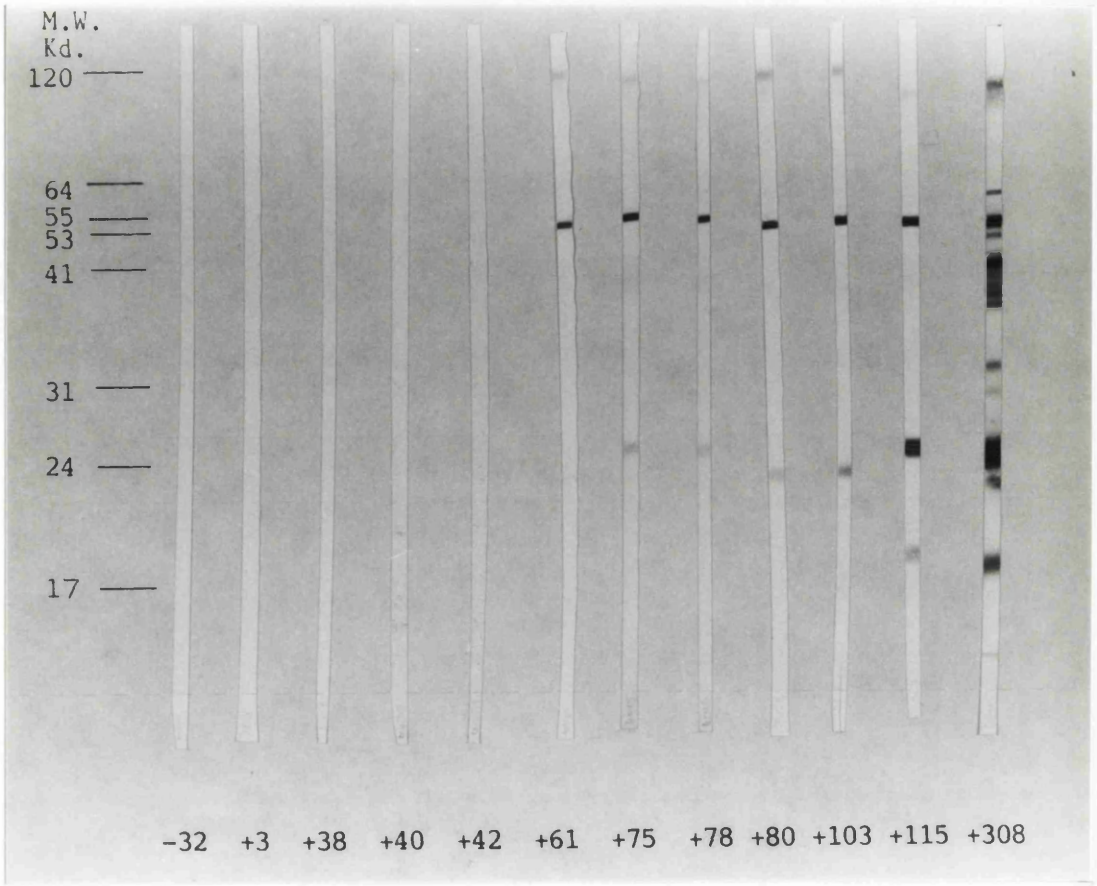


Figure 2.7. Confirmation of HIV-1 seropositivity by western blot (1:20) in serial samples from a transfusion recipient (Tx).

In addition a similar response was shown in a series of samples from a homosexual patient whose contact date was unknown but was thought to be 3-4 weeks prior to the first sample tested in the January (Fig.2.8, Table 2.14 (patient Ho)). He presented with symptoms classified as CDC stage I, i.e. that of a seroconversion illness. The first weakly reactive ELISA signal revealed p24 and gp160/120 on a WB, and the Abbott Confirmatory EIA envelope bead was negative. A sample two days later was positive by ELISA and now showed 3 bands by western blot (p24, p55, gp160/120) and a positive envelope bead on the Confirmatory EIA. There was a noticeably quicker development of all markers and the presence of antibody to all the major proteins of HIV, in this otherwise healthy individual, in comparison to the immunosuppressed patient described above.

The predominant feature is the presence of antibody to the high molecular weight glycoproteins even in those with the p24 plus p55 motif which could be associated with a false positive reaction.

Once the criteria were established, decisions regarding positivity could be made with confidence based on the available clinical information, laboratory screening and confirmatory data.

False Positives

Fig.2.9 illustrates the type of false positive reactions seen on a WB; these are samples from blood donors, many of whom have donated repeatedly and yet have shown neither an increase or change in the number of bands on a WB nor development of a genuine antibody profile to HIV-1.

B1 and B2 (Fig.2.9) are from the same donor, 9 months apart and are reactive in two of the screening tests. B3 and B4 are from the same donor, 3 months apart and are negative by the then current screening tests. However, donations prior to these have been reactive by the previous routine screening test and the policy of the BTS is to follow-up these donors until they are negative by all tests and produce no viral bands on a WB at 3 consecutive visits. This is the same situation for samples B5-B7; they are negative by ELISA in the three donations over 6 months, and the WB pattern is unaltered.

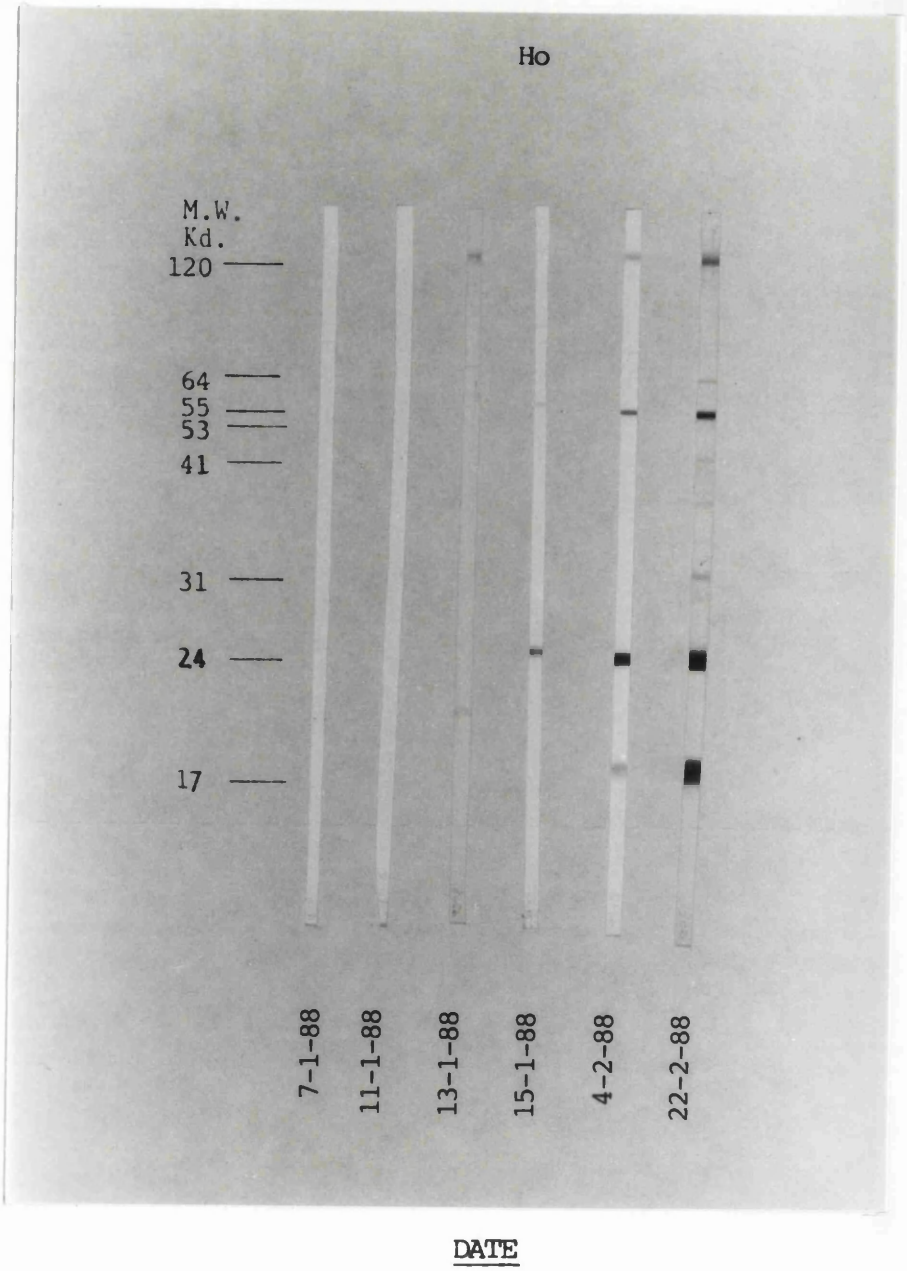


Figure 2.8. Confirmation of HIV-1 seropositivity by western blot (1:20) in serial samples from a homosexual patient (Ho).

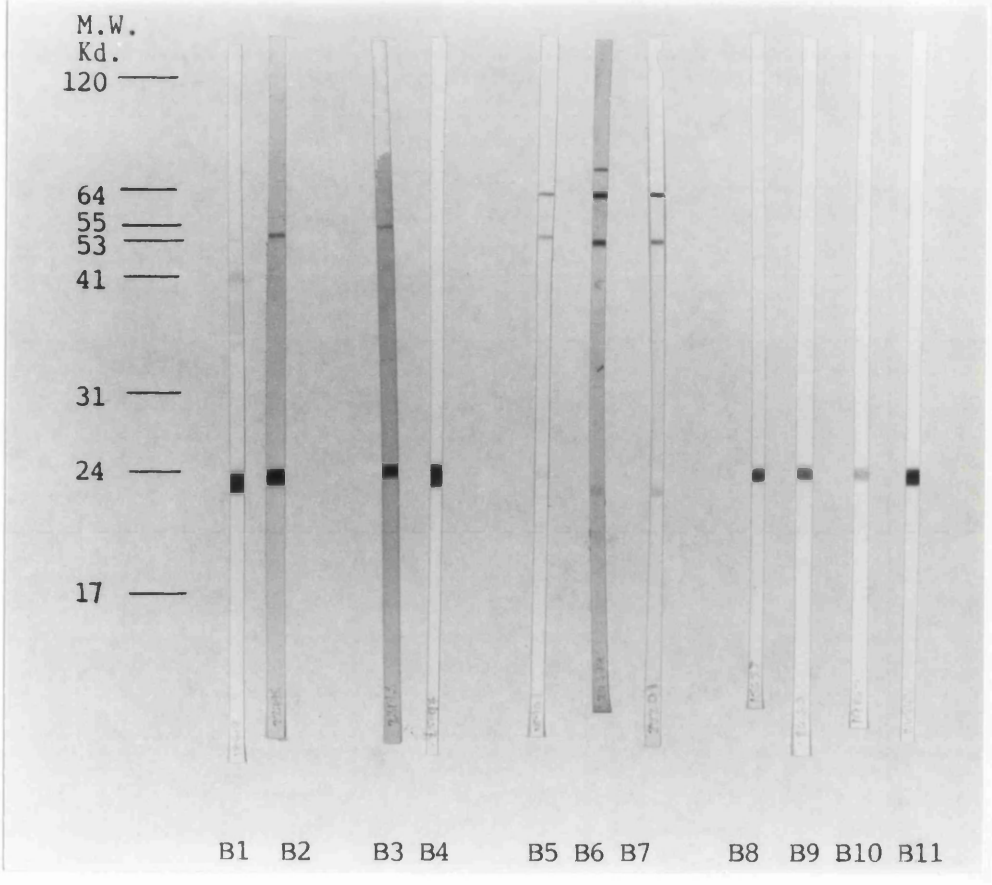


Figure 2.9. False positive reactivities on western blot (1:20)

B8-B11 show no change in WB in the same donor over a period of 18 months. However these samples are negative by all the current screening tests and would not have been blotted had they not been part of a follow-up programme of previous reactives. False positive bands have now been noted in other regions, gp41, gp160/120 to that of the pattern originally observed, i.e. a single band or a combination in the p17, p24 or p55 regions. In the routine western blots performed in 1990 on the Code 4 donors, i.e. those initially reactive on ELISA screening and repeatedly showing bands on a WB, it was found that 189 out of 392 such donors (48.2%) had bands visible, 24 of whom had 3 bands. The most common antigen to which reactivity was detected was p24 in 60.3% of cases followed by p64 (25.4%), p53, p55, p17 (18%) with only 2.6% of donors reacting with p31 (Dr. B.C. Dow, personal communication).

It was clear from the above studies that bands could be detected on WB of donor sera whether or not they reacted in a screening test. Therefore it was decided to determine the frequency of WB false positives in the normal blood donor population, i.e. a population which had been screened and shown to be ELISA unreactive. A sample of 50 blood donors (in 5 lots of 10 consecutive donors), negative by the screening test, were thus blotted at a dilution of 1:20. Thirty of the fifty (60%) were found to have bands in viral regions most commonly at p53 and/or p55 and p24. These donations passed through the system, oblivious to the presence of bands on a WB and were processed in the usual way. A western blot does not appear to be the ideal choice for confirmation of these low risk samples. The continued presence of WB bands on these follow-up samples from donors means that these donations are removed each time from the blood bank and unused. The cost to the blood bank supply is high since many individuals have continued donating in good faith, but their donation is flagged each time as a biological false positive until it is negative by all tests on three consecutive occasions. Some such donors have donated since screening began in October 1985 and very few (5%) have re-entered the blood bank pool. Their donation prior to October 1985 would never have been screened for anti-HIV 1 and would have been processed in the normal way. In addition each new

screening test picks out a new set of reactives which are then followed-up, thus increasing the number of unused donations.

Diluted Samples

An interesting difference was noted in the western blot pattern of the dilutions discussed in Table 2.5 and the pooled QC sera in Table 2.6. Fig. 2.10 illustrates what happens when single samples are diluted (in normal human serum). Antibody reactivity to a single protein remains on serial dilution. However multiple bands of low intensity were noted on the WB of the biochemistry quality control sera (Fig.2.11). This was pooled material and therefore it was assumed that a dilution effect similar to that in Fig.2.10 would be seen. It is not known why this WB pattern should be so, perhaps there is more than one infected donor in the pool.

Western Blot and Disease Progression

Follow-up samples were obtained regularly from HIV-1 Ab positive patients attending an out-patient clinic at Ruchill Hospital and in some patients a change in WB pattern was noticed as time progressed. Could the usefulness of WBs be expanded? Was a change predictive of infectivity or of disease progression? The predominant feature was a decreased reactivity to the p24 core protein and this correlated with the presence of HIV-related symptoms. This is shown in Fig.2.12 in a number of patients who first presented with symptoms.

When the time course of HIV infection was followed in individual patients, the decrease in core antibody over time was noted (Fig.2.13). It appears, therefore, that as time progresses and with the onset of symptoms, there is a resulting decrease in anti-p24. In certain (extreme) cases, only anti-env and no anti-core is visible on a WB. This correlated with a negative result in the core bead (Abbott Confirmatory EIA) in symptomatic patients, although this ELISA result becomes negative in most of these patients before a decrease in anti-p24 on the blot. The lack of sensitivity of this ELISA is useful and it plays a role as a prognostic indicator of disease

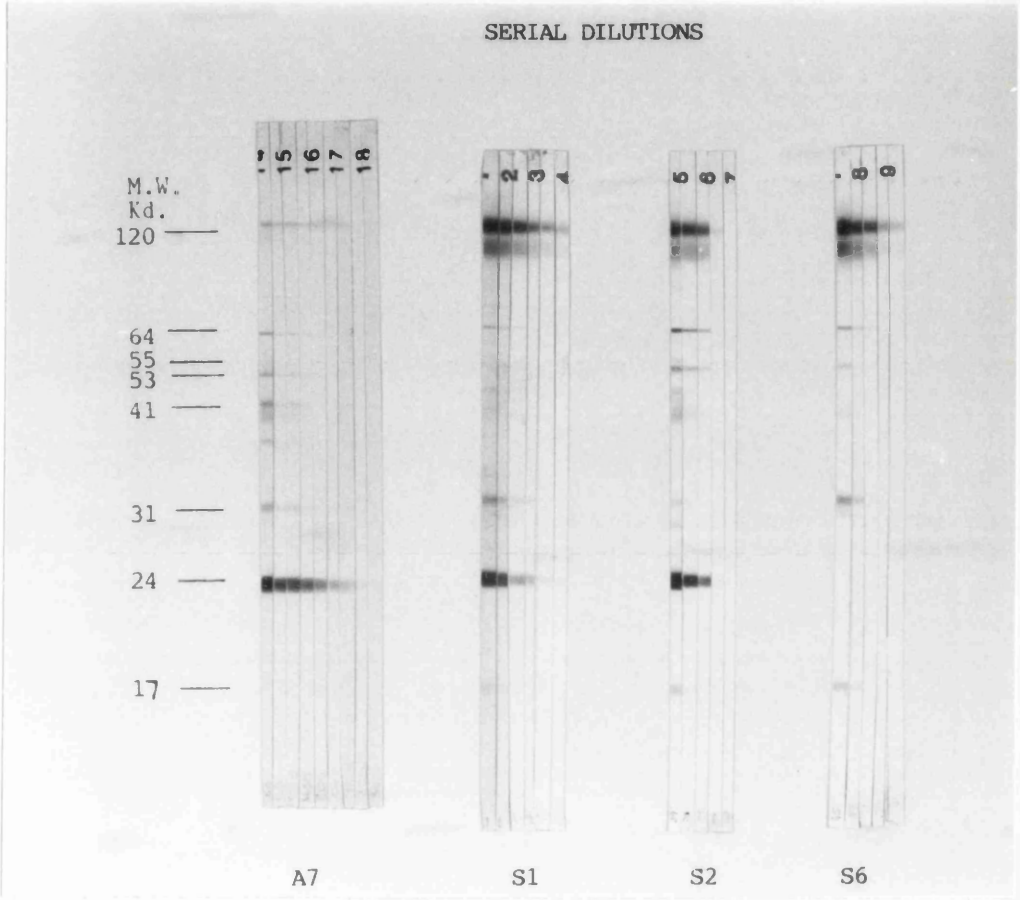


Figure 2.10. Serial dilutions by western blot (1:20).

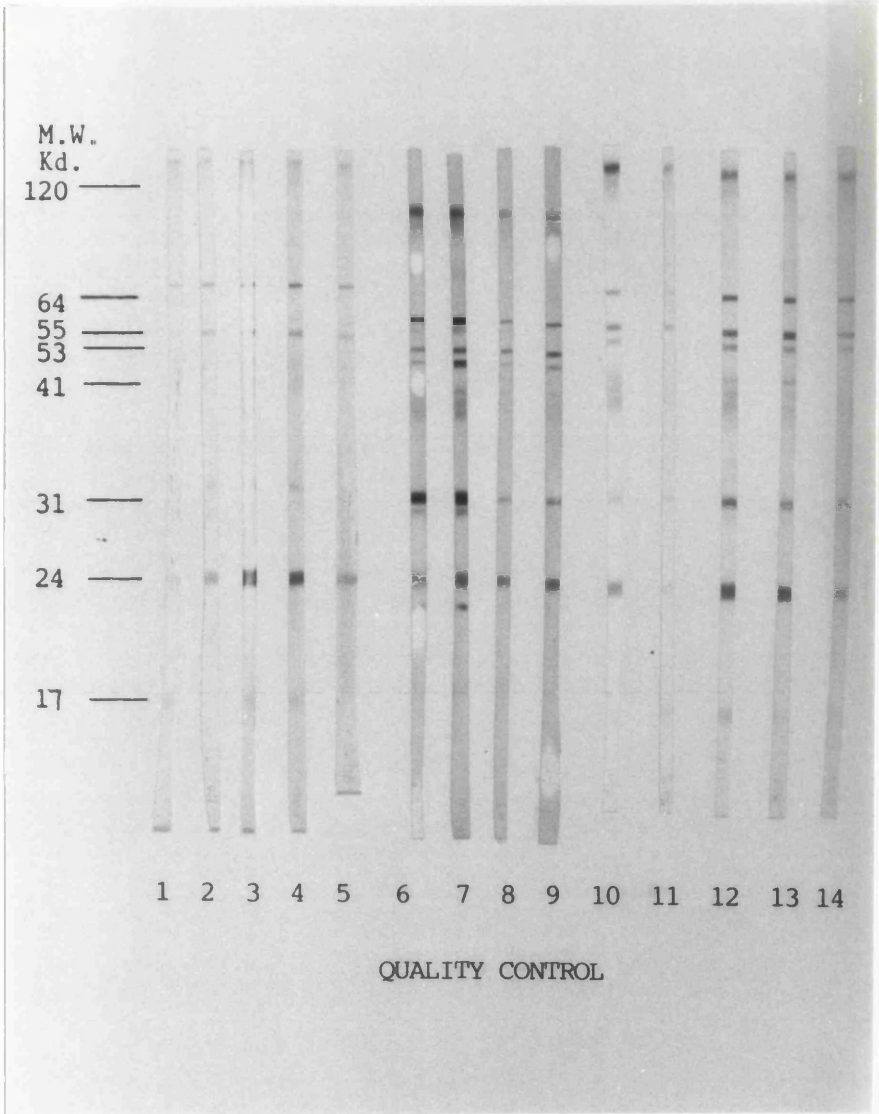


Figure 2.11 Quality control (pooled) sera by western blot (1:20)

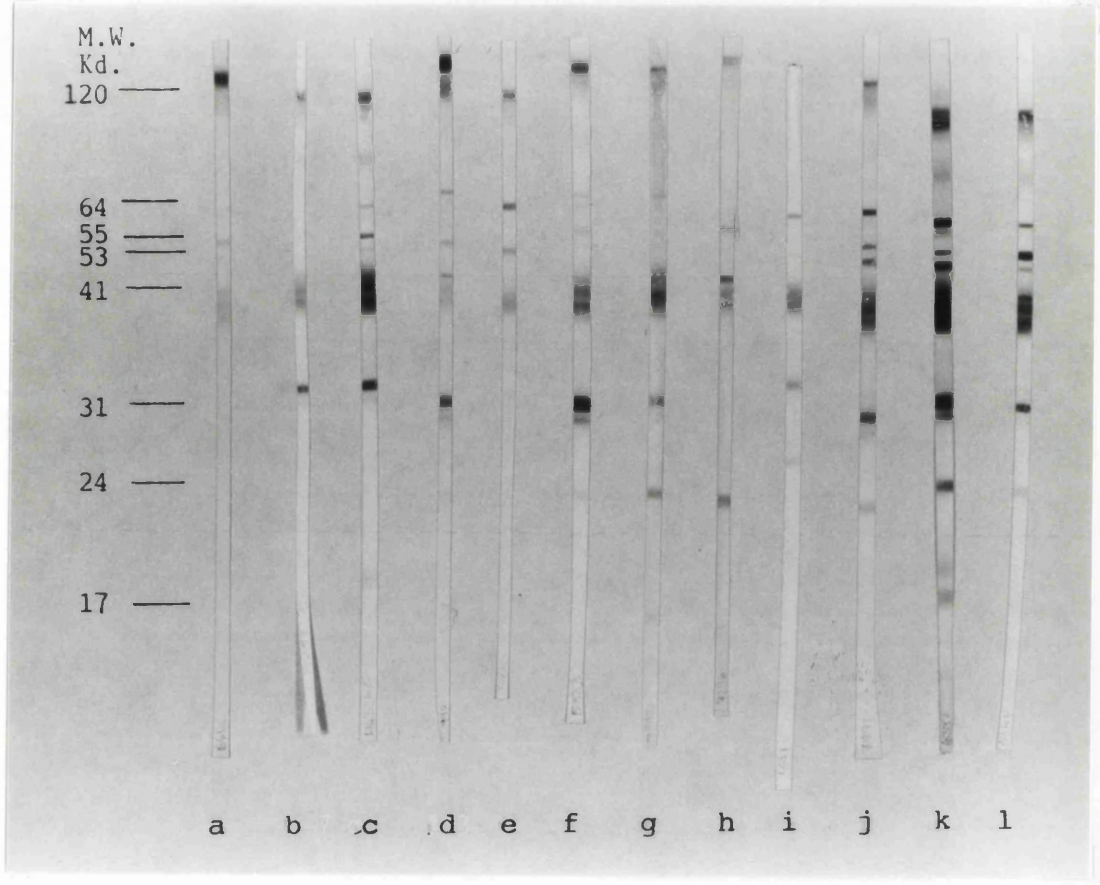


Figure 2.12 Western blot (1:100) antibody profiles from symptomatic HIV-1 seropositives

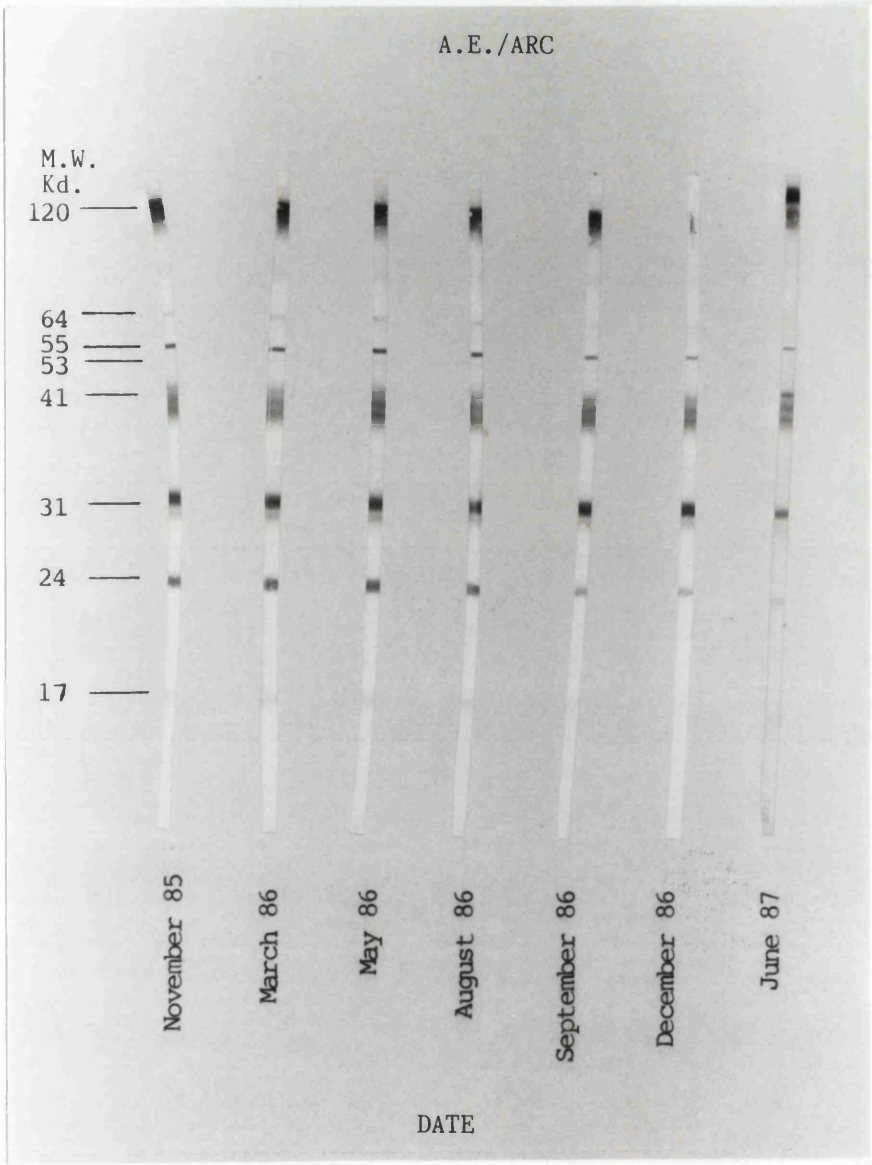


Figure 2.13 Temporal decline of HIV-1 anti-p24 observed on western blots (1:100) from a homosexual with AIDS-related complex (ARC).

progression whereas WB is an expensive and time-consuming test to perform routinely for this purpose.

Reading Western Blot Results

Laser densitometry can be used to read the developed nitrocellulose strips. This was attempted a number of years ago (courtesy of Professor H. Pennington, Department of Bacteriology, Forresterhill Hospital, Aberdeen) (data not shown). It was found not to be of significant use to justify the purchase of such expensive hardware. Two problems in particular were noted; the reader requires an even background of staining to establish a baseline, this is not always possible with the blots and secondly the area under the peak, produced when the laser beam passes through the coloured bands, is related to the width of the band rather than the depth of colour and therefore does not represent a measure of the intensity of the Ab-Ag reaction. Western blots have always been read by eye and a scoring system + to ++++ used to determine the intensity of the Ab-Ag reaction.

DISCUSSION

The need for a confirmatory test is self-evident because of the initial rate of false positives obtained when antibody screening commenced. A correct result is obviously important to a patient's physical and mental wellbeing. Therefore a confirmatory testing service has been in operation since screening began.

An ideal confirmatory test should have a high sensitivity and specificity, use an independent methodology from the screening test, examine the antibody reaction to a range of viral proteins, be able to detect all strains of HIV-1 and ideally detect all classes of antibody, i.e. IgG, IgM and IgA.

The test of preference at the HRL has been, and remains the western blot. The development of a commercial Western Blot kit for HIV-1 meant ease of use since quality control had already been performed. This is important because of the subjective nature of WB interpretation. As with many of the tests examined in this Chapter, the assay improved over the time in use at the HRL. The western blot has improved mainly due to enhanced envelope glycoprotein detection. The transfer of glycoproteins to NC has always been notoriously difficult, probably due to their bulky sugar side chains. However the higher molecular weight glycoproteins, gp160 and gp120, are now clearly distinguished as two separate bands.

The strong ELISA reactives present no problem in confirmation on any test system, showing multiple bands of intense reactivity on a WB. However the occurrence of weak ELISA reactives meant that the criteria of positivity had to be established to distinguish a genuine positive from that of a false positive (FP). The spread of HIV infection in the DA population tested in 1986 and the follow-up of the unfortunate recipient of an infected transfusion allowed the development of markers to be examined and assisted in determining a minimum expected result for a sample to be considered positive at the HRL, i.e. that of the presence of 3 viral specific bands, one of which must be the high molecular weight envelope glycoprotein.

Various criteria from different sources have been established over the years. The initial p24 and/or gp41 requirement has most

certainly been updated (126). A report by the Consortium for Retrovirus Serology Standardisation in 1988 stated that four different "standard" criteria were being used (172). These included:

(i) presence of antibodies to p24, p31 and gp41 or gp160/120 (DuPont);
(ii) presence of antibodies to at least one gene product from each of gag, pol and env (American Red Cross);
(iii) presence of antibodies to any two of p24, gp41 and gp160/120 (Association of State and Territorial Public Health Laboratory Directors (ASTPHLD), Department of Defense) and
(iv) presence of antibodies to at least p24 or p31 plus gp41 or gp160/120 (Consortium for Retrovirus Serology Standardisation.

The latter was the minimum accepted since No.(i) would exclude many of the AIDS patients in whom the anti-p24 reactivity has been reduced. However No.(i) is probably most used since this is the recommended criterion of the FDA licensed kit. In a report on the use and interpretation of WB in the serodiagnosis of HIV-1 infection, the CDC recommended the use of the ASTPHLD criterion which gave the highest percentage of positives and the lowest percentage of indeterminate results (130). The criterion described and used at the HRL in conjunction with clinical information and follow-up where indicated, has presented no problems in HIV confirmation and serodiagnosis. This criteria has been updated recently to a requirement of four bands, two of which must be gp120 and gp160 as these proteins are now routinely visually distinct as two separate bands on a developed WB. The lack of a pol gene product requirement in the HRL criteria is because antibody to these proteins developed late in the seroconversion case, patient Tx (Table 2.14).

Care has to be taken in reading the blots and making comparisons because of the variation between batches and the subjective nature of interpretation. A western blot is only as good as the antigen preparation on the strip, i.e. a high quality, clean antigen source is

a necessary requirement. A sample can react only with the proteins present on the nitrocellulose so each viral protein needs to be represented in sufficient quantity with minimal cellular components, to give a clean background. Problems were encountered when DuPont supplied strips of inferior quality from their manufacturing plant in Singapore. Interpretation was made difficult due to the presence of multiple non-viral bands and high background on colour development. It is important to note that the proteins are not in their native state as they have been disrupted with detergent. This may affect antibody binding since linear epitopes rather than conformational epitopes will be recognised. The transfer of glycoproteins from polyacrylamide gels to nitrocellulose is also notoriously difficult, resulting in smeared bands.

The occurrence of false positive bands at equivalent positions to that of the viral proteins aroused suspicion in these patients and tended to discredit WB ("Indeterminate" category). These individuals who have been followed up showed no development of markers and in many cases retained the same reactivities over a period of time. This is understandably of great concern in blood-donor screening. Donors with such indeterminate reactions are excluded from the blood bank (but not from donating) until 3 consecutive donations are completely negative by all screening and confirmatory tests. This rarely occurs, only 5% of such donors in the West of Scotland, and current proposals to change this are being considered. Genesca et al. (173) found that 20% of ELISA negative blood donors produced an indeterminate WB pattern. Recipients of such blood donations also showed indeterminate patterns and there was not always a correlation of band specificities in a donor-recipient pair. No donors or recipients seroconverted for HIV-1 and none were shown to contain HIV-1 gag and env sequences by polymerase chain reaction (PCR). Thus this FP reactivity is likely to be non-viral in origin. What are these false positive (FP) reactions? Are they HLA antigens which co-migrate with viral proteins when the disrupted viral infected cells are run on a gel? This may be the case in certain patients and has been demonstrated by Blomberg and Klasse (125). A limited amount of WB testing was performed with a mock-infected H9 cell line but with no

conclusive results as to the nature of the FP reaction (data not shown). It may be explained by cross-reaction to other retroviral sequences particularly that of the group specific antigen or gag protein(s); a legacy of ancient retroviral sequences or of those yet undiscovered. Up to June 1988 all the code 4 donors at the Regional Transfusion Centre, Law Hospital, i.e. those indeterminate or unconfirmed donors with a reactive or previously reactive ELISA result and in some cases FP western blot bands were tested and found to be HIV-2 seronegative. In the literature reference has been made to certain species of anti-human IgG conjugates and anti-species IgGs reacting with the p24 on HIV-1 western blots (125,174).

It appears that each ELISA test detects its own set of false positive reactions for one reason or another and hence it is important to use a confirmatory test with a different source of antigen from that used in the screening test otherwise the false positive ELISA reaction will also be mirrored by the confirmatory test. This has always been the practice at the HRL.

The DuPont kit has emerged as the best of the western blot assays (125) and was the first test of this nature to be licensed by the Food and Drug Administration (FDA), USA. In the early days the use of a radioimmunoprecipitation assay (RIPA) was advised to complement the western blot since it was better at detecting anti-envelope reactivity. This was important in those with an isolated anti-p24 reactivity and could be used to establish a genuine early infection from a FP. However enhanced envelope glycoprotein detection has improved the blots. Recent reports (175,176) have shown that gp41 forms multimers and viral lysate antigen preparations were found to contain this multimeric form, which has the same mobility as gp160. Anti-gp41 reacts at this site. This may give rise to false identification of reactivities. The use of monoclonal antibodies showed that true gp160 and gp120 material was present on the DuPont WBs (176). Reference laboratories rely on this quality control work being carried out at source before the kit is supplied commercially. In addition several drawbacks to the technique of RIPA, e.g. the use of radio-active label and the requirement for cultures of HIV-infected cells, would have been prohibitive in many

laboratories.

The DuPont WB is still widely used despite the introduction of recombinant immunoblot assays (RIBA). These were found not to be significantly more sensitive or specific than the viral lysate WB. But recently the RIBA strips have incorporated a recombinant protein from HIV-2, most commonly a gp36(env) epitope, e.g. Pepti-LAV 1/2 (Diagnostics Pasteur), LiaTek HIV 1 + 2 (Innogenetics N.V., Belgium) etc. This allows simultaneous testing of those samples which react on the combination ELISAs. Since the current screening tests now detect antibody to HIV-1 and HIV-2, it is necessary to confirm the presence of either anti-HIV 1 or anti-HIV 2 or both, i.e. dual infection. The viral lysate HIV-1 WB (DuPont) is the first line confirmatory test following a repeatable positive ELISA result. This is supplemented with an HIV 1+2 RIBA strip and where necessary an HIV-2 viral lysate WB. This provides a comprehensive means of identification of the infecting virus and confirmation of the antibody screen.

It was previously thought that additional information relating to stage of infection in an HIV-1 Ab positive could be read from a WB and in this way the WB would be predictive of a change in clinical status. The intensities of the bands are scored + to ++++ and the number of bands present are recorded. A number of patterns have been noted; e.g. (1) in seroconversion, high intensity reactions to few bands particularly p17, p24 and gp160, gp120, (2) high intensity reaction to all bands in the 'strong positives', generally the asymptomatic seropositives, (3) loss of anti-core antibodies generally in patients with disease progression and (4) in some cases of AIDS low intensity reactions to several proteins. In addition the pooled sera showed multiple reactivities of low intensity which was unusual. The WB has no predictive role since clinical AIDS is often manifest before a reduction in anti-core reactivity. However experience in observing such patterns enables the user to be confident in the interpretation of results.

SCREENING AND CONFIRMATION: CONCLUSIONS

The continuing advances in technology have benefitted the development and improvement of both the anti-HIV screening and confirmatory tests. This has allowed the use of alternative sources of antigen, e.g. the manufacture of recombinant antigens or synthetic peptides and the incorporation of a similar related virus, HIV-2, into the testing regime. In the future, related viruses including HTLV-I and -II may be added to the solid phase of an ELISA test such that a retrovirus screen is initially performed. Further testing with monospecific ELISAs, i.e. specific for one virus and confirmatory assays would be used to establish the nature of the reactivity of a test serum on such a screen.

The rapid improvement in sensitivity and specificity of the commercial ELISAs meant that they very quickly matched the confirmatory test, the western blot. There exists the school of thought that use of a multiple ELISA testing system could meet the needs of a confirmatory test (177). Tests of varying methodologies, e.g. competitive, direct, particle-agglutination and different sources of antigen, e.g. viral lysate, synthetic peptides, recombinant proteins, could be used in sequence. Any non-specific reaction in one test would not be repeated in a different test. The success of this procedure depends on all the ELISAs having a very high sensitivity and routinely quality controlled to maintain this. It is known in practice that different tests do detect certain samples, particularly those of genuine weak positives from a seroconversion, better than others. The use of various ELISAs of single specificity is now increasingly recommended to distinguish HIV-1 and HIV-2 seropositivity in specimens reacting in the first-line combination tests, and in this way reduce the number of samples tested on western blots or recombinant immunoblots, which are more expensive and more time-consuming to perform.

The WB has continued to be the method of choice in finally confirming seropositivity, certainly for HIV-1 at the HRL. There has been very little experience of using WB to confirm anti-HIV 2

seropositivity, as there has been no HIV-2 infection found in the population under test at the HRL. The WB has improved over time, particularly with respect to envelope glycoprotein detection, and efforts must be maintained to achieve the maximum performance from these strips. It may be necessary, in the future, to increase the sensitivity of the detection system using technologies such as radiolabelled conjugate or a chemiluminescent substrate.

It is evident that as ELISA technology is pushed to the limits of sensitivity, the confirmatory tests must keep ahead. The provision of an antibody profile and the specificity of western blotting still undoubtedly remain useful.

Another current challenge is to distinguish between anti-HIV 1 and anti-HIV 2 reactivity after an initial screen by the HIV-1/-2 combi tests. The nature of the antibody cross-reactions does make it more difficult to determine the true result. HIV-2 antibodies bind most readily to the core proteins and to a lesser degree with the envelope proteins of HIV-1 (117). However HIV-1 antibodies react mainly with the gag and pol gene products and do not recognise HIV-2 env gene products. So-called monospecific ELISAs do detect antibodies to both viruses and thus it is important to have good confirmatory systems.

There are viral lysate WB kits commercially available for both HIV-1 and HIV-2 and it is encouraging to visualise the antibody profile to back up an ELISA result. Also available are combination immunoblots where recombinant or synthetic peptides from HIV-1 and HIV-2 are painted onto a nitrocellulose support. Although more rapid than a WB they have been disappointing in use. The obvious advantage is in being able to distinguish between HIV-1 and HIV-2 in one step. However the major drawback in those kits currently available is the lack of HIV-2 specific peptides on the strip and often a positive HIV-2 Ab result is based on reactivity to a single epitope, most commonly that of the transmembrane glycoprotein gp36. It is hoped that in the future, further epitopes or peptides from HIV-2 will be included in these recombinant immunoblots and that their test peformance will improve to match or even better that of the viral lysate WB.

Minor alterations and fine tuning of tests continue to maximise the performance of ELISAs and WBs in HIV Ab screening.

CHAPTER 3

EPIDEMIOLOGY OF RETROVIRUS INFECTION IN GLASGOW AND THE WEST OF SCOTLAND

INTRODUCTION

The advent of an antibody screening test allowed the majority of those infected with HIV to be identified. The importance of screening the blood donor population for a virus which is transmitted by blood and blood products is apparent. However, alternative testing sites had to be made available to discourage members of any risk groups from using blood donating centres to determine their HIV status and therefore to reduce the chance of an infectious donation passing through the system undetected. The implementation of antibody screening at the Hepatitis Reference Laboratory (HRL) in April 1985 together with the opening of the Counselling Clinic in 1986 provided this service for Glasgow and the West of Scotland. Testing is performed on a voluntary basis.

To fulfil an epidemiological role, i.e. to assess the prevalence of HIV in the population, the laboratory is dependent on receiving samples from those individuals considered to be at risk of infection. Screening of only those individuals who wish to know their status can bias the prevalence figures in two ways; an underestimate if the majority of tests are performed on patients in the 'worried-well' category which generally comprises those at least risk, or an over-estimate when high rates are found in low numbers of the at-risk population. However, trends in prevalence amongst groups that are available for testing provide some estimate of how HIV infection is spreading in the population.

Early epidemiological studies in the USA examined the risk groups of patients with AIDS. This identified those most at risk from acquiring HIV: transfusion recipients of blood and blood products including haemophiliacs, intravenous drug abusers, homosexuals, Haitians, Hispanics and sexual contacts of these groups.

The results presented in this Chapter implicate the same groups at risk in the area of testing covered by the HRL (with the exception of Haitians and Hispanics, who have not been tested in our population).

MATERIAL AND METHODS

As a prelude to the introduction of screening of all blood donations by the Blood Transfusion Service (BTS), HIV-1 testing first began at the HRL in October 1984 using an immunofluorescence technique. Pre-prepared slides coated with LAV infected and uninfected H9 cells (Pasteur) were used on selected patients. More extensive screening became possible with the advent of an ELISA test system (Abbott, Appendix 2) in April 1985. Some retrospective testing of sera from at risk groups was performed at this time.

Samples were received from: infectious disease (ID) clinics, genito-urinary medicine (GUM) clinics, the counselling clinic, general practitioners, drug rehabilitation centres, prisons and young offenders institutions, hospital in-patients, the regional haemophilia centres and the BTS.

On introduction of routine screening an attempt was made in the laboratory to assign a risk group code or reason for testing code to all specimens sent to the laboratory for HIV antibody testing. The codes had to be simple and sufficiently specific to provide well-defined population groups. The following codes have been in use since April 1985:

DA	-	admitted drug abuser
Ho	-	homosexual
Bi	-	bisexual
CON	-	contact of risk groups
R	-	risk (unspecified)
WW	-	worried well
Hm	-	haemophiliac
VISA	-	for work abroad
INS	-	for insurance purposes

Most information was made available regarding the DAs and they

have formed the best defined group in this study.

In November 1988, the Communicable Diseases (Scotland) Unit (CDSU) produced a surveillance form seeking information from patients on risk group and the reason for testing, for all HIV tests requested in Scotland (CDS Denominator Study). This provided additional detailed information on the risk groups being tested and their contacts. The patient's details on the forms are put into a computer under code and therefore provide a database of information on all samples received for HIV testing. The clerical officers employed to do this also make follow-up telephone calls to seek missing relevant patient information if this is not included in the initial form completed by the requesting clinician.

RESULTS

A. HIV-1 SEROPREVALENCE:

TOTAL NUMBERS IN GLASGOW AND THE WEST OF SCOTLAND WITH RISK GROUP BREAKDOWN

Table 3.1 shows the total numbers screened per year and the total numbers reported HIV-1 antibody positive (including those known to be positive elsewhere). In addition 30 haemophiliacs, including both children and adults, were found to be positive before 1985 (see Chapter 5).

The HIV Reference service at the HRL covers six health board areas including Greater Glasgow, Argyll and Clyde, Lanarkshire, Ayrshire and Arran, Dumfries and Galloway and Forth Valley, with a total population estimated at 2,751,650 in June 1987 (CDS Weekly Report: back cover). In these six health boards no samples are reported positive without confirmation at the HIV Reference Unit at the Regional Virus Laboratory.

Over the 63 months, from April 1985 to the end of June 1990, a total of 23,857 specimens have been examined in the Hepatitis Reference Laboratory (HRL), Ruchill Hospital. The number of total seropositives in the population screened is **398**, a prevalence of 1.7%. However, this figure is misleading and is of little significance. The total number screened is artificially high because it includes repeat specimens from patients, reference samples from the Blood Transfusion Service (BTS), sent in triplicate and latterly multiple saliva specimens from known HIV-1 antibody positives, for a special study. The total number can also be considered artificially low as it does not include negative samples screened at laboratories in the Region carrying out their own tests.

In addition the sample population is not uniform year to year as it is influenced through publicity campaigns by the Government using television commercials, the press and bill poster advertising, leading to an increase in the numbers tested in the "worried well" category at that time, i.e. those most worried but generally at least risk

TABLE 3.1

Total number of HIV-1 seropositives detected each year from April 1985 to June 1990*

Year	Total Nos. screened	Total Nos. positive
Pre 1985	-	30
1985 (Apr-Dec)	881	66
1986	3,010	95
1987	5,228	82
1988	5,937	53
1989	5,484	44
1990 (Jan-Jun)	3,317	28
Total	23,857	398

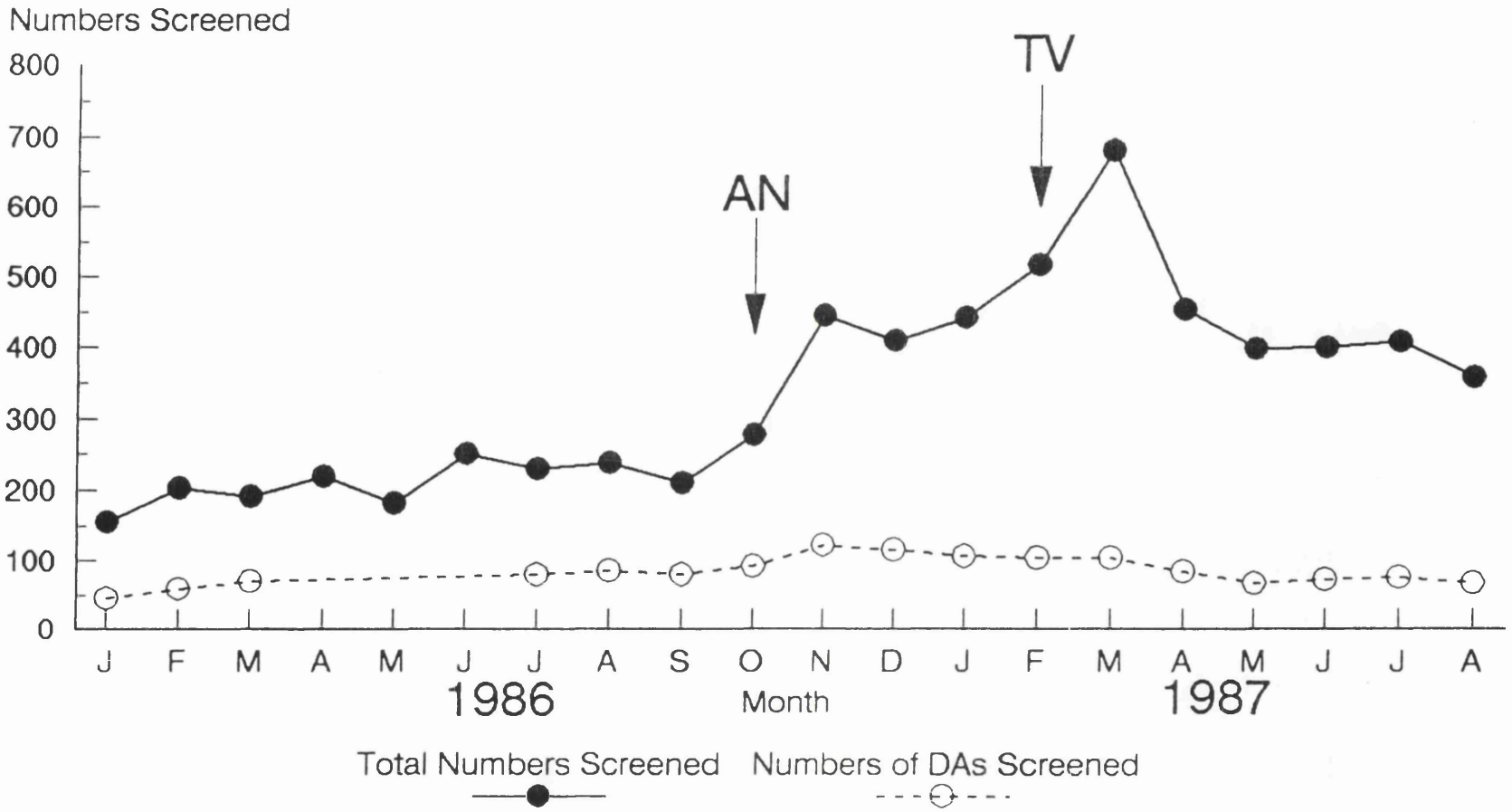
* This includes 30 haemophiliacs found to be antibody positive prior to 1985 by retrospective testing

(Fig.3.1). The number of drug abusers tested remained fairly constant during 1986 and most of 1987. It has since dropped to a new level but remains constant, currently testing an average of 43 drug abusers per month in 1990. Therefore the numerator in the prevalence quotient is very accurate as all potential positives are referred to the HRL but the denominator is inaccurate and becomes more so as increasing numbers of laboratories introduce screening. Hence the need for the CDSU Denominator Study to provide an accurate denominator for the quotient.

A breakdown of the figures in Table 3.1 demonstrates the contribution made by each risk group each year to the total number of reported seropositives (Table 3.2). These figures represent the new positive cases, i.e. only those whose sera were confirmed as positive at the HRL for the first time and had not been confirmed and diagnosed as seropositive elsewhere. To complement this are the figures in Table 3.3, showing those who have come to live in the health board areas under test but who have been diagnosed as seropositive elsewhere.

The DAs and the Hos are the major contributors to the total seropositives. The numbers of Ho and Bi seropositives per quarter and the incidence of diagnosed infection per quarter in the DAs from April 1985 to the end of June 1990 is shown in Table 3.4. An increase was noted in the Glasgow population of DAs in 1986. The prevalence of infection increased from 1.7% in the first quarter to 5.8% in the last quarter of 1986. The percentage positive remained high at the start of 1987 but subsequently decreased and has remained at this new level (less than 2%) over the following years. A further 4 seropositive Glasgow DAs in 1987 and 4 DAs in 1990 were confirmed at the HRL although initial screening was performed in two other laboratories in the city.

The Ho/Bi totals in Table 3.4 record all the confirmed Ho and Bi seropositives in Glasgow and the West of Scotland area covered by testing at the 3 laboratories in the city. A consistent number of Ho/Bi were detected each year after 1985. There was little information available regarding the prevalence of HIV-1 infection in this population until the CDS Denominator Study began.



AN:Advertising and Newspaper Publicity Campaign
TV:Television Publicity Campaign

FIGURE 3.1 Total numbers of samples sent to the HRL for HIV-1 screening and the numbers of DAs in the sampling population

TABLE 3.2 Risk group breakdown of newly diagnosed HIV seropositives in Glasgow and the West of Scotland for the period April 1985 to the end of June 1990.

Year	DA	Ho	Bi	Hm	HET	TxRecpt.	Exposure Abroad	Other	Unk	Total
1985 (Apr-Dec)	32	23#	0	4	0	0	1	0	2	62
1986	57	15	1	0	2	3	2	3	7	90
1987	32	15	1	0	0	0	2	3	10	63
1988	13	15	2**	0	1	0	6	0	1	38
1989	6	11	1	0	2	1	3	0	2	26
1990 (Jan-Jun)	7	5*	2	0	0	0	3	1	1	19
Total	147	84	7	4	5	4	17	7	23	298

* - including 1 Ho/DA
** - including 2 Bi/DA
\# - includes 2 patients found to date from 1984

TABLE 3.3 Risk group breakdown of HIV-1 seropositives, known elsewhere, but new to this reporting centre.

Year	DA	Ho	Bi	Hm	HET	TxRecpt.	Exposure Abroad	Other	Unk	Total
1985 (Apr-Dec)	0	3	0	1	0	0	0	0	0	4
1986	0	2	1	2	0	0	0	0	0	5
1987	3	10	1	0	1	0	0	0	4	19
1988	4	8*	0	0	1	0	2	0	0	15
1989	3	5	0	0	0	0	0	0	10	18
1990 (Jan-Jun)	3	4	1	0	1	0	0	0	0	9
Total	13	32	3	3	3	0	2	0	14	70

* - including 1 Ho/DA

TABLE 3.4 The number of newly diagnosed infections per quarter in high risk groups in Glasgow and the West of Scotland during the period April 1985 to June 1990.

Year and Quarter	No. DAs tested	No. DAs seropositive in Glasgow and the West of Scotland* (Greater Glasgow only)		No. Ho and Bi seropositive
1985				
A-J	154	3	(0)	6
J-S	257	5	(0)	5
O-D	195	24	(5)	10
1986				
J-M	177	8	(3)	7
A-J	222	16	(4)	3
J-S	246	9	(6)	3
O-D	329	24	(19)	3
1987				
J-M	314	14	(11)	7
A-J	224	8	(7)	7
J-S	216	4	(2)	1
O-D	170	2	(0)	1
1988				
J-M	163	2	(2)	9
A-J	163	3	(2)	2
J-S	165	4	(3)	2
O-D	138	3	(3)	4
1989				
J-M	178	2	(1)	4
A-J	136	2	(2)	2
J-S	148	2	(0)	2
O-D	114	0	(0)	4
1990				
J-M	139	0	(0)	4
A-J	99	3	(1)	3

* Prevalence can be assessed in the drug abusers and represents only those tested at the HRL. A further total of 9 DAs (8 from Greater Glasgow) tested at two other virus laboratories in Glasgow but confirmed at the HRL complement these figures to give the total of 147 shown in Table 3.2

In summary the results suggest that the pattern of infection in Glasgow has changed over the 63 months since routine screening began. In 1985, from our sampled population, HIV-1 infection was more predominant in the homosexual group than the Glasgow drug abusers. In 1986 this pattern was reversed and the prevalence of infection in the DA population increased 3.4 fold to the end of the year. In general since this time, the number of seropositive DAs has decreased (until the start of 1990), the number of Hos (including Bis) has remained constant but there has been an increased contribution from other risk groups, particularly in those exposed abroad, which includes a high percentage of African nationals.

1. SEROPOSITIVE DRUG ABUSERS

Total in Glasgow and the West of Scotland

In Table 3.2 the drug abusing seropositives contribute 49.3% of the total. The numbers are highest in 1985, 1986 and 1987 then decline.

1985

The laboratory received samples from various health boards and it was noted that in 1985 most of the seropositives were sent from the Forth Valley Health Board. Indeed only 5 out of the 32 found positive in 1985 were known to reside in Glasgow (Table 3.5). At this time serum samples were sent for testing from Cornton Vale Prison near Stirling, the only women's prison in Scotland. Fifteen out of the 21 seropositive females were from there. However, enquiries as to the home addresses of these patients revealed that 11 came from Edinburgh and 4 from Dundee. All of the remaining six seropositives lived in the West of Scotland and included one from Ayrshire and Arran Health Board area who had been in France for the previous three years and one from a drug rehabilitation centre in Tarbert who had connections with Edinburgh. The remaining four females were from

TABLE 3.5

HIV-1 seroprevalence in drug abusers tested at the Hepatitis Reference Laboratory.

Year	No. DAs tested	No. seropositives in Glasgow and West Scotland	M/F	No. seropositives Glasgow only	M/F
1985 (Apr-Dec)	606	32	11M/21F	5	1M/4F
1986	974	57	33M/24F	32	20M/12F
1987	924	28	10M/18F	20	5M/15F
1988	629	12	10M/2F	10	8M/2F
1989	576	6	6M	3	3M
1990 (Jan-Jun)	238	3	3M	1	1M
Total	3,944	138	73M/65F	71	38M/33F
*1987		4	1M/3F	4	1M/3F
*1988		1	1M		
*1990		4	2M/2F	4	2M/2F
Total		147	77M/70F	79	41M/38F

* Samples sent from two other virus laboratories in the city for confirmation.

Greater Glasgow, one of whom had been in Edinburgh and had returned to her home in Glasgow, probably early in 1985.

One of the male DAs tested positive in 1985 was known to reside in Glasgow. Two others were from another Glasgow hospital and another was in prison here, however in all 3 cases their area of residence was unknown. Another patient came from the Forth Valley area. Six of the male seropositive DAs found in this year had Edinburgh connections: three via Dumfries, one via Lanarkshire and two via a drug rehabilitation centre in Cardross, Argyll and Clyde.

1986

In 1986 the pattern changed and 56% of the DAs found seropositive in that year were Glasgow residents. In that year, three out of 15 samples found positive from Cornton Vale Prison were Glasgow residents (seven ex-Edinburgh, three ex-Dundee, one ex-Aberdeen and one unknown). A further nine seropositive female DAs were from Glasgow. Seven of the 33 male seropositives were from Edinburgh, one from the USA, five whose connections were unknown and 20 from Glasgow.

1987

The Edinburgh contribution to the total decreased further in 1987. Five of the 10 male seropositives detected at the HRL in this year were from Glasgow. The male DA referred for confirmation from another Glasgow hospital had been found dead. One male was in prison, the remaining four were sent from hospitals in the outlying health board areas covered by the HRL; one who was known to have Edinburgh connections, one from Stirling and two with connections further afield, in London and Amsterdam. Eighteen of the 21 seropositive females were from Glasgow, three of the requests were sent from Cornton Vale and the remainder were sent from several sources within the city including the counselling clinic, Ruchill Hospital, and three referred for confirmation. One other positive report from Cornton Vale was a female from Aberdeen. The two remaining seropositives were resident in health board areas in the West of Scotland.

After this time very few specimens were received from Cornton Vale Prison. Most testing of DAs was from within Glasgow via G.P's, hospital out-patients, drug rehabilitation units and the counselling clinic, which opened at Ruchill Hospital in December 1986.

The contribution of Glasgow DAs to the total continued to be high in 1987 but the total number found was reduced and the overall prevalence (3%) was less than in the previous year (Table 3.5).

1988

In 1988, 10 of the 12 seropositives at the HRL were Glasgow residents, five of whom were tested via the Counselling Clinic. One DA was in the drug rehabilitation ward at Ruchill Hospital and another had been admitted to the hospital with an acute hepatitis B infection. Two of the other three Glasgow seropositives were sent for testing from Glasgow hospitals and the remaining one was tested via the forensic laboratories. The remaining two include one male prisoner and one male DA from Lanarkshire tested via his GP.

1989

Only 6 seropositives were diagnosed in 1989, 3 of these came from health boards outwith Greater Glasgow. Two of the remaining three were patients in the drug rehabilitation ward in Ruchill and the other patient was a prisoner.

1990

When the figures for the other Glasgow laboratories are included in the total it can be seen that as many DAs were found seropositive in the first half of 1990 as were detected in total in 1989. Five out of the seven seropositives in 1990 resided in Glasgow; two of these were found deceased (probably drug related). One new infection was noted in this year in a female DA who was seronegative on testing in December 1989, but subsequently seroconverted in May 1990.

Early Infections in Glasgow Drug Abusers

Fifteen of the 32 Glasgow DAs found positive in 1986, one from

1985, and five out of 24 seropositives in 1987 had previous specimens in 1984, 1985 or 1986 that contained no detectable antibody to HIV-1 (Table 3.6). Many of the remainder showed serological evidence of recent infection by ELISA and western blot. A test for HIV-1 antigen (Abbott Diagnostics) in 16 specimens taken up to 16 months prior to the first positive antibody result was reactive in only two (four and five months prior to antibody detection). These results were unconfirmed due to insufficient sample remaining for a neutralisation test.

A total of five drug abusers (1M/4F) resident in Glasgow were detected in 1985. The earliest case was a female DA who had previously been in Edinburgh, had returned home to Glasgow and was found to be HIV-1 antibody positive in the first sample available for testing in February 1985. In the last quarter of 1985, two females and one male DA were found seropositive and were known to have shared needles with the first case mentioned above. In addition, another female DA was found retrospectively to have seroconverted during October to November 1985, but remained undetected with the screening test used at that time until a serum sample was taken a year later, in October 1986. She has now been included in the 1985 figures.

HIV-1 infection appears to have entered the drug abusing population in Glasgow during the latter part of 1985, early 1986 and subsequently spread. Seroconversions are still occurring in this risk group but they are now a rare occurrence; three DAs in 1988 and one DA in 1990 were known to have previously seronegative samples (Table 3.6). In two of these cases, several samples had been taken prior to their first seropositive results, implying that they were considered to be at high risk of acquiring HIV-1 infection, since a drug-abusing sibling was HIV-1 seropositive in both cases.

Spread of Infection

An examination of the area of residence of the Glasgow seropositive DAs shows that the majority came from the G20-G23 postcode areas (Table 3.7). These are areas long associated with drug abuse. In addition other clusters of positives have been found

TABLE 3.6

Timing of HIV-1 antibody seroconversions in drug abusers in Glasgow.

Sex/Age	Last Ab. Neg. Month/Year	First Ab. Pos. Month/Year	Time Interval (Months)
F22	10/85	11/85	1
F19	12/85	5/86	5
M20	3/86	7/86	4
M21	12/84	8/86	20
M19	5/85	10/86	17
F19	11/85	10/86	11
F16	6/86	11/86	5
M32	5/86	11/86	6
F31	7/85	11/86	16
F24	6/86	11/86	5
F18	4/86	12/86	8
M20	2/86	12/86	10
M18	7/86	12/86	5
M22	8/85	12/86	16
M19	10/85	12/86	14
F22	4/86	12/86	8
F25	9/86	2/87	5
F25	10/86	2/87	4
F23	3/87	5/87	2
F16	4/86	6/87	14
F20	11/84	12/87	37
F30	3/87	2/88	11
M24	6/86	9/88	27
M29	2/88	11/88	9
F23	12/89	5/90	5

TABLE 3.7 Post-code areas of residence of HIV-1 seropositive drug abusers in Glasgow.

Postal Code Areas	1985 (Apr-Dec)	1986*	1987	1988	1989	1990 (Jan-Jun)	Total
G1-5	-	-	5	-	-	-	5
G11-15	-	-	1	1	-	1	3
G20s	5	19	11	4	2	1	42
G30s	-	3	7	3	-	2	15
G40s	-	3	-	1	1	1	6
G50s	-	2	-	1	-	-	3
Other	-	2	-	-	-	-	2

* three patients whose post-code was unknown have not been included in the 1986 figures.

in discrete pockets around the city in areas where drug abuse itself is known to be a problem, e.g. in Castlemilk, G45 and Easterhouse, G32-34.

The first seropositives at the end of 1985 were found to have addresses in the G20s. This was also the location of at least 17 of the 32 Glasgow DAs in 1986. In this year a few seropositives were detected in other areas in Glasgow. One female patient who lived on the outskirts of Glasgow was known to have travelled into the city for drugs. In 1987 there was a greater contribution from the Gorbals area (G5) and the G30s in the east end of Glasgow. It is of interest to note the changing pattern of the origin of requests sent to the laboratory. In 1986, eight seropositives were found either in the young offenders institution at Glenochil, Stirling, or the remand unit at Longriggend, Lanarkshire. Three were from Cornton Vale Prison, 11 from Glasgow hospitals, six from G.P's and four from the drug rehabilitation units at Kilmahew House and Woodilee in Glasgow. In 1987, half the seropositives were found through either the Counselling Clinic based at Ruchill Hospital (opened in December 1986) or through the Infectious Diseases clinics there. Five specimens were sent from other Glasgow hospitals, three from G.P's, three from Cornton Vale Prison and one from Woodilee. After this time, most seropositives were detected through the Counselling Clinic, the drug rehabilitation ward at Ruchill Hospital or from other Glasgow hospitals.

In other health board areas the first HIV seropositives were found in 1985 in Forth Valley, Lanarkshire, Dumfries and Galloway and Ayrshire and Arran. In all cases, except the latter, these seropositives had connections with Edinburgh; in Ayrshire and Arran this female DA reported in 1985 had previously been in France. In 1986, two female DAs who resided in the Argyll and Clyde Health Board area were found antibody positive. They presented for testing through Cornton Vale Prison in one case and through the Infectious Diseases Unit at Ruchill Hospital in the other.

In 1986 there was considerable concern in Glasgow at the trend shown in drug abusers found positive. The number of positive DAs increased each quarter through 1986 and there was speculation that the same trend would occur here as in Edinburgh and the graph (Fig.3.2)

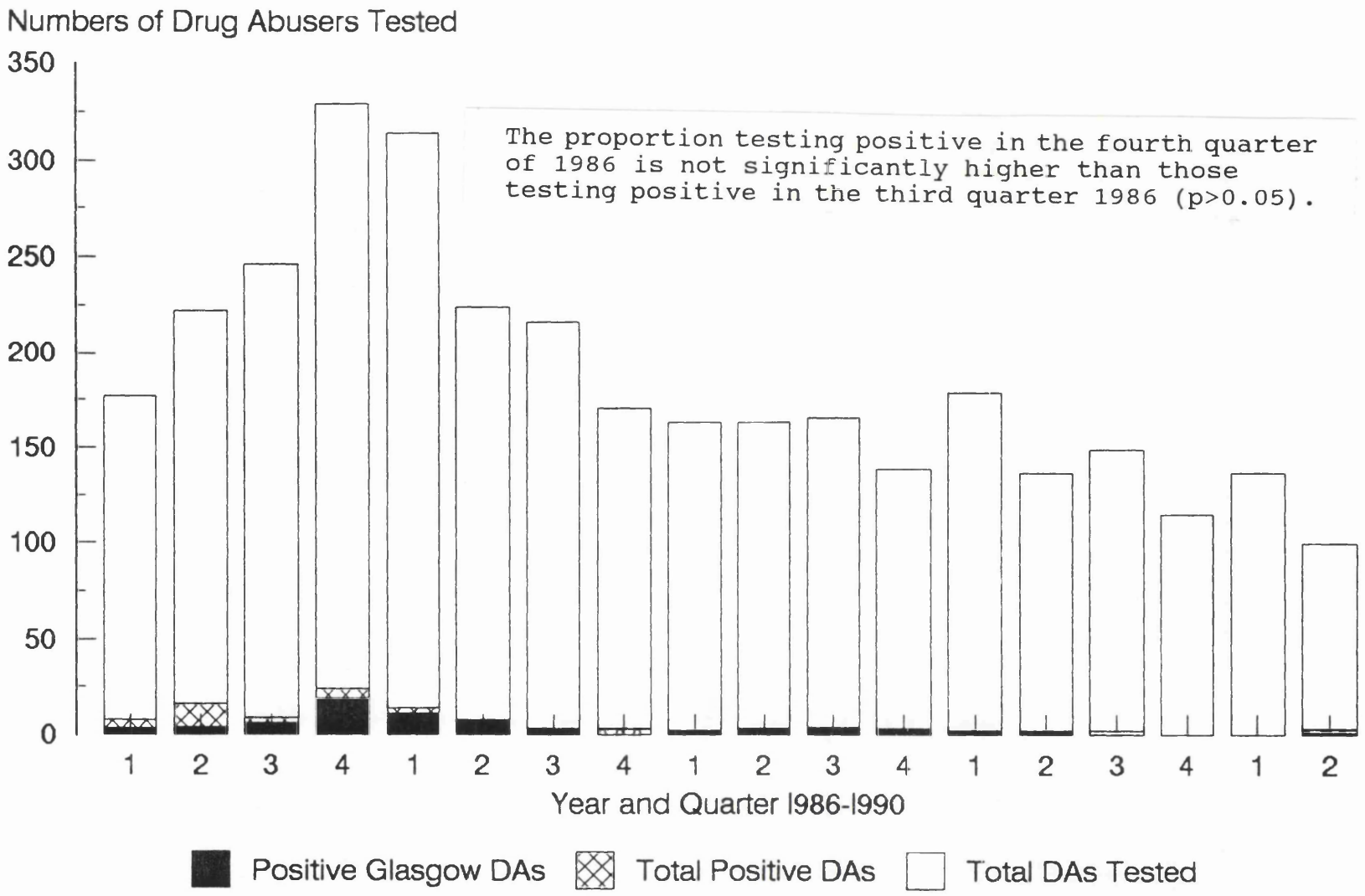


Figure 3.2 Seroprevalence in drug abusers in Glasgow and West of Scotland tested at HRL

would continue in an upward fashion. However, this has proved not to be the case and the number of seropositives diagnosed continues at a low level, on average 2-3 DAs per quarter. There has been no sustained increase in Glasgow in comparison to the Edinburgh experience.

Prior Hepatitis B Infection in Drug Abusers

Past or current exposure to hepatitis B (HBV) was found in 70% of all DAs tested and in 93% of the HIV-1 seropositives in Glasgow in 1985 (178). In the following year 69% of HIV-1 seronegative DAs and 81.2% of the seropositive Glasgow DAs had evidence of past HBV infection (179). A similar high prevalence (85.7%) was found in seropositive DAs tested in 1987.

2. SEROPOSITIVE HOMOSEXUALS/BISEXUALS

Total in Glasgow and the West of Scotland

The homosexual and bisexual population contribute 30.5% to the total positives (Table 3.2). This includes 1 Ho/DA and 2 Bi/DA. Their numbers are further swollen by the 35 seropositives in Table 3.3 who were known to be positive elsewhere but who have moved into the area or come home for treatment.

The prevalence in the Ho population (including Bi and Ho/DA) can be better assessed since the CDS Denominator Study was set-up at the end of 1988. Tests performed on Ho/Bi from Glasgow (Table 3.8) show that between 4% and 6% of those tested in 1990 are seropositive; this is more than double the prevalence in the DA population tested, however the actual figures are small.

When HIV-1 screening began in Glasgow, homosexuals were the predominant risk group found seropositive. The number of new positives decreased in subsequent years but the total pool of infection has been increased by those positives known elsewhere. New

TABLE 3.8

Homosexual and bisexual risk group screening during 1989 and 1990 (Jan-Jun).*

Year	Nos. Ho/Bi Screened	(Ho/Bi)	Nos. Positive (%)
1989			
J-M	66	50Ho/16Bi	2 (3.0)
A-J	50	40Ho/10Bi	1 (1Bi) (2.0)
J-S	63	49Ho/14Bi	2 (3.2)
O-D	60	52Ho/ 8Bi	1 (1.7)
1990			
J-M	67	52Ho/15Bi	4 (2Ho/2Bi) (6.0)
A-J	49	35Ho/14Bi	2 (1Ho 1Ho/DA) (4.1)

* The numbers represent only the testing performed at the HRL from Ho/Bi in Glasgow.

cases are still being diagnosed, about 2-3 per quarter, generally through the genito-urinary medicine clinics. In general the homosexual population are known to be a more widely travelled group, being older than most DAs and have a different socio-economic background. Eight of the antibody positives who were diagnosed elsewhere were from England, three from the USA, one from as near as Edinburgh and one from as far away as New Zealand.

Early Infection in the Ho/Bi Population

Over the first 5 years of screening only 3 cases of seroconversion were observed in homosexuals presenting for screening. Serum samples were available 6 days, 9 days and 2 months prior to the confirmed detection of anti-HIV 1. The presence of antigen was confirmed in only one patient.

The first seropositive found on screening at the HRL was a homosexual; the patient presented clinically with AIDS and had been ill since 1984. His partner was also HIV-1 antibody positive. Evidently HIV-1 infection has been in this population in the West of Scotland for a number of years prior to 1984-1985. In addition evidence of AIDS-associated infections were found on a post-mortem of a patient who had received a blood transfusion in 1983. One of the donors was a homosexual and was known to be HIV-1 seropositive on testing at the HRL in 1985. HIV-1 infection was in the blood donor population at least as early as 1983.

Ho/Bi and HIV-1 Disease

There is evidence to suggest that the homosexual population has been infected with HIV-1 for a longer period of time than the DAs. In many cases, the newly diagnosed seropositives presented with signs of progression of HIV infection.

1985

The clinical status was known in 11 out of the 23 seropositives in 1985; five were asymptomatic at presentation, four had PGL and two

had AIDS.

1986

In 1986, seven were asymptomatic at presentation: of these seven, one progressed to PGL, one to ARC, one to AIDS and four whose clinical state is unknown. Three presented with PGL, two of whom developed AIDS and have since died and one other patient presented with AIDS.

1987

In 1987, five out of the 13 whose status was known presented with AIDS all of whom died 1-19 months later, two presented with ARC, one died 13 months later with ARC, the other died 19 months later having developed AIDS. Six were asymptomatic at presentation, one of whom developed ARC 22 months later, two have progressed to PGL and the clinical state of the remaining three is unknown.

1988

Seven asymptomatic patients presented in this year, two of whom are know to have progressed. Two had a documented seroconversion illness when diagnosed, four appeared with symptoms and clinical status was unknown in the remaining four.

1989

Out of a total of 12 seropositives reported, three patients were asymptomatic at presentation, two had symptoms of progressive illness, and there was no information regarding the clinical status of the other seven patients.

1990

Three of the seven seropositives reported in this year from Glasgow and the West of Scotland appeared with symptoms; one with weight loss, fatigue etc. and the other two with recurrent chest infections. There was no clinical information given about the remaining four patients.

3. HIV-1 SEROPOSITIVE BLOOD PRODUCT AND BLOOD TRANSFUSION RECIPIENTS

The knowledge that blood product recipients were at high risk of acquiring HIV prompted the retrospective testing of haemophiliacs in 1985. Since this group of patients were monitored regularly for hepatitis, serum samples were available from up to ten years prior to this date in some cases (see Chapter 5). One hundred and eleven adults attending the Regional Haemophilia Centre at Glasgow Royal Infirmary (GRI) and 57 children attending the Royal Hospital for Sick Children (RHSC) were tested; 14 adults and 16 children were seropositive prior to 1985; two of whom have now been lost to follow-up in this area. Three of the GRI seronegative adults and one adult attending another Glasgow hospital and not previously tested here, were subsequently found to be seropositive on routine screening in 1985. There were no new cases in the haemophilia population after 1985, due to the introduction of heat-treated concentrates in late 1984 and blood donor screening in October 1985.

Blood transfusions were attributed risk factors in three patients reported in 1986 and in one patient first tested and confirmed in 1989. In the latter case, the infected transfusion was given prior to the introduction of anti-HIV 1 screening. In August 1986, two patients who became HIV antibody positive received blood products from the same donor; concentrated red blood cells in one case and platelets in the other. At the time of donation in August 1986, no antibodies to HIV were detected in the donor serum even by western blot. However 86 days later in October 1986 the donor was found to be seropositive. In addition the same donor had given blood in May 1986, but all recipients of this donation were seronegative. The infectious donation was tested for the presence of HIV antigen (Abbott) when a test became available in 1987 and gave a borderline result. Lack of specimen prevented further confirmation of this result.

4. HETEROSEXUAL HIV INFECTION INCLUDING PERSONS EXPOSED ABROAD

HIV infection in the heterosexual West of Scotland population contributed only 5 cases (1.7%) to the total new seropositives; three of the five cases are females, one whose sexual partner is African, the remaining two cases involved contact with a bisexual man. One of the seropositive males reported heterosexual contact with a risk group. In addition, of the 3 others who were known elsewhere (Table 3.3), 2 were female, one of whom had been a contact of a male drug abuser in Greece. The remaining case was a male, known in the USA, who had come home for treatment. Heterosexual contact is also specified in five of the 17 cases who were exposed abroad.

All those listed under the 'exposure abroad' column in Table 3.2 had their exposure in Africa. All except one are male. Twelve are African nationals; four Ugandans, three Tanzanians, three Zambians, one South African and one African of unknown origin. Three of the African nationals are known to have died in Glasgow and several are students who will most probably return home eventually. Table 3.9 shows the location or place of contact of these seropositives; it can be seen that both Uganda and Zambia are areas of note regarding HIV-1 infection. Male to female and female to male transmission of the virus occurs, but there is little evidence to suggest that HIV is epidemic in the heterosexual population in the West of Scotland nor that it is spreading in this area outwith sexual contacts of known risk groups.

5. HIV-1 IN OTHER RISK CATEGORIES

Four of the 7 listed under 'other' (Table 3.2) had unspecified contacts with HIV antibody positives. The remaining three are sisters, whose parents were both found to be HIV seropositive. It is not known how the parents were infected, but an older child is HIV antibody negative. The three subsequent children may have been infected through vertical transmission from mother to child. In

TABLE 3.9

Sex, age and origin of HIV-1 seropositive African nationals and West of Scotland heterosexuals infected through contact abroad.

Year	Heterosexuals: Exposure Abroad	African National
1985		F28 - Tanzania
1986	M21 - Central Africa	M28 - Zambia d
1987	M38 - Africa	
	M31 - Zambia	
1988	M37 - Africa	M41 - Uganda
	M67 - Zambia and Zimbabwe	M39 - Uganda d
	M38 - Zambia#*	M28 - Uganda
		M30 - Uganda *
		M31 - Zambia
1989		M38 - Zambia
		M29 - 'African'
		M25 - Tanzania
1990		M35 - Tanzania
		M25 - Uganda
		M35 - South Africa

* - diagnosed seropositive elsewhere
\# - infected through a blood transfusion
d - deceased

addition three children in the West of Scotland born to HIV-1 positive mothers became antibody negative 12-18 months after birth and have remained so, to date. They have not been included in the figures as they are not currently increasing the pool of infection.

6. HIV-1 SEROPREVALENCE IN THE BLOOD DONOR POPULATION

The BTS began screening every blood donation for HIV antibodies from October 14th 1985. A text of exclusion clauses was produced for every blood donor to read in order that those in any risk group or a contact of any such group would be dissuaded from donating.

Initial ELISA tests lacked specificity and were subject to false positive results. To avoid the trauma of notifying donors as such, confirmatory testing was instigated at the appropriate Reference Laboratories. The function of these Reference Laboratories was to provide additional but separate methods of testing those sera found repeatedly reactive by the BTS screening ELISAs. The HRL at Ruchill Hospital, Glasgow, serves as a Reference Laboratory for three Regional Transfusion Centres (RTC) that of Glasgow and the West of Scotland, the North of Scotland and Northern Ireland.

Table 3.10 shows the number of confirmed seropositives detected each year and the risk category of these donors from the Glasgow and West of Scotland BTS. Of these 18 positive donors, (16 male and two female), five were new donors, five had donated previously but prior to the introduction of testing and eight donors who had been tested previously and were anti-HIV negative. Less than 0.01% of all donors tested are positive and 2.5 in 100,000 donations tested are positive (Dr. B.C. Dow, personal communication).

B. PREVALENCE OF OTHER RETROVIRUSES IN GLASGOW AND THE WEST OF SCOTLAND

1. HTLV-I AND HTLV-II

A limited amount of screening for anti-HTLV 1 and anti-HTLV II

TABLE 3.10

Risk group breakdown of HIV-1 seropositive blood donors in Glasgow and the West of Scotland for the period October 1985 to June 1990.

Year	DA	Ho	HET	Other/Unk	Total
1985 Oct-Dec	-	-	-	-	0
1986	2	3	-	1	6
1987	-	1	1	1	3
1988	-	2	-	-	2
1989	2	2	1	-	5
1990 Jan-Jun	-	2	-	-	2
Total	4	10	2	2	18

has been carried out on sera from HIV-1 high risk patients and in the blood donor population.

No drug abusers have been found to be either HTLV-I or HTLV-II seropositive over four years of testing (Table 3.11). In addition anti-HIV 1 positive (n=33) and anti-HIV 1 negative (n=32) haemophiliacs were tested. No patient was found to be HTLV-I or -II seropositive.

In 1989, 5000 random blood donors were screened at the Glasgow and West of Scotland BTS, using the three test kits available at the time (Abbott, Du Pont and Serodia). The purpose of the study was to determine the specificity of anti-HTLV screening tests. Thirty-four (0.7%) were found to react repeatedly in one or more ELISA. None of the 34 were confirmed positive by either a western blot assay (DuPont and Abbott Laboratories, Chicago) or a radioimmunoprecipitation assay (RIPA) performed by Abbott Laboratories, Chicago.

There is no evidence of HTLV-I or -II infection in the blood donor population in the West of Scotland or in the IVDAs, a group considered to be at risk at this time.

2. HIV-2

Anti-HIV 2 screening has been routinely performed at the HRL since the development and launch of the combination anti-HIV 1/2 ELISAs in 1989. To date, no clinical sample has been found to contain antibody to HIV-2 in Glasgow and the West of Scotland.

TABLE 3.11

Results of antibody screening for HTLV-I/-II

Year	No. DAs Tested	HTLV-I/-II Ab. No. Positive
1987	82	0
1988	205*	0
1989	274	0
1990	40	0

* This figure includes 16 HIV-1 antibody positives

DISCUSSION

The climate of public opinion and the attitudes of those most at risk have changed over the years, whether influenced by the media or by peer pressure. At times it has been acceptable to be tested and know one's HIV status and at other times, the general feeling of those at risk has been that they don't want to know. This has influenced the total number of test requests and the number from the high risk populations coming into the laboratory.

An attempt has been made to examine the epidemiology of HIV-1 infection in Glasgow and the West of Scotland; who is infected and how many are infected? Assuming that the sample populations available for testing are representative of the populations at risk, the results presented here show the extent of HIV infection in this area up to the end of June 1990.

Several problems are associated with epidemiological surveillance of this type of disease. In the opinion of the general public there was and still is very much a stigma attached to being infected with HIV-1; it was seen to be primarily a sexually-transmitted disease and only certain members, the more undesirable numbers, of society could be infected, excepting the haemophiliacs and other blood product recipients who are regarded as innocent victims. The main problem is admitting to being a member of a risk group. The prevalence of seropositives in the different risk groups cannot be assessed when there are no baseline figures for the numbers at risk; the information presented here is predominantly about the DAs since they were the most frequently openly identified on request cards, whereas the homosexuals often did not volunteer the information regarding their risk activity or this information was not included on the request card.

Another problem in epidemiological surveillance is the possibility of double reporting, e.g. patients attending GUM clinics who are assigned an identification number can be recorded as seropositive and then appear at the Infectious Diseases (I.D.) clinics

or other as a named patient. By removing the numbers of patients known elsewhere or previously, where this information is available, from the total numbers of seropositives, an attempt has been made to keep this duplication to a minimum.

A. PREVALENCE OF HIV-1

The general pattern of infection is similar here as elsewhere in that the same risk groups can be identified as susceptible to infection, these are: intravenous drug abusers, homosexuals, bisexuals, blood product recipients, haemophiliacs and heterosexual contacts of these groups, also children of HIV-1 seropositive mothers. The variation in prevalences in the different risk groups and in different areas are influenced by the date of introduction of the virus into the community and by the frequency of behaviours promoting transmission. The overall impression in the groups at risk, who have been tested here is that the homosexuals have been infected for longer, since, to date, many have signs of progression of HIV-1 related disease and there is an indication in some cases that HIV-1 infection has been "imported" from the USA early in the epidemic. Serological evidence (Chapter 5) indicates that infection appeared in the Glasgow haemophiliac population as early as 1981 and also was "imported" from the USA. Seroconversion occurred in the DA population in Glasgow in late 1985 through 1986, and only now, four or five years on, do this group manifest signs of HIV disease. A diagnosis of AIDS has been recorded in all risk groups in the West of Scotland implying that no group, no matter the manner in which they were infected, escapes the possibility of disease.

Intravenous Drug Abusers

The most interesting data collected is that concerning the drug abusers. The DA population in the region is currently estimated at between 8-12,000 (180). The data presented here suggests that HIV-1 infection in Glasgow is not widespread, in marked contrast to the

extent of the problem in Edinburgh, where greater than 30% were found seropositive (181,182). Since routine screening began, almost 4,000 specimens from DAs have been tested at the HRL (Table 3.5). The prevalence is low,currently 1.2% of the total tested are HIV-1 antibody positive. Sampling may be a problem, since only those who wish to know their HIV-1 status can be tested. The problem may lie in those not wishing to be screened. However a larger number of positives would have been expected in this pool, if the problem was genuinely worse.

An HIV seroprevalence and behavioural study was introduced in May 1990 to screen DAs anonymously; to sample 500 DAs each year for 5 years at various sites within Glasgow and compare the behaviour of the seronegatives and the seropositives (Dr. D.J. Goldberg, CDSU, personal communication). Salivette samples and blood spots were collected and tested by GACELISA. Of over 500 screened in the first year 10 were found positive, however 3 of these were from Edinburgh and therefore should never have been included in the study, leaving 7 Glasgow seropositives (1.4%). This is further evidence that HIV-1 infection has not spread widely in Glasgow drug abusers. However HIV-1 is still spreading albeit slowly as new seroconversions are detected occasionally.

Reports in the literature in 1984-85 found varying rates of seropositivity in certain populations of DAs across Europe and the USA. Table 3.12 indicates the percentage positive in this population in different areas and in some cases, details of the increased prevalence with time as HIV infection entered and spread in the population(s) examined (101,178,179,181-189).

In the United Kingdom (UK) the total numbers of HIV infected DAs has increased as time has progressed. The total seropositives reported in the whole of the UK up to the end of June 1990 was 14,090, of which 1903 (13.5%) were DAs and 7208 (51.2%) were Ho/Bi males (145). If the Scotland total of seropositive DAs (926), to the same date, are subtracted, only 977 HIV-1 antibody positive DAs remain for the rest of the UK. Therefore Scotland has contributed 48.7% of all the seropositive DAs reported. Something different has happened in this population in Scotland compared to the rest of the UK. In the

TABLE 3.12

Early reports of the percentage of seropositive DAs in populations across the UK, Europe and the USA.

	Percentage positive	Year	Location	Reference
UK	1.5	1983	London	(183)
	2.5	1984	England	(184)
	6.4	1985	London	(184)
	51	1983-85	Edinburgh	(181)
	38	1985	Edinburgh	(182)
	0.5	1985	Glasgow	(178)
	3.2	1986	Glasgow	(179)
Europe	4	1983	Southern Bavaria	(185)
	6	1984	Southern Bavaria	(185)
	36	1984	Zurich	(101)
	11	1983	Spain	(186)
	40	1984	Spain	(186)
	48	1985	Spain	(186)
	44	1984-85	Austrian Tyrol	(187)
	6	1980	Italy	(188)
	10	1981	Italy	(188)
	15	1982	Italy	(188)
	31	1983	Italy	(188)
	53	1984	Italy	(188)
	76	1984	Italy	(188)
USA	29	1981-82	New York	(189)
	50	1984	New Jersey	(189)
	87	1984	Manhattan	(189)

14 reporting units in England, the percentage of total seropositives which were DAs ranged from 6% in the North East Thames region to 18.7% in East Anglia since reporting began, up to the end of June 1990 (Table 3.13). By comparison the figures from the Glasgow reporting unit are high, as are those from 4 out of the other 5 reporting units in Scotland. When the numbers of DA seropositives from outwith Greater Glasgow are removed from the total, the figure drops to 24%, i.e. 79 out of 328. This is still higher than any reporting unit in England. However 61% of the Scotland DA total were reported from Edinburgh and only 16% from Glasgow. Why is the situation in Glasgow so different from Edinburgh, which is situated only 70 km to the east when other indicators in this population are similar?

There was a 51% prevalence reported in the Edinburgh DAs between 1983 and 1985 (Table 3.12). Robertson et al. (181) found that HIV-1 infection entered this group late 1983, early 1984 and then spread rapidly; seroconversion was also noted in 33 of the 83 antibody positives. There was no indication to suggest that seropositivity was related to the duration of drug abuse but there was a correlation with the frequency of sharing needles and syringes. Both the frequency of sharing and injection practices led to the rapid spread of HIV-1 in Edinburgh (91% of seropositives reported frequent sharing versus 71% in the seronegatives). Testing at a separate site in Edinburgh in 1985 showed 38% were infected (182). Evidence of hepatitis B (HBV) infection (i.e. presence of hepatitis B surface antigen or antibody to hepatitis B core antigen) was found in 62% of all patients tested, and in 90% of the HIV-1 seropositives. This extent of exposure to HBV is consistent with a longer history of drug abuse and a greater frequency of sharing. Needle sharing is the predominant risk activity by which HIV-1 is spread in this population as HIV-1 seropositivity is low in their sexual contacts (190), and so a high rate of past or current HBV would not be unexpected.

A similar study in Glasgow in 1985 found 4.5% of DAs tested were HIV antibody positive (178). However the results presented here indicate that less than 1% were Glasgow based (Table 3.12). In 1986 this rose to 6% in the last quarter. However this increase was not sustained, as was expected, when other similar populations were

TABLE 3.13 Cumulative regional totals of HIV-1 seropositives detected in the UK to the end of June 1990.**

	Region	Nos. Seropositive All Risk Groups	DAs (%)
England:	Northern	343	58 (16.9)
	Yorkshire	375	46 (12.3)
	Trent	371	49 (13.2)
	East Anglia	198	37 (18.7)
	NW Thames	4,194	315 (7.5)
	NE Thames	2,383	151 (6.3)
	SE Thames	1,547	175 (11.3)
	SW Thames	361	31 (8.6)
	Wessex	296	37 (12.5)
	Oxford	380	50 (13.2)
	South Western	289	40 (13.8)
	West Midlands	432	25 (5.8)
	Mersey	178	15 (8.4)
	North Western	671	68 (10.1)
	Channel Islands	23	6 (26.1)
Wales		171	7 (4.1)
Northern Ireland		74	7 (9.5)
Scotland:	Aberdeen	45	15 (33.3)
	Dundee	235	177 (75)
	Edinburgh	1,074	565 (53)
	Glasgow	328	147*(44.8)
	Inverness	11	1 (9.1)
	SNBTS	41	16 (39)

*79 of whom were from Greater Glasgow

** Source of data: PHLS AIDS Centre - Communicable Disease Surveillance Centre, and Communicable Diseases (Scotland) Unit. Unpublished quarterly surveillance tables No.8, September June 1990 Table 9 (145) and Communicable Diseases (Scotland) Weekly Report, AIDS News Supplement A155, July 1990; 90/28 (206).

examined, e.g. in Italy (188) and Spain (186) (Table 3.12). High rates of previous expsoure to HBV were found in the HIV-1 seropositive and seronegative Glasgow DAs during 1985 and 1986 similar to that reported from the two testing sites in Edinburgh during 1985 (181,182). This suggests that Glasgow DAs were sharing needles widely at this time. The higher prevalence of hepatitis B in the HIV-1 seropositives compared to the seronegatives in both cities is consistent with greater sharing in the former and hence an increased likelihood of coming into contact with the virus.

In a study of geographical distribution of HIV and HBV among DAs in Glasgow between 1986 and the end of June 1989 the records of acute HBV and HIV infection, from 3 virus laboratories in Glasgow, were studied (M. Peedle, personal communication). Acute HBV infection was found in all 41 post-code areas studied (the post-code areas were placed in 10 groups according to geographical proximity) but predominantly in the G20-G23 areas in the North of the city and G31-34 in the East of the city. HIV infection was found in all but 4 groups (16 postal code areas). It was also highest in the areas described above for HBV, suggesting that the same risk factors are present. A greater number of DAs were screened in these post-codes than elsewhere and HIV-1 infection was present in 4.5-7% of the total DAs tested in these areas over the period 1986-June 1989.

It was noted that acute hepatitis B occurred in areas where there is as yet no HIV infection. Since HBV is spread in a similar but more efficient manner, is it only a matter of time before HIV infection appears in these drug abusing groups? One year on from this study no spread has occurred yet in these areas. However the number of DAs tested from such postcodes has been small. These areas are further out from the city centre of Glasgow and the residents are in a higher socio-economic group, perhaps high risk behaviour occurs less often. Information from the CDS surveillance database shows that HIV test requests from DAs have been received from all but five Glasgow postal codes. The majority are from the G20-G22 areas. This is the area closest to Ruchill Hospital and may reflect a higher usage of the Counselling Clinic and the Needle Exchange Centre in the hospital, where testing would be encouraged or a heightened awareness

in the local General Practitioners attending such patients.

In 1986 two general practice populations of intravenous heroin users from Edinburgh and Glasgow were studied in an attempt to explain the variation in HIV-1 seroprevalence between the two cities (191). The groups were taken to be socially comparable, similar in behaviour and representative of DAs in the community. It was found that apart from the different dates of arrival of the virus into the two communities the only other difference was that both the number of people sharing needles and syringes and the number of occasions when equipment was shared per month were significantly higher in Edinburgh.

HIV-1 entered the drug abusing population in Edinburgh during the last quarter of 1983, early 1984, and spread rapidly. Was this a reflection on the extent of sharing and frequency of sharing needles and syringes at this time or an effect of the virulence of the virus in a susceptible population? The latter proposal can only be speculated upon but there is information available regarding the former to suggest that frequent sharing occurred, with multiple contacts involved, similar to the "shooting galleries" described in the USA (181,191). An epidemic of heroin use was also noted between 1982 and 1984 which would increase the numbers in the susceptible population (192). That such an explosion of infection occurred in Edinburgh over such a short time period suggests that a group of highly infectious DAs were sharing with large numbers of other DAs and that these contacts were in turn sharing with a further set of DAs and so forth. If all this happened when virus load in any one patient was at its greatest this would help to explain the rapidity of spread. The highest titres of virus have been shown to be present early in infection (87,88) and serological evidence is suggestive of recent HIV-1 infection in a number of the seropositives in Edinburgh at this time (181).

Glasgow is a far larger city than Edinburgh in terms of population and area. It also has 3-4 times the number of injecting DAs, currently estimated at 8-12,000 (180); however the drug users are generally found in discrete areas with very little mixing between these areas. The results have shown isolated pockets, e.g. G20, G21 post-code areas which have high rates of infection. The

comparatively late arrival of the virus helped the situation here in that publicity and targeting of the risk groups was beginning. Although the actual numbers infected in 1986 are small, the increase was marked at this time.

The mechanisms of transmission of HIV-1 infection are influenced by sharing needles; whether the "works" (needle and syringe) are cleaned between contacts, the time between contacts and by certain injection practices, particularly that of "washout" which involves flushing any remaining drug left in the syringe with one's blood. However, there also has to be at least one infected person present since needle sharing among uninfected individuals is essentially safe with respect to transmitting HIV-1 infection. The infectivity of the index case also plays a role in transmission of the virus. It appears that infectivity varies between individuals and within an individual over time.

At the end of 1986, a marked increase in positive DAs was noted in Glasgow but this was not significant. There may be many reasons for this. The low number of seropositives meant that there was less chance of coming into contact with an infected DA. Perhaps a 'threshold' number of seropositives or number of potentially infectious exposure events have to occur to promote virus transmission. In Glasgow, therefore it appears that sharing practices are probably quite different from those in Edinburgh, involving smaller numbers of known contacts at any one time.

Much hearsay evidence suggests that geographically distinct pockets of drug abusers in the city do not inter-mix. In contrast, the majority of Edinburgh DAs who became seropositive all come from the same locale, and probably took drugs together, sharing the same needle and syringe.

The availability of needles and syringes has been greater since that time both through the selling of needles and syringes by pharmacists or by needle exchange. A study by Goldberg et al (193). examined the sales of needles and syringes to DAs by a single pharmacist in the G22 area from March 1987. Around 1000-2000 per month were sold. The increase in sales appeared to coincide with the decrease in HIV-1 seroprevalence in 1987; but this is likely to be

only one of the contributing factors as the downward trend was already happening. In addition, needle exchange centres were established in 1987 following the success achieved in Amsterdam with this set-up (194). This provided DAs with a clean set of 'works' in exchange for dirty needles and syringes and was also an opportunity to offer information about HIV risks. Three such centres are currently running in Glasgow.

Results from these exchange programmes established throughout England and Scotland have shown encouraging changes of behaviour. There was a worry that increasing the availability of needles and syringes would only encourage more sharing as this is associated with a feeling of community among DAs. However an educational input regarding risk behaviours and how and why this should be changed appears to have had the desired effect. The needle exchanges provided counselling about drug use, advice on safer sex, needles, syringes and condoms thereby providing the DAs with the means with which to change their behaviour. The results of a question-based survey of the DAs attending over two points in time showed that many clients had adopted low or lower risk behaviour; there had been a decrease in syringe sharing and changes in sexual behaviour, although the non-use of condoms had increased (195).

A follow-up study on a group of Edinburgh DAs has shown that in addition to a reduction in sharing episodes, with fewer people involved they are also injecting less frequently, irrespective of serological status (196). The data also showed that the risk behaviour of the seronegatives was initially less than the seropositives. These trends continued when a second follow-up of the same individuals was carried out (197). This is encouraging as it shows that DAs are capable and willing to modify their behaviour. Changes in sexual behaviour in the same group were also noted with a reduction in the number of sexual contacts per year; there was also a trend towards single, long-term relationships. This is important since these heterosexual DAs, especially those female drug abusers who prostitute to finance their drug habit, are a major reservoir for the introduction of the virus into the non-drug abusing heterosexual population. This problem is considerable in Glasgow. Goldberg et

al. (198) showed that 26 out of 35 HIV-1 seropositive female DAs, diagnosed between 1985 and 1987, engaged in regular prostitution, with two others suspected of such practices. Thus it appears that the spread of HIV-1 in Glasgow shows a similar pattern to that of other cities in the UK and Europe, where an initial small increase in spread of infection was observed which subsequently steadied and the seroprevalence remains low. However the story in Edinburgh is quite different and nowhere else has such a rapid increase been seen.

HIV-1 is still spreading in the DAs, although at a slower rate now than previously. The current incidence is low, less than 2% of those tested are positive. This is similar in other areas, even in Edinburgh where the number of new positive DAs detected per quarter has dropped from 11 in the second quarter of 1989 to 2 in the second quarter of 1990 (data extracted from CDS surveillance reports). The DAs as a group are a difficult population to manage or control, to target publicity and even to administer routine medical treatment. However, it is clear that help and advice must continue to ensure that they are constantly aware of the threat of picking up HIV-1 and to encourage behavioural change, however small. There is no room for complacency as the potential threat of igniting an explosion of HIV-1 infection in the DA population is always present. The pool of currently infected DAs could act as a potent spark and hence the need for continued monitoring of both behaviour and infection status in this population

The Homosexual and Bisexual Population

The homosexual and bisexual populations (including Ho/Bis who also abuse drugs) contribute 52% of the total seropositives in the UK and make up 81% of the cases of AIDS up to the end of June 1990 (145). In contrast to the DAs, Scotland's contribution of seropositive Ho/Bis to the UK total is only 4.2%. In Glasgow 30.5% of the recorded seropositives are Ho/Bi (Table 3.2).

The homosexual and bisexual populations are a less well-defined cohort in the West of Scotland and very often this risk activity is not admitted when HIV-1 screening is requested. Therefore less

information is readily available. However, a consistent number of homosexuals have been diagnosed seropositive each year since screening commenced. There was initially high numbers of homosexuals detected in the first year of screening so much of the spread had occurred in the early 1980s, especially through travel. The Ho, being a more widely travelled group, were in contact earlier with at-risk groups in countries where HIV-1 infection was already widespread. In subsequent years the numbers in this pool were swollen by seropositives, diagnosed elsewhere, but returning home for treatment etc. (Table 3.3).

An interesting observation was made in 1987; an increased number of test requests were received from patients, predominantly in the Ho risk group who first presented with symptoms indicative of HIV-related disease. On western blot decreased reactivity to core proteins, especially p24, was noted in these patients compared to the asymptomatics.

No major increase in numbers has been observed but new cases are being detected, on average 2-3 per quarter, predominantly via the GUM clinics situated in other hospitals in the city or through clinical presentation, usually an atypical pneumonia.

AIDS began in the USA in the homosexual population and the first case in Glasgow was a member of this group. In San Francisco and other parts of the USA, they are a large, well-publicised and open group. Many have been followed up in cohort studies to assess the risks regarding various homosexual practices, to examine changes in behaviour, length of time of progression to AIDS, etc.

Several studies have shown that the receptive partner is the one at greatest risk (199) since damage to the rectal mucosa (the lining of the rectum) is the main means by which HIV-1 can enter the blood stream. Langerhans cells in the rectal epithelium have been proposed as a vehicle for transmission of HIV-1 firstly into lymph-nodes and then into the circulation (200). Practices increasing trauma to the epithelial layer will obviously increase the risk of acquiring HIV-1. In addition, in those patients with multiple partners the chances of coming into contact with an infected person multiplies.

Publicity and advertising has been targeted at all risk groups

especially the homosexuals, whose behaviour it was believed, could be more easily influenced. A reduction in the number of cases of other sexually transmitted diseases, implying a trend towards lower risk behaviour, was noted in GUM clinics in London (201) and Stockholm in 1986 (202). However recently this trend has been reversed in homosexual populations elsewhere (203). Despite the risks being well-known and easy access to information and advice, new cases are still being detected. Once again a campaign of information must be maintained to repeatedly inform this population of the risks associated with such a lifestyle.

Blood Donors and Blood Product Recipients

A total of 30 haemophiliacs both children and adults were found positive retrospectively, the earliest being in 1981, and a further 4 (3 of whom were known to have had previous negative samples) were detected in 1985. No new infections in the haemophilacs, tested at the HRL, were diagnosed after 1985 due to the introduction of heat-treated factor concentrates which has proved to be successful in preventing transmission of the virus by this route. The haemophiliac seropositives are described and discussed more fully in Chapter 5.

Epidemiological evidence from studies in the early 1980s implicated the transfusion of blood and blood products as a means of transmission of HIV-1. Identification of infected blood donors and removal of their donations from the blood bank, was seen as an important way to prevent the spread of HIV-1 infection into the general population.

However two of the four transfusion recipients were infected as a result of a transfusion of blood products from a single infected donor in the West of Scotland after antibody screening was introduced. Transfusion of any fraction, e.g. platelets, concentrated red cells etc. of a blood donation is known to transmit infection (204). This donation was found to contain no evidence of HIV-1 antibody by ELISA or western blot. Therefore withdrawal of antibody-infected blood is insufficient on its own to completely remove the risk of transmission of infection. Potential donors have to be repeatedly and continually

dissuaded if they have indulged to any extent in any risk activity.

These were unfortunate cases, which could have been avoided by appropriate donor deferral. However it appears that some members of the population do not perceive themselves as a member of a risk group. Fourteen of the 18 blood donors found seropositive in Glasgow and the West of Scotland belong to a high risk group; 5 of the 18 were new donors and 8 of the 18 seroconverted. These persons should not be donating blood and the provision of alternative testing sites, such as the Counselling Clinic at Ruchill Hospital was to prevent this. Either these donors did not consider themselves as 'high-risk' members of the community with respect to HIV-1 infection or the appeal of absolute anonymity made them use the BTS as a source for testing.

The Heterosexual Population

The heterosexual spread of HIV-1 is becoming an important risk factor in the epidemiological surveillance of HIV-1 infection. In Africa, this is the predominant mode of spread with equal numbers of infected males and females in the sexually active age groups (134). In the USA the heterosexual epidemic is growing quickly, largely as a result of spread by DAs (205).

In the UK (excluding Scotland) heterosexual contact accounts for 6.9% of the total seropositives up to the end of June 1990 (145). This includes those whose exposure was abroad and in some cases in countries where the spread of HIV-1 is largely through heterosexual contact (World Health Organisation pattern II countries). In addition 5.8% of the AIDS cases are in this category (145).

In Scotland the figures to the end of June 1990 are similar, 7.9% of the seropositives and 7.7% of all AIDS cases specified heterosexual contact (141,206). In Glasgow, 10 out of the 298 seropositives (3.4%) had heterosexual contact, 5 persons in this country and 5 persons in Africa.

Male to female transmission occurred in 3 cases. In several studies on the risk of transmission from male to female, a variety of factors have been suggested to increase the risk of transmission, and these are described by Holmberg et al. (147). For example, duration

of relationship and frequency of contact, a history of STDs, T4 count and stage of infection in the index male, the practice of anal intercourse etc. A combination of one or more of these factors may be important in different risk groups since it is interesting to note that the prevalence in the wives of haemophiliacs and transfusion-associated infections is low in comparison to the sexual contacts of other risk groups (150). In addition no household member who is not a sexual contact of an HIV-1 infected person has been shown to seroconvert (150).

Seven cases of female to male transmission were reported, however five of these were in Africa where the mechanisms for transmission in this way operate efficiently. Female to male transmission of HIV-1 has been reported, and it is likely that the same factors as those mentioned above operate to promote transmission of infection in this direction.

The numbers found seropositive on testing at the HRL are small and to date these have been contacts of high risk groups. This lack of spread outwith the risk group contacts so far is supported by the low numbers of seropositives in the blood donor population. In addition a sero-epidemiological survey performed anonymously on Guthrie card samples from newborn babies in Scotland, to measure the extent of HIV-1 infection in females of reproductive age has revealed few seropositives, no additional cases who were not already known and no positives in the West of Scotland (207). It is perhaps too early to realise the extent of spread into the general heterosexual population. This epidemic may develop more slowly since heterosexuals change partners less often then homosexuals. HIV-1 infected prostitutes, heterosexual DAs and bisexuals are potential reservoirs for spread of infection and spread from these sources is occurring. Continued awareness of this threat is achieved by high profile advertising. An increasing number of anonymous screening and epidemiological studies are now underway to monitor the spread of infection and identify any novel at-risk groups.

The 'exposure abroad' category in Tables 3.2 and 3.5 includes 5 males who had heterosexual contact in various parts of Africa (mentioned above) 12 African nationals, 11 of whom were male, and 2

males who presented here but were known elsewhere; one of whom was a Ugandan and the other, a British citizen had received a blood transfusion in Zambia.

The CDS surveillance figures for testing African nationals reveal that over 18 months of testing, between 1989 and the end of June 1990, a total of 9 were tested, and 6 have been found positive. This includes only those samples where the information regarding nationality had been given or found on follow-up telephone calls. It is interesting to note that greater than half of those from countries in Africa with high rates of HIV-1 infection, presenting here for testing, are positive, illustrating the nature of the epidemic in the African continent.

Other and Unknown Risk Categories

The remaining seropositives to be discussed are those listed under 'other' and 'unknown'. Four of the seven others have had unspecified contacts with HIV-1 seropositives, two males with AIDS contacts, one male with a female seropositive and one female with a male seropositive. All these patients have been lost to follow-up, but it is tempting to speculate that all four may genuinely be a result of heterosexual spread of HIV.

The remaining three females are the children of HIV-1 seropositive parents aged between 5 months and 4 years. An older sibling was anti-HIV negative when tested at this time. The oldest daughter presented with pneumonia and was subsequently diagnosed with AIDS. It is unclear how the parents were infected (although drug abuse has been implicated) or which one was infected first. The mechanism of vertical transmission of HIV-1 is unknown but can occur across the placenta, perinatally or post-natally through exposure to breast milk. Intrauterine transmission is the most likely mode (148) and HIV-1 has been shown to be present in foetal tissues as early as 15 weeks gestation (208). To reduce the chances of infection post-delivery, breast feeding is contra-indicated in HIV-1 seropositive mothers as HIV-1 has been isolated from breast milk (39).

Over the 63 months of screening, to the end of June 1990, a

total of 23 seropositives have been found in whom no risk group was notified. Very little can be said about this group. It is not known whether this is a failure on the part of the requesting clinician to record some information or whether it is unwillingness on the part of the patient to admit to a risk category. Six of the 'unknowns' were female, one of whom was the wife of a seropositive male. He is also in the same category and both have been mentioned previously as the parents of three seropositive children. The threat of increased spead into the heterosexual population means that continued epidemiological surveillance is important to identify the risk groups of patients in this other/unknown category.

Seropositives Diagnosed Elsewhere

Those patients diagnosed HIV-1 antibody positive elsewhere contribute 19% to the total seropositive figures. They have been discussed in the relevant risk groups. These persons are not seropositive as a result of transmission in this area, however they are adding to the pool of infection. It is interesting to note that the homosexuals make up half of this total and this is more than double the numbers of known positive DAs. This supports the claim that Hos are more widely travelled, since in many cases, it is stated that they have come for treatment, presumably having been infected much earlier elsewhere.

B. PREVALENCE OF OTHER RETROVIRUSES

HTLV-I and HTLV-II

No persons have been found to be HTLV-I or -II seropositive in the West of Scotland. This is not too surprising since few sporadic cases occur and HTLV-I is generally confined to endemic areas of the world; south west Japan, the Caribbean and some parts of Africa (18). Very few natives of these areas reside in the West of Scotland. However, HTLV-I and -II infection have been identified in intravenous drug abusers and blood transfusion recipients in the USA, Europe and

Latin America.

HTLV-I and -II are transmitted in a similar fashion to HIV-1 and HIV-2, i.e. perinatally, sexually and parenterally by sharing contaminated needles and syringes and by transfusion of infected blood products. In endemic areas the perinatal acquisition of HTLV-I, mostly through breast feeding, is an important transmission route.

No routine screening of blood donors for HTLV-I and -II antibody is performed in the UK, at the time of writing, although it is anticipated in the future. Transmission of HTLV-I infection by blood transfusion in Japan and the USA has been associated with a 48% to 82% seroconversion rate in the recipients (209). However there have been no reports of transfusion-associated ATL thus far, but it may be too early to assess the problem due to the long incubation period to HTLV-I associated disease. Small studies in USA and Europe have been performed to assess the prevalence in the donor population. Almost all carriers of HTLV-I were found to be of Afro-caribbean or Japanese origin. The current evidence suggests that the risk of HTLV-I infection through blood transfusion is low, although not negligible in some areas.

In America, the reported prevalence of anti-HTLV I in blood donors is about 0.025% (209) similar to that for HIV-1. This is lower in European studies; in France the reported seroprevalence is 0.011% (210) and in London 0.00036% (211). In other European countries no anti-HTLV I positive blood donors were found (212). Larger scale studies require to be performed to assess the true prevalence. To further lower the risks it may be necessary to ask persons of Afro-caribbean or Japanese origin to defer from donating blood, especially locally since there are relatively fewer persons of such nationalities living in the West of Scotland.

There have been reports of HTLV infection in DAs, particularly HTLV-II infection. In 1984 Tedder et al.(26) found 4 out of 113 DAs were HTLV antibody positive, 3 of the 4 had high titres of antibody to HTLV-II. HTLV-I has also been reported in Italian drug addicts (213) and in the USA (27). In the latter study, 21 out of 23 HTLV polymerase chain reaction (PCR) positive DAs were shown to be infected with HTLV-II. No evidence of HTLV infection was found in the DA

population tested in the West of Scotland.

Dual infection with HIV-1 is not uncommon in certain risk groups and may be important in the progression to disease; indeed in one study a cohort of IVDAs from Miami, Florida, who were infected with both HIV-1 and HTLV-I/-II were three times more likely to die from AIDS during the follow-up period than those infected with HIV-1 only (214). Once in a population such as the drug abusers, these retroviruses spread in a similar fashion to, but probably less efficiently, than HIV since seroprevalence is seen to increase with age suggesting cumulative exposure. In contrast, a very low seroprevalence of HTLV-I has been found in homosexual men tested in the USA and selected from areas with a high incidence of HIV-1 infection (215). No testing has been performed on this HIV high-risk group in the West of Scotland.

Currently these retroviruses present no challenge in diagnostic screening or to medical care in the West of Scotland.

HIV-2

No persons in the sample population at the HRL since March 1989 nor at the West of Scotland Regional Transfusion Centre (RTC) since June 1990 have been found to be seropositive. There have been 12 reports of HIV-2 infection (2 in blood donors) in the UK, to the end of December 1990 (74). Nine of these 12 patients had a connection with Africa, mainly West Africa. Reports of HIV-2 infection around the world are few suggesting a limited extent of spread. Most cases have a connection with West Africa. In Europe, France and Portugal have the highest numbers (216) and it is interesting that both these countries have past colonial associations with West Africa.

HIV-2 is transmitted in a similar fashion to HIV-1. Although HIV-2 appears to be confined to certain populations at the moment it is likely that seroprevalence will increase if the virus is allowed to spread in a susceptible population or spread to new populations through increased global travel. An AIDS-like illness is the clinical outcome, however there are reports suggesting a longer incubation time to disease in HIV-2 infection (75). This may reflect

lower pathogenicity of this virus which appears to have existed in its current host, man, for much longer than HIV-1. Infection with both HIV-1 and HIV-2 can occur but it is unclear how dual infection will affect disease progression (76). Counselling, advice and donor deferral policies for HIV-1 can be extended and expanded to include those at risk for HIV-2 infection.

CONCLUSIONS

Currently HIV-1 infection in the West of Scotland is confined to members of the high risk groups defined in epidemiological studies performed at the start of the epidemic in the USA. However there is no room for complacency since HIV-1 is starting to spread outwith the confinements of these groups, a second wave of infection in the heterosexual population has begun (217), initially through sexual contacts of the high risk population and in those returning from certain areas of the world especially Africa, where the main mode of spread is by heterosexual contact.

Potential routes of transmission are many and varied since HIV-1 has been isolated from blood, semen, cervical secretions, breast-milk, cerebro-spinal fluid, tears and saliva (34-39). But the major routes are parenterally through blood or sexually via semen and to a much lesser extent, cervical secretions. The major vehicle of transmission is most probably the virus-infected cell rather than free virus (218). Virus titre is unlikely to reach sufficiently high concentration in these other body fluids to be transmissible.

There has been no evidence of any unusual modes of spread despite reports of the survival of HIV-1 in bed bugs (219) and the isolation of HIV-1 from contact lenses in 4 ARC or AIDS patients (220). Arthropod cells were found to be unable to support HIV-1 replication although the virus has been shown to bind to Drosophilia cells resulting in non-productive infection (221). In a study of routes of transmission in Belle-Glade, Southern Florida (222) there was no evidence for spread of HIV-1 by insects nor was arthropod-borne

infection responsible for spread in Africa (134). Acupuncture and the use of dirty needles was implicated in HIV-1 infection in a French male but this was not a definite association despite detailed questioning of the patient (223). Non-sexual household contacts are at negligible risk (150). Fischl et al. (224) found no risk in hugging, kissing and sharing of kitchen and bathroom facilities in household contacts of adults with AIDS. There have been reports of HIV-1 infection in health-care workers (148), either through needlestick injury or splashes of infected body fluids onto mucous membranes or non-intact skin. The incidence of cases infected this way is low but it is not zero and therefore great care must be exercised in administering medical help or in handling the body fluids from an infected patient.

It has been observed that not all contacts of a seropositive individual become infected despite frequent and repeated contact, therefore other factors must play a role in determining the infectivity of the index case and how likely they are to pass on infection and similarly in determining susceptibility to infection in the contact. These factors may relate to properties of the infecting virus strain, the host's immune response to it, genetic factors and behavioural features. There is no test for infectivity and therefore no indication as to time or duration of infectiousness. However, there is a correlation between presence of antigen in serum and virus infectivity with advanced disease stage. This suggests an increased risk in the ability to transmit HIV-1 in ARC and AIDS patients. Serum antigenaemia does not necessarily imply infectivity of virus but does indicate increased viral replication and therefore a higher circulating virus load in the bloodstream. In addition, early in infection, prior to antibody seroconversion, serum antigenaemia (85,86) and plasma viraemia (87,88) have been shown. Contacts have been infected when the index case has been in this window period implying infectious virus is present, such a case has been described in the West of Scotland (97).

Changes in immunological parameters either as a direct result of HIV-1 infection or other infections, especially a low T4 lymphocyte count may affect the rate of transmission.

Certain factors in the contact must also play a part. Any degree of immunosuppression or chronic activation of the immune system by other infectious diseases increases susceptibility (143). An increased risk of being infected by sexual contact was found in those with other sexually-transmitted diseases particularly those causing genital ulceration and bleeding thereby facilitating the entry of HIV-1 into the blood stream.

Transmission dynamics of HIV-1 vary in different geographical locations and depend on risk activity, co-factors, e.g. use of nitrites by Hos, numbers of contacts, frequency and duration of contact etc. It is therefore essential that people are constantly reminded to adopt low-risk behaviour and that advice and education are continually provided to prevent further spread of HIV-1 and other retroviruses spread in a similar way.

CHAPTER 4

MARKERS OF DISEASE PROGRESSION IN A COHORT OF HIV-1 SEROPOSITIVES

INTRODUCTION

Acquired Immune Deficiency Syndrome, AIDS, is the end stage of HIV infection, and is characterised by a number of clinical, immunological and serological markers. It may take up to 10 or 15 years after initial infection with the virus, to develop AIDS; this incubation period varies amongst individuals and it is not yet known if all infected persons will eventually progress to disease. A number of stages have been defined; these have been summarised into classification systems. Those most commonly in use are the Centre for Disease Control (CDC) classification (137) and the Walter Reed staging (136). There are 4 CDC stages labelled CDC I to CDC IV and 6 stages in the Walter Reed system. The members of the HIV seropositive cohort followed up at Ruchill Hospital, Glasgow, have been staged by the CDC classification system. In the presence of laboratory evidence of HIV-1 infection clinical signs, e.g. weight loss, lymphadenopathy, oral candidiasis etc. and laboratory measured observations , e.g. thrombocytopenia, anaemia etc., are used to define each stage of the disease. In broad terms CDC I corresponds to an acute seroconversion illness, CDC II refers to asymptomatic patients, CDC III is those who are progressing with signs of persistent generalised lymphadenopathy (PGL). CDC IV is subdivided IVA to IVE; CDC IVA and IVC_2 are the pre-AIDS or AIDS-related complex (ARC) stage and CDC IVB, IVC_1, IVD, IVE refer to full-blown AIDS, incorporating various manifestations, opportunistic infections, neurological signs and certain cancers.

There is no cure for AIDS, but a number of drugs, particularly nucleoside analogues are beneficial and available for treatment. The main drug available AZT, 3'-azido-3'-deoxythymidine, also know as Retrovir or Zidovudine (Burroughs Wellcome) is a thymidine analogue which inhibits the action of reverse transcriptase (RT) of HIV-1 by chain terminating DNA synthesis (225) and thus inhibits viral

replication. This drug was initially licensed only for use in ARC and AIDS patients. Current trials (MRC/INSERM Concorde Trial) are investigating its use at earlier stages of infection. Early intervention with AZT, despite its haematological toxicity, could prove useful in delaying the onset of further symptoms. This would allow the other medical problems encountered by these patients to be kept under control.

A number of markers indicative of immune activation and viral replication have been examined in other studies to assess their importance as indicators of clinical progression. Those most commonly measured include HIV-1 antigen, anti-p24, β_2-microglobulin (β_2-mg) neopterin, α- and γ- interferon, interleukin-2 (IL-2), T4, T8 cell counts (absolute levels and ratios) and immunoglobulin levels (IgG, IgA, etc.)(154,158). During the course of HIV-1 infection, described in Chapter 1.2, the predominant virological changes seen include loss of p24 antibody and detection of antigen, mainly p24, in the serum (104-107). Immunological disturbances such as inverted T4/T8 ratios and increased levels of immune markers, e.g. IL-2 and β_2-mg, all signify progression of disease.

In this study three serological markers, p24 antigen, anti-p24 and β_2-mg, were measured on sequential samples to examine what changes, if any, correlated with onset of symptoms and/or poor prognosis in a group of HIV-1 seropositive patients who regularly attended the Infectious Diseases (ID) clinics at Ruchill Hospital.

MATERIAL AND METHODS

STUDY POPULATION

The patients described are HIV-1 seropositives attending the ID physicians either at out-patient clinics or as in-patients at Ruchill Hospital.

TESTS

Antigen: HIV-1 antigen (Ag) was measured in serum samples using the Abbott HIV-1 Antigen EIA (Abbott Diagnostics), Appendix 2. The specificity of any positive result was confirmed by neutralisation using human anti-HIV as a blocking antibody. When positive, the antigen level was measured using the Du Pont HIV p24 Core Antigen ELISA (Appendix 2). The Du Pont assay is quantitative: optical density readings from a series of dilutions, made from a 200 pg/ml standard, are plotted on a graph. The concentration of antigen in the test sample can be read directly from the standard curve and in this way the levels of Ag in a patient's blood can be monitored.

The DuPont test specifically binds p24 Ag since the solid phase of the assay is coated with antisera of high specificity and affinity for p24. The Abbott test can detect both envelope and core antigens but is more sensitive for the core Ag. This is partly determined by the specificity of the rabbit anti-HIV conjugate used.

Antibody: Anti-p24 was measured using the Abbott HIV-1 Anti-Core EIA (Appendix 2). This assay uses identical reagents to the core bead test which is part of the ENVACOR HIV-1 EIA, described previously (Chapter 2).

β_2-microglobulin: Levels of β_2-microglobulin were measured using the Abbott β_2-microglobulin RIA (Abbott Diagnostics) a competitive radioimmunoassay for the detection and quantitation of β_2-mg in serum, plasma or urine. β_2-mg in the test samples competes

with ^{125}I-labelled β_2-mg for binding sites on the anti-β_2-mg (mouse monoclonal)-coated solid phase. The amount of bound label is inversely proportional to the concentration of β_2-mg in the test samples. A standard curve is obtained by plotting the percentage bound radioactivity versus the logarithm of the β_2-mg concentration of a set of standards. The concentration of β_2-mg in the test specimens can be determined from the graph.

TERMINOLOGY

The terms CDC II and asymptomatic, CDC III and PGL, CDC IV A and/or CDC IV C_2 (IV A/C_2) and ARC, CDC IV B and/or IV C_1 and/or IV D (IV B/C_1/D) and/or IV E and AIDS can be interchanged. The CDC terms will be used in the tables and the words ARC, AIDS etc. will be written in the text. There is a certain degree of subjective clinical interpretation and judgement on the part of the physicians in classifying the patients into disease categories and this should be taken into consideration. Patients may be symptomatic for a period of time before being staged in order to meet the criteria.

RESULTS

A. SEROLOGICAL MARKERS IN HIV-1 SEROPOSITIVES

Detection of HIV-1 Antigen

Samples from seropositive patients, predominantly in the homosexual risk category, were tested for HIV-1 antigen by EIA.

Initial assessment of a small number of patients showed a correlation between the presence of antigen (Ag) and progression of HIV-1 infection (Tables 4.1). Eighty-four per cent of symptomatic patients (PGL, ARC and AIDS) were reactive compared to 5.6% of asymptomatic patients.

For the purposes of the rest of this study, a patient described as being antigen positive is defined as having a positive Ag result, i.e. reactive by ELISA and confirmed by neutralisation, at least once, as many of the symptomatic patients have been treated with AZT. In most patients this reduced the level of serum Ag below the detection limits of the test.

Detection of HIV-1 Anti-p24

It was noted that certain patients who were confirmed HIV-1 antibody positive by ELISA and WB, were negative for core antibody. This occurred in two situations; in the initial stages of HIV-1 infection or at the advanced stages of disease. In some patients followed sequentially over time, anti-p24 became negative by EIA. This corresponded with production of antigen and in some cases a clinical change. This is illustrated in several patients in Table 4.2. Therefore an attempt was made to establish the time from loss of anti-p24 to development of antigenaemia and/or AIDS.

β_2-Microglobulin Levels in HIV-1 Seropositives

Initially a number of samples from both HIV seropositive and seronegative risk groups were tested. The results are shown in Table

TABLE 4.1

HIV-1 antigen results on a sample of patients at different stages of disease

CDC Stage	No. Reactive	No. Non-reactive	Total
CDC II (asymptomatic)	1	17	18
CDC III (PGL)	6	1	7
CDC IV (ARC and AIDS)	10	2	12
	—	—	—
Total	17	20	37

Patients at stages CDC III and CDC IV were amalgamated and the chi-squared test was performed to investigate the association of reactivity in the antigen test with stage of disease. The chi-squared test shows that there is a significant association, ($\chi^2=23.02, p<0.01$). The asymptomatic patients are associated with non-reactivity and the symptomatic patients are associated with reactivity in the antigen test.

TABLE 4.2

Loss of p24 antibody in HIV-1 seropositives

Patient	Date	p24 Ab	p24 Ag	CDC Stage
G5	12/86	+		II
	12/87	-	-	II
	4/88	-	Bd*	II
	11/88	-	+	III
	4/89	-	+	III
Mc20	7/86	-	-	II
	3/87	+	-	II
	8/87	+	Bd	II
	9/88	+	-	III
	10/88	NT#	+	III
	12/88	+	+	III
	2/89	-	+	III
M9	5/87	+	-	II
	10/88	+	-	II
	1/89	-	-	III
	12/89	NT	-	III
	3/90	-	+	III
P7	3/88	+	-	II
	8/88	+	+	IVA
	3/89	+	+	IVA
	4/89	-	+	IVA
S6	3/86	+	NT	II
	10/86	-	-	II
	1/87	-	+	III
	11/87	-	NT	IVC_2
	7/88	-	+	IVC_2
	4/89	-	+	IVC_2
T1	12/86	-	-	
	5/88	-	-	II
	10/88	(+)	-	II
	3/89	+	-	II
	8/89	-	-	II
	2/90	(+)	+	II

*Bd = Borderline
#NT = Not tested

4.3. A difference was noted in both the mean β_2-mg value and the range. The mean (plus 2 standard deviations) β_2-mg level, determined on specimens from normal individuals is 2.0mg/l for serum (Instruction Manual, β_2-Microglobulin RIA, Abbott Diagnostics). It can be seen that the mean level is higher for each group of seropositives and was greater than the value determined for the normal population. Table 4.4 shows the mean β_2-mg levels on a number of HIV seropositive patients whose CDC stage was known. A higher mean level is observed in those whose HIV-1 infection has progressed and is highest in those with AIDS. A wide range of values is obtained in 3 out of the four categories, even in those seropositives who were asymptomatic when tested. The numbers tested does reflect more than one sample from some patients.

Thus serial β_2-mg values from HIV-1 seropositives attending the ID clinics at Ruchill Hospital were measured to determine the role of β_2-mg as an indicator of disease progression.

B. ASSESSMENT OF MARKERS IN THE RUCHILL COHORT

The markers described in the earlier part of this Chapter (section A), i.e. p24 antigen (Ag), anti-p24 (Ab) and β_2-microglobulin (β_2-mg), were examined along with clinical information in a core group of patients who regularly attended the ID clinics at Ruchill Hospital and were defined as the Ruchill cohort.

The Ruchill Cohort

Up to the end of June 1989 a total of 133 patients were initially described in the cohort, 14 have been lost to follow-up. Of the remaining **119**, 19 have died during the period of the study: 16 with AIDS or HIV related conditions and 3 others, all from drug overdoses. Table 4.5 shows the breakdown of the risk groups and CDC staging at initial presentation of the members in the cohort. The homosexuals (and bisexuals) are the largest contributing risk group and presented at different stages of disease. Seventeen of the 19 patients presenting with advanced HIV disease (ARC or AIDS) were

TABLE 4.3

Mean β_2-microglobulin levels (mg/l) in seronegative and seropositive risk groups for HIV

	HIV-1 Ab Negative	HIV-1 Ab Positive
No risk	1.57	-
Homosexual	1.63 (0.085)*	3.28 (0.185)
Drug addict	1.82 (0.085)	3.48 (0.445)
Haemophiliac	2.12 (0.094)	3.11 (0.151)
No. tested	128	63
Range	0.72 - 3.8 mg/l	1.55 - 6.5 mg/l

* Standard error of the mean
Using a two sample t test with a 95% confidence level the mean β_2-microglobulin levels for each risk group are significantly different, ($p<0.05$).

TABLE 4.4

Mean levels of β_2-microglobulin (mg/l) and relationship with HIV-1 disease

Clinical Status	No. Tested*	Mean (mg/l)	Range
CDC II (Asymptomatic)	77	2.9	1.3 -> 4.7
CDC III (PGL)	25	3.0	1.0 -> 6.5
CDC IV A/C_2 (ARC)	33	3.2	2.4 -> 4.3
CDC IV $B/C_1/D/E$ (AIDS)	33	4.6	2.6 -> 8.4

*Nos. tested in each category (II -> IV) are samples taken from 26, 9, 8 and 13 patients respectively.

No statistical evaluation was performed on these results due to the small number of patients in each disease category.

homosexuals (and bisexuals). The earliest recruits in the cohort had a diagnosis of AIDS in 1985 and in one case this was backdated to 1984. The most recent member was recruited in February 1989, a homosexual man with ARC.

Table 4.6 shows the number of patients who have clinically progressed during the period of follow-up. The majority, 93 out of 119 i.e. 78.2%, of the patients were asymptomatic at presentation, one-third of these progressed, two-thirds of these were homosexuals. Sixity-one remained asymptomatic over the period of time studied (this figure includes two patients who died from drug overdoses). The most interesting patients to examine are those that progressed.

Clinical progression, CP, was noted in a total of 40 patients (Table 4.6):

- 32 progressed from being asymptomatic to PGL (n=15), to ARC (n=13) or to AIDS (n=4)
- 4 progressed from PGL to ARC (n=1) or AIDS (n=3), and
- 4 progressed from ARC to AIDS.

A breakdown of the risk groups reveals that 29 of the 40 patients are homosexuals or bisexuals (including 1 homosexual drug-abuser), 10 are drug abusers and the remaining patient, a recipient of an infected platelet transfusion.

Nine of the 13 patients presenting with AIDS were deceased by the end of June 1989, one person died within a month of presentation; the longest survived 27 months with a diagnosis of AIDS. The mean survival time was 13.3 months. The remaining 4 have survived 9 to 19 months to the end of June 1989. It is interesting to note that the longest surviving patients with AIDS at Ruchill Hospital (not included in the cohort) has been ill since 1986 and is still alive with AIDS up to the time of writing (June 1991).

Serological Markers and Their Link with Disease Stage

Two parameters, anti-p24 and p24 antigen, were examined in patients in the cohort from each disease category, at the end of June 1989, or at the time of death where appropriate (Fig.4.1). Loss of anti-p24, in this section, refers to a negative result in the Abbott

TABLE 4.5

Risk groups and CDC staging at presentation of the cohort members attending Ruchill Hospital.

Nos. Presenting at CDC Stage:	Risk Group						
	Ho/Bi	DA	Ho/DA	Tx	Het/Oth	Unk	Total
CDC II	40	47	1	1	3	1	93
CDC III	6				1		7
CDC A/C_2 (ARC)	6						6
CDC IV B/C_1/D (AIDS)	11				2		13
	63*	47*	1	1	6*	1	119

* deceased patients : 14 Ho/Bi
4 DA
1 Het/Oth

TABLE 4.6

Numbers of patients who progressed clinically during the period of follow-up to the end of June 1989

Nos. Presenting at CDC Stage:		Nos. Progressing to CDC Stage: III	IV A/C_2(ARC)	IV $B/C_1/D$(AIDS)	Total
II	93	15	13	4	32
III	7	-	1	3	4
IV A/C_2	6	-	-	4	4
		15	14	11	40

HIV-1 Anti-Core EIA. The association between loss of p24 antibodies (Ab) and appearance of p24 antigen (Ag) in serum, with advancing disease can be seen: 83.9% of asymptomatic patients were p24 Ab positive compared to 14.3% in those with AIDS. In contrast 81% of those with AIDS were antigenaemic compared to 5.4% in the asymptomatic group. Thus the majority (82%) of asymptomatic patients were p24 Ab positive and Ag negative but the reverse was true in ARC and AIDS patients where it was more usual (76%) to be p24 Ab negative/Ag positive. A dramatic change in Ab/Ag status occurs in progression from PGL to ARC or AIDS.

Those patients who progressed during the period of follow-up were examined more closely to establish which, if any, of the above markers changed first, which preceded a clinical change and what predictive role the measurement of β_2-mg played in disease progression.

Virological Progression

Virological progression, VP, defined by a loss of p24 Ab and/or production of Ag, was observed in a total of 23 patients up to the end of June 1989 in the cohort. A change in a further three patients was noted after this time, to the time of writing (two in July/August 1989 and one in February 1990) and these are included in the results giving a total of 26 patients from the cohort of 119 patients.

Five patterns related to changes in markers have been observed:

(i)	Loss of Ab pre-appearance of Ag	(13 patients)
(ii)	Loss of Ab post-appearance of Ag	(4 patients)
(iii)	Loss of Ab and appearance of Ag together	(2 patients)
(iv)	Loss of Ab alone	(3 patients)
(v)	Appearance of Ag alone	(4 patients)

Thirteen of the 19 patients (68.4%) with changes in both markers, i.e. those who became Ab-/Ag+ showed a decline in Ab prior to detection of Ag. HIV-1 antigen was detected on average 14.1 months (range 3-28 months) after the first anti-core EIA negative result.

A small percentage of these patients (4 out of 19 i.e. 21%) had detectable Ab a number of months after antigenaemia (pattern (ii)) and

The chi-squared test was performed to establish the association between HIV-1 anti-p24 status and disease stage. The chi-squared test shows that there is a significant association, (χ^2=35.82, p<0.01).
The asymptomatic patients are associated with the presence of anti-p24 and the symptomatic patients are associated with the lack of anti-p24.

The chi-squared test was performed to investigate the association between HIV-1 p24 antigen status and disease stage. The chi-squared test shows that there is a significant association, (χ^2=58.15, p<0.01).
The asymptomatic patients are associated with lack of serum p24 antigen and the symptomatic patients are associated with the presence of p24 antigen.

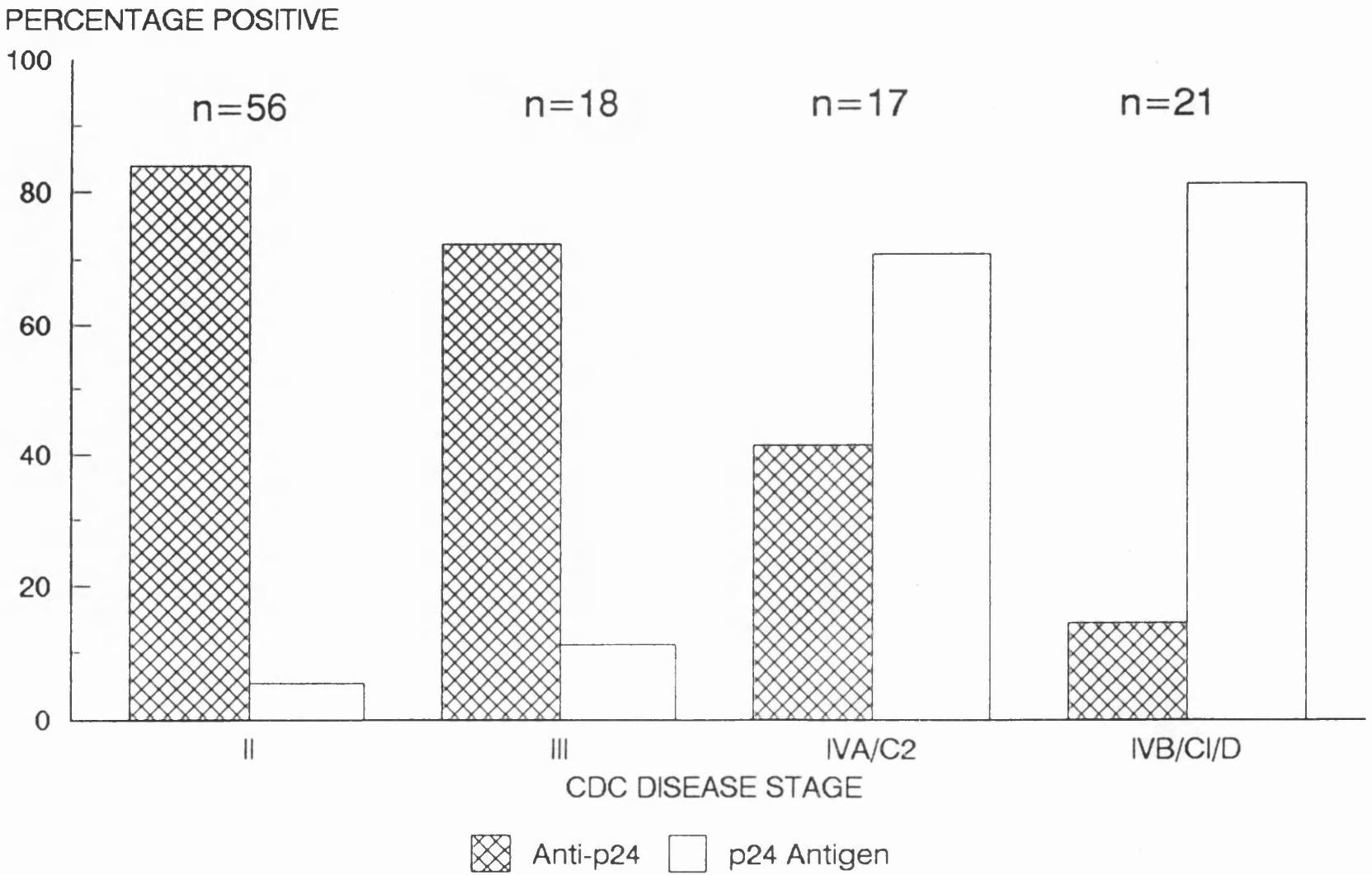


Figure 4.1 HIV-1 anti-p24 and HIV-1 antigen status in a group of patients at different stages of disease

in two patients the first changes were recorded in the same sample (pattern (iii)). Patients showing patterns (iv) and (v) may not have been followed up for a sufficient length of time since they could be potential members of patterns (i) and (ii) respectively. The above markers are associated with a bad prognosis, 15 of these patients progressed clinically and will be described below.

Clinical Progressors

Those patients identified in Table 4.5 as showing clinical progression (CP) were examined more closely to determine which markers if any changed pre- or post-observation of progressive disease. The patients have been grouped according to their clinical status to facilitate analysis of the relationship between VP and CP.

A total of 15 of the 40 patients with CP were noted to have a change in one or more of the markers discussed above (Table 4.7) over the period of follow-up, to the end of June 1989.

Group P1 Asymptomatic -> PGL (n=15)

Five of the 15 who progressed from asymptomatic to PGL became Ab negative (by ELISA) and four of these patients also developed antigen. Of the nine marker changes, five occurred after clinical diagnosis, 2 changed at the same time and 2 changed prior to diagnosis. In none of the patients did both markers change prior to the clinical change. The remaining 10 patients in this group are still Ab+/Ag-. Progression to PGL may represent a minimal clinical change in these patients.

Group P2 Asymptomatic -> ARC (n=13)

Seven of the 13 patients progressing to ARC showed virological progression (VP). Two of these patients had a documented episode of time with PGL. All 7 patients became antigenaemic, however only 5 became anti-core negative. Both markers changed prior to the clinical diagnosis of ARC in 4 patients; in the two patients retaining Ab, both were Ag positive prior to clinical change, in Mc13 this was post CP to PGL (Table 4.7). The remaining patient, P7,

TABLE 4.7

Clinical and virological progression in the Ruchill cohort to the end of June 1989.

Group	Patient	II	III	$IV_{A/C2}$	$IV_{B/C1/D}$	First Ab Neg.	First Ag Pos.
P1	F3	12/86 ---->	2/87			3/89	NA
	G5	12/86 ---->	11/88			4/87	11/88
	Mc20	3/87 ---->	9/88			2/89	10/88
	M9	3/86 ---->	1/89			1/89	3/90
	Y1	5/87 ---->	12/87			6/89	11/87
P2	B5	6/85 ---------->		9/89		NA	3/88
	G2	12/86 ---------->		4/88		6/87	11/87
	Mc10	5/87 ---------->		3/89		5/87	10/88
	Mc13	3/87 ---->	1/88 ---->	12/88		NA	9/88
	Mc18	11/85 ---------->		10/87		11/85	5/87
	P7	3/88 ---------->		8/88		4/89	8/88
	S6	3/86 ---->	1/87 ---->	11/87		10/86	1/87
P3	T2	2/86 ---------->			3/88	2/86	10/87
P5	L2		9/86 ---------->		3/88	5/86	4/87
	S13		6/85 ---------->		2/88	3/89*	3/86

NA - not applicable; * - next sample available for testing after 3/86

developed Ag as the clinical change was noted and subsequently (8 months later) lost anti-p24. Four of the remaining 6 were Ab+/Ag- and 2 were Ab-/Ag+ at presentation.

Group P3 Asymptomatic -> AIDS (n=4)

Only one of the four patients who developed AIDS from being initially asymptomatic during follow-up was noted to have VP. A negative anti-p24 EIA result and a positive p24 Ag result was recorded, 25 and 5 months respectively, prior to diagnosis of AIDS. In the other three CPs one was Ab+/Ag-, one was Ab-/Ag? (where sample size was insufficient to determine Ag status) and the remaining patient was Ab+/Ag+ at presentation.

Group P4 PGL -> ARC (n=1)

There was no recorded VP in the one patient member of this group. He was Ab-/Ag+ throughout the period of study.

Group P5 PGL -> AIDS (n=3)

Two of the three patients with CP to AIDS from PGL also had changes in both Ab and Ag status. The remaining patient was Ab-/Ag+ at presentation. In one patient, L2 (Table 4.7) a loss of anti-p24 was recorded 22 months prior to the diagnosis of AIDS and antigenaemia occurred 11 months after loss of Ab, i.e. 11 months pre-diagnosis. Production of Ag was the first recorded change in patient S13, 23 months prior to diagnosis of AIDS, anti-p24 was subsequently negative by EIA, 13 months later.

Group P6 ARC -> AIDS (n=4)

No VP was noted in the 4 patients in this group. All were Ab-/Ag+ at presentation.

Clinical Non-Progressors

Virological changes were noted in 11 of the clinical non-progressors, i.e. those patients who presented and remained in the same CDC category; eight from Group NP1, two from Group NP2, nil from

Group NP3 and one from Group NP4 (Table 4.8).

Group NP1 Asymptomatic (n=61)

A total of eight patients showed VP; in 5 patients both Ab and Ag changed and all demonstrated a pattern (i) VP, two patients lost Ab alone, i.e. pattern (iv) and one patient became Ag positive (pattern (v)). The majority of patients (48 out of 61) were Ab+/Ag- as would be expected in asymptomatic patients. Three presented Ab-/Ag- and in the remaining two patients there was insufficient sera to complete the tests.

Group NP2 PGL (n=3)

In Group NP2 patients; one patient became Ab-/Ag+, 12 months after first presentation (pattern (iii)) and the only change in the other patient was the appearance of Ag, 28 months after presentation (pattern (v)). The only other member of this group is Ab+/Ag-.

Group NP3 ARC (n=2)

No VP was observed in this group. One patient was already Ab-/Ag+ at presentation and the other remains Ab+/Ag-.

Group NP4 AIDS (n=13)

Five months after presenting with AIDS, one patient became Ab-/Ag+ (pattern (iii)). He died 8 months later. Six patients presented with Ab-/Ag+ status, the pattern most commonly associated with advanced disease and two patients showed the reverse, i.e. Ab+/Ag-. Four other patients were Ab-/Ag-.

In summary, in the groups of patients with a noted clinical change, i.e. groups P1-P6, a total of 15 patients were observed to have a change in one or more of the viral markers, anti-p24 or p24 Ag, over the period of follow-up testing at the HRL. Twelve patients changed with respect to both markers, i.e. loss of anti-p24 by EIA and production of Ag, 2 patients developed antigen only and one patient became anti-p24 negative only (Table 4.9). In only 5 of the above noted 15 patients did both markers change prior to the observation of CP. It is interesting to note that the time span of events was

TABLE 4.8 Virological changes in the Ruchill cohort who have not progressed clinically

Group	Patient	CDC stage	Date (month/year) first HIV-1 Ab positive report	p24 Ab first neg. (month/year)	p24 Ag first pos. (month/year)	Time (months)
NP1	C4	II	2/87#	9/87	1/90	28
	Mc1	II	3/87	NA	1/89	-
	M1	II	2/87	12/88	3/90	15
	M2	II	1/86	1/88*	12/88	11
	M8	II	8/87	8/87†	1/89	17
	S7	II	7/87	7/89	NA	-
	T1	II	12/86#	8/89	2/90	6
	W6	II	6/87	1/89	NA	-
NP2	M5	III	8/88	8/89	8/89	17
	S9	III	3/85	NA	7/86	-
NP4	Mc8**	IVC_1+D	9/85	2/86	2/86	0

NA = not applicable; * = next sample available for testing after 1/86;
# = C4 previous seronegative sample 9/86, T1 previous seronegative sample 4/86
† = patient presented anti-p24 negative; ** = deceased 10/86; time(months) = first Ag pos. - first Ab neg.

TABLE 4.9

Numbers of patients with virological and clinical progression subdivided by group and pattern of virological progression

	Total nos. with VP	Nos. with VP and CP	Breakdown of clinical progression groups: Group P1 II-->III	Group P2 II-->IV A/C_2	Group P3 II-->IVC_1	Group P5 III-->IVC_1
Pattern (i)	13	8	2	4	1	1
" (ii)	4	4	2	1	-	1
" (iii)	2	-	-	-	-	-
" (iv)	3	1	1	-	-	-
" (v)	4	2	-	2	-	-
Total	26*	15				

* The remaining 11 patients with VP showed no clinical progression and are described in the text.

similar. Anti-core declined on average 18.6 months (range 10-25 months) prior to diagnosis of clinical change and Ag appeared on average of 6 months (range 5-10 months) prior to the clinical progression. Loss of p24 Ab preceded Ag by a mean of 12.6 months (range 3-20 months). Four of these patients are in Group P2 and have developed ARC and one patient developed AIDS (Group P3). In addition 3 patients in Group P1 showed virological change after the clinical diagnosis of PGL. Thesedata fits with that in Fig 4.1 where the most dramatic change in marker status was observed in those patients with ARC compared to these with PGL.

In addition a further 10 patients presented with one or both markers already changed and subsequently progressed (up to 26 months later). The most rapid progression was seen in GroupP6 patients who were diagnosed AIDS within 6 months of presentation.

To complement this the remaining 15 patients with observed disease progression showed no alteration in Ab or Ag status and remained Ab+/Ag-. Ten belonged to Group P1, four to Group P2 and one to Group P3. It can be seen that the initial stages of progression, i.e. asymptomatic to PGL appear to be less associated with any viral changes and represent a minimal clinical change with respect to the ongoing pathogenic effects of the virus.

Only limited conclusions can be made regarding time(s) to progression and the significance of changes in markers. Progression of HIV-1 disease is indeed associated with changes in p24 Ab and Ag; a total of 25 out of the 40 patients (62.5%) in this cohort who developed symptoms had a change in one or both indicators. The most common pattern of VP observed was pattern (i) i.e. loss of Ab before the production of Ag. However the observation that 15 patients progressed with no viral changes and 10 (excluding Mc8 (Table 4.8)) showed viral changes and no clinical advancement means that these markers are not the absolute or only important factors involved in the disease process and are not prognostic in all patients.

No consensus of timing of the clinical and virological changes could be established to allow a putative time-course of events and therefore an indication of when disease might occur and at what stage intervention with drug therapies may be used to best effect in

delaying onset of symptoms and prolonging life.

Screening for such markers gives the clinicians more information regarding the status of the patient at any one point, particularly the measurement of Ag levels which is especially useful in patients on AZT treatment.

Serial β_2-Microglobulin Measurements

The serum concentration of β_2-mg was measured routinely in the cohort of seropositives. The results presented in Table 4.10 show some examples of patients where an apparent rise preceded or was noted at the time of onset or progression of symptoms. In some patients this increase was sustained but in others a decrease was noted. Using the same groups described in the previous section on virological markers, the β_2-mg results were as follows:

β_2-Microglobulin in Clinical Progressors

Group P1 Asymptomatic -> PGL (n=15)

Over the period of follow-up, only 3 patients showed a rise in β_2-mg concentration, in 2 patients this preceded clinical change (P5, M9) (Table 4.10) and in one patient this was after CP. Ten patients consistently had levels <3mg/l and two patients had levels >3mg/l. There was no correlation with CP.

Group P2 Asymptomatic -> ARC (n=13)

The majority of patients had average β_2-mg levels >3mg/l. Two patients had a raised level pre-CP, 3 at the time of CP and 2 patients post-CP. In 3 patients with consistently high levels this was unrelated to any clinical changes.

Group P3 Asymptomatic -> AIDS (n=4)

Two patients in this group had high levels throughout and therefore there was no correlation with disease progression. One patient showed a rise after the diagnosis of AIDS. An increase through the course of disease was noted in the remaining patient (B2,

TABLE 4.10

Serial β_2-mg concentrations in patients with a change in clinical status*

Patient	Date	β_2-mg mg/l	p24 Ab	p24 Ag	CDC Stage
M9	3/86	2.5	+	-	II
	5/87	2.2	+	-	
	6/88	3.1	(+)	-	
	10/88	3.0	(+)	-	
	1/89	NT	-	-	III
	5/89	3.1	-	-	
	12/89	2.9	-	-	
	3/90	2.4	-	+	
P5	6/87	2.95	NT	-	II
	12/87	4.7	NT	-	
	2/88	NT	NT	-	III
	3/88	4.5	NT	-	
	7/88	6.5	+	-	
	11/88	4.2	NT	-	
	3/89	2.95	+(6/89)	-	
Mc5	8/88	4.1	-	+	II
	12/88	4.0	-	+	III
	3/89	2.7	-(5/89)	-#	IVC_2(2/89)
	10/89	1.8	NT	-	
Mc13	3/87	3.5	NT	NT	II
	11/87	5.4	+	-	
	1/88	4.7	+	-	III
	9/88	NT	+	+	
	12/88	NT	NT	NT	III+IVC_2
	1/89	3.9	+	+	
B2	12/85	2.2	NT	NT	II
	1/86	NS	NS	NS	III
	8/86	2.2	NT	-	
	6/87	6.5	NT	-	?IV_A
	12/87	NT	NT	-	IV_B
	2/88	3.4	NT	-	
	4/88	5.0	NT	-	
	8/88	4.1	+	-	
	2/89	3.0	+	-	IVC_1d

* A rise in concentration is observed prior to or at clinical progression

\# On AZT treatment; NT - not tested; NS - no sample; d - deceased

Table 4.10). The largest spike in concentration occurred prior to the diagnosis of AIDS. No VP was noted in this patient. This was the first drug abuser in the cohort to develop AIDS and she died three months later.

Group P4 PGL -> ARC (n=1)

The one member of this group had a β_2-mg concentration ranging from 1.6-3.9mg/l over the period of follow-up, there was no correlation with progressing disease.

Group P5 PGL -> AIDS (n=3)

There was no association of β_2-mg levels with clinical changes in the three patients in this group. One patient had particularly high levels (between 5-8.5mg/l). He was diagnosed with AIDS within two months of presenting at the ID clinic, and his β_2-mg levels were measured after this time.

Group P6 ARC -> AIDS (n=4)

The highest levels were noted in this group; where the mean value was >6mg/l in three patients. The remaining patient had an average value of 2.9mg/l. A concentration of 3.4mg/l was noted prior to clinical change but this decreased below 3mg/l after the diagnosis of AIDS.

β_2-Microglobulin in Clinical Non-Progressors

Group NP1 Asymptomatic (n=61)

Four out of the 8 patients who had changes in virological markers (Table 4.8) had β_2-mg concentrations around 3mg/l, two of whom had levels persistently >3mg/l. The remaining four had normal levels. There was no association with VP, nor was an increase observed over the follow-up period. The majority (32) of the remaining patients all had levels consistently <2.5mg/l. Fluctuating values were noted in nine patients, where at least one value was higher than 3mg/l, in some cases this corresponded with an infection, e.g. herpes. A concentration of 3mg/l or greater was observed in 8

patients. (Four patients have not been tested).

Group NP2 PGL (n=3)

All three patients had β_2-mg concentrations within the normal range (2mg/l or less) and there was no evidence of a rise in concentration.

Group NP3 ARC (n=2)

An apparent decrease in concentration of β_2-mg was observed in one patient who became Ag negative after beginning AZT treatment.

Group NP4 AIDS (n=13)

Only 4 out of the 11 patients tested had mean values >4mg/l. The remaining seven had mean values around 3mg/l.

The main observation from the results as noted in Section A is that β_2-mg concentration is increased in those with more advanced disease. It can be seen that a value of 3mg/l is a better lower limit than 2mg/l for indication of disease since at least 56% of the asymptomatic patients (Group NP1) had values around the 2mg/l level and in all other groups the levels were higher. In most cases once the β_2-mg level rose above 3mg/l it remained high, independent of clinical status. In those groups where a clinical change was diagnosed (Groups P1-P6), no obvious changes in β_2-mg levels were observed.

A decrease in β_2-mg was noted in 3 patients in particular; one patient with ARC who had levels >3mg/l before developing AIDS showed an apparent decrease in β_2-mg after diagnosis of AIDS. Two patients, one from Group P2 and one from Group NP3 had high levels initially when they were also Ag positive. Serum antigen became undetectable in these patients once treatment with AZT was commenced and an apparent decrease in β_2-mg was observed. A contrast was also observed in the levels of β_2-mg in Group NP4 patients (those appearing with AIDS) and Group P6 patients (those developing AIDS). In the latter patients, the highest concentrations of β_2-mg were observed (>6mg/l). However in the AIDS patients, average values between

3-5mg/l were obtained.

Only 11 of the 40 patients with CP showed a rise in β_2-mg and in only 5 such patients did this precede CP therefore no correlation of CP and β_2-mg was found in the Ruchill cohort, however it should be noted that rapid progression with respect to CDC staging occurred in some patients because they had only attended the clinics once they were ill and baseline values were more difficult to ascertain. Further follow-up of those patients currently asymptomatic may yield better results regarding β_2-mg as a prognostic indicator of disease progression.

Time to Progression

Seroconversion was documented in 17 members of the cohort; 13 DAs, 3 Hos and a transfusion recipient (Table 4.11). Two of the DAs died from overdoses and have not been included. Five of the seven seropositive for 3 years (1986-1989) showed either CP (n=2), VP (n=1) or both (n=2). In contrast there was only one clinical progressor and one patient with VP in the eight patients seropositive for 2 years or less. The ages of the patients at seroconversion were similar and no correlation with progression can be made.

Summary

Six out of the 11 clinical progressors with a rise in β_2-mg concentration also had virological progression, five of whom also had changes in both viral markers. Therefore a total of only five out of the 40 patients with clinical progression demonstrated all the predicted changes in markers. However closer examination reveals that no patients in this cohort showed a change in all 3 markers prior to clinical progression, four had a change in 2 markers prior to CP and these were most commonly the virological markers.

TABLE 4.11

Clinical and virological progression in HIV-1 seroconversions in the cohort up to the end of June 1989

Patient	Risk Group	HIV-1 Ab Neg. (Month/Year)	HIV-1 Ab Pos. (Month/Year)	CP (Month/Year)	VP (Month/Year)	Age at First Ab Pos. (Years)
K4	Tx	8/86	9/86	IV A 12/87	NA	22
P6	DA	7/85	11/86	III 1/89	NA	21
D1	DA	6/86	11/86	NA (1/88)	NA (1/88)	23
G5	Ho	9/86	11/86	III 11/88	Ab- 4/87 Ag+ 11/88	24
T1	DA	4/86	12/86	NA	Ab- 8/89 Ag+ 2/90	18
F3	DA	2/86	12/86	III 2/87	Ab- 3/89	20
Mc19	DA	10/85	12/86	NA	NA	18
C4	DA	9/86	2/87	NA	Ab- 9/87 Ag+ 1/90	25
F2	DA	10/86	2/87	NA	NA	25
K1	DA	3/87	5/87	NA	NA	23
P5	DA	4/86	6/87	III 2/88	NA	17
Mc12	DA	11/84	12/87	NA	NA	20
H2	Ho	11/1/88	13/1/88	NA	NA	24
Mc15	Ho	12/9/88	21/9/88	NA	NA	35
C3	DA	2/88	11/88	NA	NA	29

Tx - Transfusion recipient; DA - Intravenous drug abuser; Ho - Homosexual; NA - not applicable

DISCUSSION

The antibody ELISA screening and confirmatory tests to detect HIV infection, described in Chapter 2, are not a test for AIDS. AIDS is the end-stage disease resulting from infection with HIV-1 (and HIV-2) and the classification of a patient as such has to fulfil a number of criteria as described by the Centre for Disease Control (CDC), Atlanta, USA. These criteria do include a positive HIV antibody test result, along with other clinical and laboratory information. In this Chapter, three tests have been examined to establish their use as indicators of advancing disease.

VIROLOGICAL CHANGES

Two important changes in serum chemistry of viral markers have been established from studies of patients at various stages of HIV-1 disease; a temporal decline or loss of core Ab and appearance of serum Ag. However, the causal relationship between the two and with the progression of disease has not been fully elucidated.

HIV-1 Antigen

HIV-1 Ag is detected in patients with advancing disease (85) and most probably represents a switch from latent or low level replication to active or high level replication of virus. Plasma viraemia has been detected throughout all stages of infection in most, if not all, patients (33,100). Higher titres of virus are present in those with more advanced disease. However the presence of Ag in serum is generally associated with the onset of the clinical symptoms of AIDS (104,105,107) and this was found in the group of patients tested in the HRL. Only free serum Ag, i.e. the quantity of Ag in excess of that bound in immune complexes, was measured in these patients. The predominant viral antigens detected in serum are viral core proteins.

The result of productive viral infection where high levels of viral protein synthesis are occurring may be sufficient on its own to induce the cytopathic effect of HIV-1 infection, thus leading to a

drop in T4 helper cells which in turn allows opportunistic infections to flourish unhindered. The appearance of detectable levels of Ag in serum may signal this switch to lytic infection and thus it is not surprising to detect Ag in the ARC and AIDS patients more often than in those with PGL or the asymptomatics.

The switch or combination of events that triggers high levels of viral protein synthesis remains to be fully elucidated. Most mechanisms involve stimulation of T cells since HIV-1 is not produced by quiescent T cells (226). This may occur in a variety of ways. Various factors in the host, e.g. certain HLA haptotypes have been associated not only with seroconversion but also with disease progression through immune activation (162). Cellular, host regulatory proteins such as nuclear factor kappa B (NF-KB) have been shown to bind to enhancer sequences in the proviral DNA and to activate transcription from the HIV-1 long terminal repeat (LTR) resulting in low level proviral transcription leading to the expression of the viral regulatory proteins tat, rev and nef (43). Induction of NF-KB is a result of immune stimulation by the gene products of other viruses, e.g. HTLV-I, herpes viruses (161), or by certain cytokines e.g. tumour necrosis factor (162) and T cell mitogens. Other DNA-binding proteins constitutively expressed in the host may also play a similar role e.g. Sp1 (227). In addition, it has been shown in vitro that different isolates of HIV-1 can vary in their replicative capacity, cytopathic effect (syncytium-inducing capacity) and host range and that these differences may affect clinical progression (163,228). Therefore a particular variant may be selected for, in vivo, which confers certain properties and affects disease outcome. Åsjö et al. (228) isolated virus strains with a higher replicative capacity in patients with HIV-related disease than those from asymptomatics. Tersmette et al. (163) showed that a more rapid progression and shorter survival time occurred in those patients with high-replicating, highly cytopathic isolates compared to either high-replicating, non-cytopathic strains or low-replicating, non-cytopathic strains. This may help to explain why some persons with AIDS in the Ruchill cohort remain antigen negative if a low-replicating, cytopathic strain is the dominant circulating strain

in vivo and why some asymptomatics develop antigen if a high-replicating, non-cytopathic strain is selected in vivo. The combination of a competent immune system able to cope with high levels of virus and the in vivo selection of non-cytopathic viral strains may retard disease progression even when serum Ag is detected.

HIV-1 Anti-p24

The other most notable serological features was that of a decline in core antibodies in symptomatic patients both by specific anti-core ELISA (Abbott) and by western blotting (92,101-103). This was observed in the patients presenting at various disease stages at Ruchill Hospital. The anti-core EIA (Abbott) is less sensitive than the western blot assay and in most cases although the EIA result was negative, core antibodies were still visible on a western blot. Western blotting is an expensive and time-consuming test to perform and it is primarily used in the initial confirmation of a positive antibody test and not in follow-up screening. However, on the occasions when a series of samples from patients showing symptoms of disease progression were blotted together, a decline in any one or more of the core antibodies, p55, p24, p17, has been observed. This lack of sensitivity of the ELISA was not considered a hinderance as it was a measurement of Ab change that was being sought, not an absolute value.

A decrease in, and loss of, anti-p24 occurs with a rise in levels of serum p24 Ag after the change from latency to productive HIV-1 infection and has been shown to precede the onset of AIDS by up to 27 months (103) and precede antigenaemia by 12-18 months (106). This may be a result of complexing the rising concentration of viral antigens released in quantity from infected cells destroyed by the virus as Ab-Ag complexes are found in AIDS patients (229). Other mechanisms must be involved; 7.9% of the ARC and AIDS patients in the Ruchill cohort were anti-p24 positive in the presence of serum antigen (Fig.4.1). It is interesting to note that decline in anti-p24 occurs in a much smaller percentage of persons with disease progression in Central Africa (230). A good immune response to p24 may be

protective against the development of AIDS or may indicate a better prognosis in infected patients. Recently Cheingsong-Popov et al. (231) found an association between antibody response at seroconversion and clinical outcome; specifically those patients with an initial low titre of anti-p24 progress more rapidly to ARC or AIDS and are more likely to develop serum antigenaemia than those with an initial high titre anti-p24 response. (Higher titres of gp120 antibody at seroconversion were also associated with clinical progression). They postulated that a high dose of infecting virus may lead to more rapid progression by binding to T cell receptors. In this way the T cell-dependent B cell response is depressed resulting in low levels of antibody production. However this would be expected to affect the antibody levels to other antigens. It is more probable that progressive defects in the immune system as a consequence of viral persistence may result in a decreased synthesis of specific anti-p24 by B lymphocytes. In those individuals retaining anti-p24 throughout disease there may be specific host factors, e.g. human leukocyte antigens which maintain immune competence in spite of high titres of viral antigen. Much remains to be understood about the immune complexities of infection with HIV-1. A discussion of the complex interadions between cells of the immune system and their role in HIV infection is beyond the scope of this thesis.

One patient in the Ruchill cohort is particularly interesting; throughout follow-up testing he had one of the highest levels of serum antigen recorded when he developed AIDS. He maintained this despite treatment with AZT. In addition he remained core antibody positive by EIA and western blot.

In a small number of patients the reappearance of anti-p24 in patients assumed to have become negative has been observed. This has been noted in some patients on anti-viral therapy and may reflect a decrease in antigen production associated with AZT treatment. In others the test OD fluctuates above and below the ELISA cut-off, making the assessment of the first anti-p24 negative result difficult. In such patients this probably illustrates a declining anti-p24 titre but it may also reflect differences in intra-assay test performance.

Decline in anti-p17 has been suggested to be an even earlier

marker of disease progression (232). This has not been measured specifically in this study, but the Abbott anti-core ELISA does detect epitopes at the carboxy terminus of the p17 protein. The loss or decline in anti-p24 was the more prominent observation on western blots.

HIV-1 Ag, Anti-p24 and the Time Course of Infection

Thus the majority of patients fit the putative time course of events in HIV-1 disease described in Chapter 1 (Fig.1.2) and discussed above, i.e. loss of Ab as Ag appears. The Ag measured in this study is free serum Ag and does not include Ag bound in complexes. The reduced sensitivity of the anti-core ELISA means a negative result may be obtained when core Ab is still present. Thus a comparable graph for the Ruchill cohort using these test results would show actual loss of Ab before Ag appears, described in the results as pattern (i).

Results of anti-p24 and p24 antigen measurements from patients in the Ruchill cohort at different stages of disease do show a strong association of antibody negative/antigen positive (Ab-/Ag+) status and AIDS and the reverse, i.e. antibody positive/antigen negative (Ab+/Ag-) status in asymptomatic patients (Fig.4.1). However this is not absolute; Ag is not detectable in 100% of AIDS patients, only 81% in the Ruchill cohort had detectable Ag at some point during follow-up and a small percentage (14.3%) retained core Ab. In this cohort all combinations of Ab and Ag have been noted in those with AIDS or developing AIDS during follow-up, i.e. Ab-/Ag+, Ab-/Ag-, Ab+/Ag+ and Ab+/Ag-. Similarly a number of asymptomatic patients (5.4%) were antigenaemic and 16% were anti-core negative when tested. Therefore these markers could never form part of the criteria for diagnosis of AIDS, but they could be prognostically useful where a change, referred to here as virological progression, might implicate onset of disease.

β_2-MICROGLOBULIN

The remaining serum marker measured in this study was the β_2-mg concentration. β_2-mg is an 11.8 Kd protein present on the surface of

all nucleated cells (233). It constitutes an invariant light chain of an immunoglobulin-type molecule and is associated with an HLA heavy chain at the cell membrane. In healthy subjects, β_2-mg turnover is constant with circulating serum levels of 2mg/l in adults, although the level does increase with age. It is eliminated via the kidneys and in the absence of any impairment of renal tubular function, which is associated with elevated levels, an increased β_2-mg level indicates an activated immune process, e.g. in various autoimmune disorders or neoplastic disease. Thus an increased serum β_2-mg concentration reflecting increased lymphocyte turnover may be a marker of immune activation in HIV-1 infection, this would lead in turn to increased viral replication. This is true of other acute viral infections especially cytomegalovirus (CMV). Higher than normal levels were observed in patients with AIDS or suspected AIDS (234) and has been shown to rise during HIV-1 infection (235). This was also found in patients at various stages of disease and from various risk groups tested at the HRL. When members of HIV-1 risk groups were tested, a higher mean β_2-mg value was noted in those who were HIV-1 seropositive. The mean levels in the seronegatives compares with that established in studies of healthy adults and thus a modified cut-off level of 3mg/l may be a better indicator in the HIV-1 seropositive patients.

In agreement with other reports (234,235) higher mean values were observed in patients with more advanced disease. It is interesting to note the broad range of values obtained in at least 3 out of the 4 disease categories. Could this be suggestive of an intercurrent acute infection or perhaps indicative of disease progression?

SEROLOGICAL CHANGES LINKED TO CLINICAL PROGRESSION

As the understanding of the disease and the pathogenic effects of the virus has increased a link between the risk of developing disease and changes in a number of measurable laboratory parameters was observed. A wide range of viral, immunological and other related markers have been measured in cohort studies to establish their

usefulness in a predictive role, either individually or as a combination of criteria. Such studies were performed to establish a model for progression to determine those HIV-1 infected persons most likely to benefit from early intervention of drug treatment. At least four laboratory markers have been shown to predict AIDS in asymptomatic, seropositive homosexual men (236). These were; T4 count, p24 Ag, serum β_2-mg and serum neopterin. Moss et al. (235) showed that the chances of developing AIDS were increased when β_2-mg was raised and T4 counts had dropped below 400 x 10^3/l (where a normal value is 800-900 x 10^3/l). An increased risk of developing AIDS is associated with the production and persistence of serum Ag (99,235,237). Allain et al. (237) found it to be a better marker than the absolute T4 count in predicting disease onset. Loss of core antibody was also noted and was linked more to the production of antigen than as a marker on its own, such that a core Ab negative, antigen positive status developing in a patient implied that they were more likely to progress to AIDS.

An attempt was made to link changes in the 3 markers described in this Chapter with the clinical progression in the Ruchill cohort to assess the prognosis of HIV-1 infection. A number of patients in the Ruchill cohort (twenty-six in total) were observed to have virological progression. Clinical progression was recorded in fifteen of these twenty-six patients. The predictive value of the Ag and Ab tests in this situation was assessed carefully. In general virological progression could be associated with clinical progression. However there were too many exceptions to this rule, e.g. some patients whose HIV-1 disease state advanced, did so without ever having detectable Ag and/or losing Ab. It appears that p24 Ab and Ag are not sufficiently reliable markers to be of clinical predictive value. In a cohort of homosexual men, de Wolf et al. (107) showed that loss of core Ab, appearance of Ag and a drop in T4 counts preceded AIDS by a mean of 21.3 (8.9), 17.7 (8.8) and 15.7 (8.9) months respectively. However the standard deviations, shown in parentheses, allow for a wide range in time-span of events. A similar time-scale could be seen in five patients in the Ruchill cohort where loss of Ab preceded serum Ag by an average 12.6 months (range 3-20 months) and Ag preceded onset of

symptoms by 5 months in 4 patients and 10 months in the remaining patient. Only one patient in the group had developed AIDS, the others had progressed to ARC. The main conclusion would be that such changes in markers would imply not only an increased likelihood of developing AIDS but also a more rapid progression. A fundamental flaw in the use of serum Ag as a marker is that it is not produced by or detectable in all patients who eventually progress.

It should be noted that more recently measurement of Ag has become less useful as a marker of progression as anti-viral therapy, e.g. AZT, can now be given to patients at earlier stages of progression rather than to AIDS patients alone. Thus a degree of clinical deterioration in association with a falling T4 count has prompted treatment in a number of patients before serum Ag has been detected. AZT inhibits the viral reverse transcriptase and the level of serum Ag, in most patients on treatment, becomes undetectable. Those patients designated Ag positive in this study had at least one serum sample reactive and confirmed in the Ag test as many of the ARC and AIDS patients have been on various dose regimes of AZT therapy, at various intervals, throughout the follow-up period. Thus some patients subsequently retested as non-reactive, in others the antigen level appeared to fluctuate around the detection limits of the test and in others AZT apparently had no effect (data not shown). However the appearance of antigen can still indicate a poor prognosis as there is evidence to indicate the emergence of AZT-resistant viral strains, so-called escape mutants, in some patients on prolonged therapy. The reduced sensitivity to AZT, <u>in vitro</u>, is associated with mutations in the reverse transcriptase gene (238). In addition a small number of patients, either asymptomatic or with PGL, have been enrolled in the MRC-INSERM Concorde trial since 1989. This is a randomised, double-blind, placebo-controlled trial to study the efficacy of AZT in reducing disease progression and prolonging life in asymptomatic or PGL patients. The identity of the patients involved is unknown to the laboratory and thus some patients will be receiving treatment which could prevent Ag production.

In the Ruchill cohort there was little evidence of an increase in β_2-mg concentration as a predictor of CP. Although this is a

non-specific marker there is an association of higher levels with more advanced disease, but only a total of 11 out of the 40 CPs had a rise in β_2-mg at some time during the course of HIV-1 infection. However the increase was noted at earlier stages of progression, i.e. from asymptomatic to PGL or ARC. This may not be surprising as it has been noted that many patients showed a rapid clinical progression after their first attendance, i.e. they had attended the clinic as symptoms began to appear and were therefore on the brink of a progressive diagnosis, and so a baseline β_2-mg concentration was obtained from one measurement only. Nevertheless there is an indication that earlier intervention with treatment may delay the onset of AIDS and prolong life. There was evidence that β_2-mg decreased during AZT treatment in these patients (239). If AZT is slowing the disease process by halting viral replication the subsequent reduction in immune stimulation, i.e. decrease in T cell and macrophage turnover of β_2-mg is mirrored by a return to normal β_2-mg concentration. Lower β_2-mg levels were noted in the patients presenting with AIDS versus those developing AIDS. Perhaps long-term AZT treatment in the AIDS patients has stabilised the disease process and the pathogenicity of the virus resulting in less immune activation and steadier β_2-mg levels. However it is probable that the destruction and resultant decrease in T4 cell numbers may play a role. It has been suggested that serum β_2-mg measurement would be a better marker than serum antigen for estimating the antiretroviral activity of drugs as not all patients produce detectable antigen (240).

The most interesting group of patients to follow will be group NP1. Four of the eight patients in this group who had progressed virologically showed higher levels than most of the asymptomatics, an additional 8 patients without VP also had raised β_2-mg levels. β_2-mg has been found to be a useful predictor of impending disease in other cohort studies (235,241,242). In seropositive homosexual men, it was strongly correlated with the risk of progression to AIDS and was the best single predictor of progression in the parameters measured in the San Francisco General Hospital cohort study (235). Similarly Fahey et al. (241), in an extensive study of eight cellular and serological indexes, found that β_2-mg (and neopterin) reflected prognosis equally

as well as T4 counts and could predict the future rate of decrease in T4 cells. A more recent study on a cohort of haemophiliacs (242) infected from the same batch of FVIII in whom the times of seroconversion were well-documented, showed that β_2-mg levels >3mg/l had been attained at least one year before symptoms developed. They concluded that although most markers measured reflected the disease process, β_2-mg concentration appeared to predict the onset of symptoms. The mechanisms responsible for the elevated levels of β_2-mg are not fully understood. Constant immune stimulation via lymphokines etc., reflected in raised levels of β_2-mg, may over time increase the production of HIV from infected cells. The production of virus may reach a threshold level, above which syncytia or giant cells form killing off infected and uninfected T4 cells, thus resulting in decreased T4 counts. At the start of the AIDS epidemic the first marker measured was the T4 cell count; the observed immune deficiency was a consequence of the reduction in this cell population. Although useful for plotting the course of disease, i.e. low counts before development of AIDS, the T4 count has never been established as a useful predictor of disease progression on its own. However, used in combination with β_2-mg concentrations a better estimate of the risk of progression can be made (154).

A variety of other markers, e.g. IgA concentration, neopterin, levels of interferon and interleukin, T8 counts etc. which reflect immune disturbances and various aspects of viral pathogenic mechanisms have been measured in other studies (241,242). Neopterin has been found to be similar in usefulness to β_2-mg (241). However it appears that no single marker can predict disease onset on its own. Predicting who will progress to AIDS, despite the numerous markers is clearly not simple and some attempt has been made in this Chapter to link the changes in laboratory parameters with clinical staging. In the Ruchill cohort no conserved or distinct pattern of change associated with clinical progression has been identified, except that loss of antibody was the first marker change in the majority of patients. There was no finite or definitive time between loss of core Ab and progression to disease, production of Ag and progression or level of serum β_2-mg during progression. In many circumstances

close, careful, clinical observation is as useful a predictor of disease progression than the results of laboratory measurements. The underlying theme is the individuality of response in the different patients. Persons respond in different ways to this viral infection, and even in the small number of patients described here, there are many exceptions to confound any rules or associations that might be made. However, general trends have been shown, e.g. production of Ag in the later stages, rise in β_2-mg etc. although these are not true for every patient. Thus most markers reflect the disease process rather than predicting onset. Appearance of change in one or more markers in a person may only indicate an increased likelihood of developing disease quicker than in persons with less evidence of virological or immunological change. Moss and Bacchetti (154) have defined an algorithm, based on five variables, for estimating the 3 year probability of progression to AIDS from their studies on the San Francisco General Hospital (SFGH) cohort of homosexual men. In this way a combination of markers are used to predict progression; this is likely to be more reliable than observations based on single measurements.

The Ruchill cohort have been followed up for a relatively short period of time; many patients only presented at clinics as symptoms appeared and these patients, mainly homosexuals, had obviously been seropositive for a number of years. Sixty-seven percent of those who progressed were homosexuals which supports the suggestion in Chapter 3 that members of this group have been infected with HIV-1 for longer than other risk groups tested in the West of Scotland except the haemophiliacs, who are discussed in Chapter 5. However, the late(r) arrival of the virus in the drug abusing members of the cohort (as discussed in Chapter 3) means that continued follow-up of these patients may yield further interesting results. As earlier intervention with drug therapies, irrespective of disease stage, is now permitted it would be interesting to see if this prolongs the progression to AIDS and prolongs life in those with AIDS compared to current estimated incubation times.

No good data regarding time to progression is available from the Ruchill cohort. Longer follow-up is required in those who

seroconverted during the period of the study. However a greater number (9 out of 16) had progressed to ARC or AIDS by the end of June 1989 in those first recorded seropositive at the HRL in 1985-86 compared to 2 out of 10 reported seropositive in 1987-88. This is not unexpected but is of limited predictive use since the dates of seroconversion are unknown. Ideally information about incubation times for progression require follow-up of a cohort in whom the dates of infection are known; studies involving transfusion recipients and some haemophiliac cohorts can be successful in this respect.

CONCLUSION

Thus as time passes, the development of AIDS has increasingly become the inevitable outcome of HIV-1 infection. Disease progression is determined by many factors and co-factors, properties of both the host and the virus, some of which have been discussed here. Viral and immunological markers indicative of viral pathogenesis and disease progression have been identified, e.g. anti-p24, p24 Ag, β_2-mg. No single marker has been found to be predictive but a combination of laboratory data and clinical observations can be used with caution and experience to assess the prognosis in an individual patient.

Studies on viral and immunological markers, as described here, leads to a greater understanding of the mechanisms of viral latency and pathogenesis also the immune system and its response to HIV-1. Such advances may help in the design of vaccines and in better targetting of anti-viral drugs. AIDS is a terminal disease and much hope for the sufferers lies in better treatment and possibly also the use of vaccine in the HIV-1 infected patients.

CHAPTER 5

HIV-1 INFECTION IN HAEMOPHILIACS, GLASGOW AND THE WEST OF SCOTLAND.

INTRODUCTION

Haemophilia, a disorder of blood clotting, is genetically inherited as a sex-linked recessive characteristic. It affects the male population predominantly and occurs only in females who are homozygous for this trait, i.e. possessing two copies of the defective gene. Heterozygous females act as carriers. One of the blood-clotting proteins (factors), is either absent or has a markedly reduced activity. This is most commonly Factor VIII (FVIII) or antihaemophilia factor which causes haemophilia A (HmA) also called classical haemophilia with an incidence of 12 in 100,000 males. Factor IX (FIX) deficiency or haemophilia B (HmB) is rarer, affecting 1 in 30,000 males. This is also known as Christmas Disease after Stephen Christmas, the first patient in whom it was described. Other blood clotting disorders exist, e.g. von Willebrand's disease, but these are less common than the above mentioned disorders.

These patients periodically require transfusion with the defective protein to complement the series of clotting factor proteins in their blood. This is in the form of a concentrate which is extracted from human plasma, pooled from 2,000 to 20,000 blood donations. The severity of the two major forms of haemophilia does vary and therefore requirement of factor concentrate is different. In severe haemophilia the <u>in vivo</u> activity of the FVIII protein is less than one per cent (moderate 1-5% and mild >5%). Thus severe haemophiliacs require greater quantities of the factor. In the United Kingdom, about 5,000 persons suffer from haemophilia, 2,000 of whom have severe haemophilia. Haemophilia can be crippling both in terms of orthopaedic disabilities, due to bleeding into joints and in social problems, e.g. in maintaining employment. Intensive treatment with blood products from an early age was shown to lead to an improved quality of life. Transfusions of freshly donated blood/plasma or fresh frozen plasma, as a hospital in-patient, was the only treatment

for these disorders until 1964 and the discovery of a method of concentrating FVIII in blood. Treatment with this product allowed the patients greater freedom since they could be treated at home. In 1973 the UK government allowed human blood products to be imported; lyophilised concentrates became readily available and the majority of supplies came from manufacturers in the USA. These commercial concentrates supplemented the locally produced FVIII.

However the Scottish National Blood Transfusion Service (SNBTS) has been self-sufficient for many years and Scottish haemophiliacs have been treated almost totally with locally produced FVIII since 1981.

In the past, transfusion of blood products has been associated with the risk of viral transmission particularly in immunosuppressed patients or in patients receiving large doses. Post-transfusion hepatitis was recognised more than ten years ago (243,244), particularly that caused by a non A non B (NANB) agent, now identified as hepatitis C virus (HCV)(245) and hepatitis B virus (HBV) from FVIII and FIX concentrates. The acute illness may be mild, even sub-clinical but the risk of long-term chronic damage to the liver, by these hepatotropic agents, may be as high as 21%(246).

A new threat, that of infection with the AIDS virus, appeared in the early 1980s when epidemiological data established that transfusion of blood and blood products represented one of the major routes of transmission of HIV. Haemophiliacs were thus identified as a high-risk population (247). Hence the blood supply had to be made safer to reduce the risk of transmission by this route. What could be done to achieve this?

Past experience with HCV and HBV identified three major ways. Firstly, donor selection; active dissuasion of members of identified high-risk groups from donating blood. Secondly, treatment with chemical agents or heat to inactivate the transmissible agent without destroying the activity of the factor concentrate. The AIDS virus was found to be extremely heat labile in the liquid phase (248), but heat treatment of factor concentrates in the liquid phase rapidly reduces their biological activity and so they are preferentially heat-treated in the lyophilised state. Lack of seroconversion in

haemophiliacs treated with heat-treated factor concentrate indicated the success of this procedure (249). Lastly, the development of sensitive and specific assays for detection of viral markers in order to exclude infected donations. There was now a real possibility that transmission by this route could be eliminated.

The haemophiliac population form a well-defined group in which to study the natural history of HIV infection, particularly since the introduction of heat-treated concentrate in October 1984 and the implementation of blood-donor screening in October 1985, has significantly reduced the likelihood of further seroconversions in this population.

MATERIAL AND METHODS

SUBJECTS

In the West of Scotland, haemophilia patients attend two centres; children up to the age of 14 years attend clinics at the Royal Hospital for Sick Children (RHSC), Glasgow and adults (15 years old and over) attend the Regional Haemophilia Centre (RHC) at Glasgow Royal Infirmary (GRI).

HIV-1 SCREENING

Antibody: Initial screening for anti-HIV 1 in serum samples was performed in 1985, using the Abbott HTLV-III EIA (Abbott Laboratories) described in Appendix 2. This was the first commercial test kit available. Seropositives were confirmed by indirect immunofluorescence at this time. Subsequent samples from these patients have been tested using one of the other screening ELISAs described in Appendix 2 and by western blotting (DuPont).

Antigen: Screening for HIV-1 antigen by both the Abbott HIV-1 antigen EIA and the Du Pont HIV p24 core antigen ELISA (Appendix 2) commenced in March 1987.

RESULTS

ANTIBODY IN HAEMOPHILIACS

Serum samples from 111 haemophiliacs registered at the RHC, GRI and from 57 haemophiliacs treated at RHSC who were regular attenders were tested for HIV-1 antibodies (Table 5.1). Retrospective samples (stored as serum at -20°C) from the 31 seropositives who have been followed up were then examined to determine the approximate dates of seroconversion, where possible, or the dates of the first antibody positive sample (Fig. 5.1).

Fifteen out of the 31 seropositives were infected with HIV-1 prior to or during 1982. No patient was found to be seropositive in samples taken prior to 1980. The earliest positive case was detected in 1981 in patient 20; patient 5 was shown to seroconvert in that year. The first sample from patient 5 in June 1981 was negative by ELISA, IF and western blotting but 4 months later, in October a follow-up sample was weakly positive by ELISA, negative by IF, and produced gag, pol and env bands, on an early version western blot (i.e. those with no high molecular weight proteins on the strips) (Table 5.2).

The latest seroconversions occurred in 1985 in four patients. Patients 7, 15 and 16 were antibody negative in the last quarter of 1984, patient 16 was also seronegative in January 1985. However, all three were seropositive when next tested, in the last quarter of 1985. Patient 25 was first found seropositive in January 1985 having been seronegative in August 1984.

A total of 9 out of the 31 patients (29%) were positive on the first specimen available for testing. Therefore the precise date of infection could not be determined. The remaining 22 patients had previous specimens, where the time interval between the last negative and the first positive specimen ranged from 104 days (patient 5) to 772 days (patient 26), excluding patients 2 and 20, where the time interval was of the order of 4 or 5 years. ELISA and western blot data indicated that 7 patients were at an early stage of HIV infection in their first positive samples (Table 5.2). Samples tested within 6

TABLE 5.1

HIV-1 seroprevalence in the haemophilia population in Glasgow and the West of Scotland.

	No. Tested	No. Positive (%)
Adults (15 years and over)	111	17* (15.3)
Children (14 years and under)	57	16* (28.1)
	168	33 (19.6)

* One adult and one child lost to follow-up

	Patient No.	1977	1978	1979	1980	1981	1982	1983	1984	1985	Age at first antibody positive serum
(a)	1						●				11
	3							●			14
	4								●		51
	6						●				15
	9						●				16
	11							●			14
	18						●				24
	28						●				5
	29						●				6
(b)	2			O					●		37
	5					O ●					15
	7								O	●	46
	8	O	O	O		O	O	O	●		31
	10	O	O			O	O	●			21
	12					O	●				30
	13					O	●				14
	14					O	●				16
	15							O	O	●	36
	16						O	O	O	O ●	19
	17	O	O	O		O	O	O	●		42
	19					O	●				13
	20	O				●					20
	21					O	O	●			10
	22					O	O ●				12
	23					O	●				7
	24						O ●				7
	25					O		O	O	●	10
	26*						O		●		8
	27*					O		●			7
	30*					O	O	●			8
	31*					O	O	●			8
Totals						2	13	7	5	4	

FIG. 5.1 History of seroconversion in serial samples from 31 haemophiliacs from Glasgow and the West of Scotland.

O = anti-HIV 1 negative
● = anti-HIV 1 positive

Patients 16 and 18 have haemophilia B, the remainder have haemophilia A.

* = Patients 26 and 27 are twin brothers and patients 30 and 31 are twin brothers.

(a) = Haemophiliacs where first available sample is HIV-1 antibody positive.

(b) = Seroconversions in haemophiliacs

TABLE 5.2

ELISA, immunofluorescence (IF) and western blot data on 7 haemophiliacs showing recent infection with HIV-1

Patient	Date of sample (month/year)	Time (in days) since previous sample	Estimate of date of seroconversion* (month/year)	ELISA OD	ELISA C/O	Result	IF	Western Blot Bands visible	Western Blot Dilution
5	(i) 6/81	161	8/81	0.045	0.133	-	-	no bands visible	1/20
	(ii) 10/81	104		0.149	0.133	(+)	-	p15,24,gp41,p55,64**	1/20
10	6/83	355	12/82	0.396	0.173	+	+	p24,31,53,64,gp160/120	1/20
13	3/82	301	10/81	0.765	0.111	+	ND	p24,31,53,64,gp160/120	1/100
17	2/84	271	10/83	0.673	0.179	+	+	p24,gp41,p53,55,64 gp160/120	1/20
19	11/82	534	2/82	0.171	0.132	(+)	ND	p24,31,gp41,p53,p55 64,gp160/120	1/20
22	(i) 1/82	241		0.092	0.133	-	-	p24	1/20
	(ii) 5/82	135		0.488	0.133	+	-	p24,31,53,64**	1/100
24	8/82	220	5/82	0.126	0.134	+/-	-	p15,24(gp41),(p55) gp160/120	1/20

* Date of seroconversion estimated as the midpoint in time between the last negative and the first positive sample.
** Early western blot which contained no high molecular weight glycoproteins.
ND Note done

months after the estimated seroconversion date showed weakly positive reactions with the tests available at this time. The estimated seroconversion date is taken as the mid-point in time between the last seronegative result and the first seropositive result. Most of the seroconversions appear to have occurred between 1981 and 1983.

Patients 16 and 18 suffered from Hm B and received FIX concentrate; the remaining 29 had HmA, 28 of whom were severe haemophiliacs. In addition, patients 7 and 12 had inhibitor, that is anti-FVIII antibodies and thus were treated with a commercial product of American origin. There are two sets of twins in this cohort; patients 26 and 27 and patients 30 and 31 (Fig.5.1).

The age of the patients at the time of their first antibody positive sample ranged from 5 to 51 years. This includes 10 children infected when 10 years of age or younger. When initial screening was performed, a higher percentage of seropositives was found in the patients attending the RHSC (28.1%) compared to 15.3% in the adults attending GRI. At the end of June 1990 the average length of time of seropositivity is 7 years (range 5 to 9 years), where 87% have been positive for a minimum of 6 years.

ANTIGEN IN HAEMOPHILIACS

No HIV-1 antigen was found in sera from 15 patients taken prior (4-18 months) to their first antibody positive sample. In addition 1983-84 samples from 20 antibody negative haemophiliacs were tested, retrospectively, for antigen as they were believed to have been treated with a batch of FVIII thought to be infected with HIV-1 (Dr. R. Madhok, personal communication). No sample contained antigen but 4 patients are now known to have seroconverted; one in 1983 (patient 10) and 3 later in 1985 (patients 7, 15 and 16). No further information on this batch or its origin is available.

Serum p24 antigen levels were monitored regularly in the 31 seropositive haemophiliacs. Over the 5 year follow-up period, 8 patients (25.8%) were found to have detectable levels of antigen repeatedly in their serum. In patient 1 the presence of HIV-1 antigen was reactive and confirmed on only one sample in January 1990.

He has subsequently tested negative. The remaining patients are antigen negative up to the end of June 1990 (patient 9 moved to London during 1988 and was antigen negative up to this time).

Antigen Positive Group

The time interval to antigenaemia ranged from 16 months to 93 months in these 8 patients (mean 48.9 months) (Table 5.3). However the precise dates of infection in these patients are unknown and so this can only represent the minimum time interval. The four patients who were HIV-1 seropositive in 1982 showed widely differing times to antigenaemia, from 22 to 92 months, i.e. in the order of 2-8 years; two patients who seroconverted in 1983 developed HIV-1 antigen almost four-and-a-half years later. Patient 8, first found seropositive in 1984, had detectable HIV-1 antigen 29 months later and the most rapid progression was observed in patient 7 who seroconverted during 1985 and had detectable serum antigen 16 months later.

A correlation between age at first antibody positive test and progression to antigenaemia was suggested. The oldest patient in this group, patient 7, aged 46 years when first found to be seropositive, progressed most rapidly to antigenaemia whereas the younger patients took 4 to 5 times longer.

The production of antigen (Ag) was associated with other changes in serum chemistry. Decline in reactivity to core proteins was demonstrated in all of these patients by ELISA (Abbott HIV-1 Anti-Core EIA, Appendix 2) and in some patients by western blotting. In 4 patients, ELISA results showed the typical profile of a loss in anti-p24 levels preceding antigenaemia by 5 and 16 months in patients 13 and 7 and by 30 and 42 months in patients 28 and 1. Patients 8 and 18 became anti-p24 negative by EIA at the same time as Ag was detected in serum. Patients 27 and 31 were more atypical in that they were anti-core positive by ELISA on developing antigenaemia (Table 5.4) and became anti-p24 negative 34 and 28 months later.

Western blot data differed: all patients showed anti-p24 on a blot at some point in time, a decrease was noted in patient 7 during 1989, patient 8 between 1988 and 1989, patient 13 and patient 31 between 1986 and 1988. Patient 13 experienced a decrease in anti-p24

TABLE 5.3

Time to development of serum antigen in antigen positive patients from their first antibody positive result.

Patient	First Antibody Positive month/year	First Antigen Positive month/year	Time to Development of Antigen (months)	Age at First Antibody Positive (years)
1	5/82	1/90	92	11
7*	11/85	3/87	16	46
8*	1/84	6/86	29	31
13*	3/82	4/86	49	14
18	4/82	2/84	22	23
27	3/83	9/87	54	7
28	11/82	8/88	69	5
31	4/83	3/88	59	8

* patient 7 deceased 3/90
patient 8 deceased 2/91
patient 13 deceased 7/87

Spearman's rank correlation coefficient = -0.81 for the association of age with time to development of antigen. This is significant at a 95% confidence level.

TABLE 5.4 Progression of HIV markers in Ag positive patients

			1984	1985	1986	1987	1988	1989	1990
1	anti-p24	WB	NS	+	-#	+	+	+	+
		ELISA	NS	+	+;-	-	-	-	-
	p24	Ag	NS	NT	-	-	-	-	+;-
7	anti-p24	WB		+	+	+	+	+	D
		ELISA		-	-	-	-	-	D
	p24	Ag		-	-	-	+;-*	+	D
8	anti-p24	WB	+	+	+	+	+	+	+
		ELISA	-	+	Bd;-	-	-	-	-
	p24	Ag	-	-	+	+	+*	+;-	-;+
13	anti-p24	WB	+	+	+	+	D		
		ELISA	Bd	-	-	-	D		
	p24	Ag	-	Bd	+	+*	D		
18	anti-p24	WB	-	-	-	-	+	+	+
		ELISA	-	-	-	-	-;+	+	+
	p24	Ag	+	+	+	+	+	+	-
27	anti-p24	WB		+	+	+	+	+	+
		ELISA	-	-	+	+	+	Bd	-
	p24	Ag			-	+	+	+	+
28	anti-p24	WB	-	-	(+)	(+)	(+)	(+)	(+)
		ELISA	+	+	-	-	-	-	-
	p24	Ag		-	NT	-	+	+	+
31	anti-p24	WB			+	NS	+	+	+
		ELISA		+	+	NS	+	+	Bd
	p24	Ag			-	NS	+;-*	-;+	+

D = deceased; NS = no sample; Bd = borderline; NT = not tested; (+) = weak band on western blot;
* = known to be on AZT treatment; # = specimen contaminated

on becoming antigen positive during 1987, this decreased further on progression of clinical illness until death (Fig.5.2). Low levels of anti-p24 were detected in patient 28 throughout the period of follow-up. The lack of anti-p24 in the 1984 and 1985 specimens may reflect the lower sensitivity of the early version western blots used at the beginning of this study. Neither patient 8 nor patient 13 had anti-p17 and a decrease in anti-p17 response was noted in patients 7 and 31. There was no evidence of change on a western blot in patient 1 and 27.

Patient 18 was unusual (Fig.5.3). Core antibodies were present in the first sample available for testing in April 1982; by the end of 1982, however, the patient became anti-p24 negative. The loss of anti-p24 preceded development of antigen by a maximum of 22 months. This status quo (anti-p24 negative, p24 antigen positive) remained until 1988, when antibodies to p24 reappeared, detected by western blot and by ELISA. The anti-p24 response has been retained through 1989 and 1990 despite the presence of serum antigen.

Anti-env response was retained and unchanged by ELISA and western blot in all patients.

Antigen Negative Group

The remaining 22 patients are HIV-1 antigen negative up to the end of June 1990. The mean duration of HIV-1 seropositivity to this time is 7 years (range 5-9 years) where 11 out of the 22 have been seropositive for 8 years or more.

An anti-p24 response was detected in all 22 patients by ELISA. However, 2 patients (patients 12 and 15) showed a weaker reactivity in the anti-p24 ELISA and this has been interpreted as a decline in core antibody. Patient 15 has since become antigen positive after June 1990.

β_2-MICROGLOBULIN IN HAEMOPHILIACS

Serum β_2-microglobulin (β_2-mg) was measured in a group of HIV-1 seropositive and seronegative haemophiliacs (Table 5.5). In

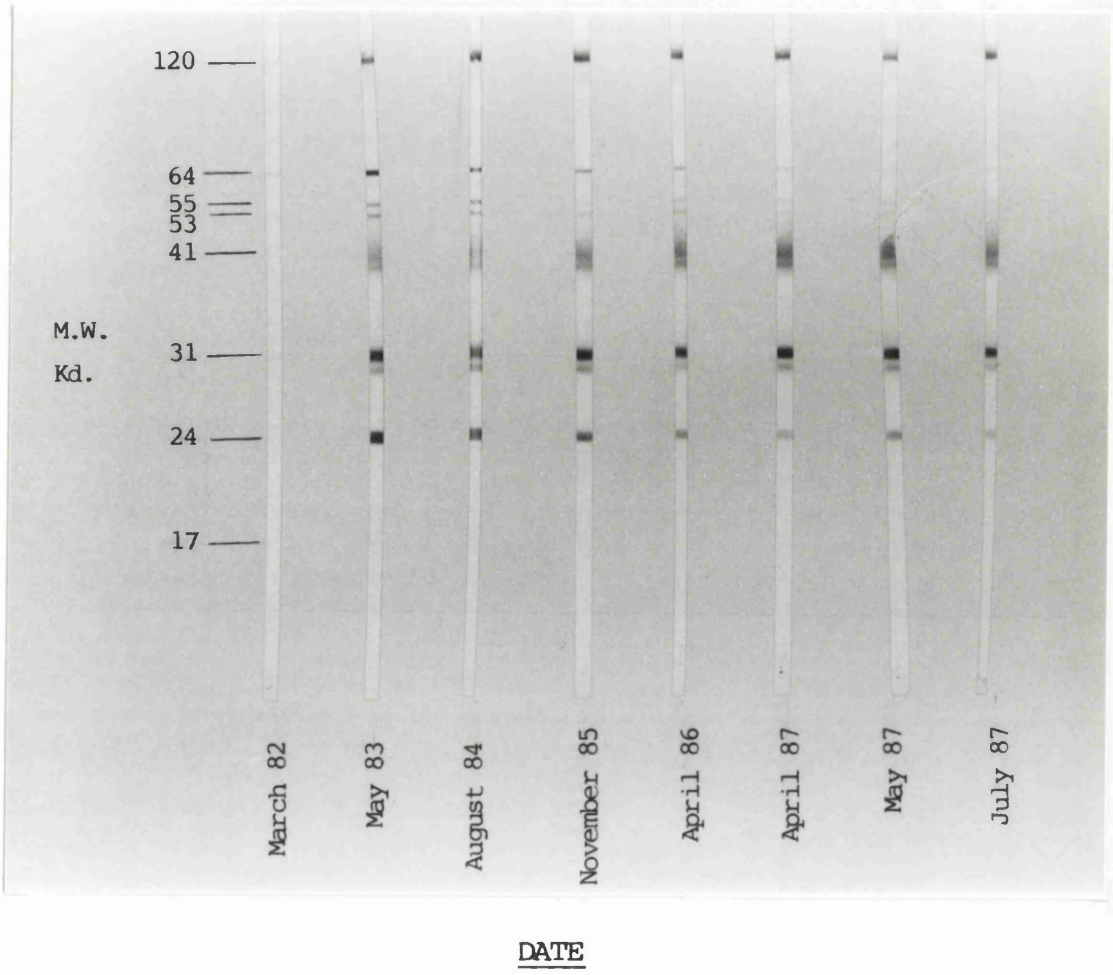


Figure 5.2 Temporal decline of HIV-1 anti-p24 observed on western blots from patient 13 up to the time of his death in July 1987.

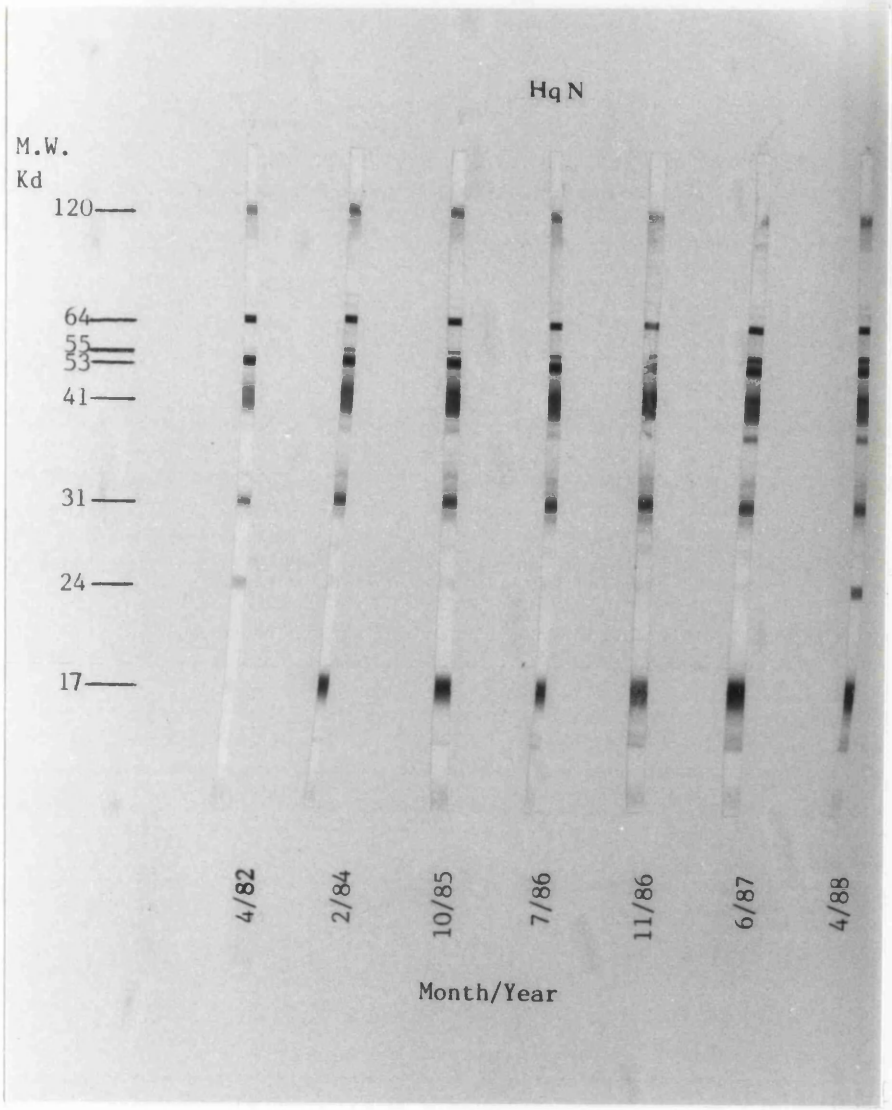


Figure 5.3 Western blot antibody profile in patient 18 showing re-eppearance of anti-p24 in April 1988.

healthy individuals, values up to 2 mg/l are accepted as normal (Abbott β_2-microglobulin RIA Test procedure, Expected Value p11). Although the ranges are wide for both groups certain differences between the two have been noted: 57% of antibody negative haemophiliacs have values ≤2 mg/l in contrast to only 7% in the antibody positive group. In this latter group 48.3% have levels ≥3mg/l but only 6.7% of the seronegative patients have levels as high as this

β_2-microglobulin levels were measured in serial samples from the HIV-1 antigen positive group and age-matched HIV-1 seropositive controls up to the end of June 1989 (Table 5.6). In 5 out of the 8 Ag negative seropositives the β_2-mg was seen to rise (≥3mg/l) 4 to 5 years after being found to be HIV-1 Ab-positive; in one patient (26) the time interval was only 2 years. However this increase was not always maintained. Three patients remained within the normal range of β_2-mg levels even 8 years after being HIV-1 Ab positive.

In the Ag positive group, β_2-mg levels have been in the normal range in 2 patients and always raised, i.e. >3mg/l in another 4 patients throughout the time period of follow-up, and therefore did not correlate with the appearance of Ag. In one of these patients the β_2-mg levels were recorded in the last few months of his life when he was Ag positive and symptomatic. An increase in level was noted in the 2 remaining patients, and this corresponded with the detection of serum Ag in both cases. The levels of β_2-mg did not appear to correlate with Ag status, nor with the age of the patient, nor the length of time seropositive. No virological progression was observed at any subsequent time in these Ag negative patients with an increasing β_2-mg concentration. However patient 17 has progressed clinically to ARC. Thus the measurement of β_2-mg levels, in the small sample of patients studied was found to have a limited association with virological progression.

Since the end of 1989, β_2-mg concentration has been measured routinely in all HIV-1 seropositive haemophiliacs. In the 25 patients with two or more values only 6 patients have an average value ≥3mg/l where 4 of these patients are members of the antigen positive group (patients 7, 8, 27 and 28). A further 5 patients have mean

TABLE 5.5

Serum β_2-microglobulin levels (mg/l) in HIV-1 seronegative and seropositive haemophiliacs

HIV antibody status	No. tested	Mean value mg/l	Range mg/l
Positive	29	3.11(0.151)*	1.55 - 4.9
Negative	30	2.12(0.094)	1.4 - 3.8

* Standard error of the mean
Using a two sample t test with a 95% confidence level the mean β_2-microglobulin level for the HIV-1 antibody positive haemophiliacs is significantly different from that of the HIV-1 antibody negative haemophiliacs, ($p<0.05$).

TABLE 5.6

Examination of serial β_2-microglobulin testing in a group of HIV-1 seropositive haemophiliacs: Ag positives versus age-matched Ag negatives#

Ag Positive Haemophiliacs			Ag Negative Haemophiliacs		
Patient No.	β_2-mg Level*	No. Years Seropositive to end of June 1990	Patient No.	β_2-mg Level*	No. Years Seropositve to end of June 1990
28	High	8	29	Normal range	8
27**	High	7	26**	Increasing	6
31**	Increasing	7	30	Increasing	7
1	Normal range	8	22	Normal range	8
13**	High	8	19	Increasing	8
18	Normal range	8	10**	Increasing	7
8**	Increasing	6	12	Normal range	8
7**	High	5	17	Increasing	6

* Designation of β_2-mg levels
Normal range <3mg/l
High >3mg/l
Increasing from <3mg/l to >3mg/l

** β_2-mg level >4mg/l recorded in one or more samples
# The patients are listed in age order from the youngest to the oldest

values >2.5mg/l, two of whom are also in the antigen positive group (patients 13 and 31).

Details of clinical status were obtained and an assessment made about the role of the above markers, anti-p24, Ag and β_2-mg, in disease progression in the adult HIV-1 seropositive haemophilacs in Glasgow and the West of Scotland.

CLINICAL STATUS

Clinical information was available on the haemophiliacs attending the RHC at GRI (Dr. G.D.O. Lowe, personal communication). Up to the end of June 1990, 22 HIV-1 seropositives attended this centre. (One individual, patient 9, moved away in 1988 and another individual, patient 23, formerly at the RHSC was now of an age to attend the adult centre). Eleven patients have become symptomatic (Table 5.7); 3 with PGL, 5 with ARC (1 deceased) and 3 with AIDS (1 deceased and 1 subsequently died in February 1991). Patient 5 developed PGL after the end of the period of follow-up, in September 1990, and then progressed to ARC (May 1991). He has been included in the group of clinical progressors. In the patients progressing to ARC and AIDS time to progression ranged from 3 years to 10 years. This represents the minimum time to progression. The first patients found seropositive (patients 5 and 20) took the longest to progress, i.e. 9 and 10 years respectively. In contrast, the last seroconverter, patient 7 in 1985, had developed ARC within 3 years. The remaining 11 are asymptomatic with one on prophylactic treatment with AZT and/or pentamidine because of a falling T4 count (and 4 others after June 1990, to the time of writing).

Clinical Progressors

p24 Antibody and Antigen

Six of the adult patients who progressed clinically showed virological changes associated with disease advancement (patients 1, 7, 8, 13, 18 and patient 15 after June 1990, Table 5.7). Five patients developed antigen and lost p24 antibody; the other patient,

Spearman's rank correlation coefficient = -0.491 for the association of age with time to development of ARC in eight patients. This is not significant at a 95% confidence level.

TABLE 5.7

Clinical and virological progression in HIV-1 seropositive haemophiliacs*

Patient No.	Asymptomatic	PGL	ARC	AIDS	First p24 Ab Negative (Month/Year)	First Ag Positive (Month/Year)	Age at First HIV-1 Seropositive Test
23	10/82 ---->	3/90			NA	NA	7
1	5/82 ---->	3/90			7/86	1/90	11
13	3/82 ---->	3/87 -->	7/87		11/85	4/86	14
5	10/81 ---->	9/90 -->	5/91		NA	NA	15
20	4/81 ---->	1/86 -->	6/90		NA	NA	20
18	4/82 ---->	4/89			2/84**	2/84	23
8	1/84 ---------->		6/88 -->	8/89	6/86	6/86	31
15	10/85 ---------->		8/89		NA	9/90	36
17	2/84 ---------->		6/89		NA	NA	42
7	11/85 ---------->		6/88 -->	9/89	NA#	3/87	46
4	4/84 ---------->		6/89 -->	8/89	NA	NA	51

NA = Not applicable
** = Patient 18 subsequently became anti-p24 positive by ELISA in April 1988
# = Patient 7 has been anti-p24 negative by ELISA since his first HIV-1 seropositive test
* Patients are listed in age order from the youngest to the oldest

patient 15, showed an apparent decline in anti-p24 and became antigen positive in September 1990, one year after being diagnosed with ARC. Where a change in both markers was noted, this occurred prior to the diagnosis of HIV-related symptoms in all 5 cases. Loss of anti-p24 preceded symptoms by 2 to 4 years in 4 patients, where one of these patients was always anti-core negative by ELISA. The remaining patient, patient 18, was most unusual and was described previously; anti-core reappeared 4 years after being undetectable by ELISA. HIV-1 antigen was detected 2 months prior to developing PGL in one patient and up to 2 years before the diagnosis of ARC in 3 other patients. In patient 18, antigen had first been detected 5 years prior to progression. The remaining 5 patients showed no changed in virological markers. All 11 patients in this group began AZT treatment at or shortly after diagnosis of HIV-related disease.

β_2-microglobulin

The β_2-mg levels in the 4 haemophiliacs (patients 7, 8, 13 and 18) who had regular measurements taken over the time span of progression show differing results (Table 5.8). In patient 7 a rise was noted after antigen was detected prior to the development of ARC; however this patient always had β_2-mg levels >3mg/l. The level increased to >4mg/l almost a year after the diagnosis of ARC. In patient 8 the concentration of β_2-mg rose above 3mg/l after becoming antigen positive and remained stable at this level although the patient progressed clinically. β_2-mg levels in patient 13 were very high, but these measurements were taken in the last few months of his life when rapid clinical progression occurred following a salmonella septicaemia as a result of a surgical procedure becoming infected. No obvious changes in β_2-mg concentration were detected in patient 18 with an average concentration of 2.4mg/l despite being symptomatic and antigen positive. In the remaining 7 patients with clinical progression routine β_2-mg levels were measured, following the diagnosis of HIV-related symptoms. All but one patient had a mean concentration $\leq$2.6mg/l. One patient (patient 20) did have a β_2-mg concentration >3mg/l prior to the diagnosis of ARC but he had been symptomatic for four years. A rise in β_2-mg concentration above

TABLE 5.8

β_2-microglobulin levels (mg/l), anti-p24, antigen (Ag) status and CDC Stage in haemophiliacs with virological progression

Patient No.	Date (Month/Year)	β_2-mg (mg/l)	Anti-p24	Ag.	CDC Stage*
7	11/85	3.1	-	-	II
	12/86	3.1	-	-	
	7/87	3.8	-	+(3/87)	
	4/88	4.8	-	NT	
	8/88	3.9	NT	- AZT(6/88)	IVA/C_2(6/88)
	5/89	7.8	-	+	
	11/89	5.9	-	Bd	IVB/C_1/D(9/89)
8	4/86	0.5	+	-	II
	6/86	2.95	-	+	
	2/87	0.5	NT	+	
	10/87	3.3	NT	NT	
	8/88	4.2	-	+	IVA/C_2(6/88)
	5/89	3.0	-	Bd AZT(9/88)	
	11/89	2.8	NT	+	IVB/C_1/D(8/89)
	1/90	3.1	-	+	
	2/90	2.8	NT	+	
	3/90	2.0	NT	-	
18	2/84	1.95	-	+	II
	10/85	1.8	-	+	
	6/87	2.7	-	NT	
	1/88	2.45	-	+	
	4/88	2.8	+	+	
	8/88	2.6	-	+	
	2/89	2.85	+	+	
	3/90	2.3	+	-	III(4/89)

* CDC II: Asymptomatic; CDC III: PGL; CDC IV A/C_2: ARC; CDC IV B/C_1/D: AIDS

3mg/l was also observed in patient 17 prior to developing ARC; this subsequently decreased.

Clinical Non-Progressors

There is no evidence of virological progression or an increase in β_2-mg levels, where more than 2 measurements have been made, in 10 out of the 11 patient members of this group despite being HIV-1 seropositive for up to 9 years.

Summary

Thus p24 antibody and antigen are the markers that have changed and usually a number of years in advance of any clinical diagnosis of HIV-1 disease in half of the total number of patients studied. This may signify a more rapid onset of disease than those with no virological progression. The predictive role of β_2-microglobulin levels in disease progression is less obvious but does appear to be associated with the disease process in half of the individuals with clinical progression. There is no evidence of rising levels in the group who have not progressed clinically even in those with other warning signs of disease onset, i.e. a falling T4 count.

Age at seroconversion has been associated with disease progression and this has been noted in this haemophiliac population. All except one of the patients over 30 years of age have progressed to symptomatic disease and have progressed more quickly than the younger patients. In the group of clinical non-progressors who have been seropositive for 7 or 8 years, a falling T4 count has been noted in 5 patients and hence prophylactic treatment started (Dr. Lowe, personal communication). These patients are the oldest members of this group (Table 5.9).

TABLE 5.9

Data on the asymptomatic haemophiliacs listed in age order from youngest to oldest

Patient No.	Date of First HIV-1 Seropositive Result (Month/Year)	No. of Years Seropositive to June 1990	AZT Commenced (Month/Year)	Age
21	2/83	7	NA	10
22	5/82	8	NA	12
19	11/82	8	NA	13
11	2/83	7	NA	14
3	2/83	7	11/90	14
6	12/82	8	1/91	15
14	11/82	8	?/91*	16
16	11/85	5	NA	19
10	6/83	7	1/90	21
12	9/82	8	10/90	30
2	3/84	6	NA	37

* Patient moved to a new centre and put on AZT some time between January-June 1991.

DISCUSSION

The emergence of another blood-borne virus put the haemophiliacs at great risk due to their requirement for transfusion of blood products, which have been pooled from up to 20,000 donors. Evidently the greater the quantities of factor concentrate used, the greater the risk of infection. Studies in the UK (250) and elsewhere (251) have demonstrated that the greatest percentage of seropositives occurs in those patients with HmA. This is also true in the West of Scotland patients where the majority, 29 out of 31 seropositives (93.5%) suffered from HmA. All HmA patients except one had severe haemophilia and were therefore large users of FVIII. Of the two HmB patients; one, patient 16, was a moderate user of FIX concentrate, the other, patient 18, was also receiving blood products in Manchester. The probable reasons for this increased prevalence in HmA over HmB are twofold. Firstly, the greater the quantities of factor concentrate used by an individual the higher the probability of being transfused with an infected batch. This was observed in a higher percentage of HIV-1 seropositives detected in the child haemophilia population who have a different treatment policy, using greater quantities of factors than the adults. Secondly, the differences in the extraction and purification of FVIII and FIV from pooled blood may affect the risk of acquiring HIV. FVIII concentrate is prepared from the cryoprecipitate of frozen-thawed plasma (252), where any viral particles complexed with immunoglobulins are also likely to be found. FIX concentrate is further purified from the supernatant of frozen-thawed plasma by adsorption on a DEAE ion-exchange column (253) and in this way HIV may be excluded from FIX concentrate.

In 1984 the use of heat-treated concentrate (60^{o}C for 72 hr.) was advocated in an attempt to prevent further infections. This was not entirely satisfactory. There were reports of seroconversions in haemophiliacs after receiving commercial heat-treated FVIII (254). However this may reflect inadequate treatment of the concentrate to reduce the infectious titre of virus since certain groups were successful in preventing further seroconversions (249). But in most

countries, by this date, the damage had been done. The indication from several reports published in 1984 of high rates of infection amongst haemophiliacs, supported this (Table 5.10).

Although the numbers did vary between different continents and between countries within these continents, the highest rates were found in the USA, where AIDS was first described. In a sample of Scottish haemophiliacs (256) including the population of patients on which this study was based, 15.6% were found to be seropositive. The present figure including children in the population of 250 haemophiliacs in the West of Scotland is 12.4%.

In this study, samples prior to 1985 were available, because of the regular monitoring of hepatitis status in these patients, so it could be shown that HIV infection was acquired in almost three-quarters (71%) of the total seropositives before the end of 1983. An increase in prevalence in the early 1980s, e.g. from 0% in 1980 to 53% in 1984 (257) was shown in other European haemophiliacs (258). A higher prevalence of seropositives was noted in the haemophiliac children (28.1%) compared to the adults (15.3%) tested at this time in the West of Scotland. This is most probably associated with the more liberal approach to FVIII usage at the RHSC with the almost exclusive use of FVIII manufactured in the U.S.A.

Detection of seropositives in this group as early as 1981 coincident with the emergence of the AIDS epidemic among homosexual men and intravenous drug abusers in the U.S.A., but not with the AIDS epidemic in the UK, implicates the import and use of foreign blood products, of American origin, at this time. In addition Cheingsong-Popov et al. (183) found 0% prevalence of anti-HIV 1 in a sample of more than 1,000 unselected blood donors from one transfusion centre in the UK in 1984, however there is evidence to suggest HIV-1 seropositivity in certain risk groups in the population in Glasgow and the West of Scotland as early as 1983 but whether such persons donated blood at this time is unknown (Chapter 3).

A positive association between the use of commercial FVIII and increased incidence of seropositivity was shown by Melbye et al. (256): 40% of haemophiliacs treated with either FVIII alone or in combination with locally produced FVIII were positive compared with

TABLE 5.10

Seroprevalence reports in other haemophilia populations in 1984

Country	Seroprevalence %	Reference
Georgia, U.S.A.	72	Ramsay et al. (255)
Massachusetts, U.S.A.	66	Kitchen et al. (251)
Denmark	59	Melbye et al. (256)
South Bavaria, Germany	53	Gürtler et al. (257)
United Kingdom	34	Cheingsong-Popov et al. (183)
West of Scotland	15.6	Melbye et al. (256)

6.7% in those using only local FVIII. Since 1981 haemophiliacs in Scotland have been treated mainly with FVIII concentrate produced by the Scottish National Blood Transfusion Service (SNBTS) at the Protein Fractionation Centre (PFC) in Edinburgh. Such practice, in addition to the different treatment policies practiced in different regions may explain the relatively low prevalence of HIV infection in Scottish haemophiliacs. However one of the seropositive cases described here received only local concentrate. This patient, 10, was first found to be seropositive in June 1983 (but seronegative in July 1982) suggesting that he was infected during 1982 or early 1983. This confirms the presence of HIV infection in the blood donor population in Scotland in 1982/83. Although an attempt was made at identifying the batch of concentrate responsible, no other haemophiliac seroconverted as a result (Dr. R. Madhok, personal communication). The remaining seropositives received significantly greater quantities of commercial concentrates than the seronegatives (Dr. R. Madhok, personal communication) and this is therefore the likely source of infection especially in those seropositive prior to the introduction of heat treated concentrate. In Edinburgh, 18 out of a cohort of 32 haemophiliacs all treated with the same infected batch of FVIII seroconverted. Cuthbert et al. (242) showed that the main difference between the two groups was that greater quantities of the FVIII were given to the seropositives.

Three cases were documented as being seropositive for the first time in the latter part of 1985. The results suggest that they were infected during 1985. This would be after the introduction of heat treated concentrate in December 1984. However, they may have received an infected batch earlier: they were recipients of an implicated batch of FVIII during 1983. Perhaps the virus remained dormant in their peripheral blood lymphocytes or in an alternative reservoir, e.g. monocytes or macrophages, (lymphocytes) having avoided the immune system, until activated by some other co-factor e.g. another virus. Perhaps repeated exposures, even in small doses, to factor concentrate, and the allogeneic proteins it contains, triggered expression of latent virus into active infection. Consequently it implies that each time a haemophiliac has treatment, he is in danger

of switching on viral expression. That T cells are antigenically primed, and proliferate on exposure to some component of FVIII concentrate has been shown in a small study by Mattheson et al. (259).

Why did some haemophiliacs become infected while others treated with the same batch have no apparent evidence of infection? Certainly some members of the population appear to be more susceptible to infection with HIV although the mechanisms involved have yet to be determined; in haemophiliacs the effect of FVIII on the immune system may play a role. Repeated exposure to pooled blood products, from many donors, is associated with the development of T4/T8 abnormalities (259). Factor VIII appears to cause immune suppression, perhaps those patients with the severest abnormalities in lymphocyte sub-populations and immune response may be at greater risk of acquiring HIV infection. Indeed Ludlam et al. (260) followed a cohort of haemophiliacs all treated with the same batch of contaminated FVIII and suggested that haemophiliacs with a helper/suppressor T cell ratio <1.5 were more susceptible to infection. Patients with haemophilia B are noted to have fewer lymphocyte subset abnormalities and a lower prevalence of antibody to HIV. There have also been reports (162,261) associating HLA phenotype particularly A1, B8, DR3, with an increased risk of seroconversion.

Other interesting questions arise: have the haemophiliacs been treated with infectious virus or have these patients synthesised antibodies to viral proteins disrupted during the preparations of concentrate and are therefore effectively vaccinated? This latter suggestion is probably unlikely since haemophiliacs do get AIDS; 50% of this cohort of adults developed signs of HIV-related disease progression.

The presence of HIV antigen in serum prior to seroconversion has been reported in haemophiliacs (86, 262) and in other risk groups (85). However in this cohort of patients with previous seronegative specimens available for testing there was no confirmed antigenaemia present. Allain et al. (86) reported 22.5% and Simmonds et al. (262) 42% of haemophiliacs with antigenaemia prior to seroconversion; Allain et al. (86) found Ag in serum up to 9 months prior to

seroconversion. However presence of antigen at this stage in infection is a transient phenomenon and the random timing of the samples in this cohort may have precluded its detection.

The presence of antigen early in infection would be expected to coincide with an acute or primary infection with the virus. There have been reports (82) to suggest that a seroconversion illness occurs less often in haemophiliacs in contrast to other risk groups (77-81). Only 1 out of 22 case records examined (Dr. Madhok, personal communication) revealed an illness consistent with primary infection.

At the opposite end of the spectrum of infection, progression of HIV disease has been associated with changes in levels of certain markers in blood. Decline in anti-core, production of HIV-1 antigen, decrease in T4 cell counts and raised serum β_2-microglobulin levels have all been associated with progression in haemophiliacs (237,242,263) as in other risk groups (104-107,235). A change in Ab and Ag status was observed in 8 out of the 31 patients (25.8%) up to the end of June 1990. Five of these patients received treatment in the early 1980s at the RHSC, and three are currently patients there, where FVIII usage has always been liberal. Therefore they probably received greater doses than the adults whose treatment tended to be given only if they had a major bleed. Such frequent treatment may promote disease advancement. In the haemophiliac children there are two sets of HIV-1 seropositive twins (patients 26 and 27 and patients 30 and 31). One brother from each pair is antigen positive but the sibling, in both cases, has shown no signs of virological progression yet. If these twins are genetically identical this suggests that co-factors other than genetic factors influence progression of illness.

No obvious pattern emerges from the 8 members of the antigen positive group as to which marker, p24 antibody or p24 antigen, changes first or incubation times heralding change. In 4 patients loss of antibody preceded antigen detection by up to 3.5 years and in a further two patients the first virological change was the appearance of antigen followed by decline and loss of anti-p24 up to 2.5 years later. In the remaining two patients an antibody negative/antigen positive, Ab-/Ag+, pattern was observed at the same sampling time.

The reappearance of anti-p24 in one of the latter patients is interesting and may demonstrate some recovery of B-cell function resulting in an increase in antibody production. This may be in response to increased viral synthesis or de novo antibody synthesis directed against a different epitope of the p24 core protein. Perhaps antibodies to certain core epitopes could not be detected by the standard screening and confirmatory tests, until some selection pressure altered the dominant circulating virus strain in this patient.

Time to production of HIV-1 antigen also varied from less than 2 years up to 8 years after HIV-1 antibody seroconversion. The most rapid time to development of antigen was observed in patient 7 who was one of the last haemophiliacs to seroconvert in 1985. In addition, he was also the oldest of the 8 patients showing virological progression. Previous reports have suggested a positive correlation of age at seroconversion with disease progression (155,158,159). This probably reflects a decrease in the capabilities of the immune system to resist the pathogenic effects of HIV-1. Prolonged factor VIII usage and clinical conditions, e.g. chronic liver disease associated with older haemophiliacs may lead to chronic antigen stimulation and/or impaired antigen processing and thus promote disease advancement.

Core Ab and p24 Ag along with other markers have frequently been used to determine rapid onset of symptomatic infection. Eleven out of the 22 patients in whom clinical information was available became symptomatic over the period of follow-up; 50% of these patients had become Ab-/Ag+, the pattern most commonly found in symptomatic patients (as discussed in Chapter 4), prior to any clinical diagnosis. One additional patient became anti-p24 negative but the remainder showed no signs of virological progression. No change in either of the viral markers was observed in the asymptomatic patients. Thus Ag production and loss of anti-core does occur in symptomatic haemophiliacs but not in all such patients. These markers may signify early progression and rapid disease onset. The changes may not necessarily precede symptoms; in most patients HIV-related signs and symptoms are present for a period of time before

a clinical diagnosis is made so as to meet the criteria of disease classification. Allain et al. (237) found that HIV-1 Ag was a better marker than absolute T4 cell counts in predicting the appearance of symptomatic infections in a group of French haemophiliacs. Eyster et al. (263) showed that p24 antigenaemia predicted a poor prognosis and was a more specific but less sensitive marker than T4 cell counts; 46% of their haemophiliac patients with AIDS never developed detectable antigenaemia, an equivalent finding in the symptomatic patients in this study. It is interesting to note that there was no suggestion of progressing disease in the asymptomatic patients using the viral markers p24 Ab and Ag. Instead the first observation, possibly indicative of some ongoing disease process was a falling T4 count (Dr. Lowe, personal communication). This was more instructive to the physicians; when this occurred prophylactic anti-viral therapy was commenced early in the hope of retarding the disease process. Continued testing for Ag is less useful in these asymptomatic patients now on AZT treatment.

β_2-microglobulin, a nonspecific marker of immune activation, was also measured in the HIV-1 seropositive haemophiliacs but was found to have little prognostic value. An increase in β_2-mg concentration was seen in a total of 9 out of the 16 HIV-1 seropositives in whom serial testing was performed before 1989; five of the 8 antigen negatives and 4 of the 8 antigen positives (2 others in the latter group repeatedly had levels higher than 3mg/l). However the increase observed in the antigen negative patients was not maintained although one of the patients is known to have developed ARC. The temporary rise in β_2-mg in the haemophiliacs may be related to other stimulatory effects on the immune system, e.,g. through the regular treatment with blood products. However a raised level still remains a feature of HIV-1 infection as only 2 out of the 30 seronegative haemophiliacs tested had levels >3mg/l.

As a predictor of disease progression, five out of the 11 haemophiliacs who developed HIV-related disease had a rise in β_2-mg concentration prior to diagnosis. In contrast no patient who remained asymptomatic showed an increasing β_2-mg concentration, even in those patients with the suspicion of clinical progression due to a

falling T4 count. Thus an association exists between raised β_2-mg concentration and progressive HIV disease. However this is not definitive or absolute as variation occurs between different patients. A recent study (242) in a cohort of well characterised Edinburgh haemophiliacs infected from a common batch of FVIII (260) examined a variety of markers of immune status including T4 and T8 cell counts and β_2-microglobulin. Serial T4 counts decreased as disease progressed, a common finding in all cohort studies and a well-established feature of the viral pathogenicity. However plasma β_2-mg concentration not only reflected the presence of clinical disease but was useful in predicting the onset of symptomatic infection. In the Edinburgh study it was persistently raised (>3mg/l) in all nine, i.e. 100%, of the symptomatic seropositives, compared to 45.4% (5 out of 11) in the Glasgow haemophiliacs. In 5 out of the 9 Edinburgh patients this rise had been noted at least one year prior to development of symptoms; this was similar in Glasgow, a rise was noted less than 1 year before the diagnosis of ARC in the relevant 5 patients. In a San Francisco cohort of homosexuals (235), β_2-mg was the single, most useful predictor of disease progression, however this was not found to be the case in the Hm patients studied here.

The time to progression of disease is more difficult to assess in patients where the date of infection is not precisely known. In the Glasgow haemophiliacs the time interval ranged from 3 years to 10 years (mean 5.9 years) in the 8 patients progressing to ARC or AIDS. This represents the minimum time in some patients and in other patients the time since the date of the estimated seroconversion. Twenty-seven per cent progressed to ARC within 6 years of their first antibody positive result or seroconversion, with 14% developing AIDS in less than 6 years. This compares with estimates when included with the rest of the UK haemophiliacs (264), in the American (155) and Swedish Hm (156), and in other risk groups, e.g. homosexuals (157). However progression in the Edinburgh haemophiliac cohort appears to be quicker; with 27.7% having developed AIDS (and a total of 55.5% with ARC or AIDS) within 5 years (242). The reasons for this are unclear and may be directly related to the quantities of the infected batch received by these individuals. The overall picture suggests that

progression to AIDS is generally slow. Such progression can be delayed still further with the early intervention of drug therapies particularly AZT which directly inhibits viral replication by inhibiting the viral reverse transciptase enzyme.

Several reports in the literature have noted older age at seroconversion as an important co-factor in clinical progression (155,158,164). This is also true of the Hm population studied here. In the United Kingdom Haemophiliac Centre survey (264) a five-fold greater risk of developing AIDS within 5 years of seroconversion was observed in patients aged over 45 at the earliest HIV-1 seropositive report. Patient 7, aged 46 years at seroconversion and who was one of the last patients to become infected developed ARC within 3 years and AIDS within 4 years (Table 5.7). The majority of the other patients over 30 years of age at initial reporting had also developed ARC or AIDS within 5 or 6 years. The younger patients took twice as long to progress. This probably reflects a reduction in both lymphocyte numbers and immune competence in the older patients after years of treatment and chronic antigenic stimulation. The role of other factors and their contribution to progression is not fully understood. Chronic liver disease, for many years a problem in Hm may contribute to AIDS-related diseases. Haemophiliacs infected with cytomegalovirus have been found to progress more rapidly (265). Infection with other viruses, particularly hepatitis C, may also have a role to play.

Determinants of progression to disease are likely to be multifactorial. Most markers reflect the disease process rather than being predictive of clinical change. The more markers that are examined, the more evident it becomes that patients vary as individuals and only general trends can be established. There is no single marker which could be used diagnostically to predict onset of symptoms with absolute certainty. However, it appears that one of the most useful parameters is the T4 count; a falling T4 count is highly indicative of impending disease and a level below 200 cells/ l is associated with a very poor prognosis and prophylactic treatment is now instigated at this stage.

HIV-1 infection in the haemophiliac population is now

essentially preventable and this would appear to be so, as no new seropositives have been detected since late 1985, after the introduction of heat-treated FVIII and blood donor screening. Sadly, however, the evidence shows that the incidence of AIDS in this group is increasing as time progresses and the time-bomb of HIV-1 infection ticks away. Hope lies in the improvement of drug therapy and the early intervention of treatment in order to maintain a reasonable quality of life in these patients.

CHAPTER 6

GENERAL DISCUSSION

> A pestilence isn't a thing made to man's measure; therefore we tell ourselves that pestilence is a mere bogy of the mind, a bad dream that will pass away. But it doesn't pass away and, from one bad dream to another, it is men who pass away .....
>
> Albert Camus, 'The Plague'.

Ten years have passed since the apparent start of the AIDS epidemic in 1981 and the deaths of the first cases of AIDS. Retrospective evidence reveals sporadic cases in the 1970s and earlier (266). The emergence of the human immunodeficiency virus family has plundered research resources, activated the minds of clinicians, epidemiologists and research scientists the world over, killed thousands and decimated certain African populations. No other virus or virus-related disease has seen such a boom in research in such a short time. Our knowledge and perspective of the HIV family and AIDS has advanced faster than any known modern infectious agent. However, many questions still remain to be answered.

Not least of all is the question of the origin of the virus. Several pointers suggest it emerged in Africa where a number of related lentiviruses in both monkeys and man have been found, e.g. SIV_{mac}, SIV_{agm}, HIV-2 etc. and perhaps others as yet undiscovered which sit close together on a phylogenetic tree. Sufficient homology exists for these viruses to be related but they are sufficiently different at the genomic level to be different types and not just variants. There is also the suggestion from the greater extent of variation in African isolates compared to American isolates that the virus has been in Africa for longer (50). One can only speculate as to how the virus got to America and elsewhere, perhaps via commercial trade in the past or more recently via sailors and travellers. In the initial stages of the epidemic, the finger was pointed at African viruses as being the progenitor of the AIDS virus. This created bad feeling between African and American medical and

scientific communities and led to an underplaying of the extent of the problem in Africa and the true extent of the problem in some areas of Africa remains unknown.

The virus had time to run its full course of infection to AIDS before the problem was evident. The initial spread of HIV-1 infection therefore occurred as a silent epidemic throughout the 1970s in Africa and America. The extent of the spread at that time become apparent in the upsurge of cases of AIDS in these continents during the late 1970s, early 1980s. The first case of AIDS in the West of Scotland was in a homosexual man whose diagnosis was back-dated to 1984. There is also evidence to suggest that HIV-1 was in the blood donor population here as early as 1983. Thus the virus was introduced into the population in the West of Scotland in the early 1980s coincident with the emergence of the AIDS epidemic in the U.S.A. How did the virus get here? Originally this was through travel to areas with high rates of infection or through the import of infected blood products.

The boundaries of international travel have expanded over the last two decades and places previously inaccessible are now open to travel for the purposes of both tourism and business. In addition greater numbers of persons are now members of the itinerant population and thus the opportunity for spread and inter-mixing of infectious agents increases.

Population groups such as the homosexuals, which comprise many professional persons in older age groups, probably have greater opportunity to travel. HIV-1 infection in the homosexual population in the West of Scotland was initially imported; a result of contact in the early 1980s with infected partners in areas with high rates of infection such as North London in the U.K. and the west coast of the United States. This was the time of gay liberation and the fight for gay rights, particularly in the U.S.A. Homosexuals could be more open and still be accepted by the community. So it was only natural that homosexuals would go and indulge in places where attitudes were more relaxed. Unfortunately conditions were also prime for the transmission of an infectious agent such as HIV-1.

In the haemophiliac population, the virus was undoubtedly

introduced by the use of contaminated factor concentrate from the U.S.A. Most transmission to haemophiliacs had occurred by 1982-83. Fortunately the requirement for imported products in Scotland is low as Scotland has its own Protein Fractionation Centre and is usually self-sufficient in FVIII and FIX. Consequently, Scotland has one of the lowest reported seroprevalence rates in haemophiliacs in the world. However, eventually even the locally produced FVIII became contaminated (260).

Seroconversion to anti-HIV 1 was observed in the drug abuser population in Glasgow and the West of Scotland from the end of 1985, through 1986 and into 1987. The drug abusers in Glasgow are generally members of the lower socio-economic groups and are therefore less likely to indulge in international travel. It was speculated that the virus arrived in Glasgow from Edinburgh in 1985 via a female drug abuser. Several of her needle-sharing contacts subsequently became seropositive in that year. Samples from these patients are currently being sequenced following PCR amplication by Dr. P. Simmonds (University of Edinburgh) to establish the relatedness of the infecting viral strain. A sequence in the fourth hypervariable region (V4) of the OMP gene could determine relatedness (52). Such molecular epidemiology may prove or disprove that the Edinburgh strain of the virus did infect the Glasgow drug abusers initially and whether the same strain is still spreading. However, it is unlikley that this was the only source of infection.

The data presented indicate that the same groups are at risk in Glasgow and the West of Scotland as elsewhere, i.e. homosexuals (including bisexuals), drug abusers and haemophiliacs. Infection of haemophiliacs was halted after 1985 and there has been no evidence to suggest that they have contributed to further spread of the virus in the area. However the same is not true for the homosexuals and the drug abusers.

The drug abusers represent almost half of the newly diagnosed seropositives in this area, and the homosexuals about one-third. The actual numbers do not reflect the dynamics of spread of HIV-1 infection. The seroprevalence in the homosexual population is difficult to assess because they are a hidden group within the

population. It is clear from continued screening that the epidemic in the homosexual population here is still being boosted by persons returning to Glasgow having been infected elsewhere. In addition evidence of the silent epidemic, resulting from transmission in the early or mid-1980s is becoming apparent in the increasing numbers of patients whose first presentation is with an opportunistic infection and full-blown AIDS.

In contrast the drug abusers have a higher profile in this area and more readily admit to their risk activity. The problem of drug abuse in Glasgow is enormous and much attention has been focused on treatment and management of such individuals in the past decade. Medical problems, from septic abscesses to heart-valve replacements, associated with drug abuse itself are a greater threat to the abusers themselves and a greater drain on medical resources at the moment than the effects of HIV-1 infection. The prevalence of HIV-1 is currently low, <2% in the population screened and the indication is that most of this is 'home-grown', i.e. a result of spread within Glasgow. To find such a low level was encouraging; perhaps the factors promoting transmission are working less efficiently in this group. However sero-epidemiological studies rely not only on appropriate sampling but also on sensitive and specific laboratory tests.

The laboratory diagnosis of infection has to be accurate, particularly when dealing with such an emotive subject as HIV. An extensive choice of screening ELISAs has been available since the first screening test was developed in 1984. This has been a huge commercial venture for many of the large pharmaceutical and chemical companies, some of whom had never before been involved in diagnostic virology but jumped on the bandwagon to develop and/or market a screening test for anti-HIV 1. However the performance of the majority of tests was and has continued to be satisfactory, both in screening high prevalence populations, i.e. high risk groups and low prevalence populations, i.e. blood donors.

There is no doubt that screening tests have greatly improved. In five years there have been three generations of assays reflecting technological advances; from viral lysate-based tests to recombinant protein-coated solid phases and more recently, amplified detection

systems, resulting in tests with a high degree of sensitivity.

Initially the screening tests were not without their problems and the specificity of an ELISA reactive sample was further tested by western blot, the confirmatory test of choice at the HRL. In no other area of diagnostic virology has so much effort been spent on establishing a confirmatory system. The performance of the commercial DuPont Western Blot assay was maximised for use in the HRL using seroconversion samples, samples from those recently infected and also false reactives found on screening low prevalence populations. Criteria were established so that genuine reactivities were distinguished from false reactives and thus HIV-1 infection could be reliably diagnosed. The advances made in screening and confirmation means that the diagnosis of HIV-1 infection is sensitive, specific and reliable. That those infected are reliably identified is supported by the results from current anonymous screening studies. A seroprevalence and behavioural study on the local Glasgow drug abusing population has confirmed the same seroprevalence rate as that in the drug abusers who were screened voluntarily. This also suggests that the extent of spread of HIV-1 infection in the Glasgow drug abusers is much less than expected from experiences elsewhere in Scotland, the U.K. and worldwide.

What is the future for screening and confirmatory tests? The current screening tests detect anti-HIV 1 and anti-HIV 2 and problems arise in distinguishing between these reactivities or in confirming a dual reaction. There is sufficient homology between the viral proteins for them to cross-react on individual ELISA systems and on the viral lysate WBs. Single-specificity ELISAs need to be developed using immunodominant epitopes that are not conserved between the viruses. If only very short sequences of amino acids are required for antibody recognition this may increase the choice of epitopes available from each virus.

Confirmation is made easier by the more recent availability of strip assays containing recombinant or synthetic peptides from both viruses. Thus ELISA reactivity can be determined in one test. However only one epitope for HIV-2 is present on the currently available tests; greater confidence in reporting HIV-2 seropositivity

would be achieved by including more HIV-2 epitopes. No marked increase in sensitivity was noted in the recombinant immunoblot strips and this may reflect the lack of conformational epitopes in such material. It is becoming increasingly more difficult to match or better the improvement in the screening test performance characteristics with the currently available confirmatory tests, even with new advances such as chemiluminescent substrates, etc. for use in WBs.

HIV-1 infection continues to spread slowly in the West of Scotland. It is interesting to note that prevalence within a risk-group varies geographically suggesting that local factors contributing to the spread of the virus do vary substantially. This is most obvious in the drug abusing communities in Glasgow and Edinburgh. In Edinburgh, high rates of infection were recorded and this was associated with an increased pool of heroin abusers in the early 1980s who frequently shared needles and syringes, with multiple contacts at each sharing episode. Conditions were perfect for the spread of the virus. Evidence from the early case control studies in the U.S.A. show that it requires only one individual, who is highly infectious and indulging in risk behaviour, to promote spread of the virus within a community — the so-called Orange County connection (267,268). This was a homosexual, Canadian, airline steward with multiple partners in different areas of the United States who was identified as the link between a number of the first cases there. It was fortunate that no similar explosion of infection occurred in the West of Scotland; this was due in part to the later arrival of the virus in this drug abusing community and also to other behavioural factors, i.e. less sharing and less often than their Edinburgh counterparts (191). Advertising, television and press publicity campaigns began in late 1986 and 1987 but it is difficult to assess whether this reached the high-risk groups and had the desired effect or whether changes in attitudes and behaviour had already begun.

The appearance of AIDS and HIV-1 infection has shaken society and its previously held values causing individuals to examine moral issues and their own lifestyle. It has brought homosexuality and drug abuse out of the closet. Sadly, social attitudes are in general not

sympathetic to such lifestyles; there is still a stigma associated with being HIV seropositive. HIV is transmitted through intimate contact, either sexually, via blood or from mother to child, however it is not selective in its target population contrary to what was originally believed. It is not a gay-person's disease and these attitudes will have to change if further spread of the virus is to be prevented. It is clear that once the virus is introduced into certain populations and given the right conditions it will spread. The dynamics of the epidemic are changing elsewhere with spread into the heterosexual population. This suggests that all sexually active adults are at risk of acquiring infection. How long before this takes off in the West of Scotland is unknown. Currently heterosexual spread here is low and is a result of contact of high risk groups; only 10 of the 298 newly diagnosed seropositives, i.e. 3.4% reported heterosexual contact. Five were infected while staying in the African continent, the other five were contacts of risk groups here, e.g. bisexual men. Recent results from an anonymous HIV survey of all babies born in Glasgow during 1990 show no evidence of HIV infection in any child indicating no general heterosexual spread at this time (207).

Factors governing the transmissibility of the virus, i.e. both the infectivity of the donor and susceptibility of the recipient may also play a role in viral pathogenesis and disease progression. Since the beginning of the epidemic, infection with HIV-1 has confronted the scientific community with several important questions. Who will progress to AIDS? - does everyone infected proceed to end-stage disease? When does progression occur? Why does progression occur? and what are the factors promoting disease advancement and viral pathogenesis and can they be halted? As time progresses the epidemic of AIDS will incur more fatalities as present studies indicate that HIV-related disease and AIDS is the eventual outcome for infected persons. This can be seen in the Glasgow and West of Scotland adult haemophiliacs studied here; 50% have now been classified with HIV-disease, a further 22.7% are on prophylactic drug therapies because of a falling T4 count. However the incubation time to disease is longer than first thought. Gloomy predictions in the

early days of the epidemic suggested not only rapid progression to AIDS, within 3 or 4 years of being antibody positive, but also a shorter lifespan, i,e. death within a year after diagnosis of AIDS. Follow-up of the adult haemophiliac population in the West of Scotland showed a minimum progression time to ARC or AIDS of 4 to 5 years in the older patients with up to 10 years in the other patients. The dates of infection of all but a few members of the Ruchill cohort are unknown and thus no assessment of time to progression of disease can be made in these patients, i.e. in the homosexual and drug abusing populations in Glasgow and the West of Scotland.

Evidence of progression was examined by detection of a change in markers which were thought to be predictive of disease progression. Two virological markers, HIV-1 Ag and HIV-1 anti-core and an immunological marker, β_2-microglobulin were examined. In the group of clinical progressors, changes in one or both viral markers was noted in a similar percentage of members in the Ruchill cohort (62.5%) and the haemophilia cohort (54.5%) although the actual numbers are small. These preceded the diagnosis of clinical change in all but one haemophilia patient. No consistency between β_2-mg levels and disease progression was found. No patient in the Ruchill cohort and only two haemophilia patients demonstrated a change in all three markers prior to clinical progression. The most common changes observed were the virological markers particularly loss of anti-p24.

No definitive profile of time to change in markers and progression of disease could be established. In both the haemophiliac adults and the Ruchill cohort no one or combination of markers was found to be always reliably predictive in determining who progressed or the time to disease progression. Although an association of loss of Ab, production of Ag, increase in β_2-mg level with clinical progression was noted, the changes observed merely reflected the immunopathogenesis of HIV-1 and the ongoing disease process. That individual patients responded in individual ways became the common theme and therefore only general trends could be established.

In practical terms, astute physicians and nursing staff who know the patients as individuals are more likely to be aware of a change

before any laboratory parameter indicates progression. The measurement of the markers described here gives extra information to the physicians rather than being predictive on their own. The future remit of Ag and β_2-mg testing is more likely to be in monitoring the effects of anti-viral therapy as a decrease in Ag and β_2-mg levels is observed in patients on AZT treatment. Monitoring of β_2-mg levels will be more successful in this respect because not all patients develop detectable serum Ag through the course of disease.

It is still a matter of debate as to which markers best predict disease onset; different markers are found to be more or less useful in the cohort studies reported in the literature but β_2-microglobulin measurement emerges as one of the best predictors (235,241). No outstanding marker was significant in our two patient cohorts. No one marker is probably sufficient on its own to predict rates of progression and Moss and Bacchetti describe an algorithm based on five variables to estimate the probability of progression (154). Trying to assess when a significant change is about to occur in such a dynamic system as the immune system especially one supporting a chronic viral infection such as HIV-1 is very difficult. Spikes of HIV-1 antigen and increases in immune markers occur in response to other stimuli, e.g. bacterial infections. The molecular switch in HIV-1 which increases the transcription and translation of the structural genes is the rev protein (the regulator of expression of virion proteins). However this involves a complex interaction involving other proteins and sophisticated feedback control loops, a discussion of which is beyond the scope of this thesis. Perhaps a threshold level of structural protein synthesis and subsequent viral release is required before any cytopathic events occur and therefore an intracellular marker of viral activation, possibly at the molecular level, may be a more specific indicator.

More information could still be extracted from our infected population in the West of Scotland. There is an advantage in the epidemic here being several years behind other populations and the hope of prolonged survival for such patients is greater as advances in treatment etc. are made. Data on T4 cell counts and other immunological parameters was also collected in both the Ruchill cohort

and the haemophiliac cohort and a start will be made to assess the role of these markers on their own and in association with the markers measured here. Most members of the Ruchill cohort were recruited as they attended the ID clinic following a seropositive result. This is not ideal and in the future a better defined cohort with clear criteria for entry is required to establish a routine sampling and screening programme for markers.

Factors promoting disease progression are many and varied involving intrinsic qualities of the virus, e.g. cytopathicity of the infecting strain and the host response to it, e.g. neutralising antibodies, integral features of the host, e.g. age, HLA phenotype etc. and behaviour patterns of the host, e.g. continuation of risk activity. All these qualities will govern the individuality of response. Some of these factors can be measured to determine the likelihood of progression but no alterations can be made to affect disease outcome. The most marked feature in the haemophilia population studied here was the association of older age at seroconversion with disease progression. Why this is so remains unclear but probably reflects some aspect of the immune system. Other factors, e.g. behaviour, can be modified and the responsibility for such rests with the individual.

A slower rate to initial progression was noted in the adult haemophilia cohort compared to the members of the Ruchill cohort in whom an estimated time of seroconversion was known. This may reflect repeated antigenic stimulation of the host with HIV-1 or other microbial agents in those drug abusers and homosexuals who continue their high risk activities which may bring them into contact with such agents. In contrast the haemophiliacs have not been re-exposed to HIV-1, after the introduction of heat-treated factor products, thus preserving a more intact immune system for longer.

Is it only a matter of time before all those infected with HIV-1 develop AIDS? This is difficult to assess as there are members of both cohorts who remain asymptomatic, but how long this will be so is unknown. A study of non-progressors may be useful to examine what features of their immune response to the virus are protective and what clues could be useful in designing treatment. Lifson et al. (269)

examined immunological characteristics in such a group of patients and found a raised T8 count may help to control viral replication and delay progression to AIDS. Further studies have shown a lack of enhancing antibodies for HIV in non-progressors. In addition, and what may be the most important feature of all is the nature of the infecting strain of virus. A less virulent strain may be associated with lack of disease progression. If this is so, could such a state be induced in persons infected with a highly virulent strain?

Much work remains to be done for greater understanding of how the pathogenic effects of the virus are achieved, how the virus maintains latency, the nature of the immune response to the virus etc. If HIV-1 is truly the cause of AIDS, a view challenged by Dr. P. Duesberg (270), then the bounty of knowledge already available about the molecular mechanisms of the virus will provide the necessary information for design and manufacture of anti-virals and vaccines.

Ten years on and man continues to pass away. In the West of Scotland, AIDS has occurred predominantly in the homosexual population (43%). However signs and symptoms of HIV-1 disease progression are now beginning to appear in the drug abusers. HIV-1 infection is still spreading in the population albeit at a slower rate. Although it is encouraging that certain indicators are changing, i.e. longer to progression of disease and those with AIDS are living longer the only hope for the HIV-1 infected persons (HIPs) and the people with AIDS (PWAs) rests with the development of suitable, effective anti-viral therapy and vaccine. Phase 1 trials of vaccine made from recombinant envelope proteins are currently underway but it is beyond the scope of this thesis to discuss vaccine design and efficacy trials.

This discussion has focused on HIV-1 and AIDS since no members of the population in Glasgow and the West of Scotland have been found seropositive for either HTLV-I, HTLV-II or HIV-2.

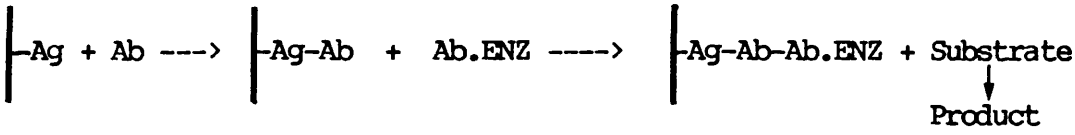
AIDS has rocked the world like no other modern epidemic. AIDS and HIV-1 infection have implications for the whole of society. A massive global input of resources and scientific research continues and slowly but surely advances are being made. It is interesting to speculate that in the future a symbiotic relationship may develop between human immunodeficiency virus and man, such that in generations

to come HIV-1 may become non-pathogenic in its host, however carriers may remain in the population and continue to contribute to spread of infection albeit at a much lower rate.

APPENDIX 1.

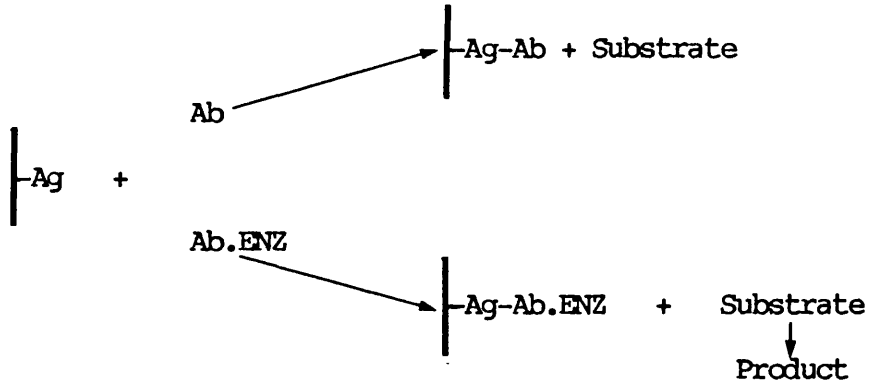
Design of ELISA tests (Mortimer et al. (108))

TYPE I: INDIRECT ASSAY



Virus-specific antibodies in a patient's serum bind to viral antigens coated onto a solid phase (e.g. polystyrene bead or microtitre well). Following a wash step a second species antibody (usually goat or rabbit) conjugated to an enzyme is added. This recognises and binds to the Fc portion of human immunoglobulin G (IgG). Any bound conjugated antibody will be detected by a colour change on the addition of substrate and this is directly proportional to the amount of antibody in the test serum. The results are read by eye or in a spectrophotometer.

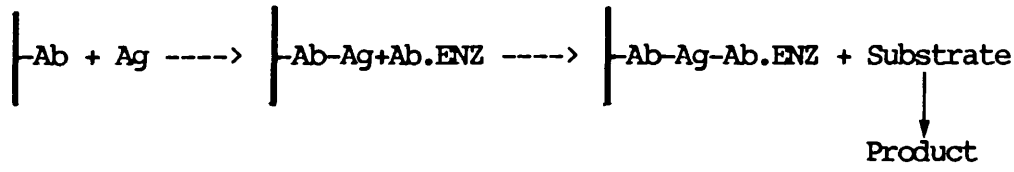
TYPE II: COMPETITIVE ASSAY



Both the test specimen and human anti-HIV-enzyme conjugate compete for binding sites on the antigen-coated solid phase. Virus-specific antibodies present in the test serum will prevent binding of the antibody conjugate and no colour change will form on the addition of substrate.

TYPE IV: SANDWICH ASSAY

Modified for the Detection of Antigen



Viral antigen in the test serum forms a bridge between immobilised antibody on the solid phase and the antibody conjugated to enzyme. A colour change is produced on the addition of substrate if antigen is present in the test serum.

Modifications (Type I and Type IV)

Modifications to enhance detection include using a detector antibody which is labelled with a biotin moiety. Enzyme is conjugated to avidin (or streptavidin) which has a high affinity for biotin. In this way a two-step detection system can be used.

APPENDIX 2

Retrovirus antibody screening and confirmatory tests, antigen tests and supplementary tests.

NAME	TYPE	SOLID PHASE	CONJUGATE	DILUTION
HIV-Screening				
Abbott Laboratories				
1st Generation:				
Abbott HTLV-III EIA	I	Viral Lysate H9/HTLV-III$_B$ Cell Line	Anti-Human IgG (Goat)-HRP*	1:400
2nd Generation:				
Abbott Recombinant HIV-1 EIA	I	rDNA# (E.coli) HIV-1 core and env antigens	Anti-Human IgG (Goat)-HRP	1:40
Abbott Recombinant HIV-1/HIV-2 EIA	I	rDNA (E.coli) HIV-1 core and env plus HIV-2 env antigens	Anti-Human IgG (Goat)-HRP	1:40
Wellcome Diagnostics				
1st Generation:				
Wellcozyme Anti-HTLV III EIA	II	Viral Lysate captured onto anti-HIV 1 coated wells	Anti-HIV (Human)-HRP	Neat
2nd Generation:				
Wellcozyme HIV Monoclonal EIA	II	Viral Lysate (CEM Cell Line) captured onto mouse monoclonal anti-HIV	Anti-HIV (Human) - HRP	Neat
DuPont:				
Dipstick Assay	I	Unknown	Unknown	1:40
Diagnostics Pasteur **(anti-HIV 2)**				
ELAVIA Ac-Ab-Ak II	I	Viral Lysate Dual Well: Viral Ag coated - cellular and serum Ag coated	Anti-Human IgG (Goat)-HRP	1:100

NAME	TYPE	SOLID PHASE	CONJUGATE	DILUTION
HTLV SCREENING				
Abbott Laboratories				
Abbott HTLV-I EIA	I	Viral Lysate HUT-102-2B cell line	Anti-Human IgG (Goat)-HRP	1:40
HIV-1 CONFIRMATION				
Abbott Laboratories				
Abbott ENVACOR HIV-1 EIA (formerly the Abbott Confirmatory EIA)	II	Dual Bead 1.rDNA#(E.coli) env antigen	Env Anti-HIV (Human)-HRP*	Neat
		2.rDNA (E.coli) core antigens	Core Anti-HIV (Human)-HRP	Neat
DuPont				
Western Blot Kit for the Detection of IgG Antibodies to HIV-1	I	Viral Lysate H9/HTLV-III_B	1.Biotinylated Anti-Human IgG (Goat) 2.Avidin-HRP	1:100
HIV-1 ANTIGEN DETECTION				
DuPont NEN				
HIV p24 core Antigen ELISA	IV	Anti-HIV (Rabbit)	1.Biotinylated Anti-HIV p24 (Human) 2.Streptavidin-HRP	Neat
Abbott Laboratories				
Abbott HIV-1 Antigen EIA	IV	Anti-HIV (Human)	1.Anti-HIV (Rabbit) 2.Anti-Rabbit IgG (Goat)-HRP	Neat

NAME	TYPE	SOLID PHASE	CONJUGATE	DILUTION
SUPPLEMENTARY TESTS				
Abbott HIV-1 Anti-Core EIA	II	rDNA (E.coli) core antigens	Core Anti-HIV (Human)-HRP	Neat
Abbott β_2-microglobulin RIA	II	Mouse monoclonal anti-β_2-microglobulin	^{125}I-labelled β_2-microglobulin	1:10

* HRP: horseradish peroxidase
\# rDNA: recombinant DNA

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