THE ROLE OF DIETARY ANTIGENS IN THE PATHOGENESIS OF EXPERIMENTAL GLOMERULONEPHRITIS

Michael John Browning, B.Sc., B.M., B.Ch.

University of Glasgow,
Department of Bacteriology and Immunology,
Western Infirmary,
Glasgow, G11 6NT.

A Thesis submitted for the Degree of Doctor of Philosophy of the University of Glasgow.

February, 1987.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

DECLARATION

Parts of this thesis have been used in the following publication:

Browning MJ & Parrott DMV. Protection from chronic immune complex nephritis by single doses of antigen administered by the intragastric route. In press in "Recent Developments in Mucosal Immunology".

ACKNOWLEDGEMENTS

I am grateful to Professor Delphine Parrott for her support throughout the project, and for her helpful criticism during the preparation of this thesis.

Experiments on the genetic basis of oral tolerance induction were performed in collaboration with Dr Allan Mowat. Electron microscopy was carried out by Tom Downie and Jane Hair, and the results were interpreted by Dr Ian More (Department of Pathology).

I am grateful to Dr Madeleine Devey and Professor M Steward, of the London School of Hygeine and Tropical Medicine, for the gift of TO mice.

Thanks also to Mr Fraser Darling and the staff of the Research and Diagnostic Facility, for the breeding and care of experimental animals; to Hector Cairns and Margaret Hardie, for the preparation of tissue sections for light microscopy; to David McCombe, for excellent developing and printing of sometimes less than excellent photographic films; to Ronnie Muirhead (Department of Pathology), for allowing me to use the cryostat; to Eric Campbell (Department of Surgery), for his assistance with urea and creatinine assays; and to Dr Tony Speekenbrink, for advice on statistics, and for assistance with computers.

Particular thanks to Eric Galloway and to Charlie McSharry, for their help and advice throughout the project, and to David Sinclair for taking on the thankless task of proof reading the thesis.

The project was carried out under the tenure of a

Fellowship from the Wellcome Trust, whose support is gratefully acknowledged.

Finally, I should like to thank my parents for their continued support throughout the project. Their friendship, encouragement and not infrequent hot meals have helped me considerably over the past three years, and it is to them that I dedicate this thesis, with my love.

SUMMARY

In this thesis I have investigated the role of dietary antigens in the pathogenesis of experimental glomerulonephritis in mice. I have identified a role of dietary antigens in two forms of glomerulonephritis. Firstly, prolonged oral administration of protein antigen was associated with the induction of glomerular IgA deposition. Secondly, intragastric administration of protein antigen was shown to protect against the subsequent induction of immune complex glomerulonephritis by repeated injections of the same antigen.

Prolonged oral administration of bovine gamma globulin (0.1%) in the drinking water of BALB/c mice was associated with the appearance of glomerular deposits of IgA in the kidneys of antigen fed mice. This association was not seen when ovalbumin was administered to the same strain of mouse in the drinking water (0.05%) or by weekly intragastric intubation to the same total dose. Prolonged oral administration of ovalbumin (0.1% in the drinking water) or of sheep erythrocytes (10^9 cells per day by intragastric intubation) was not associated with any increase in glomerular deposition of IgA in C3H/HeJ or C3H/HeOla mice. The presence of antigen deposition in the kidneys was not detected in antigen fed mice in any of the above experiments.

The role of hepatic sequestration of IgA-immune complexes in glomerular IgA deposition was investigated by the induction of experimental liver damage in mice. The administration of carbon tetrachloride to mice by weekly

intragastric intubation was associated with hepatocellular damage and the induction of chronic hepatic fibrosis. There was, however, no increase in glomerular IgA deposits in mice with experimentally induced liver damage as compared with controls.

Repeated injections of foreign protein antigen induced an immune complex glomerulonephritis characterised by predominantly mesangial deposition of IgG, C3 and the immunising antigen in TO [low], TO [high] and BALB/c mice. Electron microscopy showed mesangial expansion, with large numbers of electron dense deposits in the mesangium and subendothelial space.

Intragastric administration of single doses of protein antigen prior to the induction of glomerulonephritis in these strains of mice was associated with a reduction in the incidence and degree of glomerular immune complex deposition. The reduced incidence of immune complex glomerulonephritis was associated in antigen fed animals of all three strains with reductions in the antibody responses to the immunising antigen, and the incidence and degree of glomerular immune complex deposition correlated with the antibody titres.

This protection from antigen induced immune complex glomerulonephritis was shown to be specific for the immunising antigen. Further investigation of the specificity of the response using hapten-carrier conjugates indicated that the protective effects of antigen feeding were induced at the level of the carrier protein. The degree of protection from immune complex

glomerulonephritis conferred by single intragastric doses of antigen was dependent on the dose of antigen administered. Protection from antigen induced immune complex glomerulonephritis was not transferred by serum collected from donor mice 1 hour after administration of single intragastric doses of antigen. Protection from immune complex glomerulonephritis by antigen feeding was resistant to pretreatment with cyclophosphamide, and was not transferred by spleen cells from syngeneic donor mice rendered tolerant to the immunising antigen by prior intragastric administration of antigen. These results suggested that suppressor T cells did not play a major role in this effect.

The model of protection from antigen induced immune complex glomerulonephritis appeared to be related to the phenomenon of oral tolerance induction, which showed a similar dose-dependent relationship with regard to systemic delayed type hypersensitivity and antibody responses. Studies on the induction of oral tolerance in 11 inbred strains of mice showed marked genetic variations in the ease of oral tolerance induction. The roles of both H-2 and non H-2 genes were implicated in the induction of oral tolerance. Disparate effects of single intragastric doses of antigen administration on the cell mediated and humoral limbs of the immune response were seen in certain strains of mice, suggesting that oral tolerance induction for delayed type hypersensitivity and for antibody responses were under the control of separate mechanisms.

Thus a direct role of dietary antigen has been identified in the induction of glomerular IgA deposits,

suggesting a possible role of dietary antigens in the pathogenesis of IgA nephropathy. In addition, antigen ingestion may protect against the induction, by systemic exposure to the same antigen, of immune complex glomerulonephritis. This latter effect appeared to be mediated by an oral tolerance mechanism, acting on the humoral limb of the immune response. The exact nature of this mechanism, however, remains obscure.

ABBREVIATIONS

Abbreviations used in the text:

B cell	: bone marrow derived lymphocyte
BGG	: bovine gamma globulin
BSA	: bovine serum albumin
C3	: third component of complement
CII	: type II collagen
CCl4	: carbon tetrachloride
CFA	: complete Freund's adjuvant
CIC	: circulating immune complex
CR1	: C3b (immune adherence) receptor
DEA	: diethanolamine
DNFB	: 2,4-dinitrofluorobenzene
DNP	: dinitrophenyl
DTH	: delayed type hypersensitivity
EDD	: electron dense deposit
ELISA	: enzyme linked immunosorbent assay
EM	: electron microscopy
FIA	: Freund's incomplete adjuvant
FITC	: fluorescein isothiocyanate
GALT	: gut associated lymphoid tissue
H-2	: murine major histocompatibility complex
H & E	: haematoxylin and eosin
HEV	: high endothelial venule
HSA	: human serum albumin
HSP	: Henoch-Schonlein purpura
IgA	: immunoglobulin A
IgG	: immunoglobulin G
IgM	: immunoglobulin M
IgA-IC	: IgA-containing immune complex
^{125}I-ova	: ^{125}iodine-labelled ovalbumin
i.m.	: intramuscular
i.p.	: intraperitoneal
LPS	: lipopolysaccharide (bacterial)
mAGG	: heat aggregated mouse IgG
M315	: paraprotein of murine plasmacytoma MOPC 315
MPS	: mononuclear phagocytic system
NK	: natural killer
nms	: normal mouse serum
nrs	: normal rabbit serum
nss	: normal sheep serum
ova	: ovalbumin
PAS	: periodic acid-Schiff
PBS	: phosphate buffered saline
PFC	: plaque forming cell
poly IgA	: polymeric IgA
RES	: reticuloendothelial system
RF	: rheumatoid factor
sIgA	: secretory IgA
SRBC	: sheep red blood cell
T cell	: thymus dependent lymphocyte
UV	: ultraviolet
VBS	: veronal buffered saline

Abbreviations of measures:

weight : g = gram
mg = milligram
µg = microgram
ng = nanogram

volume : l = litre
ml = millilitre
µl = microlitre

length : mm = millimetre
nm = nanometre

molecular weight : kD = kilo Dalton

CHAPTER ONE

INTRODUCTION

1.1 GENERAL INTRODUCTION

Most forms of glomerulonephritis are believed to result from antibody deposition in the form of antigen-antibody complexes within the renal glomerulus activating secondary mediators of tissue injury (Couser & Salant, 1980). Two major mechanisms of antibody mediated tissue damage have been identified : lesions induced by antibody binding directly to constituent glomerular components (type II hypersensitivity reactions by the Coombs and Gell (1963) classification), and lesions induced by the glomerular localisation of antigen-antibody complexes involving antigens unrelated to glomerular structural components (type III hypersensitivity reactions). In the latter case, preformed antigen-antibody complexes may be deposited within the glomerulus, or complexes may form *in situ* within the glomerulus by the interaction of antibody with "planted" antigens. Although a number of extrinsic and constitutional antigens have been implicated in the pathogenesis of various forms of glomerulonephritis (summarised in Table 1), the nature of the antigen involved in most cases of human glomerulonephritis remains obscure.

Whilst dietary antigens have been implicated in a number of cases of glomerulonephritis, relatively little is known about the role of dietary antigens in the pathogenesis of glomerulonephritis.

The aim of this thesis was to extend our knowledge of the role of dietary antigens in the pathogenesis of glomerulonephritis by examining the effects of orally

TABLE 1.

ANTIGEN	DISEASE ASSOCIATION
Exogenous Antigens	
viral antigens:	
hepatitis B; measles; vaccinia.	hepatitis; measles; vaccinia in man.
lymphocytic choriomeningitis; Aleutian mink disease	associated diseases in animals
bacterial antigens:	
Group A Streptococci	post Streptococcal gn
T.Pallidum	syphilis
M.Leprae	leprosy
parasitic antigens:	
P.falciparum; P.malariae	nephrotic syndrome in malaria
Schistosoma sp	schistosomiasis
Trypanosoma sp	trypanosomiasis
microfilariae	onchocerciasis
foreign protein	serum sickness
drugs e.g. gold; penicillamine	membranous gn
Endogenous Antigens	
nuclear antigens	systemic lupus erythematosus
immunoglobulins: rheumatoid factors; anti-idiotypic antibodies	cryoglobulinaemia;
tissue/cellular antigens	membranous gn in malignancy; Goodpasture's disease Heymann nephritis
thyroglobulin	thyroiditis-nephritis

Table 1 : Antigens which have been implicated in antibody-mediated glomerulonephritis.

abbreviation : gn = glomerulonephritis

administered antigens on the induction and course of experimental glomerulonephritis in mice.

1.2 HISTORICAL PERSPECTIVE

The clinical significance of renal disease has been recognised for over 2400 years (Nicias, 415 BC). It was not until the work of Bright in the 19th century, however, that detailed accounts of the anatomy, histology and clinical symptoms associated with nephritis were described.

The association between certain infections or injections of foreign proteins and the development of glomerulonephritis was initially ascribed to toxic properties of the proteins or infectious agents. The first convincing evidence for the involvement of immune mechanisms in renal injury came from the work of Lindemann (1900), in which he injected rabbit kidney homogenates into guinea pigs. Passive transfer of serum from the immunised guinea pigs back into rabbits led to the induction of proteinuria, uraemia and death. Lindemann suggested that the "nephrolytic" serum from the guinea pigs contained (undefined) specific substances which were induced in the guinea pigs' serum by the injections of rabbit kidney. In thus describing his "nephrolytic serum", Lindemann described three of the main characteristics of antibody i.e. specificity, inducibility and presence in serum.

Contemporary with Lindemann, the association of heterologous serum therapy with a syndrome of urticarial rash, joint pain, fever, lymphadenopathy and,

occasionally, proteinuria was described in humans (Francioni, 1904), and termed "serum sickness". In their classical review of serum sickness, von Pirquet and Schick (1905) expressed the opinion that the symptoms of the disease were not induced by toxic properties of the heterologous serum proteins themselves, but by the interaction of antibody with the heterologous serum proteins giving rise to the formation of "toxic bodies". Thus the concept of immune complex mediated disease was formulated.

The focussing on the renal pathology in serum sickness started with the experiments of Longcope (1913), in which he showed that animals injected repeatedly with foreign proteins developed proteinuria associated with interstitial and glomerular lesions. Longcope's initial interpretation of these findings assumed a direct toxic action of the proteins following repeated injections. In 1918, Longcope and Rackemann described the relation between circulating antibodies and serum disease. They concluded that the presence of circulating antibody was a result of the disease, and that it heralded recovery from serum sickness.

The role of antibody in the pathogenesis of glomerulonephritis was confirmed in the 1930's (Masugi, 1934) in experiments on nephrotoxic nephritis, mediated by the direct binding of (heterologous) antibody to components in the glomerular basement membrane (Mellors, Siegal & Pressman, 1955). Unanue and Dixon showed that the severity of the lesions induced depended on the quantity

of nephrotoxic antibody present in the serum (Unanue & Dixon, 1965), and demonstrated the importance of complement activation in the induction of the renal lesions (Unanue & Dixon, 1964).

In the 1950's and 1960's, the groups of Germuth and Dixon demonstrated the relationship between the appearance of immune complexes in the circulation and the development of glomerular lesions in animal models of serum sickness. Germuth (1953), studying acute, "single shot" serum sickness in rabbits, demonstrated that the induction of renal lesions following intravenous administration of foreign protein coincided with a phase of accelerated antigen elimination, and regressed with the appearance of free circulating antibody. From the temporal relationship of these events, he concluded that the tissue lesions resulted from antigen-antibody combination in the tissues. Dixon and colleagues developed a model of chronic serum sickness, in which rabbits were injected repeatedly with a foreign protein antigen. Using this model, the authors reported the induction of glomerular lesions in rabbits, which resembled the lesions seen in human glomerulonephritis (Dixon, Feldman & Vazquez, 1961). Chronic glomerulonephritis was seen in rabbits in which the serum level of antibody was insufficient to completely eliminate the injected antigen, suggesting the formation of soluble immune complexes in antigen excess, and their deposition in the kidneys (Dixon etal, 1961). These two experimental models suggested the relationship between the presence of circulating antigen-antibody complexes and the development of glomerular lesions, and have provided the

basis for subsequent studies on the role of immune complex deposition in the pathogenesis of experimental glomerulonephritis.

In 1959, Heymann and coworkers described a nephrotic syndrome in rats injected with homogenates of autologous kidney in complete Freund's adjuvant (Heymann etal, 1959). Histologically the disease was characterised by diffuse thickening of capillary walls with little or no cellular proliferation. Edgington, Glassock and Dixon (1967) defined the antigen (termed Fx1A) involved in this model of autologous allergic glomerulonephritis as a component of the luminal brush border of proximal tubular epithelial cells. Immunofluorescent examination of the glomerular lesions showed granular deposits of antigen, IgG and C_3 similar to those found in rabbits with serum sickness nephritis, and suggestive of glomerular deposition of circulating antigen-antibody complexes (Edgington, Glassock & Dixon, 1967 & 1968).

Using a carefully controlled model of passive Heymann nephritis in isolated, perfused rat kidneys, Couser etal (1978) demonstrated that the subepithelial deposits of Heymann nephritis could be formed *in situ* by direct binding of antibody to antigen within the capillary wall, and not as a result of deposition of circulating immune complexes. Thus in Heymann nephritis, circulating antibodies to the Fx1A antigen cross-reacted directly with discontinuously distributed glomerular components to produce granular subepithelial immune deposits in the absence of circulating immune complexes (CIC). The *in situ*

formation of immune complexes by antibody binding to constituent or "planted" antigens in the pathogenesis of glomerulonephritis characterised by subepithelial deposits is now widely accepted (Couser, 1986).

Thus three basic models for antibody mediated glomerular damage have been described (Table 2) : i) lesions produced by the deposition of immune complexes derived from the circulation, as in Dixon's model of chronic serum sickness, characterised by granular mesangial or capillary immunofluorescent staining patterns and a spectrum of histological types of glomerulonephritis; ii) lesions caused by the in situ formation of immune complexes, as in Heymann nephritis, involving either constitutive or planted antigens, and characterised by a membranous nephropathy, with granular immunofluorescent staining patterns and predominantly subepithelial electron dense deposits; and iii) Masugi (nephrotoxic) nephritis, mediated by antibodies to the glomerular basement membrane, and characterised by linear fluorescent staining along the glomerular basement membrane and a progressive glomerulonephritis.

Most forms of glomerulonephritis are mediated by antigen-antibody complex deposition or formation within the glomerulus. Mechanisms of antigen-antibody complex mediated tissue damage are integral therefore to the understanding of the immunopathogenesis of glomerulonephritis.

TABLE 2.

MECHANISM OF DAMAGE	DISEASE EXAMPLE
A. Immune complex deposition	
i) Circulating immune complexes	
exogenous antigens	serum sickness gn; gn associated with infections
endogenous antigens	SLE; nephritis of malignant disease
ii) *In situ* complex formation	
endogenous fixed antigens	Heymann nephritis
endogenous planted antigens	DNA in lupus gn
exogenous planted antigens	some membranous gn; Con A nephritis
B. Anti-glomerular basement membrane antibody	Goodpasture's syndrome; Masugi (nephrotoxic) nephritis

Table 2 : Models of antibody-mediated glomerulonephritis.

abbreviation : gn = glomerulonephritis

1.3 ANTIGEN-ANTIBODY COMPLEXES AND GLOMERULAR DAMAGE

1.3.1 Formation and clearance of antigen-antibody complexes in the immune response

The role of antigen-antibody complexes as part of the normal immune response has been known for many years. The elimination of foreign antigen from the system is a basic feature of the host immune response to antigenic challenge. The role of antibody in this process is well defined. Three phases are involved in antibody mediated elimination of antigen : i) antigen-antibody complex formation; ii) activation of complement; and iii) clearance of complexes by cells of the mononuclear phagocytic system.

The first phase of antigen elimination in the primed host involves the formation of antigen-antibody complexes. The interaction of an antibody combining site with an antigenic determinant is reversible, and the immune complexes formed remain in equilibrium with free antibody and free antigen in solution. This equilibrium is determined by the nett attractive force between the antibody combining site and the antigenic determinant - the antibody "affinity" for the antigen. Thus complexes formed between low affinity antibody and antigen are less stable than complexes formed with high affinity antibody. The strength of interactive force between antibody and antigen is further influenced by the valence of the antibody i.e. the number of combining sites expressed per immunoglobulin molecule. The "avidity" of the antibody for antigen is determined by both the antibody affinity and

the valence, and represents a composite description of the overall attractive force between antibody and its antigen.

The solubility of the antigen-antibody complexes formed is affected by a number of factors, including the relative ratio of antigen to antibody present, and the activation of complement (see below). At "optimum proportions" of antigen and antibody the complexes form stable lattice structures which tend to precipitate out of solution (Marrack, 1938). In situations of both antigen excess and antibody excess the degree of precipitation of immune complexes is less and the complexes formed tend to remain in solution. The formation of immune complexes in slight to moderate antigen excess has been associated with the induction of both acute and chronic tissue damage (Germuth & McKinnon, 1957; Dixon etal, 1961).

Once formed, antigen-antibody complexes become the target for a number of secondary humoral and cellular immune mechanisms (Nydegger, 1985), which may profoundly affect the properties and fate of the antigen-antibody complexes. Of these, activation of the complement system is the most important, both in terms of clearance of immune complexes and in their immunopathogenic effects. Immune complexes involving antibodies of the IgM class and IgG1, IgG2 and IgG3 subclasses activate complement via the classical pathway.

The activation of complement has a number of biological consequences, including the promotion of inflammatory reactions through the generation of anaphylatoxins and chemotaxins, enhancement of phagocytosis, and direct

tissue damage or cytolysis through activation of the late (membrane attack) components of complement activation.

Enhanced phagocytosis of antigen-antibody complexes by complement activation is mediated largely through the generation of C3b, and its binding to the immune complex. This acts in two ways in the clearance of the antigen antibody complex by the mononuclear phagocytic system (MPS). Firstly, binding of C3b to the immune adherence (CR1) receptor on neutrophils and macrophages directly promotes phagocytosis of the complexes by these cells (Mantovani, Rabinovitch & Nussenzweig, 1972; Newman & Johnston, 1979). Secondly, adherence of complexes to erythrocyte CR1 facilitates their clearance from the circulation by cells of the MPS (Siegel, Liu & Gleicher, 1981).

Thus the formation of antigen-antibody complexes as part of the normal immune response is an important element in the elimination of antigen from the system. It has been known for many years, however, that the interaction of antibody and antigen to form antigen-antibody complexes may also be involved in the pathogenesis of a variety of pathological conditions.

1.3.2 Immune complexes and disease

The association between antigen-antibody complexes and disease was first proposed in 1905 by von Pirquet and Schick, studying serum sickness in patients receiving injections of diphtheria antitoxin. These authors observed that the toxic symptoms of serum sickness coincided with the appearance of antibody to the foreign serum, and they

proposed that the interaction of antigen and antibody to form "toxic bodies" was responsible for the induction of the disease.

At the same time, Arthus (1903) described a localised vasculitis and necrosis which appeared within hours of the intradermal inoculation of horse serum in primed rabbits. The reaction was caused by the deposition of antigen-antibody complexes in the walls of the blood vessels, where they gave rise to local inflammation (Cochrane, 1971).

Interest in the role of immune complexes in disease was maintained by the demonstration of immune complex involvement in the pathogenesis of glomerulonephritis (reviewed below). With the advent in the 1970's of sensitive assay systems for measuring immune complexes, however, there came an explosion of interest in the role of antigen-antibody complexes in disease, and in the past decade immune complex mediated tissue damage has been implicated in a wide variety of clinical disorders. It is beyond the scope of this thesis to discuss this subject further, except in the context of the role of immune complexes in the pathogenesis of glomerulonephritis.

1.3.3 Immune complexes and glomerulonephritis

The pathogenicity of antigen-antibody complexes in glomerulonephritis was demonstrated by the work of the groups of Germuth and Dixon on the mechanism of glomerulonephritis associated with acute and chronic serum sickness. Using a model of chronic serum sickness, Dixon etal (1961) injected rabbits with different amounts of

protein antigen. In all animals in which the amount of antibody formed barely neutralised the antigen injected, some degree of chronic glomerulonephritis was seen. The authors concluded that the interaction of antibody with excess amounts of antigen in the circulation was related to the development of chronic glomerulonephritis (Dixon etal, 1961). Histologically the glomerular lesions resembled the spectrum of glomerulonephritis seen in humans. Immunofluorescent examination showed granular deposits of antigen and immunoglobulin along the glomerular basement membrane. The authors proposed that the deposits represented immune complexes which had become passively trapped within the glomerulus, where they gave rise to a local inflammatory reaction leading to nephritis (Dixon etal, 1961).

Since the description of this model of chronic serum sickness nephritis, the role of immune complexes in glomerular damage has been studied extensively, and a wide range of factors which influence the formation, clearance and tissue deposition of antigen-antibody complexes has been identified. Early experiments suggested the importance in the induction of glomerular lesions of the formation of soluble circulating immune complexes by the maintenance of moderate antigen excess (Dixon et al, 1961; Andres etal, 1963). The size of the immune complexes was shown to influence their glomerular deposition and localisation (Cochrane & Hawkins, 1968; Wilson & Dixon, 1971; Cochrane, 1971), and both antigen : antibody ratio and lattice structure were shown to contribute to this effect (Haakenstad, Striker & Mannik, 1976). More recently

Gallo, Caulin-Glaser and Lamm (1981) demonstrated charge related differences in glomerular localisation of immune complexes. Christian and colleagues reported that the development of glomerulonephritis in rabbits depended on the production of non-precipitating antibody (Pincus, Haberkern & Christian, 1968), and Kuriyama (1973) showed that decreased precipitating activity of antibody, and production of low avidity antibody occurred in rabbits developing membranous glomerulonephritis after prolonged immunisation. The role of antibody affinity in the induction of immune complex disease was proposed by Soothill & Steward (1971), and was shown to affect localisation and pathogenicity of immune complex deposition in antigen induced immune complex glomerulonephritis in mice selectively bred for the production of high and low antibody affinity (Devey & Steward, 1980). From this list it can be seen that a variety of properties of immune complexes may influence their deposition in the renal glomerulus.

In addition to these intrinsic properties of immune complexes, manipulations of reticuloendothelial activity have been shown to influence glomerular deposition of aggregated proteins and immune complexes (Ford, 1975), and local vasoactive and haemodynamic factors have been implicated in the glomerular localisation of immune complexes from the circulation (Kniker & Cochrane, 1968; Cochrane, 1971). Thus a number of factors have been identified which may influence the glomerular deposition and localisation of immune complexes in the induction of

immune complex mediated glomerulonephritis.

It was originally believed that all immune complex deposition arose from passive trapping of soluble complexes formed in the circulation, and the site of final deposition depended predominantly on the size of the immune complexes involved. It is now clear that this is not the case, and immune complex formation may occur *in situ* in the glomerulus, involving discontinuously distributed endogenous glomerular antigens or planted exogenous antigens (Couser and Salant, 1980). *In situ* immune complex formation is now accepted as playing a major role in the development of subepithelial immune deposits, and may contribute also to subendothelial and mesangial immune complex formation (Couser, 1986).

In addition to the original formation of glomerular immune deposits, a process of immune deposit accretion may occur, involving interaction of the immune deposits with further unbound antigen or antibody (Ford & Kosatka, 1981), with rheumatoid factors (Ford & Kosatka, 1983) or with anti-idiotypic antibody (Zanetti & Wilson, 1983). Thus, *in situ* immune deposit accretion may contribute to glomerular deposits formed originally by trapping of immune complexes from the circulation, as well as to deposits formed *in situ*.

There is little evidence that the formation or deposition of immune complexes in the glomerulus itself produces significant tissue damage. Rather, the tissue injury consequent to the development of glomerular immune deposits is mediated by activation of secondary humoral and cellular inflammatory mechanisms.

The major humoral mediator of antibody induced glomerular damage is the complement system (Unanue & Dixon, 1964). Activation of complement may mediate tissue damage in two ways. Firstly, the generation of chemotactic factors (C3a and C5a) for polymorphonuclear leucocytes may promote neutrophil infiltration, and thus complement dependent neutrophil mediated tissue damage (Cochrane, Unanue & Dixon, 1965). More recently, a direct role for complement induced glomerular injury, mediated by the C5b-9 membrane attack mechanism has been proposed (Salant etal, 1980). Subsequent studies by this group (Groggel etal, 1983), using a model of passive Heymann nephritis, showed that C6 deficient rabbits failed to develop proteinuria, whilst normal controls had markedly increased urinary protein excretion. These findings supported a role of the late components of complement activation in the induction of glomerular damage in glomerulonephritis characterised by subepithelial immune deposits and membranous glomerulopathy.

The role of macrophages in glomerular damage has been established only in the last decade (Cotran, 1978). Mononuclear phagocytic cell mediated glomerular damage was confirmed by Schreiner et al (1978), who demonstrated the prevention of proteinuria and glomerular lesions by irradiation-induced monocyte depletion in a model of nephrotoxic nephritis in rats. Similarly, Holdsworth, Neale and Wilson (1981) demonstrated abrogation of glomerular injury in rabbits by the use of anti-macrophage antiserum. The same author demonstrated that the

accumulation of macrophages in the glomerulus was dependent on the Fc portion of immunoglobulin, but was not dependent on complement (Holdsworth, 1983).

Glomerular damage may be enhanced by intraglomerular activation of the coagulation system. Pretreatment of animals with anticoagulants (Vassalli & McCluskey, 1971) or with defibrinating agents (Naish, Evans & Peters, 1975) has been shown to prevent the progression of lesions in some models of glomerulonephritis.

Thus the role of immune complexes in the induction of glomerulonephritis is a complex process, and is influenced by the mechanism of deposition and localisation of immune complexes within the glomerulus, and by the secondary mediators of tissue damage which are activated. These factors influence both the histological characteristics of the glomerular lesions and the clinical severity of the associated disease.

Whilst much has been learnt about the pathogenesis of glomerulonephritis from the study of these models, the antigens involved in the majority of cases of human glomerulonephritis remain obscure. The intestinal tract is a major source both of antigenic stimulation and of antigen entry, and provides a potential source of antigens which may be implicated in glomerulonephritis. The following two sections review the evidence for a possible role of dietary antigens in the pathogenesis of glomerulonephritis.

1.4 THE ROLE OF DIETARY ANTIGENS IN THE PATHOGENESIS OF GLOMERULONEPHRITIS

Dietary antigens have been implicated in a wide variety of systemic diseases in recent years. A role for the mucosal immune system in the pathogenesis of IgA nephropathy has been proposed (Woodroffe etal, 1980), and clinical and experimental evidence for such an association is reviewed in section 1.5. In addition, dietary antigens have been implicated in individual cases in a number of other forms of glomerulonephritis.

A clinical association between food sensitivity and childhood nephrosis has been described by Matsumura and Kuruome (1961) and by Sandberg etal (1977). Richards, Olson and Church (1977) reported the case of a young girl with childhood nephrosis which relapsed on ingestion of eggs.

An association between membranous nephropathy and food sensitivity has been reported recently by Sandberg (1987), who described 3 children with asthma, multiple food sensitivities and membranous glomerulonephritis in whom cows' milk ingestion was associated with increased urinary protein excretion.

Gaboardi etal (1983) described the case of a 6 year old girl with coeliac disease, dermatitis herpetiformis and minimal change nephrosis, in whom the nephrotic syndrome resolved after she was put on a gluten free diet. Similar associations between gluten sensitive enteropathy and glomerulonephritis have been described by a number of other authors (De Coteau, Gerrard & Cunningham, 1973;

Davies & Davies, 1979; Katz, Dyck & Bear, 1979)

In all of the cases mentioned, the involvement of dietary antigens in glomerulonephritis was associated with clinical food sensitivity. Van der Woude etal (1983) described a 50 year old man with IgA deficiency and focal glomerulonephritis. He had no clinical history of food sensitivity, but post-prandial circulating immune complex levels were markedly raised. Jejunal biopsy was normal. Conventional treatment for nephritis proved unsuccessful. An antigen free diet was initiated and was accompanied by an improvement in glomerular filtration rate. Challenge with a range of common dietary antigens was associated with relapse, suggesting that multiple food antigens might play a role in the pathogenesis of nephritis in this patient (van der Woude etal, 1983).

Such direct evidence for a role of dietary antigens in the pathogenesis of human glomerulonephritis is rare. Whilst an association between glomerulonephritis and an aberrant immune response to dietary antigen might pass undetected in the absence of clinical sensitivity to food antigens, it must be assumed that the paucity of reported cases of glomerulonephritis involving dietary antigens reflects the underlying fact that such an association is uncommon.

1.5 IGA NEPHROPATHY

1.5.1 Clinical disease

Since its description by Berger & Hinglais in 1968, a relationship between IgA nephropathy and the mucosal

immune system has been proposed.

The term "IgA nephropathy" covers three clinical disease entities. : idiopathic IgA nephropathy (Berger's disease), the nephropathy of Henoch-Schonlein (anaphylactoid) purpura, and IgA nephropathy associated with hepatic cirrhosis. The characteristic feature of all three conditions is the predominance of IgA containing immune deposits on immunofluorescent examination of renal biopsy material. Circumstantial evidence exists in all three diseases for a role of the mucosal immune response in the immunopathology of the disease.

Idiopathic IgA nephropathy is characterised by episodic frank haematuria, commonly associated with viral infections of the respiratory or gastrointestinal tracts. It affects predominantly young adult males, and represents a chronic, slowly progressive renal disease. Henoch-Schonlein purpura (HSP) mainly affects children, and involves episodic purpural rash, arthralgia and colicy abdominal pain, associated with glomerulonephritis in up to 30% of patients (Heng, 1985). Involvement of mucosal sites is virtually invariable (Bene & Faure, 1986). As with idiopathic IgA nephropathy, acute exacerbations coincide with infections of the upper respiratory or gastrointestinal tracts. HSP is thought by many to represent a systemic juvenile variant of idiopathic IgA nephropathy.

The association between liver cirrhosis and IgA nephropathy was first identified in post mortem examination of kidneys from cirrhotic patients (Callard etal, 1975). The glomerulonephritis associated with

cirrhosis is apparently benign, although microhaematuria and proteinuria may be present (Bene & Faure, 1986).

The diagnostic feature of IgA nephropathy is the predominance of IgA containing immune deposits, usually within the mesangium, in renal biopsy material. These deposits also contain C3 in the majority of cases and, to a lesser degree, IgG and IgM. At the light microscopic level the appearance varies from light negative nephropathy through mesangial expansion and proliferation to focal or diffuse sclerosis associated with epithelial crescent formation in a minority of cases. Electron microscopy shows electron dense deposits in the mesangium, with an increase in mesangial matrix but rarely with mesangial cell proliferation (Zimmerman & Burkholder, 1975; Nakamoto etal, 1978).

1.5.2 Immunological abnormalities in IgA nephropathy

A variety of immunological abnormalities have been detected in patients with IgA nephropathy. Serum levels of IgA are commonly elevated as compared with normal individuals (Zimmerman & Burkholder, 1975; Finlayson etal, 1975; Woodroffe etal, 1980), and abnormal levels of circulating polymeric IgA (Lesavre, Digeon & Bach, 1982; Valentijn etal, 1983) and of IgA containing immune complexes (Coppo etal, 1982; Sancho etal, 1983) have been reported. Clearance of IgA containing immune complexes (IgA-IC) following food ingestion was delayed in patients with IgA nephropathy (Sancho etal 1983), which the authors suggested might be associated with impaired hepatic clearance of IgA-IC.

Analysis of peripheral blood mononuclear cells has shown increased numbers of T-α cells (Adachi etal, 1983). Egido etal (1982) reported elevated levels of IgA secretion following *in vitro* stimulation of peripheral blood B cells from patients with IgA nephropathy, and Sakai, Nomoto and Arimori (1979), reported deficient IgA specific suppressor T cell activity of peripheral blood mononuclear cells *in vitro* in patients with IgA nephropathy as compared with patients with other forms of renal disease and with healthy controls.

Several possible mechanisms, therefore, might play a role in the pathogenesis of IgA nephropathy, including abnormal synthesis of IgA, defects in the regulation of IgA production and defects in the clearance of IgA-IC from the circulation. A number of observations have suggested a mucosal origin for glomerular IgA deposits in IgA nephropathy, and these are reviewed below.

1.5.3 Association between IgA nephropathy and the mucosal immune response

Two clinical observations suggest an association between IgA nephropathy and the mucosal immune response. The first is the predominance of IgA, the major immunoglobulin isotype of mucosal immune responses, in glomerular deposits. The second is the association in IgA nephropathy between acute clinical exacerbations of nephropathy and infections at mucosal sites.

In support of a mucosal origin for IgA in immune deposits in IgA nephropathy, Andre etal (1980) reported a predominance of IgA2 in glomerular deposits in all types

of IgA nephropathy. This observation, however, has not been confirmed by other reports (Conley, Cooper & Michael, 1980; Tomino etal, 1981).

Antibodies to common dietary antigens have been identified in the serum (Woodroffe etal, 1980), in immune complexes (Sancho etal, 1983), and in renal biopsy eluates (Galla etal, 1985) of patients with IgA nephropathy. Delayed clearance of IgA-IC after food ingestion has been noted in a proportion of patients with IgA nephropathy (Sancho etal, 1983), and Coppo etal (1986) have shown a reduction in levels of IgA-IC in patients with IgA nephropathy (with normal jejunal histology) put on a gluten free diet. Thus a number of immune abnormalities involving dietary antigens have been identified in IgA nephropathy. Whether these abnormalities represent the primary defect or are simply secondary manifestations of an underlying immune abnormality, however, remains uncertain.

The association of IgA nephropathy and hepatic cirrhosis has given rise to the hypothesis that IgA nephropathy may occur as a result of deficient hepatobiliary clearance of IgA-IC containing antigens absorbed at mucosal sites, leading to deposition of such complexes within the glomerulus (Sancho etal, 1983). The role of hepatobiliary clearance of IgA-IC has been well demonstrated in rodents (Lemaitre-Coelho, Jackson & Vaerman, 1978; Orlans etal, 1978; Fisher etal, 1979). The evidence for a significant role of hepatobiliary clearance of IgA-IC in humans is less well established. Challacombe,

Greenhall & Stoker (1987), however, have reported increased levels of secretory IgA and IgA-IC in portal blood as compared with matched peripheral blood samples in patients undergoing abdominal surgery. The results suggest some role of the liver in the clearance of IgA and IgA-IC from the portal venous system in man.

Animal models of IgA nephropathy have given insights into the renal deposition of IgA-IC, and have suggested a direct association between dietary antigen, the mucosal immune system and the induction of IgA nephropathy.

1.5.4 Animal models of IgA nephropathy

The first animal model of IgA nephropathy was described by Rifai etal (1979). In this model, IgA-IC were formed *in vitro* using the BALB/c IgA secreting plasmacytoma MOPC 315 paraprotein (M315) and dinitrophenylated proteins. Injection of preformed complexes in mice led to a mild focal glomerulonephritis with mesangial deposits in all glomeruli. Similar results were obtained when dinitrophenylated proteins were inoculated into mice bearing the MOPC 315 tumour. The presence of polymeric IgA was found to be critical for renal deposition of complexes. Subsequent studies by the same author (Rifai & Millard, 1985) confirmed that only IgA-IC formed with polymeric IgA were deposited in the kidney. By covalently crosslinking monomeric IgA with a specific affinity labelling antigen, however, the same authors demonstrated that the critical factor in IgA-IC deposition in the glomerulus was the size of the complex, and that large latticed, cross linked monomeric IgA-IC were capable of

depositing in the kidney with the same immunofluorescent pattern as polymeric IgA-IC (Rifai & Millard, 1985).

The first actively induced animal model of IgA nephropathy was described by Isaacs, Miller & Lane (1981), in which mice were injected repeatedly with neutral or sulphated dextrans of different molecular weights over a period of 10 weeks. Immunofluorescent examination of the kidneys showed bright granular deposition of IgA in the glomeruli of all animals, associated with IgM in the majority of cases and C3 in 50% of experimental animals. Further studies by the same group (Isaacs & Miller, 1984) demonstrated that antigen charge and size were not important in the pathogenesis of IgA nephropathy in this model, nor in the induction of antidextran IgA responses. The authors did not, however, investigate the role or character of circulating IgA-IC in this model.

Both the above models used parenteral routes of immunisation in the induction of experimental nephropathy. In 1983 Emancipator, Gallo and Lamm described a model of experimental IgA nephropathy in which mice were fed protein antigens in their drinking water (Emancipator, Gallo & Lamm, 1983 a + b). Prolonged oral antigen administration was associated with a specific IgA response in the mice, with the subsequent codeposition of polymeric IgA and the immunising antigen in the glomerular mesangium. Similar observations were described by Genin and colleagues (1984, 1986) using a model of oral immunisation in C3H/HeJ mice. These authors, however, failed to detect circulating antigen specific IgA in the serum, or the presence of the antigen in glomerular

deposits in orally immunised animals as compared with controls. From these observations they proposed a model of antigen non-specific but isotype specific stimulation of IgA by oral immunisation which, possibly associated with defective clearance of IgA in these mice, resulted in renal deposition of IgA.

Sato, Ideura and Koshikawa (1986) demonstrated mesangial deposits of IgA in the kidneys of mice in which prolonged oral administration of ∝-lactalbumin was combined with blockade of the reticuloendothelial system (RES) by daily injections of colloidal carbon. The immunising antigen, however, was not found in the kidney of any animal. Mesangial deposits of IgA were not found in mice receiving either oral antigen alone, or reticuloendothelial blockade alone.

Thus an association between oral immunisation and the development of glomerular IgA deposits has been described in experimental animals.

The associaton between renal glomerular lesions and experimental liver damage induced by carbon tetrachloride or ethionine in rats was described by Sakaguchi etal (1964). In 1981, Gormly demonstrated the presence of glomerular IgA deposits in rats rendered cirrhotic by the administration of carbon tetrachloride (Gormly etal, 1981). The renal lesions produced were similar to those seen in cirrhosis-associated IgA nephropathy in humans, and this model would appear to provide an experimental corollary of this disease.

1.5.6 Pathogenesis of IgA nephropathy

The pathogenesis of IgA nephropathy is still unclear. An association between glomerular IgA deposits and the mucosal immune system has been proposed, and is supported by experimental evidence of glomerular IgA deposits induced by oral immunisation. An association between hepatic impairment and IgA nephropathy has been described in both man and experimental animals, and suggests that defective sequestration of polymeric IgA and IgA-IC by the damaged liver may be associated with the development of glomerular IgA deposits.

In addition to these mechanisms, Lowance, Mullins and McPhaul (1973) described an IgA-rich eluate from the kidney of a patient with IgA nephropathy which fixed to homologous renal mesangium, suggesting a possible role of IgA anti-mesangial antibodies in the pathogenesis of some cases of IgA nephropathy. More recently, Sinico etal (1986) described the presence of raised levels of IgA rheumatoid factors (RF) in the serum of patients with idiopathic IgA nephropathy, raising the possibility of a pathogenic role of IgA RF in IgA nephropathy.

In summary, the term "IgA nephropathy" is used to describe a variety of clinical entities which share the common feature of mesangial immune deposits in which IgA is the sole or predominant immunoglobulin isotype. The renal lesion varies both in its histological appearance and in its clinical severity. As the clinical condition represents a spectrum of disease, so a number of possible aetiopathological factors have been implicated. Several

pathogenic processes may be involved in the pathogenesis of IgA nephropathy and these may operate singly or in conjunction in the pathogenesis of any individual's disease. An association between IgA nephropathy and the mucosal immune response to dietary antigen has been reported, but remains to be established.

1.6 LOCAL AND SYSTEMIC EFFECTS OF ORAL ANTIGEN ADMINISTRATION

A role of the immune response to dietary antigen has thus been implicated in the pathogenesis of certain cases of glomerulonephritis. Both mucosal and systemic immune responses to dietary antigens may contribute to the disease process. It is important, therefore, in considering the role of dietary antigens in the pathogenesis of glomerulonephritis to define the local and systemic immunological effects of antigen ingestion. In the following section the immune response to ingested antigen is reviewed, with particular emphasis on those factors which may contribute to a role of dietary antigen in the pathogenesis of glomerulonephritis.

1.6.1 Normal structure and function of the gastrointestinal immune system

The gastrointestinal tract is continuously challenged by a huge antigenic load from a wide variety of sources, ranging from ingested protein antigens to potentially pathogenic micro-organisms and parasites. The extent and organisation of lymphoid tissue in the gastrointestinal tract reflects the extent and diversity of the antigenic

load borne by the gut, and involves both organised lymphoid tissues and isolated mucosal lymphoid cells.

The structure of the lymphoepithelial organs associated with the gastrointestinal tract (known collectively as the gut associated lymphoid tissues; GALT) has been reviewed recently by Parrott (1987). These structures include the tonsils, Peyer's patches, appendix, isolated lymphoid follicles and the mesenteric lymph nodes. The lymphoepithelial organs of the intestinal tract resemble other lymphoid organs in terms of internal structure. They differ from the lymphoid organs elsewhere in the body, however, in that they lack afferent lymphatics, in place of which they impinge directly on the intestinal lumen. Specialised epithelium on the luminal border of the intestinal lymphoid organs facilitates antigen sampling within the gut, which may be important in the induction of intestinal immune responses (Walker & Isselbacher, 1977).

In addition to the organised lymphoid structures of the intestinal tract, the small intestinal mucosa contains many lymphoid cells scattered throughout its length. Two major populations have been defined on the basis of their location within the mucosal lamina propria and the intestinal epithelium. The normal lamina propria is heavily infiltrated with a wide variety of lymphoid cells of both T and B cell lineages. The intestinal epithelial layer contains large numbers of intraepithelial lymphocytes (IEL) expressing predominantly T cell associated surface antigens. The functions of these cell populations is discussed in section 1.6.2.

Throughout the intestinal mucosa and GALT are found large numbers of non-specific effector and accessory cells including mast cells, natural killer (NK) cells, antigen presenting cells, macrophages, eosinophils and other polymorphonuclear leucocytes. Thus the gastrointestinal tract contains all the cellular components required for the induction and implementation of the whole spectrum of immune responses.

The origin of mucosal lymphocytes has been the subject of extensive investigation, and there is now considerable evidence that the Peyer's patches of the small intestine, and other organised lymphoid tissues of the intestinal tract, play a major role in the repopulation of intestinal mucosal lymphocytes, and thus the provision of local immune mechanisms within the intestinal tract (Craig & Cebra, 1971; Rose, Parrott & Bruce, 1976; Roux etal, 1981).

The selective migration of intestinal lymphocytes to mucosal sites, both local and distant, is clearly distinct from that of peripheral lymphocytes. Both T and B intestinal lymphocytes have the ability to traffic through mucosal lymphoid follicles (Rose etal, 1976; Phillips-Quagliata etal, 1983). Within the intestinal lymphoid follicles the lymphocytes leave the blood circulation by migrating through high endothelial venules (HEV), which contain vascular endothelium expressing GALT specific recognition factors (Gallatin, Weissman & Butcher, 1983). The mechanism of cell migration to mucosal sites is not understood. HEV are not found in mucosal tissues outside of the GALT. Autoradiographic studies (Mirski etal, 1981)

have shown that the majority of lymphoblasts emerge in the basal lamina propria. Whilst the mechanism of this migration is not known, lymphocyte migration to mucosal sites may be influenced by a number of factors including local blood flow (Ottaway & Parrott, 1980), local presence of antigen (Rose etal, 1976; Husband, 1982), and the presence of intestinal peptide hormones (Ottaway, 1984).

Thus the intestinal immune system involves a complex interaction of organised lymphoid tissues and scattered mucosal effector cells, which together provide an immunological barrier in addition to the epithelial barrier at the mucosal surface.

1.6.2 The local mucosal response to ingested antigens

The local immune response of the gastrointestinal tract involves both humoral and cellular immune mechanisms. The stimulation of antibody secretion within the intestinal tract in response to antigen challenge has been known for many years (Davies, 1922). It was not, however, until 1963 that Tomasi and Zigelbaum demonstrated that the major immunoglobulin isotype in external secretions was IgA (Tomasi & Zigelbaum, 1963). This secretory IgA (sIgA) differs from IgA in the serum in a number of respects. It is present in secretions predominantly as a dimer, containing an additional polypeptide "J" chain (Halpern & Koshland, 1970). The secreted dimeric IgA binds secretory component in the basal membrane of mucosal epithelial cells, and the complex IgA dimer-secretory component is then transported across the epithelial cell and released into the intestinal lumen (Brown, Isobe & Nakane, 1976;

Nagura, Nakane & Brown, 1979).

In addition to the direct secretion of sIgA locally into the intestinal lumen, a number of groups (Lemaitre-Coelho etal, 1978; Orlans etal, 1978; Russell, Brown & Mestecky, 1981) have demonstrated that dimeric IgA is selectively transported from the circulation across the hepatic parenchymal cell into the bile, and thence into the intestinal lumen. The mechanism of hepatobiliary transport of IgA appears to be similar to that of transport across the intestinal epithelial cells, and is dependent on the expression and binding of secretory component on the surface of the hepatocyte (Fisher etal, 1979).

The induction of secretory antibody may be stimulated both locally within the mucosa and centrally via the GALT. The requirement for antigenic stimulation in the induction of the mucosal antibody response is demonstrated by the experiments of Crabbe and colleagues (1968 & 1970), who showed that the intestinal tract of adult germ free mice contained greatly reduced numbers of IgA plasma cells as compared with normal adult mice. Exposure of germ free mice to a conventional environment led to a rapid repopulation of both lymphoid tissues and the intestinal mucosa with IgA plasma cells and precursors. This situation can be regarded as an experimental corollary of the rapid acquisition of IgA cells in the neonatal period following intestinal colonisation with commensal flora. The specificity of mucosal antibody has been demonstrated repeatedly. Davies (1922) demonstrated the presence of

specific agglutinins to Shigella in dysenteric stools. Burrows and Ware (1953) showed the presence of agglutinating "coproantibodies" in guinea pigs infected with cholera, and demonstrated the correlation of these antibodies with protection from infection. More recently, Crabbe etal (1969) demonstrated the appearance of antigen specific IgA immunocytes in the intestines of germ free mice given single doses of of horse spleen ferritin by the oral route.

The local origin of IgA in secretions has been demonstrated in a number of experimental systems, including the incorporation of labelled amino acids into IgA produced by salivary gland explants (Hurlimann & Darling, 1971) and immunohistological examination of lymphoid tissues (Crabbe, Carbonara & Heremans, 1965; Crabbe etal, 1969).

In addition to the local induction of IgA in the intestinal mucosa, antigen administered via the intestinal tract may lead to systemic immunisation. The presence of specific serum antibodies has been demonstrated following oral antigen administration of protein antigens (Rothberg, Kraft and Farr, 1967; Crabbe etal, 1969; Thomas & Parrott, 1974). The serum antibody response following oral antigen administration, however, is not a constant finding for either the detection of serum antibody or the class of serum antibody induced (reviewed by Heremans, 1974).

The role of the central lymphoid tissues of the GALT in the intestinal immune response to antigen was demonstrated by Craig and Cebra (1971). Using a model of reconstitution of irradiated rabbits with allotype labelled cells, they

demonstrated that lymphoid cells from intestinal Peyer's patches were able to serve as progenitors of IgA producing mucosal plasma cells. It was later shown that IgA precursor cells originating in Peyer's patches could migrate via the circulation not only to the intestinal mucosa, but also to other mucosal surfaces, where they gave rise to IgA secreting cells (McDermott & Bienenstock, 1979). In addition, the presence of specific IgA producing cells has been demonstrated in lymphoid tissues of the GALT and other lymphoid organs following oral and intragastric administration of SRBC (Heremans & Bazin, 1971; Andre, Bazin & Heremans, 1973).

The effector functions of mucosal antibodies have been extensively reviewed (Heremans, 1974; Crago & Tomasi, 1987) and will not be discussed in detail. Of relevance to the gastrointestinal immune response to dietary protein antigens, it has been proposed that mucosal antibodies may bind to antigens in the intestinal lumen and thus inhibit their absorption from the gut (Walker etal, 1975a). This phenomenom is discussed further in section 1.6.3.

The role of local cell mediated immune mechanisms in the gastrointestinal tract has been less extensively studied. T lymphocytes are found both within the lamina propria and the mucosal epithelium. Lamina propria T lymphocytes have been assigned a number of functions, including cytotoxic activity in response to local immunisation with allogeneic tumour cells (Davies & Parrott, 1980), and isotype specific help for IgA producing B cells (Elson etal, 1983). In addition, the

intraepithelial lymphocytes have been shown to exhibit cytotoxic (Davies and Parrott, 1981) and NK (Mowat etal 1983) activity, and to proliferate in response to mitogenic stimulation and in mixed lymphocyte reactions (Arnaud-Battandier & Nelson, 1982; Dillon & MacDonald, 1984).

Thus T lymphocytes of the intestinal mucosa are capable of mediating a variety of effector functions, including local regulation of IgA synthesis, T cell (and NK) mediated cytotoxicity and delayed type hypersensitivity reactions. In addition, all the accessory cells required for these functions are available locally within the intestinal mucosa (Parrott, 1987).

There is increasing evidence that local cell mediated immune responses within the intestinal mucosa may result in gut damage. Using a model of allograft rejection of heterotransplanted grafts of fetal intestine, Ferguson and Parrott (1973) and MacDonald and Ferguson (1976) showed that cell mediated immune responses could result in partial or subtotal villous atrophy and crypt hyperplasia. Similar mechanisms have now been implicated in other experimental models (Mowat & Ferguson, 1981 a+b) and in a number of clinical diseases involving gut damage (Ferguson & Mowat, 1980)

1.6.3 Systemic effects of antigen ingestion

In addition to the local immune mechanisms which antigen challenge at mucosal sites may invoke, oral antigen administration may be associated with a variety of systemic effects. The systemic effects of antigen

ingestion are diverse, and depend on a variety of factors, including the nature and dose of the antigen, the age, immune status and genetic background of the animal, the integrity of the gastrointestinal mucosal barrier and the immunological parameter under investigation.

There is considerable evidence that macromolecules (Wilson & Walzer, 1935; Warshaw etal, 1971; Thomas & Parrott, 1974) and even particulate material (Volkheimer & Schulz, 1968) may penetrate the mucosal epithelial barrier and gain entry to the circulation, in quantities too small to be of dietary significance but which may be of immunological importance (Bruce & Ferguson, 1986 a). Several studies (Walker etal, 1975 a; Swarbrick, Stokes & Soothill, 1979; Stokes, Swarbrick & Soothill, 1983) have shown an association between oral immunisation and reduced absorption of protein antigens through the gut. This finding led Walker and colleagues (1975 b) to propose that the induction of mucosal antibodies in the intestinal tract may inhibit the absorption of macromolecular antigens by the formation of immune complexes within the intestinal lumen, which decrease the binding of antigen to intestinal epithelial cells. Antigens complexed with antibody may then be degraded by pancreatic enzymes, reducing the antigenically active material available for absorption within the intestine. In spite of the relevance of this phenomenom of "immune exclusion" to a variety of food allergic disorders, relatively little work has been done to study the extent to which oral immunisation may inhibit antigen absorption from the gut.

Failure of antigen exclusion may lead to the induction

of a systemic antibody response. Since both antigen absorption and the presence of antibodies to dietary antigens are normal physiological events (Cunningham-Rundles, 1987), some difficulty lies in the interpretation of when absorbed antigen or antibody levels exceed those which may be considered normal. In certain diseases (e.g. coeliac disease) the presence of antibodies to dietary antigens has been linked to a pathological process. In many instances, however, the relationship between absorbed antigen, or antibody to ingested antigens and disease remains obscure. The possible association between dietary antigens and glomerulonephritis has been reviewed in sections 1.4 and 1.5.

Experimental studies on the effects of oral antigen administration on the systemic immune response have shown that, under different conditions, oral immunisation may lead to either systemic priming or to specific hyporesponsiveness (oral tolerance) (Asherson etal, 1977; Nicklin & Miller, 1983; Strobel & Ferguson, 1984; Challacombe & Tomasi, 1987).

A number of factors have been shown to influence the systemic effects of oral antigen administration, including the nature of the antigen, the dose and frequency of administration, the immune status of the animal (including prior systemic exposure to the antigen) and the age of the animal at first exposure. Enhancement of subsequent systemic immune responses to bacterial antigens following oral administration of whole bacteria has been described in a number of experimental systems including the immune

response to Streptococcus mutans (Challacombe, 1983), and to the somatic antigens of Eschericia coli (Stokes etal, 1979). The importance of the nature of the antigen in determining the outcome of the immune response to ingested antigen has been described by Challacombe & Tomasi (1987). These authors reported that oral administration of insoluble Streptococcal antigen was associated with enhancement of the systemic immune response, whilst the soluble form of the same antigen was associated with the induction of oral tolerance (Challacombe & Tomasi, 1987). The dose of antigen and the feeding schedule may also affect the systemic response to oral antigen administration. The contact sensitising agent oxazolone has been shown (Asherson, Perrera & Thomas, 1979) to induce tolerance when given in small (0.01-0.1 mg) doses by the intragastric route, whilst higher (10 mg) doses were associated with priming for subsequent systemic sensitisation. In contrast, low (μg) doses of protein antigens administered orally have been shown to prime mice, whilst higher (mg) doses led to the induction of oral tolerance (Mowat etal, 1986).

The importance of the immune status of the animal at the time of oral antigen administration has been demonstrated in a number of systems. The administration of soluble protein antigens to immunologically naive animals generally results in oral tolerance, whilst administration of similar oral doses of antigen to primed animals may enhance the systemic immune response to the antigen (Hanson etal, 1979 a; Titus & Chiller, 1981 a). Exceptions to this general rule, however, have been cited. Lafont

etal (1982) demonstrated abrogation of the antibody response by repeated oral antigen administration in parenterally immunised mice, and Bloch etal (1983) reported the induction of partial systemic tolerance in primed rats after prolonged antigen feeding.

Strobel and Ferguson (1984) have recently demonstrated the influence of the age of the animal at initial oral exposure to antigen on the subsequent systemic immune response to the antigen. Feeding of body weight related doses of ovalbumin to neonatal mice resulted in priming for both humoral and cell mediated immune responses to the antigen, whilst adult mice were rendered profoundly hyporesponsive by the antigen feeding regime (Strobel & Ferguson, 1984).

Finally the presence of the normal intestinal flora may affect the outcome of the immune response to orally administered antigen. The effects of the normal gut flora on the immune system have been described in section 1.6.2. In addition, it has been shown (Newby, Stokes & Bourne, 1980) that the presence of bacterial lipopolysaccharide (LPS) in the gut may enhance the induction of oral tolerance to contact sensitising agents in mice.

The down regulation of the systemic immune response to an antigen resulting from initial ingestion of the antigen has been known for many years (Wells, 1911; Chase, 1946). It is only over the past decade, however, that the phenomenon of oral tolerance has been studied in detail. A wide range of antigens, including contact sensitising agents (Asherson etal, 1977), soluble protein antigens

(Thomas & Parrott, 1974; Miller & Hanson, 1979; Challacombe & Tomasi, 1980) and heterologous red blood cells (Mattingly and Waksman, 1978 & 1980; Kagnoff, 1978 & 1980), have been shown to be capable of inducing systemic tolerance, affecting both humoral and cell mediated limbs of the immune response, when administered by the oral route.

The postulated mechanisms underlying the induction of oral tolerance are almost as numerous as the models for studying oral tolerance. These may be divided broadly into humoral and cellular mechanisms. Andre etal (1975) first showed that tolerance could be transferred with serum from animals which had been orally immunised, and postulated that this effect was mediated by the presence of IgA containing immune complexes. Subsequent studies confirmed the ability of serum from antigen fed animals to transfer tolerance, but indicated that this ability was mediated by the IgG containing fraction of serum (Kagnoff, 1978). Furthermore the IgG fraction of serum was shown to suppress in vitro antibody synsthesis (Chalon, Milne & Vaerman, 1979). From these and similar studies Kagnoff (1980) suggested that anti-idiotypic antibodies may be involved in the induction of oral tolerance. This theory has been developed by Cunningham-Rundles (1987) to include a role for immune complexes containing ingested antigens stimulating the induction of anti-idiotypic antibodies, which then suppress further production of the primary antibody.

All the above mechanisms require a systemic immune response to the antigen prior to serum transfer for the

induction of oral tolerance. Strobel and colleagues (1983), however, described the transfer of tolerance for DTH responses by serum from antigen fed animals collected 1 hour after initial oral antigen administration. Subsequent studies using this model have shown that the transfer of tolerance involved a serum factor of the same molecular weight as the native antigen, which could be absorbed out of the serum by antibody to the antigen (Bruce & Ferguson, 1986 a + b). Systemic administration of native, deaggregated or denatured antigen to similar concentrations as were seen in the serum 1 hour after antigen feeding, however, was not associated with the same pattern of systemic DTH and antibody responses upon subsequent parenteral immunisation (Bruce & Ferguson, 1986 a). The results suggest that the small amounts of native antigen which are absorbed following antigen ingestion may be directly responsible for the induction of oral tolerance, but that some form of gut processing of the antigen is required to render tolerogenic antigen fragments.

In addition to the humoral factors noted above, a number of cellular control mechanisms have been demonstrated in the induction of oral tolerance. Asherson etal (1977), using a model of oral tolerance to contact sensitising agents, demonstrated that suppression of DTH responses could be transferred with B cells, and postulated that oral antigen administration induced a population of suppressor B cells within the lymphoid tissues. Titus & Chiller (1981), using hapten-carrier

conjugates, demonstrated that oral tolerance was induced at the level of carrier specific helper T cells, suggesting that oral antigen administration induced anergy at the level of (carrier specific) helper T cells. T cell mediated suppressor mechanisms have been demonstrated in a number of models, and appear to present the dominant mechanism of induction of oral tolerance following oral administration of a wide range of antigens (Mattingly & Waksman, 1978; Richman etal, 1978; Miller & Hanson, 1979). Suppressor T cells have been demonstrated in the Peyer's patches and mesenteric lymph nodes earlier than in the spleens of animals following oral antigen administration (Mattingly & Waksman, 1978), suggesting that these cells might originate within mucosal lymphoid tissues. The suppressor cells induced by oral antigen administration have been shown to be radiosensitive (Hanson etal, 1979 b) and sensitive to treatment with cyclophosphamide (Strobel etal, 1983).

To summarise the effects of oral antigen administration, it is evident that the consequences of antigen ingestion are complex and diverse. The gastrointestinal immune response to antigen may involve both local and systemic effects. The local immune response involves the induction and synthesis of secretory antibodies, predominantly of the IgA class, but may also involve a variety of cellular and cell mediated immune mechanisms. Cell mediated immune responses to dietary antigens may lead to damaging hypersensitivity reactions within the intestinal mucosa, but are normally under the control of regulatory mechanisms within the GALT (Mowat &

Ferguson, 1981 a). In addition to the local mucosal response to the ingested antigen, oral antigen administration may be associated with enhancement of the systemic immune response, or with the induction of specific systemic unresponsiveness to the ingested antigen. The outcome of the response to oral antigen, however, is dependent on many factors, and involves a complex system of regulatory mechanisms.

1.7 MODIFICATION OF THE INDUCTION AND COURSE OF GLOMERULONEPHRITIS BY ORAL ANTIGEN ADMINISTRATION

Whilst much is known about the effects of antigen feeding on the systemic immune response to antigen with respect to such parameters as antibody production and delayed type hypersensitivity reactions, relatively little is known about the effects of dietary antigen on the course and progression of pathogenic systemic immune responses to antigen.

The progression of autoimmune renal disease in mice can be influenced indirectly by a number of dietary manipulations (Levy & Morrow, 1983; Kelley, 1986), including restrictions of dietary fat and protein content. These effects do not depend on the antigenic nature of the dietary components, and are thus immunologically non-specific modulators of the systemic immune response.

Direct evidence that dietary antigen might alter the course of glomerulonephritis was reported by Devey and Bleasdale (1984), using a model of the induction of chronic immune complex glomerulonephritis in low affinity

antibody producing mice by daily parenteral injections of protein antigen. Low affinity TO mice were given antigen (HSA, 0.05% w/v) in their drinking water for 7 days prior to the induction of nephritis by daily injections of the same antigen. Antigen feeding led to a reduced incidence of immune complex glomerulonephritis, in spite of high levels of circulating immune complexes which persisted in the circulation. Serum antibody levels in antigen fed animals, however, were lower than in control mice.

This was the first report to suggest that antigen feeding may alter the course and progression of a pathogenic systemic hypersensitivity reaction. Further data are required to confirm these observations, and to evaluate the potential role of oral antigen administration in the management of hypersensitivity disease.

1.8 AIMS OF THE THESIS

There is growing evidence that the mucosal immune response of the gastrointestinal tract to dietary antigens may be implicated in the pathogenesis of systemic diseases, including certain forms of glomerulonephritis. In addition, the gastrointestinal immune response to fed antigen may have profound immunomodulatory effects on the subsequent systemic immune response to the antigen, and oral antigen administration may affect the course of antigen specific immune complex disease. Dietary antigens may, therefore, both be implicated directly in the immunopathogenesis of glomerulonephritis, and may modulate the course of a nephropathogenic systemic immune response.

In this study I sought to investigate this apparently

paradoxical role of dietary antigen in the pathogenesis of glomerulonephritis by extending studies on the effects of administration of antigens by the oral route on the pathogenesis of experimental glomerulonephritis. The aims of the study were threefold :

i) to extend previous observations on the systemic effects of oral antigen administration, with particular reference to the genetic basis of control of oral tolerance, the relationship between the dose of oral antigen and the induction of oral tolerance, the role of gut processing of antigen in the induction of oral tolerance, the absorption of antigen from the intestinal tract, and the effects of oral immunisation on the subsequent systemic elimination of antigen.

ii) to establish the role of dietary antigens in the pathogenesis of experimental IgA nephropathy in normal mice and in mice rendered liver damaged by administration of carbon tetrachloride.

iii) to investigate the effects of single oral doses of antigen on the course and induction of immune complex glomerulonephritis in susceptible strains of mice, and to examine the factors which affect the modulation by oral antigen administration of antigen induced immune complex glomerulonephritis, and the mechanism(s) underlying the modulation of antigen induced immune complex glomerulonephritis by oral antigen administration.

CHAPTER TWO

MATERIALS AND METHODS

2 MATERIALS AND METHODS

2.1 Animals : Inbred mice used throughout the study were bred in the Research and Diagnostic Facility of the Department of Bacteriology and Immunology, University of Glasgow, from stock originally obtained from commercial breeders.

TO [high] and [low] strains of mice were bred from high and low affinity antibody producing mice obtained as a gift from Prof. M Steward and Dr. M Devey (London).

All mice were maintained from weaning on a standard mouse diet (Biosure Ltd, UK) which was free from ovalbumin.

2.2 Antigens : Ovalbumin (Grade V; Sigma, UK) (ova), bovine gamma globulins (BGG) (Cohn fractions II and III; Sigma, UK) and human serum albumin (HSA) (Behring, W. Germany) were used as protein antigens. Sheeps' blood, 50% in sterile Alsever's solution (Gibco, Scotland) was used as a source of sheep red blood cells (SRBC).

2.3 Cyclophosphamide : Cyclophosphamide (Endoxana; WB Pharmaceuticals, UK) was dissolved in sterile distilled water prior to use, and was administered to mice by intraperitoneal inoculation.

2.4 Phenobarbitone : Phenobarbitone (Gardenal sodium; May and Baker Ltd, UK) was administered to mice in solution in their drinking water.

2.5 Oral antigen administration : antigen was administered by the oral route by two methods :

i) By per oral intragastric intubation using a rigid

hollow feeding tube adapted from a 21 gauge steel hypodermic needle. Protein antigens were dissolved in distilled water, and 0.2 ml final volume of antigen solution per mouse was injected slowly via the feeding tube. Control animals received 0.2 ml distilled water by the same route.

SRBC for oral administration were washed 4-6 times in sterile saline. After the final wash the cell concentration was adjusted to 5×10^9 SRBC / ml with saline. Mice received 10^9 SRBC in 0.2 ml saline by intragastric intubation. Control animals received 0.2 ml saline by intragastric intubation.

ii) Via the drinking water. Protein antigens were dissolved in tap water to the desired concentration prior to administration. Control animals received plain tap water.

2.6 Administration of carbon tetrachloride : Carbon tetrachloride (CCl_4) (BDH Chemicals Ltd, UK) was dissolved in vegetable oil prior to administration of 0.1 ml of the resulting solution by per oral intragastric intubation. Control animals received 0.1 ml vegetable oil alone by the same route.

2.7 Parenteral immunisation : Mice were immunised by the inoculation of 100 µg ova in 0.05 ml complete Freund's adjuvant (CFA) into one rear footpad. CFA was prepared by incorporating ova (4 mg/ml) in aqueous solution into an equal volume of Bacto-H37 Ra complete adjuvant (Difco Ltd, USA), by the syringe method as described by Herbert (1973), to form a water-in-oil emulsion. The resulting

mixture was shaken on a mechanical shaker until the emulsion remained as a discrete droplet when dropped onto water.

2.8 Induction of chronic immune complex glomerulonephritis Chronic immune complex glomerulonephritis was induced in mice by daily intraperitoneal inoculation of 0.3 mg ova in 0.2 ml sterile saline for up to 120 inoculations. In the majority of experiments a standard regime of 60 (58 - 62) antigen injections was used for the induction of immune complex glomerulonephritis.

2.9 Collection and transfer of serum : Serum for passive transfer was obtained from antigen fed animals or water fed controls. Donor mice were fed either 25 mg ova in 0.2 ml distilled water or 0.2 ml distilled water alone by gastric intubation, and were bled out (section 2.11) 10 or 60 minutes later. Sera from donor groups were pooled, and 0.8 ml pooled donor serum was transferred by intraperitoneal inoculation into each recipient mouse. Recipient animals were immunised one week after serum transfer with 100 µg ova in CFA as described above.

2.10 Collection and transfer of spleen cells : Donor mice were fed either 25 mg ova in 0.2 ml distilled water or 0.2 ml distilled water by intragastric intubation as above. One week later, mice were killed by cervical dislocation and their spleens removed into RPMI 1640 (Appendix 1). The spleens were teased apart using a scalpel blade, and a single cell suspension prepared by repeated passage through a Pasteur pipette. The cells were washed twice in

RPMI 1640 and then passed twice over glass wool to remove any remaining clumps. The cells were washed and adjusted to 5 x 10^8 viable lymphocytes per ml. 10^8 spleen cells in 0.2 ml RPMI 1640 were transferred by intravenous inoculation via the tail vein into recipient animals. Recipients were immunised either with 100 µg ova in CFA immediately after cell transfer (oral tolerance experiment) or with 0.3 ml ova in 0.2 ml sterile saline by intraperitoneal inoculation followed by similar daily inoculations (induction of experimental nephritis).

2.11 Bleeding of mice : Two methods were used :

i) Venepuncture of the retro-orbital plexus using heparinised microcapillary tubes (Hawksley Ltd, UK). Heparinised blood was separated by centrifugation and the plasma stored at -20°C until use.

ii) Mice used as serum donors or undergoing laparotomy for the removal of kidneys for histological examination were bled out by transection of the inferior vena cava distal to the renal veins. After clotting, the serum was separated by centrifugation and stored at -20°C until use.

In both cases mice were anaesthetised by ether inhalation throughout the procedure. Mice which were bled out were killed by cervical dislocation immediately after bleeding, whilst still under anaesthetic.

2.12 Heat aggregation of ovalbumin : Heat aggregation of ova for footpad testing was performed by the method of Titus and Chiller (1981 b). Briefly, ova (20 mg/ml) in sterile saline was heat aggregated at 70°C in a water bath

for 1 hour with intermittent shaking. The resulting solution was cooled, spun at 2000 rpm for 10 minutes at 4 ^{o}C, and the pellet was resuspended in saline to 10 times its original volume. The resulting heat aggregated ova was stored in aliquots at -20^{o}C until use. Prior to administration, the solution was thawed and placed in an ultrasonicator for 20 - 30 minutes to break down large aggregates. The resulting solution for inoculation had a uniformly cloudy appearance.

2.13 Preparation of mouse anti-ova antiserum : Mouse anti-ova antiserum, for use as standard in ELISA systems, was prepared by immunisation of mice with 100 µg ova in CFA into one rear footpad. Three weeks later the mice were boosted with 0.3 mg ova in 0.2 ml sterile saline by intraperitoneal injection. Ten days later the mice were bled out, and the serum separated and pooled. Aliquots of pooled mouse anti-ova antisera were stored at -20^{o}C until use.

2.14 Preparation of rabbit anti-ova antiserum : Rabbit anti-ova antiserum was prepared by immunising a rabbit with 1 mg ova in CFA by deep intramuscular injection. After 4 weeks the rabbit was further immunised with 1 mg ova in incomplete Freund's adjuvant (Difco, USA; prepared as for CFA, section 2.7) by intramuscular inoculation. After a further 4 weeks the rabbit was boosted with 1 mg ova in saline i.m. The rabbit was bled 2 weeks later and the serum separated and stored at -20^{o}C.

2.15 Preparation of rabbit anti-DNP-BGG antiserum : Rabbit anti-DNP-BGG antiserum was prepared by the immunisation of a rabbit with DNP-BGG, according to the protocol in section 2.14.

2.16 Affinity purification of rabbit anti-ova antibody : The gamma globulin fraction of rabbit anti-ova was prepared by ammonium sulphate precipitation of 8 ml of rabbit anti-ova antiserum as described by Hudson and Hay (1976). Affinity purification of rabbit anti-ova was performed by passage of the gamma globulin fraction over a cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals Ltd) column to which ova had been covalently bound, followed by elution of the bound antibody. Six grams of dry gel was swollen and washed according to the manufacturer's instructions. Ovalbumin (5 mg per ml of swollen gel, in NaHCO3 coupling buffer ; Appendix 1) was coupled to the gel by end-over-end mixing for 2 hours at room temperature. Remaining active groups were blocked with Tris-HCl (pH 8 ; Appendix 1) for 2 hours. The gel was packed in a 60 ml syringe, washed alternately with acetate buffer (pH 4 ; Appendix 1) and coupling buffer and equilibrated with 10 volumes phosphate buffered saline (PBS). The sample was applied and the column run until the protein concentration of the eluate (by absorbance at 280 nm) had returned towards zero. The column was then eluted with glycine-HCl (0.1M, pH 2.5) and protein containing fractions were pooled and the pH brought up to pH 8 by addition of solid Tris. The pooled fractions were then concentrated by dialysis against solid polyethylene glycol

20000 and dialysed at 4°C overnight against PBS. The protein concentration was estimated by absorbance at 280 nm, using an extinction coefficient of 1.35 per mg rabbit IgG per ml. The affinity purified rabbit anti-ova was stored in aliquots at -20°C.

2.17 Alkaline phosphatase conjugation of rabbit anti-ova : Five mg alkaline phosphatase (bovine intestinal, type VII; Sigma, UK; 1170 units enzyme activity per mg protein) was spun for 10 minutes at 4°C at 150 g. The supernatant was discarded and the pellet was resuspended in 1 ml PBS containing 1.67 mg affinity purified rabbit anti ova. The mixture was dialysed for 48 hours at 4°C against 2 changes of PBS. Gluteraldehyde (specially purified, Grade 1, 25% in aqueous solution; Sigma, UK) was added to a final concentration of 0.2%. The resulting mixture was left at room temperature for 4 hours before extensive dialysis against PBS (3 x 2 litre changes) followed by 0.05M Tris buffered saline (pH 8.0) containing 0.001M MgCl2 (2 x 2 litre changes). The antibody-enzyme conjugate was stored in aliquots at -20°C until use. The optimum working dilution (1/1000) in ELISA systems was determined by chequerboard analysis.

2.18 Preparation of mouse IgG : Mouse IgG was prepared by ammonium sulphate precipitation of normal mouse serum followed by ion exchange chromatography using diethylaminoethyl (DEAE) cellulose. The gamma globulin fraction from 8 ml pooled normal mouse serum was prepared by ammonium sulphate precipitation as described in 2.16. The precipitate was redissolved and dialysed against the

starting buffer (0.005M phosphate buffer, pH 8; Appendix 1) and centrifuged to remove any protein which precipitated at this low ionic strength. Diethylaminoethyl cellulose (DE 32, 15 grams dry weight; Whatman Co Ltd) was washed and precycled according to the manufacturer's instructions, and the resulting slurry was packed in a glass column. The column was equilibrated with 0.005M phosphate buffer until the pH and ionic strength of the eluate were equal to those of the starting buffer. The gammaglobulin fraction of serum was applied and the column run in an ionic gradient at pH 8 using 0.005-0.3M phosphate buffers (Appendix 1). Fractions of eluate were collected and their protein content estimated by absorbance at 280 nm. Protein containing peaks were pooled and checked for purity by immunoelectrophoresis against anti-mouse IgG and anti-mouse whole serum antisera. The initial protein peak contained IgG only, and this was concentrated on an Amicon filter (Amicon Corp., USA), and dialysed against PBS + 0.02% Na azide (pH 7.4) and spun down to remove any precipitate. The absorbance (280 nm) of the supernatant was measured, and the concentration of IgG was estimated using an extinction coefficient of 1.5 per mg mouse IgG per ml. The concentration was then adjusted to 330 µg/ml with PBS-azide.

2.19 Preparation of heat aggregated mouse IgG : Two ml of purified mouse IgG (330 µg/ml) were heat aggregated at 63^{o}C for 30 minutes in a water bath. The solution was stored in aliquots at -70^{o}C until use.

2.20 Preparation of heat aggregated mouse IgG standards for immune complex assay : 200 µl heat aggregated mouse IgG (mAGG; 330 µg/ml) were incubated with 350 µl veronal buffered saline (VBS) + 0.05% Tween 20 (Appendix 1) + 60 µl fresh normal human serum for 30 minutes at 37 ^{o}C in a water bath to activate complement. Doubling dilutions of "activated" mAGG from 100-0.75 µg mAGG /ml were applied to wells in duplicate in the preparation of a standard curve for quantitation of immune complex assays.

2.21 Preparation of hapten-carrier conjugates :

i) DNP-ova : 2 g ova were dissolved in 10 ml 10% (w/v) aqueous NaHCO3. 390 µl of 30% (w/v) 2,4-dinitrofluorobenzene (DNFB; BDH Chemicals Ltd, UK) in ethanol were added with constant stirring. The resulting solution was mixed in an end-over-end rotator for 3 hours at room temperature in the dark. The solution was spun at 2500 rpm (MSE Mistral centrifuge) for 10 minutes to remove aggregates, and the supernatant was passed down a Sephadex G25 (Pharmacia Fine Chemicals Ltd, UK) column, equilibrated with normal saline, to separate the DNP-ova conjugate from unreacted DNFB. The hapten-protein conjugate was collected as the first yellow-coloured eluate peak. Conjugate fractions were pooled and dialysed overnight against normal saline at 4^{o}C. The protein concentration was estimated by the Biuret technique (section 2.22) against standard dilutions of ova in saline. The DNP content was estimated using a UV spectrophotometer by absorbance of light at 360 nm against standard dilutions of DNP-lysine. The DNP : ova ratio was

calculated from the relative molar concentrations (assuming molecular weight of ova = 45 kD). The resulting solution had a DNP : ova ratio of 6.85 : 1.

ii) DNP-BGG : 1 g BGG was dissolved in 5 ml 0.1M Na_2CO_3, and 100 µl of 10% DNFB in ethanol added as described above. The solution was stirred constantly for 2 hours at room temperature, and the pH of the solution was kept alkaline by dropwise addition of 0.1 M Na_2CO_3 to inhibit precipitation of the protein. The solution was centrifuged, and the supernatant was passed down a Sephadex G25 column to separate the hapten-carrier conjugate from unreacted DNFB. The conjugate was dialysed overnight at $4^{o}C$ against normal saline. The protein content of the resulting solution was estimated by the Biuret technique, and the DNP content by absorbance of light at 360 nm as described above. The relative molar concentration of the conjugate (DNP : BGG) was 4 : 1 (assuming molecular weight of BGG = 160 kD).

2.22 Biuret method of protein estimation : The protein content of hapten-carrier conjugates was estimated by the Biuret technique of protein estimation. 1 ml 3N NaOH was added to 2 ml protein solutions (test and standards) in normal saline, and the resulting mixture was placed in a boiling water bath for 5 minutes. The solutions were cooled in an ice bath, and 1 ml 2.5% (w/v) $CuSO_4.5H_2O$ was added and mixed. After 5 minutes at room temperature the solutions were spun down at 2500 rpm (MSE Mistral centrifuge) for 15 minutes, and the colourimetric reaction of the supernatants was read at 555 nm in a UV

spectrophotometer blanked on normal saline treated as above. The protein content of the sample was extrapolated from a standard curve prepared from known concentrations of native protein.

2.23 ^{125}I labelling of ova : Ovalbumin was labelled with ^{125}Iodine (^{125}I) using Iodogen (1,3,4,6-tetrachloro-3a,6a-diphenyl glycouril; Pierce Warriner Ltd, UK) according to the manufacturer's instructions. Briefly, 10 µg of Iodogen in 75 µl chloroform were added to a 5 ml glass test tube and dried under nitrogen gas. The tube was given a light wash with PBS and dried prior to use. 100 µg ova in 25 µl PBS was added to 1 mCi (37 MBq) ^{125}I in 50 µl PBS and transferred to the Iodogen coated tube. The reaction was allowed to proceed for 15 minutes, with intermittent shaking, after which the reaction was stopped by the removal of the mixture from the reaction vessel. The mixture was passed down a Sephadex G25 (Pharmacia Fine Chemicals) column which had been equilibrated with PBS + 0.1% bovine serum albumin (BSA; Sigma) to remove unreacted ^{125}I. Eluate fractions were estimated for radioactivity in an LKB bench top gamma counter. Two peaks of activity were obtained. The fractions from the first (protein bound) peak were pooled, and the protein content was estimated by absorbance at 280 nm in a UV spectrophotometer, by extrapolation against standard dilutions of ova in PBS + 0.1% BSA (blanked on PBS + 0.1% BSA alone). The binding efficiency of the reaction, estimated from the ratio of protein bound radioactivity to total radioactivity of the mixture prior to removal of unreacted ^{125}I, was less than

10%.

2.24 Clearance of ^{125}I-ova from the circulation : The rate of clearance of ^{125}I-ova from the circulation of mice was estimated by the radioactive content of samples of venous blood taken at intervals following the intravenous inoculation of 200 ng ^{125}I-ova. ^{125}I-ova solution was diluted in PBS to 1 μg/ml. Mice were injected with 0.2 ml of the resulting solution into the tail vein, and 50 μl venous blood samples were taken by retro-orbital venepuncture into heparinised capillary tubes (Hawksley Ltd, UK) at 10 minutes and 1, 2, 3, 4, 6, 8 and 24 hours after the inoculation of ^{125}I-ova. The radioactive content of the samples was estimated by 30 second counts in an LKB 1282 Compu gamma counter using a window of 35 - 100 KeV. The initial (10 minute) sample was set as 100%, and counts of subsequent samples expressed as a percentage of this total in the construction of clearance curves.

2.25 Estimation of delayed type hypersensitivity reactions Delayed type hypersensitivity (DTH) reactions were estimated by the increase in footpad thickness following local antigen challenge in primed mice. Mice had the thickness of one rear footpad measured (sum of 3 separate measurements) using a "Pocotest" micrometer (Carobronze Ltd) prior to challenge with 100 μg heat aggregated ova (section 2.12) in 0.05 ml saline inoculated into the same footpad. Twenty four or 48 hours after challenge the footpad thickness was measured as before, and the increase in footpad thickness was taken as an estimate of DTH response. In experiments involving inbred mice, ovalbumin-

specific footpad responses were calculated by subtracting the response of immunised mice to challenge with saline alone from the responses of experimental mice to challenge with ova in saline. In TD mice, due to the relative scarcity of these mice, this control was not used, and results were expressed as gross change in footpad thickness following intradermal challenge with ova. The results of DTH responses of immunised mice were expressed either as increase in footpad thickness in mm, or by expressing the responses of test animals as a percentage of the mean of water fed, immunised control responses. For statistical analysis of results, unpaired Student's t test was performed on the raw data.

2.26 Estimation of antibody titres : Ovalbumin specific antibody titres were estimated by enzyme linked immunosorbent assay (ELISA). For the estimation of anti-ova antibodies in experiments on the induction of oral tolerance in inbred strains of mice (section 3.2.1), 96 well flat-bottomed microtitre plates (Flow Laboratories Ltd, UK) were coated with 120 µl per well of ova (1 mg/ml) in carbonate/bicarbonate buffer (Appendix 1). The plates were incubated overnight at $4^{o}C$ and then washed 3 times in wash buffer (Appendix 1) and shaken dry. Plasma samples from test and control experimental animals were diluted 1/1000 in serum diluent (wash buffer + 10% normal rabbit serum) and applied in triplicate (100 µl per well). A standard curve was prepared by application in triplicate of tenfold dilutions from 10^{-2} - 10^{-6} of standard mouse anti-ova antiserum (section 2.13) in serum diluent as

above. The plates were incubated at 37°C for 3 hours in a moist box. After incubation the plates were washed x3 in wash buffer and shaken dry. Sheep anti-mouse IgG (Serotec Ltd, UK) was diluted 1/2500 in serum diluent, and applied at 100 µl per well. The plates were incubated at 37°C for 3 hours as above before being washed x3 in wash buffer. Alkaline phosphatase conjugated rabbit anti-goat IgG (Miles Scientific Ltd, UK) was diluted 1/1000 in serum diluent and 100 µl per well applied. The plates were incubated overnight at 4°C and washed x3 in wash buffer. Phosphatase 104 substrate (Sigma, UK) diluted 1 mg/ml in 10% diethanolamine (DEA; Appendix 1) was applied at 100 µl per well as visualising agent. The colorimetric reaction was stopped by the addition of 50 µl 3N NaOH per well, and the reaction read at 405 nm using a single channel or multichannel ELISA reader (Flow Labs Ltd, UK).

In all subsequent experiments a modification of the technique was used as follows : plates were coated with ova 100 µg/ml in carbonate/bicarbonate buffer (120 µl per well) and incubated overnight at 4 °C before washing. Samples and standards were applied in triplicate as above, and the plates were incubated at 37°C for 2 hours. After washing, affinity purified goat anti-mouse IgG - alkaline phosphatase conjugate (Northeast Laboratories Ltd) was applied, diluted 1/5000 in serum diluent, and plates were incubated for 2 hours at 37°C. After washing, substrate solution was added to wells as above. The colorimetric reaction was stopped by the addition of 3N NaOH.

Appropriate dilutions for reagents were ascertained by

Replicate assay of 8 randomly selected samples on 2 separate occasions gave anti-ova antibody titres (ELISA units) with between-assay correlation (r) of 0.95.

chequerboard analysis. The assay was specifically inhibited by ova, but not by BSA or by normal mouse serum at test dilutions.

Anti-ova antibody titres in individual animals were obtained by extrapolation of optical density (405 nm) against the straight line phase of the standard curve by computer analysis. All standard curves used for the extrapolation of antibody titres had straight line correlation coefficients (r) of greater than 0.94. The Wilcoxon rank sum test was used to compare results of experimental groups.

Several batches of standard mouse anti-ova were used in the course of these studies. The same batch of mouse anti-ova was used as standard in groups of experiments which were reported together. The use of different mouse anti-ova standards in different groups of experiments, however, means that antibody titres cannot be compared directly between experiments which have been reported separately.

2.27 Estimation of circulating ovalbumin : Measurement of circulating ovalbumin levels was performed by ELISA. Flat-bottomed microtitre plates (Flow Labs Ltd, UK) were coated (120 µl per well) with 10 µg/ml affinity purifed rabbit anti-ova (section 2.16) in carbonate/bicarbonate buffer.

For experiments on the uptake of antigen from the gastrointestinal tract (section 3.3) plates were incubated at 4°C for 40 hours. The plates were washed as described in 2.26, and serum samples diluted 1/10 in serum diluent (wash buffer + 20% normal rabbit serum) were applied in triplicate. Standard dilutions of ova (1 µg/ml - 10 ng/ml)

in serum diluent + 10% normal mouse serum (nms) were applied similarly. Plates were incubated for 1 hour at 37^{o}C.

For experiments on estimation of circulating antigen after the induction of nephritis (section 6.3.3) plates were coated overnight at 4^{o}C, and samples were applied in triplicate diluted 1/50 in wash buffer + 10% normal rabbit serum (nrs). Standards of ova (1 µg/ml - 10 ng/ml) were diluted in serum diluent + 2% nms and applied as above. Plates were incubated for 2 hours at 37^{o}C before being washed x3 in wash buffer.

In both sets of experiments alkaline phosphatase-conjugated, affinity purified rabbit anti-ova (section 2.17) was added at 1/1000 dilution in serum diluent. After 2 hours incubation at 37^{o}C the plates were washed for the final time and the substrate applied as described in 2.26. The reaction was stopped by the addition of 50 µl 3N NaOH per well, and the plates read as described previously.

Optimum conditions for each assay system were worked out by chequerboard analysis. Serum ovalbumin concentrations of individual samples were calculated by extrapolation from the standard curve. The assay system was specific for ovalbumin, and showed no cross reactivity with bovine serum albumin, mouse serum albumin or bovine gamma globulin in the concentration ranges tested.

2.28 Immunofluorescent examination of kidneys : Whole kidneys for immunofluorescent examination were removed immediately after killing mice, and were placed in isopentane (BDH Chemicals Ltd, UK) in boiling tubes, and

snap frozen in acetone cooled with dry ice. The kidneys were stored at -70^{O}C until use.

For processing, the kidneys were mounted in OCT mounting medium (Miles Scientific Ltd, UK) and frozen to cryostat chucks under a stream of carbon dioxide gas under pressure. Four micrometer sections were cut at -20^{O}C in a Bright cryostat. Sections were mounted onto clean microscope slides and maintained at -20^{O}C until use.

Unfixed cryostat sections were stained directly for the presence of immunoglobulin isotypes IgG, IgA and IgM and for C3, and indirectly for ova, DNP and bovine IgG, using immunofluorescent techniques. Sections for direct immunofluorescent examination were thawed and air dried, and washed in PBS + 0.01% Na azide for 10 minutes. The sections were overlayed with PBS-azide + 20% normal sheep serum (nss) for 20 minutes, and washed in PBS-azide for 20 minutes with shaking. Sections were stained directly using isotype specific FITC-labelled goat anti-mouse IgG (1/100), IgA (1/40), IgM (1/60) or C3 (1/60) (Cappel Laboratories, Philadelphia, USA) or FITC-labelled sheep anti-mouse IgA (1/40) or anti-mouse immunoglobulins (1/20) (Serotec Ltd, UK), diluted in PBS-azide + 20% nss for 1 hour at room temperature. After staining, sections were washed for 90 minutes in PBS-azide, over a magnetic stirrer. Sections were mounted under floating coverslips with glycerol + 10% PBS-azide and sealed with clear nail varnish. All antisera used were checked for specificity by immunoelectrophoresis against normal mouse serum.

Sections for indirect immunofluorescence were air dried and washed as above. For indirect staining for ova and for

DNP, sections were overlayed with PBS-azide + 20% nss, and washed as described for direct immunofluorescence. Sections were stained with rabbit anti-ova (1/30) or rabbit anti-DNP-BGG (1/30) for 45 minutes, washed for 1 hour, and stained with FITC-labelled sheep anti-rabbit IgG (1/40) (Serotec Ltd, UK) for 45 minutes before washing and mounting as described above. All dilutions of antisera were prepared in PBS-azide + 20% nss. For indirect staining for bovine IgG, the sections were air dried and washed as before, and overlayed with PBS-azide + 20% normal donkey serum. The sections were washed, and stained with sheep anti-bovine IgG (1/20 in PBS-azide) for 45 minutes. The sections were washed, and stained with FITC-labelled donkey anti-sheep IgG (1/40 in PBS-azide + 20% normal donkey serum) (Serotec Ltd, UK) for 45 minutes, before being washed and mounted as described above.

The rabbit anti-ova serum was ovalbumin specific as determined by double diffusion analysis against ova, BSA, HSA and mouse serum. Rabbit anti-DNP-BGG reacted with BGG, DNP-BGG and DNP-ova, but not with ova, on double diffusion analysis. On immunofluorescent staining, the antibody fixed to kidney sections from mice in which immune complex deposition had been induced by DNP-ova, but did not fix to kidney sections of mice in which immune complex deposition had been induced by ova alone. It was therefore assumed that the antibody was DNP specific in this assay system. The sheep anti-bovine IgG antiserum showed no cross reaction with mouse IgG in either double diffusion analysis or in immunofluorescent staining.

Slides were viewed blind and in random sequence, using a Leitz Ortholux fluorescence microscope, under ultraviolet transillumination at 490 nm using a mercury light source. The site and intensity of immunofluorescent stain was noted and scored 0, trace, +, ++ or +++ (Plates 1 - 5). As trace amounts of immunoglobulins were found commonly in normal (uninjected) TO mice, in experiments on the induction of immune complex glomerulonephritis (Chapters 5 & 6), sections scored 0 or trace were taken as being negatively stained for that parameter. Sections scored +, ++, or +++ were considered as being positively stained.

An immunofluorescence score for each kidney section was obtained by the summation of the staining scores for each staining parameter. Immunofluorescence scores were used in the analysis of the degree of immune complex deposition seen in different experimental groups.

An immunofluorescence index for each staining parameter was obtained by calculating the mean immunofluorescence score for the individual staining parameters within a single experimental group.

2.29 Light microscopic examination : Renal and hepatic tissue for light microscopic examination was fixed in formal saline prior to embedding in wax. Four micrometer sections were cut, dewaxed and stained by haematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) (kidney sections), and by H&E, PAS, reticulin and Masson techniques (liver sections). Sections were examined using a Leitz SM-Lux microscope.

2.30 Electron microscopic examination : 1 mm cubes of renal tissue were fixed in 2% glutaraldehyde in phosphate buffer, rinsed, and osmicated in osmium tetroxide for 1 hour. The tissue was then rinsed in buffer and dehydrated through graded alcohols, propylene oxide and resins, and finally polymerised in Araldite resin mixture overnight at 60^{o}C.

80 - 90 nm sections were cut, mounted on copper grids and double stained using lead and uranium. The sections were examined in a Phillips 301G electron microscope.

2.31 Estimation of circulating immune complexes : Conglutinin binding circulating immune complexes (CIC) were estimated by ELISA. Ninety six well flat bottomed microtitre plates (Flow Labs Ltd, UK) were coated with purified conglutinin (Shield Immunologicals, Dundee) at 10 µg/ml in carbonate/bicarbonate buffer and incubated overnight at 4^{o}C. The plates were washed x3 in VBS-Tween (Appendix 1). Test sera were diluted 1/10 in VBS-Tween + 10% nrs and applied in duplicate, 100 µl per well. A standard curve was prepared from "activated" mAGG as described in section 2.20. The plates were incubated for 2 hours at 37^{o}C, and washed x3 in VBS-Tween. Alkaline phoshatase-conjugated protein A (Sigma, UK) was applied at 1/1000 dilution in VBS-Tween + 10% nrs, 100 µl per well, and the plates were incubated for a further 2 hours at 37^{o}C. After a final wash the substrate solution was added as described previously. The reaction was stopped by the addition of 50 µl 3N NaOH per well. The plates were read

at 405 nm. The assay was inhibited by the addition of excess mAGG, and partially inhibited by purified mouse IgG, but was not inhibited by normal mouse serum at concentrations used. Results were calculated by extrapolation from the standard curve (Figure 1). On the basis of this curve, a lower limit of sensitivity for the assay (accounting for sample dilution) was defined as 15 µg/ml mAGG binding equivalents. In the analysis of results, samples giving values greater than 15 µg/ml mAGG were considered as showing significant binding activity.

2.32 Estimation of renal function : Renal function of mice was estimated by the measurement of serum urea and creatinine.

Serum urea was measured by the Bertholet technique. Twenty µl of serum were added to 200 µl urease (Sigma, UK) solution (Appendix 1) in glass tubes and incubated for 5 minutes at 50 ^{o}C in a water bath. One ml phenol colour reagent (Appendix 1) and 1 ml alkaline hypochlorite solution (Appendix 1) were added, mixed and incubated for 5 minutes at 50 ^{o}C as before. Eight ml distilled water were added to each tube. A standard curve was prepared from standard dilutions of urea (BDH Chemicals Ltd, UK) treated as above. The colorimetric reaction was read at 630 nm in a UV spectrophotometer. Sample values were obtained by extrapolation from the straight line standard curve.

Serum creatinine was measured as described by Bartels and Bohmer (1971), using commercial reagents (Boeringer Mannheim, W Germany), according to the manufacturer's

instructions. Briefly 70 µl sera or creatinine standard (177 µmol/l) were mixed with 700 µl picric chloride (17.5 mmol/l) in 0.03N NaOH and mixed. Thirty seconds later the absorbance at 490 nm was measured in a spectrophotometer. Exactly 2 minutes later the absorbance was read again, and the change in absorbance with time was taken as a measure of serum creatinine, which was calculated from the equation creatinine (µmol/l) = 177 x $\frac{d\ A\ (sample)}{d\ A\ (standard)}$

where d A is the change in absorbance at 490 nm with time.

In both assay systems quality control was checked by the use of a sample of known urea and creatinine concentration ("Precinorm"; Boehringer Mannheim, Germany).

2.33 Statistics : Unpaired Student's t test was used in comparison of results from experimental groups where results were assumed to follow a normal distribution. The Wilcoxon rank sum test was used in the analysis of non parametric results. Fisher's exact test was used in the comparison of results of immunofluorescent staining in antigen fed and control mice.

FIGURE 1

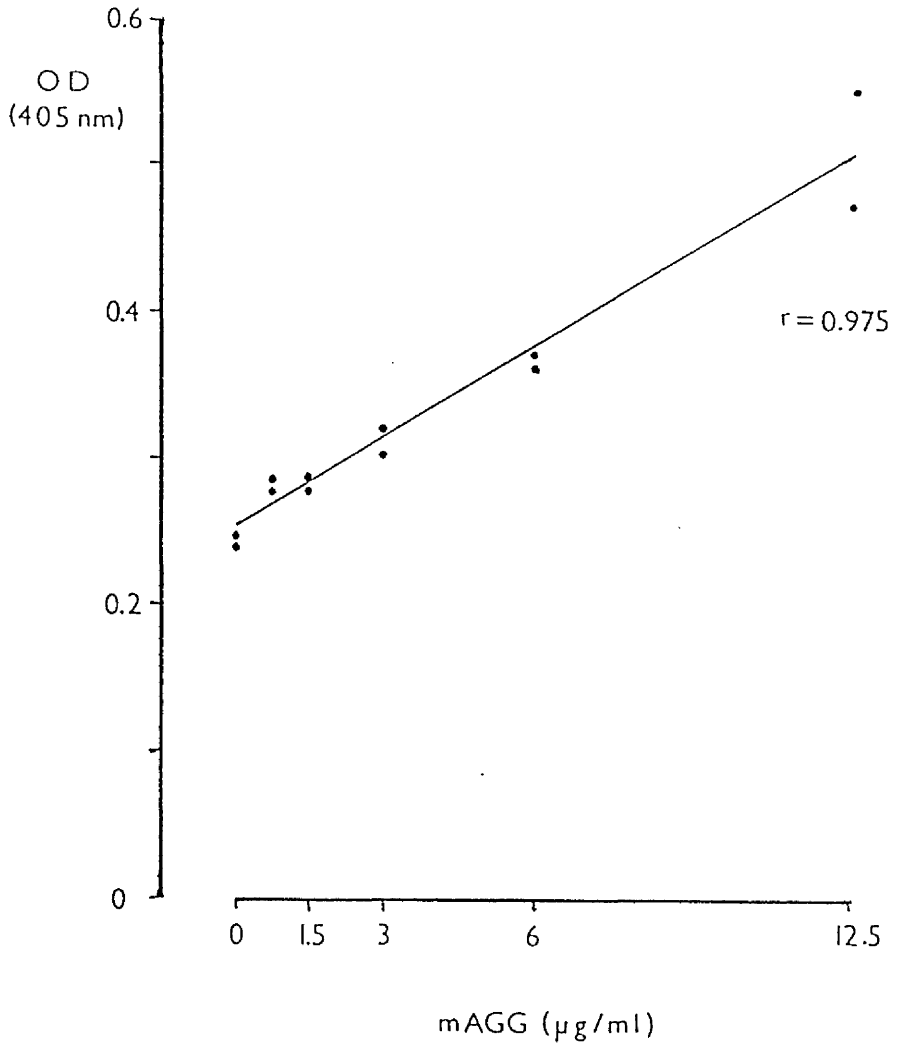

Figure 1 : Standard curve of conglutinin binding ELISA for detecting circulating immune complexes. Standards were prepared by doubling dilution of heat aggregated mouse IgG (mAGG) which had been incubated with normal human serum for 30 minutes at 37^{o}C to activate complement. Results are expressed as absorbance of light at 405 nm (OD 405 nm) following incubation with protein-A-alkaline phosphatase conjugate, and visualisation using Sigma phosphatase 104 substrate.

PLATES 1-5 : Sections of kidney from TO [low] mice, injected repeatedly with ova, showing the range of immunofluorescent staining seen in experiments on antigen induced immune complex glomerulonephritis. All sections stained indirectly for the presence of ova using immunofluorescent techniques. (Magnification x150).

Plate 1 : negative glomerular staining.

Plate 2 : trace glomerular staining.

Plate 3 : glomerular paramesangial and capillary staining; scored +.

Plate 4 : Marked mesangial immunofluorescent staining ; scored ++.

Plate 5 : Intense mesangial staining ; scored +++.

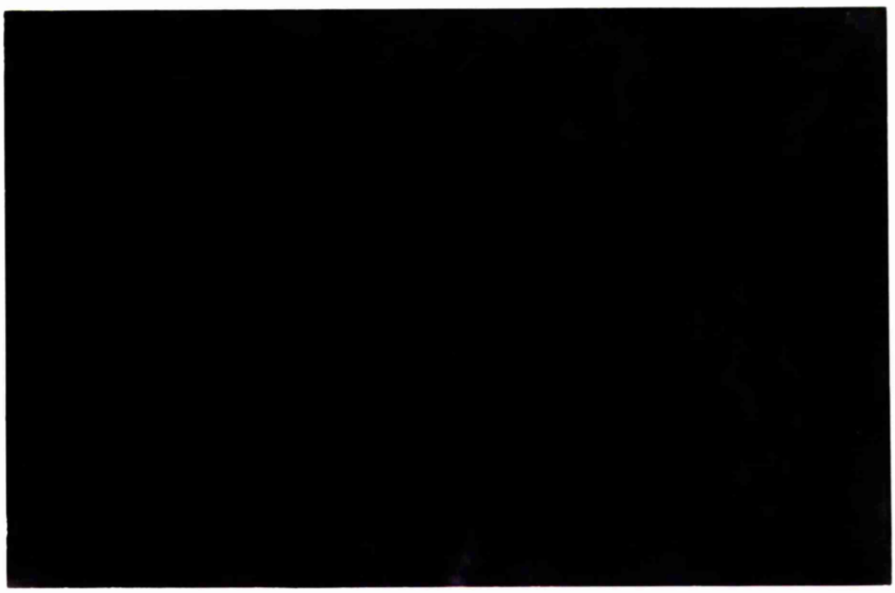

Plate 1

Plate 2

Plate 3

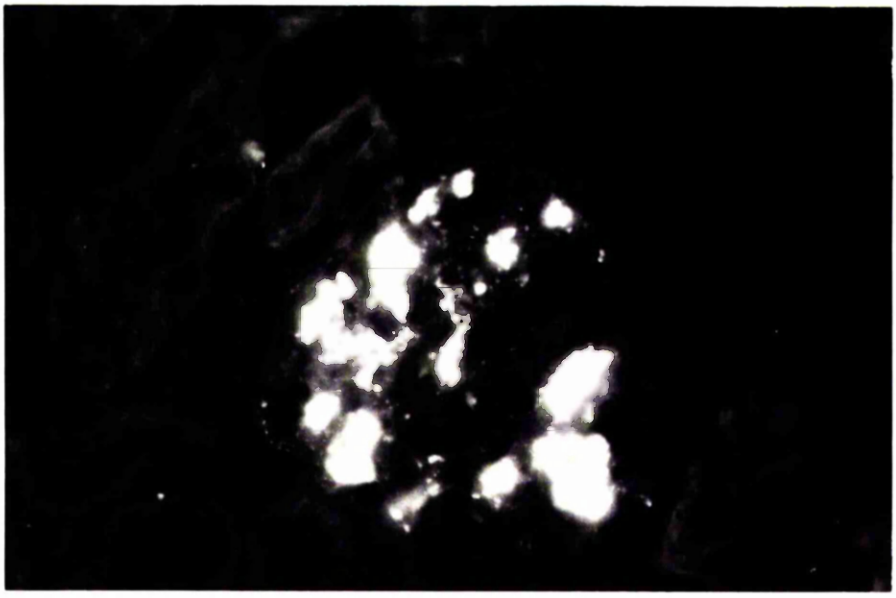

Plate 4

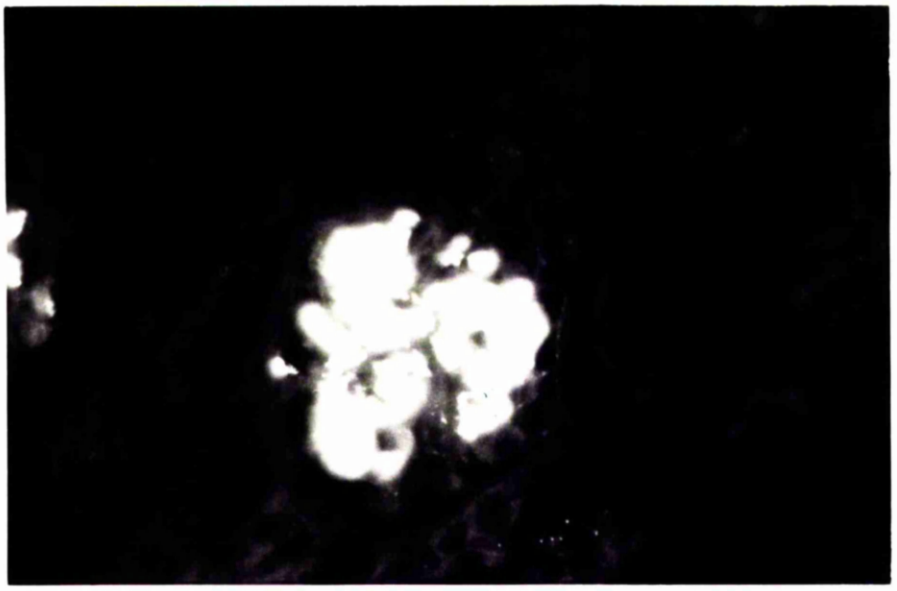

Plate 5

CHAPTER THREE

SYSTEMIC EFFECTS OF ORAL ANTIGEN ADMINISTRATION

3.1 INTRODUCTION

Immunopathological disease to extrinsic antigens is dependent upon both antigen entry and a disordered immune response to the antigen. The gastrointestinal immune response to ingested antigen may affect both antigen absorption from the intestinal lumen and the systemic immune response to the antigen (section 1.6). It is important in any study of the immunopathogenic effects of dietary antigens in systemic disease, therefore, to first define the physiologic effects of dietary antigens on the systemic immune response to those antigens.

In this chapter I extend studies on the genetic basis and dose dependent nature of the induction of oral tolerance, on the absorption of protein antigen from the gastrointestinal lumen, and on the effects of oral immunisation on the systemic handling of antigen.

3.2 ORAL TOLERANCE

3.2.1 Differences in the induction of oral tolerance in inbred strains of mice

The effects of single doses of ovalbumin administered by the intragastric route on the subsequent induction of systemic antibody and delayed type hypersensitivity (DTH) responses to parenteral immunisation with the same antigen were studied in 11 inbred strains of mice of 5 major histocompatibility complex (H-2) haplotypes.

Materials and Methods

Animals : Eight week old male mice of the following inbred strains were used : BALB/c ($H\text{-}2^d$), DBA/2 ($H\text{-}2^d$), BALB/k ($H\text{-}2^k$), B10.BR ($H\text{-}2^k$), CBA ($H\text{-}2^k$), C3H/HeOla ($H\text{-}2^k$),

BALB/b ($H-2^b$), C57BL/10 ($H-2^b$), C57BL/6-bg (Beige) ($H-2^b$), A ($H-2^a$) and NIH ($H-2^q$).

Oral antigen administration : Groups of mice were given single 2 mg or 25 mg doses of ovalbumin by intragastric intubation. Control animals received water by the intragastric route.

Parenteral immunisation : Fourteen days after intragastric antigen administration the animals were immunised with 100 µg ova in CFA into one rear footpad.

Estimation of antibody response : Twenty one days after parenteral immunisation the mice were bled and the plasma separated. Ovalbumin specific plasma antibody levels were estimated by ELISA as described in section 2.26.

Estimation of DTH response : Following bleeding, the contralateral rear footpad thickness was measured and the footpad challenged by the intradermal inoculation of 100 µg heat aggregated ova in 0.05 ml saline. Twenty four hours later the footpad thickness was measured again, and the change in footpad thickness was used as an estimate of DTH response.

Results

Results of DTH responses, as estimated by change in footpad thickness following intradermal footpad challenge, in control (water fed, immunised) mice of 11 inbred strains of mice are are shown in Table 3. A wide variation in the degree of DTH responses was seen between mice of the different strains. To allow for direct comparison of results between strains, DTH responses 3 weeks after parenteral immunisation in mice fed 2 or 25 mg ova prior

to immunisation are expressed as percentages of the mean control DTH responses for each of the strains tested (Figure 2). The results showed a wide variation in the effects on the DTH responses of antigen feeding prior to immunisation between the strains tested. Feeding with 2 mg ova prior to immunisation had no significant effect on the DTH response in BALB/k, C3H/HeOla, BALB/b, C57BL/10 and NIH strains of mice. In BALB/c, DBA/2, B10.BR, CBA, Beige and A strains, however, prior feeding with 2 mg ova was associated with significantly reduced DTH responses to ova as compared with control responses. In all strains, with the exception of B10.BR and BALB/b mice, DTH responses of mice fed 25 mg ova were significantly reduced as compared with controls, and were reduced as compared with animals of the same strain fed 2 mg ova. In no strain of mouse was the DTH response of antigen fed animals significantly increased as compared with control responses at either dose of oral antigen tested. Thus reductions in systemic DTH responses to ova were seen following intragastric administration of 25 mg ova in 9 of 11 strains tested. Intragastric administration of 2 mg ova, however, was associated with significant reductions in DTH responses in only 6 of these strains.

Antibody responses to parenteral immunisation in the same mice are shown in Figure 3 for $H\text{-}2^d$ and $H\text{-}2^k$ strains of mice, and in Figure 4 for $H\text{-}2^b$, $H\text{-}2^a$ and $H\text{-}2^q$ strains. As with DTH responses, there was marked variability between the antibody responses both between control mice of the different strains, and in the effects of feeding 2 mg or 25 mg ova prior to immunisation in the different

strains tested. Oral administration of 2 mg ova prior to immunisation was associated with significant reductions in antibody titre as compared with control responses in BALB/c, BALB/k, CBA, C3H/HeOla, Beige, A and NIH mice as compared with controls. Following feeding with 25 mg ova, significant reductions in antibody titre were seen in all strains except BALB/b and B10.BR. In no strain of mouse was antigen feeding at the doses tested associated with a significant increase in the antibody response to parenteral immunisation.

Table 4 summarises the effects of intragastric antigen administration on subsequent systemic immune responses in 11 inbred strains of mice. In each strain of mouse the reduction in DTH response correlated with the reduction in the antibody response in mice fed 25 mg ova by intragastric intubation. Relative DTH and antibody responses of mice of the 11 strains given 25 mg ova intragastrically showed a highly significant overall correlation ($r = 0.83$; $p = 0.0015$). This correlation between relative DTH and antibody responses was less marked in mice fed 2 mg ova prior to systemic immunisation ($r = 0.15$; $p = 0.65$), and "splitting" of oral tolerance induction for DTH and for antibody responses was seen in mice of several strains fed 2 mg ova. In DBA/2 and B10.BR mice fed 2 mg ova, significant reductions were seen in DTH but not in antibody responses. Conversely in mice of the BALB/k, C3H/HeOla and NIH strains, feeding with this dose of antigen was associated with significant reductions in antibody but not in DTH responses as compared with

controls. The disparate effects of intragastric administration of 2 mg ova on the two limbs of the systemic immune response were particularly marked in DBA/2 and NIH mice. Thus, whilst intragastric administration of 25 mg ova affected the subsequent cell mediated and humoral limbs of the immune response roughly equally, administration of 2 mg ova by intragastric intubation revealed differences between the induction of oral tolerance for DTH and for antibody responses in certain of the 11 inbred strains tested.

Mice of 5 different H-2 haplotypes were investigated. Two $H\text{-}2^{d}$ strains tested (BALB/c and DBA/2) showed highly significant reductions in both DTH and antibody responses after feeding with 25 mg ova prior to immunisation. Four $H\text{-}2^{k}$ strains (BALB/k, B10.BR, C3H/HeOla & CBA) and three $H\text{-}2^{b}$ strains (BALB/b, C57BL/10 & Beige) showed marked interstrain variability in the effects of antigen feeding on the systemic immune response to antigen. Mice of the A strain ($H\text{-}2K^{k}$ / $H\text{-}2D^{d}$) showed reductions in DTH and antibody responses similar in magnitude to those of the two $H\text{-}2^{d}$ strains tested and to those of NIH ($H\text{-}2^{q}$) mice. Included in the study were three strains of mice congenic for the BALB background and differing only at the H-2 gene complex (BALB/c, $H\text{-}2^{d}$; BALB/k, $H\text{-}2^{k}$; BALB/b, $H\text{-}2^{b}$). These three strains of mice differed widely in their ease of oral tolerance induction for both DTH and for antibody responses. Genetic differences in oral tolerance induction have been shown, therefore, in mice bearing the same H-2 haplotype but differing in non H-2 background, and in mice of the same background but differing in H-2 haplotype.

3.2.2 Oral tolerance in TO [high] and TO [low] mice

Similar studies were carried out to examine the induction of oral tolerance in TO [high] and TO [low] strains of mice, which were used extensively in subsequent experiments.

Materials and Methods

Animals : Eight week old male mice were used.

Oral antigen administration : Groups of mice were given single 2 mg or 25 mg doses of ova by intragastric intubation as in the previous experiment. Control mice received water alone.

Parenteral immunisation : Fourteen days after antigen feeding mice were immunised with 100 µg ova in CFA to one rear footpad.

Estimation of antibody responses : Mice were bled 21 days after immunisation. Anti-ova antibody titres were estimated by ELISA.

Estimation of DTH responses : Following bleeding, mice were challenged with 100 µg heat aggregated ova in 0.05 ml saline into the contralateral rear footpad and the increment in footpad thickness measured 24 hours after challenge was used as an estimate of DTH response.

Results

Results of DTH responses 3 weeks after immunisation in TO [high] and TO [low] mice fed 2 mg or 25 mg ova or water alone prior to immunisation with ova are shown in Figure 5. Feeding with 2 mg ova did not significantly affect the DTH responses to parenteral immunisation in either strain of mice. Feeding with 25 mg ova, however, was associated

with reductions in DTH response of 77% and 87% as compared with control (water fed, immunised) animals in TO [high] and TO [low] mice respectively.

Antibody titres in the same mice are shown in Figure 6. In TO [high] mice feeding with neither 2 mg nor 25 mg ova had any effect on the antibody response to parenteral immunisation. In TO [low] mice, feeding with 25 mg ova, but not with 2 mg ova was associated with a significant (58%) reduction in the ovalbumin specific antibody titre as compared with controls.

3.2.3 The effect of oral antigen dose on the induction of oral tolerance

The previous experiments on the induction of oral tolerance showed differences in the effects of feeding 2 mg or 25 mg ova on subsequent DTH and antibody responses to parenteral immunisation in certain strains of mice. To further examine the effect of the dose of oral antigen on the induction of oral tolerance, TO [low] mice were given varying single doses of ova by intragastric intubation prior to systemic immunisation.

Materials and Methods

Animals : Male TO [low] mice at 8 weeks of age were used.

Oral antigen administration : Groups of mice were given single oral doses of 1 mg, 2 mg, 10 mg, 25 mg or 40 mg ova by intragastric intubation. Control animals received water alone by the same route.

Parenteral immunisation : Fourteen days after antigen feeding mice were immunised with 100 µg ova in CFA into one rear footpad.

Estimation of antibody titre : Mice were bled 21 days after immunisation and anti-ova antibody titres were estimated by ELISA.

Estimation of DTH responses : Following bleeding, mice were challenged with 100 μg heat aggregated ova into the contralateral rear footpad. The increment in footpad thickness 24 hours after challenge was taken as an estimate of the DTH response.

Results

Antigen feeding with varying amounts of ova prior to systemic immunisation with ova was associated with a dose dependent reduction in DTH responses (Figure 7) and in antibody responses (Figure 8) as compared with responses of control animals. Significant reductions in DTH responses were seen after feeding with 10 mg, 25 mg or 40 mg ova, with reductions of 47%, 87% and 88% respectively as compared with control DTH responses.

A similar dose / response pattern was seen in the anti-ova antibody responses of antigen fed animals. Feeding with 2 mg ova was associated with a significant reduction (27%) in antibody titre as compared with controls, and highly significant reductions in antibody titre were seen following feeding with 10 mg, 25 mg or 40 mg ova (47%, 62% and 63% reductions respectively). Intragastric administration of 40 mg ova had no greater effect on the DTH and antibody responses of antigen fed animals than was seen following intragastric administration of 25 mg ova. Percent reductions in DTH and antibody responses were of a similar order of magnitude within each dosage group.

3.2.4 Serum transfer of oral tolerance

The appearance of antigen in the circulation of animals has been demonstrated after antigen feeding (Thomas & Parrott, 1974; Swarbrick etal, 1979). Passive transfer of serum 60 minutes after antigen feeding has been shown to induce immunological tolerance to the fed antigen in recipient mice (Strobel etal, 1983). Subsequent data from this group have suggested that this effect is mediated by the presence of small amounts of absorbed antigen in the serum (Bruce & Ferguson, 1986 b). My own data (section 3.3) showed that peak levels of antigen were detected in the serum 10 - 15 minutes after antigen feeding. In the following experiment I sought to examine the effects of passive transfer of serum 10 minutes after antigen feeding on the subsequent systemic immune response.

Materials and Methods

Animals : Male and female BALB/c mice at 8 weeks of age were used as serum donors. Male mice of the same age and strain were used as serum recipients.

Collection and transfer of serum : Donor mice were fed 25 mg ova dissolved in 0.2 ml distilled water, or 0.2 ml distilled water alone by intragastric intubation, and were bled out 10 minutes after antigen administration. Sera from groups of antigen fed and control (water fed) mice were pooled, and 0.8 ml pooled donor serum was transferred to naive recipient animals by intraperitoneal inoculation (section 2.9).

Parenteral immunisation : Seven days after serum transfer, recipients of antigen fed or control serum were immunised

with 100 μg ova in CFA into one rear footpad. Antibody titres and DTH responses to ova were estimated as described previously 3 weeks after immunisation.

Results

Results of DTH responses 24 and 48 hours after antigen challenge in recipients of serum from ova fed or water fed (control) donors are shown in Figure 9. DTH footpad responses were lower in recipients of serum from ova fed donors than in recipients of serum from water fed donors at 24 hours (31% reduction) and 48 hours (19% reduction) after antigen challenge. In neither instance, however, was the reduction in DTH response significant.

Ovalbumin specific antibody responses in the same animals 3 weeks after immunisation are shown in Figure 10. No difference in antibody response was seen between recipients of serum from water fed and recipients of serum from ova fed animals. Thus serum transfer 10 minutes after intragastric antigen administration did not transfer oral tolerance for either DTH or antibody responses, in spite of peak levels of circulating antigen at this time.

3.2.5 Cell transfer of oral tolerance

To study whether orally induced tolerance to an antigen could be transferred by sensitised spleen cells from donor mice rendered tolerant to the antigen by prior intragastric antigen administration, spleen cells from ova fed mice or water fed controls were transferred to naive syngeneic recipients prior to immunisation of the spleen cell recipients with ova.

Materials and Methods

Animals : Male BALB/c mice at 8 weeks of age were used as spleen cell donors and recipients.

Oral antigen administration : Donor mice were fed 25 mg ova or water alone by intragastric intubation.

Spleen cell transfer : Seven days after intragastric administration, ova fed and water fed donor mice were killed and the spleens removed. Single cell suspensions of spleen cells were prepared as described in section 2.10. Recipient mice were inoculated by the intravenous route with 10^8 pooled donor spleen cells from ova fed or water fed (control) donors.

Parenteral immunisation : Immediately after spleen cell transfer, recipient mice were immunised with 100 μg ova in CFA to one rear footpad. Three weeks after immunisation mice were bled and challenged with antigen as described previously. Anti-ova antibody titres were estimated by ELISA. The increase in footpad thickness 24 and 48 hours after antigen challenge was taken as an estimate of DTH response.

Results

DTH footpad responses were significantly lower in recipients of spleen cells from ova fed donors than in recipients of spleen cells from control (water fed) donors at both 24 and 48 hours after antigen challenge (Figure 11; 48% and 74% reduction respectively). The antibody responses in the same mice, however, showed no difference between recipients of spleen cells from ova fed or from water fed donors (Figure 12). Transfer of spleen cells from mice rendered tolerant by intragastric intubation of

antigen was thus capable of transferring partial tolerance for DTH responses but not for antibody responses.

3.3 SYSTEMIC ABSORPTION OF ANTIGEN FROM THE INTESTINAL TRACT

There is increasing evidence that, following antigen ingestion, a proportion of the antigen penetrates the intestinal epithelial barrier and gains entry to the circulation in an antigenically active form (Thomas & Parrott, 1974; Bruce & Ferguson, 1986 b). Whilst the proportion of ingested antigen absorbed in this way is small, it may be important in the induction of systemic immune responses to antigen. In the following experiment I sought to determine the kinetics of systemic absorption of a protein antigen following intragastric administration of that antigen.

Materials and Methods

Animals : Adult male BALB/c mice were used.

Oral antigen administration : The animals were starved of food for three hours prior to the administration of antigen, but had free access to drinking water during this period. Mice were given single 50 mg doses of ova in 0.2 ml distilled water by intragastric intubation.

Estimation of plasma ovalbumin levels : Venous blood samples (100 µl) were taken by retro-orbital venous plexus puncture immediately prior to and at 15, 30, 45, 60 and 90 minutes following antigen administration. Plasma levels of ova were determined by ELISA as described in section 2.27

Results

There was a rapid appearance of small (ng) amounts of ova in the circulation following intragastric ova administration (Figure 13). Peak circulating levels were seen at 15 minutes after intragastric administration, and fell away rapidly thereafter.

Subsequent experiments using bleedings from individual animals (as opposed to sequential bleeding of mice) indicated that peak circulating levels of antigen were seen 10 - 15 minutes after intragastric antigen administration (data not presented). The levels of absorbed antigen varied between experiments, however, and in one experiment no absorbed antigen was detected following intragastric administration of 50 mg ova per mouse. Overall these results indicate that small but variable amounts of antigen appear rapidly in the circulation following the intragastric administration of protein antigen in solution to mice.

3.4 THE EFFECT OF ORAL IMMUNISATION ON SYSTEMIC ANTIGEN ELIMINATION

The mononuclear phagocytic system (MPS) is the major site of clearance of antigen from the circulation (DiLuzio & Morrow, 1971). Stimulation of the MPS has been shown to abrogate both systemically induced (Yoshikai etal, 1981) and orally induced (Mowat & Parrott, 1983) tolerance. In the following experiments I have investigated whether differences in the ease of induction of oral tolerance might be associated with differences in the clearance of

antigen from the circulation, and have examined whether prior oral antigen administration affected the rate of clearance of circulating antigen.

3.4.1 Systemic antigen elimination in naive mice

To study the role of MPS clearance of antigen in the induction of oral tolerance, the clearance of antigen from the circulation was studied in two strains of mice which differed in their ease of induction of oral tolerance. The BALB/c and BALB/k strains were chosen, as previous experiments had suggested that these strains differed markedly in their ease of oral tolerance induction.

Materials and Methods

Animals : Male and female mice of the BALB/c and BALB/k strains were used at 12 weeks of age.

Measurement of systemic antigen elimination : Systemic antigen elimination was estimated by the rate of clearance of ^{125}I-ova (expressed as % counts of 10 minute sample) from the circulation following the intravenous inoculation of 200 ng ^{125}I-ova, as described in section 2.24. This dose was chosen to fall within the range of antigen absorbed into the circulation following a single 50 mg intragastric dose of antigen.

Results

Following the intravenous inoculation of ^{125}I-ova into naive recipients, the levels of radioactivity in the blood fell progressively over the 24 hour period from the time of inoculation. The fall in log % counts following inoculation showed a linear relationship with log time

post inoculation, with a highly significant degree of correlation. Correlation coefficients (r) for the relationship between log % counts and log time were 0.990 for BALB/c males and females, 0.970 for BALB/k males and 0.985 for BALB/k females. A representative antigen elimination curve is shown in Figure 14, for the clearance of ^{125}I-ova from the circulation of naive BALB/k male and female mice.

3.4.2 Systemic antigen elimination following oral antigen administration

To study whether oral antigen administration affected the systemic elimination of antigen, the clearance of ^{125}I-ova from the circulation was studied in mice fed antigen in their drinking water for 4 weeks, and in water fed control mice.

Materials and Methods

Animals : Male and female BALB/c and BALB/k mice were used at 8 weeks of age.

Oral immunisation : Mice received 0.05% (w/v) ova in their drinking water (equivalent to approximately 2.5 mg per mouse per day) for 28 days. The mice used in the previous experiments were used as water fed, age and sex matched controls.

Measurement of systemic antigen elimination : On day 29 the mice were given 200 ng ^{125}I-ova by intravenous inoculation, and the clearance of antigen from the circulation was estimated as described in the previous experiment.

Results

Oral administration of 0.05% ova in drinking water for 28 days prior to the intravenous inoculation of ^{125}I-ova had no effect on the clearance of antigen from the circulation, as compared with antigen elimination in water fed control mice. Representative antigen clearance curves are shown in Figures 15 and 16 for antigen fed and control BALB/k and BALB/c male mice respectively. There was no difference in the rate of clearance of ^{125}I-ova in antigen fed as compared with control mice. Antigen levels fell over 24 hours from the time of inoculation, and the decrease in % counts followed a log - log linear relationship, with correlation coefficients (r) of 0.993 (BALB/c male), 0.995 (BALB/c female), 0.993 (BALB/k male) and 0.998 (BALB/k female).

3.5 CONCLUSIONS

The results of the experiments described in this chapter confirm the observation that single doses of antigen administered by the intragastric route may lead to a reduction in systemic DTH and antibody responses upon subsequent parenteral immunisation, as compared with responses of water fed controls. Of 11 inbred and 2 other strains of mice examined, no strain of mouse showed an increase in DTH or antibody response suggestive of systemic priming following oral antigen administration.

Two strains of mice (BALB/b and B10.BR) were identified in which antigen feeding was not associated with significant reductions in systemic DTH or antibody responses. In one other strain (T0 [high]) antigen feeding

at the higher (25 mg) dose used was associated with a reduction in systemic DTH response but not in the antibody response. "Split" oral tolerance was seen following intragastric administration of 2 mg ova prior to immunisation in DBA/2 and NIH mice.

In all strains of mice, with the exceptions of BALB/b, B10.BR and TO [high], feeding with 25 mg ova prior to immunisation was associated with reductions in both DTH and antibody responses as compared with controls. The results suggested that the effect of antigen feeding on the systemic immune response was dependent not only the genetic basis of the animal, but also on the dose of oral antigen given. This observation was confirmed by dose / response studies in TO [low] mice, in which increasing doses of oral antigen up to 25 mg were associated with increasing reductions in DTH and antibody responses as compared with water fed controls. No further reduction in DTH and antibody responses was seen when the dose of oral antigen was increased beyond 25 mg antigen in this strain of mouse.

Passive transfer of spleen cells from antigen fed animals to naive recipients was associated with a reduction in the systemic DTH responses, as compared with recipients of control spleen cells, following subsequent systemic immunisation. Spleen cell transfer from antigen fed animals, however, had no effect on the antibody responses of mice to systemic immunisation.

Transfer of serum from antigen fed animals 10 minutes after antigen feeding had no effect on systemic antibody or DTH responses in spite of peak levels of circulating

antigen in the serum at this time.

The rate of clearance of intravenously inoculated antigen from the circulation did not differ between BALB/c and BALB/k mice, and oral administration of antigen in the drinking water for 4 weeks prior to intravenous antigen administration did not affect the rate of clearance of antigen from the circulation in either of these strains.

TABLE 3.

Strain	24 hour increase in footpad thickness (mm)
BALB/c	0.376 + 0.028
DBA/2	0.255 + 0.028
BALB/k	0.257 + 0.038
B10.BR	0.242 + 0.032
CBA	0.107 + 0.009
C3H/HeOla	0.253 + 0.050
BALB/b	0.286 + 0.047
C57BL/10	0.084 + 0.012
Beige	0.481 + 0.037
A	0.433 + 0.034
NIH	0.273 + 0.027

Table 3 : Systemic delayed type hypersensitivity responses in control (water fed) mice of 11 inbred strains, 3 weeks after immunisation with 100 μg ova in CFA. Results are expressed as mean specific increments in footpad thickness (mm) 24 hours after challenge with 100 μg ova in saline + 1 standard error.

TABLE 4.

Strain	Dose of antigen	DTH response	Ab response
BALB/c	2 mg ova ig	52 + 6	52 + 5
	25 mg ova ig	15 + 3	10 + 3
DBA/2	2 mg ova ig	31 + 15	136 + 23
	25 mg ova ig	14 + 4	18 + 11
BALB/k	2 mg ova ig	82 + 10	60 + 12
	25 mg ova ig	54 + 7	33 + 13
B10.BR	2 mg ova ig	49 + 14	65 + 8
	25 mg ova ig	59 + 17	62 + 5
CBA	2 mg ova ig	19 + 7	59 + 10
	25 mg ova ig	16 + 10	9 + 3
C3H/HeOla	2 mg ova ig	57 + 9	52 + 12
	25 mg ova ig	44 + 10	35 + 19
BALB/b	2 mg ova ig	95 + 13	121 + 12
	25 mg ova ig	100 + 15	80 + 10
C57BL/10	2 mg ova ig	113 + 50	124 + 9
	25 mg ova ig	36 + 23	54 + 6
Beige	2 mg ova ig	29 + 8	44 + 10
	25 mg ova ig	16 + 10	37 + 12
A	2 mg ova ig	37 + 12	74 + 12
	25 mg ova ig	10 + 5	27 + 8
NIH	2 mg ova ig	107 + 9	26 + 13
	25 mg ova ig	26 + 10	10 + 5

Table 4 : The effects of antigen feeding on systemic delayed type hypersensitivity (DTH) and antibody (Ab) responses to parenteral immunisation in 11 inbred strains of mice. Results, expressed as a percentage of the mean of control (water fed, immunised) mice, represent mean + 1 standard error DTH and Ab responses 3 weeks after immunisation with 100 μg ova in CFA, in mice fed 2mg or 25 mg ova by intragastric (ig) intubation 14 days prior to immunisation.

FIGURE 2

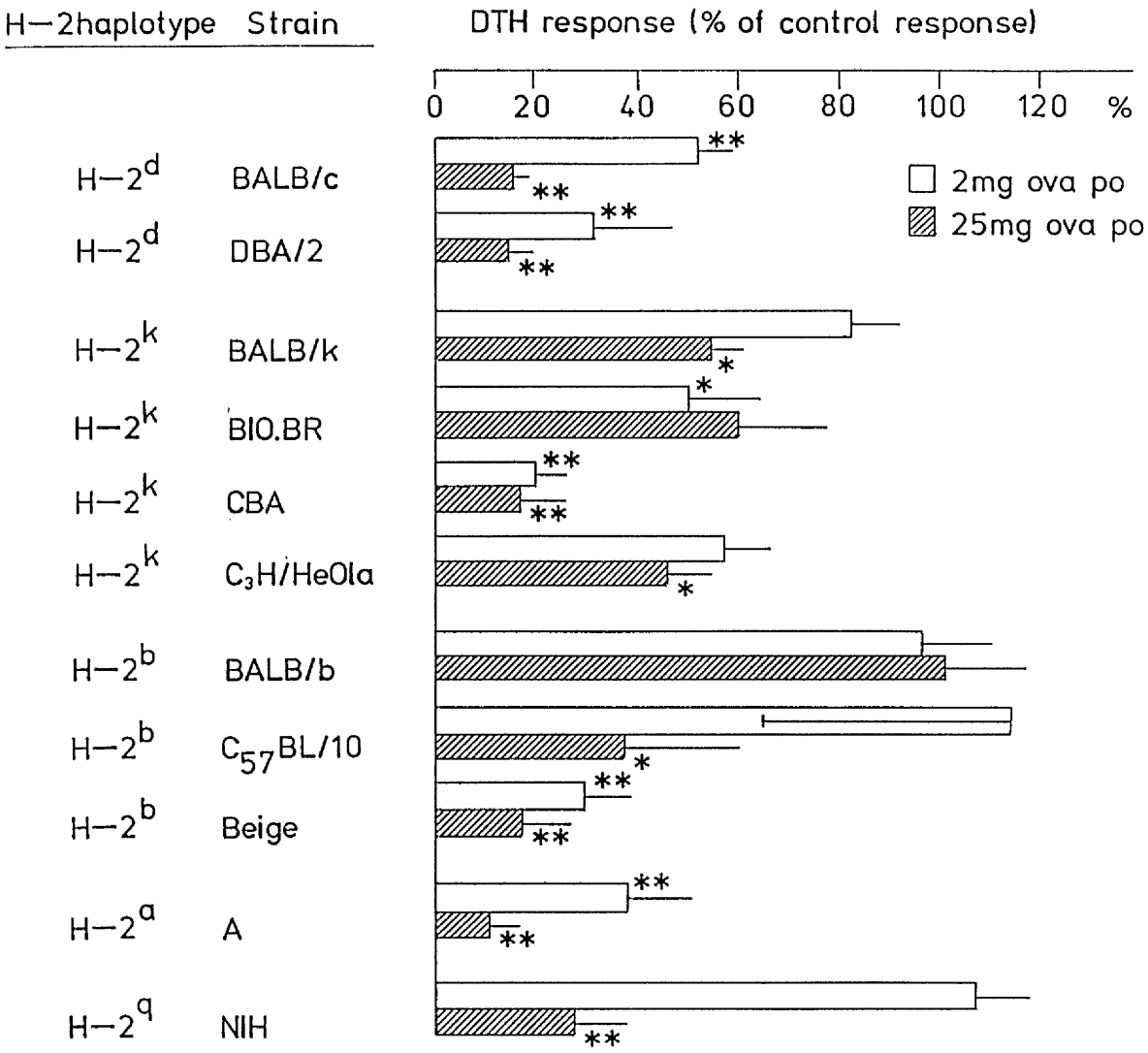

Figure 2 : Systemic delayed type hypersensitivity (DTH) responses 3 weeks after immunisation with 100 µg ova in CFA in mice of 11 inbred strains given 2 mg or 25 mg ova by intragastric intubation 14 days prior to immunisation. Bars represent mean specific increments in footpad thickness, expressed as percent of mean control (water fed, immunised) response, 24 hours after challenge with 100 µg ova in saline + 1 standard error.

* $p < 0.05$; ** $p < 0.01$ (Student's t test) as compared with control responses.

FIGURE 3

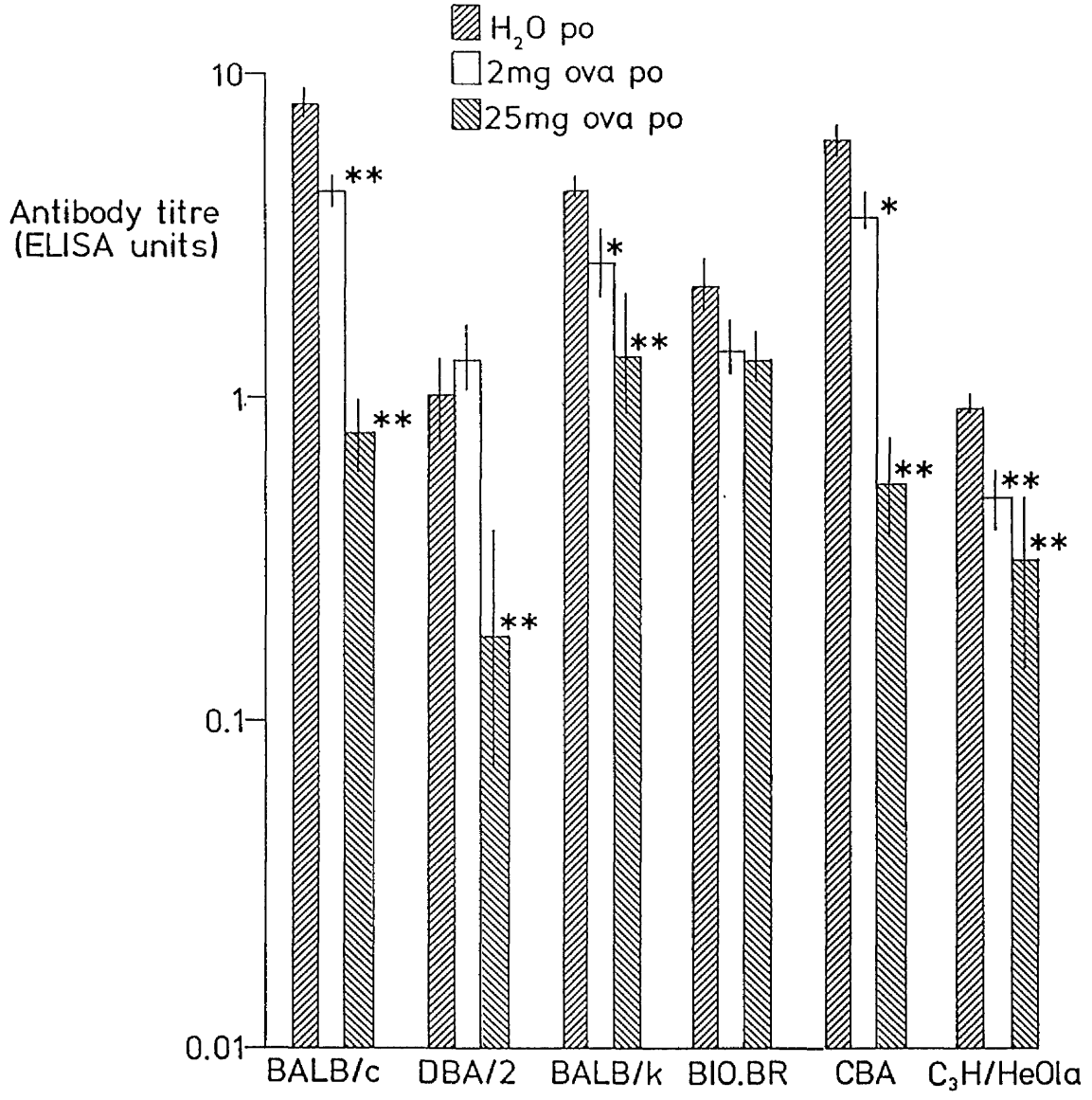

Figure 3 : Serum anti-ova antibody titres 3 weeks after immunisation with 100 µg ova in CFA in mice of 6 inbred strains given 2 mg or 25 mg ova, or water alone (control), by intragastric intubation 14 days prior to immunisation. Bars represent mean antibody titre (ELISA units) + 1 standard error.

* $p < 0.05$; ** $p < 0.01$ (Wilcoxon rank sum test) as compared with control responses.

FIGURE 4

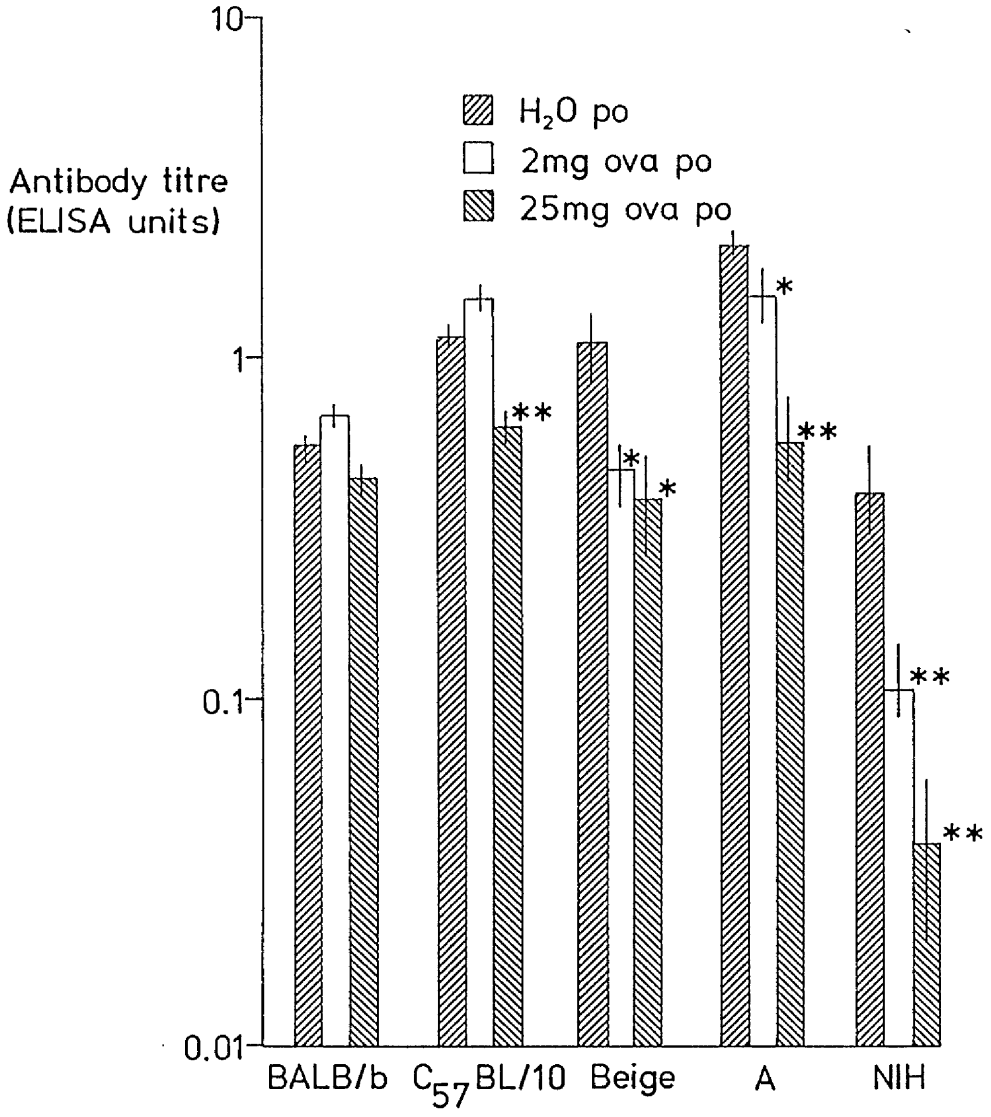

Figure 4 : Serum anti-ova antibody titres 3 weeks after immunisation with 100 µg ova in CFA in mice of 5 inbred strains given 2 mg or 25 mg ova, or water alone (control), by intragastric intubation 14 days prior to immunisation. Bars represent mean antibody titre (ELISA units) + 1 standard error.

* $p < 0.05$; ** $p < 0.01$ (Wilcoxon rank sum test) as compared with control responses.

FIGURE 5

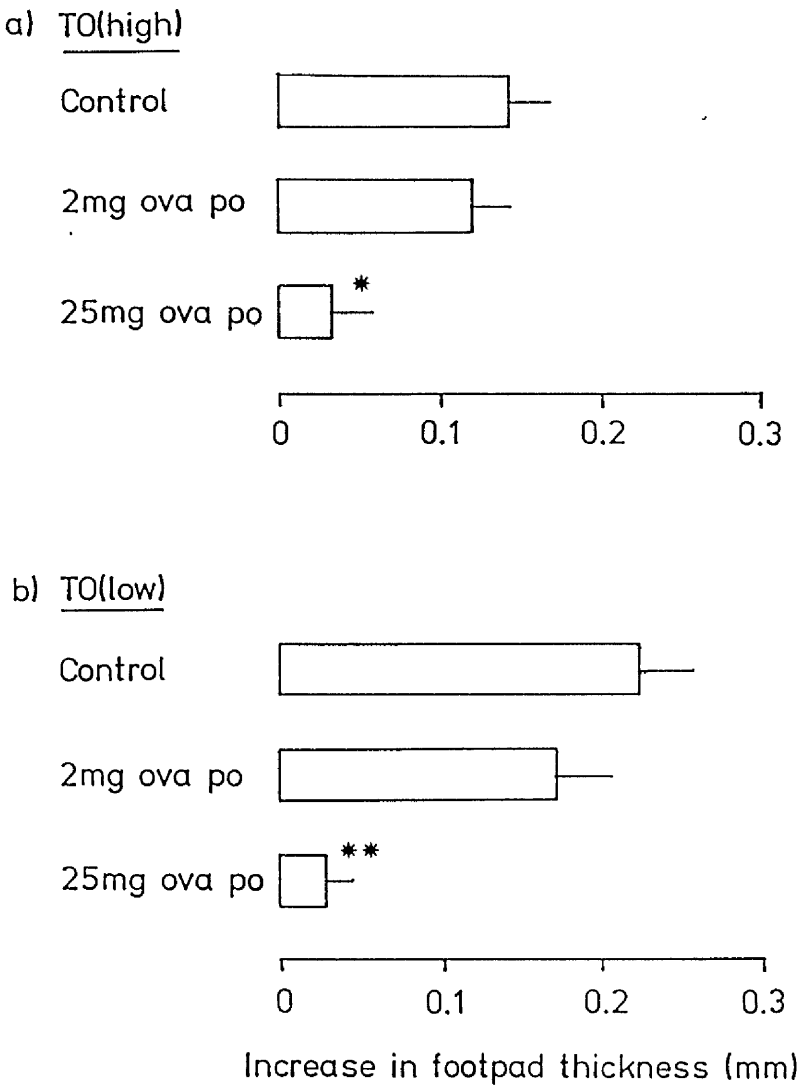

Figure 5 : Systemic delayed type hypersensitivity responses 3 weeks after immunisation with 100 µg ova in CFA in TO [high] (Figure 5a) and in TO [low] (Figure 5b) mice given 2 mg or 25 mg ova, or water alone (control), by intragastric intubation 14 days prior to immunisation. Bars represent mean increments in footpad thickness (mm) 24 hours after challenge with 100 µg ova in saline + 1 standard error.

* $p < 0.05$; ** $p < 0.01$ (Student's t test) as compared with control responses.

FIGURE 6

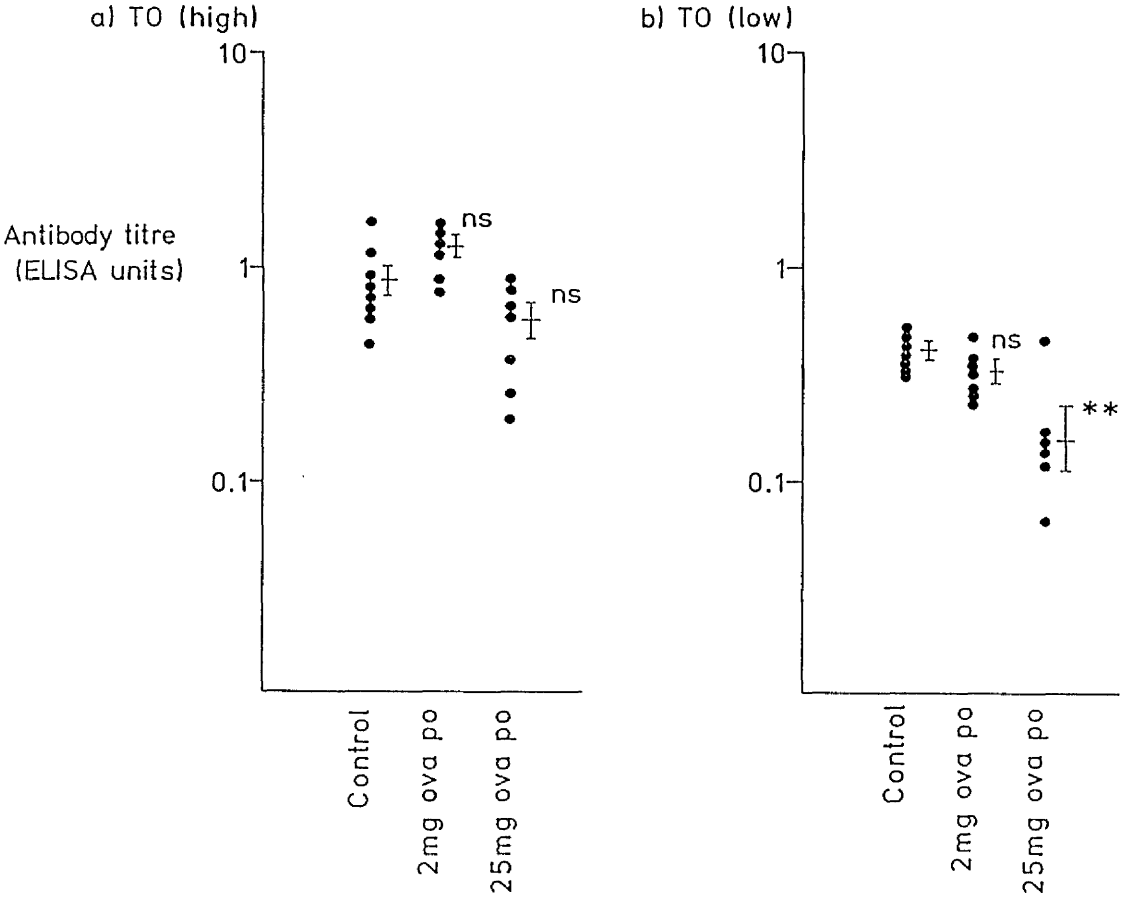

Figure 6 : Serum anti-ova antibody titres 3 weeks after immunisation with 100 µg ova in CFA in T0 [high] (Figure 6a) and T0 [low] (Figure 6b) mice given 2 mg or 25 mg ova, or water alone (control), by intragastric intubation 14 days prior to immunisation. Circles represent antibody titres (ELISA units) of individual experimental mice. Bars represent group mean ± 1 standard error.

** $p < 0.01$ (Wilcoxon rank sum test) as compared with control responses.

FIGURE 7

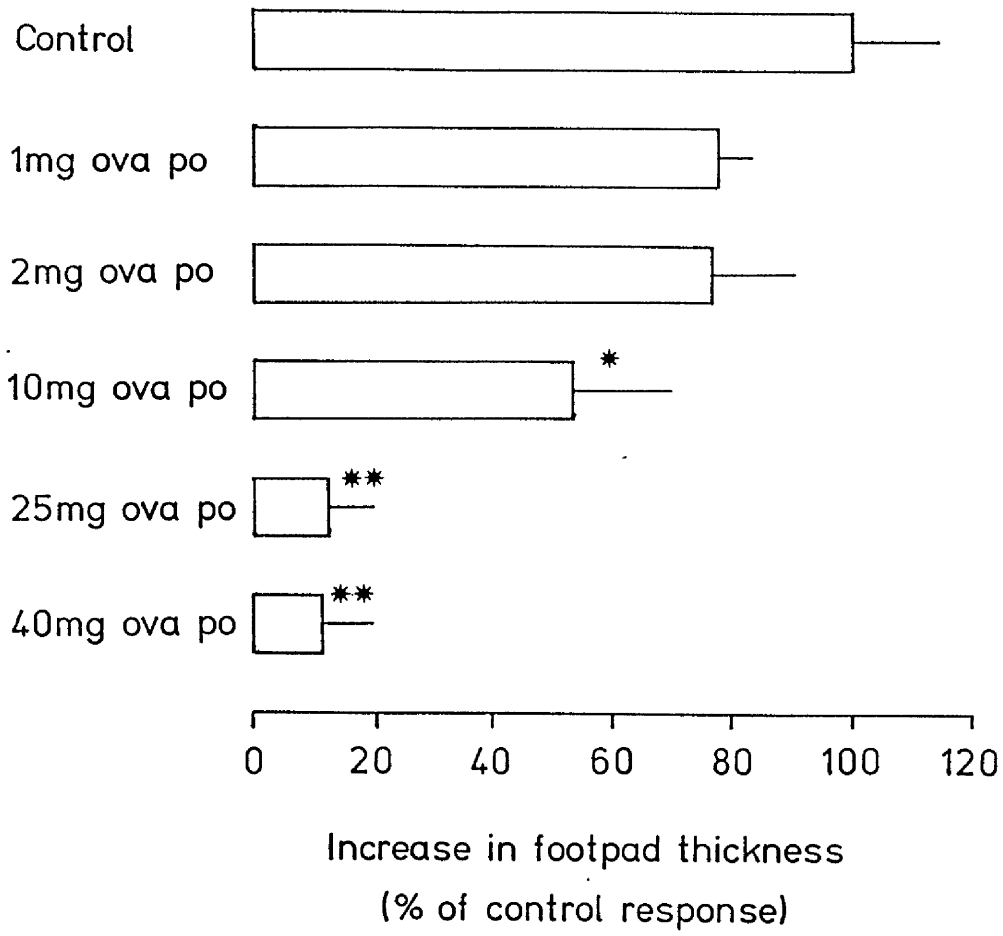

Figure 7 : Systemic delayed type hypersensitivity responses 3 weeks after immunisation with 100 µg ova in CFA in TO [low] mice given 1 mg, 2 mg, 10 mg, 25 mg or 40 mg ova, or water alone (control), by intragastric intubation 14 days prior to immunisation. Bars represent mean increments in footpad thickness, expressed as percentage of the mean control response, 24 hours after challenge with 100 µg ova in saline + 1 standard error.

* $p < 0.05$; ** $p < 0.01$ (Student's t test) as compared with control responses.

FIGURE 8

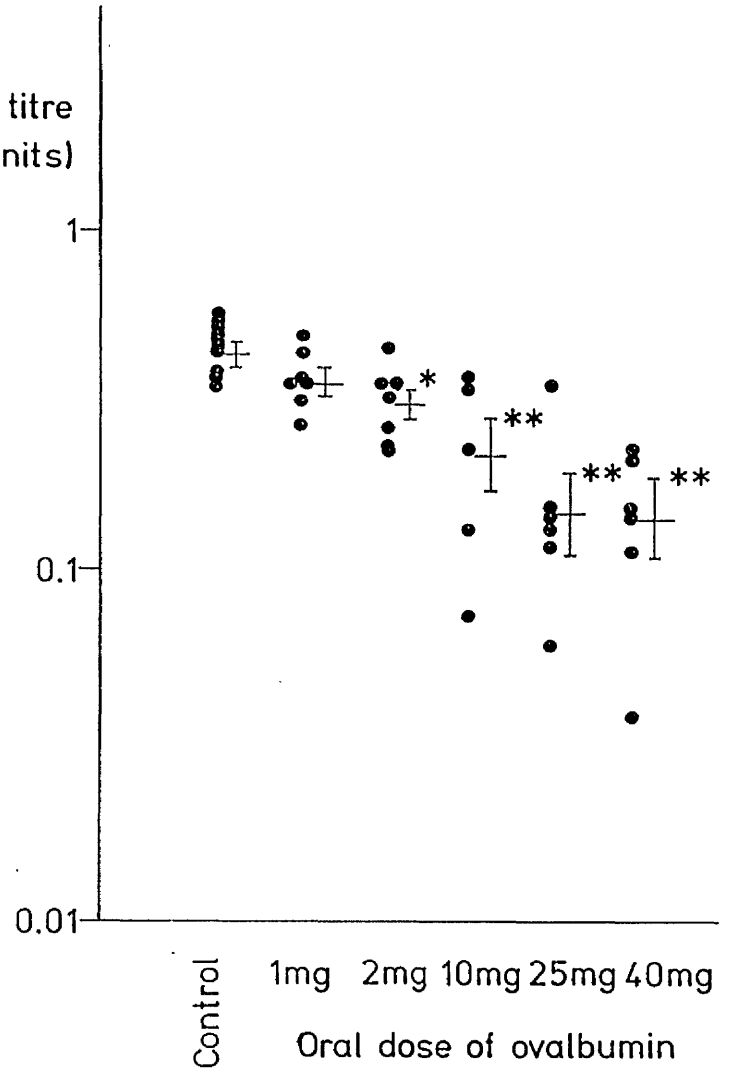

Figure 8 : Serum anti-ova antibody titres 3 weeks after immunisation with 100 μg ova in CFA in TO [low] mice fed 1 mg, 2 mg, 10 mg, 25 mg or 40 mg ova, or water alone (control), by intragastric intubation 14 days prior to immunisation. Circles represent antibody titres (ELISA units) of individual experimental mice. Bars represent group mean ± 1 standard error.

* $p < 0.05$; ** $p < 0.01$ (Wilcoxon rank sum test) as compared with control responses.

FIGURE 9

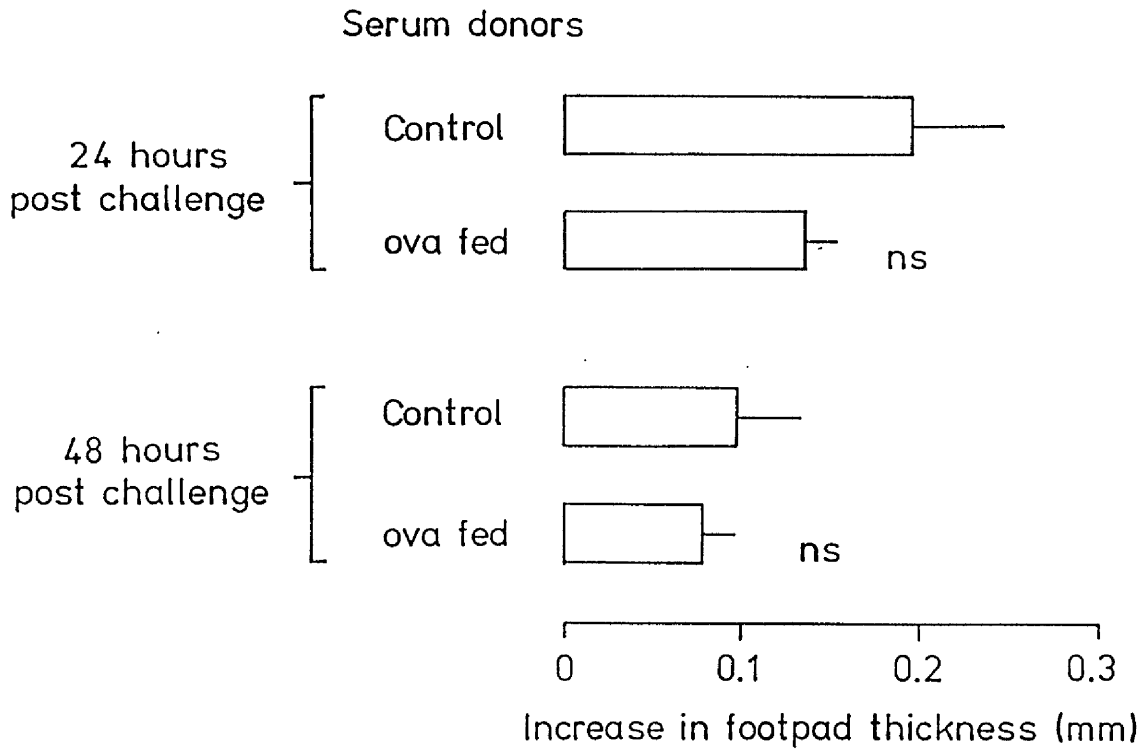

Figure 9 : Systemic delayed type hypersensitivity responses 3 weeks after immunisation with 100 µg ova in CFA in TO [low] mice receiving 0.8 ml pooled serum, collected 10 minutes after intragastric administration of 25 mg ova, or of water alone (control), to donor mice, and passively transferred 7 days before immunisation of serum recipients. Bars represent mean increments in footpad thickness (mm) 24 and 48 hours after challenge with 100 µg ova in saline + 1 standard error.

FIGURE 10

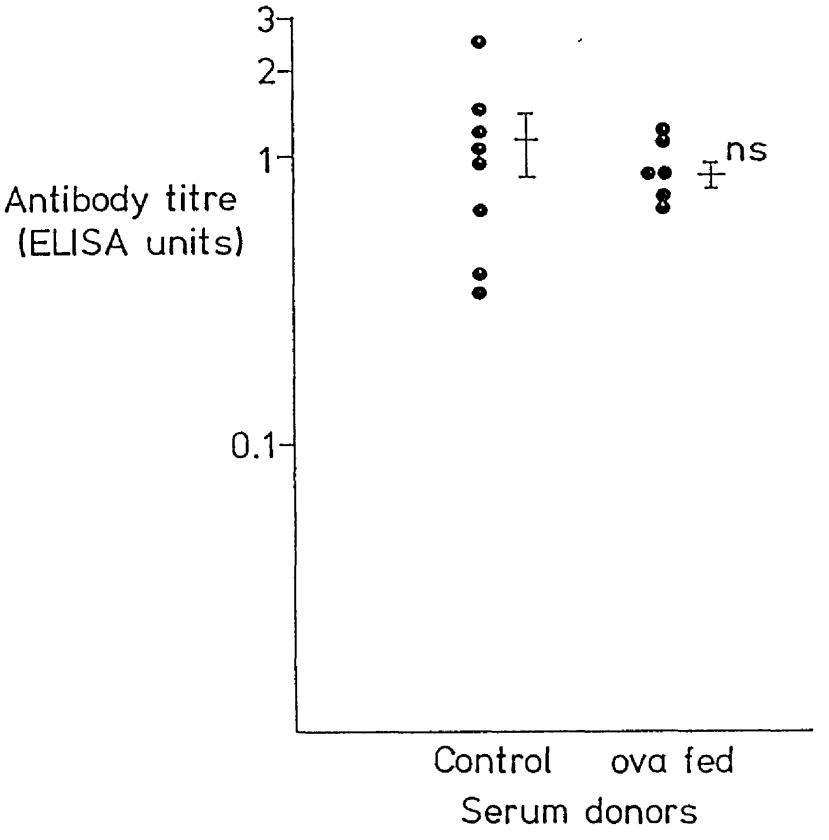

Figure 10 : Serum anti-ova antibody titres 3 weeks after immunisation with 100 µg ova in CFA in T0 [low] mice receiving 0.8 ml pooled serum, collected 10 minutes after intragastric administration of 25 mg ova, or of water alone (control), to donor mice, and passively transferred 7 days before immunisation of serum recipients. Circles represent antibody titres (ELISA units) of individual experimental mice. Bars represent group mean ± 1 standard error.

FIGURE 11

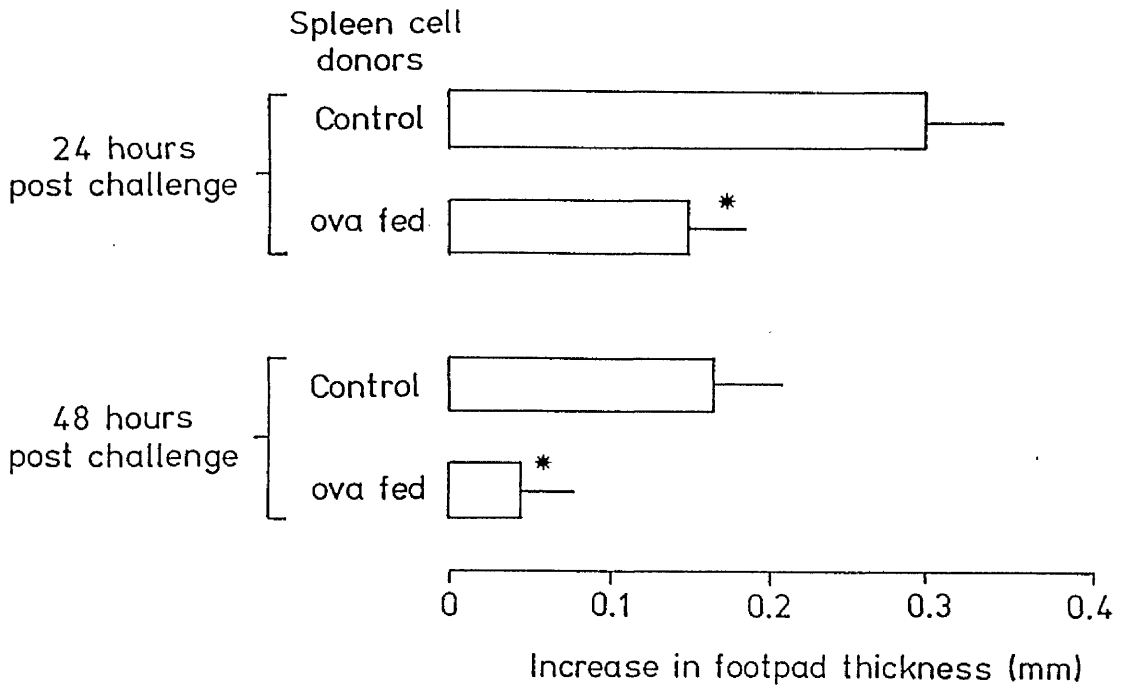

Figure 11 : Systemic delayed type hypersensitivity responses in BALB/c mice receiving by intravenous inoculation 10^8 spleen cells from donor mice rendered tolerant to ova by intragastric administration of 25 mg ova, or from donor mice receiving intragastric water alone (control). Spleen cell recipients were immunised with 100 µg ova in CFA immediately after spleen cell transfer, and delayed type hypersensitivity responses were estimated 3 weeks after immunisation, by intradermal challenge with 100 µg ova in saline into one rear footpad. Bars represent mean increments in footpad thickness (mm) 24 and 48 hours after challenge + 1 standard error.

* $p < 0.05$ (Student's t test) as compared with control responses.

FIGURE 12

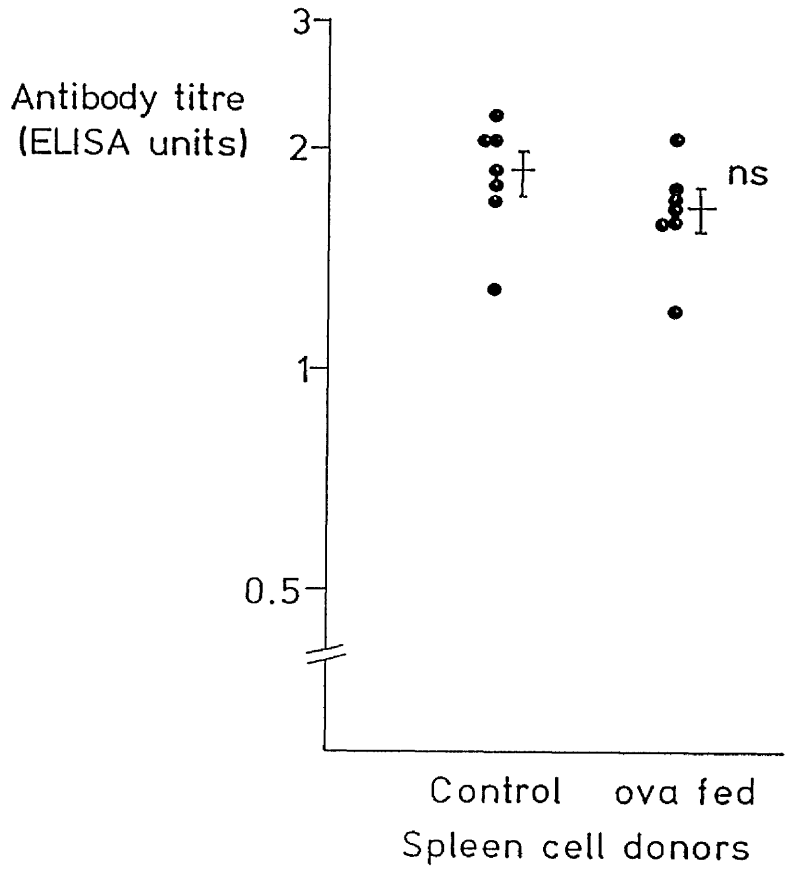

Figure 12 : Serum anti-ova antibody titres in mice receiving by intravenous inoculation 10^8 spleen cells from donor mice rendered tolerant to ova by intragastric administration of 25 mg ova, or from donor mice receiving intragastric water alone (control). Spleen cell recipients were immunised with 100 µg ova in CFA immediately after spleen cell transfer, and serum antibody responses were estimated 3 weeks after immunisation. Circles represent antibody titres (ELISA units) in individual experimental mice. Bars represent group mean ± 1 standard error.

FIGURE 13

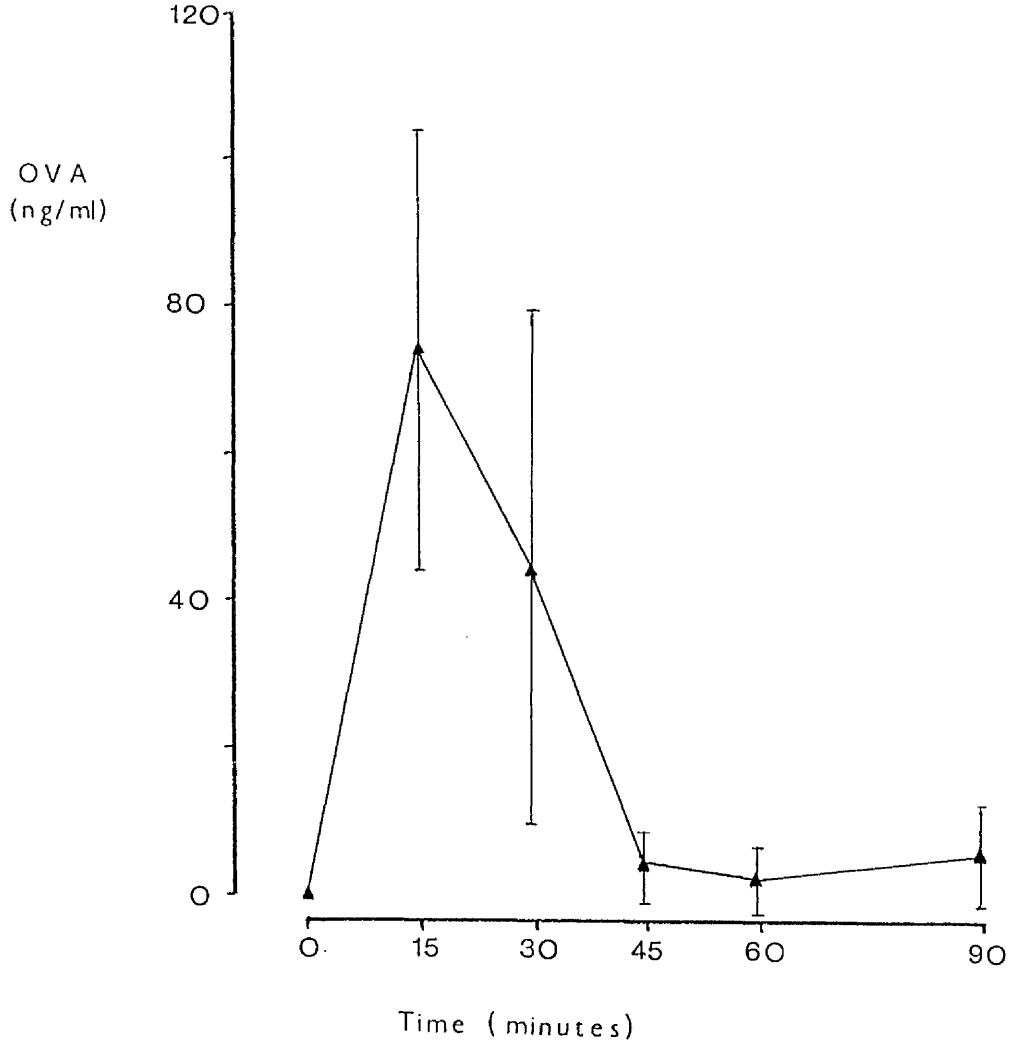

Figure 13 : Antigen absorption from the intestinal tract. Sequential serum levels of ova (ng/ml) in naive mice, following the intragastric administration of single, 50 mg doses of ova (mean ± 1 standard error).

FIGURE 14

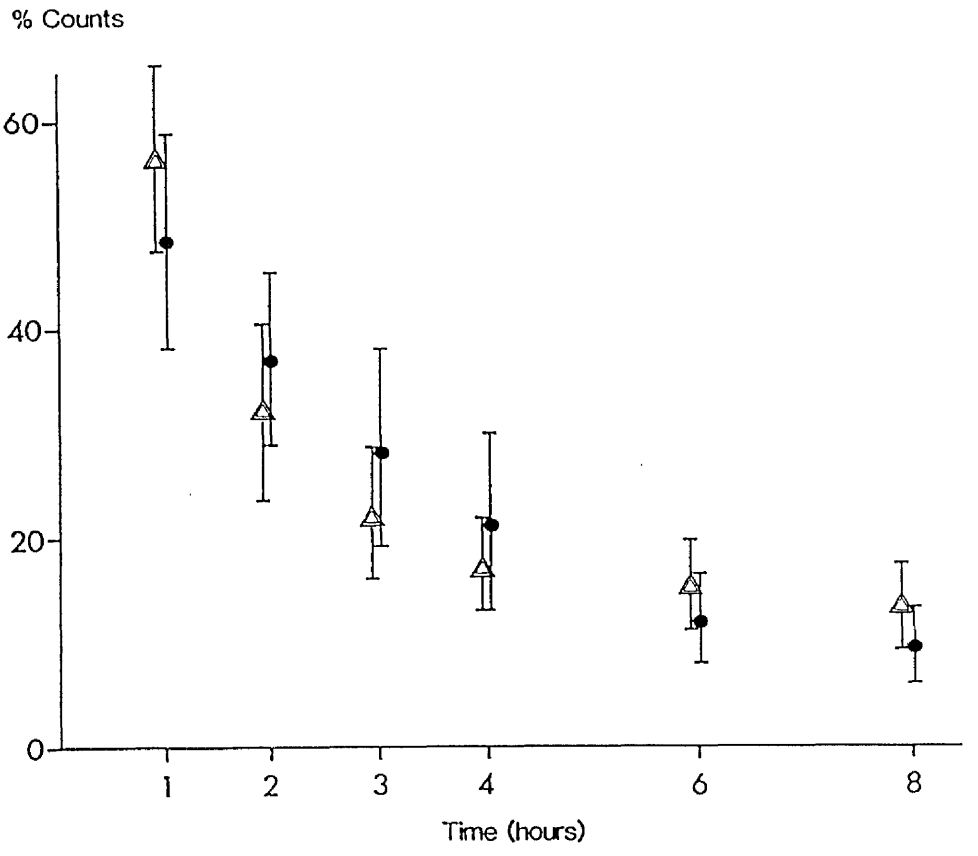

Figure 14 : Clearance of ^{125}I-ova from the circulation of naive male (circles) and female (triangles) BALB/k mice following the intravenous inoculation of 200 ng ^{125}I-ova. Results are expressed as percent of mean 10 minute counts per minute (% counts) $\pm$ 1 standard error.

FIGURE 15

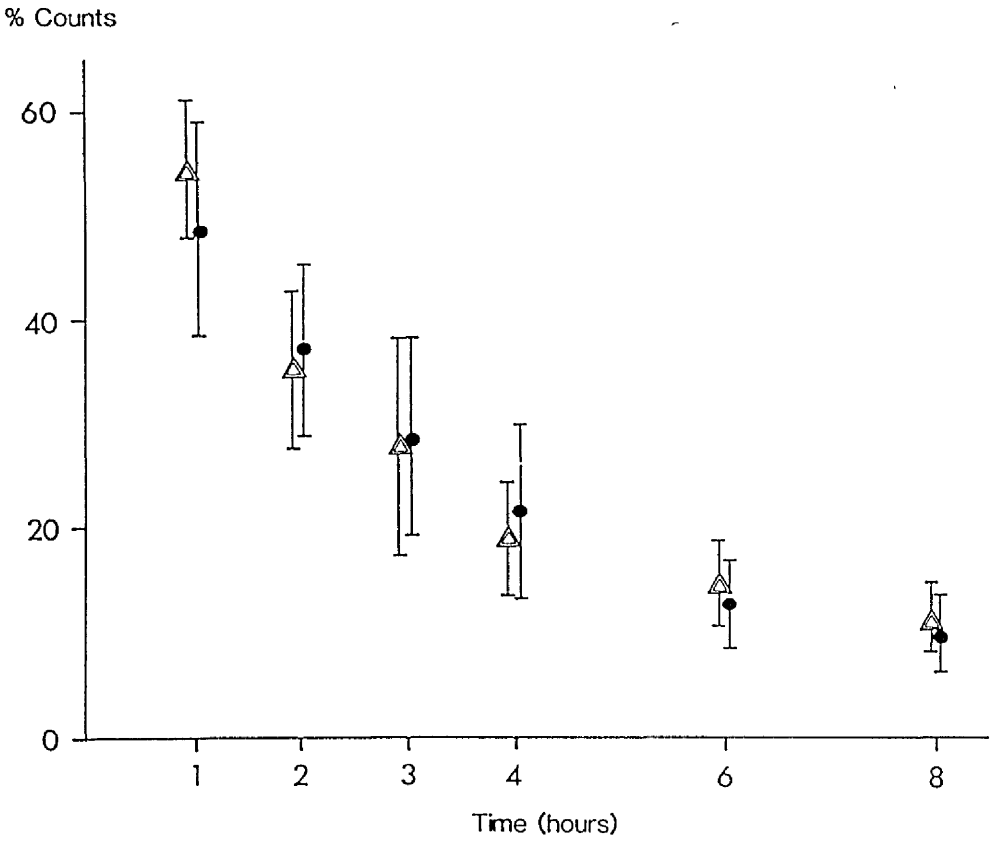

Figure 15 : Clearance of ^{125}I-ova from the circulation of male BALB/k mice receiving ova (0.05%) in the drinking water for 4 weeks (triangles), and in age matched naive controls (circles), following the intravenous inoculation of 200 ng ^{125}I-ova. Results are expressed as percent of mean 10 minute counts per minute (% counts) $\pm$ 1 standard error.

FIGURE 16

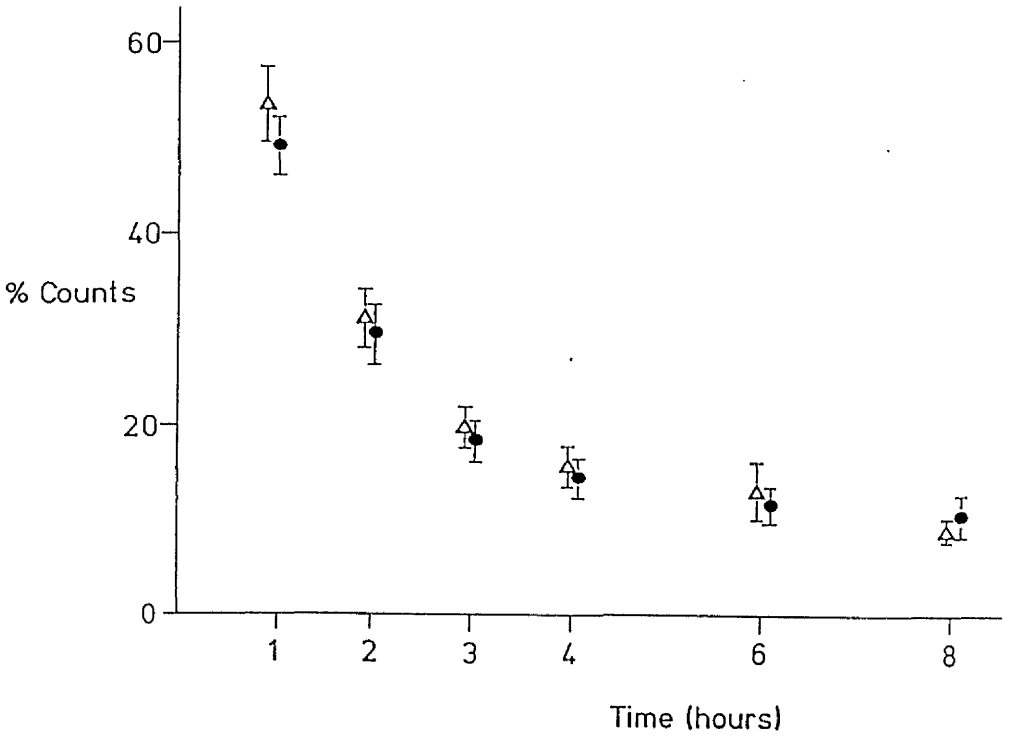

Figure 16 : Clearance of ^{125}I-ova from the circulation of male BALB/c mice receiving ova (0.05%) in the drinking water for 4 weeks (triangles), and in age matched naive controls (circles), following the intravenous inoculation of 200 ng ^{125}I-ova. Results are expressed as percent of mean 10 minute counts per minute (% counts) $\pm$ 1 standard error.

CHAPTER FOUR

ORAL IMMUNISATION AND THE INDUCTION OF EXPERIMENTAL IGA NEPHROPATHY

4.1 INTRODUCTION

In 1983 Emancipator, Gallo and Lamm described a model of experimental IgA nephropathy induced by oral immunisation in mice. BALB/c mice were given protein antigens in their drinking water for 14 weeks. The regime resulted in oral immunisation of antigen fed animals, with increased numbers of antigen-specific IgA producing plasma cells at mucosal sites and specific IgA antibodies in the serum. The majority of mice developed glomerular mesangial immune deposits involving codeposition of IgA and the immunising antigen (Emancipator etal, 1983 a + b). The report presented the first direct evidence for an association between dietary antigen and IgA deposits in IgA nephropathy, and suggested a basis for further study, both of the mechanisms and determinants of glomerular immune deposits in IgA nephropathy, and of the relationship between dietary antigen and glomerulonephritis.

In a separate model of experimental IgA nephropathy in rats rendered cirrhotic by the administration of carbon tetrachloride (CCl4), Gormly etal (1981) proposed that defective hepatic sequestration of IgA immune complexes in cirrhotic animals allowed the passage of gut associated antigens in antigen-antibody complexes into the systemic circulation, resulting in increased levels of polymeric IgA and IgA-IC in the circulation and the deposition of such complexes in the kidney.

In the following chapter I have examined the effects of oral immunisation and of experimental liver damage on the

induction of experimental IgA nephropathy in mice, with a view to extending observations of Emancipator etal (1983 a + b) and Gormly etal (1981) on the role of dietary antigens in the pathogenesis of glomerulonephritis.

4.2 ORAL IMMUNISATION AND IGA NEPHROPATHY

4.2.1 The effect of administration via the drinking water of BGG to mice

It has been reported that administration of protein antigen via the drinking water may be associated with oral immunisation and the presence of mesangial IgA deposits in mice (Emancipator etal, 1983 a + b). I sought to reproduce the induction of IgA glomerular immune deposits in mice by the administration of the antigen BGG in their drinking water.

Materials and Methods

Animals : 8 week old male BALB/c mice were used.

Oral antigen administration : Two groups of mice were given BGG (0.1% w/v) in their drinking water for 12 weeks. In one group the antigen solution was prepared fresh and changed daily. In the other group the antigen solution was changed every third day. A third group of mice, acting as controls, received plain drinking water.

Sacrifice and tissue processing : After completion of the antigen administration regime, all mice were maintained with free access to plain drinking water and food for a further 48 hours. The mice were then bled out, and the right kidneys were taken for immunofluorescent examination as described in section 2.28. Unfixed cryostat sections were stained for the presence of IgG, IgA, IgM and C3 by

direct immunofluorescence, and for bovine IgG by indirect immunofluorescence.

Results

The results of immunofluorescent examination of unfixed cryostat kidney sections from mice given BGG via their drinking water for 12 weeks are shown in Table 5. Trace amounts of IgG were found in the kidneys of over 50% of mice examined. There was no difference in staining patterns for IgG between control and BGG fed mice. IgM was detected in all kidney sections examined. Again there was no difference in the patterns of staining between control and BGG fed mice. No IgA was detected in 5 of 6 control mice (Plate 6). In 1 of 6 control mice, trace amounts of IgA were detected. In kidney sections from all of 11 BGG fed mice, however, IgA was detected ($p < 0.001$; Fisher's exact test) in the mesangium and segmentally in capillary loops (Table 5; Plate 7). There was no difference in the degree of IgA staining between mice fed BGG changed daily and mice fed BGG which was changed every third day. The presence of C3 was detected in trace amounts in only 1 BGG fed mouse, and was not detected in any control animal. The presence of bovine IgG was not detected in the kidney of any control or antigen fed mouse. Whilst these experiments indicated an association between oral antigen administration and glomerular IgA deposits, it should be noted that the amounts of glomerular IgA seen in antigen fed mice were no greater than were seen in "normal" mice of similar age of other strains (Plate 8).

4.2.2 Oral administration of ovalbumin via the drinking water or intermittently by gastric intubation

The administration of antigen via the drinking water is likely to provide a persistent antigenic stimulation at the intestinal mucosa. To investigate whether such persistent antigenic stimulation of the intestinal mucosa was required for the induction of experimental IgA nephropathy by oral antigen administration, mice were given ova either via their drinking water or intermittently by weekly intragastric intubation to the same total dose of ova.

Materials and Methods

Animals : Female BALB/c mice were used at 6-8 weeks of age.

Oral antigen administration : One group of mice received ova (0.05% w/v) via the drinking water for 14 weeks. A second group of mice had free access to plain drinking water, but were given 16 mg ova in 0.2 ml distilled water by intragastric intubation at weekly intervals for 14 weeks (15 doses). The intragastric dose of antigen was calculated to give the same total dose of ova over the antigen feeding period, based on the assumption that mice receiving ova in their drinking water had an average daily fluid intake of 5 ml per mouse. A group of control animals had free access to plain drinking water and received 0.2 ml distilled water by intragastric intubation at weekly intervals.

Sacrifice and tissue processing : On completion of the antigen feeding regime, all animals were maintained for a

further 48 hours with free access to plain water and food. The mice were bled out, and the right kidneys removed for immunofluorescent examination and the left kidneys for light microscopic examination (section 2.29).

Immunofluorescence : Methanol fixed cryostat sections were stained for the presence of IgG and IgA by direct immunofluorescence and for ova by indirect immunofluorescence as described in section 2.28.

Results

Light microscopy : No pathological changes were observed by light microscopy in the kidneys of any animal.

Immunofluorescence : Trace amounts of IgG were detected in a proportion of kidneys of animals from all groups as described above. There was no difference in the staining pattern for IgG between control animals and mice of either ova fed group. Trace amounts of IgA were detected in the kidneys of 1 of 6 control animals, 3 of 8 animals receiving ova in the drinking water and in 1 of 6 animals receiving ova by gastric intubation. Trace amounts of ova were noted only in 2 (of 8) animals receiving ova in their drinking water. None of the differences in staining patterns noted above were significant.

4.2.3 Oral antigen administration in C3H/HeJ mice

The presence of an immunogenetic abnormality of mucosal immunity in patients with IgA nephropathy has been proposed (Berthoux etal, 1979). The C3H/HeJ strain of mice expresses two traits which may be of relevance in investigating this hypothesis. Firstly they have unusually high levels of IgA production following oral immunisation

(Kiyono etal, 1980), and secondly they are deficient in the induction of oral tolerance to SRBC (Kiyono etal, 1982). To test the possible role of these genetic traits in the induction of experimental IgA nephropathy by oral immunisation, C3H/HeJ mice were given ovalbumin or SRBC by the oral route for prolonged periods. Congenic C3H/HeOla mice do not express these abnormalities, and were used as experimental controls in the following experiments.

Materials and Methods

Animals : 8 week old male C3H/HeJ mice received ova or SRBC as described. 8 week old male C3H/HeOla were used as controls for oral administration of ova. 8 week old female C3H/HeOla mice were used as controls for SRBC feeding.

Oral antigen administration : One group each of C3H/HeJ and C3H/HeOla mice received ova (0.1% w/v) in their drinking water for 14 weeks. One group of each strain was maintained on ordinary drinking water but was given 10^9 SRBC in 0.2 ml saline daily by intragastric intubation for 14 weeks. A third group of C3H/HeOla mice was maintained on ordinary drinking water throughout. All groups had free access to normal diet.

Sacrifice and tissue processing : After completion of the antigen feeding regime, or 24 hours after the final dose of SRBC, the mice were bled out. The right kidneys were removed for immunofluorescent examination and the left kidneys for light microscopy.

Immunofluorescence : Unfixed cryostat sections were stained for the presence of IgG, IgA, IgM, C3 and ova (water maintained HeOla, and ova fed HeJ and HeOla) as described previously. Sections from SRBC fed mice were

stained for the presence of IgA, C3 and mouse immunoglobulins (section 2.28).

Results

Light microscopy : No changes were observed by light microscopy in any animal.

Immunofluorescence : Trace amounts of IgG were found in the kidneys of 2 of 4 water fed HeOla, 4 of 6 ova fed HeOla and in 4 of 6 ova fed HeJ mice. Small amounts (trace or +) of IgM were found in the kidneys of all mice examined. IgA was detected in 2 of 4 water fed HeOla, 3 of 6 ova fed HeOla and in 5 of 6 HeJ mice. C3 was detected in trace amounts in the glomeruli of one ova fed HeJ mouse. Ova was not detected in the kidney of any mouse examined. No significant differences in immunofluorescent staining were detected between the three groups for any target parameter.

In mice which had been fed SRBC, 1 of 7 HeOla and 2 of 7 HeJ mice had trace amounts of IgA in the kidney, with one other HeJ mouse scoring + for the presence of IgA. C3 was detected in trace amounts in 2 SRBC fed HeOla and 1 SRBC fed HeJ mice. The kidneys of all mice examined showed positive staining for mouse immunoglobulins. There were no significant differences in staining patterns for any target parameter between the two groups.

4.3 EXPERIMENTAL CIRRHOSIS AND GLOMERULAR IGA DEPOSITS

The association between hepatic cirrhosis and glomerular IgA deposits in humans (Callard etal, 1975) suggests that defective hepatic clearance of polymeric IgA

and IgA-IC may result in glomerular IgA immune deposits (Woodroffe etal, 1980). The induction of experimental cirrhosis in rats following repeated administration of carbon tetrachloride (CCl4) has been described (McLean, McLean & Sutton, 1969). Using this model, Gormly etal (1981) described the presence of IgA glomerular deposits in the kidneys of rats rendered cirrhotic by the administration of CCl4. I was interested in seeing whether the induction of hepatic cirrhosis by CCl4 in mice was associated similarly with the presence of glomerular IgA deposits.

Materials and Methods

Animals : 8 week old male BALB/c mice were used.

Induction of cirrhosis : Experimental liver damage was induced by the intragastric administration of CCl4. Experimental animals were given sodium phenobarbitone (0.5 g/l) in their drinking water for 10 days prior to the initial administration of CCl4, to increase the hepatotoxic effect of CCl4 administration (Garner & McLean, 1969). Groups of experimental animals were given weekly intragastric doses of 4 µl or 16 µl CCl4 dissolved in 0.1 ml vegetable oil. The doses used were chosen on a weight-for -weight basis to span the dose range which caused cirrhosis in rats (McLean etal, 1969). Control animals were given 0.1 ml vegetable oil by intragastric intubation at weekly intervals.

Sacrifice and tissue processing : CCl4 treated and control mice were killed on day 6 following the 8^{th} intragastric dose, and similarly following the 15^{th} dose (control and lower dose CCl4 treated mice). Mice receiving the higher

dose of CCl_4 appeared chronically unwell after 8-10 doses of CCl_4, and were sacrificed 6 days after the 10^{th} intragastric dose.

The right kidneys were taken for immunofluorescent examination, and the left kidneys and specimens of hepatic tissue were taken for light microscopy as described in sections 2.28 and 2.29 respectively.

Immunofluorescence : Methanol fixed cryostat sections of kidneys for immunofluorescent examination were stained for the presence of IgG, IgA, IgM and C3.

Results

Induction of cirrhosis : Administration of CCl_4 to mice at the doses used was associated with the induction of liver damage. The gross appearance of the livers of CCl_4 treated mice was generally normal, although livers from CCl_4 treated mice were noted to have a "gritty" texture on cutting. No ascites nor gross splenomegaly was noted. On microscopic examination, the livers from CCl_4 treated mice showed regenerative activity, with the hepatocytes showing large, pleomorphic nuclei. No regenerative nodules were seen. Reticulin stain showed fibrous septum formation indicative of chronic liver damage in CCl_4 treated mice but not in controls (Plates 9 & 10). The degree of fibrosis varied with the dose and duration of CCl_4 administration.

Renal histology and immunofluorescence : No glomerular lesions were seen on light microscopy. Immunofluorescent examination of kidneys from all CCl_4 treated mice of both dosage groups showed negative or trace only staining for

IgG, IgA, IgM and C3 after 8 and 15 intragastric doses in control and CCl4 treated mice of the lower dosage group, and after 8 and 10 weeks in mice of the higher dose CCl4 treated group. There was no difference in the staining patterns for any target parameter between CCl4 treated mice of either dosage group and control mice.

4.4 CONCLUSIONS

Prolonged administration of BGG (0.1%) in the drinking water of BALB/c mice was associated with the deposition of small amounts of IgA, but not of IgG, IgM, C3 or the immunising antigen in the kidneys of antigen fed mice. Prolonged administration of ovalbumin (0.05%) in the drinking water to the same strain of mice, however, was not associated with an increase in glomerular IgA deposition as compared with controls maintained on plain drinking water. Similarly, intermittent administration of ovalbumin by intragastric intubation to the same total dose had no effect on glomerular immune deposits in antigen fed mice as compared with controls. Administration of ovalbumin (0.1%) via the drinking water to C3H/HeJ mice, which are known to be high producers of IgA, also had no effect on glomerular immune deposits as compared with both water fed and ova fed C3H/HeOla controls.

The possibility that defective oral tolerance might be associated with enhanced oral immunisation resulting in increased levels of glomerular immune deposits was investigated by prolonged administration of SRBC to C3H/HeJ mice. Again no increase in glomerular IgA deposits was seen in SRBC fed C3H/HeJ mice as compared with SRBC

fed C3H/HeOla controls.

The results of the experiments described above confirm the observation that prolonged antigen feeding may be associated with glomerular IgA deposits in mice, and demonstrate a potential relationship between dietary antigens and IgA nephropathy. The results also demonstrate, however, the limitations of the model, and suggest that the relationship between dietary antigens and glomerular IgA deposits may be dependent on a number of factors, including the strain of animal studied, the antigen used, and the dose and frequency of administration.

Studies on the effects of CCl4 induced liver damage in mice demonstrated that CCl4 given by the intragastric route to mice was associated with the induction of hepatic cellular damage and chronic hepatic fibrosis. The lack of regenerative nodule formation in this model, however, fails to meet the histological criteria required for the diagnosis of cirrhosis. In spite of histological evidence of liver damage, no increase was seen in the levels of glomerular IgA deposits in CCl4 treated mice. In view of the association between hepatic cirrhosis and glomerular IgA deposits in humans and the demonstration of increased levels of IgA deposits in rats rendered cirrhotic by administration of CCl4, interpretation of these results must be guarded.

TABLE 5.

			Immunofluorescence score		
	Group	n	-	trace	+
IgG					
	Control	6	3	3	0
	BGG-1	6	2	4	0
	BGG-3	5	2	3	0
IgA					
	Control	6	5	1	0
	BGG-1	6	0	6	0
	BGG-3	5	0	4	1
IgM					
	Control	6	0	1	5
	BGG-1	6	0	1	5
	BGG-3	5	0	0	5
C3					
	Control	6	6	0	0
	BGG-1	6	5	1	0
	BGG-3	5	5	0	0

Table 5 : Results of immunofluorescent staining for IgG, IgA, IgM and C3 of sections of kidney from BALB/c mice fed BGG in the drinking water changed daily (BGG-1) or changed every third day (BGG-3), and from control mice given plain drinking water.

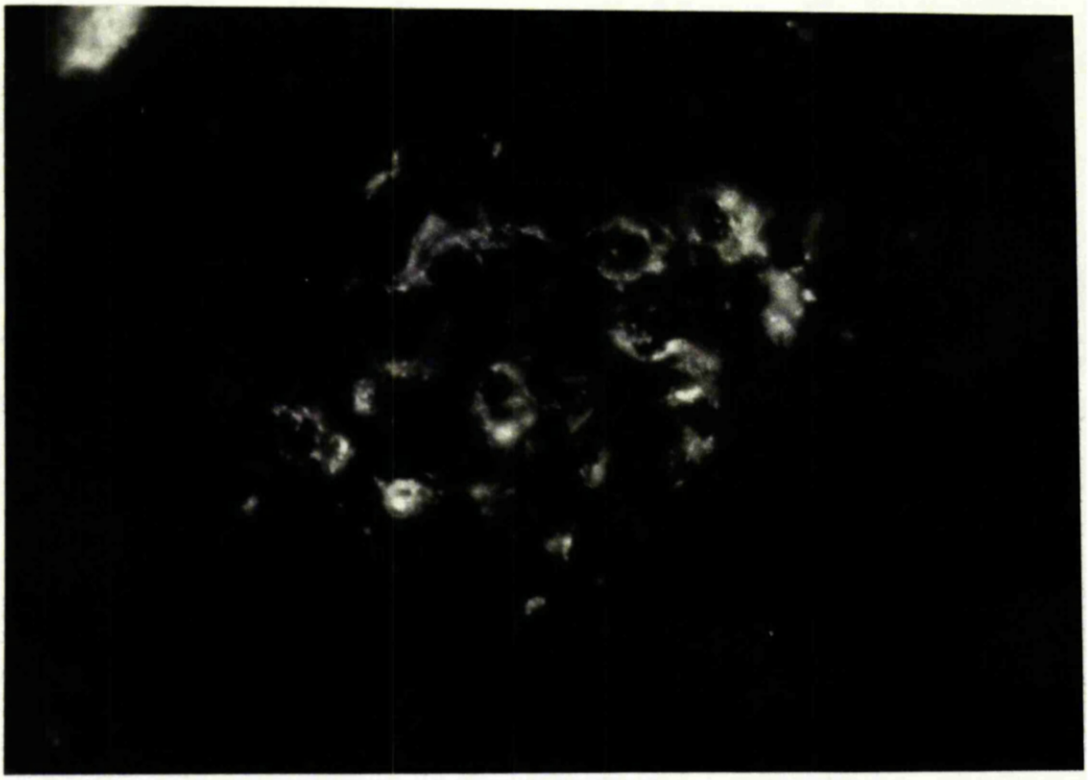

Plate 8

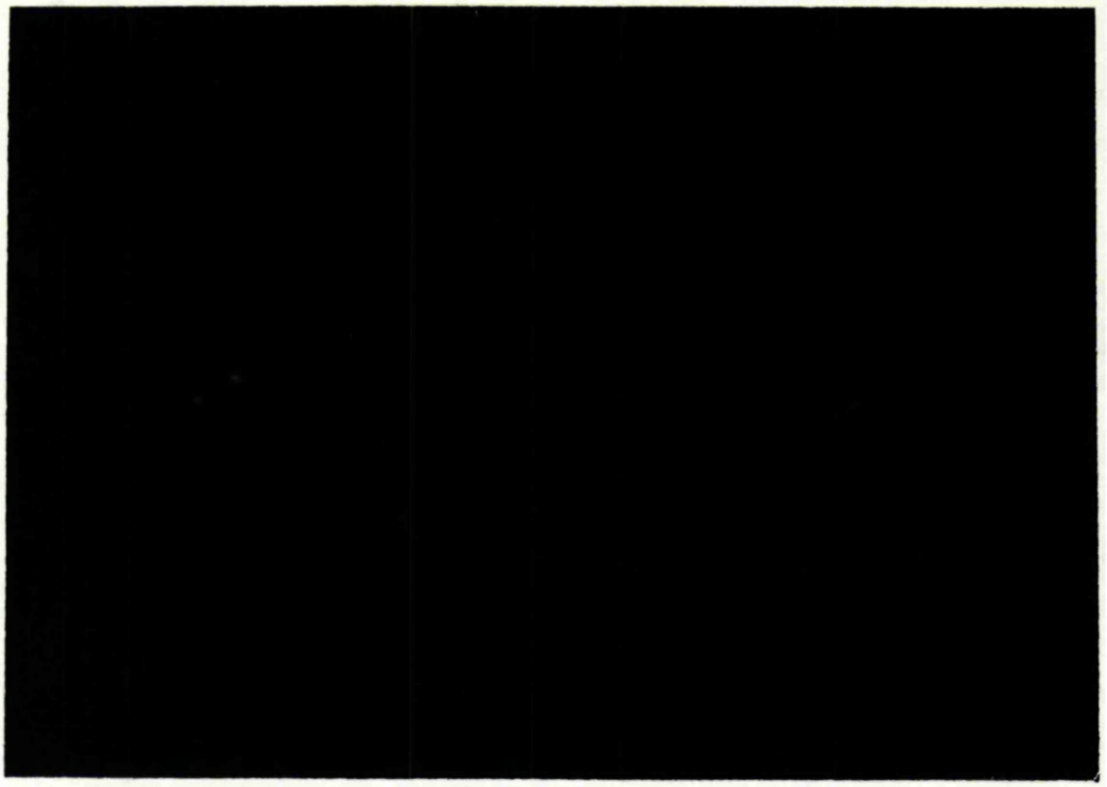

Plate 6

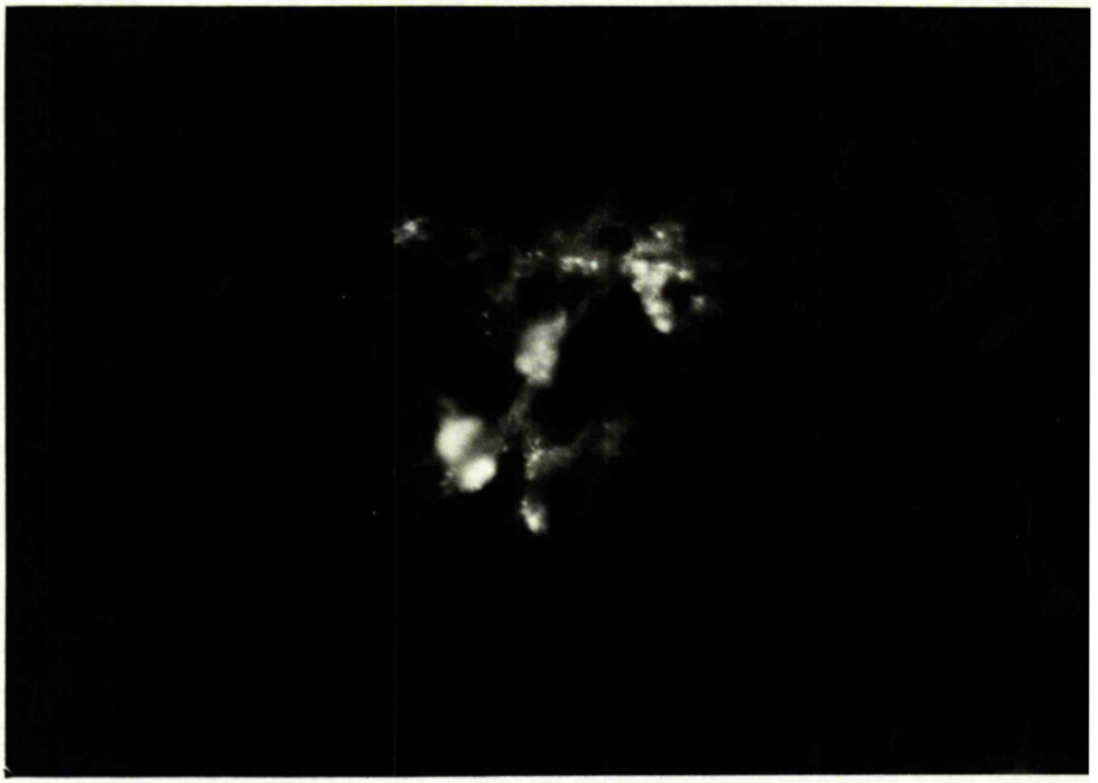

Plate 7

PLATE 6 : Section of kidney from control (water fed) BALB/c mouse stained by direct immunofluorescence for the presence of IgA. No glomerular IgA was detected. (Magnification x150).

PLATE 7 : Section of kidney from BALB/c mouse given ova in the drinking water for 12 weeks. Section, stained by direct immunofluorescence for IgA, shows the presence of glomerular mesangial IgA deposition. (Magnification x150).

PLATE 8 : Section of kidney from "normal" TO [low] mouse at 8 months of age, stained for the presence of IgA by direct immunofluorescence, shows the presence of glomerular IgA deposition. (Magnification x150).

PLATE 9 : Liver section from control mouse given vegetable oil by weekly intragastric intubation for 14 weeks, showing normal hepatic architecture. (Reticulin stain; magnification x33).

PLATE 10 : Liver section from mouse given 16 µl carbon tetrachloride by weekly gastric intubation for 10 weeks, showing fibrous septum formation. (Reticulin stain; magnification x33).

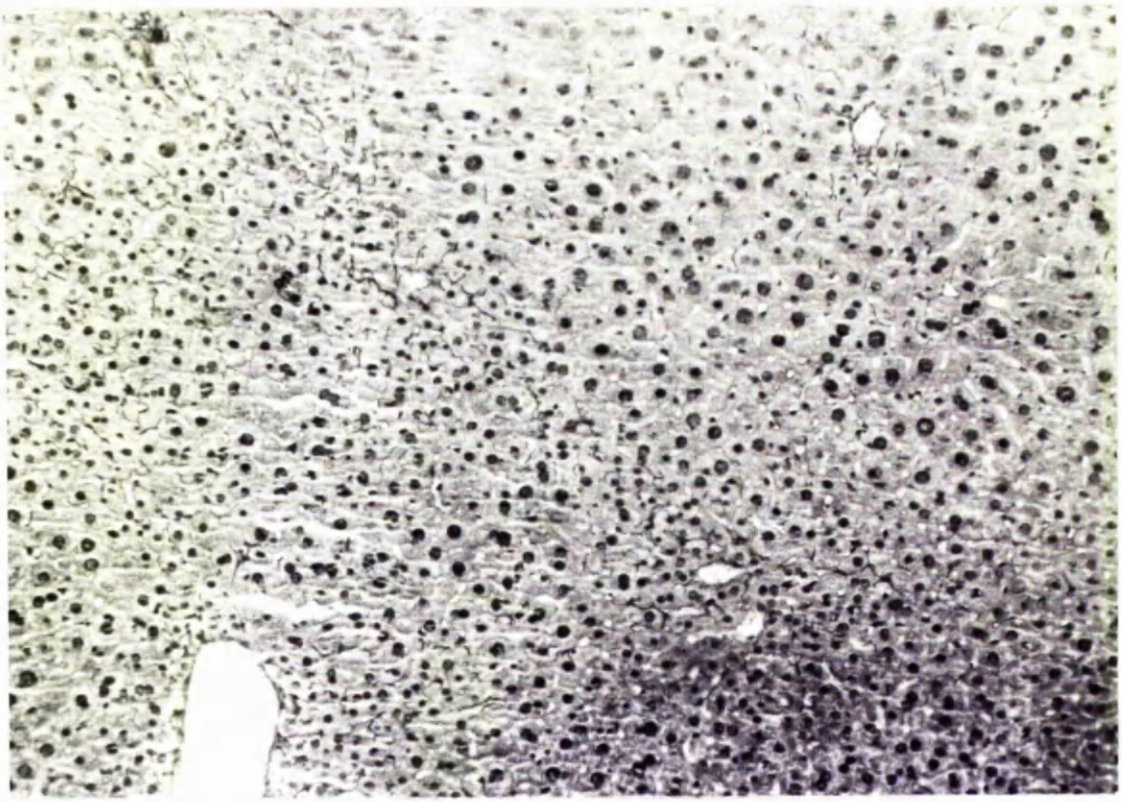

Plate 9

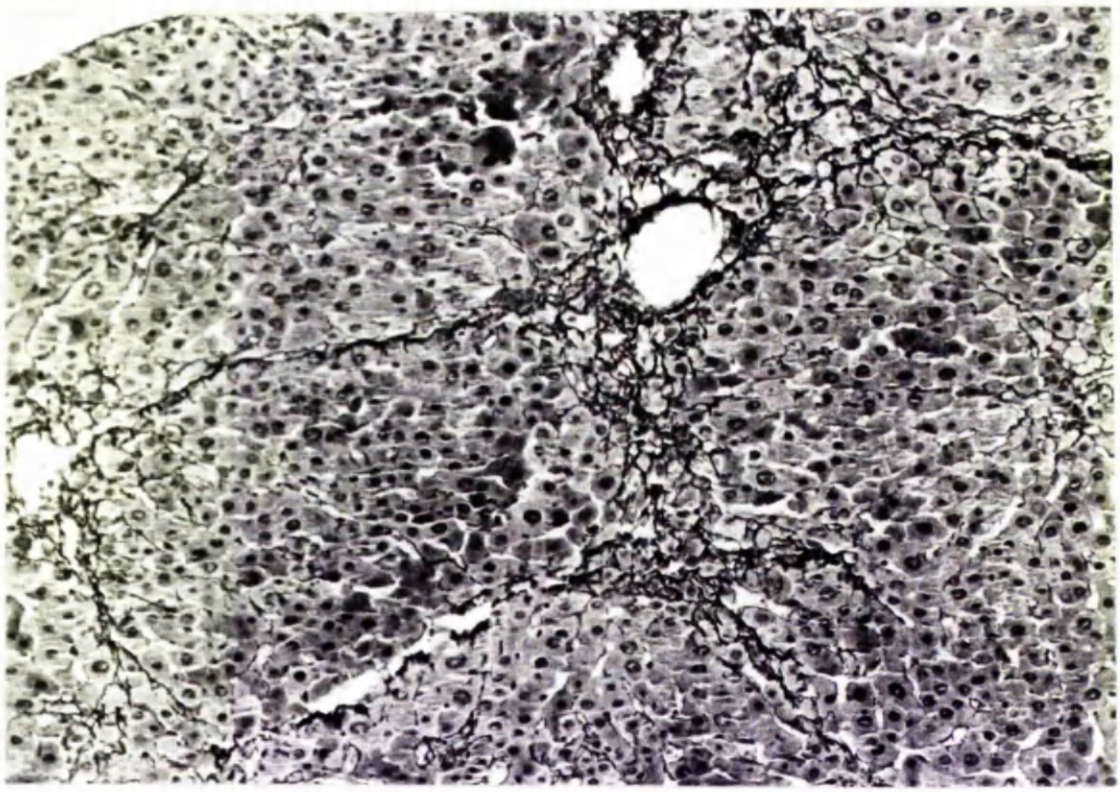

Plate 10

CHAPTER FIVE

THE EFFECTS OF ORAL ANTIGEN ADMINISTRATION ON THE INDUCTION OF EXPERIMENTAL IMMUNE COMPLEX GLOMERULONEPHRITIS

5.1 INTRODUCTION

The absorption of antigen from the gastrointestinal tract can lead to the production of circulating antibodies, which may contribute to the pathogenesis of certain diseases. Oral antigen administration, however, can also lead to a state of systemic hyporesponsiveness to the antigen, which may protect against damaging hypersensitivity reactions both locally and systemically.

In the following two chapters I have investigated the immunomodulatory effects of oral antigen administration on a pathogenic systemic hypersensitivity reaction : the induction of chronic immune complex glomerulonephritis by repeated injections of antigen in susceptible strains of mice.

In this chapter the model of antigen induced immune complex glomerulonephritis which was used in the subsequent experiments is defined. The induction of immune complex glomerulonephritis was investigated in several strains of mice, to establish susceptible strains suitable for further study. The effects of prior intragastric antigen administration on the induction and course of immune complex glomerulonephritis by repeated injections of the same antigen were examined in these strains of mice.

5.2 INDUCTION AND COURSE OF IMMUNE COMPLEX GLOMERULONEPHRITIS IN TO [LOW] MICE

The genetically controlled production of low affinity antibody to protein antigens is associated with

susceptibility to the development of immune complex disease in experimental animals. Low affinity antibody producing TO mice have been shown previously to be susceptible to the induction of immune complex disease by repeated injections of protein antigen (Devey & Steward, 1980). In this experiment I sought to determine the course and induction of immune complex glomerulonephritis by daily injections of ova in TO [low] mice (bred without selection for antibody affinity from a stock of low affinity antibody producing mice).

Materials and Methods

Animals : Male TO [low] mice were used at 8-10 weeks of age.

Induction of IC glomerulonephritis : Mice were injected daily for 30, 60, 90 or 120 days with 0.3 mg ova in 0.2 ml sterile saline by intraperitoneal inoculation. This dose of antigen for injection was chosen on the basis of previous observations on the induction of immune complex glomerulonephritis in low affinity TO mice (Devey & Steward, 1980), in which the majority of mice demonstrated glomerular immune complex deposition after 41-44 antigen injections. I wished to study the effects of both fewer and more antigen injections on glomerular immune complex deposition and renal morphology in TO [low] mice. Controls for this experiment were provided by uninjected age and sex matched mice.

Sacrifice and tissue processing : Twenty four hours after the final antigen injection the mice were bled out. The right kidney was taken for immunofluorescent examination and the left kidney for light microscopic examination.

Immunofluorescence : Unfixed cryostat sections were stained directly for IgG, IgM, IgA and C3, and stained indirectly for ovalbumin (section 2.28)

Renal function : Renal function was assessed by the measurement of serum levels of urea and creatinine (section 2.32).

Results

Light microscopy : Kidney sections from uninjected control mice showed normal renal morphology (Plate 11). Sections of kidneys from mice receiving 30 daily injections of antigen showed no focal or diffuse glomerular lesions. In mice receiving 60 daily antigen injections, a mild degree of mesangial expansion was seen in a proportion of kidney sections, but no focal or diffuse glomerular lesions were seen. Following 90 daily antigen injections the mesangial expansion was more marked, and focal interstitial nephritis was present in 4 of 6 mice. In mice receiving 120 daily antigen injections, there was marked mesangial expansion in 3 of 4 mice (Plate 12), and a lesser degree of mesangial expansion in the remaining mouse. The urinary space was reduced in these animals. Little or no cellular proliferation was seen in the glomeruli of any antigen injected mouse. Interstitial nephritis was present in all 4 mice receiving 120 injections of antigen.

Immunofluorescence : Results of immunofluorescent staining of unfixed cryostat kidney sections are shown in Table 6, for antigen injected mice and uninjected, age matched controls. IgG, C3 and ova were not detected in control mice at the start of the experiment, although a proportion

of control mice showed small amounts of deposition of IgG and C3 at the end of the experiment (i.e. after 120 days). Deposits of IgA were found in a proportion of controls at each time point, and deposits of IgM were virtually universal in control mice, and increased in degree and intensity with time. Ova was not detected in the kidney of any control mouse.

In antigen injected mice receiving 30 daily injections of antigen, 5 of 7 mice showed predominantly mesangial deposition of IgG and ova, and 4 of these mice also showed deposition of C3. The incidence and degree of glomerular immune deposits increased with increasing numbers of antigen injections, such that glomerular immune deposits of IgG, IgA, IgM and ova were seen in 100% of experimental animals and C3 in 75% of animals after 120 daily antigen injections (Plates 13-15).

In an attempt to quantitate the deposition of immunoglobulin, complement and antigen in the kidneys of experimental groups of animals, an immunofluorescence index was produced for each experimental group for IgG, IgA, IgM, C3 and ova in the kidneys. Individual kidney sections were scored 0, +, ++ or +++ on the degree and intensity of fluorescent stain present. The scores for each stain were summed and divided by the number of animals in the experimental group to give the immunofluorescence index. Results of immunofluorescent indices are shown in Table 7. There was a progressive increase in the degree of immunofluorescent staining for IgG, IgM, C3 and ova of kidney sections from antigen injected mice with increasing numbers of antigen

injections, suggesting increasing levels of immune complex deposition in the kidneys of these mice. The immunofluorescence indices for IgA were affected little by repeated systemic antigen injections. The degree of glomerular immune complex deposition in uninjected control mice also increased with time, but to a lesser degree. After 120 daily injections the immunofluorescence indices for IgG, C3 and ova were markedly increased as compared with age matched control mice. The increase in immunofluorescence index for IgM was less marked, and there was no difference in the immunofluorescence indices for IgA between control and antigen injected animals.

The results suggest that daily injections of antigen in TO [low] mice were associated with the induction of glomerular immune complex deposits. These deposits were localised in the mesangial areas and, to a lesser extent, in the glomerular capillary loops, and consisted of IgG, the immunising antigen and C3, with lesser involvement of IgM.

Renal function : Serum urea and serum creatinine levels in the same mice are shown in Table 8. There was no increase in either serum urea or serum creatinine in antigen injected mice as compared with age and sex matched, uninjected controls. The serum urea and creatinine levels of all experimental mice remained within normal limits.

5.3 THE EFFECT OF ORAL ANTIGEN ADMINISTRATION ON THE INDUCTION OF IMMUNE COMPLEX NEPHRITIS

5.3.1 The effect of oral antigen on the induction of immune complex nephritis in TO [high] and TO [low] mice

The previous experiment demonstrated that daily injections of antigen in TO [low] mice induced a nephropathy characterised by predominantly mesangial glomerular immune complex deposition. Sixty daily injections of antigen were asociated with marked deposition of IgG, IgM, C3 and antigen in the kidneys of the majority of mice, and this was chosen as a suitable end point to examine the effect of oral antigen on the induction of immune complex nephritis in TO [high] and TO [low] mice.

Materials and Methods

Animals : Male TO [high] and TO [low] mice at 8 weeks of age were used.

Oral antigen administration : Mice were given 25 mg ova in 0.2 ml distilled water by intragastric intubation as described previously. Control mice received 0.2 ml distilled water by the same route.

Induction of IC nephritis : Fourteen days after intragastric intubation, groups of antigen fed and water fed mice were started on a regime of daily intraperitoneal injections of 0.3 mg ova in 0.2 ml sterile saline for 60 injections. A second group of water fed mice of each strain was given 0.2 ml sterile saline by daily intraperitoneal injection for 60 injections.

Sacrifice and tissue processing : Twenty four hours after the final antigen injection the mice were bled out. The right kidney was taken for immunofluorescent examination and the left kidney for light microscopic examination.

Immunofluorescence : Unfixed cryostat sections were stained directly for the presence of IgG and C3 and indirectly for ova.

Estimation of antibody titres : Serum antibody levels were estimated by ELISA as described previously.

Results

Light microscopy : No focal or diffuse glomerular lesions were seen by light microscopy.

Immunofluorescence : Results of immunofluorescent examination of kidneys are shown in Table 9. Marked deposition of IgG, C3 and ova was seen in the kidneys of the majority of water fed animals which had received repeated injections of antigen of both TO [high] and TO [low] strains (Plate 16). In animals of both strains which had been given a single 25 mg dose of ova by intragastric intubation prior to the induction of nephritis by daily injections of ova, the kidneys were virtually free from IgG, C3 or ova deposition (Plate 17). The results indicated a marked decrease in the incidence of immune complex deposition in the kidneys of antigen fed mice. No IgG, C3 or ova was detected in the kidneys of animals injected repeatedly with sterile saline alone.

Antibody titres : Ovalbumin specific antibodies were not detected in the serum of any saline injected controls. Serum antibody titres from antigen injected animals are shown in Figure 17. In antigen fed animals of both strains

which had been injected repeatedly with ova, the antibody titres were significantly reduced as compared with water fed, antigen injected controls.

5.3.2 The effect of oral antigen on the induction of immune complex nephritis : electron microscopic studies

In the previous experiment oral antigen administration prior to the induction of immune complex nephritis by daily injections of the same antigen led to a marked reduction in glomerular immune complex deposition following the induction of nephritis by repeated daily injections of the same antigen.

In the following experiment I sought to examine at the electron microscopic level the induction of immune complex glomerulonephritis, and the effects of antigen feeding on the induction of nephritis in TO [low] mice.

Materials and Methods

Animals : Two groups each of 5 male TO [low] mice were used at 8 weeks of age.

Oral antigen administration : One group of mice was given 25 mg ova by intragastric intubation. The control group received water alone by the same route.

Induction of nephritis : Fourteen days after antigen feeding, immune complex nephritis was induced by daily injections of 0.3 mg ova by intraperitoneal inoculation. Twenty four hours after the 60th antigen injection the mice were killed by cervical dislocation under ether anaesthesia. The right kidney was taken for immunofluorescent examination and processed as described previously. The left kidney was taken for electron microscopic

examination.

Electron microscopy : Transmission electron microscopy was carried out as described in section 2.30.

Immunofluorescence : Unfixed cryostat sections of kidney were stained directly for IgG and C3 and indirectly for ova by immunofluorescent staining.

Results

Immunofluorescence : Four of 5 water fed animals showed positive immunofluorescent staining for IgG, C3 and ova following 60 daily antigen injections (Table 10). In the remaining water fed mouse, trace amounts of IgG and ova were detected. Three of 5 antigen fed mice were positive for IgG and C3, of which 2 were also positive for ova. In the other two antigen fed mice, C3 and ova were not detected, although trace amounts of IgG were present in the kidneys. In this experiment, therefore, the incidence of immune complex deposition was not significantly reduced in mice which had received ova by gastric intubation prior to the induction of glomerulonephritis by repeated injections of ova. The degree of immune complex deposition, as estimated by the immunofluorescence scores, however, was reduced in antigen fed mice as compared with controls.

Electron microscopy : Results of electron microscopic examination of sections of kidney from water fed and antigen fed animals are presented in Table 11. Electron dense deposits (EDD) were found in large numbers in the mesangium of 4 of 5 water fed animals (Plates 18 & 19), and were associated with mesangial expansion. In addition,

subendothelial EDD were found in lesser amounts in 3 of the 5 mice. Inflammatory cells and platelets were present in the glomerular capillary loops of the majority of animals in this group. In mice which had received ova by intragastric intubation prior to the induction of nephritis, mesangial EDD were detected in 4 of 5 animals, and subendothelial EDD in 3 of 5 mice. The degree of EDD seen in antigen fed mice was generally less than was seen in the water fed controls. In one antigen fed mouse no EDD were seen (Plate 20). Foot processes in the glomeruli of mice of both experimental groups appeared intact.

Individual mice of both groups were assigned an EM score, rated from 0 - 3, on the basis of the amounts of electron dense deposition. EM scores in antigen fed mice were, generally, lower than those of water fed mice. Overall, the EM scores and the immunofluorescence scores correlated with a high degree of significance (Figure 18), indicating that the amounts of electron dense material seen on electron microscopy were closely related to the degree of immune complex deposition as determined by immunofluorescence. Based on this result, I assumed that the immunofluorescent techniques used for the detection of glomerular immune deposits represented a sensitive and reliable method for the detection of immune complex deposition within the glomerulus.

5.3.3 The induction of immune complex nephritis, and the effects of oral antigen on the induction of nephritis, in inbred strains of mice

The susceptibility of selectively bred TO mice to the induction of immune complex nephritis has been reported previously (Devey and Steward, 1980). In order to extend observations on susceptibility to immune complex disease and on the effects of oral antigen on the induction of immune complex nephritis, I investigated the induction of immune complex nephritis in mice of 3 inbred strains which differed in their susceptibility to oral tolerance induction, and examined the effects of oral antigen administration on the subsequent induction of nephritis and on the antibody responses in these strains of mice.

I have shown previously that BALB/c, B10.BR and BALB/b mice vary markedly in their ease of oral tolerance induction to ova. In the following experiments I wished to examine the induction of immune complex glomerulonephritis by repeated antigen injections in these strains of mice, and to investigate the relationship between oral tolerance induction and protection from immune complex glomerulonephritis by antigen feeding.

Materials and Methods

Animals : Male mice of the BALB/b, BALB/c and B10.BR strains were used at 8 weeks of age.

Oral antigen administration : Mice were given 25 mg ova or water alone by gastric intubation.

Induction of nephritis : Fourteen days after antigen feeding, immune complex nephritis was induced by 60 daily antigen injections as described previously. Twenty four

hours after the final antigen injection mice were bled out. The right kidney was taken for immunofluorescent examination and the left kidney for light microscopic examination. Uninjected age and sex matched animals of the same inbred strains were used as controls.

Estimation of antibody titres : Serum antibody levels in experimental and control animals were estimated by ELISA.

Results

Light microscopy : No focal or diffuse glomerular lesions were seen in the kidneys of antigen fed or water fed animals of any strain examined.

Immunofluorescence : Unfixed cryostat sections were stained directly for the presence of IgG and C3 and indirectly for ova. Results of immunofluorescent examination of kidneys of water fed and antigen fed experimental mice and uninjected age and sex matched controls are shown in Table 12 for mice of BALB/b, BALB/c and B10.BR inbred strains.

The kidneys of BALB/b mice from all three experimental groups were all negative on immunofluorescent examination for the presence of IgG, C3 and ova.

In BALB/c mice, uninjected controls showed no immunofluorescent staining for IgG, C3 or ova. Water fed, injected animals, however, showed positive staining for IgG and ova in all animals examined, and for C3 in 5 of 7 animals. Feeding with ova prior to the induction of immune complex nephritis resulted in a marked reduction in immunofluorescent staining, with only 2 of 7 animals showing positive staining for IgG, C3 and ova.

Two of 5 uninjected B10.BR mice had IgG deposition in the kidney, of which one had concomitant staining for C3. Ova was not detected in the kidney of any control mouse. A similar proportion of mice from both the water fed and ova fed experimental groups showed positive staining for IgG as compared with uninjected controls, and one mouse from the ova fed group also showed positive staining for C3. Ova was not detected in the kidney of any experimental mouse. There were no significant differences in staining patterns between the three groups.

The results indicated that BALB/c mice were susceptible to the induction of immune complex complex glomerulonephritis, and that prior antigen feeding was associated with a decreased incidence of glomerular immune deposits in this strain. BALB/b and B10.BR mice were not susceptible to the induction of immune complex glomerulonephritis as tested, although B10.BR mice showed "spontaneous" glomerular immune deposits in a proportion of mice.

Antibody titres : Ovalbumin specific antibodies were not detected in the sera of any uninjected control animals from the three inbred strains tested. Antibody titres for water fed and ova fed experimental animals of BALB/b, BALB/c and B10.BR strains are shown in Figure 19. There was no difference in antibody titre between water fed and ova fed BALB/b mice. Mice of the BALB/c and B10.BR strains which had been given ova by intragastric intubation prior to the induction of immune complex nephritis by daily injections of ova, however, showed significant reductions in antibody titres as compared with water fed, antigen

injected mice of the same strain.

5.4 CONCLUSIONS

Repeated injections with protein antigens may result in the induction of a chronic immune complex glomerulonephritis in susceptible strains of mice. TO [low] mice injected daily with ovalbumin showed glomerular immune complex deposition affecting predominantly the mesangium after 30 antigen injections. Continued daily antigen injections were associated with a progressive increase in the levels of glomerular immune complex deposits of predominantly IgG, C3 and ova. Electron microscopic studies confirmed the presence of mesangial electron dense deposits (EDD), with lesser amounts of EDD in the subendothelial space in a proportion of cases. Light microscopy showed only mild increase in mesangial matrix after 60 injections of antigen. Following 90 and 120 injections the mesangial expansion was more marked, with reduction in the urinary space. No focal or segmental glomerular lesions were seen. Renal insufficiency was not seen in any mouse.

Administration of single doses of oral antigen prior to the induction of nephritis almost completely ablated the glomerular deposition of immune complexes in TO [high] and TO [low] mice. This effect was associated with reduction in ovalbumin specific antibody titres in ova fed mice as compared with water fed controls. Whilst only a limited reduction in glomerular immunofluorescent staining and electron dense deposits was seen in antigen fed mice which were studied at the electron microscopic level, the levels

of EDD seen within the glomeruli correlated highly significantly with the degree of glomerular immunofluorescent staining.

Attempts to induce immune complex nephritis in inbred strains of mice yielded varying results. BALB/b and B10.BR mice were not susceptible to the induction of immune complex nephritis as tested. A proportion of B10.BR mice, however, appeared to have a low grade immune complex nephritis in the absence of extrinsic antigen challenge. In BALB/c mice deposition of IgG, C3 and antigen was seen in the glomeruli of almost all antigen injected control animals, suggestive of the induction of immune complex glomerulonephritis. Oral administration of single doses of antigen prior to the induction of nephritis in BALB/c mice was associated with a marked reduction in the incidence of glomerular immune complex deposition.

In all three strains of mice susceptible to the induction of IC nephritis, reductions in the incidence of nephritis in antigen fed animals were associated with reductions in the systemic antibody response to the antigen.

TABLE 6.

Group	Time (days)	n	Percent of sections showing positive staining				
			IgG	IgA	IgM	C3	ova
uninjected	0	5	0	40	100	0	0
uninjected	60	5	20	40	80	20	0
uninjected	120	5	40	80	100	20	0
injected	30	7	71	71	100	57	71
injected	60	7	86	57	86	57	86
injected	90	6	83	100	100	67	83
injected	120	4	100	100	100	75	100

Table 6 : The induction and progression of antigen induced immune complex glomerulonephritis in T0 [low] mice. The incidence of positive glomerular immunofluorescent staining for IgG, IgA, IgM, C3 and ova of kidney sections from mice receiving 30 - 120 daily i.p. injections of 0.3 mg ova, and in uninjected, age matched control mice.

TABLE 7.

Group	Time (days)	n	Immunofluorescence Index IgG	IgA	IgM	C3	ova
uninjected	0	5	0	0.40	1.00	0	0
uninjected	60	5	0.20	0.60	0.80	0.20	0
uninjected	120	5	0.40	1.00	1.40	0.20	0
injected	30	7	0.71	0.71	1.00	0.57	0.71
injected	60	7	1.43	0.57	1.00	1.14	1.29
injected	90	6	1.67	0.83	1.17	1.33	1.83
injected	120	4	2.25	1.00	2.25	1.75	2.25

Table 7 : The induction and progression of antigen induced immune complex glomerulonephritis in T0 [low] mice. The degree of glomerular deposition of IgG, IgA, IgM, C3 and ova in kidney sections from mice receiving 30 - 120 daily i.p. injections of 0.3 mg ova, and in uninjected, age matched controls. The immunofluorescence index represents the group mean immunofluorescence score for each staining parameter.

TABLE 8.

Number of injections	serum urea (mMol/l)	serum creatinine (µMol/l)
0	7.57 + 0.31	72.3 + 4.3
30	5.25 + 0.51	68.2 + 6.1
60	4.90 + 0.39	60.4 + 8.7
controls	6.48 + 0.32	61.5 + 5.8
90	6.40 + 0.22	60.4 + 5.9
120	5.74 + 0.56	52.9 + 9.1
controls	5.94 + 1.43	52.1 + 9.2

Table 8 : The induction and progression of antigen induced glomerulonephritis in TO [low] mice : renal function. Estimation of serum urea (mMol/l) and of serum creatinine (µMol/l) in mice receiving 30 - 120 daily i.p. injections of 0.3 mg ova, and in uninjected, age matched control mice. Results represent group mean + 1 standard error.

TABLE 9.

Strain	Group	n	Percent of sections showing positive staining		
			IgG	C3	ova
TO[high]	H2O ig. ova ip	6	83	50	50
TO[high]	ova ig. ova ip	6	17*	0	0
TO[high]	H2O ig. sal ip	4	0	0	0
TO[low]	H2O ig. ova ip	7	86	57	86
TO[low]	ova ig. ova ip	5	0**	0	0**
TO[low]	H2O ig. sal ip	5	0	0	0

* $p < 0.05$; ** $p < 0.01$ (Fisher's exact test) as compared with water fed, antigen injected mice of the same strain.

Table 9 : The effect of intragastric antigen administration on the induction of immune complex glomerulonephritis in TO [high] and in TO [low] mice. The incidence of positive glomerular immunofluorescent staining for IgG, C3 and ova of kidney sections from mice receiving 60 daily i.p. injections of ova subsequent to the intragastric (ig) administration of single 25 mg doses of ova or of water alone, and in mice receiving 60 daily i.p. injections of saline subsequent to intragastric administration of water alone.

TABLE 10.

Animal	Group	Immunofluorescent staining IgG	C3	ova	Immuno-fluorescence score
1	H20 ig	++	+	++	5
2	H20 ig	+	+	+	3
3	H20 ig	tr	-	tr	1
4	H20 ig	++	+	++	5
5	H20 ig	+	+	+	3
6	ova ig	+	+	-	2
7	ova ig	tr	-	-	0
8	ova ig	+	+	+	3
9	ova ig	tr	-	-	0
10	ova ig	+/++	+/++	+/++	4

Table 10 : Results of glomerular immunofluorescent staining for IgG, C3 and ova of kidney sections from TO [low] mice receiving 60 daily i.p. injections of ova subsequent to the intragastric (ig) administration of single 25 mg doses of ova, or of water alone.

TABLE 11.

Animal	Group	EM findings	EM score
1	H2O ig	copious mesangial and subendothelial EDD	3
2	H2O ig	many mesangial and subendothelial EDD	2
3	H2O ig	very few EDD. Slight mesangial interposition	0
4	H2O ig	copious mesangial EDD	3
5	H2O ig	copious mesangial and subendothelial EDD	3
6	ova ig	mesangial EDD present	1
7	ova ig	mesangial EDD present	1
8	ova ig	mesangial and subendothelial EDD	2
9	ova ig	no EDD seen	0
10	ova ig	copious mesangial + few subendothelial EDD	3

Table 11 : The incidence and localisation of glomerular electron dense deposits (EDD), as determined by electron microscopic (EM) examination, in kidneys of TO [low] mice receiving 60 daily i.p. injections of ova subsequent to the intragastric (ig) administration of single 25 mg doses of ova, or of water alone.

TABLE 12.

Strain	Group	n	Percent of sections showing positive staining		
			IgG	C3	ova
BALB/b	H20 ig. ova ip	7	0	0	0
BALB/b	ova ig. ova ip	7	0	0	0
BALB/b	controls	5	0	0	0
BALB/c	H20 ig. ova ip	7	100	71	100
BALB/c	ova ig. ova ip	7	28**	28	28**
BALB/c	controls	5	0	0	0
B10.BR	H20 ig. ova ip	7	28	0	0
B10.BR	ova ig. ova ip	7	43	14	0
B10.BR	controls	5	40	20	0

** p = 0.01 (Fisher's exact test) as compared with water fed, antigen injected mice of the same strain.

Table 12 : The effect of intragastric antigen administration on the induction of immune complex glomerulonephritis by repeated antigen injections in BALB/b, BALB/c and B10.BR mice. The incidence of positive immunofluorescent staining for IgG, C3 and ova of kidney sections from mice receiving 60 daily i.p. injections of ova subsequent to the intragastric (ig) administration of single 25 mg doses of ova or of water alone, and in uninjected, age matched control mice.

FIGURE 17

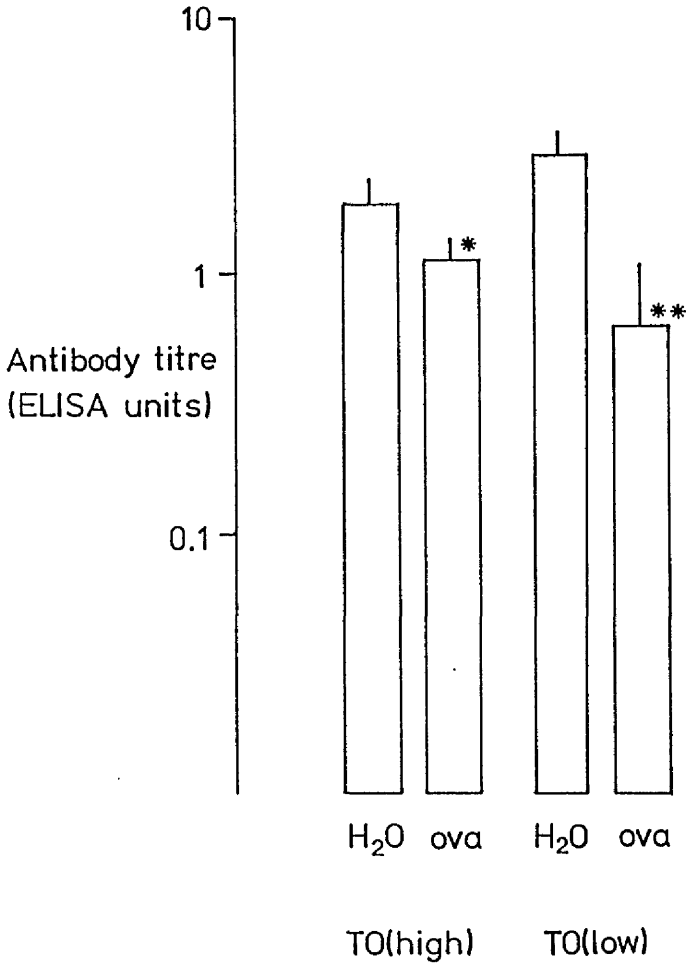

Figure 17 : Serum anti-ova antibody titres after 60 daily i.p. injections of ova in T0 [high] and in T0 [low] mice, given 25 mg ova or water alone by intragastric intubation 14 days before starting the regime of daily antigen injections. Bars represent mean antibody titres (ELISA units) + 1 standard error.

* $p = 0.05$; $p < 0.01$ (Wilcoxon rank sum test) as compared with water fed mice of the same strain.

FIGURE 18

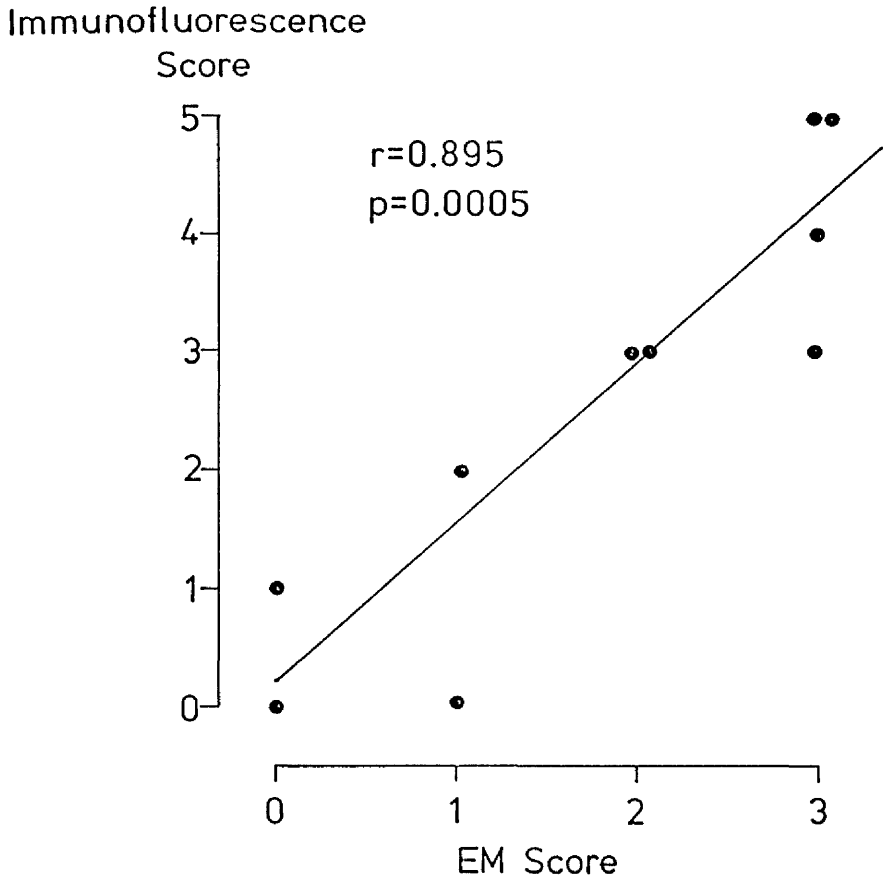

Figure 18 : The relationship between the degree of immunofluorescent staining (immunofluorescence score) and the extent of electron dense deposition seen on electron microscopy (EM score) in antigen fed and water fed TO [low] mice receiving 60 daily i.p. injections of ova.

FIGURE 19

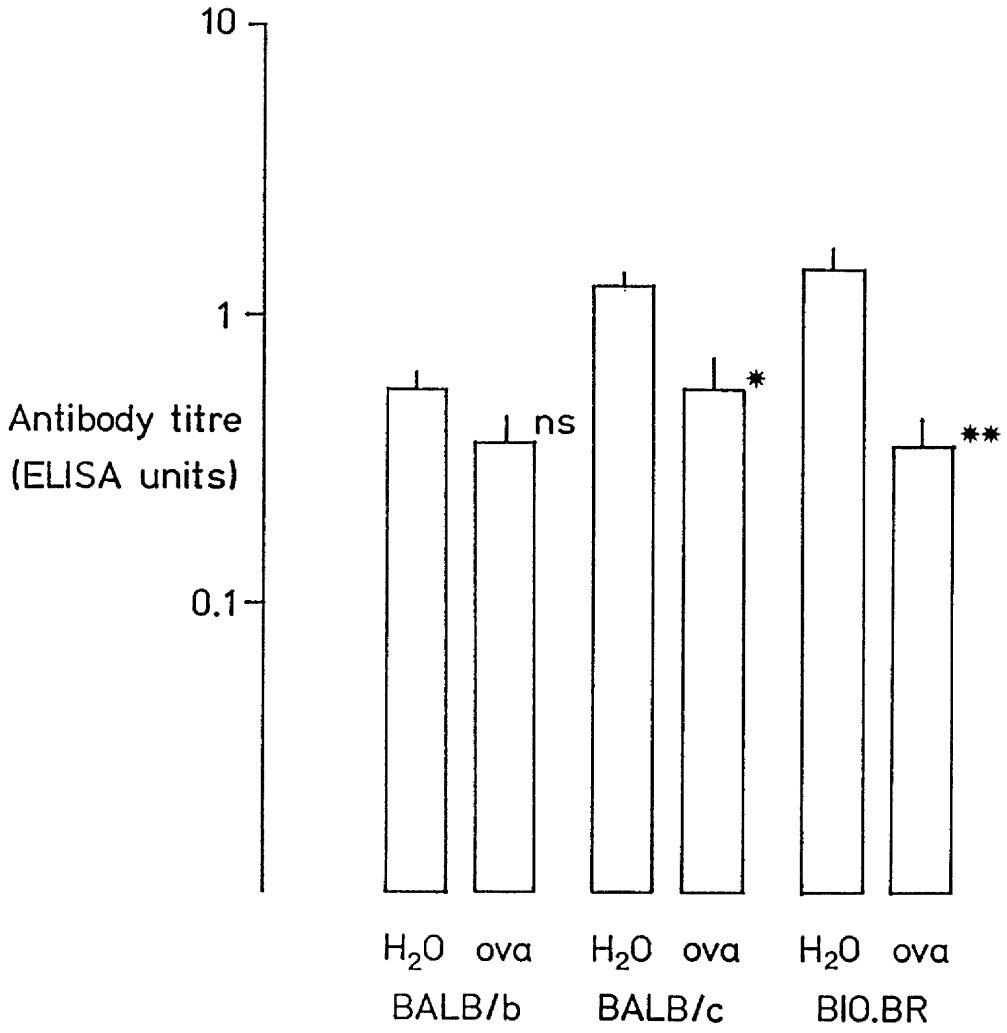

Figure 19 : Serum anti-ova antibody titres after 60 daily i.p. injections of ova in BALB/b, BALB/c and B10.BR mice, given 25 mg ova or water alone by intragastric intubation 14 days before starting the regime of daily antigen injections. Bars represent mean antibody titres (ELISA units) + 1 standard error.

* $p < 0.05$; ** $p < 0.01$ (Wilcoxon rank sum test) as compared with water fed mice of the same strain.

PLATE 11 : Glomerulus from uninjected, control TO [low] mouse, showing normal glomerular appearance. (Haematoxylin & eosin; magnification x135).

PLATE 12 : Glomerulus from TO [low] mouse receiving 120 daily i.p. injections of 0.3 mg ova. The section shows glomerular mesangial expansion, but with little or no cellular proliferation. (Haematoxylin & eosin; magnification x135).

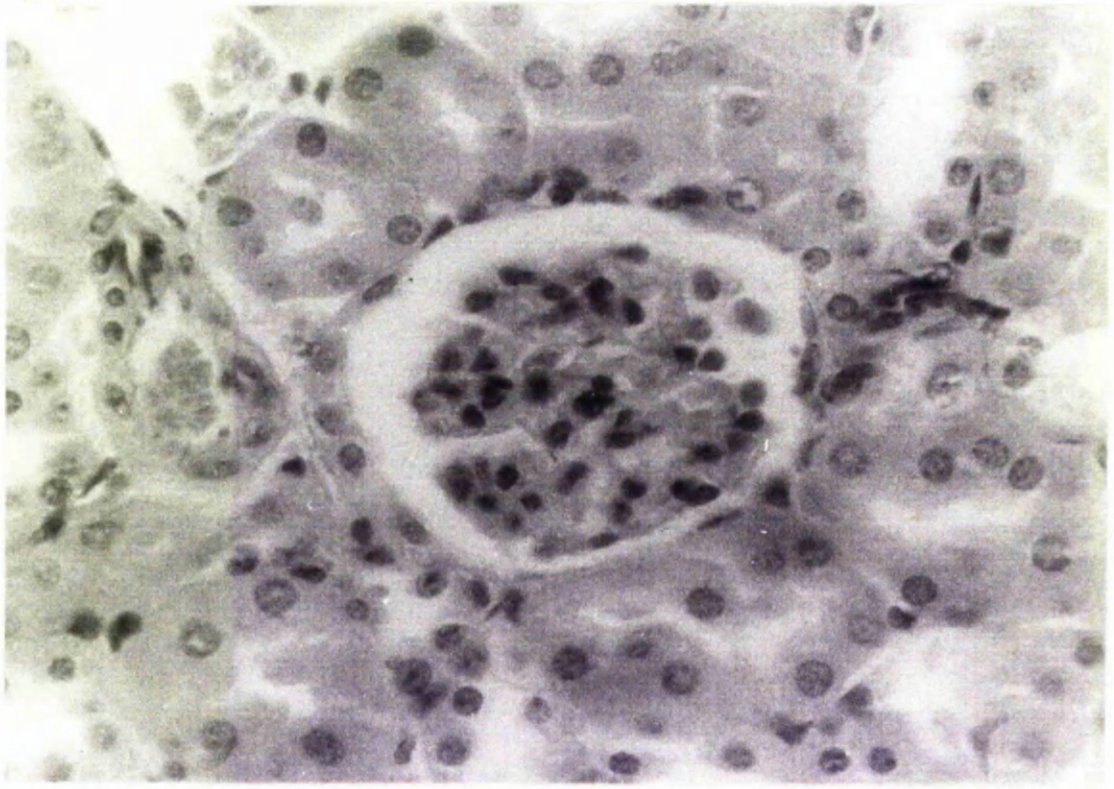

Plate 11

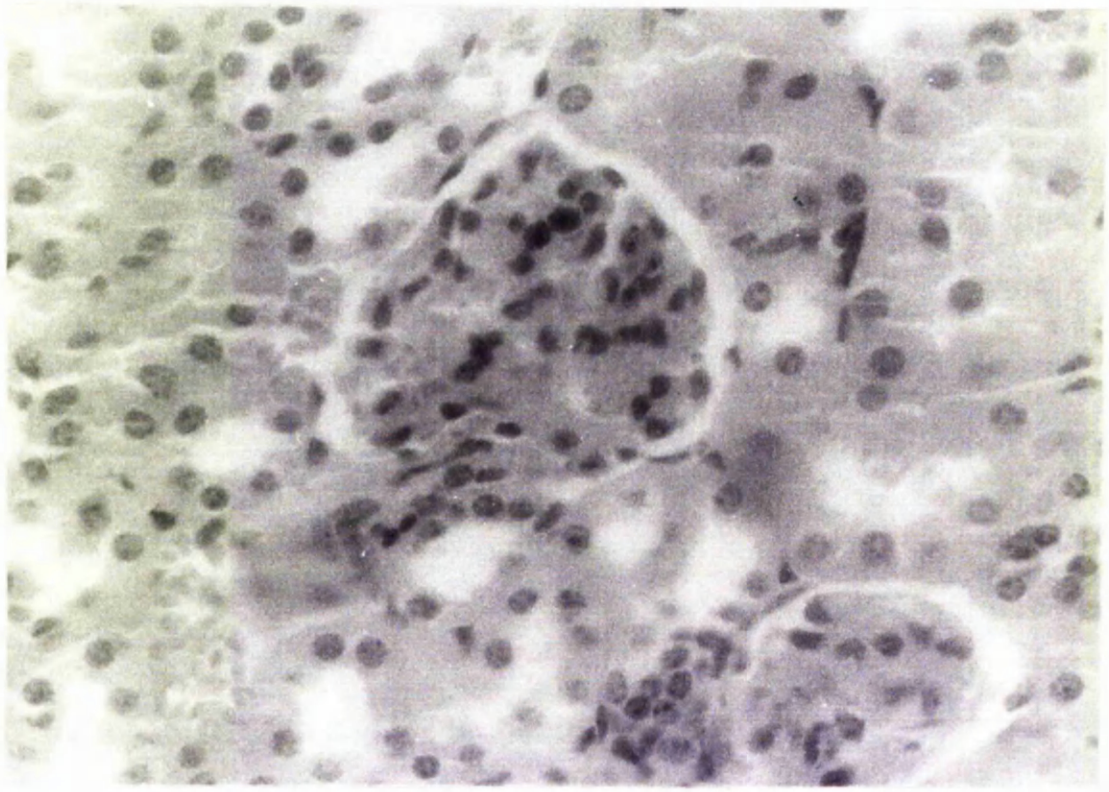

Plate 12

PLATES 13-15 : Sections of kidney from TO [low] mice receiving 60 daily i.p. injections of 0.3 mg ova, stained by immunofluorescent techniques.

Plate 13 : presence of glomerular mesangial staining for mouse IgG. (Magnification x 150).

Plate 14 : presence of glomerular mesangial staining for C3. (Magnification x150).

Plate 15 : presence of glomerular mesangial staining for ova. (Magnification x150).

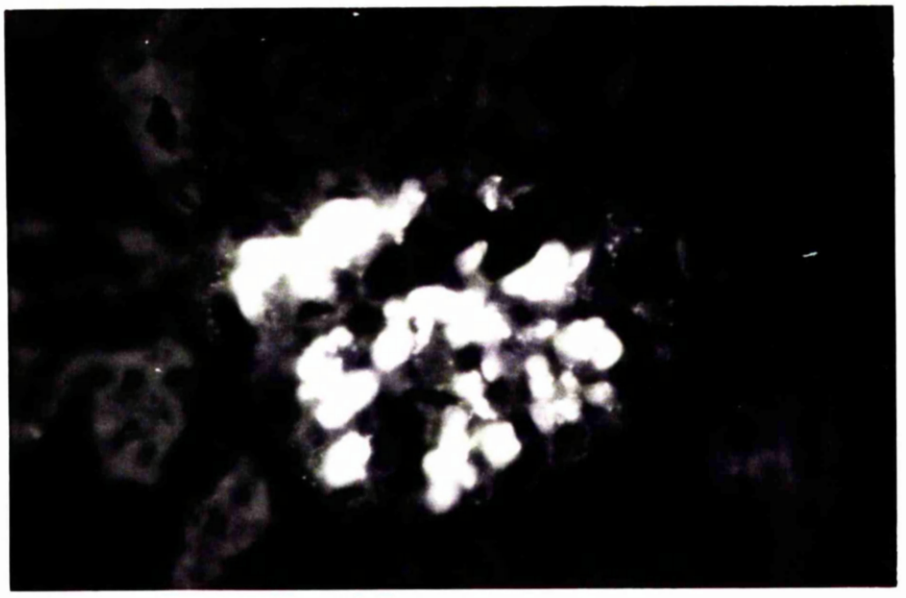

Plate 13

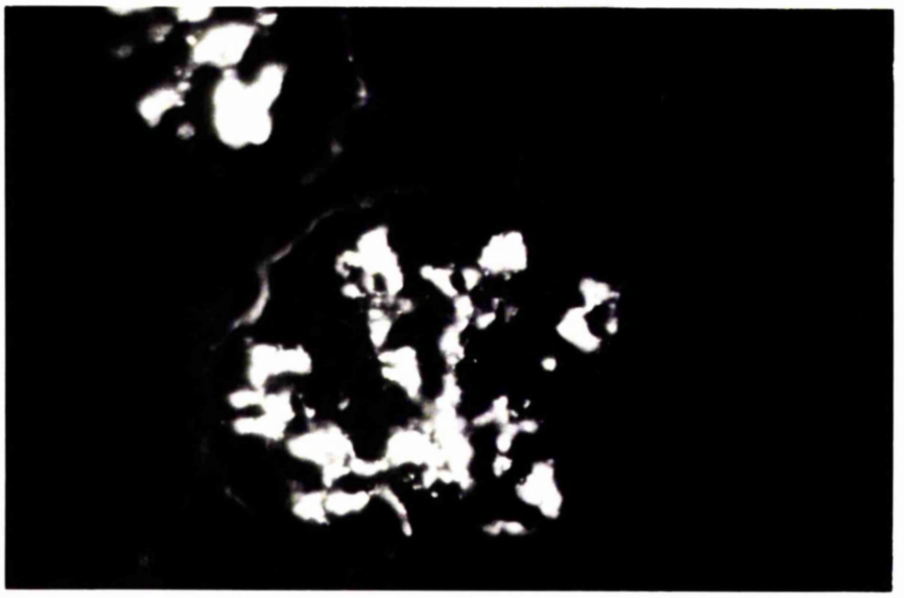

Plate 14

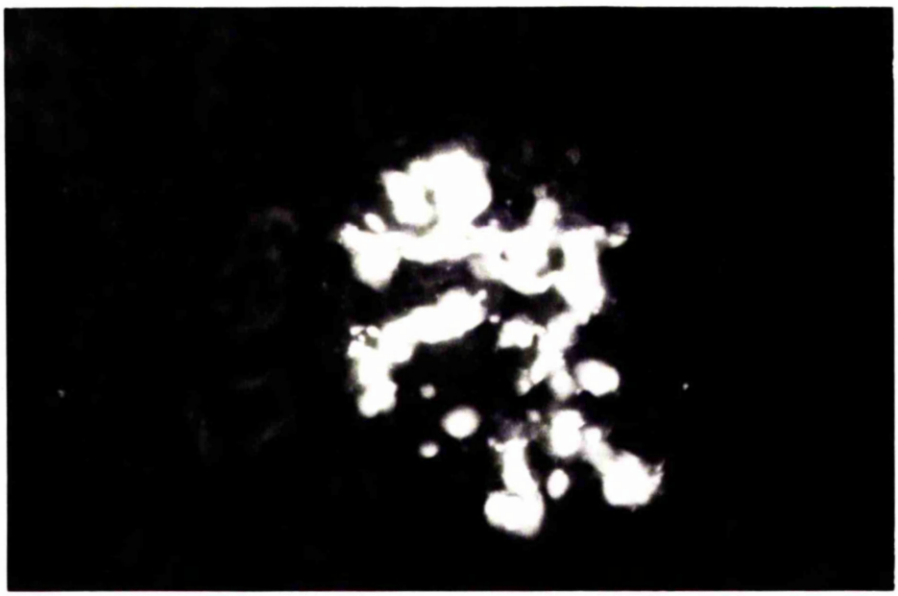

Plate 15

PLATE 16 : Kidney section from water fed TO [low] mouse receiving 60 daily injections of ova. Section, stained indirectly for ova, shows marked glomerular mesangial and capillary loop immunofluorescent staining. (Magnification x150).

PLATE 17 : Kidney section from TO [low] mouse given 25 mg ova by intragastric intubation prior to receiving 60 daily injections of ova. Section, stained indirectly for ova, shows no glomerular immunofluorescent staining. (Magnification x150).

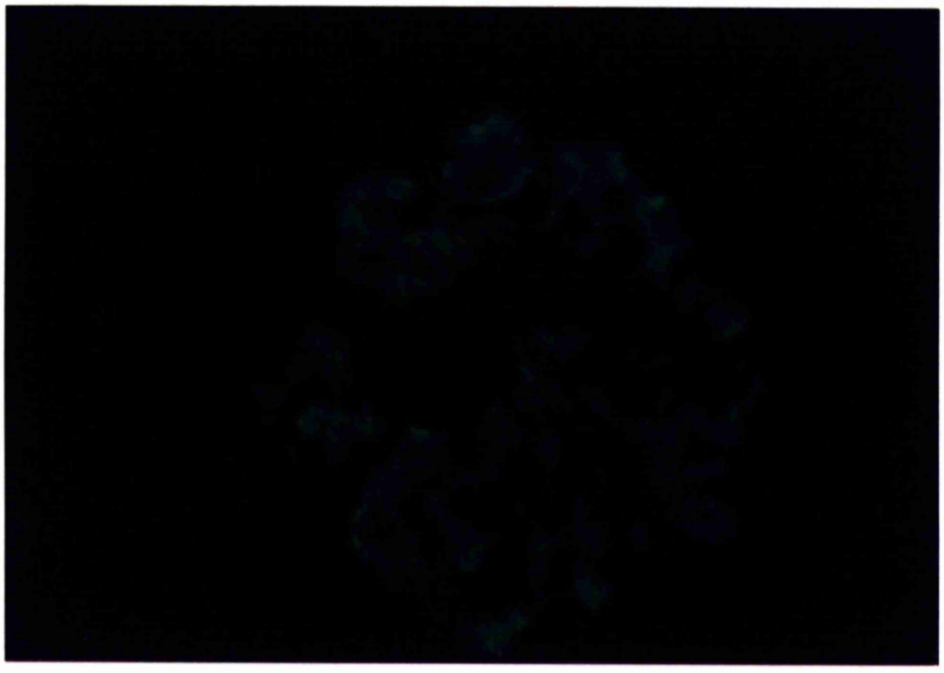

Plate 16

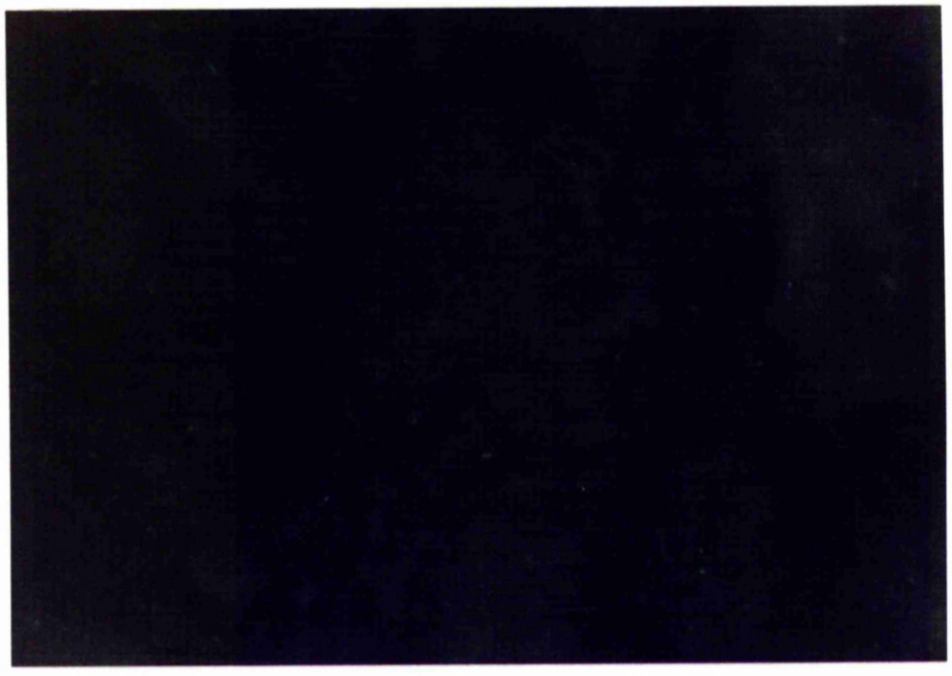

Plate 17

PLATE 18 : Electron micrograph of glomerulus of water fed TO [low] mouse receiving 60 daily injections of ova. Section shows mesangial expansion, with electron dense deposits (arrowed) in the mesangium and backing into the subendothelial space. Polymorphonuclear leucocytes (PMN) and lymphocytes (L) were seen within the capillary loops. (Magnification x1300).

PLATE 19 : Electron micrograph of renal mesangium from water fed TO [low] mouse receiving 60 daily injections of ova. Section shows a mesangial cell (M), with electron dense deposits (arrowed) in the mesangial matrix. (Magnification x7500).

PLATE 20 : Electron micrograph of kidney section from TO [low] mouse given 25 mg ova by intragastric intubation prior to receiving 60 daily injections of ova. Section shows renal tubules (T) and glomerulus. Mesangium (M) appears normal, with few electron dense deposits present. No inflammatory cells were seen. (Magnification x1300).

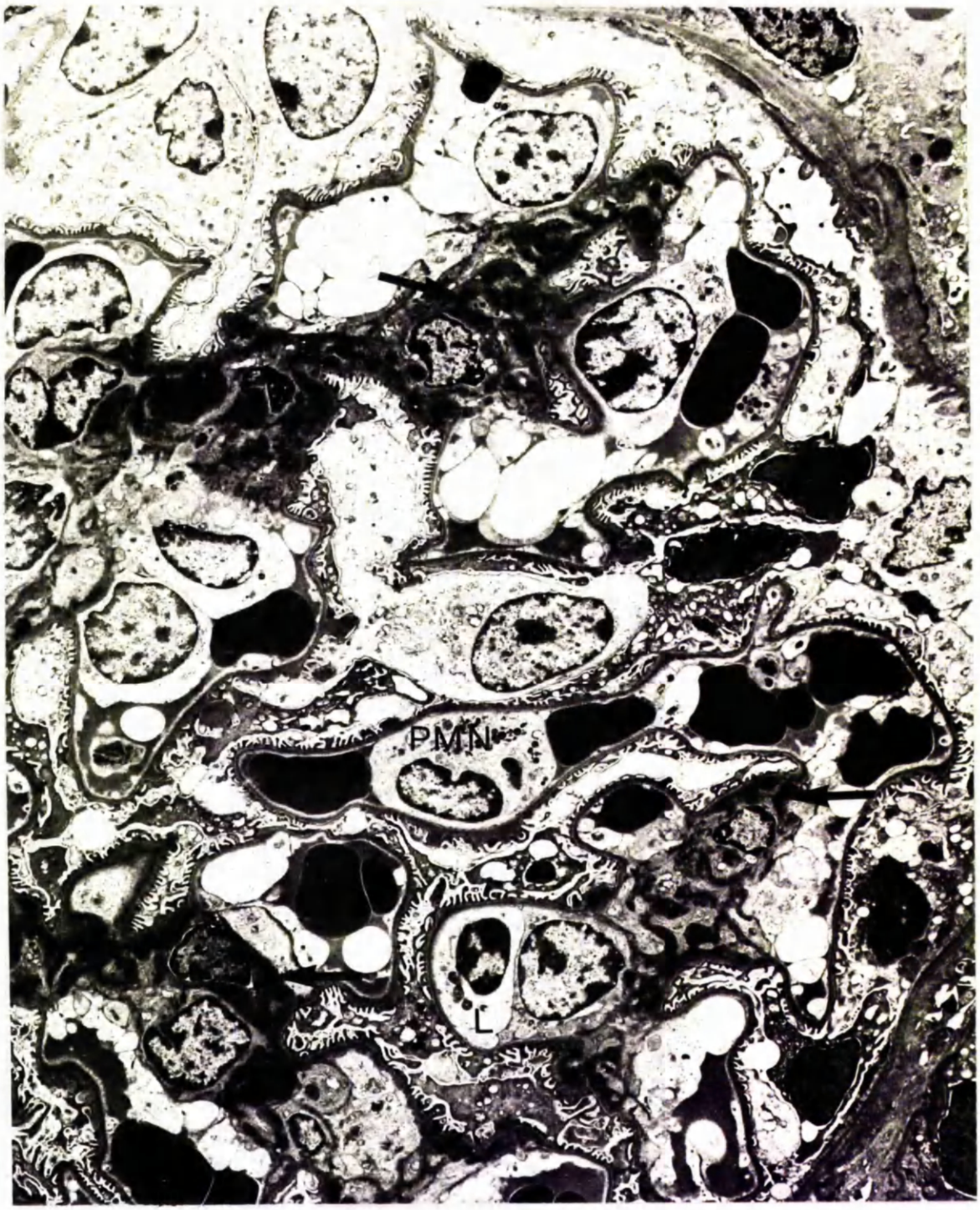

Plate 18

Plate 19

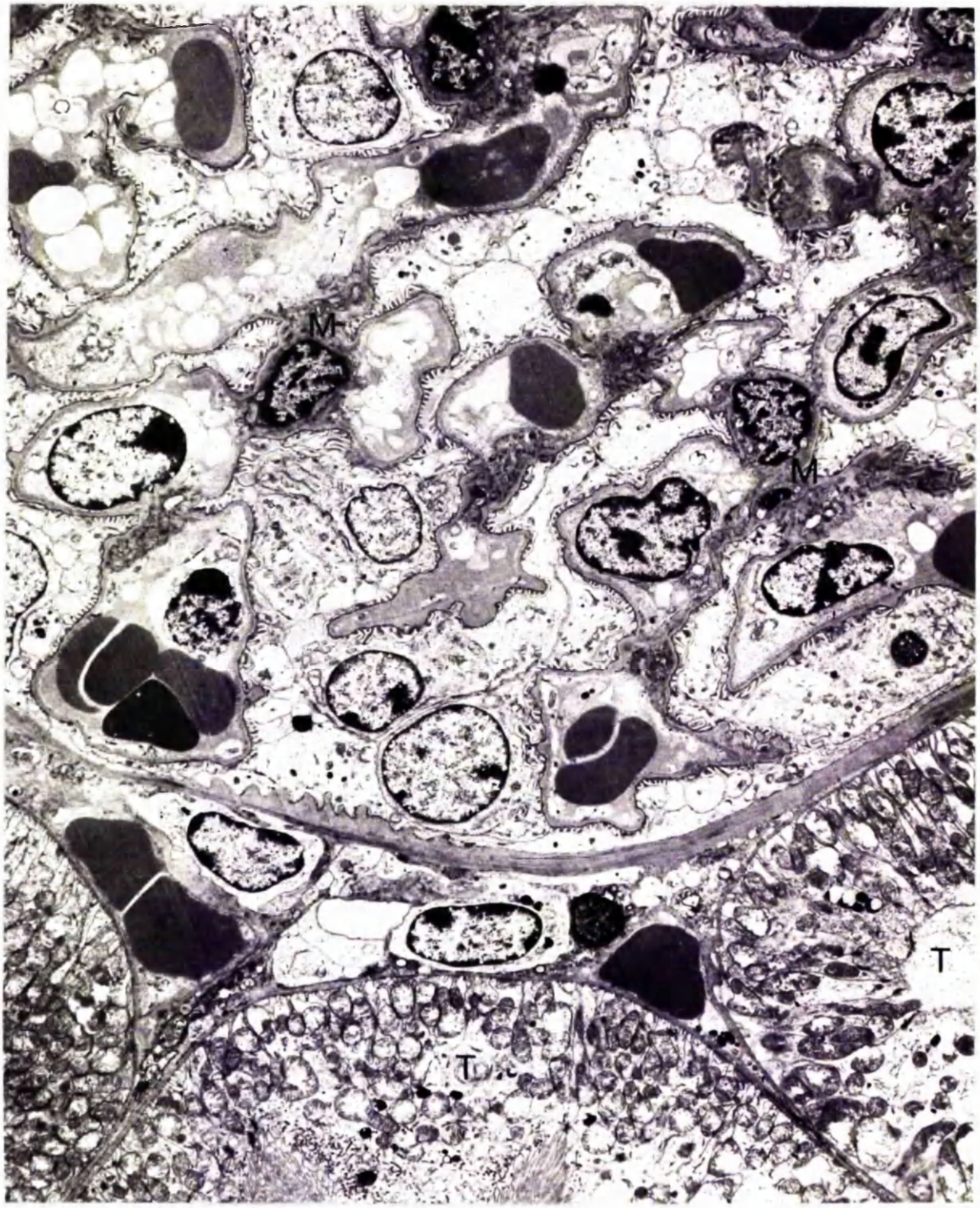

Plate 20

CHAPTER SIX

PROTECTION FROM IMMUNE COMPLEX GLOMERULONEPHRITIS BY ORAL ANTIGEN ADMINISTRATION

6.1 INTRODUCTION

In the previous chapter, the induction of immune complex glomerulonephritis by repeated injections of antigen was defined in TO [low] mice and other inbred strains. The administration of single doses of antigen by the oral route prior to the induction of nephritis in susceptible strains was associated with a reduced incidence of immune complex glomerulonephritis and with reduced systemic antibody responses in antigen fed animals as compared with water fed controls.

In this chapter I have investigated the effects of oral antigen administration on antigen induced immune complex glomerulonephritis with respect to the factors which limit, and the mechanisms which underlie protection from immune complex nephritis by intragastric antigen administration.

6.2 SPECIFICITY FOR THE IMMUNISING ANTIGEN

6.2.1 Oral administration of different protein antigens

To investigate the specificity of protection from immune complex glomerulonephritis by oral antigen administration, groups of mice were fed different protein antigens prior to the induction of immune complex glomerulonephritis by daily injections of ovalbumin.

Materials and Methods

Animals : Male TO [low] mice were used at 8 weeks of age.

Oral antigen administration : Groups of mice were given single 25 mg doses of ova or HSA or BGG by intragastric intubation. Control animals received distilled water by

the same route.

Induction of nephritis : Fourteen days after antigen feeding, immune complex nephritis was induced by daily injections of 0.3 mg ova by intraperitoneal inoculation for 60 days. Twenty four hours after the final antigen injection the mice were killed and the right kidneys taken for immunofluorescent examination.

Results

Results of immunofluorescent examination of unfixed cryostat kidney sections are shown in Table 13. Kidneys from water fed animals all showed glomerular deposition of IgG, which was associated with the presence of C3 and ova in 75% of cases examined. Feeding with single 25 mg doses of ovalbumin prior to the induction of immune complex nephritis by repeated injections of ovalbumin was associated with the absence of IgG, C3 and ova from all kidney sections examined. Oral administration of similar doses of HSA or BGG, however, had no abrogative effect on the glomerular deposition of immune complexes in the kidneys in antigen fed as compared with water fed controls, with 5 of 7 mice from both HSA and BGG fed groups showing the presence of IgG, C3 and ova in the glomerulus.

The results indicate that protection from immune complex glomerulonephritis is specific for the immunising antigen.

6.2.2 Hapten-carrier studies

The use of hapten-carrier conjugates allows the disection of the immune response at the cellular level by

dissociating the B cell and thymus-dependent limbs of the antibody response to the hapten. To examine further the specificity of protection from immune complex nephritis by oral antigen administration, the hapten dinitrophenyl (DNP) was conjugated to carrier proteins which were then administered by the oral route prior to the induction of immune complex nephritis by injections of DNP-ova.

Materials and Methods

Animals : Male TO [low] mice at 6-8 weeks were used.

Oral antigen administration : Groups of mice were fed single 25 mg doses of protein antigen or dinitrophenylated proteins containing 25 mg carrier protein by intragastric intubation according to the regime defined in Table 14. Control animals received distilled water by the same route.

Induction of nephritis : Mice of all experimental groups were injected daily from days 0 - 59 with DNP-ova (0.3 mg protein) by intraperitoneal inoculation. Twenty four hours after the final injection the mice were bled out and the right kidneys taken for immunofluorescent examination. Anti-ova antibody responses were estimated as described in section 2.26.

Results

Immunofluorescence : Unfixed cryostat sections of kidney were stained for the presence of IgG, C3, ova and DNP. The percent of kidney sections showing positive immunofluorescent staining for IgG, C3, ova and DNP are shown in Table 15. Deposition of IgG, C3, ova and DNP was seen in the kidneys of all water fed animals following the

induction of nephritis. Feeding with DNP-BGG prior to the induction of nephritis had no effect on the incidence of glomerular deposition of these parameters. One of 7 mice given ova, and 2 of 7 mice given DNP-ova by the intragastric route prior to the induction of nephritis showed no glomerular immune complex deposition. Only prior feeding with ova plus DNP-BGG was associated with a significant reduction in the incidence of glomerular immune complex deposition as compared with water fed controls, with 3 of 7 mice showing no deposition of IgG, C3, ova or DNP, and 1 other showing negative staining for C3 and DNP ($p < 0.05$; Fisher's exact test).

Examination of the degree of immunofluorescent staining, as indicated by the immunofluorescence scores, however, showed significant reductions in the degree of immunofluorescent staining in kidneys from mice fed ova, DNP-ova and ova plus DNP-BGG ($p < 0.01$, $p < 0.05$ and $p < 0.01$ respectively; Wilcoxon rank sum test). Immunofluorescence indices for the 4 parameters examined in the 5 experimental groups are shown in Table 16. Induction of nephritis in water fed animals produced a marked glomerular deposition of all 4 parameters. Immunofluorescence indices in animals fed DNP-BGG prior to the induction of nephritis were slightly reduced as compared with these. Feeding with ova, DNP-ova and ova plus DNP-BGG prior to the induction of nephritis was associated with marked reductions, as compared with water fed controls, in the immunofluorescence index to all 4 target parameters.

Antibody titres : The anti-ova antibody titres of mice of

the 5 experimental groups are shown in Figure 20. Repeated intraperitoneal injections of DNP-ova in water fed animals resulted in a systemic antibody response to ova. Anti-ova antibody responses in mice fed ova, DNP-ova, DNP-BGG or ova plus DNP-BGG were reduced in comparison with water fed animals (mean percent reductions of 45%, 34%, 24% and 78% respectively). These reductions in antibody titre were significant only in mice fed ova plus DNP-BGG ($p < 0.01$; Wilcoxon rank sum test).

Analysis of the relationship between the anti-ova antibody titres and the degree of glomerular immune complex deposition (immunofluorescence score) showed a significant correlation between these two parameters ($r = 0.623$; $p < 0.0001$)

6.3 THE EFFECT OF THE DOSE OF ORAL ANTIGEN ON THE INDUCTION OF IMMUNE COMPLEX NEPHRITIS

In the previous chapter it was shown that single 25 mg oral doses of ova could protect against the induction of immune complex nephritis by repeated systemic injections of ova. In earlier experiments I demonstrated that the reductions in DTH and antibody responses seen following intragastric antigen administration were dependent on the dose of antigen administered. In order to examine whether protection from immune complex glomerulonephritis was dependent on the dose of oral antigen administered, groups of mice were fed different doses of antigen prior to the induction of nephritis. The presence of glomerular immune deposits was investigated, and their relation to serum

antibody, circulating free antigen and circulating immune complex levels was examined.

Materials and Methods

Animals : Male TO [low] mice were used at 8 weeks of age.

Oral antigen administration : Groups of mice were fed single 1 mg, 10 mg or 25 mg doses of ova by intragastric intubation. Control animals received distilled water by the same route.

Induction of nephritis : Fourteen days after oral antigen administration, the mice were started on a regime of daily intraperitoneal inoculations of 0.3 mg ova for 60 injections. A second group of water fed animals, to act as negative controls in serological assays, was injected daily with 0.2 ml sterile saline by the intraperitoneal route.

Twenty four hours after the final injection, the mice were bled out. The blood was allowed to clot at room temperature, and the serum was separated and stored in aliquots at $-20^{o}C$ for antibody and free ova assays (sections 2.26 and 2.27), and at $-70^{o}C$ for immune complex assay (section 2.31). The right kidneys were removed for immunofluorescent examination. Unfixed cryostat sections were stained for the presence of IgG, C3 and ova.

Results

6.3.1 Induction of nephritis :

Results of immunofluorescent examination of kidneys from mice fed 1 mg, 10 mg or 25 mg ova or water alone prior to the induction of immune complex nephritis are shown in Table 17.

Five of 6 water fed animals showed renal deposition of

IgG and ova, with 4 of 6 mice showing the presence of C3 in the kidney. Feeding with 1 mg ova prior to the induction of nephritis had no effect on the renal deposition of IgG, C3 or ova. In animals fed 10 mg ova, whilst small amounts of IgG were seen in 4 of 6 kidneys examined, only 2 of 6 mice had deposition of C3, and no mouse showed the presence of ova in the kidney. In mice fed 25 mg ova prior to the induction of nephritis one animal out of seven had deposition of IgG and ova only.

Thus the degree of protection from immune complex glomerulonephritis was related to the dose of antigen administered by the intragastric route.

6.3.2 Systemic antibody response :

Levels of anti-ova antibodies in water fed, saline injected controls were negligible. Anti-ova antibody titres in antigen injected mice are shown in Figure 21. Antigen fed animals showed a dose-dependent reduction in anti-ova antibody titres as compared with water fed mice, with mean percent reductions of 19%, 58% and 64% for animals fed 1 mg, 10 mg and 25 mg ova respectively.

The presence of glomerular immune deposits (defined by the codeposition of at least 2 target parameters of immune complex deposition) correlated with anti-ova antibody titre. Eleven of 14 mice in which the anti-ova antibody titre was greater than 0.2 ELISA units had glomerular immune deposits, whilst only 1 of 11 mice with anti-ova antibody titres below this level had glomerular immune deposits ($p < 0.001$; Fisher's exact test).

6.3.3 Free circulating antigen :

The presence of free circulating antigen in the serum of water fed and antigen fed mice following the induction of nephritis was detected by antigen specific ELISA. No free antigen was detected in the serum of any mouse injected with saline alone (data not presented). Levels of free circulating ova in antigen injected mice are shown in Figure 22. Free antigen was detected in the serum of only 1 of 8 water fed control mice. In animals fed 1 mg ova prior to the induction of nephritis free antigen was detected in 3 of 8 sera at the lower limit of sensitivity of the assay. Four of 7 mice fed 10 mg ova, and 8 of 9 mice fed 25 mg ova had detectable serum levels of ovalbumin. Only in mice fed 25 mg ova prior to the induction of nephritis by daily ova injections were levels of free serum ova significantly raised as compared with water fed controls ($p < 0.05$; Wilcoxon rank sum test). There was, however, a marked variation in the levels of free circulating ova within this group.

Analysis of the relationship between the anti-ova antibody titres and the levels of free circulating ovalbumin in these mice showed a significant inverse correlation ($r = 0.616$; $p < 0.0002$)

6.3.4 Circulating immune complexes

The presence of conglutinin binding circulating immune complexes was investigated using a solid phase ELISA. Circulating immune complexes were not detected in the serum of any saline injected mouse. Of 31 sera from antigen injected mice of the four experimental groups,

only four sera had detectable conglutinin binding levels, and the results are listed in Table 18. The remaining 27 sera all gave values below 15 μg / ml mAGG, which was considered to be the lower limit of sensitivity of the test (as defined in section 2.31).

6.4 THE EFFECTS OF PASSIVE SERUM TRANSFER ON PROTECTION FROM IMMUNE COMPLEX NEPHRITIS

Strobel and colleagues (1983) demonstrated the presence of a serum factor capable of passively transferring oral tolerance 60 minutes after antigen feeding. To examine whether protection from immune complex nephritis could be transferred similarly by serum following antigen feeding, serum was passively transferred from antigen fed animals to naive recipients prior to the induction of immune complex nephritis.

Materials and Methods

Animals : 8-10 week old male and female TO [low] mice were used as serum donors. Eight week old male TO [low] mice were used as serum recipients.

Collection and transfer of serum : Serum was collected by bleeding out donor mice 60 minutes after the intragastric administration of 25 mg ova per mouse, or of distilled water alone as described in section 2.9. 0.8 ml pooled donor serum was passively transferred to naive recipient mice by intraperitoneal inoculation.

Induction of nephritis : Seven days after serum transfer, recipient mice were started on a regime of daily intraperitoneal injections of 0.3 mg ova. Twenty four hours after the 60th antigen injection the animals were

bled out. The right kidneys were taken for immunofluorescent examination. Serum antibodies to ova were estimated as described previously.

Results

Immunofluorescence : Unfixed cryostat sections were stained for the presence of IgG, C3 and ova. Results of immunofluorescent examination of kidney sections from recipients of serum from water fed and from ova fed donors are shown in Table 19. Glomerular immune complex deposition was seen in the kidneys of the majority of mice from both experimental groups. There was no difference in either the percent of sections showing positive immunofluorescent staining, nor in the degree of immunofluorescent staining (data not presented) between the two groups. The results indicate that transfer of serum 60 minutes after antigen feeding did not confer protection from immune complex glomerulonephritis to recipient mice.

Antibody titres : Anti-ova antibody titres in the same mice are shown in Figure 23. There was no difference in antibody responses between recipients of serum from water fed donors and recipients of serum from ova fed donors.

6.5 THE ROLE OF SUPPRESSOR T CELLS IN PROTECTION FROM IMMUNE COMPLEX NEPHRITIS

A number of mechanisms have been implicated in the suppression of systemic antibody and DTH responses following oral antigen administration (reviewed in section 1.6.3). Of these the best characterised is the induction

of a population of suppressor T lymphocytes. In order to assess whether a similar mechanism might operate in protection from immune complex nephritis by oral antigen administration, the induction and role of suppressor T cells in protection from immune complex nephritis after antigen feeding was studied in mice pretreated with cyclophosphamide, and in suppressor cell transfer studies.

6.5.1 Induction of suppressor T cells

The induction of suppressor T cells in mice is specifically inhibited by administration of cyclophosphamide at a dose of 100 mg/ kg body weight (Schwatrz, Askanase & Gershon, 1978). Cyclophosphamide, given at this dose prior to intrasgatric administration of antigen, has been shown to abrogate the induction of oral tolerance for both DTH and antibody responses (Mowat etal, 1982). To investigate the possible role of the induction of a population of suppressor T cells in protection from immune complex glomerulonephritis, mice were given cyclophosphamide prior to antigen feeding.

Materials and Methods

Animals : Male TO [low] mice were used at 8 weeks of age.

Administration of cyclophosphamide : The mice were separated into 4 experimental groups. Two groups were injected with cyclophosphamide (100 mg/ kg) in 0.2 ml sterile distilled water per mouse by intraperitoneal inoculation on day 0. The remaining two groups received 0.2 ml sterile distilled water by the same route.

Oral antigen administration : Two days later, one group each of cyclophosphamide treated and water treated mice

were given single 25 mg doses of ova by intragastric intubation. The remaining two groups received distilled water by the same route.

Induction of nephritis : Fourteen days after antigen feeding, all mice were started on a regime of daily intraperitoneal inoculations of 0.3 mg ova, for a total of 60 injections. Twenty four hours after the final antigen injection the mice were bled out and the right kidneys were taken for immunofluorescent examination.

Results

Unfixed cryostat sections of kidney were stained for the presence of IgG, C3 and ova. The results of immunofluorescent examination of kidney sections are shown in Table 20. Five of 6 mice which had been treated with distilled water prior to intragastric administration of water showed glomerular deposition of IgG, C3 and ova, following the induction of nephritis by daily injections of ova. In water treated animals which received ova by intragastric intubation, glomerular deposits of IgG, C3 and ova were seen in 2 out of 5 mice examined. Mice which had been treated with cyclophosphamide prior to intragastric administration of ova showed a significantly reduced incidence of glomerular immune deposits as compared with water treated, water fed animals, with only 2 of 7 mice showing positive immunofluorescent staining for IgG, and 1 of 7 showing positive staining for C3 and ova. Water fed mice which had been pretreated with cyclophosphamide showed a similar incidence of deposition of IgG, C3 and ova as untreated water fed animals. The results indicated that pretreatment with cyclophosphamide

did not ablate the protective effects of antigen feeding on the induction of immune complex glomerulonephritis.

6.5.2 Spleen cell transfer

To investigate whether an established population of suppressor cells could inhibit the induction of nephritis by daily antigen injections, adoptive transfer of spleen cells from animals rendered tolerant to the immunising antigen to naive syngeneic recipients was carried out prior to the induction of nephritis. Using the same experimental protocol, I have shown previously the transfer of tolerance for DTH responses by adoptive spleen cell transfer from antigen fed donors (section 3.2.5).

Materials and Methods

Animals : Eight week old female BALB/c mice were used as spleen cell donors. Eight to 10 week old female mice acted as spleen cell recipients.

Induction of tolerance : Donor mice were rendered tolerant to ovalbumin by the administration of single 25 mg doses of ovalbumin by the intragastric route. Control donor animals received distilled water alone by the same route.

Spleen cell transfer : Seven days after the induction of tolerance, donor mice were killed. Suspensions of pooled spleen cells were prepared as described in section 2.10. Recipient mice received 10^8 pooled spleen cells from antigen fed or water fed donors by intravenous inoculation.

Induction of nephritis : Four hours after spleen cell transfer, all recipient mice were injected with 0.3 mg ova by the intraperitoneal route, and similarly thereafter for

a total of 60 daily antigen injections. Twenty four hours after the final antigen injection the mice were bled out and the right kidneys were taken for immunofluorescent examination. Anti-ova antibody titres were estimated by ELISA.

Results

Immunofluorescence : Results of immunofluorescent examination of unfixed cryostat sections of kidney from recipients of spleen cells from ova fed (tolerised) and water fed (control) donors are shown in Table 21. Following the induction of nephritis, kidney sections from the majority of recipients of both tolerised and control spleen cells showed glomerular deposition of IgG, C3 and ova. There was no difference in the incidence nor in the degree of immunofluorescent staining between the two groups.

Antibody titres : Antibody responses to daily injections of ova in recipients of tolerised and control spleen cells are shown in Figure 24. There was no difference in the antibody titres between recipients of spleen cells from tolerised or from control donor mice.

6.6 CONCLUSIONS

Single doses of protein antigen administered by the intragastric route have been shown to be capable of inhibiting the subsequent induction of immune complex glomerulonephritis by repeated injections of the same antigen. In this chapter I have shown that this effect is specific for the immunising protein antigen. Similar

studies using a hapten-carrier conjugate system indicated carrier specific reductions in the degree of glomerular immune complex deposition following intragastric administration of various hapten / carrier combinations. Significant reductions in carrier-specific antibody responses, however, were only seen following separate oral administration of both carrier and hapten conjugated to a heterologous protein carrier.

The degree of protection from immune complex nephritis conferred by single intragastric doses of antigen was shown to be dependent on the dose of antigen administered to the intestinal tract. Similar dose dependent reductions were seen in the systemic antibody titres induced by repeated parenteral antigen injections following intragastric antigen administration. Overall the presence and degree of immune complex nephritis correlated with the antibody titre to the immunising antigen. Circulating antigen was detected 24 hours after the final antigen injection in antigen fed, but not in water fed animals, and the levels of circulating antigen varied in relation to the dose of antigen administered and inversely with serum antibody titres. Conglutinin binding circulating immune complexes were not consistently demonstrated in animals of any experimental group.

Attempts to transfer inhibition of the induction of immune complex nephritis by serum transfer following intragastric antigen administration were unsuccessful. Similarly serum transfer from antigen fed donors had no effect on the subsequent systemic antibody responses to the immunising antigen.

The role of suppressor T cells in the inhibition of induction of immune complex glomerulonephritis was investigated by pretreatment of mice with doses of cyclophosphamide which selectively inhibit the induction of suppressor T cells, and by transfer of spleen cells from mice rendered tolerant to the immunising antigen by the oral administration of antigen. Cyclophosphamide had no abrogative effect on the inhibition of induction of immune complex nephritis by prior intragastric administration of the immunising antigen, suggesting that the induction of a population of suppressor T cells is not a major factor in the inhibition of induction of nephritis in this model. Similarly the transfer of spleen cells from mice rendered tolerant to the immunising antigen failed to transfer protection from the induction of immune complex glomerulonephritis.

The results support the hypothesis that the inhibition of induction of immune complex glomerulonephritis by intragastric antigen administration is associated with the reduction in the antibody response to the immunising antigen, and that the induction of a population of suppressor T cells does not play a major role in this effect. The mechanism of inhibition of induction of immune complex glomerulonephritis by prior intragastric administration of antigen, however, remains obscure.

TABLE 13.

Group	n	Percent of sections showing positive staining		
		IgG	C3	ova
H2O ig	4	100	75	75
ova ig	5	0**	0*	0*
HSA ig	7	86	71	71
BGG ig	7	71	71	86

* $p < 0.05$; ** $p < 0.01$ (Fisher's exact test) as compared with water fed mice.

Table 13 : The specificity for the immunising antigen of protection from antigen induced immune complex glomerulonephritis. The incidence of positive glomerular staining for IgG, C3 and ova of kidney sections from TO [low] mice given single 25 mg intragastric (ig) doses of ova, HSA, BGG or water alone prior to receiving 60 daily i.p. injections of ova.

TABLE 14.

Experimental protocol				
Group	Day -14	Day -7	Days 0 - 59	Day 60
A	-	H2O ig	DNP-ova ip	mice bled and sacrificed
B	-	ova ig	"	"
C	-	DNP-ova ig	"	"
D	-	DNP-BGG ig	"	"
E	ova ig	DNP-BGG ig	"	"

Table 14 : Experimental protocol for the investigation of the specificity of protection by antigen feeding from antigen induced immune complex glomerulonephritis, using hapten-carrier conjugates.

TABLE 15.

Group	n	Percent of sections showing positive staining			
		IgG	C3	ova	DNP
A	8	100	100	100	100
B	7	86	57	86	86
C	7	71	71	71	71
D	7	100	100	100	100
E	7	57	43*	57	43*

* $p < 0.05$ (Fisher's exact test) as compared with water fed, antigen injected controls (Group A).

Table 15 : The effect of intragastric administration of antigen on the induction of immune complex glomerulonephritis by repeated antigen injections : definition of the specificity of the response using hapten-carrier conjugates. The incidence of positive glomerular immunofluorescent staining for IgG, C3, ova and DNP of sections of kidney from TO [low] mice receiving 60 daily i.p. injections of DNP-ova subsequent to the intragastric administration of water (Group A), ova (Group B), DNP-ova (Group C), DNP-BGG (Group D) or ova + DNP-BGG (Group E).

TABLE 16.

Group	n	Immunofluorescence Index			
		IgG	C3	ova	DNP
A	8	2.125	2.00	2.125	2.125
B	7	1.00	0.71	1.14	0.86
C	7	1.29	1.00	1.43	1.14
D	7	1.71	1.71	1.86	1.71
E	7	0.86	0.43	0.86	0.71

Table 16 : The effect of intragastric administration of antigen on the induction of immune complex glomerulonephritis by repeated antigen injections : definition of the specificity of the response using hapten-carrier conjugates. The degree of glomerular deposition of IgG, C3, ova and DNP in kidney sections from TO [low] mice receiving 60 daily injections of DNP-ova subsequent to the intragastric administration of water (group A), ova (Group B), DNP-ova (Group C), DNP-BGG (Group D) or ova + DNP-BGG (Group E).

TABLE 17.

Group	n	Percent of sections showing positive staining		
		IgG	C3	ova
H20 ig	6	83	67	83
1 mg ova ig	6	83	67	83
10 mg ova ig	6	67	33	0**
25 mg ova ig	7	14*	0*	14*

* $p < 0.05$; ** $p < 0.01$ (Fisher's exact test) as compared with water fed, antigen injected mice.

Table 17 : The dose-dependent nature of protection by antigen feeding from antigen induced immune complex glomerulonephritis. The incidence of positive glomerular staining for IgG, C3 and ova in kidney sections from T0 [low] mice receiving 60 daily i.p. injections of ova subsequent to the intragastric (ig) administration of single 1 mg, 10 mg or 25 mg doses of ova, or of water alone.

TABLE 18.

Animal number	CIC level (µg/ml mAGG)
H20 . 1	47
1 mg ova . 8	35
10 mg ova . 1	72
10 mg ova . 5	24

Table 18 : Conglutinin binding circulating immune complex (CIC) levels in TO [low] mice given 1 mg, 10 mg or 25 mg ova or water alone by intragastric intubation prior to receiving 60 daily i.p. injections of 0.3 mg ova. Results presented are for individual mice of different experimental groups in which CIC levels were greater than 15 µg/ml mAGG equivalents. Results of less than 15 µg/ml mAGG equivalents were considered to fall below the lower limit of sensitivity for the assay, and are not presented.

TABLE 19.

Group	n	Percent of kidneys showing positive staining		
		IgG	C3	ova
Control serum ip	7	86	86	71
Ova fed serum ip	7	86	86	100

Table 19 : The role of absorbed antigen in protection by antigen feeding from antigen induced immune complex glomerulonephritis. The incidence of positive glomerular staining for IgG, C3 and ova of kidney sections from TO [low] mice receiving by passive transfer 0.8 ml pooled serum collected 60 minutes after the intragastric administration to donor mice of 25 mg ova or of water alone, prior to the induction in serum recipients of immune complex glomerulonephritis by 60 daily i.p. injections of ova.

TABLE 20.

Group	n	Percent of sections showing positive staining		
		IgG	C3	ova
H20 ip. H20 ig	6	83	83	83
H20 ip. ova ig	5	40	40	40
Cy ip. ova ig	7	28	14*	14*
Cy ip. H20 ig	4	100	50	100

* $p < 0.05$ (Fisher's exact test) as compared with water treated, water fed control mice.

Table 20 : The effects of pretreatment with cyclophosphamide on protection by antigen feeding from antigen induced immune complex glomerulonephritis. The incidence of positive glomerular staining for IgG, C3 and ova in sections of kidney from TO [low] mice treated with cyclophosphamide (Cy) or with water by intraperitoneal (ip) inoculation 2 days prior to the intragastric (ig) administration of single 25 mg doses of ova or of water alone, followed by the induction of immune complex glomerulonephritis in all mice by 60 daily i.p. injections of ova.

TABLE 21.

Group	n	Percent of sections showing positive staining		
		IgG	C3	ova
Control cells iv	7	71	57	86
Tolerised cells iv	7	57	43	86

Table 21 : The effects of spleen cell transfer from orally tolerised or from control mice to naive recipients on the subsequent induction of immune complex glomerulonephritis by repeated antigen injections. The incidence of positive glomerular immunofluorescent staining for IgG, C3 and ova of sections of kidney from BALB/c mice receiving by intravenous inoculation 10^8 spleen cells from orally tolerised or control donors prior to the induction of immune complex glomerulonephritis by 60 daily i.p. injections of ova.

FIGURE 20

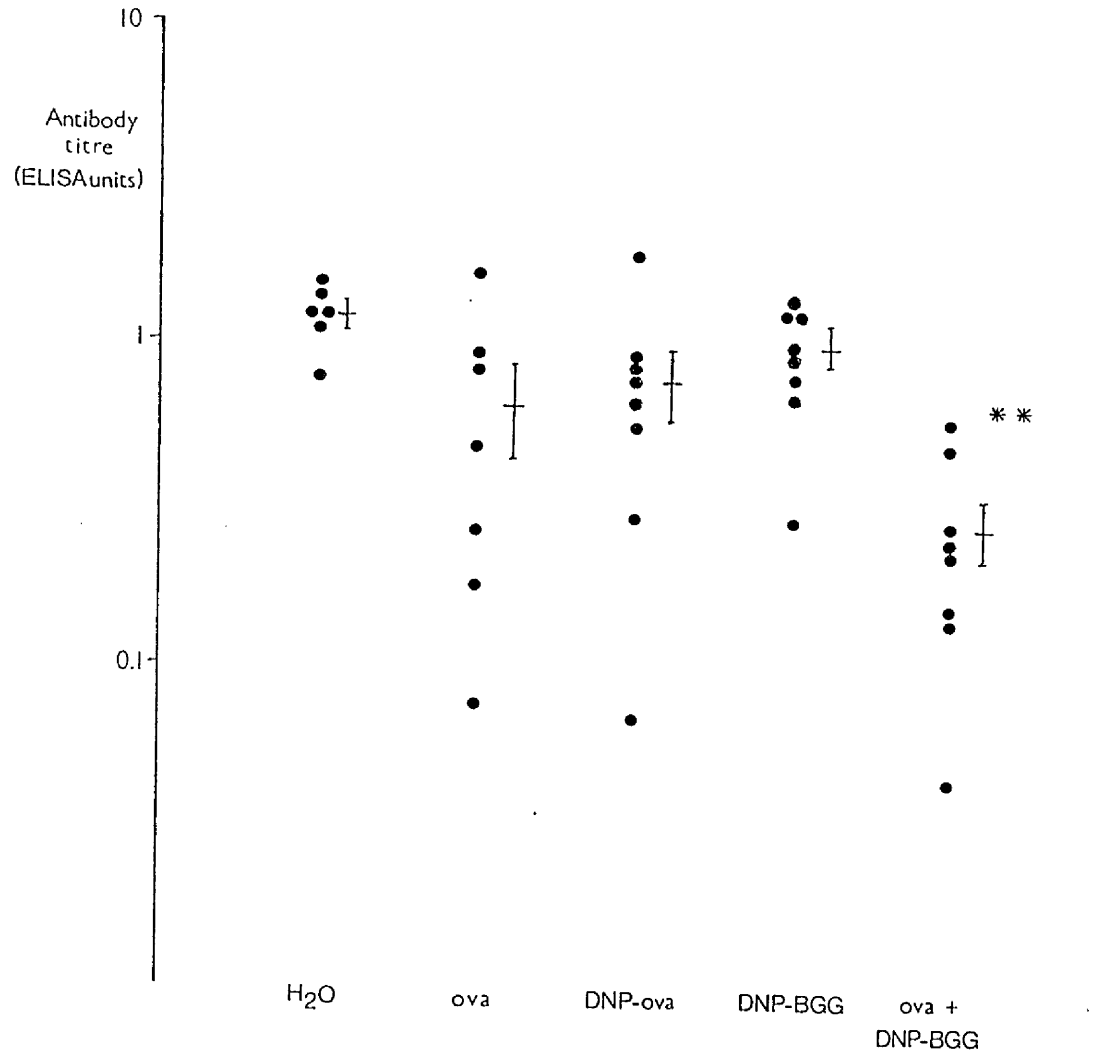

Figure 20 : Serum anti-ova antibody titres after 60 daily i.p. injections of DNP-ova in T0 [low] mice given ova, DNP-ova, DNP-BGG, ova + DNP-BGG or water alone before starting the regime of daily antigen injections. Circles represent antibody titres (ELISA units) in individual experimental mice. Bars represent group means ± 1 standard error.

** $p < 0.01$ (Wilcoxon rank sum test) as compared with water fed mice.

FIGURE 21

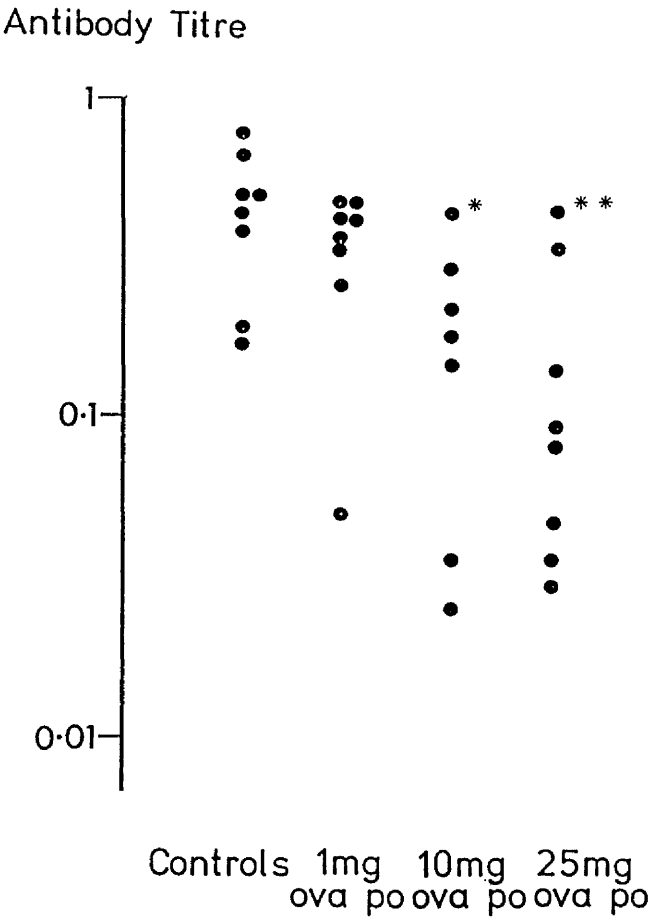

Figure 21 : Serum anti-ova antibody titres after 60 daily i.p. injections of ova in TO [low] mice given 1 mg, 10 mg or 25 mg ova, or water alone (controls), by intragastric intubation 14 days before starting the regime of daily antigen injections. Circles represent antibody titres (ELISA units) of individual experimental mice.

* $p < 0.05$; ** $p < 0.01$ (Wilcoxon rank sum test) as compared with water fed mice.

FIGURE 22

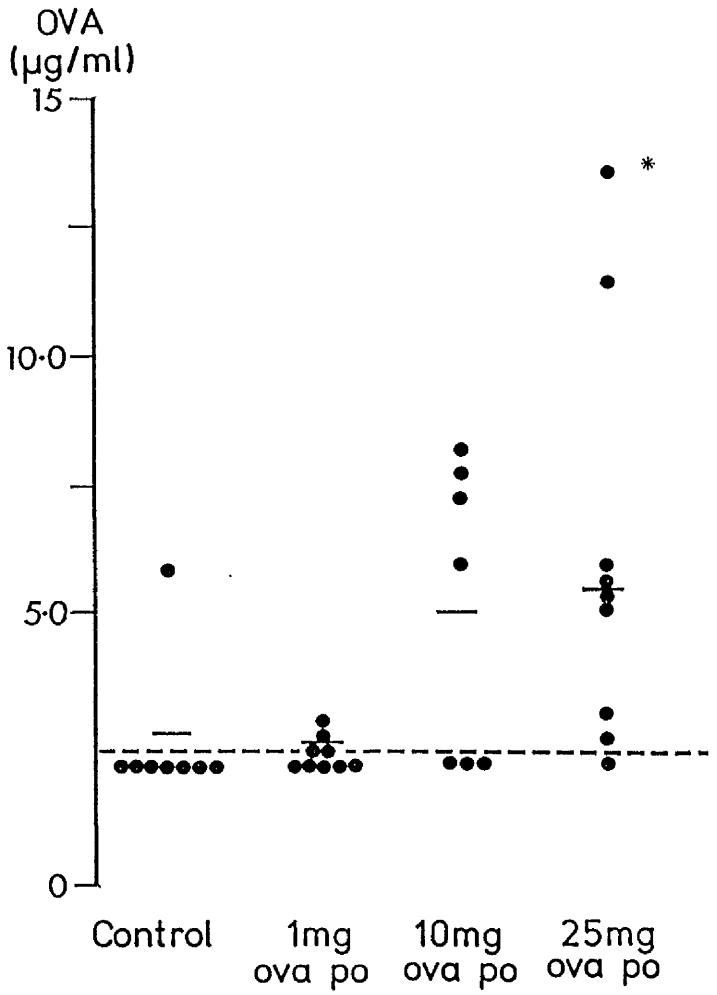

Figure 22 : Serum ova (µg/ml) levels 24 hours after the 60th daily i.p. injection of ova in TO [low] mice given 1 mg, 10 mg or 25 mg ova, or water alone (control), by intragastric intubation 14 days before starting the regime of daily antigen injections. Circles represent serum ova levels in individual experimental mice.

* $p < 0.05$ (Wilcoxon rank sum test) as compared with water fed mice.

FIGURE 23

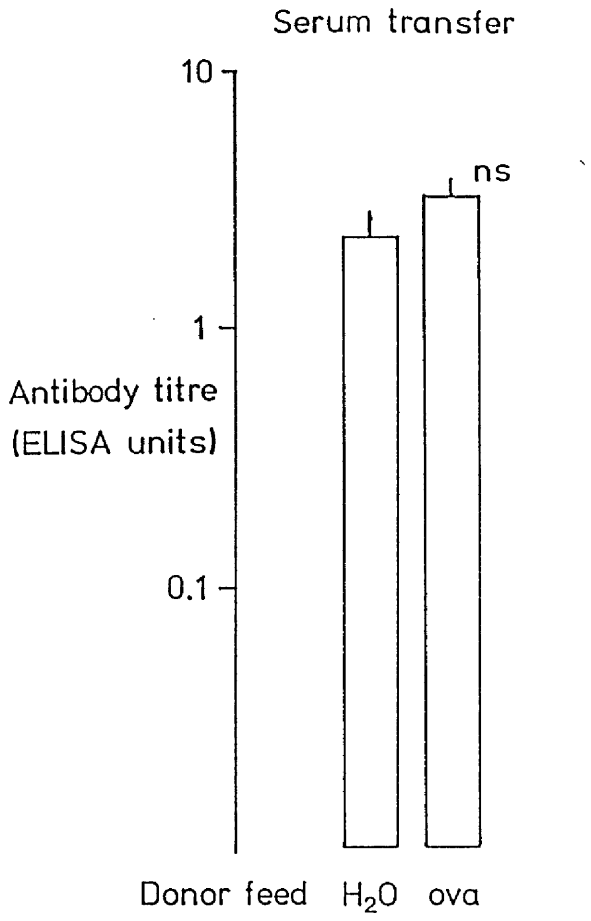

Figure 23 : Serum anti-ova antibody titres after 60 daily i.p. injections of ova in TO [low] mice receiving 0.8 ml pooled serum collected 60 minutes after the intragastric administration of 25 mg ova or of water alone to donor mice, and passively transferred 7 days before immunisation of serum recipients. Bars represent group mean antibody titre (ELISA units) + 1 standard error.

FIGURE 24

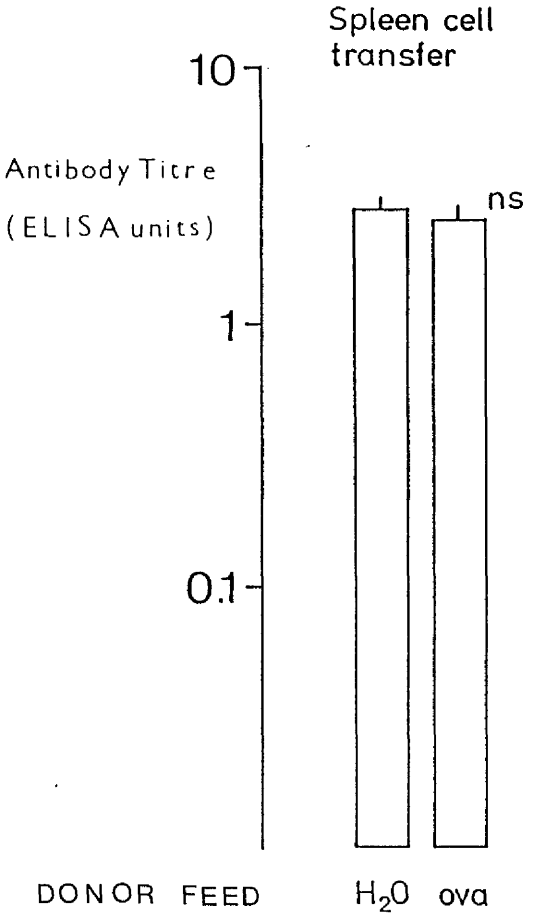

Figure 24 : Serum anti-ova antibody titres after 60 daily i.p. injections of ova in BALB/c mice receiving by intravenous inoculation 10^8 spleen cells from donor mice rendered tolerant to ova by intragastric administration of 25 mg ova, or from donor mice receiving intragastric water alone. Spleen cell recipients started the regime of daily antigen injections 4 hours after spleen cell transfer. Bars represent group mean antibody titre (ELISA units) + 1 standard error.

CHAPTER SEVEN

DISCUSSION

7.1 INTRODUCTION

In this thesis I have attempted to identify and evaluate the role of dietary antigens in the pathogenesis of experimental glomerulonephritis. Two major questions have been addressed : i) can dietary antigens be implicated directly in the pathogenesis of experimental glomerulonephritis? and ii) can oral antigen administration alter the induction and course of systemically induced immune complex mediated glomerulonephritis?

In addressing the first of these questions, the effects of prolonged oral antigen administration were observed on glomerular immune deposits in the kidneys of mice. Prolonged administration of BGG in the drinking water to BALB/c mice was associated with the appearance of glomerular deposition of IgA in the kidneys of antigen fed mice. Administration of ovalbumin in the drinking water or by intermittent intragastric intubation to the same strain of mouse, however, was not associated with an increase in glomerular IgA deposits. Similarly the administration of ovalbumin via the drinking water, or of SRBC by daily intragastric intubation had no effect on the levels of glomerular IgA deposits in C3H/HeOla and in LPS insensitive, congenic C3H/HeJ mice. The role of the liver in clearing potentially pathogenic immune complexes was investigated in mice with experimentally induced liver damage. Administration of intragastric doses of carbon tetrachloride (CCl_4) to mice was associated with the induction of chronic liver damage and hepatic fibrosis.

The induction of chronic liver damage, however, was not associated with increased levels of glomerular immune deposits in CCl4 treated mice.

In investigating the role of dietary antigens in the pathogenesis of systemically induced glomerulonephritis, I have examined the effects of intragastric antigen administration on the induction and course of antigen induced glomerulonephritis in susceptible strains of mice. In all such strains examined, the intragastric administration of single doses of antigen prior to the induction of immune complex glomerulonephritis was associated with a decreased incidence of glomerular immune complex deposition. This protection from immune complex glomerulonephritis by prior intragastric antigen administration was specific for the immunising antigen. Studies using hapten-carrier conjugates demonstrated that protection from immune complex nephritis by intragastric antigen administration was induced at the level of the carrier protein. The degree of protection from immune complex glomerulonephritis seen following intragastric administration of single doses of antigen was related directly to the dose of antigen administered. Serum antibody titres to the immunising antigen after the induction of nephritis showed a similar dose dependent reduction in antigen fed mice, and the antibody titres correlated with the degree of glomerular immune deposits. The mechanism underlying protection from immune complex glomerulonephritis by intragastric antigen administration is not clear. Investigation of the possible role of suppressor T cells suggested that these cells did not play

a major role in this effect.

Thus two possible roles have been identified for dietary antigens in the pathogenesis of experimental glomerulonephritis. i) Oral antigen administration may be implicated directly in the induction of glomerular IgA deposits in experimental IgA nephropathy. ii) Antigen feeding may also be implicated in protection from subsequent systemic induction of immune complex glomerulonephritis by the same antigen. In the following sections the experimental evidence for these associations is discussed in relation both to previous scientific observations and to human disease.

Both of these models appear to involve the induction of an intestinal immune response to the antigen, and the intestinal response to antigen is relevant to this discussion. The similarity of protection from antigen induced immune complex glomerulonephritis and the induction of immunological tolerance by intragastric antigen administration is obvious. Observations on the induction of oral tolerance have been extended, therefore, where relevant to the present studies. In addition I have studied the absorption of protein antigen from the gastrointestinal lumen, and the rate of clearance of antigen from the circulation. I shall discuss the results of these experiments on the intestinal response to ingested antigens and their relevance to the present studies before discussing the role of dietary antigens in the pathogenesis of glomerulonephritis.

7.2 SYSTEMIC EFFECTS OF ORAL ANTIGEN ADMINISTRATION

Oral antigen administration may be associated with a variety of systemic effects, including the absorption of small amounts of antigen into the circulation (Thomas & Parrott, 1974), the induction of serum antibodies to the antigen (Crabbe etal, 1969) and the induction of a state of systemic hyporesponsiveness to the antigen (Chase, 1946).

The induction of oral tolerance following oral or intragastric administration of protein antigens has been demonstrated previously (Thomas & Parrott, 1974; Richman etal, 1978; Challacombe & Tomasi, 1980). Whilst a number of factors which influence the induction of oral tolerance have been identified, the genetic basis of oral tolerance has not been extensively investigated.

I have demonstrated genetic differences in 11 inbred strains of mice in the induction of oral tolerance to a protein antigen. In all the strains tested, parenteral immunisation with antigen in CFA was associated with the induction of DTH and antibody responses in antigen fed animals as well as in water fed controls. Administration of single 25 mg intragastric doses of ovalbumin were associated with significant reductions in both DTH and antibody responses in 9 of the 11 strains tested. Following administration of single 2 mg intragastric doses of ovalbumin prior to parenteral immunisation, the reductions in DTH and antibody responses were less consistent, and disparate reductions in DTH and antibody responses were seen in a number of strains tested. The

results of these experiments confirm that single intragastric doses of antigen are capable of inducing oral tolerance, and indicate the dose-dependent nature of oral tolerance and the genetic variability in the ease of oral tolerance induction.

Stokes etal (1983) described differences in the induction of oral tolerance with respect to the systemic antibody response to ovalbumin in 4 inbred strains of mice fed ovalbumin, and Tomasi etal (1983) demonstrated differences in the ease of induction of oral tolerance in mice of the CBA, BALB/c and DBA/2 strains fed varying doses of human gamma globulin by the intragastric route. It was not possible on the basis of these experiments to draw any conclusions as to the nature of the genetic basis of these differences in oral tolerance induction.

The phenomenon of "split" oral tolerance induction for DTH and antibody responses has been described previously (Mowat etal, 1982). The bisection of the immune response in this way suggests that the induction of oral tolerance for the humoral and cell mediated limbs of the immune response may be under the control of separate mechanisms. This bisection of the immune response also occurs in tolerance induced by other routes (Mitchison, 1964), and is not, therefore, a peculiarity of oral tolerance.

My studies identified two strains of mice (BALB/b & B10.BR) in which oral tolerance induction to ovalbumin was deficient. Relative deficiencies in oral tolerance induction to protein antigens have been described previously in B10.BR mice (Tomasi etal, 1983), and in SWR

mice (Stokes etal, 1983) and NZB mice (Cowdery, Curtin & Steinberg, 1982).

Induction of oral tolerance was investigated in inbred mice of 5 different H-2 haplotypes in the present study, and the results allow some interpretation of the role of genes of the major histocompatibility complex in the induction of oral tolerance. Of 3 $H-2^b$ strains examined, one strain (BALB/b) showed defective oral tolerance for ovalbumin for both DTH and antibody responses, one strain (Beige) showed significant reductions in DTH and antibody responses following intragastric administration of both 2 mg and 25 mg ovalbumin, and one strain (C57BL/10) showed significant reductions in DTH and antibody responses only after the higher intragastric dose of antigen. A similar but less marked variability in the ease of induction of oral tolerance was seen in 4 strains of $H-2^k$ mice.

Thus different strains of mice of the same H-2 haplotype vary in their ease of oral tolerance induction. These findings extend the observations of Tomasi etal (1983), who described differences in the ease of induction of oral tolerance with respect to lymph node cell proliferative responses in 5 $H-2^k$ strains of mice.

The inclusion of congenic mice differing only at the H-2 complex in the present study allowed for further evaluation of the genetic basis of oral tolerance. Mice congenic for the BALB background but differing at the H-2 complex (BALB/c, $H-2^d$; BALB/b, $H-2^b$; BALB/k, $H-2^k$) differed widely in their ease of induction of oral tolerance for both DTH and antibody responses.

These results indicate that H-2 genes are involved in

the induction of oral tolerance, but that neither H-2 genes alone nor non H-2 genes alone can explain the genetic differences observed in the induction of oral tolerance. In this respect induction of tolerance by the oral route would appear to be similar to systemic induction of tolerance, in which both H-2 and non H-2 genes have been implicated (Ranges & Azar, 1979).

Studies on the induction of oral tolerance in TO [high] mice indicated that this strain was deficient in the induction of oral tolerance for antibody responses but not for DTH responses following the administration of 25 mg ova. No such deficiency was seen in the induction of oral tolerance in TO [low] mice. Study of the dose-dependent nature of oral tolerance in this strain showed comparable reductions in DTH and antibody responses for each dose of antigen given by the intragastric route, and confirmed the dose-dependent nature of the induction of oral tolerance.

Similar dose dependent reductions in DTH and antibody responses have been described recently in C3H/HeOla and C3H/HeJ mice fed varying doses of ova (Mowat etal, 1986). Other reports (Mowat etal, 1982; Saklayen etal, 1984) have cited disparate effects of feeding small (mg) quantities of protein antigens on different limbs of the immune response, and oral administration of microgram quantities of protein antigens has been associated with systemic priming in mice (Mowat etal, 1986). Clearly the dose of antigen administered by the oral route greatly influences the systemic effects of ingested antigen, and the effects of similar oral doses of antigen varies widely between

different strains of animal and for different parameters of the immune response.

The induction of suppressor T cells has been demonstrated following oral antigen administratrion in Peyer's patches (Mattingly & Waksman, 1978; Ngan & Kind, 1978), in mesenteric lymph nodes (Challacombe & Tomasi, 1980) and in the spleen (Miller & Hanson, 1979; Richman etal, 1978).

Spleen cell transfer from orally tolerised mice to syngeneic naive recipients has been shown to transfer suppressor cell mediated, orally induced tolerance for cell mediated immune responses (Miller & Hanson, 1979) and for antibody responses (Richman etal, 1978; Ngan & Kind, 1978). I have confirmed the ability of spleen cells from antigen fed animals to confer tolerance for DTH, but not for antibody (IgG) responses by adoptive spleen cell transfer from orally tolerised mice to naive syngeneic recipients. As transfer of tolerance for antibody responses by sensitised spleen cells has been demonstrated previously, the failure to transfer tolerance for antibody responses in the present study most likely reflects differences in the experimental protocol.

Once again the results suggest that the mechanisms governing oral tolerance induction for the humoral and cellular limbs of the immune response are under separate control. Recent evidence has suggested that a population of Lyt-1^{+} suppressor T cells may mediate orally induced suppression of lymphocyte proliferative responses in mice fed BSA (Silverman etal, 1983), whilst a population of Lyt-2^{+} suppressor cells has been implicated in the

mediation of oral tolerance for antibody responses to ova (Challacombe, 1985).

The present studies on the induction of oral tolerance to protein antigens in mice have extended the data on oral tolerance with respect to genetic variations in the induction of oral tolerance and the roles of H-2 and non H-2 genes in oral tolerance induction. The dose dependent nature of oral tolerance induction has been demonstrated in TO [low] mice, and the disparate effects of certain oral doses of protein antigen on the humoral and cell mediated limbs of the immune response have been demonstrated in several strains of mice.

The presence of antigen in the circulation following intragastric antigen administration has been described previously in experimental animals (Thomas & Parrott, 1974; Swarbrick etal, 1979). Thomas & Parrott (1974) showed that the peak of radioactivity seen 1 hour after intragastric administration of radiolabelled BSA to rats eluted in the same fraction as native BSA after passage of the serum through a Sephadex G100 column.

Strobel and colleagues (1983) demonstrated the transfer of oral tolerance for DTH responses by serum collected from mice 60 minutes after the intragastric administration of single doses of ova. Further work by this group showed that the serum factor responsible for the transfer of tolerance contained "immunoreactive" antigen, and was removed from the serum by anti-ova antiserum (Bruce & Ferguson, 1986 a + b). The results suggested that the factor in serum responsible for the transfer of oral

tolerance was absorbed antigen itself, which had been processed by the gut in such a way as to render it tolerogenic.

My studies on the kinetics of antigen absorption from the intestinal tract demonstrated peak circulating levels of antigen 10-15 minutes after intragastric antigen administration. If the induction of oral tolerance were due to the presence of absorbed antigen in the circulation, then the degree of tolerance induced by serum transfer at 10 minutes (i.e. during the peak of circulating antigen following intragastric administration) should be greater than the degree of tolerance conferred by serum transfer 60 minutes after intragastric antigen administration. Using the same protocol as Strobel etal (1983), serum transfer 10 minutes after intragastric antigen administration had no significant effect on the subsequent antibody or DTH response of recipient animals. The transfer of oral tolerance for DTH responses by serum collected 60 minutes after intragastric antigen administration in the same strain of mouse and using the same protocol has recently been demonstrated in our department (Lamont, unpublished observations). In this experiment, serum transfer 60 minutes after antigen feeding was associated with a reduction of 74% ($p < 0.01$) in the DTH responses of recipient mice, but had no effect on the antibody responses of these animals (Lamont, personal communication).

Several possible explanations exist for the failure of serum collected 10 minutes after antigen feeding to transfer oral tolerance. Firstly, the amount of antigen

absorbed from the gut shows a wide range of variation between individual animals (Stokes etal, 1983; present studies), and may thus result in between-experiment variations in the amount of absorbed antigen transferred in the serum. The use in these experiments of pooled serum from large numbers of donor mice, however, should have minimised any such effect. Whilst the tolerogenic serum factor appears to be related to the ingested antigen, the precise nature of this factor remains to be determined (Bruce & Ferguson (1986 a). It is possible that the peak of circulating antigen seen 10 - 15 minutes after intragastric antigen administration consisted of antigen or antigenic fragments in a form which retained their antibody-binding capacity, but did not act in the induction of tolerance. Alternatively the results may suggest the requirement for some form of "gut processing" of antigen to render tolerogenic fractions of antigen, as has been suggested by Bruce & Ferguson (1986 a). Investigation of the content of the peak of absorbed antigen in terms of antigen fragments, and their relationship to the induction of tolerance, would help in assessing these possibilities.

The experiments of Strobel etal (1983) and Bruce & Ferguson (1986 a + b) indicated the importance of absorbed antigen in the induction of oral tolerance. The absorption from the intestine of small amounts of antigen, and the systemic handling of absorbed antigen, may influence the induction of oral tolerance. Modulation of the function of the mononuclear phagocytic system (MPS) has been shown to

influence both systemic (Yoshikai etal, 1981) and oral (Mowat & Parrott, 1983) induction of tolerance. I wished to study whether differences in the ease of oral tolerance induction were associated with differences in systemic clearance of antigen by the MPS, and whether oral antigen administration affected the rate of clearance of antigen from the system. Using a similar model to Stokes etal (1983), I found no difference in the rate of antigen clearance between BALB/c and BALB/k mice of either sex maintained on plain drinking water or given ova (0.05%) in the drinking water for 4 weeks prior to intravenous inoculation with 200 ng ^{125}I-ova. The results suggested that differences in the ease of induction of oral tolerance in these strains of mice were not associated with differences in mononuclear phagocyte activity.

The results also support previous observations (Hanson etal, 1979 b; Stokes etal, 1983) that antigen feeding does not influence systemic elimination of antigen. Similar results have been reported with regard to systemic clearance of antigen in TO mice (Devey & Bleasdale, 1984). These authors, however, have described defective clearance of immune complexes from the circulation of antigen fed TO mice receiving daily injections of antigen (Devey & Bleasdale, 1984). The ability of these mice to clear aggregated rabbit IgG from the circulation was normal. The results suggested that impaired clearance of immune

complexes was not due to defective mononuclear phagocytic cell function, but that the differences in immune complex clearance patterns might have been due to differences in the nature of the complexes formed in antigen fed animals as compared with controls (Devey and Bleasdale, 1984).

7.3 ORAL IMMUNISATION AND IGA NEPHROPATHY

Clinical and histopathological observations have suggested an association between the mucosal immune system and glomerular IgA deposits in IgA nephropathy, and have prompted the hypothesis that glomerular IgA deposits may appear consequent to a mucosal immune response. In 1983 Emancipator, Gallo and Lamm described an experimental model of IgA nephropathy associated with oral immunisation by prolonged administration of protein antigens in the drinking water of BALB/c mice. The resulting glomerulopathy was characterised by codeposition of IgA and the immunising antigen in the glomerular mesangium. The model represented the first direct association between renal lesions and oral immunisation, and suggested a basis for the investigation of the role of dietary antigens in the pathogenesis of experimental glomerulonephritis. I have used this model to study further the effects of oral antigen administration on the induction of glomerular immune deposits.

BALB/c mice given BGG (0.1%) in their drinking water for 12 weeks were found to have increased levels of glomerular IgA deposits in their kidneys as compared with water fed controls. These deposits were located

predominantly in the mesangial areas, in a similar distribution as is seen in human IgA nephropathy (Bene & Faure, 1986). Administration of ova (0.05%) in the drinking water, or by weekly gastric intubation to the same total dose had no effect on the levels of glomerular immune deposits of immunoglobulin in antigen fed mice.

C3H/HeJ mice produce high levels of serum IgA following oral immunisation with protein antigens (Kiyono etal, 1980), and would thus appear to represent a suitable model for the investigation of the role of oral immunisation in the induction of glomerular IgA deposits. Feeding these mice with ova (0.1%) in the drinking water for 14 weeks was not associated with any increase in the levels of glomerular IgG, IgA or C3 deposits as compared with water fed and antigen fed congenic C3H/HeOla mice. C3H/HeJ mice are also deficient in the induction of oral tolerance to SRBC (Kiyono etal, 1982), and thus provided a model for investigating the role of oral tolerance in the association between oral immunisation and glomerular IgA deposits. No differences were detected in the levels of glomerular IgA deposits between C3H/HeJ mice and C3H/HeOla controls after prolonged intragastric administration of SRBC, indicating that deficient oral tolerance induction was not associated with glomerular IgA deposition after oral antigen administration. This is not surprising, as Challacombe and Tomasi (1980) have demonstrated the simultaneous induction of oral tolerance and the secretory IgA response by the same antigen feeding regime, demonstrating the independence of the mucosal antibody response from control by an oral tolerance mechanism.

I have confirmed that oral administration of protein antigens to mice may be associated with glomerular IgA deposits, as reported previously by Emancipator etal (1983 a + b). Unlike these authors, however, I have only observed this association in BALB/c mice which were fed BGG in their drinking water for prolonged periods. Furthermore, I was unable to detect the deposition of the immunising antigen in the kidneys of experimental mice.

Similar experiments have been carried out by other groups, with varied results. Devey and colleagues were unable to detect increased levels of IgA deposition in the kidneys of mice fed BSA (0.05%) in the drinking water for prolonged periods. Prolonged feeding with cows' milk proteins via the drinking water, however, was associated with the deposition of IgA, casein and β-lactoglobulin in a proportion of mice which had been systemically sensitised to cows' milk proteins prior to commencing antigen feeding (Devey, personal communication). Similarly Sato and colleagues (1986) were unable to induce glomerular IgA deposits in ddY mice fed high doses of α-lactalbumin for prolonged periods unless simultaneous blockade of the reticuloendothelial system was performed. Genin, Sabatier & Berthoux (1984), however, reported that intragastric administration of 20 mg ferritin prior to the administration of ferritin (0.1%) in the drinking water of C3H/HeJ mice was associated with increased levels of glomerular IgA deposits as compared with C3H/eB mice treated similarly, and with water fed C3H/HeJ controls. Without the initial intragastric dose of ferritin, oral

immunisation was not achieved, and the association between oral antigen administration and glomerular IgA deposits was not seen (Genin etal, 1984). More recently this group has described similar results following oral immunisation with BSA in C3H/HeJ mice (Genin etal, 1986). In contrast to the reports of Emancipator's group (Emancipator etal, 1983 a + b), Genin and colleagues were unable to demonstrate the presence of deposition of the immunising antigen in the kidneys of antigen fed mice (Genin etal, 1984 + 1986). The absence of antigen in glomerular IgA deposits reported by Genin and coworkers is in keeping with my own findings. Finally, Bene and colleagues have reported their inability to demonstrate an association between oral immunisation and glomerular immune deposits in guinea pigs (Bene & Faure, 1986).

Clearly, then, the relationship between oral antigen administration and glomerular IgA deposits is complex. A number of factors can be identified which may influence glomerular IgA deposition following oral antigen administration. The strain and species of animal may be important. Emancipator etal (1983) and my own work has demonstrated the relationship between oral immunisation and glomerular IgA deposits in BALB/c mice, and Genin etal (1984 + 1986) have shown IgA glomerular deposits in C3H/HeJ mice, but not in C3H/eB mice, following oral administration of protein antigen. The failure of Bene and colleagues to demonstrate glomerular IgA deposits associated with oral immunisation in guinea pigs may be due to the form of IgA produced following oral immunisation. The requirement for polymeric IgA in

determining the glomerular deposition of IgA-IC has been demonstrated by Rifai & Millard (1985). The amount and predominant molecular form of IgA in the serum thus influences its glomerular deposition. Serum IgA in the mouse is predominantly polymeric, whereas in the guinea pig the proportion of polymeric to monomeric IgA is much reduced as compared with the mouse (Vaerman, 1973). The guinea pig, therefore, may not provide a suitable model for the investigation of glomerular IgA deposition.

In addition to differences in the strain of animal, the nature of the antigen appears to be important. Both BGG and ferritin have been implicated in the association between oral immunisation and glomerular IgA deposition. Oral administration of foreign albumins has been less successful in the induction of glomerular IgA deposits (present studies; Devey, unpublished observations). In the present study no association was seen between oral administration of SRBC and glomerular deposition of IgA. Studies on the induction of serum antibodies by oral immunisation have met with variable success. Crabbe and colleagues (1969) demonstrated the presence of IgA antibodies to ferritin in the serum of germ free C3H mice following oral immunisation with ferritin. Heremans and Bazin (1971) showed a predominance of IgA in the serum antibody response to SRBC using a similar experimental model. Studies in conventional animals have less consistently identified serum antibodies following oral immunisation (Rothberg etal, 1967; Strannegard & Yurchison, 1969; Thomas & Parrott, 1974; Devey and

Bleasdale, 1984; Challacombe, 1983). In their studies, Emancipator etal demonstrated the presence of serum IgA antibodies to the immunising antigen (Emancipator etal, 1983 a). In a similar model, however, Genin and colleagues were unable to detect antigen specific IgA in the serum, but did report elevated levels of total serum IgA in antigen fed C3H/HeJ mice.

The passage of IgA antibodies into the circulation is, obviously, a prerequisite for the glomerular deposition of these antibodies. The role of hepatic sequestration of intestinally derived IgA is discussed in section 7.4. The passage of IgA antibodies into the circulation in complex with antigen might lead to the failure of detection of such antibodies by conventional antibody assay systems. Circulating IgA-IC have not been studied in any of the models of induction of glomerular IgA deposits by oral immunisation. The detection of IgA-IC following antigen ingestion in healthy individuals (Paganelli etal, 1979), and the presence of elevated levels of IgA-IC in many patients with IgA nephropathy (Coppo etal, 1982; Sancho etal, 1983) make this an area for further research in the association between antigen ingestion and glomerular IgA deposition.

As a model for human IgA nephropathy, experimental IgA nephropathy induced by oral immunisation has a number of limitations. Firstly, the lack of consistency of the association remains to be adequately explained. Secondly, none of the authors who have reported an association between oral antigen administration and glomerular IgA deposits have identified any clinical manifestation of

glomerular damage nor any glomerular lesions on light microscopy. Thirdly, the extent of glomerular IgA deposition in the experimental model is generally slight. As scoring of the sections is based on subjective interpretation, such minor differences in staining intensity may be difficult to score reliably. Variations in the intensity of staining of individual glomeruli within the same kidney section further complicate the issue, as the scoring of the sections is based on an overall impression of the staining intensities of a number of individual glomeruli. This criticism may be offset by the reading of the sections blind and, where possible, by an independent observer. My major criticism of the model is that the presence of glomerular deposits of IgA (and other immunoglobulin isotypes) is a common finding in "normal" mice (Markham, Sutherland & Mardiney, 1973; Table 6), and further complicates interpretation of the slides. The use of stock mice which are free from glomerular immune deposits is therefore an important factor if further studies are to be undertaken using this model.

7.4 IGA GLOMERULAR DEPOSITS IN EXPERIMENTAL LIVER DAMAGE

The association between glomerular lesions characterised by IgA deposition and cirrhosis of the liver was first described by Callard etal (1975), and has been reported widely since. The description of an experimental model of liver cirrhosis induced by the administration of CCl4 to rats (MacLean etal, 1969) has provided a model for the study of the association between glomerular immune

deposits and experimental cirrhosis (Gormly etal, 1981).

The administration of CCl4 to rats produces an acute centrilobular necrosis, but spares the periportal zones and sinusoidal lining cells (Butler, 1979). The acute lesion resolves within 7 - 10 days. Repeated administration of CCl4 prior to resolution of the acute lesion, however, results in the rapid induction of experimental cirrhosis (MacLean etal, 1969). The absence of regenerative nodule formation in this model precludes the description of the lesion as true cirrhosis.

The application of this model to the study of renal lesions associated with experimental liver damage was first described by Sakaguchi and colleagues. Their study (Sakaguchi et al, 1964) demonstrated that glomerular lesions similar to those seen in humans with cirrhosis of the liver (Sakaguchi etal, 1965) could be reproduced experimentally in rats in which liver damage had been induced by treatment with CCl4 or ethionine. These authors, however, did not investigate the composition of immune deposits involved in the lesions. The association between IgA glomerular deposits and experimental liver damage was described by Gormly etal (1981) in Lewis rats rendered "cirrhotic" by the twice weekly administration of CCl4 by inhalation for 10 weeks. In this report, rats developed clinical and histological evidence of chronic hepatic damage, with marked hepatic fibrosis, 5 weeks after the start of CCl4 treatment. Glomerular mesangial IgA and C3 deposits were detected after 5 weeks, but were maximal in animals killed 20 and 40 weeks after the start of CCl4 treatment. To investigate whether a similar

relationship between glomerular IgA deposits and experimental liver damage could be demonstrated in mice, I administered CCl4 to mice by weekly gastric intubation for periods of up to 15 weeks. This regime resulted in the induction of chronic liver damage in CCl4 treated mice, with hepatic fibrosis seen in mice on the higher (16 μl/ week) dosage regime. Experimental liver damage was not associated with increased levels of deposition of any immunoglobulin isotype or of C3 in the kidneys of these mice.

The failure to detect increased levels of glomerular immune deposits in mice with CCl4 induced liver damage may be explained in several ways. The degree of liver damage induced may have been insufficient to reveal an association between experimental liver damage and glomerular deposition of IgA or IgA-IC in the mouse. Gormly etal (1981) described the presence of ascites and splenomegaly after 5 weeks in all CCl4 treated rats. I did not observe either ascites or gross splenomegaly in any CCl4 treated mouse. The degree of hepatic fibrosis described in rats treated with CCl4 (Gormly etal, 1981) was greater than was seen in the mice in my study. Thus the degree of liver damage induced in mice appeared to be less than that seen in rats in which CCl4 induced liver damage was associated with IgA deposits. This difference may have resulted from differences in the frequency of administration of CCl4, or may suggest a relative insensitivity to the hepatotoxic effects of CCl4 in mice as compared with rats.

Defective sequestration of polymeric IgA and IgA-IC by the cirrhotic liver has been proposed as the underlying abnormality in IgA nephropathy associated with cirrhosis (Woodroffe etal, 1980). The importance of hepatobiliary clearance of IgA-IC has been demonstrated by the rapid appearance of mesangial IgA deposits in experimental cholestasis following ligation of the bile duct (Emancipator, Gallo, Razaboni & Lamm, 1983). As acute CCl4 induced liver injury selectively affects the centrilobular hepatocytes (Butler, 1979), the preservation of periportal cells might allow for continued hepatobiliary clearance of IgA-IC until the severity of the liver damage was such that the whole lobule was affected. As yet, studies on hepatobiliary clearance function in experimental liver damage are lacking. Further studies on the interrelationship between liver damage, hepatobiliary clearance of IgA-IC and glomerular IgA deposits would be helpful in increasing our understanding of the clinical relationship between cirrhosis and IgA nephropathy.

A recent report (Sato etal, 1986) has suggested that reticuloendothelial clearance of IgA-IC may play a role in protecting against glomerular deposition of IgA immune complexes. In this study the combination of blockade of the reticuloendothelial system (RES) and prolonged administration of high doses of α-lactalbumin in the drinking water of ddY mice was associated with an increased incidence of glomerular IgA deposits. This association was not seen in mice fed α-lactalbumin alone, or following RES blockade alone (Sato etal, 1986). The description of the ddY strain of mice as a model of

spontaneous IgA nephropathy (Imai etal, 1985), however, suggests that the report of Sato etal (1986) is subject to the same criticisms as have been expressed above concerning other models of IgA nephropathy in mice.

7.5 THE INDUCTION OF CHRONIC ANTIGEN-ANTIBODY COMPLEX GLOMERULONEPHRITIS IN MICE

The induction of glomerular lesions in experimental animals by repeated injections of foreign protein antigens has been known for many years. The involvement of deposition of antigen-antibody complexes in the mediation of glomerular injury in these models is well established (reviewed by Unanue & Dixon, 1967). The description of antigen induced immune complex glomerulonephritis in selectively bred TO mice (Devey & Steward, 1980; Devey & Bleasdale, 1984) appeared to present a suitable model for the investigation of the effects of oral antigen administration on the induction and course of antigen induced immune complex glomerulonephritis.

Daily injections of 0.3 mg ova in TO [low] mice for up to 120 days were associated with mesangial and capillary loop deposition of IgG, IgM, C3 and ova, suggestive of glomerular immune complex deposition. Electron microscopy showed mesangial expansion with copious mesangial electron dense deposits (EDD) in the majority of mice, and subendothelial deposits in a proportion of animals. These findings contrast with the description of predominantly subepithelial deposits in antigen induced glomerulonephritis in TO mice selectively bred for low

antibody affinity (Steward etal, 1981). The mice used in my study were not bred selectively for the production of low affinity antibody. The differences in localisation of immune deposits within the glomerulus between these studies was most likely due to differences in antibody affinity. This may have arisen either as a result of the non-selective breeding for antibody affinity of the mice used in my study, or as a result of affinity maturation of antibody with increasing numbers of antigen injections (Devey etal, 1982).

None of the experimental mice in my study developed renal dysfunction as estimated by serum urea and creatinine measurement. As serum urea and creatinine do not become elevated until glomerular function has become substantially compromised, the use of a more sensitive estimate of glomerular function, e.g. glomerular filtration rate, might have shown the development of renal insufficiency. Devey and Steward (1980) have described impaired renal function in low affinity antibody producing TO mice receiving 41-44 daily antigen injections. As discussed above, however, differences in the site of immune complex deposition between selectively bred, low affinity TO mice and the mice used in the present study suggest that these models may not be directly comparable.

I was unable to estimate urinary protein excretion in these experiments. Attempts to collect random urine samples proved unsuccessful due to inconsistency of collection. Initial attempts to estimate urinary protein in such samples by the use of "Multistix" (Ames, UK) suggested significant urinary protein excretion in all

control as well as experimental mice tested, and the reliability and sensitivity of this method was considered questionable.

Whilst there was little clinical evidence of glomerular damage in the model used in the present study, it has been termed, for the purposes of this thesis, antigen induced immune complex glomerulonephritis.

On the basis of the preceding experiments, a standard regime of 60 daily injections of 0.3 mg protein antigen was chosen as a suitable method for the induction of immune complex glomerulonephritis in susceptible mice. Using this model, the majority of T0 [high] and BALB/c mice developed an immune complex glomerulonephritis, whilst BALB/b and B10.BR mice were not susceptible to the induction of immune complex nephritis. B10.BR mice, however, showed higher levels of "spontaneous" immune complex deposition than was seen in other strains. The presence of such "spontaneous" immune complex deposition in the kidneys of "normal" mice has been ascribed to vertically transmitted or neonatally acquired persistent viral infections (Oldstone & Dixon, 1969).

Interstrain variations in the nephritogenicity of heterologous proteins in inbred mice have been described previously (Iskander etal, 1982), and support a genetic basis for susceptibility to antigen induced immune complex glomerulonephritis.

7.6 PROTECTION FROM ANTIGEN INDUCED IMMUNE COMPLEX GLOMERULONEPHRIIIS BY ORAL ANTIGEN ADMINSTRATION

Experiments have shown that antigens administered by the oral route may be implicated directly in the pathogenesis of experimental IgA nephropathy (Emancipator etal 1983 a+b; Genin etal, 1984 + 1986; present studies). Oral antigen administration, however, can also lead to a state of systemic hyporesponsiveness to the antigen, which may protect against damaging hypersensitivity reactions both locally (Mowat & Ferguson, 1981 a) and systemically (Devey and Bleasdale, 1984).

Devey and Bleasdale (1984) described a decrease in the incidence of antigen induced immune complex glomerulonephritis in mice fed 0.05% HSA in their drinking water for 7 days prior to the induction of nephritis by daily injections of HSA.

Using a similar model, I have shown that single 25 mg intragastric doses of ovalbumin administered prior to the induction of nephritis by daily injections of ovalbumin may result in a decreased incidence and severity of glomerulonephritis, as estimated by immunofluorescent and electron microscopic techniques, in antigen fed mice. By including in these experiments a number of strains of mice which varied in their ease of oral tolerance induction, I hoped to study the relationship between protection from antigen induced glomerulonephritis and oral tolerance. BALB/b and B10.BR mice, deficient in oral tolerance induction, were not susceptible to the induction of immune complex glomerulonephritis as tested. TO [low], TO [high]

and BALB/c mice were susceptible to antigen induced glomerulonephritis. Prior antigen feeding was associated with a reduced incidence of glomerular immune deposits in all of these strains. This reduction in the incidence of glomerular immune deposits in antigen fed mice was associated with reduced antibody titres to the immunising antigen in mice of all three strains of mice.

It is interesting to note that significant reductions in antibody titre were seen in TO [high] and B10.BR mice in these experiments. These animals had been shown in previous experiments (Chapter 3) to be deficient in the induction of oral tolerance for antibody responses. This disparity in the effects of intragastric antigen may reflect the use of native antigen, as opposed to antigen in CFA, to achieve systemic immunisation in the induction of immune complex glomerulonephritis. The results suggest that the use of potent adjuvants to stimulate systemic immune responses may overcome the immunoregulatory effects of oral antigen administration in some strains of mice. A similar observation has been noted previously (Saklayen etal, 1984) with respect to the oral induction of tolerance subsequent to parenteral immunisation (Lafont etal, 1982). The use of CFA in parenteral immunisation was required in experiments on oral tolerance for the induction of delayed hypersensitivity to the antigen. Induction of delayed hypersensitivity by immunisation with native antigen alone was poor, and of insufficient intensity to allow for footpad testing (data not presented).

In a similar experimental model, Thompson and Staines

(1986) have described delayed onset and reduced severity of collagen induced arthritis in rats fed soluble type II collagen (CII) or glutaraldehyde-polymerised CII prior to the induction of arthritis by immunisation with CII in incomplete Freund's adjuvant (FIA). This model differs from the present work in two major ways. Firstly, Thompson and Staines have used oral antigen administration to reinforce self tolerance to an auto-antigen which immunisation with the antigen in FIA is able to overcome. Secondly, serum antibody titres to the immunising antigen were not affected by prior intragastric antigen administration. This is in contrast to the protective effects of antigen feeding in antigen induced immune complex glomerulonephritis, and probably reflects differences in the pathogenesis of the experimental lesions.

The prospect of therapeutically altering the course of a pathological process by so simple a means as oral antigen administration is clearly of importance, and demands further attention. Using the model of protection from antigen induced immune complex glomerulonephritis by intragastric administration, I have investigated some of the factors which may limit the protective effects of oral antigen administration and the mechanisms whereby these effects may be mediated. To control against possible interstrain differences in these effects, all the experiments, with the exception of spleen cell transfer, were carried out in TO [low] mice. As this strain is not inbred, I used BALB/c mice in experiments involving spleen

cell transfer. Both these strains had been shown previously to be susceptible to the induction of immune complex glomerulonephritis, and to exhibit protection from immune complex nephritis by prior antigen feeding.

It was important initially to define the immunological specificity of the protective effects of intragastric antigen administration on the induction of immune complex glomerulonephritis. Dietary influences on the progression of autoimmune renal disease have been described previously (Levy and Morrow,1983; Kelley, 1986). These reports have been concerned primarily with immunologically non-specific effects.

My experiments demonstrated that protection from immune complex glomerulonephritis by antigen feeding is specific for the immunising antigen. Thompson and Staines (1986) similarly have demonstrated the specificity of the protective effects of intragastric antigen administration in their model of experimental arthritis in rats.

I have extended these studies, by the use of hapten-carrier conjugates, to define the level of induction of protection from immune complex glomerulonephritis by antigen feeding. Significant reductions were seen in the degree of immune complex deposition following intragastric administration of the carrier alone, the hapten-carrier conjugate, and the carrier plus the hapten conjugated to a heterologous carrier. The results suggested that the protective effects of intragastric antigen administration are induced at the level of the carrier molecule, and support previous observations of Titus & Chiller (1981 a). These authors demonstrated that mice given ova by

intragastric intubation showed markedly reduced anti-hapten plaque forming cell (PFC) responses to hapten conjugated to ova upon subsequent immunisation with hapten conjugated to ova, but gave normal anti-hapten PFC responses on immunisation with the hapten conjugated to keyhole limpet haemocyanin (Titus & Chiller, 1981 a).

In my own study, the intragastric administration of carrier plus hapten conjugated to a heterologous carrier had an apparently synergistic effect on both the degree of glomerular immune complex deposition and on the anti-ova antibody response. This result is intriguing. As the intragastric antigen administration involved two non-cross reactive carriers (see Table 13), the effect cannot be explained in terms of an additive carrier-specific increase in tolerance. An alternative explanation is that the hapten may itself be capable of inducing tolerance to the hapten-carrier complex. In order to explain the reductions seen in anti-ova antibody responses, this effect would have to be mediated in a carrier non-specific (but presumably hapten specific) way. Such an association is without precedent, but is supported by the reduction, albeit slight, in both the degree of immune complex deposition and the anti-ova antibody titre seen following intragastric administration of DNP-BGG prior to immunisation with DNP-ova. The simplest explanation of these results is that the observation was invalid. Both the ova fed group and the DNP-ova fed group contained a single "high responder" mouse, with respect to the anti-ova antibody response. Exclusion of these animals from the

analysis of data would have resulted in significant reductions in the anti-ova antibody responses in the ova fed and DNP-ova fed groups ($p=0.01$, both groups), and the mean antibody titres of these groups would then have approached that seen in mice fed ova plus DNP-BGG. It is thus considered likely that the apparently synergistic effect of feeding ova plus DNP-BGG was an artefact of experimental sampling. Repetition of the experiment would be useful in determining whether the observed effect is consistent.

Administration of a range of different single intragastric doses of ova to mice prior to the induction of immune complex glomerulonephritis was associated with a dose dependent reduction in the incidence of immune complex deposition. The reduction in the incidence of immune complex deposition closely mirrored the reductions seen in antibody titres and delayed hypersensitivity responses seen in the induction of oral tolerance in mice of the same strain receiving the same intragastric doses of antigen. Both the incidence and the degree of immune complex deposition correlated with the anti-ova antibody titre in these mice. The results suggested that glomerular deposition of immune complexes was related to the intensity of the anti-ova antibody response, and that protection from antigen induced glomerulonephritis was related to the reduction in antibody titre to the immunising antigen consequent to intragastric antigen administration.

The model of glomerulonephritis used in these studies was suggestive of the deposition of circulating immune

complexes in the kidneys. Raised levels of circulating immune complexes have been demonstrated previously in the majority of high and low affinity TO mice receiving 41-44 daily injections of antigen (Devey & Steward, 1980). I did not detect raised levels of conglutinin binding circulating immune complexes in the sera of antigen fed or water fed TO [low] mice after 60 antigen injections. This result may reflect the choice of assay system rather than the absence of immune complexes from the sera of these mice. The different physical and biological properties of the range of antigen-antibody complexes which may be formed *in vivo* can greatly influence their detection by different immune complex assay systems (Lambert etal, 1978). Devey, Taylor & Steward (1980) reported that levels of circulating conglutinin binding immune complexes decreased with increasing numbers of antigen injections in mice injected repeatedly with protein antigen. It is thus possible that the use of a conglutinin binding assay system was inappropriate for the detection of immune complexes in this experimental system. In the absence of comparative studies, it is not possible to discuss the sensitivity of the assay system used. The use of a range of assay tecniques covering the detection of immune complexes of different physical and biological properties has been recommended for the study of circulating complexes in immune complex mediated disease (Lambert etal, 1978).

Devey and Bleasdale (1984) demonstrated the persistence of low molecular weight complexes in the circulation of

antigen fed TO [low] mice which had been injected repeatedly the same antigen. Small latticed complexes have been shown not to deposit in the glomeruli (Haakenstad, Striker & Mannik, 1982), and the formation of such complexes may have contributed to the reduction in the incidence of glomerular immune complex deposition in antigen fed mice.

The presence of free circulating antigen in the serum 24 hours after the final antigen injection was found in antigen fed mice but not in controls. Early studies (Germuth & McKinnon, 1957; Dixon etal, 1961) suggested that the formation of soluble complexes in antigen excess was associated with tissue deposition of the complexes and with the induction of disease. Boyns and Hardwicke (1968), however, described the lack of chronic renal disease in rabbits injected repeatedly with varying doses of antigen calculated to maintain a situation of persistent antigen excess. In the present study, levels of circulating free antigen were highest in the group of mice showing the least degree of glomerular immune complex deposition, and showed an inverse correlation with the antibody titre. My findings are, therefore, in keeping with those of Boyns and Hardwicke (1968) in this respect. The results suggest impaired clearance of antigen from the circulation in antigen fed mice. This has not been investigated in the present study, but Devey & Bleasdale (1984) have described delayed clearance of antigen in mice which had been fed HSA prior to receiving daily injections of the same antigen.

The similarity between protection from immune complex

glomerulonephritis and the induction of oral tolerance suggested that similar mechanisms might underlie both phenomena. In an attempt to identify the mechanisms involved in protection from immune complex glomerulonephritis by antigen feeding, I have examined the effects of serum transfer and the role of suppressor T cells on protection from immune complex glomerulonephritis induction by antigen feeding.

The transfer of serum 60 minutes after intragastric administration of antigen has been shown to split the induction of tolerance to the antigen by selectively transferring tolerance for DTH but not for antibody responses (Strobel etal, 1983). Serum transfer according to the same experimental protocol had no affect on the induction of immune complex glomerulonephritis by daily antigen injections starting one week after serum transfer. The results indicate that protection from immune complex glomerulonephritis by intragastric antigen administration is independent of the mechanisms which mediate suppression of DTH responses following antigen feeding.

The induction of suppressor T cells has been implicated in the mediation of oral tolerance with respect to both antibody responses and DTH responses (Mattingly & Waksman, 1978; Richman etal, 1978; Hanson etal, 1979 b). The report that cyclophosphamide administered to mice at a dose of 100 mg/kg selectively inhibits the induction of suppressor T cells (Schwatrz etal, 1978) suggested a suitable means for the investigation of the role of suppressor T cells in protection from immune complex glomerulonephritis by

antigen feeding. Cyclophosphamide had no abrogative effect on the reduction of the incidence of immune complex deposition seen in mice given 25 mg ova prior to the induction of immune complex nephritis. Thus the mechanism underlying protection from immune complex glomerulonephritis appeared to be resistant to cyclophosphamide, and thus independent of the induction of a population of suppressor T cells. This is in contrast to previous reports on the effects of cyclophosphamide on the induction of oral tolerance. Pretreatment of mice with this dose of cyclophosphamide was shown to abrogate the induction of oral tolerance for both DTH and antibody responses (Mowat etal, 1982). The same authors have shown that cyclophosphamide mediated abrogation of oral tolerance to dietary antigen may be associated with the induction of cell mediated immune responses to the antigen within the intestinal mucosa (Mowat & Ferguson, 1981 a).

The transfer of suppression of DTH and of antibody responses by adoptive spleen cell transfer from antigen fed donors to naive recipients has been shown in a number of models (Richman etal, 1978; Ngan and Kind, 1978; Miller & Hanson, 1979). I was unable to demonstrate any reduction in the incidence or degree of immune complex deposition in the glomeruli of mice receiving spleen cells from antigen fed donors as compared with recipients of control cells following the induction of nephritis. The experimental protocol used had been shown in earlier experiments to transfer suppression of DTH responses in the same strain of mouse. Thus investigation of the possible role of suppressor T cells by two methods failed

to identify a significant role for these cells in protection from immune complex glomerulonephritis by intragastric antigen administration.

In summary, I have demonstrated that single doses of antigen administered by the intragastric route may be associated with reductions in the incidence and degree of glomerular immune complex deposition in the kidneys of mice undergoing a regime of daily antigen injections, which has been shown previously to induce immune complex glomerulonephritis in susceptible strains of mice.

This effect of intragastrically administered antigen was specific for the immunising antigen, and the degree of protection from immune complex glomerulonephritis varied with the dose of intragastric antigen administered. Similar dose dependent reductions were seen in the antibody titres of antigen fed mice. In all cases where significant reductions in the incidence of glomerular immune complex deposition were seen in antigen fed mice, these were accompanied by similar reductions in antibody titres. Significant correlations were demonstrated between the antibody response to the immunising antigen and the degree of immune complex deposition.

Protection from immune complex glomerulonephritis by antigen feeding was not mediated by suppressor T cells, and was independent of the oral induction of tolerance for cell mediated immune responses. The relation of the antibody titres to the incidence of glomerular immune complex deposition in antigen fed mice suggests that the reductions in immune complex deposition and in the

antibody responses are related, and may share a common mechanism of induction. The mechanism underlying the phenomenon of protection from antigen induced immune complex glomerulonephritis by intragastric antigen administration, however, remains obscure.

7.7 THE ROLE OF DIETARY ANTIGEN IN THE PATHOGENESIS OF GLOMERULONEPHRITIS

In this thesis I have examined the role of dietary antigens in the pathogenesis of experimental glomerulonephritis. The work has identified two areas in which antigen administered by the oral route may influence the induction and course of experimental glomerulonephritis. The implications of these findings have been discussed in relation to previously reported experimental work. In this section I shall discuss the implications of my findings in relation to human disease.

In spite of an earlier report of an association between food sensitivity and childhood nephrosis (Matsumura & Kuruome, 1961), interest in an association between immune reactions at mucosal sites and glomerulonephritis was first stimulated by the description of a clinical nephropathy characterised by mesangial deposits of IgA (Berger & Hinglais, 1968).

The demonstration of an association between antigen ingestion and IgA glomerular deposits in the present study confirms a relationship between the mucosal immune system and glomerular deposits of IgA, and suggests a possible role for dietary antigens in the pathogenesis of IgA nephropathy.

In spite of the clinical association between infections and acute exacerbations in IgA nephropathy, specific antigens have been identified rarely in glomerular immune deposits. Clarkson has recently suggested that mesangial IgA deposits in IgA nephropathy may result from deposition of complexes of IgA in the absence of specific antigens (Clarkson etal, 1984). Such a model would not rule out a mucosal origin for IgA involved in glomerular immune deposits.

I should like to suggest an alternative model involving specific immune responses at mucosal sites as a possible pathogenetic mechanism in IgA nephropathy. I propose that in patients with IgA nephropathy an as yet undetermined primary defect in IgA synthesis or regulation at mucosal sites is associated with increased passage of antigen-specific polymeric IgA (poly-IgA) antibodies and IgA-IC into the circulation, with subsequent deposition within the renal glomerulus. The antibodies involved would be of multiple specificities, including antibodies to commensal flora and to common dietary antigens. The "steady state" intestine of such patients would thus be a low grade but persistent source of poly-IgA and IgA-IC, which could deposit (and be cleared) by the renal mesangium. Any acute event at a mucosal surface, such as a respiratory or gastrointestinal infection, would be associated with showers of poly-IgA and IgA-IC appearing in the circulation, resulting in an increase in glomerular IgA, and an acute nephritic episode.

Such a model is supported by the finding of elevated

levels of poly-IgA (Lesavre etal, 1982; Valentijn etal, 1983) and IgA-IC (Coppo etal, 1982; Sancho etal, 1983) in serum from patients with IgA nephropathy, the lack of detection of specific antigens in glomerular immune deposits (Bene & Faure, 1986), and the clinical association between infections at mucosal sites and acute exacerbations of nephritis. Furthermore this model is consistent with the finding of recurrence of IgA deposits in renal allografts in patients with IgA nephropathy (Berger etal, 1975). The mouse model may serve to underline the importance of polymeric IgA in IgA nephropathy. The requirement for polymeric IgA in glomerular deposition of IgA-IC has been demonstrated by Rifai & Millard (1985). In the mouse (as opposed to man) serum IgA is found predominantly in the polymeric form (Vaerman, 1973). Thus the low grade IgA deposition in the kidneys of "normal" mice of some strains may be analogous to the "steady state" situation in human IgA nephropathy. Similarly glomerular IgA deposition associated with oral immunisation in the mouse may be analogous to the clinical exacerbations seen in association with infections at mucosal sites.

This model does not take account of hepatic sequestration of poly-IgA and IgA-IC. The nature of the model proposed, however, might suggest chronic overload of the hepatobiliary clearance of IgA, resulting in deficient clearance of IgA-IC from the circulation.

Clearly this model does not explain all cases of IgA nephropathy. Defective hepatobiliary and mononuclear phagocytic clearance clearance of IgA in cirrhosis of the

liver would represent a separate aetiopathological mechanism of IgA nephropathy. In this model too, however, mucosal surfaces may be inferred as a major source of poly-IgA and IgA-IC, and dietary antigens may thus be implicated in IgA nephropathy associated with hepatic cirrhosis. IgA antibodies to mesangial antigens (Lowance etal, 1973) and, more recently, IgA rheumatoid factors (Sinico etal, 1986) have been implicated in the pathogenesis of some cases of IgA nephropathy. The role and extent of involvement of these mechanisms in IgA nephropathy remains to be established, but they would represent models of IgA nephropathy independent of the one proposed.

In summary, I propose a role for intestinal antigens, including dietary antigens, in the pathogenesis of IgA nephropathy associated with hepatic cirrhosis, and in a proportion of cases of idiopathic IgA nephropathy. Failure to identify individual antigens in the aetiopathogenesis is explained by the involvement of multiple antigens at mucosal surfaces. Furthermore the requirement for the presence of antigen in glomerular immune deposits is not absolute, as poly-IgA complexes themselves may be capable of glomerular deposition. Such a model for the aetiopathogenesis of IgA nephropathy is supported both by studies of the human disease and by animal models of IgA nephropathy.

In addition to the proposed role of dietary antigens in the pathogenesis of IgA nephropathy, a second major effect of dietary antigen in the pathogensis of experimental

glomerulonephritis has been identified. Intragastric administration of antigen has been shown to be associated with protection from antigen induced immune complex glomerulonephritis. The relation of this phenomenon to human disease is speculative, but the observations may be of relevance in two respects. Firstly, whilst a number of individual cases have been reported in which dietary antigens were implicated directly in the pathogenesis of glomerulonephritis, the incidence of such reports is very low. In view of the number and range of antigens which have been implicated in human (and animal) glomerulonephritis it is, perhaps, surprising that so few cases of food related glomerulonephritis have been described. The continuous antigenic stimulation of the intestinal mucosa and the access of luminal antigens to the circulation would suggest an ideal situation for chronic antigen-antibody formation, and the induction of immune complex disease in a proportion of individuals. That this does not occur suggests the presence of a mechanism to protect against chronic immune complex disease induced by ingested antigens. Hepatobiliary clearance represents such a mechanism for the disposal of mucosally derived complexes involving IgA. Oral tolerance to dietary antigens has been shown to protect against potentially damaging cell mediated immune response to dietary antigens within the intestinal mucosa (Mowat & Ferguson, 1981 a). From this it may be inferred that the intestinal immune response to dietary antigens, by the induction of oral tolerance, may also protect against damaging systemic hypersensitivity reactions to

intestinally derived antigens. The evidence presented in this thesis would support such a proposal.

The second implication of the protective effects of antigen ingestion lies in the therapeutic potential of oral antigen administration in the treatment of systemic hypersensitivity diseases. Of particular importance in this respect is whether antigen ingestion can influence the course of an ongoing pathogenic hypersensitivity reaction. Observations on the induction of oral tolerance in primed animals have suggested that prior systemic sensitisation with an antigen may inhibit the subsequent induction of oral tolerance (Hanson etal, 1979 a; Titus & Chiller, 1981 a). The reported models of protection from systemic hypersensitivity disease by antigen feeding (Devey & Bleasdale, 1984; Thompson & Staines, 1986; present study) have all adopted the administration of antigen prior to the induction of the hypersensitivity state. Investigation of the effects of oral antigen administration on the course of ongoing systemic hypersensitivity reactions in primed animals is, therefore, of importance in establishing the therapeutic potential of oral antigen therapy in systemic hypersensitivity diseases.

The relevance of such a model to human disease remains to be established. Barnes etal (1987) have described recently the failure in humans of oral administration of erythrocyte membrane antigen to suppress subsequent antibody responses to the Rhesus (D) antigen following intravenous infusion of whole red blood cells. Further

studies, however, are required, both in man and in animals, before the protective effects of antigen feeding on hypersensitivity diseases can be evaluated.

The prospect that so simple a manoeuvre as antigen ingestion may alter the course of systemic hypersensitivity diseases is an exciting one. One limitation of this model is the specificity of the protective effects of antigen feeding. In view of the antigen specific nature of the model, the therapeutic use of oral antigen therapy would be restricted to diseases in which a causative antigen has been identified. Nevertheless, protection from systemic hypersensitivity disease by antigen feeding is a potentially important mechanism, and merits further investigation.

CHAPTER EIGHT

REFERENCES

8 REFERENCES

Adachi M, Yodoi J, Masuda T, Takatsuki K & Uchino H. (1983). Altered expression of lymphocyte Fc ∝ receptor in selective IgA deficiency and IgA nephropathy. Journal of Immunology 131: 1246-1251.

Andre C, Bazin H & Heremans JF. (1973). Influence of repeated administration of antigen by the oral route on specific antibody-producing cells in the mouse spleen. Digestion 9: 166-175.

Andre C, Heremans JF, Vaerman JP & Cambiaso CL. (1975). A mechanism for the induction of immunological tolerance by antigen feeding : antigen-antibody complexes. Journal of Experimental Medicine 142: 1509-1519.

Andre C, Berthoux FC, Andre F, Gillon J, Genin C & Sabatier JC. (1980). Prevalence of IgA2 deposits in IgA nephropathies. New England Journal of Medicine 303: 1343-1346.

Andres GA, Seegal BC, Hsu KC, Rothenberg MS & Chapeau ML. (1963). Electron microscopic studies of experimental nephritis with ferritin-conjugated antibody. Localization of antigen-antibody complexes in rabbit glomeruli following repeated injections of bovine serum albumin. Journal of Experimental Medicine 117: 691-704.

Arnaud-Battandier F & Nelson DL. (1982). Immunologic characteristics of intestinal lymphoid cells of the guinea pig. Gastroenterology 82: 248-253.

Arthus M. (1903). Injections repetees de serum de cheval chez le lapin. Comptes Rendus de la Societe de Biologie 55: 817-820.

Asherson GL, Zembala M, Perera MACC, Mayhew B & Thomas WR. (1977). Production of immunity and unresponsiveness in the mouse by feeding contact sensitizing agents and the role of suppressor cells in the Peyer's patches, mesenteric lymph nodes and other lymphoid tissues. Cellular Immunology 33: 145-155.

Asherson GL, Perera MACC & Thomas WR. (1979). Contact sensitivity and the DNA response to high and low doses of oxazolone : low dose unresponsiveness following painting and feeding and its prevention by pretreatment with cyclophosphamide. Immunology 36: 449-459.

Barnes RMR, Duguid JKM, Roberts FM, Risk JM, Johnson PM, Finn R, Hardy J, Napier JA & Clarke CA (1987). Oral administration of erythrocyte membrane antigen does not suppress anti-Rh(D) antibody responses in humans. Clinical and Experimental Immunology 67: 220-226.

Bartels H & Bohmer M. (1971). Eine Mikromethode zur Kreatininbestimmung. Clinica Chimica Acta 32: 81-85.

Bene MC & Faure G. (1986). IgA nephropathy. In Clinics in Immunology and Allergy. ed Carpenter CB. Vol 6, pp 307-329. London: WB Saunders.

Berger J & Hinglais N. (1968). Les depots intercapillaires d'IgA-IgG. Journal d'Urologie et Nephrologie 74: 694-695.

Berger J, Yaneva H, Nabarra B & Barbanel C. (1975). Recurrence of mesangial deposition of IgA after renal transplantation. Kidney International 7: 232-241.

Berthoux FC, Genin C, Gagne A, Le Petit JC & Sabatier JC. (1979). HLA Bw35 antigen and mesangial IgA glomerulonephritis : a poor prognosis marker?. Proceedings of the European Dialysis and Transplant Association 16: 551-555.

Bloch KJ, Perry R, Bloch M & Walker WA. (1983). Induction of (partial) systemic tolerance in primed rats subjected to prolonged oral administration of antigen. Annals of the New York Academy of Sciences 409: 787-788.

Boyns AR & Hardwicke J. (1968). The relationship between circulating soluble antigen-antibody complexes and the production of chronic glomerulonephritis in the rabbit. Immunology 14: 367-378.

Brown WR, Isobe Y, Nakane PK. (1976). Studies on translocation of immunoglobulins across intestinal epithelium. II. Immunoelectron microscopic localization of immunoglobulins and secretory component in human intestinal mucosa. Gastroenterology 71: 985-995.

Bruce MG & Ferguson A. (1986 a). Oral tolerance to ovalbumin in mice. Studies of chemically modified and of "biologically filtered" antigen. Immunology 57: 627-630.

Bruce MG & Ferguson A. (1986 b). The influence of intestinal processing on the immunogenicity and molecular size of absorbed, circulating ovalbumin in mice. Immunology 59: 295-300.

Burrows W & Ware LL. (1953). Studies on immunity to Asiatic cholera . VII. Prophylactic immunity to experimental cholera. Journal of Infectious Diseases 92: 164-174.

Butler WH. (1979). Experimental liver injury. In Pathology of the liver. ed McSween RNM, Anthony PP & Schener PJ. Ch 3, pp 55-67. Edinburgh: Churchill Livingstone.

Callard P, Feldmann G, Prandi D, Belair MF, Mandet C, Weiss Y, Druet P, Benhamou JP & Bariety J. (1975). Immune complex type glomerulonephritis in cirrhosis of the liver. American Journal of Pathology 80: 329-340.

Challacombe SJ & Tomasi TB. (1980). Systemic tolerance and secretory immunity after oral immunization. Journal of Experimental Medicine 152: 1459-1472.

Challacombe SJ. (1983). Salivary antibodies and systemic tolerance in mice after oral immunization with bacterial antigens. Annals of the New York Academy of Sciences 409: 177-193.

Challacombe SJ. (1985); quoted in Tomasi TB & Challacombe SJ. (1987). Oral tolerance. In Food Allergy and Intolerance. ed Brostoff J & Challacombe SJ. Ch 14, pp 255-268. London: Bailliere Tindall.

Challacombe SJ, Greenhall C & Stoker TAM. (1987). A comparison of IgA in portal and peripheral venous blood. Immunology 60: 111-116.

Challacombe SJ & Tomasi TB. (1987). Oral tolerance. In Food Allergy and Intolerance. ed Brostoff J & Challacombe SJ. Ch 14, pp 255-268. London: Bailliere Tindall.

Chalon MP, Milne RW & Vaerman JP. (1979). In vitro immunosuppressive effect of serum from orally immunized mice. European Journal of Immunology 9: 747-751.

Chase MW. (1946). Inhibition of experimental drug allergy by prior feeding of the sensitizing agent. Proceedings of the Society of Experimental Biology and Medicine 61: 257-259.

Clarkson AR, Woodroffe AJ, Bannister KM, Lomax-Smith JD & Aarons I. (1984). The syndrome of IgA nephropathy. Clinical Nephrology 21: 7-14.

Cochrane CG, Unanue ER & Dixon FJ. (1965). A role of polymorphonuclear leukocytes and complement in nephrotoxic nephritis. Journal of Experimental Medicine 122: 99-116.

Cochrane CG & Hawkins D. (1968). Studies on circulating immune complexes. III. Factors governing the ability of circulating complexes to localize in blood vessels. Journal of Experimental Medicine 127: 137-154.

Cochrane CG. (1971). Mechanisms involved in the deposition of immune complexes in tissues. Journal of Experimental Medicine 134: 75s-89s.

Conley ME, Cooper MD & Michael AF. (1980). Selective deposition of immunoglobulin A1 in immunoglobulin A nephropathy, anaphylactoid purpura nephritis, and systemic lupus erythematosus. Journal of Clinical Investigation 66: 1432-1436.

Coombs RRA & Gell PGH. (1963). Classification of allergic reactions responsible for clinical hyperseisitivity and disease. In Clinical Aspects of Immunology. ed Gell PGH & Coombs RRA. Ch 20, pp 575-596. Oxford: Blackwell.

Coppo R, Basolo B, Martina G, Rollino C, de Marchi M, Giacchino F, Mazzuco G, Messina M & Piccoli G. (1982). Circulating immune complexes containing IgA, IgG and IgM in patients with primary IgA nephropathy and with Henoch-Schonlein nephritis. Correlation with clinical and with histologic signs of activity. Clinical Nephrology 18: 230-239.

Coppo R, Basolo B, Rollino C, Roccatello D, Martina G, Amore A & Piccoli G. (1986). Dietary gluten and primary IgA Nephropathy. New England Journal of Medicine 315: 1167-1168.

Cotran RS. (1978). Monocytes, proliferation and glomerulonephritis. Journal of Laboratory and Clinical Medicine 92: 837-840.

Couser WG, Steinmuller DR, Stilmant MM, Salant DJ & Lowenstein LM. (1978). Experimental glomerulonephritis in the isolated perfused rat kidney. Journal of Clinical Investigation 62: 1275-1287.

Couser WG & Salant DJ. (1980). In situ immune complex formation and glomerular injury. Kidney International 17: 1-13.

Couser WG. (1986). In situ formation of immune complexes and the role of complement activation in glomerulonephritis. In Clinics in Immunology and Allergy. ed Carpenter CB. Vol 6, pp 287-306. London: WB Saunders.

Cowdery JS, Curtin MF & Steinberg AD. (1982). Effect of prior intragastric antigen administration on primary and secondary anti-ovalbumin responses of C57BL/6 and NZB mice. Journal of Experimental Medicine 156: 1256-1261.

Crabbe PA, Carbonara AO & Heremans JF. (1965). The normal intestinal mucosa as a major source of plasma cells containing γA-immunglobulin. Laboratory Investigation 14: 235-248.

Crabbe PA, Bazin H, Eyssen H & Heremans JF. (1968). The normal microbial flora as a major stimulus for proliferation of plasma cells synthesizing IgA in the gut. International Archives of Allergy and Applied Immunology 34: 362-375.

Crabbe PA, Nash DR, Bazin H, Eyssen H & Heremans JF. (1969). Antibodies of the IgA type in intestinal plasma cells of germfree mice after oral or parenteral immunization with ferritin. Journal of Experimental Medicine 130: 723-744.

Crabbe PA, Nash DR, Bazin H, Eyssen H & Heremans JF. (1970). Immunohistochemical observations on lymphoid tissues from conventional and germ-free mice. Laboratory Investigation 22: 448-457.

Crago SS & Tomasi TB. (1987). Mucosal antibodies. In Food Allergy and Intolerance. ed Brostoff J & Challacombe SJ. Ch 9, pp 167-189. London: Bailliere Tindall.

Craig SW & Cebra JJ. (1971). Peyer's patches : an enriched source of precursors for IgA-producing immunocytes in the rabbit. Journal of Experimental Medicine 134: 188-200.

Cunningham-Rundles C. (1987). Failure of antigen exclusion. In Food Allergy and Intolerance. ed Brostoff J & Challacombe SJ. Ch 12, pp 223-236. London: Bailliere Tindall.

Davies A. (1922). An investigation into the serological properties of dysentery stools. Lancet ii: 1009-1012.

Davies MDJ & Parrott DMV. (1980). The early appearance of specific cytotoxic T cells in murine gut mucosa. Clinical and Experimental Immunology 42: 273-279.

Davies MDJ & Parrott DMV. (1981). Cytotoxic T cells in small intestine epithelial, lamina propria and lung lymphocytes. Immunology 44: 367-371.

Davies MG & Davies PG. (1979). Dermatitis herpetiformis, glomerulonephritis and HLA-DRw3. Lancet ii: 911.

De Coteau WE, Gerrard JW & Cunningham TA. (1973). Glomerulitis in dermatitis herpetiformis. Lancet ii: 679-680.

Devey ME & Steward MW. (1980). The induction of chronic antigen-antibody complex disease in selectively bred mice producing either high or low affinity antibody to protein antigens. Immunology 41: 303-311.

Devey ME, Taylor J & Steward MW. (1980). Measurement of antigen-antibody complexes in mouse sera by conglutinin, C1q and rheumatoid factor solid phase binding assays. Journal of Immunological Methods 34: 191-203.

Devey ME, Bleasdale K, Collins M & Steward MW. (1982). Experimental antigen-antibody complex disease in mice : the role of antibody levels, antibody affinity and circulating antigen-antibody complexes. International Archives of Allergy and Applied Immunology 68: 47-53.

Devey ME & Bleasdale K. (1984). Antigen feeding modifies the course of antigen-induced immune complex disease. Clinical and Experimental Immunology 56: 637-644.

Dillon SB & MacDonald TT. (1984). Functional properties of lymphocytes isolated from murine small intestinal epithelium. Immunology 52: 501-509.

Di Luzio NR & Morrow HS. (1971). Comparative behaviour of soluble and particulate antigens and inert colloids in reticuloendothelial-stimulated or depressed mice. Journal of the Reticuloendothelial Society 9: 273-287.

Dixon FJ, Feldman JD & Vazquez JJ. (1961). Experimental glomerulonephritis. The pathogenesis of a laboratory model resembling the spectrum of human glomerulonephritis. Journal of Experimental Medicine 113: 899-920.

Edgington TS, Glassock RJ & Dixon FJ. (1967). Autologous immune-complex pathogenesis of experimental allergic glomerulonephritis. Science 155: 1432-1434.

Edgington TS, Glassock RJ & Dixon FJ. (1968). Autologous immune complex nephritis induced with renal tubular antigen. I. Identification and isolation of the pathogenetic antigen. Journal of Experimental Medicine 127: 555-571.

Egido J, Blasco R, Sancho J, Lozano L, Sanchez-Crespo M & Hernendo L. (1982). Increased rates of polymeric IgA synthesis by circulating lymphoid cells in IgA mesangial glomerulonephritis. Clinical and Experimental Immunology 47: 309-316.

Elson CO, Weiserbs DB, Ealding W & Machelski E. (1983). T-helper cell activity in intestinal lamina propria. Annals of the New York Academy of Sciences 409: 230-237.

Emancipator SN, Gallo GR & Lamm ME. (1983 a). Experimental IgA nephropathy induced by oral immunization. Journal of Experimental Medicine 157: 572-582.

Emancipator SN, Gallo GR & Lamm ME. (1983 b). IgA-immune complex renal disease induced by mucosal immunization. Annals of the New York Academy of Sciences 409: 171-176.

Emancipator SN, Gallo GR, Razaboni R & Lamm ME. (1983). Experimental cholestasis promotes the deposition of glomerular IgA immune complexes. American Journal of Pathology 113: 19-26.

Ferguson A & Parrott DMV. (1973). Histopathology and time course of rejection of allografts of mouse small intestine. Transplantation 15: 546-554.

Ferguson A & Mowat AMcI. (1980). Immunological mechanisms in the small intestine. In Recent Advances in Gastrointestinal Pathology. ed Wright R. pp 95-103. London: WB Saunders.

Finlayson G, Alexander R, Juncos L, Schlein E, Teague P, Waldman R & Cade R. (1975). Immunoglobulin A glomerulonephritis : a clinicopathologic study. Laboratory Investigation 32: 140-148.

Fisher MM, Nagy B, Bazin H & Underdown BJ. (1979). Biliary transport of IgA : role of secretory component. Proceedings of the National Academy of Sciences USA 76: 2008-2012.

Ford PM. (1975). The effect of manipulation of reticuloendothelial system activity on glomerular deposition of aggregated protein and immune complexes in two different strains of mice. British Journal of Experimental Pathology 56: 523-529.

Ford PM & Kosatka I. (1981). A mechanism of enhancement of immune complex deposition following in situ immune complex formation in the mouse glomerulus. Immunology 43: 433-439.

Ford PM & Kosatka I. (1983). In situ immune complex formation in the mouse glomerulus : reactivity with human IgM rheumatoid factor and the effect on subsequent immune complex deposition. Clinical and Experimental Immunology 51: 285-291.

Francioni C. (1904). La malattia da siero. Sperimentale ; quoted in Albini B, Brentjens JR & Andres GA. (1979). The Immunopathology of the Kidney. London: Edward Arnold.

Gaboardi F, Perletti L, Cambie M & Mihatsch MJ. (1983). Dermatitis herpetiformis and nephrotic syndrome. Clinical Nephrology 20: 49-51.

Galla JH, Russell MW, Hammond D, Spotswood M & Mestecky J. (1985). Environmental antigens (EAg) in IgA nephropathy (IgAN). Kidney International 27: 210 (Abstract).

Gallatin WM, Weissman IL & Butcher EC. (1983). A cell-surface molecule involved in organ-specific homing of lymphocytes. Nature 304: 30-34.

Gallo GR, Caulin-Glaser T & Lamm ME. (1981). Charge of circulating immune complexes as a factor in glomerular basement membrane localization in mice. Journal of Clinical Investigation 67: 1305-1313.

Garner RC & McLean AEM. (1969). Increased susceptibility to carbon tetrachloride poisoning in the rat after pretreatment with oral phenobarbitone. Biochemical Pharmacology 18: 645-650.

Genin C, Sabatier JC & Berthoux FC. (1984). IgA mesangial deposits in C3H/HeJ mice after oral immunization. Proceedings of the European Dialysis and Transplant Association 21: 703-708.

Genin C, Laurent B, Sabatier JC, Colon S & Berthoux FC. (1986). IgA mesangial deposits in C3H/HeJ mice after oral immunization with ferritin or bovine serum albumin. Clinical and Experimental Immunology 63: 385-394.

Germuth FG. (1953). A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. Journal of Experimental Medicine 97: 257-282.

Germuth FG & McKinnon GE. (1957). Studies on the biological properties of antigen-antibody complexes. I. Anaphylactic shock induced by soluble antigen-antibody complexes in unsensitised normal guinea pigs. Bulletin of the Johns Hopkins Hospital 101: 13-44.

Gormly AA, Smith PS, Seymour AE, Clarkson AR & Woodroffe AJ. (1981). IgA glomerular deposits in experimental cirrhosis. American Journal of Pathology 104: 50-54.

Groggel GC, Adler S, Rennke HG, Couser WG & Salant DJ. (1983). Role of the terminal complement pathway in experimental membranous nephropathy in the rabbit. Journal of Clinical Investigation 72: 1948-1957.

Haakenstad AO, Striker GE & Mannik M. (1976). The glomerular deposition of soluble immune complexes prepared with reduced and alkylated antibodies and with intact antibodies in mice. Laboratory Investigation 35: 293-301.

Halpern MJ & Koshland ME. (1970). Novel subunit in secretory IgA. Nature 228: 1276-1278.

Hanson DG, Vaz NM, Rawlings LA & Lynch JM. (1979 a). Inhibition of specific immune responses by feeding protein antigens. II. Effects of prior passive and active immunization. Journal of Immunology 122: 2261-2266.

Hanson DG, Vaz NM, Maia LCS & Lynch JM. (1979 b). Inhibition of specific immune responses by feeding protein antigens. III. Evidence against maintenance of tolerance to ovalbumin by orally induced antibodies. Journal of Immunology 123: 2337-2343.

Heng MCY. (1985). Henoch-Schonlein purpura. British Journal of Dermatology 112: 235-240.

Herbert WJ. (1973). Mineral oil adjuvants and the immunization of laboratory animals. In Handbook of Experimental Immunology 2nd Edition. ed. Weir DM. Appendix 2. Oxford: Blackwell.

Heremans JF & Bazin H. (1971). Antibodies induced by local antigenic stimulation of mucosal surfaces. Annals of the New York Academy of Sciences 190: 268-275.

Heremans JF. (1974). Immunoglobulin A. In The Antigens. ed Sela M. Vol II, pp 365-522. New York: Academic Press.

Heymann W, Hackel DB, Harwood S, Wilson SGF & Hunter JLP. (1959). Production of nephrotic syndrome in rats by Freund's adjuvant and rat kidney suspensions. Proceedings of the Society for Experimental Biology and Medicine 100: 660-664.

Holdsworth SR, Neale TJ & Wilson CB. (1981). Abrogation of macrophage-dependent injury in experimental glomerulonephritis in the rabbit. Journal of Clinical Investigation 68: 686-698.

Holdsworth SR. (1983). Fc dependence of macrophage accumulation and subsequent injury in experimental glomerulonephritis. Journal of Immunology 130: 735-739.

Hudson L & Hay FC. (1976). Practical Immunology. pp 1-5. Oxford: Blackwell.

Hurlimann J & Darling H. (1971). In vitro synthesis of immunoglobulin-A by salivary glands from animals of different species. Immunology 21: 101-111.

Husband AJ. (1982). Kinetics of extravasation and redistribution of IgA-specific antibody-containing cells in the intestine. Journal of Immunology 128: 1355-1359.

Imai H, Nakamoto Y, Asakura K, Miki K, Yasuda T & Miura AB. (1985). Spontaneous glomerular IgA deposition in ddY mice : an animal model of IgA nephritis. Kidney International 27: 756-761.

Isaacs K, Miller F & Lane B. (1981). Experimental model for IgA nephropathy. Clinical Immunology and Immunopathology 20: 419-426.

Isaacs K & Miller F. (1984). Dextran induced IgA nephropathy. In IgA mesangial nephropathy. ed D'Amico G, Minetti L & Ponticelli C. pp 45-50. Basel: Karger.

Iskandar SS, Jennette JC, Wilkman AS & Becker RL. (1982). Interstrain variations in nephritogenicity of heterologous protein in mice. Laboratory Investigation 46: 344-354.

Kagnoff MF. (1978). Effects of antigen-feeding on intestinal and systemic immune responses. III. Antigen-specific serum-mediated suppression of humoral antibody responses after antigen feeding. Cellular Immunology 40: 186-203.

Kagnoff MF. (1980). Effects of antigen feeding on intestinal and systemic immune responses. IV. Similarity between the suppressor factor in mice after erythrocyte-lysate injection and erythrocyte feeding. Gastroenterology 79: 54-61.

Katz A, Dyck RF & Bear RA. (1979). Celiac disease associated with immune complex glomerulonephritis. Clinical Nephrology 11: 39-44.

Kelley VE. (1986). Dietary effects on the progression of autoimmune glomerulonephritis. in Clinics in Immunology and Allergy. ed Carpenter CB. Vol 6, pp 367-381. London: WB Saunders.

Kiyono H, Babb JL, Michalek SM & McGhee JR. (1980). Cellular basis for elevated IgA responses in C3H/HeJ mice. Journal of Immunology 125: 732-737.

Kiyono H, McGhee JR, Wannemuehler MJ & Michalek SM. (1982). Lack of oral tolerance in C3H/HeJ mice. Journal of Experimental Medicine 155: 605-610.

Kniker WT & Cochrane CG. (1968). The localization of circulating immune complexes in experimental serum sickness. The role of vasoactive amines and hydrodynamic forces. Journal of Experimental Medicine 127: 119-136.

Kuriyama T. (1973). Chronic glomerulonephritis induced by prolonged immunization in the rabbit. Laboratory Investigation 28: 224-235.

Lafont S, Andre C, Andre F, Gillon J & Fargier MC. (1982). Abrogation by subsequent feeding of antibody response, including IgE, in parenterally immunized mice. Journal of Experimental Medicine 155: 1573-1578.

Lambert PH, Dixon FJ, Zubler RH, Agnello V, Cambiaso C, Casali P, Clarke J, Cowdery JS, McDuffie FC, Hay FC, MacLennan ICM, Masson P, Muller-Eberhard HJ, Penttinen K, Smith M, Tappeiner G, Theofilopoulos AN & Verroust P. (1978). A WHO collaborative study for the evaluation of eighteen methods for detecting immune complexes in serum. Journal of Clinical and Laboratory Immunology 1: 1-15.

Lemaitre-Coelho I, Jackson GDF & Vaerman, JP. (1978). High levels of secretory IgA and free secretory component in the serum of rats with bile duct obstruction. Journal of Experimental Medicine 147: 934-939.

Lesavre P, Digeon M & Bach JF. (1982). Analysis of circulating IgA and detection of immune complexes in primary IgA nephropathy. Clinical and Experimental Immunology 48: 61-69.

Levy JA & Morrow WJW. (1983). Dietary regulation of the autoimmune process in murine lupus. Immunology Today 4: 249-250.

Lindemann W. (1900). Sur le mode d'action de certains poisons renaux. Annales d'Institut Pasteur 13: 49-59.

Longcope WT. (1913). The production of experimental nephritis by repeated proteid intoxication. Journal of Experimental Medicine 18: 678-703.

Longcope WT & Rackemann FM. (1918). The relation of circulating antibodies to serum disease. Journal of Experimental Medicine 27: 341-358.

Lowance DC, Mullins JD & McPhaul JJ. (1973). Immunoglobulin A (IgA) associated nephritis. Kidney International 3: 167-176.

MacDonald TT & Ferguson A. (1976). Hypersensitivity reactions in the small intestine. 2. Effects of allograft rejection on mucosal architecture and lymphoid cell infiltrate. Gut 17: 81-91.

Mantovani B, Rabinovitch M & Nussenzweig V. (1972). Phagocytosis of immune complexes by macrophages. Different roles of the macrophage receptor sites for complement (C3) and for immunoglobulin (IgG). Journal of Experimental Medicine 135: 780-792.

Markham RV, Sutherland JC & Mardiney MR. (1973). The ubiquitous occurrence of immune complex localization in the renal glomeruli of normal mice. Laboratory Investigation 29: 111-120.

Marrack JR. (1938). The Chemistry of Antigens and Antibodies. London: HMSO.

Masugi M. (1934). Uber die experimentelle Glomerulonephritis durch das spezifische Antinierenserum. Ein Betrag zur Pathogenese der diffusen Glomerulonephritis. Beitrage zur Pathologischen Anatomie und zur Allgemeinen Pathologie 92: 429-466.

Matsumura T & Kuruome T. (1961). The role of allergy in the pathogenesis of the nephrotic syndrome. Japanese Journal of Pediatrics 14: 921.

Mattingly JA & Waksman BH. (1978). Immunologic suppression after oral administration of antigen. I. Specific suppressor cells formed in rat Peyer's patches after oral administration of sheep erythrocytes and their systemic migration. Journal of Immunology 121: 1878-1883.

Mattingly JA & Waksman BH. (1980). Immunologic suppression after oral administration of antigen. II. Antigen-specific helper and suppressor factors produced by spleen cells of rats fed sheep erythrocytes. Journal of Immunology 125: 1044-1047.

McDermott MR & Bienenstock J. (1979). Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory and genitial tissues. Journal of Immunology 122: 1892-1898.

McLean EK, McLean AEM & Sutton PM. (1969). Instant cirrhosis. An improved method for producing cirrhosis of the liver in rats by simultaneous administration of carbon tetrachloride and phenobarbitone. British Journal of Experimental Pathology 50: 502-506.

Mellors RC, Siegel M & Pressman D. (1955). Analytic pathology. Histochemical demonstration of antibody localization in tissues , with special reference to the antigenic components of kidney and lung. Laboratory Investigation 4: 69-89.

Miller SD & Hanson DG. (1979). Inhibition of specific immune responses by feeding protein antigens. IV. Evidence for tolerance and specific active suppression of cell-mediated immune response to ovalbumin. Journal of Immunology 123: 2344-2350.

Mirski SE, McDermott MR, Befus AD & Bienenstock J. (1981). Selective localization of mesenteric lymphoblasts in mucosal tissues : effects of altering the number of donor lymphoblasts. Immunology 43: 669-675.

Mitchison NA. (1964). Induction of immunological paralysis in two zones of dosage. Proceedings of the Royal Society 161 B: 275-292.

Mowat AM & Ferguson A. (1981 a). Hypersensitivity reactions in the small intestinal mucosa. 5. Induction of cell-mediated immunity to a dietary antigen. Clinical and Experimental Immunology 43: 574-582.

Mowat A McI & Ferguson A. (1981 b). Hypersensitivity reactions in the small intestine. 6. Pathogenesis of the graft-versus-host reaction in the small intestinal mucosa of the mouse. Transplantation 32: 238-243.

Mowat AMcI, Strobel S, Drummond HE & Ferguson A. (1982). Immunological responses to fed protein antigens in mice. I. Reversal of oral tolerance to ovalbumin by cyclophosphamide. Immunology 45: 105-113.

Mowat AMcI & Parrott DMV. (1983). Immunological responses to fed protein antigens in mice. IV. Effects of stimulating the reticuloendothelial system on oral tolerance and intestinal immunity to ovalbumin. Immunology 50: 547-554.

Mowat AMcI, Tait RC, MacKenzie S, Davies MDJ & Parrott DMV. (1983). Analysis of natural killer effector and suppressor activity by intraepithelial lymphocytes from mouse small intestine. Clinical and Experimental Immunology 52: 191-198.

Mowat AMcI, Thomas MJ, MacKenzie S & Parrott DMV. (1986). Divergent effects of bacterial lipopolysaccharide on immunity to orally administered protein and particulate antigens in mice. Immunology 58: 677-683.

Nagura H, Nakane PK & Brown WR. (1979). Translocation of dimeric IgA through neoplastic colon cells in vitro. Journal of Immunology 123: 2359-2368.

Naish PF, Evans DJ & Peters DK. (1975). The effects of defibrination with ancrod in experimental allergic glomerular injury. Clinical and Experimental Immunology 20: 303-309.

Nakamoto Y, Asano Y, Dohi K, Fujioka M, Iida H, Kida H, Kibe Y, Hattori N & Takeuchi J. (1978). Primary IgA glomerulonephritis and Schonlein-Henoch purpura nephritis : clinicopathological and immunohistological characteristics. Quarterly Journal of Medicine 47: 495-516.

Newby TJ, Stokes CR & Bourne FJ. (1980). Effects of feeding bacterial lipopolysaccharide and dextran sulphate on the development of oral tolerance to contact sensitizing agents. Immunology 41: 617-621.

Newman SL & Johnston RB. (1979). Role of binding through C3b and IgG in polymorphonuclear neutrophil function: Studies with trypsin-generated C3b. Journal of Immunology 123: 1839-1846.

Ngan J & Kind LS. (1978). Suppressor T cells for IgE and IgG in Peyer's patches of mice made tolerant by the oral administration of ovalbumin. Journal of Immunology 120: 861-865.

Nicias; quoted in Thucydidis. (415 B.C.). Historiae VII, 15. Oxford: Scriptorum Classicorum Bibliotheca Oxoniensis.

Nicklin S & Miller K. (1983). Local and systemic immune responses to intestinally presented antigen. International Archives of Allergy and Applied Immunology 72: 87-90.

Nydegger UE. (1985). A place for soluble immune complexes in clinical immunology. Immunology Today 6: 80-82.

Oldstone MBA & Dixon FJ. (1969). Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. I. Relationship of antibody production to disease in neonatally infected mice. Journal of Experimental Medicine 129: 483-505.

Orlans E, Peppard J, Reynolds J & Hall J. (1978). Rapid active transport of immunoglobulin A from blood to bile. Journal of Experimental Medicine 147: 588-592.

Ottaway CA & Parrott DMV. (1980). Regional blood flow and the localization of lymphoblasts in the small intestine of the mouse. I - Examination of normal small intestine. Immunology 41: 955-961.

Ottaway CA. (1984). In vitro alteration of receptors for vasoactive intestinal peptide changes the in vivo localization of mouse T cells. Journal of Experimental Medicine 160: 1054-1069.

Paganelli R, Levinsky RJ, Brostoff J & Wraith DG. (1979). Immune complexes containing food proteins in normal and atopic subjects after oral challenge and effect of sodium cromoglycate on antigen absorption. Lancet i: 1270-1272.

Parrott DMV. (1987). The structure and organisation of lymphoid tissue in the gut. In Food Allergy and Intolerance. ed Brostoff J & Challacombe SJ. Ch 1, pp 3-26. London: Bailliere Tindall.

Phillips-Quagliata JM, Roux ME, Arny M, Kelly-Hatfield P, McWilliams M & Lamm ME. (1983). Migration and regulation of B-cells in the mucosal immune system. Annals of the New York Academy of Sciences 409: 194-203.

Pincus T, Haberkern R & Christian CL. (1968). Experimental chronic glomerulitis. Journal of Experimental Medicine 127: 819-832.

Ranges GE & Azar MM. (1979). Inheritance of tolerance susceptibility to human γ-globulin in congenic mice. Journal of Immunology 123: 1151-1154.

Richards W, Olson D & Church JA. (1977). Improvement of idiopathic nephrotic syndrome following allergy therapy. Annals of Allergy 39: 332-333.

Richman LK, Chiller JM, Brown WR, Hanson DG & Vaz NM. (1978). Enterically induced immunologic tolerance. I. Induction of suppressor T lymphocytes by intragastric administration of soluble proteins. Journal of Immunology 121: 2429-2434.

Rifai A, Small PA, Teague PO & Ayoub EM. (1979). Experimental IgA nephropathy. Journal of Experimental Medicine 150: 1161-1173.

Rifai A & Millard K. (1985). Glomerular deposition of immune complexes prepared with monomeric or polymeric IgA. Clinical and Experimental Immunology 60: 363-368.

Rose ML, Parrott DMV & Bruce RG. (1976). Migration of lymphoblasts to the small intestine. II. Divergent migration of mesenteric and peripheral immunoblasts to sites of inflammation in the mouse. Cellular Immunology 27: 36-46.

Rothberg RM, Kraft SC & Farr RS. (1967). Similarities between rabbit antibodies produced following ingestion of bovine serum albumin and following parenteral immunization. Journal of Immunology 98: 386-395.

Roux ME, McWilliams M, Phillips-Quagliata JM & Lamm ME. (1981). Differentiation pathway of Peyer's patch precursors of IgA plasma cells in the secretory immune system. Cellular Immunology 61: 141-153.

Russell MW, Brown TA & Mestecky J. (1981). Role of serum IgA. Hepatobiliary transport of circulating antigen. Journal of Experimental Medicine 153: 968-976.

Sakaguchi H, Dachs S, Mautner W, Grishman E & Churg J. (1964). Renal glomerular lesions after administration of carbon tetrachloride and ethionine. Laboratory Investigation 13: 1418-1426.

Sakaguchi H, Dachs S, Grishman E, Paronetto F, Salomon M & Churg J. (1965). Hepatic glomerulosclerosis. An electron microscopic study of renal biopsies in liver diseases. Laboratory Investigation 14: 533-545.

Sakai H, Nomoto Y & Arimori S. (1979). Decrease of IgA-specific suppressor T cell activity in patients with IgA nephropathy. Clinical and Experimental Immunology 38: 243-248.

Saklayen MG, Pesce AJ, Pollak VE & Michael JG. (1984). Kinetics of oral tolerance : study of variables affecting tolerance induced by oral administration of antigen. International Archives of Allergy and Applied Immunology 73: 5-9.

Salant DJ, Belok S, Madaio MP & Couser WG. (1980). A new role for complement in experimental membranous nephropathy in rats. Journal of Clinical Investigation 66: 1339-1350.

Sancho J, Egido J, Rivera F & Hernando L. (1983). Immune complexes in IgA nephropathy : presence of antibodies against diet antigens and delayed clearance of specific polymeric IgA immune complexes. Clinical and Experimental Immunology 54: 194-202.

Sandberg DH, McIntosh RM, Bernstein CW, Carr R & Strauss J. (1977). Severe steroid-responsive nephrosis associated with hypersensitivity. Lancet i: 388-390.

Sandberg D. (1987). Food sensitivity : the kidney and bladder. In Food Allergy and Intolerance. ed Brostoff J & Challacombe SJ. Ch 43a, pp 755-767. London: Bailliere Tindall.

Sato M, Ideura T & Koshikawa S. (1986). Experimental IgA nephropathy in mice. Laboratory Investigation 54: 377-384.

Schreiner GF, Cotran RS, Pardo V & Unanue ER. (1978). A mononuclear cell component in experimental immunological glomerulonephritis. Journal of Experimental Medicine 147: 369-384.

Schwartz A, Askenase PW & Gershon RK. (1978). Regulation of delayed-type hypersensitivity reactions by cyclophosphamide-sensitive T cells. Journal of Immunology 121: 1573-1577.

Siegel I, Liu L & Gleicher N. (1981). The red cell immune system. Lancet ii: 556-559.

Silverman GA, Peri BA, Fitch FW & Rothberg RM. (1983). Enterically induced regulation of systemic immune responses. II. Suppression of proliferating T cells by an Lyt-1^{+}, 2^{-} effector T cell. Journal of Immunology 131: 2656-2661.

Sinico RA, Fornasieri A, Oreni N, Benuzzi S & D'Amico G. (1986). IgA rheumatoid factor in IgA mesangial nephropathy. 6th International Congress of Immunology (Abstract).

Soothill JF & Steward MW. (1971). The immunopathological significance of the heterogeneity of antibody affinity. Clinical and Experimental Immunology 9: 193-199.

Steward MW, Collins MJ, Stanley C & Devey ME. (1981). Chronic antigen-antibody complex glomerulonephritis in mice. British Journal of Experimental Pathology 62: 614-622.

Stokes CR, Newby TJ, Huntly JH, Patel D & Bourne FJ. (1979). The immune response of mice to bacterial antigens given by mouth. Immunology 38: 497-502.

Stokes CR, Swarbrick ET & Soothill JF. (1983). Genetic differences in immune exclusion and partial tolerance to ingested antigens. Clinical and Experimental Immunology 52: 678-684.

Strannegard O & Yurchison A. (1969). Formation of agglutinating and reaginic antibodies in rabbits following oral administration of soluble and particulate antigens. International Archives of Allergy and Applied Immunology 35: 579-590.

Strobel S, Mowat AMcI, Drummond HE, Pickering MG & Ferguson A. (1983). Immunological responses to fed protein antigens in mice. II. Oral tolerance for CMI is due to activation of cyclophosphamide-sensitive cells by gut-processed antigen. Immunology 49: 451-456.

Strobel S & Ferguson A. (1984). Immune responses to fed protein antigens in mice. 3. Systemic tolerance or priming is related to age at which antigen is first encountered. Pediatric Research 18: 588-594.

Swarbrick ET, Stokes CR & Soothill JF. (1979). Absorption of antigens after oral immunisation and the simultaneous induction of specific systemic tolerance. Gut 20: 121-125.

Thomas HC & Parrott DMV. (1974). The induction of tolerance to a soluble protein antigen by oral administration. Immunology 27: 631-639.

Thompson HSG & Staines NA. (1986). Gastric administration of type II collagen delays the onset and severity of collagen-induced arthritis in rats. Clinical and Experimental Immunology 64: 581-586.

Titus RG & Chiller JM. (1981 a). Orally induced tolerance. Definition at the cellular level. International Archives of Allergy and Applied Immunology 65: 323-338.

Titus RG & Chiller JM. (1981 b). A simple and effective method to assess murine delayed type hypersensitivity to proteins. Journal of Immunological Methods 45: 65-78.

Tomasi TB & Zigelbaum S. (1963). The selective occurence of γ1A globulins in certain body fluids. Journal of Clinical Investigation 42: 1552-1560.

Tomasi TB, Barr WG, Challacombe SJ & Curran G. (1983). Oral tolerance and accessory-cell function of Peyer's patches. Annals of the New York Academy of Sciences 409: 145-163.

Tomino Y, Endoh M, Nomoto Y & Sakai H. (1981). Immunoglobulin A1 in IgA nephropathy. New England Journal of Medicine 305: 1159-1160.

Unanue E & Dixon FJ. (1964). Experimental glomerulonephritis. IV. Participation of complement in nephrotoxic nephritis. Journal of Experimental Medicine 119: 965-982.

Unanue ER & Dixon FJ. (1965). Experimental glomerulonephritis. V. Studies on the interaction of nephrotoxic antibodies with tissues of the rat. Journal of Experimental Medicine 121: 697-714.

Unanue ER & Dixon FJ. (1967). Experimental glomerulonephritis. Immunological events and pathogenetic mechanisms. Advances in Immunology 6: 1-90.

Vaerman JP. (1973) ; quoted in Heremans JF. (1974). Immunoglobulin A. In The Antigens. ed Sela M. Vol II, pp 365-522. New York: Academic Press.

Valentijn RM, Kauffmann RH, Brutel de la Riviere G, Daha MR & van Es LA. (1983). Presence of circulating macromolecular IgA in patients with hematuria due to primary IgA nephropathy. American Journal of Medicine 74: 375-381.

van der Woude FJ, Hoedemaeker PJ, van der Giessen M, de Graeff PA, de Monchy J, The TH & van der Hem GK. (1983). Do food antigens play a role in the pathogenesis of some cases of human glomerulonephritis?. Clinical and Experimental Immunology 51: 587-594.

Vassalli P & McCluskey RT. (1971). The pathogenetic role of the coagulation process in glomerular diseases of immunologic origin. Advances in Nephrology 1: 47-63.

Volkheimer G & Schulz FH. (1968). The phenomenon of persorption. Digestion 1: 213-218.

von Pirquet CE & Schick B. (1905). Die Serumkrankheit. Leipzig: Deuticke. Translated by Schick B. (1951). Serum Sickness. Baltimore: Williams & Wilkins.

Walker WA, Wu M, Isselbacher KJ & Bloch KJ. (1975 a). Intestinal uptake of macromolecules. III. Studies on the mechanism by which immunization interferes with antigen uptake. Journal of Immunology 115: 854-861.

Walker WA, Wu M, Isselbacher KJ & Bloch KJ. (1975 b). Intestinal uptake of macromolecules. IV. The effect of pancreatic duct ligation on the breakdown of antigen and antigen-antibody complexes on the intestinal surface. Gastroenterology 69: 1223-1229.

Walker WA & Isselbacher KJ. (1977). Intestinal antibodies. New England Journal of Medicine 297: 767-773.

Warshaw AL, Walker WA, Cornell R & Isselbacher KJ. (1971). Small intestinal permeability to macromolecules. Transmission of horseradish peroxidase into mesenteric lymph and portal blood. Laboratory Investigation 25: 675-684.

Wells HG. (1911). Studies on the chemistry of anaphylaxis. (III). Experiments with isolated proteins, especially those of the hen's egg. Journal of Infectious Diseases 9: 147-171.

Wilson CB & Dixon FJ. (1971). Quantitation of acute and chronic serum sickness in the rabbit. Journal of Experimental Medicine 134: 7s-18s.

Wilson SJ & Walzer M. (1935). Absorption of undigested proteins in human beings. IV. Absorption of unaltered egg protein in infants and children. American Journal of Diseases of Children 50: 49-54.

Woodroffe AJ, Gormly AA, McKenzie PE, Wootton AM, Thompson AJ, Seymour AE & Clarkson AR. (1980). Immunologic studies in IgA nephropathy. Kidney International 18: 366-374.

Yoshikai Y, Miake S, Matsumoto T, Nomoto K & Takeya K. (1981). Effect of stimulation and blockade of mononuclear phagocyte system on the induction of suppressor T cells of delayed footpad reaction to SRBC in mice. Immunology 43: 241-247.

Zanetti M & Wilson CB. (1983). Participation of auto-anti-idiotypes in immune complex glomerulonephritis in rabbits. Journal of Immunology 131: 2781-2783.

Zimmerman SW & Burkholder PM. (1975). Immunoglobulin A nephropathy. Archives of Internal Medicine 135: 1217-1223.

APPENDIX 1

BUFFERS AND SOLUTIONS

9 BUFFERS AND SOLUTIONS

Preparation of buffers and solutions :

Saline (physiological) : 0.15M

8.7 g NaCl

Dissolve to 1 litre in distilled water.

Phosphate buffered saline : 0.15M, pH 7.2

8 g NaCl
0.2 g KCl
1.15g Na2HPO4
0.2 g KH2PO4

Dissolve to 1 litre in distilled water

RPMI 1640

20 ml RPMI 1640 concentrate (x10) (Gibco, Scotland)
180 ml Hepes bicarbonate solution (2.65 g Hepes + 0.85 g NaHCO3 per litre in distilled water)
5N NaOH

Add RPMI to Hepes bicarbonate. Adjust pH to ca. 7.2 with 5N NaOH.

Affinity purification of rabbit anti-ova

Coupling buffer : NaHCO3 (0.1M), pH 8.3, containing NaCl (0.5M)

8.4 g NaHCO3
29.2 g NaCl
1N HCl

Dissolve in 800 ml distilled water. Adjust pH to 8.3 with HCl. Add distilled water to 1l.

Tris-HCl : 0.1M, pH 8.0

12 g Tris(hydroxymethyl)aminomethane
1N HCl

Dissolve in 500 ml distilled water. Adjust pH to 8.0 with 1N HCl. Add distilled water to 1l.

Acetate buffer : 0.1M, pH 4.0, containing NaCl (0.5M)

41 ml 0.2M acetic acid (11.5 ml/l distilled water)
9 ml 0.2M Na acetate (27.2 g Na acetate.3H2O/l distilled water)
2.9 g NaCl

Mix solutions, and dissolve NaCl. Make up volume to 100 ml with distilled water.

Preparation of mouse IgG :

Phosphate buffers : pH 8.0, 0.005M - 0.3M

0.5M phosphate buffer prerared by:

7.8 g NaH2PO4.H2O dissolved to 100 ml in distilled water
71 g Na2HPO4 dissolved to 1 l in distilled water

The two solutions were mixed to pH 8.0 (approximately 50 ml and 950 ml respectively).

0.3M and 0.005M solutions were prepared by dilution in distilled water.

Gradient ionic strength buffer was produced as described by Hudson & Hay. (1980). Practical Immunology 2nd Ed. pp 171-175. Oxford: Blackwell.

Veronal buffered saline

8.5 g NaCl
0.375 g Na Barbitone
0.575 g Barbitone

Dissolve in distilled water to 1 litre.

ELISA buffers

Carbonate:bicarbonate coating buffer : pH 9.6

1.59 g Na2CO3
2.93 g NaHCO3
0.2 g NaN3

Dissolve in distilled water to 1 litre.

Wash buffer : PBS-Tween

0.5 ml Tween 20
0.2 g NaN3

Dissolve in 1 litre PBS.

VBS-Tween : 0.5 ml Tween 20 dissolved in 1 litre VBS.

Substrate buffer : 10% DEA, pH 9.8

100 ml diethanolamine
800 ml distilled water
0.2 g NaN3
1N HCl

Mix diethanolamine and distilled water. Dissolve NaN3. Adjust pH to 9.8 with 1N HCl. Adjust volume to 1 l with distilled water.

Estimation of serum urea

Urease solution :

30 U	urease
1 g	EDTA
1N	NaOH

Dissolve urease to 1.5 U/ml in distilled water. Add EDTA. Adjust pH to 6.5 with 1N NaOH. Adjust volume to 100 ml. Store at $-20^{\circ}C$ in aliquots.

Phenol colour reagent :

5 g	phenol
25 mg	Na nitroprusside

Dissolve phenol in 80 ml distilled water. Dissolve Na nitroprusside , and adjust volume to 100 ml with distilled water.

Hypochlorite solution

1 ml	hypochlorite
2.5 g	NaOH

Dissolve NaOH in 80 ml distilled water. Add hypochlorite, and adjust volume to 100 ml with distilled water.