IMMUNOHISTOLOGICAL OBSERVATIONS ON FOETAL PANCREAS AND ISOLATED PANCREATIC ISLET TRANSPLANTATION IN RATS

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A thesis submitted for the degree of

Doctor of Medicine in the University of Glasgow

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Glasgow

April 1989

It is only fitting that this thesis

should be dedicated to my parents,

Carmella and Jim, who supported and

encouraged me from the very beginning

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LIST OF ABBREVIATIONS

ALS	Anti-lymphocyte serum
CSII	Continuous subcutaneous insulin infusion
EB	Ethidium bromide
F1	First cross
FDA	Fluorescein diacetate
HBSS	Hank's balanced salt solution
Ia	I region asociated antigens
IFN-gamma	Interferon-gamma
IL-2	Interleukin 2
IL-2R	Interleukin 2 receptor
J/m^2	Joules per square metre
Kg	Kilograms
LEW	Lewis
mg	Milligrams
MHC	Major histocompatibility complex
ml	Millilitres
mmol	Millimoles
mU	Milliunits
ng	Nanograms
NK	Natural killer cells
PZI	Protamine zinc insulin
SEM	Standard error of the mean
$T_{c/s}$	T cells with cytotoxic/suppressor phenotype
U	Units
ug	Micrograms
um	Microns
UV	Ultraviolet

ACKNOWLEDGEMENTS

The work described in this thesis was carried out during my tenure as Wellcome Fellow in the Department of Surgery, Western Infirmary, Glasgow and grateful thanks are due to the Wellcome Trust for providing the necessary resources to enable this work to be done.

Thanks are also due to the following.

Expert technical help was provided by Colin Hughes, Veronica Graham and John Kennedy in the Department of Surgery Laboratories.

Mr J A Stewart, processed and stained all paraffin sections.

Dr Ian More and Mr Tom Downie showed great skill in the production of electron micrographs.

Mr Andrew Bradley and Ms Eleanor Bolton provided considerable practical help and advice. All kidney transplants were performed by Ms Bolton.

Mr Stuart Macpherson provided encouragmeent and psychological support.

Dr Harry Mosely, provided and calibrated ultraviolet equipment and offered helpful advice.

Dr Graham Beastall performed all insulin measurements.

Miss Yvonne Galbraith showed great patience and considerable secretarial expertise in transforming rough text into printed copy.

Lastly, I thank Professor W D George for providing encouragement and allowing a transplanter to commandeer his Department's laboratory resources.

SUMMARY

Although it is now rare for a patient to succumb to the acute metabolic derangements of diabetes mellitus, diabetic patients still have a reduced life expectancy and many develop disabling complications. The balance of the available evidence suggests that near-physiological control of glucose metabolism at an early stage in the disease would prevent many of the microangiopathic and neuropathic changes otherwise seen. At present, whole organ or segmental pancreatic transplantation offers the most physiological system of insulin administration available, but the hope for the future is that isolated islet or foetal pancreas transplantation will become a clinical reality.

As well as requiring only a relatively minor procedure for effective transplantation, an advantage that isolated islets and foetal pancreas have over whole organ or segmental pancreas grafts is the ability to survive in vitro allowing storage and a potential opportunity for immunomodulation.

Immunomodulation prior to transplantation aims to reduce immunogenicity by altering the antigen content of a graft. Following the realisation of the particular importance of MHC class II positive cells in antigen presentation, much attention has been focussed on methods of removing these cells.

Although foetal tissues generally express much lower levels of MHC antigens than adult organs, rat foetal pancreas allografts are rapidly rejected and immunomodulation of this tissue has been particularly difficult to achieve.

Experiments described in this thesis confirm the low levels of MHC antigen expression in the freshly isolated rat

foetal pancreas. However, removal from the foetal environment resulted in an increased antigen expression in foetal pancreas maintained in tissue culture and stimulation with interferon-gamma (IFN-gamma) demonstrated the ability of the foetal pancreas to synthesise and express MHC antigens.

DA foetal pancreas, which is rapidly rejected when transplanted to the renal subcapsular site of PVG recipients, responded to allografting by demonstrating a marked increase in MHC class I antigen expression on all cell types. This increased class I expression included exocrine cells which had been shown to remain negative in isografts. In allografts, MHC class II antigens were expressed de novo on duct epithelium and exocrine cells whilst endocrine cells remained resistant to the induction of class II expression. Isograft class II expression was unchanged.

Class II expression on exocrine cells and duct epithelium (which are absent in purified islet preparations) would be of importance if these cells were then able to function as antigen presenting cells and this could provide one possible explanation for the comparative resistance of foetal pancreas to immunomodulation.

The function of foetal pancreas transplants can be affected by factors other than graft rejection and serial immunohistological examination offers an alternative method of graft assessment. Using this technique, Cyclosporin A treatment was found to prolong the survival of DA allografts in PVG and Lewis recipients, although subsequent discontinuation of

Cyclosporin A was followed by rapid rejection. Cyclosporin A was also found to have a profound effect on the antigen expression of foetal pancreas allografts with the pattern of expression coming to resemble that of the isograft. In addition, both the magnitude and phenotype of the cellular infiltrate was also affected.

Induction of antigen expression on the cells of rejecting allografts is probably a consequence of the local release of lymphokines and may precede any evidence of cellular damage by several days. Supportive evidence for a local release of lymphokine was provided by the observed increase in antigen expression on the allograft recipient's renal tubular cells adjacent to the transplanted foetal pancreas. This induced expression on renal tubular cells occurred only when rejection was present and although some evidence of a very limited graft-versus-host reaction was seen in parent strain to F1 transplant experiments, renal tubular-antigen induction appeared to be a consequence of the local release of lymphokine rather than as a result of graft-versus-host-mediated damage to the recipient's renal tubules.

Evidence that IFN-gamma represented a possible candidate as a lymphokine of particular importance in the induction of antigen expression was provided by observing a pattern of changes in the pancreas glands of adult rats given systemic IFN-gamma which was similar to that seen in allografted foetal pancreas. However, as induction of class II antigen expression was not seen on exocrine cells in IFN-gamma treated adult

pancreas, de novo expression of class II antigens by exocrine cells in allografts must depend on the presence of either higher levels of IFN-gamma or some additional factor.

Systemic IFN-gamma administration was followed by an increase in the number of class II positive interstitial cells within the adult pancreas. One possible explanation for this increase is the conversion of class II negative interstitial cells to class II positive cells in situ. If this were the case, treatment of unstimulated isolated islets with class II specific antibodies and complement may not remove all potentially class II positive cells, rendering immunomodulation incomplete.

Although class II positive cells within a graft have a particularly important role in antigen presentation, recipient class II positive cells can provide an alternative route for antigen presentation. The relative importance of these two pathways varies with the tissue, strain and species being considered. As the depletion of donor-strain class II positive interstitial cells from DA foetal pancreas by interim-hosting did not prevent subsequent rejection by PVG recipients, antigen presentation by recipient cells may be important in this particular instance. Antigen presentation by other cells within the foetal pancreas is another less likely possibility.

Direct labelling of class II positive cells within isolated islets, using specific monoclonal antibodies, provided a method for investigating the mechanisms of action of various in vitro manipulations designed to lower immunogenicity. Using

this technique prolonged tissue culture was shown to produce a progressive decrease in the numbers of class II positive cells while irradiation with 900J/m^2 UVB did not.

Immunohistological observations of foetal pancreas and isolated islets therefore allowed the demonstration of changes in antigen expression under a variety of circumstances and provided an alternative to functional assessment as a means of evaluating foetal pancreas graft survival.

1. INTRODUCTION AND HISTORICAL REVIEW

Clinical pancreatic transplantation, particularly foetal pancreas and isolated islet transplantation is still a long way from becoming established as a safe and satisfactory treatment for Diabetes Mellitus. However, the ineffectiveness of standard insulin therapy in preventing the long-term complications associated with this disease has provided a spur to research initiatives around the world whose common aim is the development of a more effective form of replacement therapy for pancreatic beta cell dysfunction.

Much of the experimental work has focussed on methods of transplanting a source of insulin production capable of establishing near-physiological glucose homeostasis. Of additional interest to transplant immunologists is the possiblity that the immunogenicity of pancreatic tissue might be modified in some way that would allow subsequent allografting or even xenografting without the requirement for the levels of recipient immunosuppression associated with other types of organ transplantation. Before further examining potential mechanisms by which these goals may be attained it is first of all necessary to consider the condition that pancreatic islet and foetal pancreas transplantation would seek to treat.

DIABETES MELLITUS

Historical Aspects of Diabetes Mellitus

Diabetes mellitus has a long and fascinating history. Although early accounts of the production of 'overabundant

urine' were contained in the Ancient Egyptian writing dating from 1550BC, discovered in Luxor 1862 (Ebbell 1937), the first clear description of what we would now refer to as Type 1 Diabetes Mellitus (National Diabetes Data Group 1979) has been attributed to the physician Aretaeus who practiced in the mountainous regions of Asia Minor near the River Euphrates at the end of the first century AD. In addition to providing a vivid account of the 'melting down of the flesh and limbs' into urine, Aretaeus postulated that the derivation of the word diabetes was from the Greek word for a siphon (Wrenshall, Hetenyl and Feasby 1962).

However, Aretaeus and his near contemporary Galen were of the opinion that the disorder was one of the kidneys (Henschen 1969) and such was their authority that this remained the prevailing opinion for the next 1500 years. The attention of European medical scholars was only turned away from the kidneys in the latter half of the 17th century by the description by Thomas Willis, Physician to Charles II, of the sweet taste of the urine of diabetic sufferers and his belief that 'an affection of the blood rather than of the kidneys' was to blame (Willis 1679). Willis, however, thought the sweetness due to a combination of salts and acids (Poulsen 1982, Drury, 1985) and it was another hundred years before Mathew Dobson demonstrated that the sweetness of the urine was due to the presence of fermentable sugar (Dobson 1776, Wrenshall et al 1962, Poulsen 1982). Dobson also described the serum of a diabetic patient as being 'opaque, resembling cheese whey and being sweetish to the

taste' and concluded that the excess sugar primarily existed in the blood and was not formed in the kidney (Drury 1985).

Dobson's discoveries generated immense interest among the erudite scholars of the day and prompted Claude Bernard to carry out experiments on the storage of sugar within the body. However, instead of finding an internal 'assimilator' organ as he had expected, Bernard discovered that the liver stored or manufactured sugar and then released this into the blood stream. He concluded in 1848 that the fundamental defect in diabetes was faulty nerve control of the liver resulting in the production of an excess of carbohydrate (Poulsen 1982, Drury, 1985). This view did not remain unchallenged and Pavy of Guy's Hospital, London, who had studied with Bernard, took the opposite view, regarding diabetes as a disorder of underutilisation (Pavy 1878).

In 1889, the central role of the pancreas in diabetes mellitus was firmly established following the experiments and observations of Oscar Minkowski (Von Mering and Minkowski 1890). While investigating the role of the pancreas in the utilisation of fats, the observation by a laboratory servant of polyuria in a pancreatectomised dog prompted Minkowski to test for and find sugar in the urine. When the pancreas was removed from three more dogs, all became diabetic (Houssay 1952).

Confirmation that the diabetes was due to a lack of some sort of internal secretion rather than absence of pancreatic enzymes in the gut was provided by Hedon in 1893 (Bliss 1983) (and also confirmed by Minkowski (Minkowski 1893) by leaving

part of the still-vascularised pancreas in a subcutaneous position after removing the rest of the organ and finding that the animal only became diabetic when this remnant was subsequently excised.

The search for the internal secretion that was missing in diabetes was now on and the observation by Laguesse in 1893 (Laguesse 1893) of the granular content of the islands of cells described by Paul Langerhans twenty four years earlier (Langerhans 1869), made these Islets of Lagerhans the likeliest candidate for the source of the 'internal secretion'.

Further evidence that Langerhans' islets played a central role in carbohydrate metabolism was provided by Szobolev who demonstrated the atrophy of the exocrine pancreas with preservation of islet tissue and the absence of diabetes following ligation of the pancreatic duct (Szobolev 1902). At around the same time E.L. Opie of John Hopkins Hospital described histological changes in the islet cells of patients dying of diabetes (Opie 1900).

The next 20 years saw feverish activity directed towards the extraction of the all-important internal secretion from the pancreas. Banting and Best working with MacLeod and Collip in Toronto are usually credited with the first successful attainment of this objective (Banting & Best 1922), although several other workers could be considered to have a prior claim (Zuelzer 1908, Scott 1912, Kleiner 1919, Paulesco 1921).

Work proceeded apace in Toronto and Banting and Best quickly moved from experimenting with pancreactectomised dogs to

treating seriously ill diabetic patients (Bliss 1983). Interestingly, some of Banting's dogs received extract derived from the pancreas of foetal calves obtained at the local abbatoir and although this extract successfully lowered the dogs' blood glucose, such was the demand for insulin for the treatment of patients that Banting turned his attention to the more readily available adult beef pancreas (Bliss 1983). This idea of using foetal pancreas was probably based on the observation made by Laguesse in 1907, that in the pancreas of new-born and foetal animals the islet cells were at a more advanced stage of development than the related acinar tissue (Cheymol 1972), an observation which is central to the use of foetal pancreas in experimental and clinical transplantation.

Complications of Diabetes Mellitus

The discovery and subsequent rapid clinical application of insulin had a dramatic effect on the treatment of diabetic patients. Prior to the 1920's, a diagnosis of diabetes in a young patient amounted to a virtual death sentence with even the most strenuous dietary manipulations only postponing the inevitable end by two or three years (Bliss 1983).

For the first 10 years following insulin's discovery, the treatment of ketoacidosis and infection remained the major problems and although an increased prevalence of arterial disease was noted in early reports, high levels of cholesterol in the diet were thought to be responsible (Joslin 1928). Descriptions of diabetic retinopathy and neuropathy had appeared

in the pre-insulin literature (reviewed by McMillan 1975) but it was only when diabetics had been treated with insulin for a number of years that the extent of the problem became apparent (Root et al 1939, Clawson and Bell 1949). During the 1940's many reports of the increased frequency of hypertension, arterial, eye and kidney disease (Reviewed by Larsson, Lichtenstein, Ploman 1952) brought about a gradual realisation that many problems remained to be solved in the management of diabetes. Despite the increasing sophistication of insulin therapy over the years, large vessel and small vessel disease remain major causes of mortality and morbidity in patients with diabetes mellitus. The average life expectancy of Type 1 diabetics diagnosed before the age of 30 is only 29 years beyond the date of diagnosis, with only half of such patients reaching 50 years of age (Alberti and Hockaday 1983). Whereas, large vessel disease (macroangiopathy) accounts for nearly three-quarters of all deaths from diabetes in Western countries (West 1978) microangiopathic lesions are extremely important causes of disability and much attention in recent years has been focussed on possible ways of reducing the severity of these complications.

Diabetic nephropathy is a particularly important problem, affecting 40-50 percent of diabetics at some stage (White 1956, Anderson et al 1983, Moloney et al 1983) and accounting for nearly one quarter of all new dialysis patients (Rao et al 1980). Those patients who develop nephropathy account for almost all the increase in mortality associated with diabetes

while those fortunate enough to escape renal damage have a similar mortality to the rest of the population (Christiansen et al 1985). The risk of developing nephropathy, although at first increasing with the duration of disease, reaches a peak at 20-25 years with those surviving 35 years of diabetes having a very low risk of subsequently developing nephropathy (Anderson et al 1983). If nephropathy was related only to the degree of control of the diabetic state, a linear increase in the incidence of renal complications in relation to time would be expected. The peaking and subsequent decline in incidence rates suggests that a subgroup of patients exists which is more at risk of developing renal disease and this may relate to either genetic or environmental influences (Moloney et al 1983, Krolewski et al 1985). If this subgroup could be identified any treatment with potential in preventing this complication could be instituted at an early stage.

Proteinuria may be such a marker but the onset of clinical albuminuria, represented by a positive Albustix test represents a relatively advanced stage in the progression of renal damage (Mogensen 1986). Quantitative measurements of lesser degrees of albuminuria (microalbuminuria) can detect less advanced renal damage but once present the risk of subsequent clinical nephropathy is 80-100% (Mogensen 1986).

Commonly accompanying nephropathy, retinopathy is found in 60-80 per cent of long standing diabetics (Knowles et al 1965, Waldhausl et al 1985), with diabetic eye disease being the main cause of blindness in the UK between the ages of 20 and 65

(Alberti and Hockaday 1983). Retinopathy, in addition to having serious implications for the quality of life of diabetics presents the researcher with a complication assessable by fairly non-invasive techniques which can be repeated to monitor the effect that a change in treatment may be having on microangiopathic lesions. In addition, there is a close association between diabetic nephropathy and retinopathy and the finding of microalbuminuria has strong predictive powers with respect to proliferative retinopathy (Mogensen 1986).

Another complication of diabetes, peripheral neuropathy, has also been extensively investigated and an incidence rate in reported studies which varies from 20-35 per cent probably reflects differences in diagnostic criteria and techniques of assessment (White 1956, Knowles et al 1965, Waldhausl et al 1985). The measurement of motor nerve conduction velocities in the ulnar, peroneal and tibial nerves has provided another area where progression or improvement of complications can be studied non-invasively (Dahl-Jorgensen et al 1986, Winegard 1986).

The complications of diabetes mellitus continue to have a profound effect on the lives of those afflicted with the disorder and present researchers with a continuing challenge to find and develop a more effective means of treating diabetes than conventional insulin therapy currently provides.

Relationship of Diabetic Complications to Glycaemic Control

The relationship or otherwise of microvascular, macrovascular and neurological complications to the level of

control of blood glucose has been one of the most contentious areas of debate in modern medicine. A full account of the argument for and against the importance of blood glucose control is beyond the scope of this introduction and instead a brief review of the major areas of evidence will be presented.

Retrospective and epidemiological studies

This type of study has sought to identify subgroups of patients in whom control of diabetes was either 'good' or poor' and compare the incidence of complications, or alternatively compare the tightness of control in a group of patients with complications with a group without complications. Allocation to 'good' or 'poor' subgroups has therefore been retrospective and based on fairly imprecise indicators such as the frequency of severe hyperglycaemia at clinic visits (Krowlewski et al 1985) or the actual number of out-patient attendances (Deckert, Poulsen and Larsen 1979). Nevertheless, in reviews by Tchobroutsky (1978) and Schiffrin (1986) the balance of evidence from retrospective studies has been found to strongly favour an association between poor control and increased incidence and severity of complications.

One of the largest studies, involving four thousand Belgian diabetics (Pirart 1977) found a strong correlation between poor control and severity of nephropathy, retinopathy and neuropathy. Krowlewski et al (1985) also found an association between nephropathy and more severe hyperglycaemia although the existence of other influencing factors has been previously noted. Further evidence for an association between

complications and poor control has been provided by many similar studies (Wilson, Root and Marble 1951, Hardin et al 1956, Jarret and Keen 1976, Howard Williams et al 1984) and the particular importance of good control in the first few years of treatment has been emphasised (Caird, Pirie and Ramsell 1969).

However, retrospective studies are by their nature non-randomised and most have been carried out at a time when appropriate methods of monitoring metabolic control and achieving normoglycaemic were not available. It is also quite possible that the association of 'poor control' with more severe complications may merely identify a subgroup of patients with more severe diabetes and conclusions regarding the effect of restoring normoglycaemia on the incidence and progression of complications can not be drawn.

Animal experiments

Studies in animals with chemically-induced diabetes have provided further evidence that microvascular complications may be related to glycaemic control.

In alloxan-diabetic dogs treated with insulin and studied over five years a group with stricter control of blood glucose levels developed lesser degress of retinopathy and nephropathy than an equivalent group whose diabetic control was deliberately poor (Engerman, Bloodworth and Nelson 1977) although once retinal changes had developed their progression proved difficult to halt (Engerman and Kern 1987).

Near-normalisation of carbohydrate metabolism in previously diabetic rats is possible following pancreas

transplantation (Orloff et al 1986) and studies have been carried out on the effects of restoration of normoglycaemia on microvascular complications.

The progression of an early manifestation of renal damage, mesangial thickening, has been halted and at times reversed following both islet (Mauer et al 1974, 1975) and pancreatico-duodenal transplantation (Weil et al 1975) in diabetic rats and arteriolar thickening has also been prevented by islet transplantation (Gray and Watkins 1976).

The effect of restoration of a normal glycaemic environment on the progression of glomerular basement membrane thickening does not appear to be so clear cut. When islet transplantation was carried out in rats after seven months of diabetes, the glomerular basement membrane changes failed to reverse (Steffes et al 1979) although previous work had demonstrated a normalisation of urinary albumin excretion when transplantation was carried out at this time (Mauer et al 1978). Similar experiments in mice demonstrated that glomerular basement membrane changes were only reversed if mice were transplanted early (three weeks) after diabetes was induced rather than late (seven months) (Cuthbertson and Mandel 1987).

Excessive endothelial cell replication has been shown to represent an early manifestation of retinal disease in diabetic mice and can be reversed by early transplantation of islets (Naeser and Anderson 1983) although this particular change was not reversible in mice transplanted late. Cuthbertson and Mandel (1987) demonstrated that the response of the capillary

basement membrane in the eye of diabetic mice was different from that of the glomerular basement membrane with late transplant being just as effective as early transplantation.

That changes in microvasculature of animals do not correspond exactly to the changes in humans has been emphasised from the earliest studies (Engerman et al 1977) but the evidence from animal studies at least suggests that microangiopathic changes may be preventable or reversible if treatment is begun at an early enough stage.

Prospective clinical studies using exogenous insulin

Better control of blood glucose with near normalisation of levels over prolonged periods became possible with the introduction of more frequent self-monitoring of blood glucose and more physiological delivery systems such as continuous subcutaneous insulin infusions (CSII) (Pickup et al 1978). It was only with the development of such systems that meaningful prospective randomised controlled trials could be carried out, prior to this all that was available for study was varying degrees of less than ideal control.

With regard to diabetic retinopathy, no definite conclusions can be made on the basis of the evidence available at present. In trials comparing CSII with conventionally treated controls transient worsening of retinopathy has been a consistent and worrying feature (Steno Study Group 1982, Kroc Collaborative Study Group 1984, Dahl-Jorgensen et al 1985) with the appearance of cotton wool exudates in the first few months of treatment, a feature normally indicative of focal retinal

ischaemia (Wybar 1974). However, later review of the same groups of patients showed that any transient worsening had resolved with some overall improvement in retinopathy in the intensively treated group in two of the studies (Kroc 1985, Dahl-Jorgensen et al 1986). In the third study the improvement at two years in the CSII treated group was described as only marginal at most and this was thought to have been due to the advanced nature of the background retinopathy at entry to the trial (Lauritzen et al 1985). In a fourth recently reported study comparing conventional insulin therapy with CSII, after three years follow-up there was similar progression of retinopathy in both groups. However, the interpretation of this result is complicated by the fact that metabolic control improved in the conventionally treated group as well as the CSII treated group (Olsen et al 1987). Other smaller studies in which background retinopathy was advanced at the time of entry to the trial have also failed to show any improvement following intensive insulin treatment (Van Ballegooie et al 1984, Bell et al 1985, Verrilo et al 1986).

The timing of the introduction of intensive insulin therapy also appears to be of the utmost importance when diabetic nephropathy is being considered. By the time renal damage has progressed to the stage of failing glomerular function and Albustix-positive proteinuria, the process culminating in end-stage renal failure has probably become self-perpetuating and cannot be influenced by the degree of metabolic control (Viberti et al 1983). If techniques of near-

normalisation of glucose metabolism such as CSII and pancreas transplantation are to influence the course of diabetic nephropathy it is essential that diabetic patients at risk should be treated early. Microalbuminuria (detectable by quantitative methods) at rest occurs at an early stage in the progression of renal changes but its presence usefully predicts eventual renal damage (Mogensen 1986) and an increase in urinary albumin in response to exercise may be an even earlier indicator (Viberti et al 1981) although this has been disputed (Feldt-Rasmussen, Baker and Deckert 1985). Another feature of early renal changes in diabetic patients is glomerular hyperfiltration and this may be important in the pathogenesis of diabetic nephropathy (Christenson 1986).

Intensive insulin therapy has been shown to reverse some of these early changes with abnormally elevated glomerular filtration rates being reduced by a period of CSII treatment (Beck-Nielsen et al 1985, Christensen et al 1986, Dahl-Jorgensen et al 1986). A reduction in urinary albumin excretion following CSII would seem to depend on how advanced the renal damage was at the outset of treatment with patients in the Kroc Study (1984 and 1985) who showed a reduction of albuminuria with CSII tending to have lower initial levels of albumin excretion than those in the Steno study who failed to show the same improvement (Steno 1986). However, even if albumin excretion rates did not improve in the CSII patients in this latter study, the functional albumin clearance increased in the conventionally treated group while remaining basically unchanged with CSII

treatment (Feldt-Rasmussen, Mathiesen, Deckert 1986).

Two years of CSII has also been shown to improve peripheral nerve function as measured by motor nerve conduction velocity even if the motor nerve conduction velocity was within the normal range at the start of the study (Pietri, Ehle and Raskin 1980, Service et al 1985, Dahl-Jorgensen et al 1986). Autonomic nerve function has also been shown to improve following a similar period of CSII (Lauritzen et al 1985) and sensory nerve function measured by vibration sensory threshold improved when a two year regimen of more intensive management was instituted (Holman et al 1983).

Therefore, there is a definite suggestion from studies employing systems of insulin delivery resulting in near normoglycaemia that some of the very early changes of diabetic microangiopathy and neuropathy may be favourably influenced. However such is the complexity of normal insulin secretion and the multiplicity of factors influencing it (Waldhausl 1986) that no system of exogenous insulin administration could be hoped to mimic this completely and only pancreatic transplantation in some form could be hoped to provide a near-physiological replacement.

Clinical pancreas transplantation and diabetic complications.

At present there are insufficient long-term survivors with functioning pancreas grafts to make any clear statements regarding the effect of pancreas transplantation on diabetic complications. Until now transplants have been carried out in patients with advanced complications most of whom have also

required simultaneous or prior kidney transplants for end-stage diabetic nephropathy (Sutherland & Moudry 1987a). As a result although successful pancreas transplantation has been shown to result in the disappearance of the need for insulin and the normalisation or near-normalisation of metabolic abnormalities (Pozza et al 1985), the evidence that pancreas transplantation can influence established complications of diabetes is sparse (Sutherland and Moudry 1987b).

The only direct evidence for a beneficial effect of pancreas transplantation on the secondary lesions of diabetes comes from studies of glomerular basement membrane thickening in kidneys transplanted to diabetic recipients. Although an uncommon cause of graft failure in the first 10 years following transplantation there is a gradual progression in glomerular basement membrane and mesangial thickening (Mauer et al 1983, 1986). In a small group of patients studied by Bohman et al (1985), five of six diabetic patients receiving a kidney graft alone had electron microscopic evidence of glomerular basement membrane thickening within two to three years whereas both patients with simultaneous pancreas and renal transplants seemed to be protected from these changes. Similar findings have recently been reported by the Minneapolis group for a group of nine patients with longstanding pancreas grafts (Bilous et al 1988). Indirectly supporting these findings is evidence that restoration of a normoglycaemic environment may even reverse nephropathic changes demonstrated by regression of fairly advanced glomerulosclerosis in a kidney transplanted from a

diabetic donor to a non-diabetic recipient (Abouna et al 1983) although this rather surprising finding requires confirmation.

Therefore although by piecing together evidence from diverse sources a pattern emerges suggesting that glycaemic control is indeed important, the case for pancreas transplantation or any other form of treatment having a beneficial effect on the secondary complications of diabetes must be regarded as being 'non-proven'. If such treatments are going to have a significant effect it is likely that their early application will be essential.

CURRENT STATUS OF CLINICAL PANCREAS TRANSPLANTATION

Whole Organ and Segmental Pancreas Grafts

Over the last 22 years, pancreas transplantation has evolved from an essentially experimental procedure with a low success rate to an operation with results now beginning to approach that of other solid organ transplants (Sutherland and Moudry 1987).

Since the first human pancreas transplant was carried out in 1966 (Kelly et al 1966), more than 1,000 pancreatic transplants have been carried out world wide (Sutherland and Moudry 1987). Frequent complications related to leakage of exocrine secretions and the apparent poor performance of azathioprine and steroids as immunosuppressants were major factors in the early disappointing experience and this led some workers to discontinue pancreas transplant programmes and

temporarily turn their attention instead towards clinical islet transplantation (Sutherland et al 1980).

Interest in pancreas transplantation was rekindled in the 1980's by two important developments. Firstly, the use of a technique of duct obliteration with the synthetic polymer Neoprene avoided the necessity of incorporating a drainage procedure to deal with the exocrine secretions and this made the operation both simpler and safer (Dubernard et al 1978). The second innovation was the introduction of the potent immunosuppressive Cyclosporin A. This agent particularly when used in combination with azathioprine and prednisolone resulted in significantly improved graft survival with an associated improvement in mortality (Sutherland & Moudry 1987).

With the introduction of Cyclosporin A, prednisolone dosages have been reduced and this has allowed a reassessment of various techniques of dealing with the exocrine secretions (Sutherland et al 1982). Concern that in the long-term, the fibrosis associated with duct injection (Blanc-Brunat et al 1983) will lead to impaired endocrine function, has resulted in a number of centres using alternative methods of duct drainage. The most common techniques in use today are drainage into the small intestine using a Roux en Y and drainage into the bladder, with both techniques having a similar success rate to that of duct injection (approximately 40% one year graft survival) (Sutherland & Moudry 1987). A more recent development allows drainage of the venous outflow from the pancreas into the portal circulation with the exocrine secretions from the paratopically

placed pancreas draining into the stomach. This more physiological situation may confer benefits in the long-term (Calne 1984).

Following improvements in immunosuppressive regimens and in techniques of dealing with the exocrine secretions, it has become apparent that many early failures were due to vascular thrombosis due to the inherent low blood flow through the graft (Calne et al 1980, Groth et al 1982). The construction of an arterio-venous fistula between the splenic vessels distal to the pancreas has been shown to improve blood flow through the graft and may reduce the incidence of thrombosis (DuToit et al 1981, Duffy & Calne 1984) although antiplatelet or anticoagulant agents may still be required (Duffy & Calne 1984).

Most patients transplanted with a pancreas have had either simultaneous or previous kidney grafts. Although patient survival is higher in patients transplanted prior to end-stage diabetic nephropathy, pancreas graft survival is significantly poorer in this group (Sutherland & Moudry 1987). Part of the reason for this difference is related to the increased difficulty of diagnosing rejection when a kidney from the same donor is not present to act as a 'barometer.' Urinary amylase estimation, when the bladder is used for exocrine drainage (Sollinger et al 1985) and the observation of an accumulation of 111-Indium labelled platelets during rejection offer additional means of diagnosing rejection (Jurewicz et al 1985) and if a drainage catheter is used to temporary exteriorize the pancreatic juice when pancreatico-enterostomy techniques are

used, cytological examination of the juice may provide early evidence of rejection (Brattstrom et al 1980).

Although the advances described above have allowed pancreas transplantation to become a safer, more effective operation, results will have to be considerably better before there is more widespread application of this procedure as a treatment for diabetes mellitus. With whole organ and segmental pancreas transplantation, it is likely that life-long immunosuppression will be required and transplantation is only logical if the complications of immunosuppression do not prove to be greater than the diabetic complications the transplant hopes to prevent.

The possibility of avoiding or at least reducing the amount of immunosuppression required by in vitro immunomodulation of the tissue prior to transplantation is one of the most attractive prospects being currently considered. The prospect of achieving this aim seems more likely to be attainable for isolated islets and foetal pancreas than vascularised organ grafts.

Isolated Islet and Foetal Pancreas Transplantation

Whereas whole organ or segmental pancreas transplantation, has been, until recently, an operation with worrying levels of morbidity, clinical islet transplantation has been described as being a 'safe but ineffective procedure' (Largiader 1977).

Islet Transplantation

Encouraging results of experiments in rodents using islets prepared from adult, neonatal and foetal sources raised hopes of similar success in clinical islet and foetal pancreas transplantation (Ballinger and Lacy 1972, Leonard, Lazarow and Hegre 1973, Kemp et al 1973, Brown et al 1974, Matas et al 1976). However, isolation and separation of islets from the more fibrous human pancreas proved to be more difficult and early clinical experiments involved unpurified, collagenase-dispersed pancreatic fragments injected into the portal vein (Sutherland et al 1980). Although encouraging results from previous autograft operations in patients with near-total pancreactectomy seemed to validate the techniques used, none of seven diabetic renal allograft recipients receiving dispersed pancreatic tissue allografts in a similar way became insulin free although small rises in urinary C-peptide levels and a temporary decrease in insulin requirements were observed in some patients (Sutherland et al 1980). Other investigators too had a similarly disappointing experience and of 74 islet allotransplant procedures identified by the Human Pancreas and Islet Registry up to 1980, in only one case was there convincing evidence of successful transplantation from a non-fetal source (Sutherland 1981). Largiader et al (1980) had transplanted pancreatic fragments via the portal vein in four patients and into the spleen in three. Of the seven recipients, one patient who had received both kidneys and intrasplenically injected pancreatic fragments from a two and a half year old donor

eventually became insulin independent. Full doses of insulin were required for the first six months following transplantation but thereafter requirements for exogenous insulin gradually decreased and finally at eight months the patient became insulin free. However, some 10 months later, this patient rejected both kidneys and at the same time became hyperglycaemic, requiring insulin before dying one further month later (Sutherland 1982). Although no intrasplenic islet tissue was identified at autopsy this would be in keeping with rejection of the pancreatic tissue one month prior to death. More recently Alejandro et al (1987) documented prolonged survival of islets in four patients who were receiving immunosuppression for a previous or simultaneous renal transplant. Increased plasma C-peptide levels were found in all patients but although some reduction in insulin requirements was possible no patient became insulin free.

It has been suggested that the failure of early clinical islet transplantation has been due to insufficient numbers of islets transplanted to possibly inappropriate sites with an increased susceptibility to both rejection and the unfavourable environment created by corticosteroid administration (Sutherland et al 1980).

More recent experiments in larger mammals have shown that techniques developed for islet separation have the potential to produce sufficient tissue to show a clinical effect (Gray et al 1986). That similar techniques of separation have been successfully applied to the human pancreas suggest that isolation of sufficient numbers of human islets should be

possible (Gray et al 1984). Problems of rejection however have still to be overcome and this is complicated by the fact that the most potent immunosuppressive agent, Cyclosporin A, may be toxic to the pancreatic beta cell (Andersson et al 1984, Gunnarson et al 1984, Hahn et al 1986).

Foetal pancreas transplantation

Human foetal pancreas has attracted interest as an alternative source of endocrine tissue for transplantation, partly because of the initial problems of isolating sufficient numbers of islets from the adult pancreas. A capacity for growth and differentiation into a pure endocrine organ, and the possibly lower immunogenicity of foetal pancreas were additional attractive features to early transplanters (Valente et al 1980). Viable foetal pancreas can be kept in culture for extended periods (Farkas and Joo 1984, Jones and Turtle 1985) and this allows selective survival and proliferation of islet tissue and atrophy of associated exocrine elements (Mandel et al 1982). In addition to offering opportunities for in vitro modification of the graft immunogenicity (discussed later) long-term culture offers a suitable means of storing islet tissue until sufficient material is available for transplantation with cryopreservation of tissue being a suitable alternative (Chastan et al 1980, Brown et al 1980, Sandler et al 1982). The capacity for growth and differentiation of foetal human pancreas has been demonstrated histologically and functionally following transplantation to the nude mouse (Maitland, Parry and Turtle 1980, Tuch, Ng and Turtle 1985, Sandler et al 1985, Hullet et al

1987) and illustrates the potential advantage of transplanting immature tissue, even if a period of several months of residence in the diabetic recipient is required before the beta cells are able to respond satisfactorily to a glucose challenge (Tuch et al 1985).

The most encouraging reports of human foetal pancreas transplantation were both published in 1980. Valente et al transplanted human foetal pancreases to intramuscular sites in 10 patients. In a further two patients, tissue from four foetal pancreases was injected via a percutaneous catheter into the portal vein after a preliminary four day period of tissue culture. One of the patients with intramuscular placement of foetal pancreas was able to achieve 'adequate glycaemic homeostasis' after five months and both patients who received intraportal injections were able to reduce their insulin dose for a short time.

Chastan et al (1980) transplanted pancreas tissue from foetuses of nine weeks gestation following a period of cryopreservation with subsequent culture for five days. In one patient, following transplantation of fragments representing 21 pancreases to the pectoral muscle, insulin was discontinued after 62 days and the patient remained insulin-free for 12 months although his response to glucose stimulation and stress was abnormal.

In a further study by Tuch et al (1986), four patients received foetal pancreas of 14-20 weeks gestation which had been cultured for 6-21 days. Although no patients showed any

metabolic evidence of functioning islet tissue, one patient who was already receiving cyclosporine and prednisolone as immunosuppression for a previous renal transplant developed a mass at a site where pancreas fragments had been injected. Thirteen months after the original transplant this mass was biopsied and found to contain a heavy infiltrate of small lymphocytes but with some residual pancreatic duct structures and endocrine cells present. Although very small quantities of insulin could be extracted from one area of the tissue, immunoperoxidase staining of histological sections showed alpha and delta cells only with absence of the insulin-containing beta cells. However one further patient was later reported to have small numbers of beta cells as well as alpha and delta cells surviving 12 months after transplantation (Tuch et al 1987a).

No other studies have reported complete success with patients becoming insulin-independent for a significant period of time following foetal pancreas transplantation although Groth et al (1980) and Farkas et al (1987) showed some elevation of C-peptide levels suggestive of increased insulin production while patients of Hu et al (1985) showed a marked reduction in insulin requirements following transplantation with cultured foetal pancreas, with one dose of anti-human thymocyte globulin being given to supplement the traditional Chinese herbal medicine used for immunosuppression. However, Usadel et al (1980) reported no change in C-peptide levels or insulin requirements in one patient with foetal pancreas transplanted to an intramuscular site.

This limited experience with human foetal pancreas transplantation raises several interesting points. Firstly, in the two patients described above who became insulin-independent, a delay of two months and five months occurred before insulin could be discontinued suggesting that either foetal pancreas must undergo a mandatory period of maturation before it can respond adequately to a glucose stimulus or that in these cases insufficient pancreatic tissue was transplanted (or the site was inappropriate) and function was delayed until the tissue had grown. Secondly, neither of these two patients were receiving immunosuppression and if foetal pancreas had indeed survived in these patients this raises the intriguing possibility that in some way the immunogenicity of the pancreas at the time of transplantation was insufficient to provoke a complete rejection response. It is interesting to note in this context that transplantation had been preceeded in both cases by a relatively short period of tissue culture with cryopreservation also being used in one of the cases (Chastan et al 1980). Finally, the finding of surviving duct structures and alpha and delta cells in the apparent absence of beta cells in a lymphocyte-infiltrated mass (Tuch et al 1986) raises fears that a recurrence of 'auto-immune' activity may be an additional problem affecting the transplantation of islet tissue.

The evidence therefore for the effectiveness of both islet and foetal transplants is at present very sparse and only when further experience has been accrued, employing recent developments based on animal work, will the potential benefit of

these forms of transplantation be assessable. Questions related to rejection and especially to recurrence of auto-immune damage can only be fully answered from more extensive clinical trials.

Ethical considerations in foetal tissue transplantation

Although problems of donor supply affect vascularised pancreas and isolated islet transplants in a similar way to other organs, the transplantation of foetal tissue has its own particular ethical difficulties (Sutherland et al 1981, Gray and Morris 1987). The recent use of foetal substantia nigra transplants in patients with Parkinson's disease (Pearce 1988) has brought the question of transplantation of foteal tissue into the area of public debate. The ethics surrounding this have been eloquently summarised in a leading article in the British Medical Journal (Gillon 1988) and it would appear that much of the opposition to the use of foetal tissue stems from those who have moral objections to abortion under any circumstances. It is possible, however, for even those people who feel that abortion is morally wrong to be able to accept that transplanting the tissues from already aborted foetuses could be morally acceptable. Careful safeguards will be required to ensure that guidelines already approved are understood by the public and carefully adhered to by the medical profession (Supplementary Annual Report of the General Medical Council 1988).

Technical, immunological and ethical problems all remain to be resolved before transplantation of pancreatic beta cells can be expected to make any real impact on the devastating toll

of morbidity and mortality currently inflicted on diabetic patients. Vascularised organ grafts appear to be the most successful source of beta cells at present but problems of supply, the need for a major operation and life-long immunosuppression, make it unlikely to be the best possible solution in the long-term. Short of preventing diabetes in the first instance or 'curing' it before the damage is irreversible, the transplantation of isolated islets or foetal pancreas would seem to offer the best hope for the future. The likelihood that transplantation of this tissue would involve a fairly minor procedure makes it more suitable for the patients who are likely to benefit the most. The possible additional advantage of being able to reduce or avoid immunosuppression by modifying the immunogenicity of the graft is an exciting prospect which will now be discussed in detail.

IMMUNOGENICITY OF FOETAL PANCREAS AND ISOLATED PANCREATIC ISLETS IN RODENTS

Inherent Immunogenicity

Following the encouraging results of transplantation of ovary, parathyroid and thyroid allografts (Linder 1961, Russel & Gittes 1959, Perloff et al 1978) it was hoped that pancreatic islets would also enjoy the apparent advantage of the lower immunogenicity possessed by endocrine tissue. Disappointingly, however, islets transplanted across strong histocompatibility barriers were rapidly rejected (Reckard and Barker 1973, Finch

and Morris 1976) and compared poorly with other organs in terms of survival when transplanted in rats (Nash, Peters & Bell 1977).

When it was shown that transplanted syngeneic foetal pancreas could successfully reverse diabetes in rats (Brown et al 1974) it was hoped that the added advantage of immaturity would result in a lower capacity to evoke an immune response as suggested by earlier experience with skin grafts (Billingham & Silvers 1964, Wachtel & Silvers 1972, Heslop, Carter and Hornibrook 1973). However, foetal pancreas was also found to be rapidly rejected (Spence, Perloff and Barker 1979, Garvey, Morris & Millard 1979, Mullen & Shintaku 1980) and in fact appeared, to be more immunogenic than isolated islets (Simeonovic, Agostino and Lafferty 1984), perhaps as a result of associated lymphoid tissue transplanted with the foetal pancreas. That foetal tissue per se was not infact more immunogenic was established by the demonstration that islet precursors (proislets) isolated from foetal pancreas would survive when transplanted to a non-immunosuppressed recipient across a major histocompatibility barrier thus implying that some other component of the foetal pancreas was the more important immunogenic stimulus (Simeonovic & Lafferty 1982). In a later series of experiments Simeonovic & Lafferty (1987) showed that foetal pancreas was universally rejected in all strain combinations examined but proislet survival was possible in some experimental groups and this seemed to be related more to the specific strains used than the degree of

histocompatibility.

Proislets have a similar immunogenicity to adult islets (Prowse et al 1983) and more recent evidence suggests that the apparent susceptibility to rejection of islets in early experiments may have been at least partly due to contaminating structures in relatively unpurified islet preparations. Purified islets, carefully separated from lymph nodes, ductal and vascular structures and exocrine cells are much less susceptible to rejection in mice than unpurified preparations and of the contaminating structures, lymphoid tissue appears to be the most immunogenic (Gotoh et al 1986). When endothelial cells and exocrine structures are added to preparations of purified islets there is no effect on immunogenicity but rejection is accelerated by the addition of donor-strain splenocytes (Gores et al 1986).

Purity of preparation and the amount of associated lymphoid tissue present, however, would not appear to account for the described increased immunogenicity of islolated islets compared with vascularised pancreatic grafts (Perloff et al 1980, Sutherland et al 1980, Reckard et al 1981), as vascularised pancreatic grafts, by their nature would contain a greater number of associated lymphoid cells. The rapidity of islet rejection has prompted speculation that the mechanisms involved differ from those affecting vascularised organs and antibody-mediated and macrophage-mediated rejection have been suggested as possibilities (Naji, Barker & Silvers 1979, Schwitzer, Leiter and Evans 1984, Nash and Bell 1979). However,

an early infiltration of lymphocytes is seen on histological examination of intraportal islet allografts and cell-mediated immunity would appear to be the most important mechanism involved (Charles et al 1984). Why the kinetics of rejection should be more rapid in isolated islets remains uncertain and may relate in some way to the manner in which the islets are exposed to the recipient's immune system. The site of transplantation has a modifying effect on rejection. Thus islet xenografts and allografts transplanted to the renal subcapsular site survive for longer than islets injected intraportally (Lacy et al 1982, Yasunami et al 1983, Sullivan et al 1987, Reece-Smith et al 1981a) although the relative degrees of immunoprivilege of these two sites seems to depend on the degree of histocompatibility with the intraportal site having immunological advantages for transplants between congenic mouse strains (Gores et al 1987) in addition to having the well recognised metabolic advantage of insulin-delivery to the portal system (Reece-Smith, McShane & Morris 1982).

Immunomodulation by tissue culture

Pancreatic tissue, does not therefore appear to enjoy any consistent immunological advantage by virtue of its endocrine nature. Isolated islets and foetal pancreas however have the ability to survive in appropriate systems of tissue culture and thus manipulations designed to reduce immunogenicity can be carried out in vitro.

In 1957 Snell suggested that 'donor lymphocytes carried over into the graft' were of prime importance in initiating rejection and that removal of such 'passenger leukocytes' would be a more readily attainable objective than reducing the antigenicity of each individual component of the graft. Most of the investigators endeavouring to reduce the immunogenicity of pancreatic tissue have accepted as their paradigm the hypothesis that transplantation antigens, as isolated molecules, are themselves weak immunogens and donor strain leukocytes are the main determinants of immunogenicity (Lafferty et al 1983). According to this hypothesis, two separate signals are required for effective triggering of alloreactive T-lymphocytes. One signal is provided by the binding of antigen to the T cell receptor but a second signal from a metabolically active stimulating cell is required. In the case of the passenger leukocyte, both signals can be provided by the same cell (Lafferty and Simeonovic 1984).

The potential importance of passenger leukocytes in the context of endocrine transplantation was first shown by Lafferty et al (1975) who demonstrated prolongation of mouse thyroid allografts by a 12 day period of culture in a high oxygen environment, conditions thought to be unfavourable to leukocyte survival (Anderson, Hellung-Larsen and Sorensen 1968) and later showed that allografts were rejected if donor strain leukocytes were injected (Talmage et al 1976). Early reports of prolongation of survival of rat islet allografts following relatively brief periods of culture (Kedinger et al 1977) led to

an explosion of interest in immunomodulation of islets and foetal pancreas in vitro as a means of avoiding recipient immunosuppression.

Culture in high oxygen concentrations

A seven day period of culture at high oxygen tension has allowed successful transplantation of islets between MHC-disparate mouse strains, although aggregation of the islets into clusters was necessary prior to culture in order to avoid oxygen toxicity to the endocrine cells (Bowen, Andrus and Lafferty 1980). The beneficial effect of culture at high oxygen tension for seven days was found to extend to rat to mouse islet xenografts which showed prolonged survival despite the accumulation of lymphoid aggregates around the transplanted islets (Lacy, Davie and Finke 1980). Similar results were found for rat islet allografts (Tucker et al 1983). However this apparent immunomodulating effect of high concentrations of oxygen is not seen in all strain combinations examined. Bradley et al (1986) only achieved a 50% survival rate following the transplantation of BALBc mouse islets to CBA/J recipients. When transplantation between outbred mice was used as a model for the genetic variability within the human population, culture of islets for 7-10 days at high oxygen concentrations resulted in only a 45% acceptance rate even when donor pretreatment with cyclophosphamide was used. A twelve day course of recipient immunosuppression using cyclosporine was required to achieve 100% acceptance (Simeonovic, Prowse and Lafferty 1986).

Foetal pancreas has proved to be much more difficult to modify in high oxygen tensions than isolated islets. Periods of culture as long as 17-23 days were required before mouse allografts would be accepted (Simeonovic et al 1980, Collier and Mandel 1983, Georgiou and Mandel 1987) and even then, in only a proportion of allografts was survival prolonged. The increased difficulty experienced in conditioning foetal pancreas tissue has been thought to be due to the lymphoid aggregates intimately associated with the foetal organ (Simeonovic et al 1980) and the finding of prolonged survival in only a proportion of an experimental group may relate to individual differences in the amounts of lymphoid tissue transplanted with the pancreas.

Xenografts of mouse foetal pancreas cultured in high oxygen concentrations have also been carried out, but were only successful when recipient rats were immunosuppressed with steroids (Thompson, Bowen and Burton 1987).

In addition to foetal pancreas being more difficult to condition than isolated islets, rat foetal pancreas would appear to be more difficult to modify than mouse grafts. Fourteen days of culture in 95% oxygen was found to be insufficient to allow survival of rat allografts in a study carried out by Bowen et al (Bowen, Thompson and Burton 1987). Various techniques of recipient immunosuppression were added to supplement the in vitro immunomodulation and weekly doses of methylprednisolone found to be the only effective regimen. However, although the dose of cyclosporin used was adequate this was discontinued after four days and a longer course of this agent may have

produced different results. Unfortunately it was difficult to know to what extent the in vitro culture conditions contributed to the survival of pancreas in rats receiving methyl prednisolone as no steroid-treated animals received uncultured grafts.

Culture at low temperature

Other techniques of tissue-culture have been used which avoid the potential toxic effects of high oxygen concentrations. Following the discovery that lymphocytes maintained at 22°C for four days lose their ability to stimulate a mixed lymphocyte culture (Opelz and Terasaki 1974), rat islets were allografted by the portal venous route after a seven day culture period at a similar temperature (24°C). Prolongation of survival was seen although a single dose of anti-lymphocyte serum was also administered (Lacy, Davie & Finke 1979). Rat islets cultured in a similar way were also able to survive when transplanted to mouse recipients and the central importance of passenger leukocyte inactivation or elimination was suggested by the observed rapid rejection following the injection of donor splenocytes (Lacy, Davie & Finke 1981). The immunomodulatory effect of low temperature culture has also allowed prolonged survival in rat recipients of both MHC-incompatible rat islets and even monkey islets without the need for ALS administration, (Tze & Tai 1983, Ricordi et al 1988) although transplantation to an immunoprivileged site was necessary for some strain combinations studied (Ricordi et al 1988).

Culture for prolonged periods

The detrimental effects of high oxygen concentrations and low temperature culture on the endocrine cells of mouse foetal pancreas are evident when marginal volumes of tissue are transplanted (Mandel and Koulmanda 1985). Other less toxic systems have been investigated including culture at more conventional temperatures in an atmosphere of 5% carbon dioxide and air. However, culture under these conditions for 10 days was found to be ineffective in prolonging islet survival between outbred mouse strains (Anderson and Buschard 1977) despite evidence that even seven days of culture in air at 37^{o}C was sufficient to reduce the passenger leukocyte component of islets (Rabinovitch et al 1982, Serie et al 1983, Gebel et al 1983). Extending the period of culture to 21 days allowed prolongation of survival of mouse islets between strains with only minor antigen differences (Georgiou & Mandel 1987) and culture for even longer periods was also found to have beneficial effects regarding the immunogenicity of allografts but led to a gradual loss of islet viability (Andersson 1982). Again the additional lymphoid tissue associated with foetal tissue seemed to militate against an immunomodulatory effect of culture, with 21 days of culture in air at 37^{o}C appearing to be ineffective for rat foetal pancreas allografts (Garvey et al 1980a).

Purity of preparation, particularly with regard to contamination by lymphoid elements, does indeed appear to have an important bearing on the effectiveness or otherwise of in vitro treatments. When highly purified rat islets are

transplanted across a major histocompatibility barrier, culture in air at 37°C for only three days allows prolonged survival (Selawry et al 1984). Culture-isolated neonatal rat islets also survived as allografts following 10 days in vitro under conventional conditions (Serie and Hegre 1985) with results comparable to other techniques of isolation similarly designed to reduce lymphoid contamination (Yasunami et al 1983).

Culture in nutritionally poor medium

Another technique of immunomodulation by tissue culture which may be applicable to islet transplantation has been reported recently. A very short period of culture (16-20 hours) in a nutritionally poor medium and a low oxygen environment has been shown to prolong mouse thyroid allografts transplanted across both major and minor histocompatibility barriers. Again, the important effect of this culture system seems to be an inactivation or elimination of passenger leukocytes with immunogenicity capable of being restored by an injection of fresh donor peritoneal exudate cells (Moreland and Mullbacher 1987).

Treatment with antibody directed against passenger leukocytes

The indirect nature of much of the evidence suggesting that the important effect of culture is passenger leukocyte elimination does not rule out the possible role of other potential benefits of culture (such as the decreased expression of histocompatibility antigen on endocrine cells themselves as suggested by Woehrle et al (1986)). More direct evidence that removal of passenger leukocytes alone will suffice has been

provided by showing allograft survival following treatment of islets with complement and antibody directed against Ia determinants in mice (Faustman et al 1981). Ia antigen is present in large amounts on passenger leukocytes but generally thought to be absent or present in only very small amounts on endocrine cells and by using immunofluorescent techniques, two morphologically distinct populations of Ia positive cells have been identified in mouse islet tissue (round cells and dendritic cells). When only the dendritic cells were eliminated, using a specific anti-dendritic cell antibody and complement, long-term survival was seen in 89% of allografts (Faustman et al 1984). However, this still allows for the possibility that other non-dendritic, Ia positive cells may also play a part in antigen presentation and that the anti-dendritic cell antibody merely reduced the total number of Ia positive cells to a level which was no longer immunogenic. These findings therefore seemed to prove that the removal of passenger leukocytes alone would allow allograft survival.

However, the results of other experiments suggest that the overall picture is more complex. When strong responder rat strains such as Lewis were used as recipients, anti-Ia antibody and complement treatment of islets prior to transplantation was not sufficient to allow consistent prolongation of allograft survival and a 7 day period of culture at 24^{o}C in addition to recipient immunosuppression was required before 100% graft survival was achieved (Terasaka et al 1986). If the only effect of culture at low temperatures is the elimination of passenger

leukocytes then either the antibody and complement failed to penetrate to all of the Ia positive cells in the graft or some of the antigen presenting cells within the islets were Ia negative (at least at the time of antibody treatment). Presentation of antigen by host cells may have been involved and subsequently inhibited by immunosuppression of the recipient but this would not explain the synergistic effect of the period of low temperature culture.

Other techniques used to reduce islet immunogenicity

Ultraviolet irradiation

Islet immunogenicity has also been reduced by treating isolated islets in culture with ultraviolet (UV) irradiation. Following the observation that donor-specific unresponsiveness to subsequent islet transplantation could be induced by the transfusion of UVB-irradiated donor-strain blood (Lau, Reemtsma and Hardy 1983) it was found that in certin rat strain-combinations, direct UV irradiation of the isolated islets alone would allow their indefinite survival (Lau, Reemtsma and Hardy 1984a). With islet allografts to 'high responder' rat strains, however, a short course of recipient immunosuppression was required in addition to UV treatment before indefinite survival could be obtained (Lau, Reemtsma and Hardy 1984b). Ultraviolet-irradiation also prolonged the survival of islets transplanted across a species barrier (monkey to mouse) when recipients were additionally treated with anti-lymphocyte serum but indefinite survival was not achieved (Chabot et al 1987).

The effects of UV irradiation also seem to be directed towards the Ia positive passenger leukocyte component of the graft. For obvious reasons, research into the effects of ultraviolet wavelengths on cell populations has been focussed mainly on the skin. Among epidermal cells, a population of bone-marrow derived, Ia antigen positive dendritic cell is found. These so-called Langerhans' cells have properties similar to dendritic cells found elsewhere and play an important part in antigen presentation (Wolff and Stingl 1983). Exposure of skin to UVB irradiation alters its antigen presenting ability (Bergstresser and Streilein 1983). Although actual numbers of Langerhans' cells do not appear to decline (Aberer et al 1981), except when very high doses are used (Odling, Halliday and Muller 1987), there is functional impairment of antigen presentation with loss of surface markers possibly including Ia expression (Aberer et al 1981, Krueger and Emam 1984) although this has been disputed (Lau et al 1983, Czernielewski et al 1984).

The effect on Ia expression seems at least in part to depend on when this is measured in relation to exposure to UV treatment. Peripheral blood mononuclear cells have normal levels of Ia expression immediately after irradiation but this declines to low levels between 4 and 8 hours later (Gruner et al 1986). This finding may explain the unmodified rejection experienced by rat islets if transplantation is undertaken without allowing a 24 hour period of 'resting' following UV irradiation (Hardy et al 1984). However, Czernielewski et al (1984) found no diminution in the numbers of Ia positive cells

in an irradiated epidermal cell suspension regardless of the interval before measurement and the most important effect of UV irradiation is probably the functional impairment of antigen presentation whether or not Ia antigen levels are altered.

Cryopreservation

An alternative to tissue culture as a means of storage of islets prior to transplantation is cryopreservation (Warnock et al 1987). Recent reports suggest that this method too may reduce immunogenicity as shown by prolongation of rat islets in mouse recipients although some recipient immunosuppression was still required (Coulombe, Warnock and Rajotte 1987).

Mechanical barriers

Even if the intrinsic immunogenicity of islet tissue is left unaltered, rejection might be prevented by avoiding any contact between the transplanted tissue and the recipient's immune mechanisms. Early attempts to construct mechanical barriers or chambers which would still allow diffusion of nutrients and insulin were rather disappointing and have been assessed by Theodorou and Howell (1979). The development of new materials has led to a reawakening of interest in this concept and alginate-polylysine encapsulated rat islets have survived for prolonged periods in mice (O'Shea and Sun 1986). Grafts eventually failed due to gradual death of islet cells from a lack of nutrients as these capsules became overgrown but retransplantation from the same strain of donor again led to prolonged reversal of diabetes (O'Shea and Sun 1986) ruling out any possibility that immune mechanisms were involved in graft failure.

REDUCING THE HOST RESPONSE TO FOETAL PANCREAS AND ISOLATED ISLETS

It is possible that through a greater understanding of the mechanisms of rejection and further developments in the immunomodulation of isolated islets and foetal pancreas, allotransplantation or even xenotransplantation could be achieved without the need for recipient immunosuppression. However, from the available evidence, a more realistic hope would be that by reducing the immunogenicity of the graft, the level of immunosuppression necessary to induce unresponsiveness to donor tissue could be minimised, although some form of recipient treatment would probably still be required. Techniques that have been used to modify the recipient's immune response in rodent experiments have included both specific and non-specific measures.

Specific immunosuppressive techniques

Induction of tolerance

In general, it is difficult to induce donor-specific unresponsiveness in adult animals. However, the administration of donor-strain bone marrow or liver extracts as a source of antigen, coupled with various regimens of anti-lymphocyte serum administration prolonged the survival of islet and foetal pancreas allografts in certain mouse and rat strains although permanent acceptance of grafts was not achieved (Panijayanond and Monaco 1974, Vialettes et al 1978, Mullen 1980&1981).

Similar results were obtained when donor-strain red blood cells treated with an anti-Ia antibody and complement were given to recipients prior to subsequent islet transplantation with long-term acceptance of mouse allografts without the need for any further recipient treatment. The elimination of Ia positive cells from donor blood used to induce tolerance was a critical step as untreated red blood cells failed to induce a tolerant state (Faustman et al 1982).

More complicated systems have also been used to induce donor-specific tolerance to subsequent islet allografts. In rats with long-surviving renal allografts (following a 14 day course of cyclosporine) a state of tolerance exists which will allow transplantation of isolated islets from the same strain of donor (Gray et al 1984). If a similar state of tolerance was found to exist in the clinical situation, islets from the same donor, stored by cryopreservation, could later be transplanted to the diabetic recipient of a renal transplant.

The method used to induce tolerance appears to be important in determing the future of transplants from the same donor strain, as when tolerance to heart allografts had been obtained by passive enhancement, subsequent islet grafts were rejected in an unmodified manner (Nash, Peters and Bell 1978, Perloff et al 1981).

Tolerance to a particular donor strain has also been achieved by inducing a chimeric state using total body irradiation and bone marrow transplantation. Prolonged survival of adult islets and foetal pancreas in rat allotransplants

(Britt et al 1982, Mullen and Shibukawa 1982) and more recently neonatal pancreas in mice (Iwai et al 1987) has been obtained by this method. It would appear that when the potential risks of irradiation and graft versus host disease were balanced against the likely benefit of the graft, the clinical applications of this technique would be limited. However it has been suggested that following irradiation of the recipient, foetal pancreas and haemotopoeitc stem cells from the liver of the same donor could be transplanted (Iwai et al 1987).

Passive enhancement

Enhancement protocols using donor-specific antibody which have been effective for other organ transplants have had only a limited degree of success in rodent islet transplantation. Results for islet transplantation have been very dependent on the degree of histocompatibility (Finch and Morris 1976, Nash Peters & Bell 1977, Morris et al 1980) and passive enhancement of foetal pancreas appears to be ineffective (Garvey, Millard & Morris 1980, Mullen 1980).

As foetal pancreas and isolated islets are transplanted without an immediate blood supply it is perhaps not surprising that anti-donor antibodies do not effectively block antigen recognition by the host.

Non-specific immunosuppression

One of the earliest approaches to the immunosuppression of experimental islet transplantation was to target therapy against host lymphocytes. Anti-lymphocyte serum (ALS) was used to

attempt to modify the host response to islet allografts. Again the effectiveness of this agent was very dependent on the degree of histocompatibility between donor and recipient with a short course of ALS significantly extending the survival of DA islets in MHC-compatible ACI recipients but proving ineffective when strongly histo-incompatible strains were used (Reckard and Barker 1973). If ALS was continued indefinitely, permanent acceptance of islet allografts transplanted across a minor histocompatibility barrier was possible (Gray & Watkins, 1974).

Treatment with ALS was also found to be the most effective of a number of immunosuppressive regimens in preventing foetal pancreas rejection, allowing indefinite survival of F1 foetal pancreas in DA recipients (Garvey, Millard and Morris 1980b).

An alternative method of modifying the host lymphocyte response has recently been described (Hahn et al 1987). By using an antibody directed against the Interleukin 2 receptor (IL-2R), the clonal expansion of activated T lymphocytes is inhibited either by merely blocking the receptor or by eliminating IL-2R positive cells. Moderate prolongation of rat islets across a major histocompatibility barrier has been achieved using this antibody in association with a subtherapeutic dose of Cyclosporin A.

Following speculation that macrophages were especially important in isolated islet rejection, immunosuppressive therapy has also been targeted against this cell-type (Nash & Bell, 1979). Using silica which is selectively toxic to macrophage populations, prolonged survival of rat islets from F1 donors has

been achieved in parent-strain recipients (Nash et al 1980).

Other more non-specific agents that have been assessed in experimental islet transplantation have included cyclophosphamide, procarbazine, azathioprine and prednisolone but at best only slight or moderate prolongation of survival has been obtained in weak strain combinations (Morris et al 1980, Mullen 1980, Marquet & Heysteck 1975, Bell et al 1980).

Cyclosporin A

Cyclosporin A has proved to be a potent immunosuppressive agent both in clinical and experimental organ transplantation. A dose of 10mg/kg/day given for seven or 14 days is sufficient to completely suppress rejection of DA kidneys by the strong-responder Lewis strain (Homan et al 1980). However, in this same strain combination modification of the host response to transplanted islets has proved to be much more difficult, emphasising the apparent increased susceptibility to rejection of this tissue. Even with doses of Cyclosporin A as high as 40mg/kg/day only a very slight prolongation of intraportally or intrasplenically transplanted DA islet survival was seen in Lewis recipients (Morris et al 1980).

The results of islet transplantation in other strain combinations carried out at about this time emphasised the importance of the degree of histocompatibility. Rynasiewicz et al (1980) found that a dose of 20mg/kg given on a continuous daily basis allowed survival of islets transplanted across a minor barrier, but not a major barrier, for as long as the Cyclosporin A was continued. However, many of the rats given

this dose of Cyclosporin A died and at autopsy areas of consolidation and abscess formation were found in their lungs suggesting either increased susceptibility to infection or microaspiration of the olive oil used as a vehicle for oral Cyclosporin A administration.

Interestingly, at variance with the results for isolated islets, prolonged survival of vascularised segmental pancreas grafts was possible across the same major histocompatibility barrier using a similar or slightly lower dose of Cyclosporin A (Rynasiewicz et al 1980). Similarly the prolongation of survival of vascularised pancreas grafts in the DA to Lewis combination was seen to be significantly better than that seen in isolated islet transplants (Morris et al 1980).

The site of transplantation has also been shown to influence the effectiveness of Cyclosporin A treatment. Again using the DA to Lewis combination, the Oxford group showed moderately prolonged survival of islets if the renal subcapsular rather than intraportal site was used and no rejection was seen when the dose of Cyclosporin A was increased to 40mg/kg/day (Reece-Smith et al 1981b). Unfortunately at this dose level early deaths were frequent and thought to be due to infection.

Since the report by Garvey et al (1980b) it has generally been accepted that only marginal prolongation of foetal pancreas allografts by Cyclosporin A is possible. In this study, in which Cyclosporin was administered orally in doses of 10, 20 or 40mg/kg/day for 14 days, foetal pancreas rejection was diagnosed on the basis of hyperglycaemia. However, it has been reported

elsewhere that even in recipients of foetal pancreas isografts, restoration of normoglycaemia could take as long as four weeks (Morris et al, 1980), and diagnosis of rejection was further complicated by the administration of insulin for 8 days after foetal pancreas transplantation. Interestingly, histological examination of supposedly failed foetal pancreas allografts at 14 days frequently demonstrated well-developed insulin-containing islets despite a slight to moderate mixed cellular infiltrate.

Although, the evidence from these earlier studies of Cyclosporin A in rat islet transplantation suggested that islets were more difficult to prolong than vascularised pancreas a recent report has questioned this. Dibelius et al (1986), using the DA to Lewis combination, found that on a dosage regimen of 30mg/kg given on days 0, 1 and 2 only, mean survival time of islets was 90 days compared with only 37 days for vascularised pancreas. Possible explanations for this seemingly contradictory study are firstly that isolation of islets was simply by meticulous hand-picking of neutral red stained islets and this was said to result in lower levels of contamination by lymphoid tissue. Also by not using Ficoll, the possibility of the immunogenicity of islets being increased by exposure of antigens in some way by this agent, was avoided. Lastly, although the amount of Cyclosporin A administered was very small, by giving it by intramuscular injection, a depot effect was achieved with Cyclosporin A blood levels of 90ng/ml recorded 70 days after transplantation (Dibelius et al 1986).

The importance of purity of preparation has similarly been reported by Terasaka et al (1986) who showed that hand-picked WF islets in Cyclosporin A-treated Lewis recipients survived for much longer than islets isolated by Ficoll gradient separation.

Cyclosporin A has also been shown to have some effect in prolonging the survival of islets in hamster to rat xenografts and between outbred mice although high doses of the drug and the use of immunoprivileged sites have been necessary (Nakajima et al, 1985, Selawry, Whittington and Fajaco 1986, Simeonovic, Prowse and Lafferty 1986). Survival has also been shown in outbred dog allografts when Cyclosporin A has been maintained for long periods with the dose adjusted to give fairly high trough levels (Alejandro et al 1985, Kneteman, Alderson and Scharp 1987). When Cyclosporin A was eventually stopped after several months most of the successful grafts were retained (Alejandro et al 1987).

Before Cyclosporin A immunosuppression is applied to clinical islet transplantation it would be desirable to find ways of lowering the dose or limiting the duration of administration of this agent. Alejandro et al (1987) were able to obtain long-term survival of dog islets with much lower doses of Cyclosporin A when islets were pretreated with anti-Ia monoclonal antibody and improved survival of rat islets transplanted to a strong-responder strain was seen when anti-Ia antibody treatment or culture at low temperature was added to a 3 day course of Cyclosporin A (Terasaka et al 1986).

It would therefore appear that Cyclosporin A may possibly become a useful agent in clinical islet and perhaps even foetal pancreas transplantation particularly when coupled with strategies of immunomodulation of donor tissue. However, toxic effects of Cyclosporin A may prove to be the eventual stumbling block.

Cyclosporin A toxicity

Problems related to Cyclosporin A nephrotoxicity and hepatotoxicity have been recognised for several years (Calne et al 1981, Klintmalm, Iwatsuki and Starzl 1981, Myers, Ross and Newton 1984). A more recently observed interference with glucose metabolism has particular relevance to the transplantation of isolated islets and foetal pancreas.

A high dose of Cyclosporin A (50mg/kg/day) given to rats by daily gavage for as little as seven days has been shown to induce fasting hyperglycaemia and hypoinsulinaemia with evidence of severe degranulation of native pancreatic beta cells (Helmchen et al 1984). A lesser degree of glucose intolerance has been seen using doses as low as 10 or 15mg/kg/day and this was associated with a significant decrease in insulin content and beta cell volume within the native pancreas (Yale et al 1985, Hahn et al 1986b). These acute toxic effects appear to be reversible as glucose metabolism recovered soon after Cyclosporin A was discontinued with return to normal responses within a few weeks (Hahn et al 1986a, Eun et al 1987b).

Further evidence of a toxic effect of Cyclosporin A on pancreatic beta cell function has come from in vitro studies

using therapeutically-relevant concentrations of the drug in the culture medium surrounding isolated islets (Nielsen, Mandrup-Poulsen and Nerup 1986, Robertson 1986) although cultured human foetal pancreas was not affected (Tuch et al 1987b).

Transplanted isolated islet and vascularised pancreas grafts, both in experimental models and clinical transplantation, have also been shown to be at risk of Cyclosporin A toxicity (Basadonna, Kakizaki and Merrel 1986, van Schilfgaarde et al 1986, Otsu et al 1987, Gunnarsson et al 1984) and the effects appear to be correlated with mean trough levels of the drug (van Schilfgaarde et al 1987).

Interestingly, isolated islets (in autografts) were more sensitive to Cyclosporin A, if exposed to the drug in the first few days after transplantation (Basadonna et al 1986) and this may be related to the reported detrimental influence that Cyclosporin A has on the vascular ingrowth of transplanted pancreatic islets (Rooth et al 1989). This vascular effect may also be relevant to the study by Reece-Smith et al (1981c) which described islets first transplanted beneath the renal capsule of a syngeneic host prior to subsequent transplant of the kidney bearing the graft to a Cyclosporin A-treated allogeneic recipent. This strategy allowed prolonged survival of allogeneic islets which was not seen in the same strain combination when islets were allografted directly. Similarly, the improved results of transplanting vascularised pancreas compared with isolated islets in Cyclosporin A-treated recipients could also be explained by this effect on vascularisation (see above).

The fact that Cyclosporin A can have adverse effects on glucose metabolism should be borne in mind when assessment of the results of pancreas islet and foetal pancreas transplantation is based on biochemical evidence alone. Although Cyclosporin A is reportedly non-toxic to foetal beta cells (Tuch et al 1987b) (perhaps because the immature beta cells are unable to take up the drug), the in vivo effects on function and proliferative capacity are unknown. The addition of histological assessment in the monitoring of experimental islet and foetal transplants will help to differentiate immunological rejection from possible toxic effects of immunosuppressive therapy.

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) ANTIGENS IN RAT PANCREAS AND ITS RELEVANCE TO TRANSPLANTATION

Major histocompatibility complex (MHC) antigens play an important role in the regulation of the immune response. Antigens such as viral or tumour proteins are recognised only in association with host MHC molecules by a process known as MHC restriction. In contrast, T lymphocytes can react directly with allogeneic class I or class II MHC antigens in transplanted tissue without the need for presentation in association with host MHC antigens, explaining why MHC incompatibilities form a major barrier to successful transplantation of organs and tissues.

Class I MHC molecules serve as targets for cytotoxic T cell responses and have a widespread tissue distribution with only a few cell types appearing to have no, or very low levels of, expression (Daar et al 1984). Class II antigens are much more restricted and are expressed primarily by cells involved in immune reactions with very high levels detectable on dendritic cells and more variable amounts present on cells of the monocyte/macrophage lineage. Induction of antigen-specific T helper cell activity requires the recognition of antigen in association with class II surface molecules (Weinberger et al 1980).

Levels of class I and class II MHC antigen on various cell types are known to vary from species to species (eg human but not rat vascular endothelium cells normally express class II molecules [Fabre et al 1987]) and also within a species according to age, with lower levels present during neonatal and foetal life perhaps being important for the development of tolerance to self antigens (Unanue et al 1984).

The major class II positive component of grafts such as foetal pancreas and islets is the dendritic cell and much of the work on immunomodulation of these tissues has concentrated on removing this immunogenic antigen presenting cell prior to transplantation. However, neither class I or class II expression is static and levels of expression are subject to both positive and negative regulation by a variety of influences. Induction of class II antigens on non-bone marrow derived cell types is now well-recognised but the functional

consequences of this phenomenon in terms of antigen presentation is as yet unknown.

The presence or absence of class I and class II MHC antigens and the amount and location of these molecules on the various components of transplanted organs is likely to be important in determining the fate of the graft and in influencing the effector mechanisms of the host immune response.

MHC Disparities and Rejection

The functional importance of MHC gene loci in the control of immune responses has been emphasised in previous reviews (Bach, Bach and Sondel 1976, Halloran, Wadgymar and Autenried 1986). Control of strong transplantation antigens, susceptibility to disease, immune cell alterations and levels of serum complement components were all said to be influenced by MHC loci. It was also known at that time that different subpopulations of T-lymphocytes responded to different MHC antigens, with cytotoxic T cells (Tc) reacting to what would now be referred to as class I molecules and T helper cells (T_h) being stimulated by class II antigens (Bach et al 1976).

In vitro studies have shown that antigen receptors on T_h cells may be triggered either by autologous (self) MHC class II determinants modified by foreign antigen (including allogeneic class I MHC antigens) or by foreign class II determinants alone or in combination with nominal antigen (Sredni and Schwartz 1981). Activation of allo-specific T_h cells is generally recognised as being an important stage in the induction phase of

the rejection response and the ability of allogeneic MHC class II positive cells in the transplanted tissue to directly stimulate these cells emphasises the particularly important contribution they make to the immunogenicity of the graft. Even if the MHC class II antigens expressed on the cells of a graft are compatible with recipient class II these cells can still function as antigen presenting cells (APC) for donor class I differences as shown by rejection of mouse islets (class I incompatible) which contained passenger cells which were class II positive even although this was compatible with recipient class II antigen (Morrow et al 1983). In fact it has been demonstrated that class II disparity alone rarely led to rejection of islets as class I differences appeared to be necesssary to act as targets for rejection on the class II negative beta cells (Morrow et al 1983).

As discussed previously, removing class II positive antigen presenting cells from donor tissue prior to transplantation has an important effect on reducing graft immunogenicity. However, graft acceptance has not always been obtained following this manoeuvre. Using congenic mice where donor and recipient strains were disparate for class I but not for class II, Gores et al (1986) found that depleting islets of class II positive cells prior to transplantation to the renal subcapsular site did not prolong islet allograft survival. This finding was similar to that of previous studies which had shown that culture of thyroid and parathyroid grafts only resulted in prolonged survival if donor and recipient rats were MHC-

incompatible (Bartlett et al 1983, Naji, Silvers and Barker 1981), and that culture at 24°C and anti-lymphocyte serum prolonged the survival of only MHC incompatible Lewis islets and not MHC compatible Wistar Furth islets in BB recipients (Woehrle et al 1986).

The failure of culture to prolong certain MHC compatible endocrine grafts has been suggested to be due to the ability of host cells to process and present minor donor-strain antigens in an immunogenic form (Silvers et al 1984). However, in a series of experiments on foetal mouse pancreas, culture under high oxygen tension led to prolonged allograft survival in some strain combinations examined including four which were MHC compatible and had only minor antigen differences (Georgiou & Mandel 1986). Similarly La Rosa and Talmage (1985) have shown that when minor antigen differences only were present in cultured mouse thyroid allografts, rejection did not always occur. It has been suggested that some minor antigens are more potent inducers of an immune response than others and that whether rejection occurs or not will depend on the precise strain combinations used (Zhu et al 1985, La Rosa and Talmage 1985).

Taken together the above studies show that although removing class II positive passenger cells may allow prolonged survival in certain situations, antigen presentation by host cells can at times be sufficiently effective to result in rejection. The effect of removing donor strain class II positive cells from grafts is likely to be very dependent on the

strain combination used and this reflects both the varying potency of minor antigens and the particular strength of immune response characteristic of the recipient strain used. Regarding the transplantion of human islets or foetal pancreas the effectiveness or otherwise of strategies designed to remove donor class II positive cells will depend on the, as yet unknown relevance of this indirect method of antigen presentation to the clinical situation.

Antigen presentation by MHC Class II positive cells

It is now firmly established that MHC class II positive cells with dendritic morphology are of central importance in antigen presentation and their presence in allografts as passenger leukocytes represents a potent stimulus to the recipient's immune mechanisms. The minimal requirement for a cell to have antigen-presenting properties is the expression of class II antigen on its surface membrane, but whether this in itself is sufficient to enable any cell to present antigen is not so clear (Pober et al 1986).

Rejection of skin grafts following replacement of donor-strain passenger dendritic cells by those of recipient strain in radiation/bone marrow chimeras, suggested that dendritic cells were not the only cell types capable of presenting antigen and inducing rejection, (Schroeder et al 1975, Stuart, Beck-Maier and Melvold 1984). However, in this situation antigen presentation by recipient cells using the alternative pathway may play a part (Woodward, Shigekawa & Frelinger 1982).

More convincing evidence for antigen-presentation by non-bone marrow derived cells has come from in vitro studies. Endothelial cells in particular have come under scrutiny in this regard and early reports suggested a possible antigen presenting ability (Hirschberg et al 1975, Hirschberg, Bergh and Thorsby 1980, Burger et al 1981), although contamination by antigen presenting cells of bone marrow origin may not have been rigorously ruled out (Pober et al 1986). A wide variety of other non-bone marrow derived cells have been shown to be capable of presenting simple antigens in vitro under certain circumstances. Liver sinusoidal cells, isolated astrocytes and epithelial cells of intestinal origin (when fully differentiated and expressing class II antigens) have all been shown to be capable of presenting antigen (Rubinstein, Roska & Lipsky 1987, Fontana, Fierz & Wekerl 1984, Bland & Warren 1986). Incompletely differentiated cells from intestinal crypt epithelium although unable to present antigen under normal circumstances, will do so if they are induced to express class II antigens by interferon gamma (Bland & Warren 1986) as will fibroblasts treated in a similar way (Umetsu et al 1986). Thyroid epithelial cells from patients with Graves disease aberrantly express class II antigen and could consequently present certain peptide antigens but only if further catabolism or processing of antigen was not required (Londei et al 1984). The importance of Class II expression as a prerequisite for antigen presentation was further elegantly demonstrated by experiments in which murine L-cells developed antigen presenting

abilities following transfer of class II genes. (Malissen et al 1984). However, the ability of these various cells to present simple antigens involved mainly memory responses and is not necessarily relevant to the induction of a primary allogeneic response following transplantation.

Both dermal fibroblasts and endothelial cells have been shown to have the ability to provoke a primary allogeneic response from naive T cells in vitro although the response to fibroblasts was much less marked than to endothelial cells even if levels of class II antigen were increased by exposure to interferon gamma (Pober et al 1983). The response to smooth muscle cells was also less than the response to endothelial cells suggesting that some additional feature of endothelial cells other than class II MHC expression accounted for their enhanced immunogenicity (Pober et al 1986). In addition to the induction of a proliferative response, endothelial cells have been shown to be capable of generating a cell-mediated cytotoxic response from antigen-presenting cell-depleted lymphocytes cocultured with them. Also rat endothelial cells stimulated to express class II MHC antigen by exposure to interferon gamma in culture, are effective in recipient sensitisation resulting in more rapid rejection of heart allografts (Hayry et al 1986). These findings would suggest that of the other cell types present in grafted tissue, endothelial cells in particular may represent an additional source of immunogenicity. It is also likely that this immunogenicity will depend on expression of class II antigen by the endothelial cells and will therefore be

dependent on the animal model used and whether induction of class II expression has occurred. Although other cell types constitutively or potentially expressing class II antigen would seem likely to have a lesser ability to present antigen, a possible contribution to overall immunogenicity can not be completely ruled out based on the available evidence at present.

Factors influencing MHC expression

Levels of both class I and class II MHC antigen expression vary from cell type to cell type and from species to species and are subject to regulation by many different influences. Interferon gamma is the most potent inducer of both class I and class II antigens and interferons alpha and beta, enhance the expression of class I antigens but do not appear to be involved in the regulation of class II expression (Reviewed by Halloran, Wadgymar and Autenried 1986, Koene, de Waal and Bogman 1986). The effect of these and other mediators on MHC expression during immune responses will help in the MHC-restricted recognition of foreign antigens and will allow amplification of the response by means of a positive feedback loop (Janeway et al 1984). There is some evidence that even 'resting' levels of class I and class II MHC antigens depend on the presence of circulating soluble mediators in 'physiological amounts' and that culture in serum-free medium will lead to an eventual reduction in antigen expression (Beller & Ho 1982, Ball et al 1984). In addition, larger doses of Cyclosporin A have been shown to decrease MHC antigen expression on endothelial cells in the skin and kidneys

of normal dogs and in the kidneys of normal mice (Groenewegen et al 1985, Halloran et al 1986).

In vivo influences on class II MHC antigen expression have been studied more frequently than class I changes, probably because of the possible implications that increased class II expression may have for immunologically-induced disease. Also these changes are easier to demonstrate due to the very restricted basal levels of expression of class II antigens and the marked increase often seen following stimulation (Halloran et al 1986). Increased levels of expression are seen following exposure to either locally released or circulating lymphokines under a wide variety of circumstances. These include transplantation (discussed below), graft versus host disease (Mason, Dallman & Barclay 1981), in areas of normal epithelium adjacent to tumours especially when lymphocyte infiltrates are present (Rognum, Brandtzaeg & Thorud 1983), in regenerating liver mainly in Kupffer cells (Jonjic et al 1987) and in various disease states particularly when autoimmune in nature (Bottazzo et al 1983).

Although class II MHC antigen expression is increased in this way under a number of different circumstances these have in common an elevation of interferon gamma levels either locally or systemically.

Interferon gamma and class II expression

It is generally accepted that interferon gamma is the most important lymphokine capable of inducing class II MHC antigen expression, although there is recent evidence that other similar

but distinct lymphokines may have analogous effects (Groenewegen et al 1986).

When recombinant interferon gamma is given systemically to mice or rats there is a general increase in the levels of class I and class II MHC expression throughout the body with especially marked increases in cells which express low resting levels (Skoskiewicz et al 1985, Leszczynski et al 1986, Hayry et al 1986). An accompanying increase in the number of class II positive dendritic cells in organs such as the liver and pancreas could represent either conversion of previously class II negative cells to positive cells or an influx of dendritic cells from elsewhere (Skoskiewicz et al 1985, Hayry et al 1986). That both of these mechanisms play a part is suggested by the findings of an increase in the number of class II positive Langerhans cells in cultures of skin exposed to interferon gamma (Berman et al 1985) and also the observed decrease in dendritic cell numbers in lymph nodes and spleens of animals treated with this lymphokine (Skoskiewitcz et al 1985).

In vitro, interferon gamma also induces a marked increase in class II expression on a number of different cell types including endothelial cells and it has been shown that the immunogenic capacity of such cells is proportional to the extent of MHC expression (Ferry et al 1987). The effects of interferon gamma can be abolished both in vitro and in vivo by corticosteroids (Snyder & Unanue 1982, Leszczynski et al 1986, Hayry et al 1986), prostaglandins of the E series (Snyder, Beller & Unanue 1982) and alpha-fetoprotein (Lu, Changelian &

Unanue 1984). Cyclosporin A has also been found to modify levels of antigen expression following transplantation and this is discussed below.

At a molecular level, interferon gamma would appear to act at the level of DNA transcription (Collins et al 1984, Wong et al 1985) and possibly also on protein synthesis (Bishop et al 1986) and induction of class II expression could therefore be prevented by agents which act at these levels or which inhibit the production and release of interferon gamma.

Transplantation and MHC expression

Confirmation of the dynamic nature of MHC antigen expression and the inducibility of these antigens by immunological stimuli was first demonstrated in the transplant setting in mouse skin allografts (Dallman & Mason 1983, de Waal et al 1983). Since these early reports, changes in antigen expression in rejecting grafts in both experimental and clinical transplantation have been described for many other tissues including kidney (Hall et al 1984, Benson, Colvin & Russell 1985, Bishop et al 1985, Milton, Spencer & Fabre 1986, Fuggle et al 1986, Armstrong et al 1987) pancreas (Steiniger, Klempnauer, Wonigeit 1985), heart (Milton & Fabre 1985, Rose et al 1986, Forbes et al 1986) and liver (Engemann et al 1986, So et al 1987).

Transplantation not only increases the level of expression of MHC antigens in the tissues described above but de novo expression of both class I and class II antigens are seen on

some cell types normally negative for these antigens. In the rat, myocardial cells which express little or no class I antigen and no class II antigen, are reported to strongly express both these antigens within five days of allografting (Milton & Fabre 1985), and similarly pancreatic acinar cells which have levels of both these classes of MHC antigens normally undetectable by immunohistological techniques show positive labelling for class I and class II by day six following transplantation (Steiniger et al 1985). Other class I positive cells which do not normally express class II antigen in detectable amounts and are induced to express this following transplantation include duct epithelium in pancreas transplants and Kupffer cells in liver allografts (Steiniger et al 1985, Engemann et al 1986). The kinetics of this induced antigen expression have been shown to vary according to the particular organ studied and these differences may be relevant to the variation in rapidity of rejection observed in different transplanted organs (Milton et al 1986, Nash et al 1977).

The situation in clinical transplantation is obviously more difficult to interpret. In particular, there is debate as to whether changes in antigen expression can be correlated with rejection and possibly act as an early indicator of this process. In clinical renal transplantation, reports demonstrating an increase in HLA-DR (class II) expression on tubular cells several days before deterioration in renal function suggested that this may be of value in the early detection of rejection, although levels of this class of antigen

often remained elevated after rejection had been successfully controlled (Bishop et al 1985). In a more recent study, Fuggle et al (1986) described three different patterns of induction of class II antigen on renal tubules and found that fluctuating levels of expression in individual patients paralleled changes in the extent of cellular infiltration. However, although an association with rejection was found no absolute correlation was possible. Similarly the increased expression of class I MHC antigens on hepatocytes in human liver transplants was associated with rejection in patients taken as a group but no direct correlation could be obtained in individual patients (So et al 1987). With human cardiac allografts a correlation between class I induction and a first episode of rejection was demonstrated but in subsequent biopsies class I levels often remained elevated with no other evidence of rejection (Suitters et al 1987).

Although the association of increased MHC antigen expression with rejection may not be close enough to be of value in providing an early indicator of this process, this change in the levels of detectable surface antigen is likely to be of relevance in allograft rejection. Induction of class I (and possibly class II) antigens will increase the level of available targets for the effector phase of the immune response, while an increase in the level of class II expression (bearing in mind the possible role of class II positive bone-marrow derived cells in antigen presentation) will have effects on the afferent phase as well. Induction of MHC antigen could therefore be

anticipated to lead to an amplification of the rejection response by positive feedback mechanisms.

The influence of Cyclosporin A on MHC antigen expression

Cyclosporin A was originally shown to inhibit the release of interferon gamma by T lymphocytes (Reem, Cook and Vilcek 1983) and when added to mixed lymphocyte cultures (MLC) it reduced the ability of supernatants from these cultures to induce class II antigen expression on cultured endothelial cells (Groenewegen et al 1985). However, if the Cyclosporin A was added to the supernatant, no inhibitory effect was seen, suggesting that its action was on the production and release of lymphokine rather than directly on the endothelial cells (Groenewegen et al 1985). This is further supported by the demonstrable inability of Cyclosporin A to prevent the interferon gamma-induced expression of class II antigen on cultured human monocytes, although an effect on antigen presentation was seen (Snyder, Wright & Ting 1987). In rat kidney and heart allograft experiments Cyclosporin A was shown to effectively inhibit the induction of class II expression on the transplanted organs despite the presence of substantial cellular infiltrates (Milton et al 1986), and in a similar way the increased class II expression on kidney tubules in graft-versus host disease and on bronchial epithelium in lung allografts was prevented by this drug (Halloran et al 1986, Romaniuk et al 1987). In the clinical situation an effect on class II antigen expression is suggested by the description by

Fuggle et al (1986) of a much lower incidence of class II expression on renal tubules in patients treated with Cyclosporin A than in patients receiving azathioprine and prednisolone.

The observed reduction in antigen expression in the transplanted organ has been suggested to be a contributory factor in the effectiveness of Cyclosporin A as an immunosuppressant (Groenewegen et al 1985, Halloran et al 1986) and an assessment of the levels of MHC expression in the different organs transplanted and the effects of immunosuppression on these levels may help to provide an insight into methods of preventing rejection.

Pancreatic MHC antigen expression

The pattern of expression of MHC antigen on the various component cells of the pancreas has been described for rodents and man. Normally, Class I MHC antigen is expressed by duct epithelial cells, islet cells and vascular endothelium but not by exocrine cells although there is some interspecies variation in the levels of expression (Hart et al 1983, Parr, Oliver & King 1982, Steiniger et al 1985, Pujol-Borrell 1986). Class II MHC antigen expression in the resting state is restricted to a population of cells scattered among both the endocrine and exocrine components of the pancreas with many having dendritic morphology. Beta cells are generally described as being class II negative at rest (Faustman et al 1980, Parr et al 1980, Baekkeskov et al 1981, Hart and Fabre 1981, Alejandro et al 1982, Rabinovitch et al 1982, Hart et al 1983, Gebel et al 1983,

Steiniger et al 1984), although this has recently been questioned following the demonstration of weak expression of class II antigen on endocrine cells of isolated islets. This antigen expression was detected using one specific high-titre antibody and was only seen in rats of the $RT1^C$ genotype (Ulrichs & Muller-Ruchholtz 1985, Ulrichs et al 1987). As this antigen expression was demonstrated on isolated islets it is possible that during either the digestion stage or during Ficoll separation antigens hitherto hidden were revealed or possibly cells which labelled positively for insulin and class II antigen were actually class II positive phagocytic cells which had taken up fragments of dead beta cells as described by Pipeleers et al (1987).

Whether or not pancreatic beta cells normally express class II MHC antigens in functionally significant amounts is of importance when considering islet allografts and their immunomodulation, particularly when anti-class II antibodies are used to pre-treat the graft.

Another cell within the islet expressing class II antigen constitutively, at least in certain species, is the endothelial cell. Class II positive endothelial cells have been demonstrated inside human and pig islets (Alejandro et al 1982, Nocera et al 1986, Shienvold, Alejandro and Mintz 1986) but endothelium in dog, rat and mouse islets does not normally appear to express class II antigen at detectable levels (Gebel et al 1983, Shienvold et al 1986, Hart & Fabre 1981, Parr et al 1980).

Although exocrine cells and beta cells are probably class II negative at rest, under certain circumstances these can become class II positive. In patients with chronic pancreatitis expression of class II MHC antigen can sometimes be detected on exocrine and ductal cells (Pujol-Borrell 1986). In animal models of auto-immune diabetes such as the BB rat and the NOD mouse spontaneous class II expression on beta cells has been described (Dean et al 1985, Hanafusa et al 1987). With human diabetes mellitus, if the pancreases of newly-diagnosed diabetics are examined while some beta cells remain intact, class II expression accompanied by increased amounts of class I expression is seen in islets still containing insulin. Alpha and delta cells within these islets are said to remain class II negative (Bottazzo et al 1985, Foulis, Farquharson & Hardman 1987).

In in vitro experiments using isolated islets, beta cells appear to be very resistant to the induction of class II antigen expression using soluble mediators. Levels of interferon gamma which produce an increase in class II antigen expression on other pancreatic cells and greatly enhance the levels of expression of class I antigen on endocinre cells from human pancreas fail to stimulate class II expression on beta cells unless lymphotoxin or tumour necrosis factor is added to the medium (Pujol-Borrel et al 1986, 1987). In intact mice when high doses of interferon gamma are given over several days a marked increase in MHC antigen expression is seen throughout the pancreas by the sixth day. Unlike human exocrine pancreatic

cells those of the mouse remained class I negative and only focal patches of class II expression were noted. Beta cells remained class II negative (Skoskiewicz et al 1985). Mouse islets isolated using collagenase digestion and Ficoll separation and exposed to in vitro interferon gamma were originally described as being resistant to the induction of class II expression (Campbell et al 1985) but when higher concentrations were employed and a longer observation period used to allow for latency in antigen induction, beta cell class II expression was seen after five days exposure to this lymphokine (Wright et al 1986). This observation was later extended to include isolated human beta cells cultured as dispersed cells with interferon gamma (Wright et al 1987). Wright et al (1987) stated that although other lymphokines were synergistic with interferon in antigen induction unlike Pujol-Borrell et al (1987) they found that interferon gamma on its own was effective.

Induction of class II antigen expression has also been described on rat beta cells under special circumstances. Islet cells from a line of BB rats with a high incidence of spontaneous diabetes can be stimulated to express class II antigen in vitro using gamma interferon alone, an effect not seen when the islets are obtained from a line with a low incidence of spontaneous diabetes (Walker et al 1986). Also, induction of class II antigen expression has been demonstrated following exposure to gamma interferon of a rat beta cell tumour line (van Vliet et al 1987).

In interpreting these findings the possibility that methods used to isolate the islet cells may in some way alter the antigen expression should be borne in mind and this may explain the apparent difference between in vitro and in vivo findings. Alternatively, some inhibitory mechanism may exist in vivo and the removal of the islets from this milieu may allow stimulation of antigen expression to proceed. It would appear, therefore, that beta cells contain all the necessary intracellular machinery to generate and express class II molecules on their surface membrane. Their resistance to the induction of expression of this antigen has probably been acquired as a protective mechanism and may help to protect these cells from the immunological damage seen in type I diabetes mellitus, where the aberrant class II expression by these cells may be important in the pathogenesis of this disease.

MHC antigen expression in immature tissues

During foetal maturation, two additional immunological considerations are extant. Firstly, the developing embryo must avoid eliciting an allograft response from its mother with whom it shares only half its genetic complement and secondly, tolerance to self-antigen needs to be acquired. Modification of MHC antigen expression may play a part in achieving these objectives

Although early reports suggested that class I MHC antigens were present at a very early stage of embryological development these studies depended on indirect evidence from observed

rejection of skin grafts in class I disparate mice or involved the use of polyclonal antibodies which may have lacked the necessary specificity (Patthey & Edidin 1973, Warner & Spannaus 1984). Using monoclonal antibodies and DNA probes, Ozato et al detected class I mRNA from the ninth day and surface class I MHC antigens from the tenth day of gestation but no earlier in mouse embryos (Ozato, Wan & Orrison 1985). During the later stages of gestation class I expression remained at much lower levels than found in adults and similar findings have been demonstrated in human foetal tissues (Ozato et al 1985, Sewell et al 1986).

The time of onset of class II MHC expression would appear to vary depending on which tissues and species are being studied. In the skin of human foetuses at 15 to 19 weeks gestation, class II positive cells are scarce and in some biopsies completely absent whereas other tissues including pancreas contain larger numbers of these cells although still fewer than adult tissues (Hofman, Danilovs & Taylor 1984, Danilovs et al 1982). Developing mice including neonates have very few class II positive cells anywhere except the thymus (Lu, Beller & Unanue 1980, Unanue et al 1984) and antigen presentation by noenatal accessory cells is consequently inefficient (Lu, Calamai & Unanue 1979, Nadler, Klingenstein & Hodes 1980). It has been suggested that decreased class II expression in foetal and neonatal tissues is related to the existence of suppressor mechanisms, perhaps involving prostaglandins (Snyder et al 1982), present in vivo, and stimulation of class II expression by lymphokines has been

demonstrated on normally negative neonatal monocytes from mice and humans in vitro (Unanue et al 1984, Stiehm et al 1984). Similarly class I MHC expression can be induced on mouse tissues by interferons alpha, beta and gamma several days in gestation before levels would normally be detectable (Ozato et al 1985).

In the rat, class II positive cells have been detected in lymphoid tissue closely associated with the pancreas at 17 days gestation. At this time, the exocrine and endocrine components of the pancreas are said not to contain these cells except perhaps in areas close to lymphoid collections. Even in fully developed newborn rats only very low numbers of class II positive cells are detected (Fujiya et al 1985) and this is in contrast to larger animals including humans where class II positive cells are seen in close association with islets in 12-24 week foetuses with large clusters of class II positive lymphoid cells also being described (Danilovs et al 1982).

Therefore, although in general class II antigen expression appears to occur later than class I expression in embryological development, class II positive cells may be present in sufficient numbers to contribute to tissue immunogenicity in human pancreases at the time of gestation normally considered suitable for foetal pancreas transplantation.

SUMMARY OF THE HISTORICAL REVIEW

It is now rare for a patient with diabetes mellitus to succumb to the acute metabolic derangements induced by a lack of insulin. However, although in the last sixty years the quality of exogenous insulins available and the sophistication of administration regimens have improved greatly, diabetic patients still have a reduced life expectancy and many are destined to develop disabling complications.

The evidence linking poor-control of blood glucose with the development of diabetic complications is not completely conclusive, but it seems likely that normalisation of blood glucose levels at an early stage in the disease would prevent many of the microangiopathic and neuropathic changes otherwise seen. Control of insulin secretion is normally influenced by a multitude of factors and no system of exogenous insulin administration could be hoped to mimic this completely. Once a person's own pancreatic beta cells have been destroyed, the transplantation of functioning human or animal insulin-producing cells, in some form, is the only hope of restoring physiological glucose control.

The transplantation of isolated islets or foetal pancreatic tissue has an advantage over whole organ transplantation as the surgical procedure itself would be relatively minor and could probably be repeated several times if required. Of additional advantage is the ability of islets and foetal pancreas to survive in tissue culture therefore providing

an opportunity for modifying the immunogenicity of the tissue by in vitro treatment thereby allowing transplantation with no or reduced levels of immunosuppression.

Based on theories suggesting that the major barrier to transplant acceptance is the presence of class II positive antigen presenting cells within the graft, various methods of reducing this component have been investigated. Strategies of immunomodulation based on the removal of class II positive cells are of particular interest in pancreatic islet transplantation as it would appear that islet endocrine cells are constitutively class II negative and are extremely resistant to the induction of expression of this class of MHC antigen.

Initial experiments with rodent islet allografts disappointingly failed to demonstrate the lower immunogenicity associated with other endocrine grafts. Indeed, transplanted islets at first appeared to be rejected in an accelerated manner, more rapidly even than whole organ pancreas grafts. It has since become apparent that it is not the endocrine cells themselves that are responsible for this increased immunogenicity and stricter purification of islet preparations, especially the elimination of lymphoid cells, helps to reduce susceptibility to rejection. However, purification alone does not prevent rejection and various additional strategies have been employed. These have included efforts to remove class II positive passenger leukocytes by tissue culture in various environments selectively disadvantageous to leukocytes, such as high oxygen tensions and low temperatures, or using antibodies

directed against MHC class II antigens. Although some of these techniques have shown promise, results have been very variable and are on many occasions species and strain dependent with recipient immunosuppression often being required.

Of the immunosuppressive agents used in rodent islet and foetal pancreas transplantation experiments, the response to anti-lymphocyte serum has been very variable and initial results with Cyclosporin A left little ground for optimism. However, at least for islets, more recent results have shown an encouraging beneficial effect when meticulously purified islet preparations are used. Unfortunately Cyclosporin A, in addition to its nephrotoxicity and hepatotoxicity, has an adverse effect on pancreatic beta cell function and this is likely to limit its usefulness as the sole form of immunosuppression in islet cell transplantation. A combined approach, whereby the immunogenicity of the pancreatic tissue is reduced by in vitro treatments, allowing a reduction in recipient immunosuppression, would seem to offer most hope for the future.

Although removal of MHC class II positive passenger leukocytes from pancreatic tissue has not, on its own, always resulted in graft acceptance, it is clear that MHC class I and class II antigen expression on different cell types is influenced by transplantation and probably is of importance in both the afferent and efferent limbs of the immune response. Presentation of antigen by recipient cells using the indirect pathway may prove to be important in human islet and foetal pancreas transplantation and the possibility of antigen-

presentation by non-bone marrow derived cells of donor origin should also be considered.

Regarding foetal tissues, MHC class I and class II antigen expression has been shown to be different from that of the adult animal, and a knowledge of this difference in expression may be useful in predicting the likelihood of rejection occurring following transplantation. Experiments using various tissues in a number of different species have shown that both MHC class I and class II antigen expression are regulated by a variety of different influences. Increased levels of antigen expression following transplantation will increase the level of available targets for the effector phase of the immune response while at the same time will have potentially important effects on the afferent phase leading to an amplification of response.

By examining both MHC class I and class II antigen expression in adult pancreas, isolated islets and in foetal pancreas under a variety of circumstances including allografting and Cyclosporin A administration, it may be possible to obtain an increased understanding of the mechanisms of rejection and of potential areas for investigating methods of lowering graft immunogenicity and influencing the rejection response.

Although antigen expression in any tissue is subject to a degree of interspecies variation, if these limitations are accepted the findings of animal experiments may still be relevant to the clinical situation.

The rat, with its well characterised antigen expression and well established techniques of foetal pancreas and islet

transplantation, is a particularly convenient animal model for these experiments. In addition, the availability of monoclonal antibodies specific for polymorphic determinants differing in readily available strains of laboratory rat, allows differentiation of donor and recipient strain antigen expression.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMALS

DA(RT-1^a), PVG(RT-1^c), Lewis (RT-1^l) and (DAxPVG) F1 hybrid rats were obtained from the inbred colonies housed in the animal facility at the Department of Surgery, Western Infirmary, Glasgow. PVG-rnu/rnu (RT-1^c) were obtained from Harlan Olac Limited. The rnu gene was backcrossed from an outbred population onto the inbred PVG line in 1977. This line has since undergone 12 backcrosses and 12 intercrosses altogether and is now firmly established (AG Peters, Harlan Olac Ltd, personal communication).

Foetal rats of 17 days gestation were obtained from time-mated pairs with the morning following an overnight mating being defined as day 0. Successful mating was confirmed by the identification of spermatozoa in vaginal smears.

Animals were on standard rat diet with water ad libitum.

2.2 SURGICAL PROCEDURES

2.2.1 Anaesthesia

Rats were anaesthetised using several different techniques depending on the duration of anaesthesia required. A short period of anaesthesia, sufficient to allow blood sampling or intravenous injection was induced by administering 5% halothane in oxygen or ether until consciousness was lost. For more prolonged periods of anaesthesia this method of induction was followed by maintenance with an intraperitoneal injection of 0.7-1.2mls of 7.5% chloral hydrate in saline. The dose of chloral hydrate varied depending on the body weight of the rat and the particular strain.

2.2.2 Foetal pancreas transplantation

Pregnant rats (17 days) and recipient rats were anaesthetised and their anterior abdominal walls shaved and then cleaned with chlorhexidine in 70% alcohol. Using a midline incision, foetuses were removed from the uterus and placed in a petri-dish containing ice-cold saline. Almost the entire foetal pancreas was then quickly removed and kept in cold Hank's balanced salt solution until transplanted. The left kidney of the recipient animal was exposed through a midline abdominal incision with bowel retracted to the right. The anterior aspect of the renal capsule was picked up using jeweller's forceps and a longitudinal incision made in it taking care not to damage the underlying renal cortex. The renal capsule medial to this incision was gently separated from the kidney surface using a blunt probe to create a pocket. Foetal pancreas glands were then placed under the capsule which was allowed to settle back in place without suturing. The midline wound was then closed in two layers using 3/0 chromic catgut sutures (Ethicon Ltd).

2.2.3 Nephrectomy

Animals showing evidence of reversal of diabetes following foetal pancreas transplantation had their left kidney (bearing the pancreas grafts) removed four weeks after the return of normoglycaemia. The previous midline incision was re-opened and the left kidney was mobilised. The vascular pedicle and ureter were then tied using a single 3/0 silk suture (Ethicon Ltd) and the kidney and attached pancreas graft excised.

2.2.4 Retransplantation

Kidneys bearing viable pancreas grafts were retransplanted following a period of residence in an interim host. The midline wound of the rat acting as interim host was re-opened and the left kidney, renal vessels and ureter were mobilised. This procedure was repeated in the recipient rat, and after placing occlusive clips across the renal artery and vein, the renal vessels and ureter were divided and the kidney removed. Next the interim host rat was heparinised (100 units per rat) and the kidney with attached pancreas was excised and immediately transferred to a Petri-dish containing ice-cold saline.

The kidney and attached pancreas were then transplanted to the left orthotopic site with end to end anastomosis of the renal artery and vein with interrupted sutures of 10/0 polypropylene (Ethicon Ltd). The donor ureter was then anastomosed end-to-end to the recipient ureter using a similar technique of suturing. Total ischaemic times of the kidney during transplantation were approximately 25 minutes and during this time the graft was kept cool by frequent local applications of cold saline. Following completion of the anastomoses, clips were removed and the abdomen closed in two layers as described previously.

2.2.5 Intravenous injection

Intravenous injections were performed under a short general anaesthetic using ether. To avoid extravascular leakage the large dorsal vein of the penis was used.

2.2.6 Intraperitoneal injection

Insulin and interferon gamma were injected intraperitoneally while the unanaesthetised rat was restrained by an assistant.

2.2.7 Oral administration

Cyclosporin A was administered to anaesthetised rats via a fine plastic tube which was carefully guided down the oesophagus to the stomach. Misplacement of the tube was avoided by observing the tip of the tube displacing the stomach towards the anterior abdominal wall.

2.2.8 Blood sampling

Blood samples were obtained from anaesthetised rats by amputating a thin slice from the tip of the tail. 0.3ml of blood was collected in a 0.4ml plastic centrifuge tube (Beckman RIIC, Limited). The serum was then separated by centrifugation in a microcentrifuge.

2.3 ISLET ISOLATION

The technique of islet isolation was based on collagenase digestion (Moskalewski 1965) with retrograde perfusion of the pancreatic ducts (Lacy and Kostianovsky 1967) followed by hand picking of neutral-red stained islets (Dibelius et al 1986).

Through a midline incision the abdominal aorta of the donor rat was dissected out together with a suitable portion of inferior vena cava. Clips were placed on both renal arteries

and the aorta was cannulated using a 3FG plastic cannula, tied in place with 3/0 silk, with the cannula tip lying below the vessels supplying the pancreas. 50mls of neutral-red solution was then injected through the cannula until the pancreas became uniformly pink. Circulated neutral-red solution was allowed to drain from the venous system through an opening made in the inferior vena cava.

The common bile duct (CBD) was then isolated and the lower end occluded with a clip applied across the point of entry to the duodenum. A 2FG plastic cannula was then inserted into the CBD just below the hilum with the tip pointing towards the duodenum. 10mls of collagenase in HBSS (2.5mg/ml) were then slowly injected to distend the pancreas. The pancreas was then excised, placed with 10mls of HBSS in a 100ml beaker on ice and chopped with scissors into pieces 1-2mm in size. The fragments were then transferred to a 30ml test tube and allowed to settle. The supernatant containing fat particles was discarded and the pancreatic fragments incubated with 10mls of fresh collagenase solution (2.5ml/ml) at 37^{o}C in a 25ml conical flask.

The digesting fragments were then left undisturbed for 30 minutes then shaken by hand and a pinch of DNAase added. Further static digestion for five minutes followed by shaking was repeated twice with more DNAase added at 40 minutes. After a further five minutes of static digestion the reaction was terminated by washing three times with ice cold HBSS.

The resulting digestate consisted of neutral-red stained clean islets, vascular and lymphoid fragments and single cells

along with occasional undigested portions of gland. This was then screened using a dissecting microscope and side illumination against a black background and purified islets were picked out using a micropipette and transferred to HBSS on ice. This picking was repeated twice to improve purity of the final preparation.

2.4 CHEMICALS

2.4.1 Hank's balanced salt solution

Sterile Hank's balanced salt solution (HBSS) was supplied by Flow Laboratories Ltd, Irvine, Scotland.

2.4.2 Collagenase

Type V collagenase (derived from cultures of clostridium histolyticum) was obtained from Sigma Chemicals Ltd, Poole, Dorset, supplied as lyophilized powder.

2.4.3 DNAase

Deoxyribonuclease 1 was obtained from Sigma Chemicals Ltd.

2.4.4 Streptozotocin

Streptozotocin was supplied by Upjohn Co, Kalamazoo, USA and was dissolved in acetate buffer (pH 4.5) at a concentration of 20mg/ml.

2.4.5 Neutral Red Solution

Neutral red dye was obtained from BDH Chemicals Ltd,

Poole, Dorset and was dissolved in saline in a concentration of 0.08mg/ml.

2.4.6 Cyclosporin A

Cyclosporin A was gifted by Sandoz Ltd, Basle, Switzerland and made up in olive oil to a concentration of 7.5mg/ml.

2.4.7 Insulin

Protamine Zinc Insulin (100U/ml) was obtained from Wellcome Medical Division, Crewe, Cheshire and was diluted 1 in 10 in saline immediately prior to administration.

2.4.8 Interferon gamma

Rat recombinant interferon gamma was obtained as a gift from PH van der Meide, TNO Primate Center, Rijswijk, The Netherlands.

2.5 LABORATORY TECHNIQUES

2.5.1 Viability testing

Fluorescein diacetate (FDA) (Sigma Chemicals Ltd) in a stock solution of 5mg/ml in acetone and ethidium bromide (EB) (BDH Chemicals Ltd) prepared as a stock solution of 100ug/ml in HBSS were used for viability testing. 3ul of FDA stock solution and 1ml of EB stock solution were added to isolated islets suspended in 1ml of HBSS on a plastic petri dish. The islets were then transferred onto glass slides and examined under UV light, with a Leitz fluorescence microscope using a x 10 objective.

2.5.2 Tissue culture

Standard tissue culture medium contained RPMI 1640, 10% foetal calf serum, 2mM L-glutamine and 100U/ml Penicillin and 100ug/ml streptomycin (all from Gibco Ltd). Foetal pancreas was placed on a Millipore filter supported in the gas liquid interface by a platform of surgical gelatin (Sterispon, Allen and Hanburys Ltd, Greenford, Middlesex). Falcon 1008 hydrophobic petri-dishes were used.

2.5.3 Ultraviolet irradiation

Ultraviolet (UV) irradiation was carried out within a laminar flow cabinet to maintain sterility. Islets suspended in tissue culture medium in an open petri-dish were constantly stirred using a magnetic stirrer. A total dose of 900 Joules/m^2 of UVB was delivered over 15 minutes using calibrated Westinghouse FS-20 sunlamps.

2.5.4 Glucose measurements

Blood glucose measurement was by BM-test (Boehringer Corporation [London] LTD) using the glucose-oxidase/peroxidase reaction and the Reflolux meter. Readings outside the range of the meter were obtained using a laboratory kit incorporating the glucose oxidase/peroxidase reaction (Boehringer Mannhein GmBH Diagnostica).

2.5.5 Insulin measurement

Stored samples were analysed in batches using a Radio-immunoassay Kit (Radiochemicals, Amersham, Buckinghamshire). A rat insulin standard was used (Novo Laboratories, Denmark).

2.6 HISTOLOGY

2.6.1 Cryostat sections

Tissue samples were embedded in OCT compound (Tissue-Tek, BDH Ltd), snap frozen in liquid nitrogen and 6u cryostat sections cut at -20^{o}C onto gelatinised multispot slides (CA Hendley, Essex).

2.6.2 Immunoperoxidase staining of cryostat sections

Sections were fixed in acetone at room temperature for 10 minutes, then washed in DAB (Dulbecco A+B salt solution). Excess moisture was dried from around each section then 50ul of primary monoclonal antibody, appropriately diluted, were applied to each section. After incubation at room temperature for 45 minutes in a humidified chamber, slides were washed three times in DAB then 50ul of the secondary antibody were applied. Ten per cent normal rat serum was added to this peroxidase-conjugated rabbit anti-mouse immunoglobulin to absorb out any cross-reacting antibody. After a further 30 minutes incubation at room temperature, the slides were washed three times in DAB, and incubated with the peroxidase substrate, consisting of 0.6mg/ml 3,3' diaminobenzidine tetrahydrochloride (Sigma Chemical Co) plus 0.01% hydrogen peroxide. Slides were then

washed, counterstained lightly with Harris's haematoxylin (BDH Ltd) and dehydrated and cleared in alcohols and xylene, then mounted in DPX mountant (BDH Ltd).

2.6.3 Immunoperoxidase staining of paraffin sections

Paraffin sections were processed in the Department of Pathology, Western Infirmary, Glasgow. Sections were stained with Haematoxylin and Eosin or labelled with a polyclonal antiserum to insulin, using an immunoperoxidase method similar to 2.6.2.

2.6.4 Immunofluorescent labelling of isolated islets

Isolated fresh or cultured islets were labelled with monoclonal antibodies using a similar technique to that described in 2.6.2. Islets in groups of 50 were withdrawn from HBSS or tissue culture medium and washed three times with DAB salt solution. Incubation in a 10ml test tube with the primary antibody (in a similar dilution to the immunoperoxidase method) was continued for 90 minutes at 4^{o}C before further washing with DAB. A fluorescein-conjugated rabbit anti-mouse immunoglobulin containing 10% normal rat serum was used as the second antibody with incubation at 4^{o}C for 60 minutes. Following further washing with DAB islets were transferred to glass slides for examination under UV light with a Leitz fluorescence microscope using x25 and x40 objectives.

2.6.5 Antibodies

Monoclonal mouse anti-rat antibodies used to characterise MHC antigen expression were as follows:-

Antibody	Specificity	Reference
MRC OX18	MHC class I	Fukumoto, McMaster & Williams (1982).
MRC OX6	MHC class II-I/A like antigen	McMaster & Williams (1979)
MN4-91-6	Polymorphic determinant of class I (DA [$RT1^a$] positive, PVG [$RT1^c$] negative)	Milton & Fabre (1985)
F17-23-2	Polymorphic determinant of class II (DA [$RT1^a$] positive, PVG [$RT1^c$] negative)	Hart & Fabre (1981) Milton & Fabre (1985)

MRC OX21, a mouse monoclonal antibody against human C3b inactivator (Hsiung et al 1982) was used as a negative control. Secondary polyclonal antisera used to detect the presence of the primary antibodies, were peroxidase-conjugated and FITC-conjugated rabbit anti-mouse immunoglobulins (Dako Ltd, High Wycombe).

Monoclonal mouse anti-rat antibodies used to detect rat leukocytes were as follows:-

Antibody	Specificity	Reference
MRC OX1	Leukocyte common antigen	Sunderland et al (1979)
MRC OX8	CD8 positive cytotoxic/suppressor T cells, majority of natural killer cells	Gilman, Rosenberg & Feldman, 1982, Dallman & Mason 1982, Cantrell et al 1982
W3/25	CD4 positive helper T lymphocytes, some macrophages	Brideau et al 1980 Barclay 1981
MRC OX19	Peripheral T lymphocytes	Dallman & Mason 1982
MRC OX39	IL-2 receptor	Paterson et al 1988

2.6.6 Morphometric analysis of cellular infiltrate

The area of each immunoperoxidase-labelled tissue section infiltrated by leukocytes of a particular phenotype was determined by morphometric analysis using the point counting technique (Aherne & Dunnil 1982, McWhinnie et al 1986). Sections were examined at a magnification of 400 through a microscope eyepiece graticule engraved with a grid bearing either 121 intersections or 745 intersections. For each field the number of positively stained cells superimposed by an intersection was counted and the area of the field occupied by cells of a particular phenotype was calculated as:-

$$\% \text{ area of infiltrate} = \frac{\text{no. of positive grid intersections}}{\text{total no. of grid intersections}} \times 100$$

Most sections were counted with the 745 point graticule but if there was a heavy infiltrate, the section was counted with the 121 point graticule. Ten consecutive fields were counted for each section, so that for all sections the total number of points observed was well in excess of that required to maintain the accuracy of the point counting technique (Aherne & Dunnill 1982).

All counts were carried out by one observer who was not aware of the experimental group from which the section had been obtained.

2.7 ELECTRON MICROSCOPY

Electron microscopy processing of isolated pancreatic islets was carried out in the Electron Microscopy Laboratory of the Department of Pathology, Western Infirmary, Glasgow. Ultrasections of 80nm thickness were examined in a Philips 301 Electron Microscope after contrast enhancement using Uranium and Lead.

2.8 PHOTOGRAPHY

Photomicrographs of cryostat and paraffin sections were obtained using Kodachrome 25 (daylight) film and an appropriate blue filter. Processing to 35mm slides was carried out by Kodak laboratories and colour prints were generated from the original 35mm slides by Co-Lab, Coventry.

2.9 STERILISATION

Glassware and instruments were sterilised by autoclave. Solutions were either obtained pre-sterilised or passed through a millipore filter, pore size 0.45u. Plastic-ware and millipore filters were sterilised by gamma irradiation.

3. EXPERIMENTAL CHAPTERS

CHAPTER 1

MHC ANTIGEN EXPRESSION IN ADULT AND FOETAL DA RAT PANCREAS

INTRODUCTION

The celllular distribution of MHC class I and class II antigen expression in rat, mouse and human tissues share many similarities. However, differences with potentially important implications for organ transplantation do exist (Harris & Gill, 1986). For example, vascular endothelium in the rat does not constitutively express class II antigens whereas in man class II antigen is readily detectable on unmodified endothelial cells (Reviewed by Koene et al, 1986).

Historically, MHC class I antigens have been described as having a 'ubiquitous' distribution with class II antigen expression being more restricted. This concept, while broadly true, required modification in the light of more recent descriptions of absent class I expression on several different rat and human cell types including pancreatic exocrine cells (Hart et al 1983, Daar et al 1984, Steiniger et al 1985).

Levels of MHC antigen expression are in general much lower in foetal tissues than in adult tissues (Ozato et al 1985, Sewell et al 1986). Although class I MHC antigens can be detected on foetal tissue by the ninth or tenth day of gestation in mice, the degree of expression remains at much lower than adult levels until some time after birth, and in developing mice and rats, the very few class II positive cells present have a

tissue distribution quite different from that of adult animals (Fujiya et al 1985).

The pattern of MHC antigen expression in a given tissue at the time of transplantation may be important in determining whether that tissue is seen as foreign and in influencing the nature and intensity of the immune response which it provokes. Although quantitative analysis provides useful information regarding the kinetics of MHC antigen expression (Milton et al 1986) this technique does not allow localisation of antigen to specific cell types within a complex tissue such as pancreas, composed, as it is, of both endocrine and exocrine elements. In the experiments to be described, antigen expression was assessed on a qualitative basis with negativity being taken to represent absence of a particular antigen or present at levels below that detected by the method used. By using cryostat tissue sections rather than isolated cells, manipulations of tissue were kept to a minimum in the hope of avoiding any artefactual alteration in antigen expression.

MATERIALS AND METHODS

Adult rat pancreas glands were obtained from 8-20 week old male DA rats. Following excision under general anaesthesia, the whole pancreas gland was snap-frozen in liquid Nitrogen and stored at -70^{o}C. Foetal pancreas glands were excised from 17 day gestation DA foetuses and treated in a similar manner. In addition several foetal pancreas glands were fixed in buffered formalin and embedded in paraffin.

DA foetal pancreases were also cultured in RPMI 1690 with 10% heat inactivated foetal calf serum, 2mmol L-glutamine, streptomycin (100ug/ml) and penicillin 100U/ml, supported in the gas-liquid interface by a raft of surgical gelatin with a gas phase of 5% CO_2 in air. Recombinant rat interferon gamma (IFN-gamma) was added to the culture medium in half of the experiments at a concentration of 1000U/ml. Culture medium was renewed every two days. After seven days the foetal pancreas glands were harvested from culture and snap-frozen in groups of eight, and stored at -70°C. For comparison (see later) pancreas glands were removed from newborn DA rats and similarly treated.

Cryostat sections were taken from multiple levels of each of five blocks per experimental group and labelled with monoclonal antibodies to class I MHC antigens (MRC OX18, MN4-91-6) and to class II MHC antigens (MRC OX6, F17-23,2) using the indirect immunoperoxidase method and 3,3' diaminobenzidine tetrahydrochloride substrate. MRC OX-21 was used as a negative control. Sections were lightly counterstained with Harris' haematoxylin and examined at magnifications of 100 x, 250 x and 400 x and photographs taken using a standard technique.

Paraffin sections (6um) of foetal pancreas were stained with haematoxylin and eosin.

RESULTS

Adult rat pancreas

The pattern of MHC class I and class II expression in DA adult rat pancreas is summarised in Table 1.1.

TABLE 1.1

MHC ANTIGEN EXPRESSION IN ADULT DA RAT PANCREAS

	Class I	Class II
Islet cells	++	-
Duct epithelium	++	-
Exocrine cells	-	-
Vascular endothelium	++	-
Vascular smooth muscle	-	-

Examination of appropriately labelled cryostat sections of adult pancreas revealed a striking difference in the distribution of MHC class I and class II antigen expression. In class I labelled sections, islets were readily identified as uniformly positive, rounded collections of cells amidst a background of negative exocrine cells. Among these exocrine cells were many linear areas of positive staining representing small pancreatic ducts and possibly capillary endothelium (Plate 1.1). Vascular endothelium in medium sized vessels was strongly positive for class I antigen but smooth muscle in the walls of these vessels was class I negative (Plate 1.2). The pattern of antigen expression identified by MRC OX18 labelling was identical to the pattern with the MN4-91-6 monoclonal antibody.

Sections labelled with antibodies specific for MHC class II antigens showed an entirely different pattern of staining. Only a scattered population of interstitial dendritic cells (some contained within islets) was shown to be class II positive. All other pancreatic cells were class II negative, as was the vascular endothelium in medium sized vessels (Plates 1.3 and 1.4). The pattern of antigen expression identified by both class II monoclonal antibodies was again identical.

Foetal rat pancreas

The rat foetal pancreas at 17 days gestation is seen on histological examination to consist of a collection of endocrine cells forming immature islets, scattered areas of duct epithelium, developing acinar tissue and occasional lymphoid

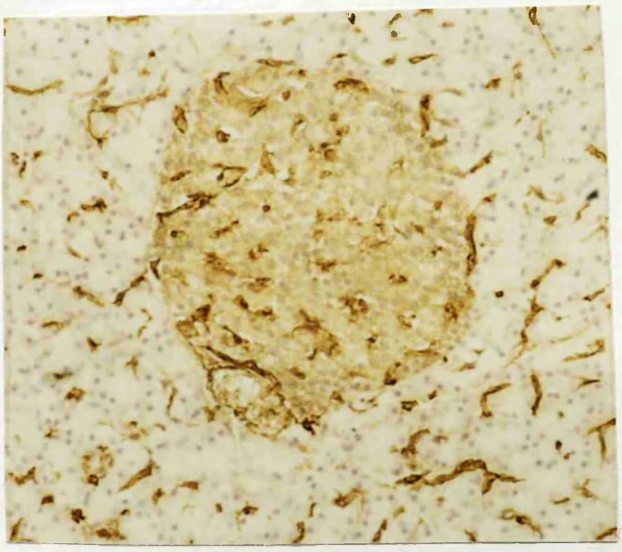

PLATE 1.1. Cryostat section of adult rat pancreas labelled with MRC OX18 which labels MHC class I antigens (x900). Note rounded collection of positive cells representing a large islet of Langerhans. Linear areas of positive staining scattered among negative exocrine cells represent small pancreatic ducts and possibly capillary endothelium.

PLATE 1.2. Cryostat section of medium sized vessel from adult rat pancreas (x900). Labelling with anti-class I MRC OX18 demonstrates strongly positive vascular endothelium (arrowed) and class I negative vascular smooth muscle.

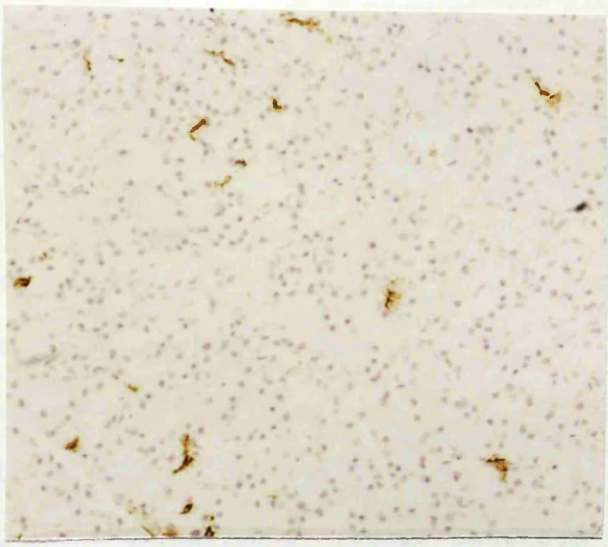

PLATE 1.3. Cryostat section of adult rat pancreas labelled with MRC OX6 which labels MHC class II antigens (x900). Only a few scattered interstitial cells are shown to be class II positive. Note the dendritic morphology of some of the positive cells.

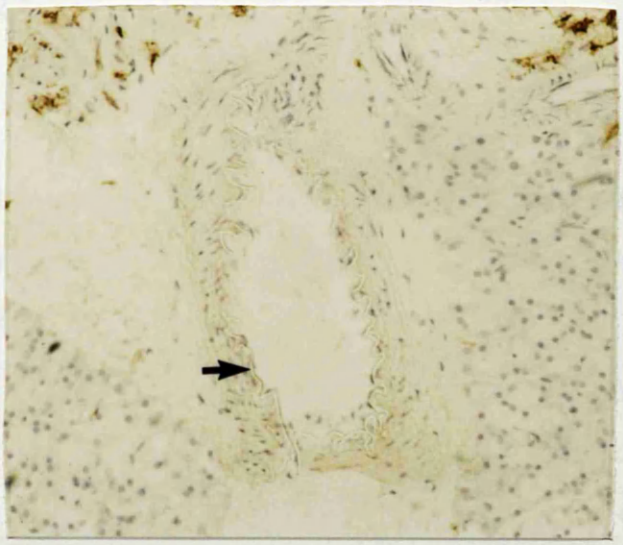

PLATE 1.4. Cryostat section of medium sized vessel from adult rat pancreas labelled with MRC OX6 (x900). Vascular endothelium (arrowed) and smooth muscle are class II negative.

aggregates (Plates 1.5 and 1.6).

There was very little evidence of detectable MHC class I expression in any of the sections examined. Only very occasional class II positive cells were present scattered amongst the developing pancreas (Plates 1.7 and 1.8).

Seven day cultured foetal pancreas (control)

Histological examination of foetal pancreas cultured for seven days revealed a central core of necrotic debris with a peripheral crust of well preserved pancreatic tissue. Class I antigen expression was more marked in the duct epithelium and endocrine cells of cultured foetal pancreas than in the neonatal rat pancreas (Plates 1.9 and 1.10). Class II positive cells were similar in number in cultured foetal pancreas, freshly isolated specimens and neonatal pancreas (Plates 1.11, 1.8 and 1.12).

Seven day cultured foetal pancreas (IFN-gamma treated)

IFN-gamma added to the culture medium produced a slight increase in the MHC class I labelling on islet cells and duct epithelium in cultured foetal pancreas, particularly noticeable on the duct cells (Plates 1.13A and 1.13B, compared with Plate 1.9). A much more obvious change in the pattern of class II antigen expression was noted with de novo induction on duct epithelium. However, islets remained class II negative (Plate 1.14).

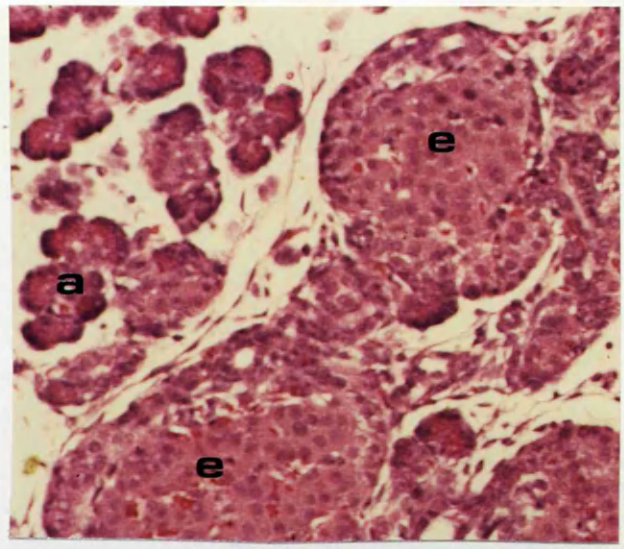

PLATE 1.5. Paraffin section of rat foetal pancreas at 17 days gestation stained with haematoxylin and eosin (x1400). Note collections of endocrine cells (e) and smaller areas of developing acinar tissue (a).

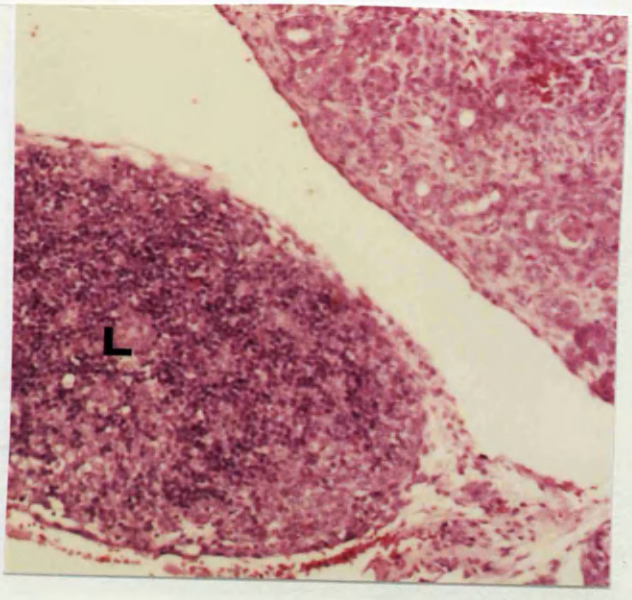

PLATE 1.6. Paraffin section of rat foetal pancreas at 17 days gestation stained with haematoxylin and eosin (x900). Note large collection of closely-associated lymphoid tissue (L).

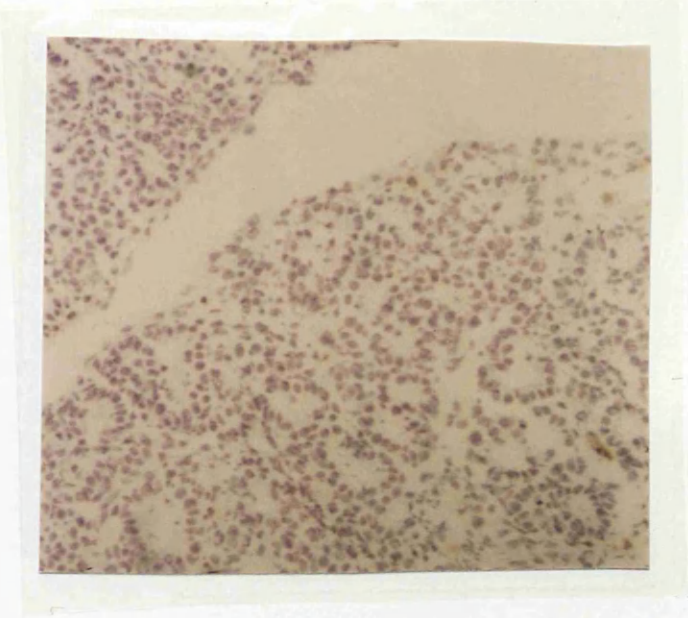

PLATE 1.7. Cryostat section of rat foetal pancreas labelled with MRC OX18 (anti-class I) (x900). There is no evidence of class I labelling on any cell-type.

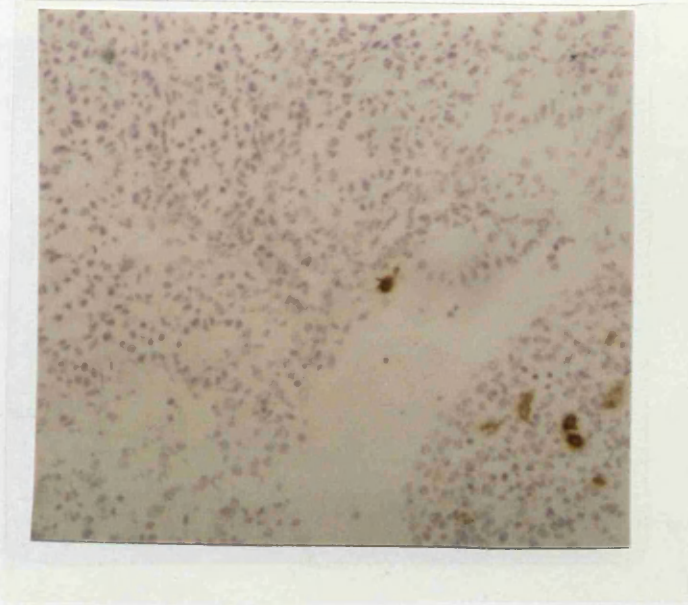

PLATE 1.8. Cryostat section of rat foetal pancreas labelled with MRC OX6 (anti-class II) (x900). Only very occasional class II positive cells are seen scattered amongst the developing pancreas.

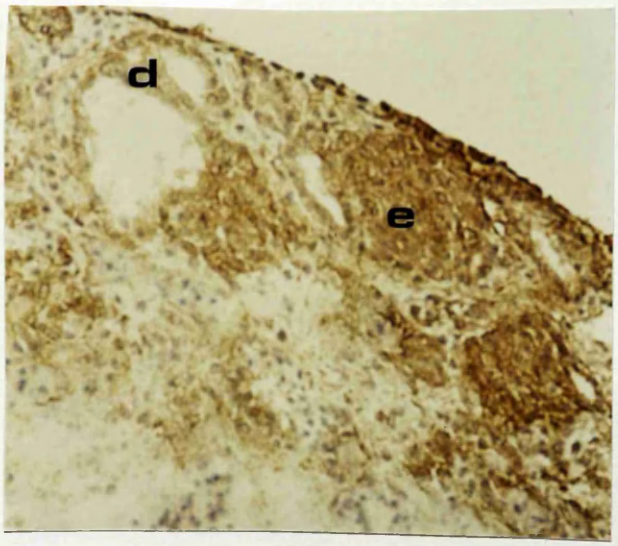

PLATE 1.9. Cryostat section of 7 day cultured rat foetal pancreas labelled with the anti-class I antibody, MRC OX18 (x900). Class I positive duct epithelium (d) and endocrine cells (e) are readily identifiable.

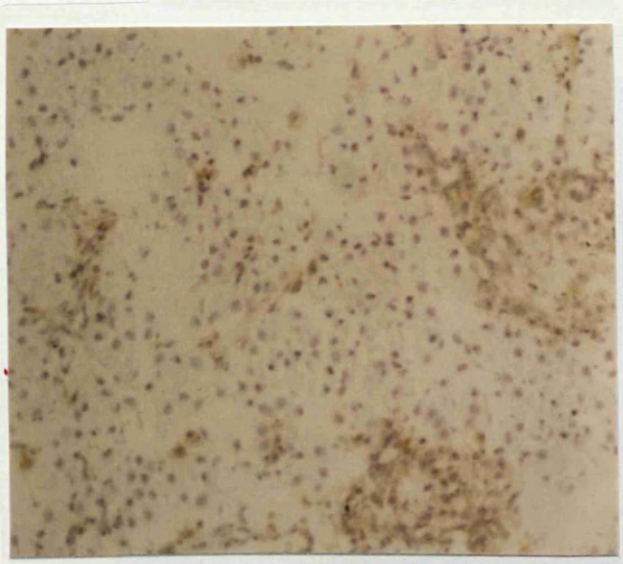

PLATE 1.10. Cryostat section of neonatal rat pancreas labelled with MRC OX18 (x900). Very little evidence of class I labelling is seen.

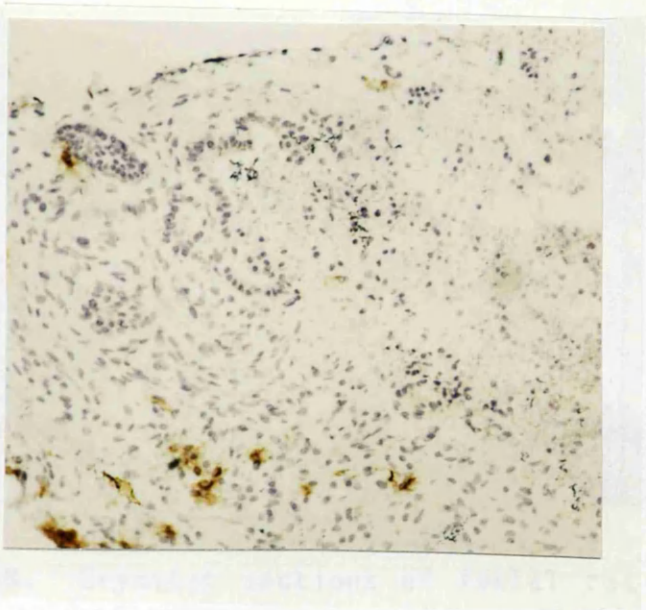

PLATE 1.11. Cryostat section of 7 day cultured foetal pancreas labelled with MRC OX6 (anti-class II) (x900). Numbers of class II positive cells are similar to those in freshly isolated foetal (Plate 1.8) and neonatal pancreas (Plate 1.12).

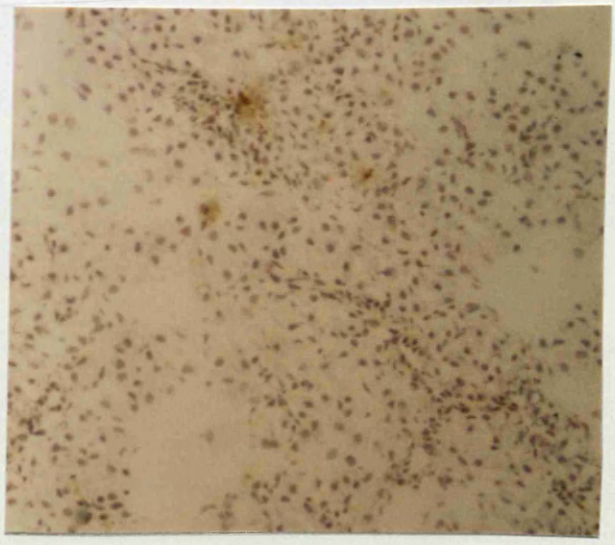

PLATE 1.12. Cryostat section of neonatal rat pancreas labelled with MRC OX6 (x900).

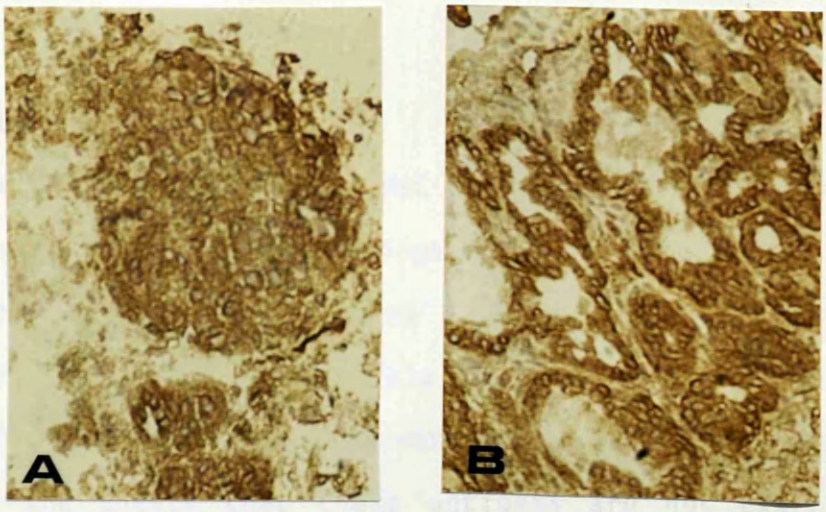

PLATE 1.13 A&B. Cryostat sections of foetal rat pancreas cultured with interferon-gamma. Sections have been labelled with MRC OX18 (anti-class I) (x900). Note slight increase in class I labelling of islet cells (A) and more marked increase in class I labelling on duct epithelium (B).

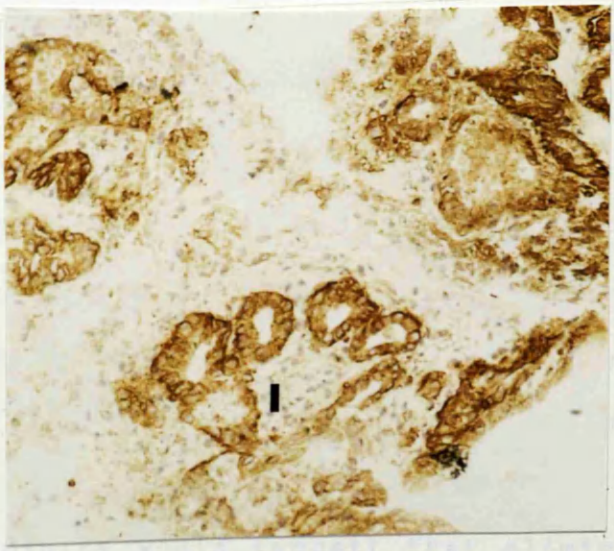

PLATE 1.14 Cryostat section of foetal rat pancreas cultured with interferon gamma. Sections have been labelled with MRC OX6 (x900). Note de novo expression of class II antigens on duct epithelium. Islets (I) remain negative except for a few contained interstitial cells.

DISCUSSION

The observed pattern of MHC antigen expression in the DA adult pancreas confirms the findings of previous authors in this and other rat strains (Hart et al 1983, Steiniger et al 1985). The absence of detectable levels of class I expression on pancreatic exocrine cells and vascular smooth muscle helps to reaffirm the thesis that these antigens are not expressed ubiquitously. However, the implications of these findings for pancreatic or islet transplantation are not immediately obvious as the endocrine cells themselves express MHC class I antigens to such an extent that identification of islets on a non-counterstained antibody labelled section is possible by merely observing characteristic distribution of the class I positive cells.

MHC class II antigen expression is thought to be an essential requirement for antigen-presenting cells (Pober et al 1986). The finding that class II expression in adult rat pancreas is limited to a scattered population of interstitial cells (many having dendritic morphology) is again in agreement with most previous studies (Hart et al 1983, Steiniger et al 1985, Shienvold et al 1986). If class II antigen expression does represent the major barrier to successful allografting, then these results would suggest that eliminating this population of cells prior to transplantation would result in a significant diminuition in graft immunogenicity. The observation of class II positive dendritic cells within the islets themselves helps to explain why unmodified isolated

islets still elicit a rejection response when transplanted across a major histocompatibility barrier (Reckard and Barker 1973, Finch & Morris 1976).

No evidence of class II MHC antigen expression was seen on endocrine cells in any of the tissue sections examined. When immunofluorescent techniques using monoclonal antibodies with particular reactivity (high-titred anti I-E) have been used by other workers, pancreatic endocrine cells in the isolated islets of certain rat strains appeared to be class II positive (Ulrichs and Muller-Rucholtz 1985, Ulrichs et al 1987). This apparent discrepancy may reflect variable class II expression in a strain dependent manner or may indicate different sensitivity or specificity of the antibody used. Changes brought about during the various stages of islet isolation may also alter antigen expression in some way and in addition, uptake of insulin by non-beta phagocytic cells could further add confusion to the interpretation of double-labelled preparations (Pipeleers et al 1987).

In non-cultured foetal DA rat pancreas, the findings of very low levels of both class I and class II MHC antigen expression was somewhat surprising in view of the reported rapidity of rejection in allograft experiments with this tissue (Garvey et al 1979). In order to try and determine whether these immature cells possessed the necessary intracellular machinery for the manufacture and expression of these antigens but were prevented from doing so by the presence of an 'inhibitor' or the absence of a 'stimulator', foetal pancreas

glands were cultured for seven days with and without IFN-gamma (a potent inducer of both class I and class II antigen expression [Halloran et al 1986]) and the patterns of antigen expression compared with that of newborn rat pancreas. In this way the foetal pancreas was allowed to grow to the developmental age of the newborn rat pancreas, being removed from the foetal environment for the last seven days of development.

When cultured without IFN-gamma for seven days the distribution of MHC antigens came to resemble the adult pattern of expression rather than the foetal or neonatal pattern. This would suggest that the capacity for class I expression is present in the immature rat pancreas and that removal from some inhibitory influence present in the foetal environment (or stimulation by some unidentified factor in the culture medium) is sufficient to allow these antigens to be expressed.

Without IFN-gamma in the medium class II antigen expression was little changed. As culture for seven days has been shown to reduce the passenger leukocyte content of islets (Rabinovitch et al 1982, Serie et al 1983, Gebel et al 1983), it is possible that any conversion of class II negative dendritic cells to class II positive cells, due to their removal from the foetal environment, may have been balanced by a selective loss of these leukocytes in tissue culture.

IFN-gamma produced a modest increase in foetal pancreas class I expression but a more striking effect of this lymphokine was the induction of strong de novo MHC class II antigen expression on pancreatic ducts of the developing pancreas.

Thus, although the very low levels of antigen expression present in freshly isolated foetal pancreas might suggest a correspondingly low level of immunogenicity, the results of these experiments would indicate that under certain circumstances (such as allotransplantation) foetal pancreas has, or can rapidly acquire, the capacity for high levels of both class I and class II antigen expression and would be likely to be able to provide a strong immunogenic stimulus.

SUMMARY

1) MHC class I antigen expression in adult rat pancreas was not detectable on exocrine cells or vascular smooth muscle.

2) MHC class II antigen expression in adult rat pancreas was restricted to a population of interstitial cells scattered among the exocrine and endocrine cells.

3) Rat foetal pancreas constitutively expresses levels of MHC antigen much lower than adult rat pancreas.

4) Following culture of foetal pancreas in standard medium MHC class I antigen expression was greater than in freshly isolated neonatal pancreas.

5) Culture with IFN-gamma produced a slight increase in class I expression and a more pronounced increase in class II expression compared to standard culture conditions.

CHAPTER 2

MHC ANTIGEN EXPRESSION IN DA FOETAL PANCREAS ISOGRAFTS AND ALLOGRAFTS

INTRODUCTION

MHC antigen is not a static phenomenon and the constitutive pattern of distribution in a tissue is influenced by a variety of different factors (reviewed by Fabre et al 1987). The changing pattern of antigen expression following transplantation was described initially for rat and mouse skin allografts (Dallman & Mason 1983, de Waal et al 1983) and in later studies similar findings were described for many other transplanted tissues (see Section 1). Information regarding pancreatic antigen expression following transplantation has so far been restricted to that derived from experiments with vascularised adult pancreas transplants in the rat (Steiniger et al 1985) and no previous study has examined changes in antigen expression in foetal pancreas (or any other foetal tissue) following transplantation.

It is likely that the level of MHC antigen expression has an important bearing on the mechanisms of graft rejection. Particularly in view of the finding reported in the previous chapter that foetal pancreas possesses low levels of basal antigen expression with higher levels induced following in vitro stimulation, analysis of changes in antigen expression may help to explain the reasons for the reported rapid rejection of foetal pancreas following allografting.

MATERIALS AND METHODS

Foetal pancreas glands were obtained from 17 day gestation DA foetuses. Following removal under sterile conditions, a total of four donor pancreas glands were transplanted without delay to the left renal subcapsular site of each recipient as described by Brown et al (1974).

Recipients were male DA rats in isograft experiments and male PVG rats in allograft experiments. Recipient animals were sacrificed at days 4,6,8,10 (two to five rats in each group at each time point) and a portion of the recipient kidney with the attached foetal pancreas graft was excised and snap-frozen to be stored at -70^{o}C prior to processing. Cryostat sections were cut at multiple levels through each block with a portion of recipient kidney included along with the grafted pancreas in each section. Sections were subsequently labelled with antibodies identifying monomorphic MHC class I and class II determinants: MRC OX18 and MRC OX6 respectively. In addition monoclonal antibodies used to differentiate between donor and recipient MHC class I and II antigens were MN4-91-6 (NDS 54) which reacts with a polymorphic determinant of MHC class I present in DA but not PVG strain rats and F17-23-2, which reacts with a polymorphic determinant of MHC class II I/A antigen present in DA but not PVG rats. A polyclonal guinea-pig anti-insulin antibody was used to identify insulin-containing endocrine cells. Again, an indirect immunoperoxidase method was used with 3,3' diaminobenzidine tetrahydrochloride as the substrate. Sections lightly counterstained with Harris'

haematoxylin were examined with a light microscope and appropriate photographs taken.

RESULTS

Isografts and allografts showed evidence of rapid initial vascularisation and by day 4 there was evidence on histological examination of tissue maturation with proliferation and dilatation of ducts and an increase in the proportions of insulin containing endocrine tissue (Plates 2.1 and 2.2). Allografts examined from day six onwards, however, showed obvious rejection characterised grossly by oedema and loss of vascularity and histologically by the appearance of a heavy mononuclear cell infiltrate and progressive tissue destruction. In none of the four allograft recipients examined at day 10, could any recognisable pancreatic elements be identified.

Antigen expression in DA foetal pancreas isografts and allografts

The changes in DA foetal pancreas antigen expression following isograft and allograft transplantation are shown in Tables 2.1 and 2.2. Information regarding the presence or absence of antigen expression in the three major components of foetal pancreas (islet cells, duct epithelium and exocrine cells) was readily obtained from appropriately labelled cryostat sections. By using monoclonal antibodies reacting with polymorphic determinants of MHC class I and class II antigens (MN4-91-6) and F17-23-2 respectively) which label DA but not PVG

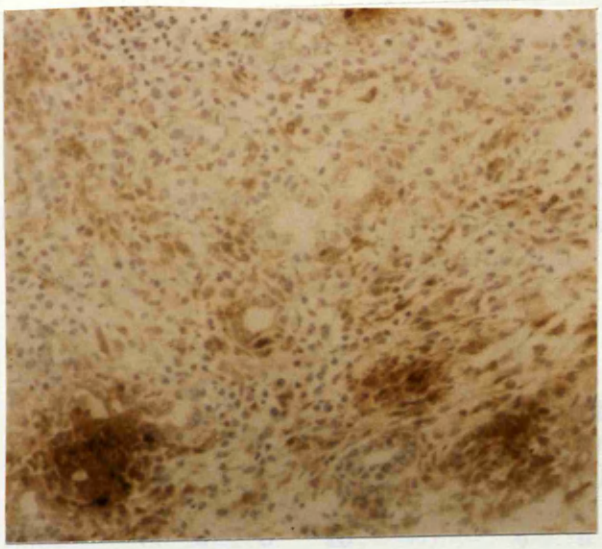

PLATE 2.1. Cryostat section of 4 day foetal pancreas isograft labelled with an anti-insulin antibody using the immunoperoxidase method (x900). Note clusters of positively labelled cells representing developing endocrine tissue.

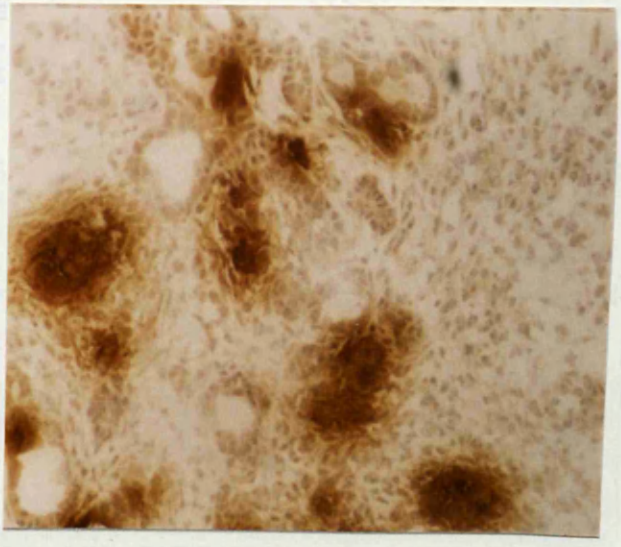

PLATE 2.2. Cryostat section of 4 day foetal pancreas allograft (DA to PVG) labelled with an anti-insulin antibody (x900). Note initial evidence of graft growth with many areas of insulin-containing endocrine tissue.

TABLE 2.1

CLASS I MHC ANTIGEN EXPRESSION IN DA RAT FOETAL PANCREAS FOLLOWING TRANSPLANTATION TO THE RENAL SUBCAPSULAR SITE[1]

Graft components	DA into DA isografts (days)[2]				DA into PVG allografts (days)			
	4	6	8	10	4	6	8	10
Islet cells	+	++	++	++	++	++	++	ND[3]
Duct epithelium	+	++	++	++	++	++	++	ND
Exocrine cells	-	-	(-)[4]	(-)	+	++	(++)	ND

1) Immunoperoxidase labelled sections were assessed without knowledge of the experimental group to which the graft belonged. Graft components were graded: 0 = no staining, + = light staining and ++ = strong staining.

2) 2-5 grafts per group examined at each time point.

3) ND - not detectable. Grafts appeared severely rejected with no recognisable pancreatic elements remaining.

4) From day 8 onwards very few exocrine cells were still identifiable.

TABLE 2.2

CLASS II MHC ANTIGEN EXPRESSION IN DA RAT FOETAL PANCREAS TRANSPLANTATION TO THE RENAL SUBCAPSULAR SITE[1]

Graft components	DA into DA syngeneic grafts (days)[2]				DA into PVG allografts (days)			
	4	6	8	10	4	6	8	10
Islet cells	-	-	-	-	-	-	-	ND[3]
Duct epithelium	-	-	-	-	+	++	++	ND
Exocrine cells	-	-	-	-	-	++	(++)	ND

1) Immunoperoxidase labelled sections were graded: 0 = no staining, + = light staining and ++ = strong staining.

2) 2-5 grafts per group examed at each time point.

3) ND - not detectable. Grafts severely rejected.

antigens, unequivocal determination of donor strain MHC antigen expression was possible. Antigen expression was graded as absent, weak or strongly positive. Blood vessels were only rarely identified in sections and in most cases proved to be of recipient origin, therefore no comment could be made regarding possible changes of antigen expression in donor strain blood vessels.

Class I MHC antigens

In DA isografts, a progressive increase in MHC class I antigen expression by islet cells and duct epithelium occurred. Although by day 10 both endocrine cells and duct epithelium were strongly class I positive, exocrine cells remained negative throughout (Plates 2.3A and 2.3B). Allografts of DA foetal pancreas showed a similar pattern of class I staining to that seen in isografts, with the notable exception that by day 6 the rejecting allografts showed strong staining of exocrine cells (Plate 2.4). A further difference between isografts and allografts was the more rapid development of strong staining of duct epithelium in allografts.

Class II MHC antigens

Sequential examination of DA foetal pancreas grafts showed more striking differences in class II MHC antigen expression between isografts and allografts. In DA foetal pancreas isografts, both the endocrine and exocrine structures remained class II negative in marked contrast to DA foetal pancreas

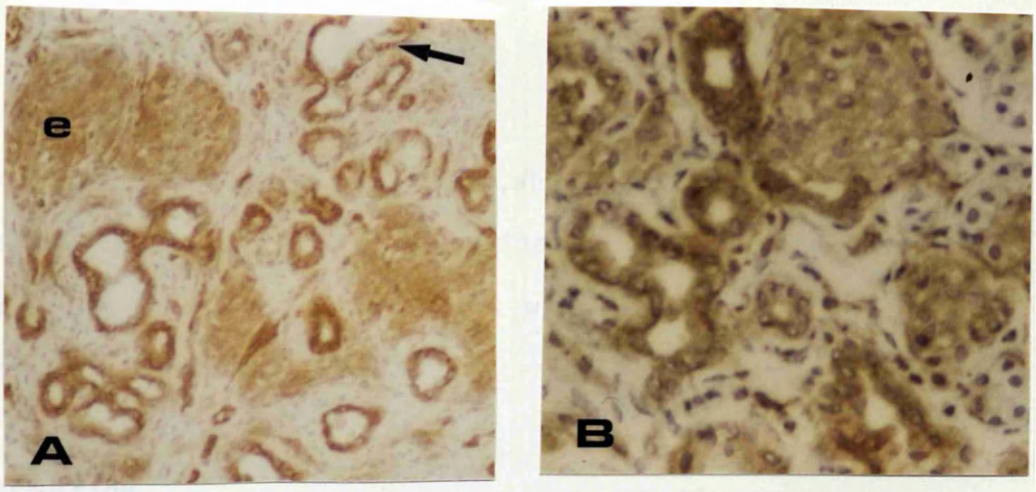

PLATE 2.3. Cryostat sections of DA foetal pancreas isograft after 6 days (A) and 10 days (B). Sections have been labelled with MN4-91-6 (anti class I). Note positive staining of developing islets (e) and duct epithelium (arrowed) with exocrine cells uniformaly negative. In the day 10 isograft (B) (x1400) very few exocrine cells remain compared with the day 6 isograft (A) (x900).

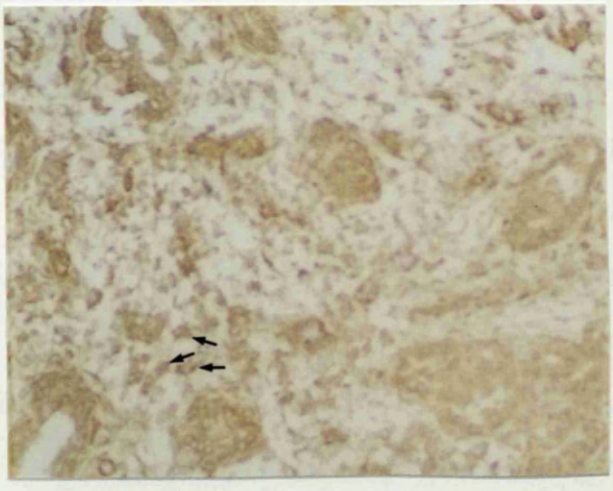

PLATE 2.4. Cryostat section of day 6 DA to PVG rejecting foetal pancreas allograft (x900). The section has been labelled with MN4-91-6 which is selective for class I of DA origin but will not label class I on infiltrating PVG cells. Note evidence of graft rejection with disruption of tissue architecture. Remaining recognisable duct epithelium and islet tissue is class I positive and evidence of class I expression on exocrine cells can also be seen (arrowed).

transplanted into PVG recipients where, by day six, there was strong staining of both duct epithelium and exocrine cells with F17-23-2, although islet cells were consistently class II negative (Plates 2.5 and 2.6).

DISCUSSION

DA foetal pancreas transplanted to PVG recipients showed evidence of rapid rejection on both gross inspection and histological examination. This rejection was accompanied by changes in MHC antigen expression in the transplanted tissue especially class II expression. The pattern of MHC expression in rejecting foetal pancreas allografts was of particular interest in view of the reported differences in immunogenicity between some types of foetal and adult tissue allografts (Billingham & Silvers 1964, Heslop et al 1973). Although at the time of transplantation there was no constitutive MHC class II expression by either the endocrine or exocrine elements of foetal pancreas, during the course of unmodified rejection marked induction of class II MHC antigens on both duct epithelium and residual exocrine cells was seen. Endocrine cells within the rejecting foetal pancreas became class I positive but were never observed to express detectable class II MHC antigens.

This pattern of MHC induction is comparable to that described for adult vascularised rat pancreas allografts during rejection (Steiniger et al 1985) and clearly demonstrates that the exocrine components of foetal pancreas possess the capacity

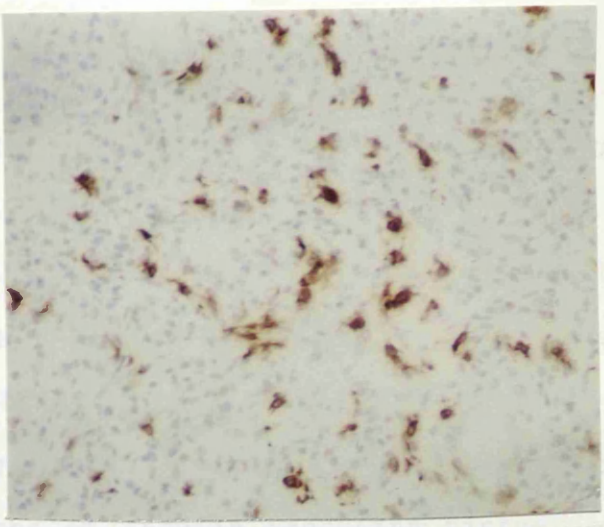

PLATE 2.5. Cryostat section of 6 day DA foetal pancreas isograft labelled with F17-23-2 (anti-class II) (x900). Only a scattered population of densely staining interstitial cells are class II positive.

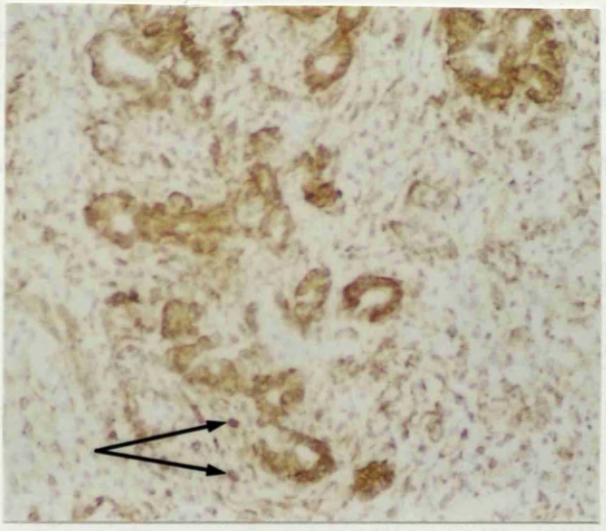

PLATE 2.6. Cryostat section of 6 day DA to PVG foetal pancreas allograft labelled with F17-23-2. This antibody is selective for class II of DA (donor) origin and will not label recipient-strain infiltrating cells. Duct epithelium and scattered exocrine cells (arrowed) are seen to express class II antigens. Evidence of rejection is apparent with disruption of tissue architecture.

to express MHC antigens in response to the stimulus of the host immune response. The presence of exocrine cells with the ability to express class II MHC antigens in foetal pancreas represents a potentially important difference between foetal pancreas and isolated islet cell preparations. This may partly account for the reported differences in immunogenicity between these two sources of insulin producing tissue and for the relative resistance of foetal pancreas to immunomodulation by organ culture prior to transplantation (Simeonovic et al 1980, Garvey et al 1980a). Interestingly, crude mouse islet preparations containing lymphoid aggregates, vascular tissue and duct fragments appear to be significantly more immunogenic than those which have been highly purified (Gotoh et al 1986).

The changes in antigen expression seen following isograft and allograft transplantation are similar to those described in the previous chapter for control and IFN-gamma-treated cultured foetal pancreas and suggest a possible role for this lymphokine in the altered antigen expression seen in the allografted pancreas.

SUMMARY

1) DA foetal pancreas allografts are rapidly rejected by unmodified PVG recipients.

2) MHC class I antigen expression is increased on the islet cells and duct epithelium of isografts and on the islet cells, duct epithelium and exocrine cells of unmodified allografts.

3) MHC class II antigen expression remains unchanged in isografts. In allografts, de novo class II expression is induced on duct epithelium and exocrine cells but not islet cells.

CHAPTER 3

THE EFFECT OF CYCLOSPORIN A ON DA FOETAL PANCREAS ALLOGRAFTS

INTRODUCTION

The fungal metabolite Cyclosporin A is now firmly established as a potent immunosuppressant with proven efficacy in a broad range of clinical and experimental organ transplantations. However, the prevention of rejection of transplanted pancreas, particularly islets and foetal pancreas in the rat would seem to be more difficult to achieve. Even doses as high as 40mg/kg/day have been reported to produce only very slightly prolonged survival of DA islets transplanted to Lewis (LEW) recipients (Morris et al 1980). Results from vascularised pancreatic grafts have been a little more encouraging (Rynasiewicz et al 1980, Morris et al 1980) but only a marginal prolongation of survival was reported for foetal pancreas transplants (Garvey et al 1980b).

A more recent study using the DA to Lewis strain combination has suggested that the pessimism generated by the results of the earlier studies may have been misplaced. Using highly purified hand-picked islet preparations with Cyclosporin A given intramuscularly in a dose of 30mg/kg/day for the first three days, Dibelius et al (1986) have shown prolonged survival of transplanted islets with results better than for vascularised grafts in the same strain combinations. If Cyclosporin A does indeed prolong the survival of vascularised pancreas and

isolated islets in strong responder strain combinations, then the absence of an effect on foetal pancreas survival is puzzling and demands a reappraisal of the available evidence.

In the study by Garvey et al (1980) survival of foetal pancreas grafts was assessed using functional criteria based on the restoration of euglycaemia in rats with chemically-induced diabetes. Mullen and Shintaku (1980) have emphasised the delay before diabetes could be expected to be improved when foetal pancreas glands are transplanted, with several weeks sometimes necessary before the foetal pancreas matures sufficiently to cope with a hyperglycaemic challenge. In addition Cyclosporin A has toxic effects on pancreatic beta cell function, shown in vitro (Nielsen et al 1986, Robertson et al 1986) and in vivo (Gunnarsson et al 1984, Basadonna et al 1986, van Schilfgaarde et al 1986, Otsu et al 1987) and it may also inhibit the vascular ingrowth required when free grafts such as islets and foetal pancreas are transplanted (Rooth et al 1988).

To overcome some of these difficulties, assessment of graft survival in this study has been based on gross inspection and histological examination with measurement of insulin content of grafts also carried out in certain instances. When these methods of assessment are employed the use of diabetic recipients carries no advantage and the diabetes may influence host immune responses (Mullen and Shintaku 1980). Therefore non-diabetic recipients were used, and the DA to PVG strain combination was again chosen to allow for unequivocal determination of donor strain antigen expression with the same monoclonal antibodies used in previous experiments.

MATERIALS AND METHODS

Experimental Groups

DA foetal pancreas glands (17-day gestation) were transplanted to the renal subcapsular site of male non-diabetic recipient rats. Four glands per recipient were transplanted to each animal in the following groups.

1) PVG unmodified (n=18)
2) PVG given continuous daily Cyclosporin A (n=32)
3) PVG given 14 days Cyclosporin A (n=6)
4) LEW unmodified (n=4)
5) LEW given continuous daily Cyclosporin A (n=15)

Cyclosporin A Administration

Cyclosporin A was given by gavage once a day in a dose of 15mg/kg/day.

Graft Assessment

Recipient animals were sacrificed at intervals from 4 to 40 days after transplantation. The gross appearance of the transplanted pancreas was noted and in most cases a portion of the recipient kidney with the attached foetal pancreas graft was excised and processed for both monoclonal antibody labelling of cryostat sections (as previously described) and Haematoxylin and Eosin staining and polyclonal anti-insulin labelling of paraffin sections.

Insulin content

In three of the animals in Group 1 and 5 of the animals from Group 2 the insulin content of the transplanted pancreas was measured. Animals in Group 1 were sacrificed 14 days after transplantation and in Group 2 after 40 days. Following sacrifice the left kidney with attached transplanted pancreas was excised, chilled in liquid nitrogen and crushed to a powder. The powdered tissue was carefully washed into a 25ml glass container filled with phosphoric acid alcohol and extraction allowed to occur for 24 hours at $4^{o}C$. Aliquots of filtered supernatant were diluted in a phosphate albumen buffer 1:4 and stored at $-20^{o}C$. Immunoreactive insulin concentration was subsequently measured using a radioimmunoassay. Results were expressed as insulin content per kidney and compared with measurements from the right kidney (which had not received a graft) for each animal. Measured insulin content of the right kidney was subtracted from the measured content of the left kidney plus graft to give a value for the insulin content of the transplanted pancreas.

Quantitative Analysis of Cellular Infiltrate

Cryostat sections from three groups of recipients of foetal pancreas grafts sacrificed at six days post-transplant were labelled with the following monoclonal antibodies using the immunoperoxidase technique.

MRC OX1 - Leucocyte Common Antigen

MRC OX8 - Cytotoxic/suppressor T cells and NK cells

W3/25 - T helper cells and some macrophages

MRC OX19 - T cells and thymocytes

MRC OX39 - IL-2 receptor

MRC OX21 - Negative control

Morphometric analysis using the point counting technique (see Section 2) was used and results expressed as percentage of area infiltrated by positive cells.

RESULTS

Sixteen of 54 rats treated with Cyclosporin A died between the third and thirty-fifth day post-transplantation (median 9 days). At autopsy, evidence of marked weight loss and lung changes consistent with microaspiration of the olive oil vehicle was consistently found. In all cases there was apparently normal growth of pancreatic grafts. Lewis rats appeared to be particularly susceptible to the adverse effects of Cyclosporin A with a mortality of 53% compared with 20% in PVG recipients.

These 16 rats were not included in further analysis.

Effect of Cyclosporin A on Foetal Pancreas Allograft Survival

Group 1

Gross examination of unmodiifed allografts from day six onwards revealed obvious evidence of rejection with oedema and loss of graft vascularity. Rejection was confirmed

histologically and in none of the four grafts examined at day 10 was there any recognisable pancreatic elements remaining (Plate 3.1)

Group 2

By contrast, DA foetal pancreas allografts transplanted into PVG recipients receiving Cyclosporin A showed evidence of early growth and maturation of grafts. All grafts examined histologically at 35 days (n=5) or 40 days (n=4) after transplantation into Cyclosporin A treated recipients showed scattered areas of islet tissue amid a background of prominent dilated ducts (Plate 3.2A). The presence of numerous areas of insulin containing endocrine tissue was confirmed in all the long surviving allografts (day 35 and 40) by anti-insulin antibody labelling of paraffin sections (Plate 3.2B).

The remainder of animals in this group were either sacrificed at intervals from day 6 to 21 and the pattern of antigen expression determined (n=11) or sacrificed at day 40 for measurement of graft insulin content (n=5). No gross evidence of rejection was seen in any of these animals.

Group 3

In 6 PVG recipients of DA allografts, Cyclosporin A treatment was stopped after 14 days. In all grafts, histological examination revealed evidence of marked rejection with no recognisable pancreatic elements surviving beyond 10 days after cessation of immunosuppression.

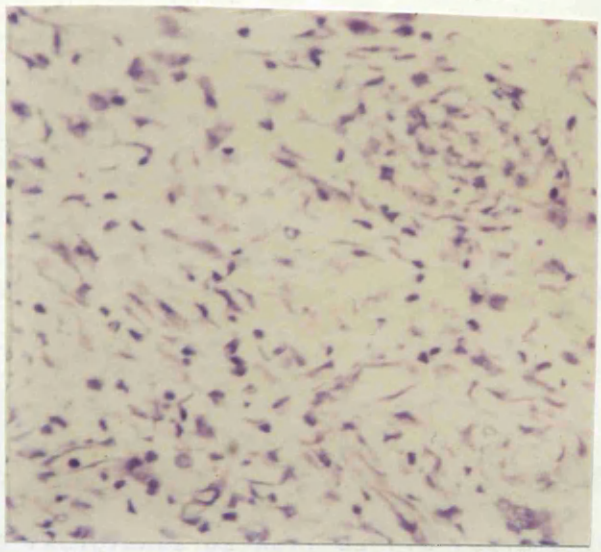

PLATE 3.1. Paraffin section of unmodified rejecting DA to PVG foetal pancreas allograft at day 10 stained with H+E (x900). No recognisable pancreatic elements remain.

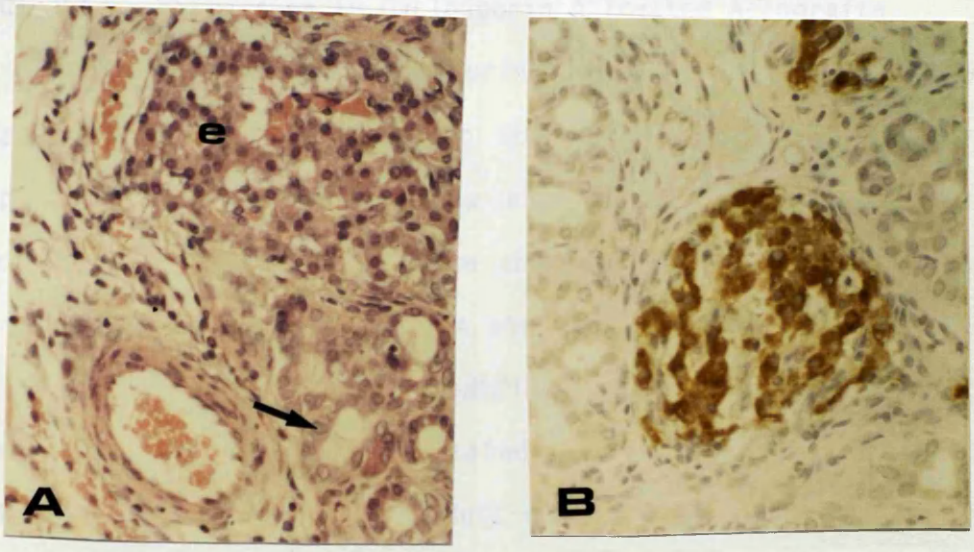

PLATE 3.2. Paraffin sections of DA foetal pancreas allograft after 40 days in a PVG recipient treated with Cyclosporin A. Haematoxylin and eosin staining (A) (x900) shows evidence of well preserved morphology with recognisable endocrine areas (e) and duct epithelium (arrowed). Labelling with an anti-insulin antibody (B) (x900) confirms the presence of insulin-containing endocrine tissue.

Group 4

In none of four Lewis recipients of DA allografts was any evidence of surviving pancreas detected 10 days after transplantation.

Group 5

Cyclosporin A effectively prevented rejection of DA foetal pancreas in Lewis recipients as well as PVG recipients. In all seven surviving Lewis rats sacrificed at day 40, grafts appeared well vascularised and healthy. Histological confirmation of this absence of rejection was obtained in all cases with appearances identical to Plates 3.2A and 3.2B).

MHC Antigen Expression in Cyclosporin A Treated Allografts

Administration of Cyclosporin A resulted in graft antigen expression closely resembling that of isografts with class I expression on endocrine cells and duct epithelium but not exocrine cells (Plate 3.3). The characteristic pattern of class II induction on duct epithelium and exocrine cells demonstrated by F17-23-2 labelling on unmodified allografts (see Chapter 2) was absent in Cyclosporin A treated allografts (Plate 3.4) in which, like syngeneic grafts, duct epithelium and exocrine cells remained class II negative even in allografts examined 35 and 40 days after transplantation.

Within 14 days of transplantation, in Cyclosporin A treated allografts only very occasional interstitial cells expressing donor strain class II antigens could be seen, the remainder having presumably been destroyed in situ or migrated

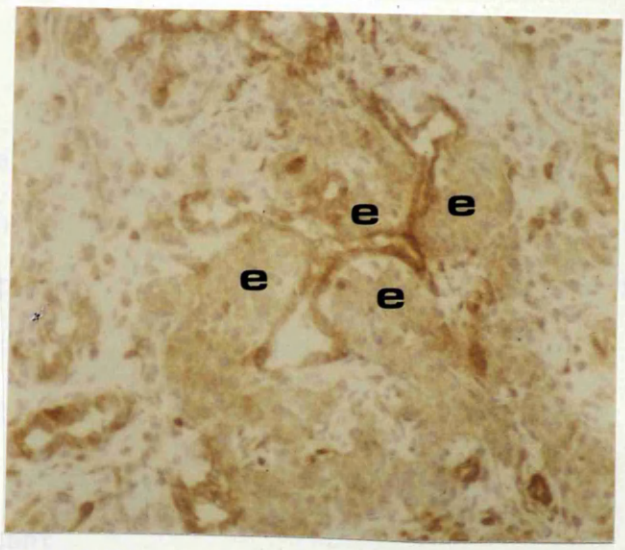

PLATE 3.3. Cryostat section of 6 day DA foetal pancreas allograft from a PVG recipient treated with Cyclosporin A (x900). The section has been labelled with MN4-91-6 which labels class I antigen of DA strain but not PVG strain. Note class I positive groups of endocrine cells (e) and duct epithelium. Exocrine cells are class I negative.

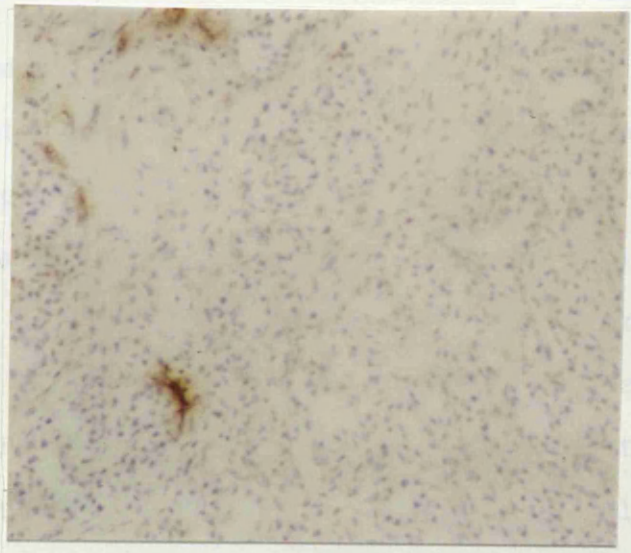

PLATE 3.4. Cryostat section of 6 day DA foetal pancreas allograft from a PVG recipient treated with Cyclosporin A (x900). The section has been labelled with F17-23-2 which labels class II antigen of DA strain but not PVG strain. Only very occasional interstitial cells are class II positive.

from the graft.

Examination of the rejecting DA foetal pancreas allografts in PVG recipients whose Cyclosporin A was discontinued after 14 days, revealed strong staining of duct epithelium with the F17-23-2 antibody within six days of stopping therapy.

Insulin Content

No insulin was detected in any of the three grafts from unmodified allograft recipients in group 1 (limit of detection = 0.25mU/graft).

By contrast the five grafts from the Cyclosporin A treated allograft recipients (Group 2) in which insulin was measured had insulin contents between 5.7 and 434.8mU/graft (median 26.7mU/graft).

Magnitude and Phenotype of Mononuclear Cellular Infiltrate

DA foetal pancreas allografts were excised from unmodiifed and Cyclosporin A treated recipients six days after transplantation and the magnitude and phenotype of the mononuclear cell infiltrate was determined by morphometric analysis of immunoperoxidase labelled cryostat sections. The results of this analysis, expressed as the percentage area of graft infiltrated by cells, is shown in Table 3.1 (a group of PVG recipients of PVG foetal pancreas isografts is included for comparison).

Cyclosporin A affected both the magnitude and the phenotype of the cellular infiltrate. Day six rejecting

TABLE 3.I

MAGNITUDE AND PHENOTYPE OF CELLULAR INFILTRATE IN RAT FOETAL PANCREAS TRANSPLANTS[1]

Experimental Group	MRC OX-1 (all leukocytes)	MRC OX-8 ($T_{c/s}$, NK NK cells)	W3/25 (T_h, macropahges	MRC OX-19 (T lympho cytes)
DA to PVG untreated	43 (40-47)	12 (10-12)	14 (13-19)	2 (1-2)
DA to PVG CyA (15mg/kg/day)	10 (10-10)	1 (1-1)	7 (5-8)	1 (1-1)
PVG to PVG	7 (6-7)	<1	4 (4-5)	<1

1) Grafts excised 6 days after transplantation were examined by morphometric analysis of immunoperoxidase labelled cryostat sections.

2) Results are median and range of 3 grafts examined.

allografts showed a heavy OX-1 positive (leukocyte common antigen) mononuclear infiltrate (median 43%) whereas allografts from day six Cyclosporin A treated recipients showed a markedly reduced infiltrate (median 10%) which was only slightly greater than that observed in day six syngeneic foetal pancreas transplants.

Phenotypic analysis of the infiltrate showed that rejecting allografts contained a much heavier infiltrate of OX-8 positive cells ($T_{c/s}$ and NK cells) than Cyclosporin A treated grafts and an increased number of W3/25 positive cells (T_h and macrophages). In both the rejecting and Cyclosporin A treated allografts the percentage of OX-19 positive (pan T cell) cells was low, indicating the presence of large numbers of NK cells (MRC OX-8 positive, MRC OX-19 negative) and macrophages (W3/25 positive, MRC OX-19 negative) and/or weakly labelled MRC OX-19 positive cells which were not apparent using the immunoperoxidase staining technique. Labelling sections with MRC OX-39 (IL-2 receptor) showed that rejecting allografts contained scattered, strongly positive cells (approximately 1% area infiltrated) whereas these cells were virtually absent from Cyclosporin A treated allografts and syngeneic grafts.

DISCUSSION

These experiments have shown that Cyclosporin A, when given in a dose of 15mg/kg/day on a continuous daily basis, will prevent the rejection of DA foetal pancreas transplanted to the renal subcapsular site of PVG and Lewis recipients. By using

histological examination and direct quantification of graft insulin content rather than functional criteria as measurements of graft viability the possible confusing effects of graft immaturity and the beta cell toxicity of Cyclosporin A have been avoided.

This perceived beneficial effect of Cyclosporin A in rat foetal pancreas transplantation would appear to be at variance with results reported by Garvey et al (1980). In their study, however, a failure to reverse hyperglycaemia in diabetic recipients was interpreted as evidence of graft rejection rather than functional immaturity of the transplanted pancreas and the possibility that grafts survived until at least day 14 (when Cyclosporin A was stopped) was not excluded. Continuous administration of Cyclosporin A appears to be necessary for prolonged foetal pancreas allograft survival as withdrawal of this drug after 14 days resulted in rapid rejection of all grafts. Although in a previous report by Dibelius et al (1986) three days Cyclosporin A was sufficient to prolong the survival of islet allografts, the method of administration (intramuscular injection) appeared to result in a depot effect with moderate levels of the drug detectable in the blood some 70 days later.

As well as prolonging graft survival, Cyclosporin A markedly altered the pattern of MHC expression within allografts to one resembling that of isografts and prevented induction of class II MHC antigen expression on the exocrine components of the graft. This effect of Cyclosporin A is consistent with the inhibited MHC induction described in other transplanted organs

(Milton et al 1986) and might be expected to contribute to this drug's immunosuppressive action by preventing an increase in the target antigen density within the graft.

Cyclosporin A also limited the mononuclear cellular infiltration of foetal pancreas allografts and, in particular, prevented the infiltration of the numerous MRC OX-8 positive cells seen in rejecting grafts. This finding, which is in agreement with a report that Cyclosporin A treatment prevents the accumulation of MRC OX-8 positive cells in rat renal allografts (McWhinnie, Dallman & Morris 1987), suggests that MRC OX-8 positive cells may play a role in graft rejection. The absence of a significant increase in the number of MRC OX19 positive cells in the cellular infiltrate of rejecting foetal pancreas grafts would be consistent with the suggestion that a small number of activated T cells initiates the rejection response and that tissue damage is mediated by the recruitment of large numbers of non-specific effector cells such as MRC OX-8 positive NK cells and W3/25 positive macrophages. However, it is not possible to exclude the alternative explanation that graft damage is mediated principally by a small number of activated T cells and that large numbers of non-specific cells are recruited as a secondary response to tissue injury.

The high mortality in the experimental groups treated with Cyclosporin A was a worrying observation and has been reported previously (Rynasiewicz et al 1980). However toxicity may have been related more to the method of administration resulting in microaspiration of the olive oil vehicle than a particular pharmacological effect of the Cyclosporin A itself.

Although, rejection would appear to be more difficult to prevent in foetal pancreas allografts than in renal allografts, Cyclosporin A does show some promise in this regard. Continuous administration may be necessary, however, and in view of the reported toxicity of this agent it would be desirable to limit the total dose administered. Combining techniques of in vitro graft immunomodulation with lower doses of Cyclosporin A may eventually prove beneficial in preventing foetal pancreas rejection and merits further investigation.

SUMMARY

1) Cyclosporin A prevents the rejection of DA foetal pancreas in PVG and Lewis recipients.

2) Stopping Cyclosporin A after 14 days results in rapid rejection of foetal pancreas allografts.

3) Cyclosporin A treatment of allograft recipients alters antigen expression in foetal pancreas grafts to a pattern resembling that of isografts.

4) Cyclosporin A affects both the magnitude and phenotype of the cellular infiltrate.

5) In grafts treated with Cyclosporin A only very occasional donor-strain class II positive interstitial cells remain after 14 days.

CHAPTER 4

INDUCTION OF MHC ANTIGEN EXPRESSION ON RECIPIENT RENAL TUBULES ADJACENT TO FOETAL PANCREAS ALLOGRAFTS

INTRODUCTION

In normal PVG strain kidney, weak class I antigen expression can be detected on all tubular cells using the previously described monoclonal antibody labelling techniques. MHC class II expression is limited to the basal portions of the cells of the proximal convoluted tubules.

In the course of examining the distribution of MHC antigen expression in foetal pancreas allografts, unambiguous induction of both class I and class II MHC antigen expression was observed on the tubules of the renal cortex adjacent to rejecting foetal pancreas allografts.

Several possible explanations can be postulated to account for this apparent increased expression of recipient MHC antigens. Firstly, release of lymphokines, such as IFN-gamma by activated infiltrating cells in the region of the renal tubules en route to the foreign pancreatic tissue, could lead to induction of recipient strain antigen expression. Secondly, adsorption of shed antigen from damaged pancreatic cells by renal tubular cells could produce a labelling pattern suggesting induction of antigen expression by tubular cells. A further possibility suggested by the occasional finding of closely-associated lymphoid tissue on histological examination of foetal

pancreas is that the observed induction of renal tubular MHC antigen expression may have been in response to a localised graft-versus-host reaction.

To examine these hypotheses a study of labelling patterns of recipient kidney tubules was conducted using polymorphic monoclonal antibodies specific for donor strain antigens in addition to monomorphic monoclonal antibodies which labelled both donor and recipient strain antigen. This technique therefore allowed unequivocal differentiation of donor and recipient strain antigen expression.

In considering the possibility of a localised graft-versus-host reaction being responsible, further experiments were carried out in which foetal pancreas glands from DA donors were transplanted into (DAxPVG) F1 recipients (giving the potential for graft versus host reaction but not for graft rejection) and from (DAxPVG) F1 donors into PVG recipients (giving the potential for graft rejection but not a graft-versus-host reaction.

MATERIALS AND METHODS

Foetal pancreas transplants were carried out in an identical manner to that described in previous chapters.

As the induction of both MHC class I and class II antigen expression on renal tubules was originally observed to be maximal on the sixth post-transplant day, this was chosen as the time of sacrifice in most instances. In order to exclude the possibility that the kinetics of antigen expression would be

different when graft-versus-host reaction without rejection was present animals in this group were also sacrificed at days 4, 8, 10, 12 and 14.

Following sacrifice cryostat sections of recipient kidney with adjacent foetal pancreas grafts were labelled with the same monoclonal antibody technique as previously described. The polymorphic antibodies labelling class I and class II antigens of DA strain were MN4-91-6 and F17-23-2 respectively. MRC OX-18 and MRC OX-6 labelled class I and class II antigens of both donor and recipient strain.

Groups

1) DA to PVG (n=5) sacrificed day 6.
2) DA to DA (n=3) sacrificed day 6.
3) DA to (DAxPVG) F1 (n=12) sacrificed days 4-14.
4) (DA x PVG) F1 to DA (n=4) sacrificed day 6.

RESULTS

All sections examined from animals in Group 1 showed obvious evidence of induction of both class I and class II MHC antigen expression on the renal tubules immediately underlying the rejecting allograft, up to a total distance of approximately three to four tubular diameters (Plates 4.1 and 4.3). This increased renal tubular expression was only seen using the monomorphic anti-class I (MRC OX-18) and anti-class II (MRC OX-6) antibodies and not the polymorphic anti-class I (MN4-91-6) and anti-class II (F17-23-2) antibodies (Plates 4.2 and 4.4)

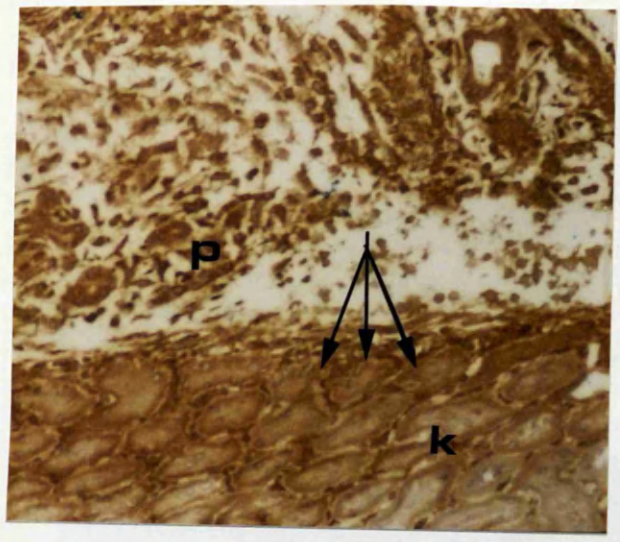

PLATE 4.1. Cryostat section of rejecting DA foetal pancreas transplant (p) and adjacent renal cortex (k) 6 days after transplant (x1400). The section has been labelled with MRC OX18 (anti-class I). Note strong induction of class I antigen expression on recipient renal tubular cells (arrowed) adjacent to rejecting allograft.

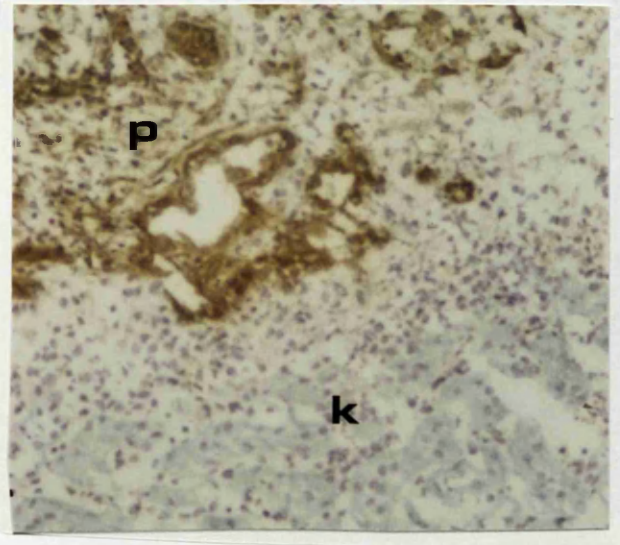

PLATE 4.2. Cryostat section of rejecting DA foetal pancreas transplant (p) and adjacent renal cortex (k) 6 days after transplant (x1400). The section has been labelled with MN4-91-6 (anti-class I) which labels DA antigen but not PVG antigen. Note absence of class I expression of donor origin on renal tubular cells confirming that tubular induction in Plate 4.1 is not due to adsorbed donor MHC antigen.

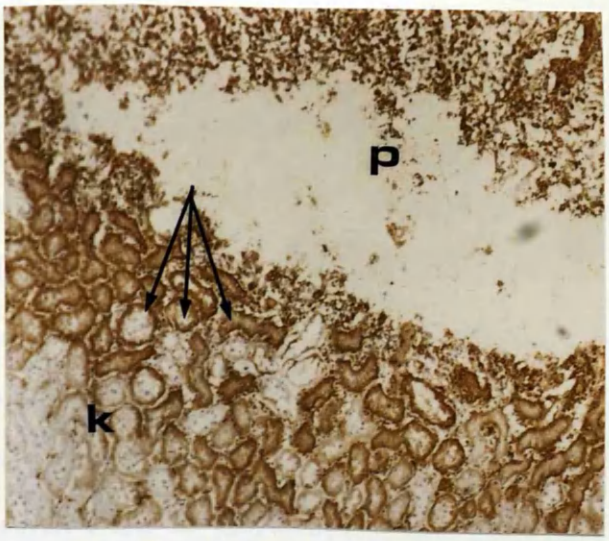

PLATE 4.3. Cryostat section of rejecting DA foetal rat pancreas transplant (p) and adjacent renal cortex (k) 6 days after transplant (x900). The section has been labelled with MRC OX6 (anti-class II). Note evidence of strong induction of class II antigens on recipient tubular cells (arrowed) adjacent to rejecting allograft.

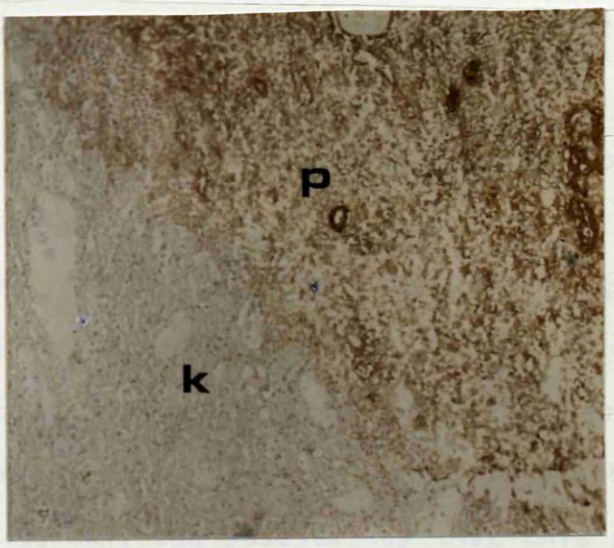

PLATE 4.4. Cryostat section of rejecting DA foetal pancreas transplant (p) and adjacent renal cortex (k) 6 days after transplant (x900). The section has been labelled with F17-23-2 which labels only DA strain class II. Note absence of class II expression of donor origin on renal tubular cells, confirming that tubular induction in Plate 4.3 is not due to adsorbed donor MHC antigen.

indicating that it represented induction of recipient strain MHC antigens, rather than adsorption of donor MHC antigens shed from the graft. In the DA to DA isografts (Group 2) there was no evidence of increased renal tubular expression of either class I or class II antigens thus excluding the possibility that the induced antigen expression was secondary to the trauma of the grafting procedure.

In the DA to (DAxPVG) F1 combination (Group 3) at no time was any induction of renal tubular antigen expression detected even in areas of kidney adjacent to lymphoid tissue transplanted with the foetal pancreas. On the other hand, in the (DAxPVG) F1 to DA combination (Group 4), the renal tubules adjacent to the transplanted pancreas in all four animals sacrificed at day six showed unequivocal evidence of induced expression of MHC class I and class II antigens.

Therefore, whereas a localised graft-versus-host reaction in the absence of rejection does not lead to the induction of antigen expression on recipient kidney tubules, allograft rejection without any graft-versus-host reaction is sufficient to induce antigen expression on the underlying tubules. However, although a localised graft-versus-host reaction is not sufficient on its own, the possibility that this reaction is additive or synergistic with rejection in inducing tubular antigen expression, has not been excluded.

An additional observation lends support to the suggestion that a limited local graft-versus-host reaction does in fact take place. Of the Group 3 animals (with the potential for

graft versus host reaction but not graft rejection), in one of four animals sacrificed at day six and in one of three sacrificed at day eight, although there was no evidence of tissue damage, induction of MHC class II antigen expression was observed on several pancreatic ducts in small areas of the graft immediately adjacent to lymphoid collections, while ducts elsewhere in the same cryostat section remained class II negative (Plates 4.5 and 4.6). This effect appeared rather weak, however, with some ducts containing both class II positive and class II negative cells.

DISCUSSION

Induction of MHC antigen expression within rejecting foetal pancreas grafts occurs in association with a heavy mononuclear cell infiltrate and is presumably mediated by IFN-gamma and other lymphokines released by activated graft infiltrating cells. It is likely that induction of MHC antigen expression on the allograft recipient's renal tubules is due to the same mechanism with either diffusion of released lymphokines from the transplanted pancreas to the underlying kidney or release of lymphokines within the kidney itself from activated infiltrating cells en route to the overlying pancreas allograft.

The finding that graft infiltrating cells and their lymphokines were able to induce MHC class II antigens in the recipient renal cortex but not in the endocrine cells within the graft is of note and emphasises the apparent resistance of islet cells to class II inducing stimuli (Pujol-Borrell et al 1986 and

1987).

A localised graft-versus-host reaction was not required for induction of antigen expression on recipient tubules and when the potential for this reaction without graft rejection was present (Group 3), no induction of MHC antigen expression was seen on recipient tubular cells. Although induction of MHC class II antigen expression was seen on pancreatic ducts close to lymphoid collections in a few examples of parent strain to F1 transplants, this effect was very localised and appeared unlikely to significantly effect graft immunogenicity. If, as has been suggested (Simeonovic et al 1984), lymphoid tissue, closely associated with the foetal pancreas does in fact significantly contribute to graft immunogenicity it would seem more likely that this is due to an increase in available target antigens for recipient effector mechanisms rather than increased antigen expression on the foetal pancreas itself as a consequence of a localised graft-versus-host reaction.

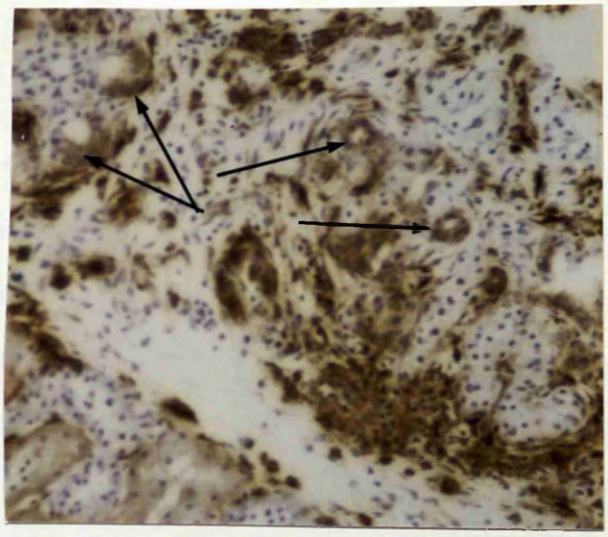

PLATE 4.5. Cryostat section of day 6 DA to (DAxPVG)F1 foetal pancreas transplant, labelled with MRC OX6 (anti-class II) (x1400). In an area of pancreas close to a collection of lymphoid tissue (not shown) evidence of class II expression on duct epithelial cells can be seen (arrowed).

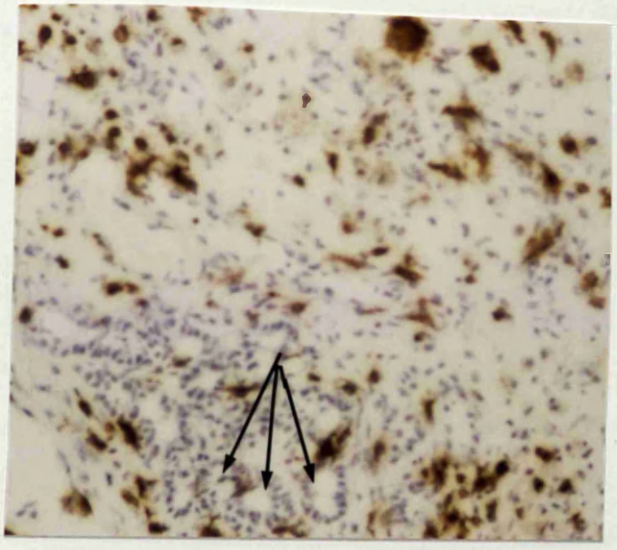

PLATE 4.6. Cryostat section from day 6 DA to (DAxPVG)F1 foetal pancreas transplant (same animal as Plate 4.5), labelled with MRC OX6 (x1400). Elsewhere in the same section duct epithelium remains class II negative (arrowed).

SUMMARY

1) An increase in recipient strain MHC class I and class II antigen expression is induced on the renal tubular cells immediately adjacent to a foetal pancreas allograft.

2) This induced expression was a consequence of the rejection response rather than a manifestation of a localised graft-versus-host reaction.

3) There is suggestive evidence that a very limited graft-versus-host reaction may occur in relation to areas of associated lymphoid tissue transplanted along with the foetal pancreas.

CHAPTER 5

THE EFFECT OF INTERFERON GAMMA ADMINISTRATION ON MHC ANTIGEN EXPRESSION IN THE ADULT RAT PANCREAS

INTRODUCTION

Tissue levels of MHC class I and class II antigen expression vary, in both extent and distribution, to a considerable degree under a variety of different circumstances (reviewed by Fabre et al 1987). Modification of antigen expression may play an important part in the regulation of immune responses and following transplantation the increase in both class I and class II antigen expression may help to determine the fate of the graft. In particular, increased class II antigen expression has been shown to result in an increased antigen-presenting ability of cultured endothelial cells with the immunogenic capacity of these cells seeming to be proportional to the extent of MHC antigen expression (Ferry et al 1987).

It has been suggested that the observed increase in antigen expression following transplantation represents a response to local release of lymphokines and although other similar but distinct lymphokines may have analogous effects (Groenewegen et al 1986), interferon-gamma (IFN-gamma) is thought to be particularly important (Halloran et al 1986, Milton et al 1986b). Supporting the notion that increased antigen expression reflects the local response to a lymphokine

is the observed distribution of the increased antigen expression on host renal tubular cells following subcapsular foetal pancreas transplantation (Chapter 4).

The reduction in class II antigen expression in animals treated with Cyclosporin A (Milton et al 1986b, Romaniuk et al 1987b) may at least in part be due to a decrease in stimulation by IFN-gamma as, although Cyclosporin A has no direct effect on decreasing the MHC expression of cultured cells (Bishop et al 1986), it has been shown that this agent will inhibit the release of IFN-gamma by T-lymphocytes (Reem et al 1983).

Systemic administration of recombinant IFN-gamma to mice and rats produces a general increase in the levels of class I and class II MHC antigen expression throughout the body, with induction of antigen expression being particularly marked in cells which normally express low levels of these antigens in the resting state (Skoskiewicz et al 1985, Leszczynski et al 1986, Hayry et al 1986). This response to IFN-gamma, as well as differing according to the tissue examined is also subject to a considerable degree of inter-species variation (Skoskiewicz et al 1985). In the experiments described below alterations in the antigen expression in the various components of adult rat pancreas are examined, following systemic administration of IFN-gamma.

MATERIALS AND METHODS

Experiment 1

Male DA rats weighing 200-250g were injected intraperitoneally with IFN-gamma each day for six days. Following preliminary studies using various doses, a standard dose of 1 x 10^6U/kg was found to produce maximal changes in MHC antigen expression and this dose was used throughout.

Rats were sacrificed on days 6, 9 and 21 (three at each time point) and cryostat sections of pancreas were labelled with anti-class I monoclonal antibodies (MRC OX18 and MN4-91-6) and anti-class II monoclonal antibodies (MRC OX6 and F17-23-2). Staining was carried out using the immunoperoxidase methods and diaminobenzidine substrate with a light counter stain of Harris' haematoxylin. Sections were then examined using a light microscope and MHC class I and class II antigen expression on the different cellular components of the rat pancreas was compared for each time point and appropriate photographs taken.

Experiment 2

A single intraperitoneal dose of rat recombinant IFN-gamma was administered to male DA rats weighing 200-250g. Rats were killed three days later, the pancreas excised and 5 micron thick cryostat sections prepared as previously described. Subsequently, labelling with MRC OX6 monoclonal antibody (anti-class II) was carried out with diaminobenzidine used as a substrate in the immunoperoxidase technique.

From each frozen block of pancreas, four sections were

taken in a random manner, and 20 high power fields examined in each section. Counting was carried out blindly on coded slides to avoid observer bias and the code was only broken when all counting was complete. The number of OX 6 positive cells with dendritic morphology was counted with the eventual result expressed as class II positive cells per mm^2.

Groups were as follows, with three animals in each group.

Group 1 - Control

Group 2 - 1 x 10^4U/kg IFN-gamma

Group 3 - 1 x 10^5U/kg IFN-gamma

Group 4 - 1 x 10^6U/kg IFN-gamma

RESULTS

Experiment 1

MHC class I antigen expression

When OX18 and MN4-91-6 labelled slides were examined, a marked increase in class I antigen expression in response to IFN-gamma administration was apparent throughout the adult rat pancreas by day six. All exocrine and endocrine cells had become class I positive with membrane labelling of exocrine cells being particularly prominent and resulting in a lacy pattern of staining. Islets could be picked out on labelled sections as more lightly stained homogeneous collections of positively-labelled cells with much less prominent membrane staining. Duct epithelium, vascular endothelium and vascular

smooth muscle were all strongly positive for class I expression (Plates 5.1 and 5.2).

By day nine all the previously positively-labelled pancreatic structures continued to express class I antigens although staining of vascular smooth muscle was much less marked (Plates 5.3 and 5.4).

By day 21, exocrine cells were only very lightly stained but a lacy pattern of membrane staining persisted. Islets were prominent with the endocrine cells now expressing class I antigens to a greater extent than the background exocrine cells and in this respect resembling the sections from the untreated control animals, although the actual intensity of class I labelling of islet cells remained greater than in controls. (Plate 5.5).

MHC class II antigen expression

Examination of OX6 and F17-23-2 labelled sections revealed a very different response to stimulation by intraperitoneal IFN-gamma. Endocrine, exocrine and smooth muscle cells all failed to show evidence of induction of MHC class II antigen expression at day 6, 9 and 21. However, vascular endothelium did become strongly class II positive by day 6 although this was a very short lived effect which could no longer be observed in the day 9 sections (Plates 5.6 and 5.7).

Experiment 2

The effect of various doses of intraperitoneal IFN-gamma on the density of class II positive dendritic cells in sections

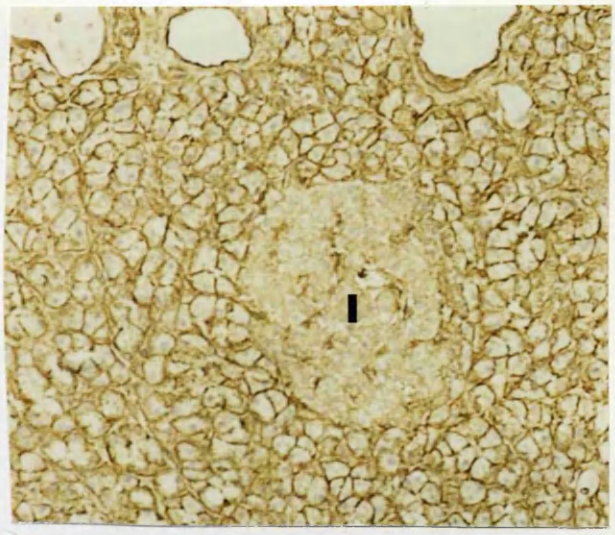

PLATE 5.1. Cryostat section of adult rat pancreas from a DA rat treated with daily intraperitoneal injections of IFN-gamma for 6 days then killed. Section has been labelled with MN4-91-6 (anti-class I) (x900). All cell types (endocrine and exocrine) have become class I positive with membrane staining less marked in islets (I).

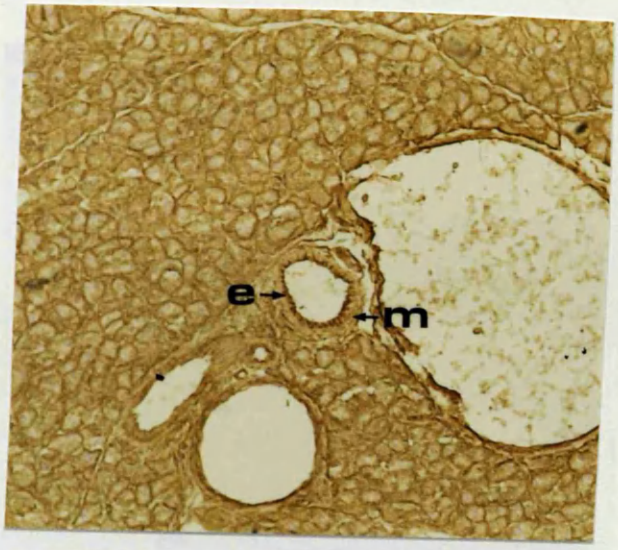

PLATE 5.2. Cryostat section of adult rat pancreas from the same animal as Plate 5.1. Section has been labelled with MRC OX18 (anti-class I) (x900). Pattern of labelling is similar to that of MN4-91-6 although staining is more intense. Note vascular endothelium (e) and smooth muscle (m) are strongly positive for class I expression.

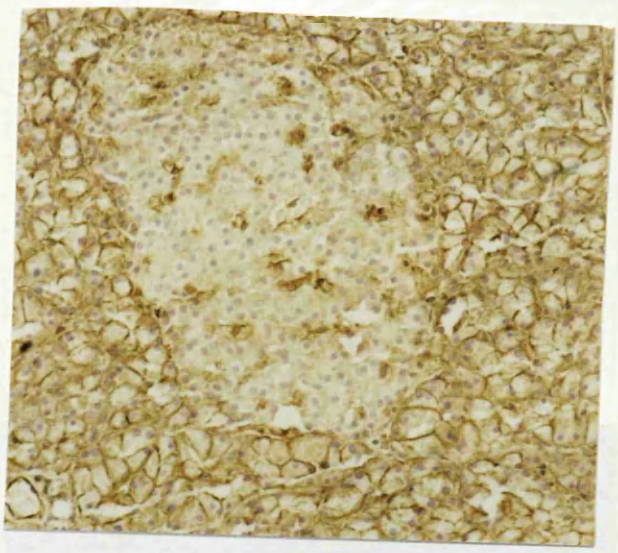

PLATE 5.3. Cryostat section of adult rat pancreas from a DA rat treated with daily intraperitoneal injections of IFN-gamma for 6 days and then killed 3 days later. Section has been labelled with MN4-91-6 (anti-class I) (x900). Note all cell types remain class I positive with similar appearances to Plate 5.1.

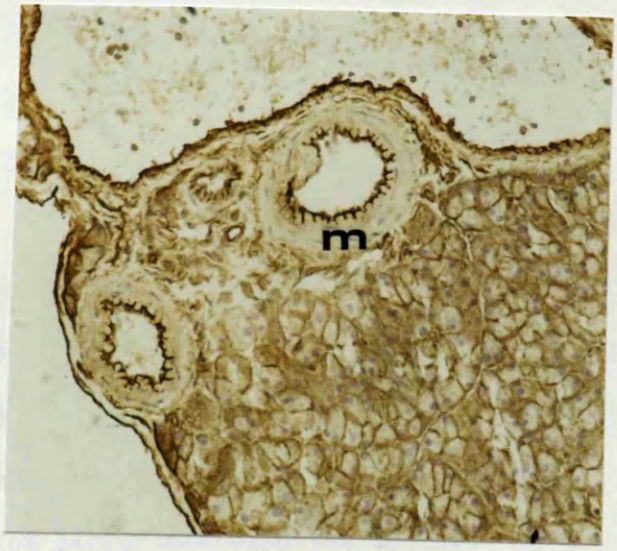

PLATE 5.4. Cryostat section of adult rat pancreas from the same animal as Plate 5.2. Section has been labelled with MRC OX18 (anti-class I (x900). Note staining of vascular smooth muscle is much less marked (m).

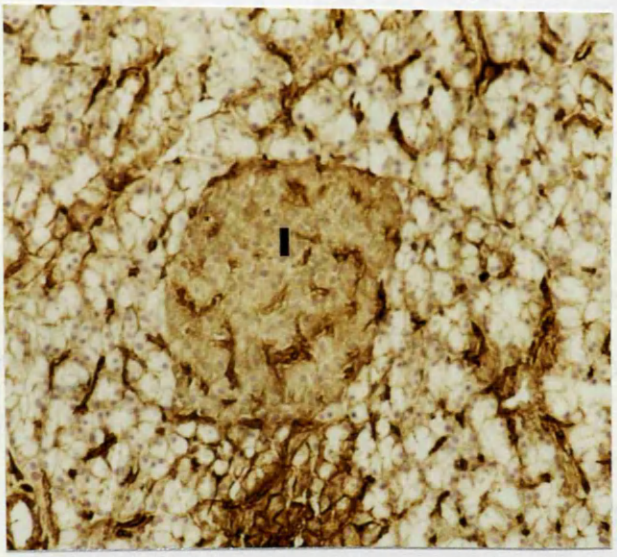

PLATE 5.5. Cryostat section of adult rat pancreas from a DA rat treated with daily intraperitoneal injections of IFN-gamma for 6 days and then killed 15 days later. Section has been labelled with MN4-91-6 (anti-class I) (x900). Exocrine cells are now only very lightly stained. Note that endocrine cells within the islets (I) are now expressing class I antigens to a greater extent than the background exocrine cells.

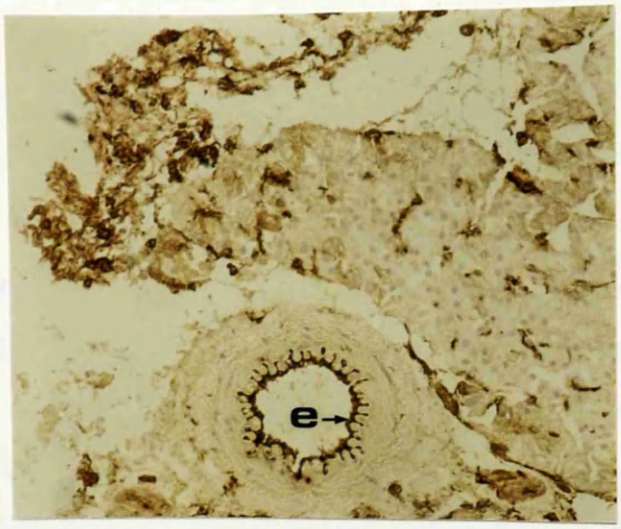

PLATE 5.6. Cryostat section of adult rat pancreas from a DA rat treated with daily intraperitoneal injections of IFN-gamma for 6 days then killed. Section has been labelled with MRC OX6 (anti-class II) (x900). Note evidence of class II antigen induction on vascular endothelium (e), with other cell types other than interstitial cells remaining class II negative.

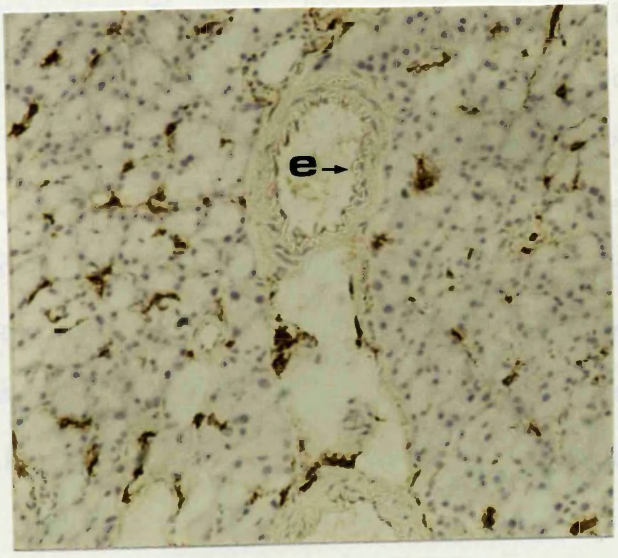

PLATE 5.7. Cryostat section of adult rat pancreas from a DA rat treated with daily intraperitoneal injections of IFN-gamma for 6 days and then killed 3 days later. Section has been labelled with MRC OX6 (anti-class II) (x900). Note that vascular endothelium (e) has already returned to a class II negative state.

of adult rat pancreas is shown in Table 5.1.

There was a progressive increase in the number of class II positive cells with increasing doses of IFN-gamma. Following the breaking of the coding system, when slides from each of the four groups were compared directly, this evenly distributed increase in the numbers of class II positive dendritic cells was apparent on visual inspection (Plates 5.8A, 5.8B and 5.8C).

DISCUSSION

These experiments, show that administration of IFN-gamma produces profound alterations in both the distribution and intensity of MHC class I and class II antigen expression in the adult rat pancreas. The marked increase in MHC class I antigen expression throughout the adult pancreas in response to a six-day course of IFN-gamma emphasises the dynamic nature of MHC expression. All pancreatic cell-types expressed class I antigens strongly following stimulation, with the previously negative exocrine cells appearing to be particularly responsive to the IFN-gamma, with prominent membrane labelling a striking feature. This class I expression by DA rat pancreatic exocrine cells is an example of interspecies variation in antigen expression contrasting with the reported persistent class I negativity of pancreatic exocrine cells in the IFN-gamma treated mouse (Skoskiewitz et al 1985).

Following discontinuation of IFN-gamma, levels of class I antigen expression declined only gradually in exocrine,

TABLE 5.1

EFFECT OF INTRAPERITONEAL INTERFERON-GAMMA ON THE NUMBERS OF CLASS II POSITIVE DENDRITIC CELLS IN ADULT RAT PANCREAS

Group	Dose of interferon gamma	Class II positive dendritic cells/mm^2
1	0	3.35 (2.78-3.93)*
2	1 x 10^4U/kg	8.65 (7.1-10.15)
3	1 x 10^5U/kg	12.63 (11.63-13.78)
4	1 x 10^6U/kg	19.80 (17.6-22.7)

*Results are expressed as the mean of three values with the range

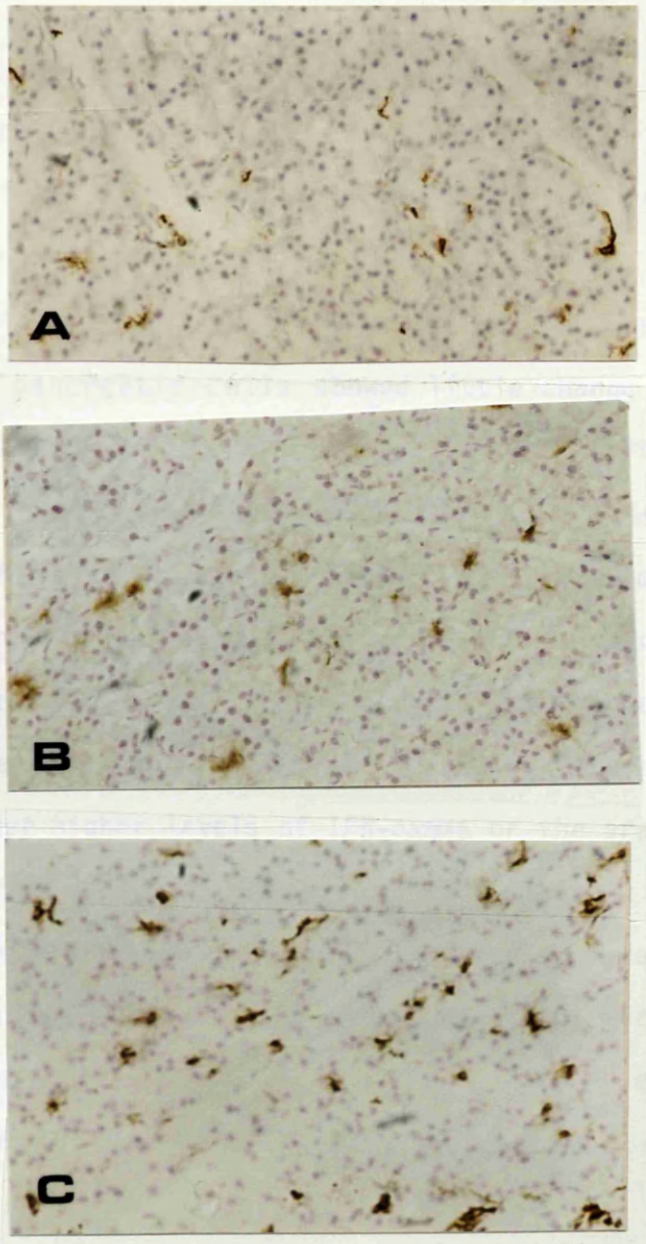

PLATE 5.8. Cryostat sections of adult rat pancreas from DA rats given various single intraperitoneal doses of IFN-gamma and killed 3 days later. Sections have been labelled with MRC OX6 (anti-class II) (x900). Note visually apparent increase in positive cells with increasing doses of IFN-gamma. A) Group 2 (1×10^4U/kg) B) Group 3 (1×10^5U/Kg) C) Group 4 (1×10^6U/Kg).

endocrine and vascular smooth muscle cells and even 15 days after the withdrawal of treatment, class I antigen expression was still in excess of basal values.

In contrast to the marked increase in class I antigen expression, pancreatic cells showed little change in class II antigen expression following IFN-gamma treatment, with endocrine and exocrine cells remaining class II negative. This persistent negativity of exocrine cells contrasts with the reported patchy increases in class II expression seen on mouse exocrine cells (Skoskiewitz et al 1985) and suggests that the class II expression seen on exocrine cells in foetal pancreas allografts requires either higher levels of IFN-gamma or the presence of an additional factor. Changes in class II antigen expression within the rat pancreas were restricted to an apparent increase in the population of class II positive interstitial cells, and a transient change in class II expression on vascular endothelium. The effect of IFN-gamma on the class II expression of vascular endothelial cells would seem to be dependent on continuing levels of circulating IFN-gamma. Although vascular endothelium had become strongly class II positive by day 6, within three days of discontinuing therapy all endothelial cells were class II negative.

The observation of a dose dependent apparent increase in numbers of class II positive dendritic cells, three days after administration of IFN-gamma, is in agreement with previous reports showing a similar effect of IFN-gamma on mouse pancreas (Skoskiewitz et al 1985) and on the livers, kidneys and hearts

of DA rats (Leszczynski et al 1986). Although no definite conclusions can be drawn regarding the lineage from which these cells are derived, the change in the number of class II positive cells following the administration of a single dose of 10^6U/kg of IFN-gamma, represents a sixfold increase and there are several possible sources for this expansion in numbers. Firstly, increased synthesis of cells within an IFN-gamma stimulated bone marrow may occur but would appear unlikely to take place over such a short period of time. Secondly, local proliferation of cells could be considered but the absence of an observed increase in the numbers of mitotic figures within the pancreas would tend to oppose this theory. More likely reasons for increased numbers of class II positive cells within the pancreas are either recruitment of cells from a circulating pool, as suggested by Skoskiewicz et al (1985) or induction of class II antigen expression on interstitial cells already present (but class II negative in the unstimulated state). Support for this concept of induction of class II antigen expression in previously-negative interstitial cells is provided by a report by Steeg, Johnson and Oppenheim (1982) which described increased expression of class II antigens in macrophages exposed to IFN-gamma in vitro.

If conversion of class II negative cells (already present in the pancreas) to class II positive cells can occur, this would have important implications for techniques of immunomodulation used in pancreatic islet and foetal pancreas transplant experiments. Passenger leukocyte depletion by

treating pancreatic tissue in vitro with anti-class II antibody and complement, might be expected to be ineffective in altering immunogenicity if a population of class II negative cells existed that had the potential to convert to class II positivity with antigen-presenting properties following transplantation. The existence of such a population of cells may be the explanation for the failure of in vitro anti-class II antibody and complement treatment to consistently prolong the survival of islet allografts in strong-responder rat strains (Terasaka et al 1986) with improvement in results following other techniques of immunomodulation.

SUMMARY

1) A marked and persistent increase in MHC class I antigen expression is seen on endocrine cells, duct epithelium and vascular endothelium in the pancreas glands of adult rats treated with systemic IFN-gamma. De novo expression of class I antigens is seen on exocrine cells and vascular smooth muscle.

2) Changes in MHC class II antigen expression are restricted to a transient de novo expresion on vascular endothelium and a dose dependent increase in positively labelled interstitial cells.

CHAPTER 6

IMMUNOGENICITY OF RAT FOETAL PANCREAS ALLOGRAFTS:- THE ROLE OF INTERSTITIAL CLASS II POSITIVE CELLS

INTRODUCTION

Much of the experimental work on the avoidance of rejection of pancreatic grafts in animal models has involved techniques of lowering graft immunogenicity. By removing or modifying the class II positive passenger leukocyte component of the graft prior to transplantation, a reduction in the antigen presenting abilities of the transplanted tissue has been sought. Techniques employed have included tissue culture, antibody treatment and ultraviolet irradiation and these have been discussed previously.

However, the relative importance of host-derived and recipient-derived cells in antigen presentation remains to be established. Lafferty originally proposed a two-signal hypothesis in which class II positive cells of donor origin were of prime importance in providing the crucial second signal involved in the afferent phase of the rejection response. (The first signal being the binding of antigen to the potentially responsive lymphocyte). Therefore the removal of 'passenger leukocytes' would result in lowered graft immunogenicity (Lafferty et al 1983). At first, retransplantation experiments using rat kidney allografts appeared to confirm the importance of host cells in antigen presentation with grafts depleted of

donor strain passenger leukocytes avoiding rejection when retransplanted (Lechler and Batchelor 1983). However, it later became clear that host cells could, in certain circumstances, serve in place of donor-strain passenger leukocytes and provide an alternative pathway for allostimulation (Sherwood, Brent & Rayfield 1986).

The theoretical basis of retransplantation experiments is the observed reduction in the number of donor-type interstitial dendritic cells in transplanted organs following transplantation, as described in rat kidney allografts (Hart & Fabre 1981b) and human and rat liver transplants (Gouw et al 1987, Gassel et al 1987). Following retransplantation to a second recipient antigen presentation by cells from the original donor would no longer be possible and therefore for rejection to occur, antigen presentation would have to be by an alternative pathway. Results of this type of experiment have been inconclusive (Hart, Winnearls & Fabre 1980, Markmann et al 1987, Miura et al 1987, Schang et al 1987) and would appear to depend on the strain combination and particular tissue transplanted.

As reported in previous chapters, when DA rat foetal pancreas grafts are transplanted to Cyclosporin A treated PVG recipients, within 14 days almost all interstitial class II positive cells of donor origin have disappeared. The set of experiments described below investigates the importance of donor strain class II positive cells in foetal pancreas rejection by examining whether depletion of passenger leukocytes by interim hosting in cyclosporin-treated or congenitally athymic PVG

recipients would lower the immunogenicity of the foetal pancreas sufficiently to prevent rejection when it was subsequently retransplanted to an unmodified PVG recipient.

MATERIALS AND METHODS

Transplantation of foetal pancreas and composite grafts

Foetal pancreas transplants to the left renal subcapsular site were performed using a standard technique as described previously. In some groups, 35 days later, composite grafts consisting of recipient kidneys plus the transplanted foetal pancreas, were retransplanted to the left orthotopic sites of animals syngeneic with the primary recipient with end to end anastomosis of the renal vessels and ureter.

Graft assessment

Cryostat sections of grafts resident in the primary host for 35 days were examined following labelling with donor-specific monoclonal antibodies. MHC class I and class II expression of donor-origin was determined.

Thirty five days after primary transplantation and fourteen days after retransplantation, recipient animals were sacrificed and the gross appearance of the transplanted pancreas was noted and paraffin sections stained with Haematoxylin and Eosin and labelled with polyclonal anti-insulin antibodies were examined.

Cyclosporin A treatment

Rats received 15mg/kg/day of cyclosporin A in olive oil via a gastric tube for 35 days.

Antibodies

F17-23-2 monoclonal antibody which reacts with a polymorphic determinant of MHC class II I/A antigen present in DA but not PVG rats and MRC OX6 which identifies a monomorphic class II determinant present in both DA and PVG rats.

Groups

1) DA foetal pancreas → PVG (CyA treated, n=3)

2) DA foetal pancreas → PVG-rnu/rnu (congenitally athymic, n=3)

3) PVG foetal pancreas → PVG → PVG (n=3)

4) DA foetal pancreas → PVG (CyA treated) → PVG (n=5)

5) DA foetal pancreas → PVG (congenitally athymic) → PVG (n=5)

RESULTS

Foetal pancreas transplants in Cyclosporin A-treated and congenitally athymic PVG recipients

Foetal pancreas grafts in group 1 and group 2 animals examined 35 days after transplantation were seen to be healthy and well vascularised. Well preserved pancreatic tissue with insulin-rich areas of endocrine cells were demonstrated in paraffin sections from all three animals in both groups.

Donor-strain, class II positive, graft interstitial cells were entirely replaced by leukocytes of recipient origin, in

both Cyclosporin A treated (group 1) and congenitally athymic PVG-rnu/rnu (group 2) rats. Allograft MHC antigen expression in congenitally athymic rat recipients resembled that seen in Cyclosporin A treated recipients (see Chapter 3) except that some small class II positive tubular structures were visible, representing small ducts or possibly capillary endothelium (Plates 6.1A and 6.1B).

Survival of retransplanted foetal pancreas allografts in secondary recipients

In group 3, PVG foetal pancreases retransplanted with the underlying kidney to a second PVG recipient 35 days after transplantation to the original PVG host showed histological evidence of graft viability with insulin rich areas of endocrine cells visible in sections taken from animals sacrificed 14 days after secondary transplantation (Plates 6.2A and 6.2B). This confirmed that grafts were not significantly damaged by the retransplant procedure itself.

In groups 4 and 5, all retransplanted foetal pancreas grafts were promptly rejected by the secondary hosts, despite the apparent absence of DA strain class II positive interstitial cells within the pancreas at the time of retransplant. All grafts examined, at day 14 after retransplant were completely rejected with no recognisable pancreatic elements remaining (Plates 6.3A and 6.3B).

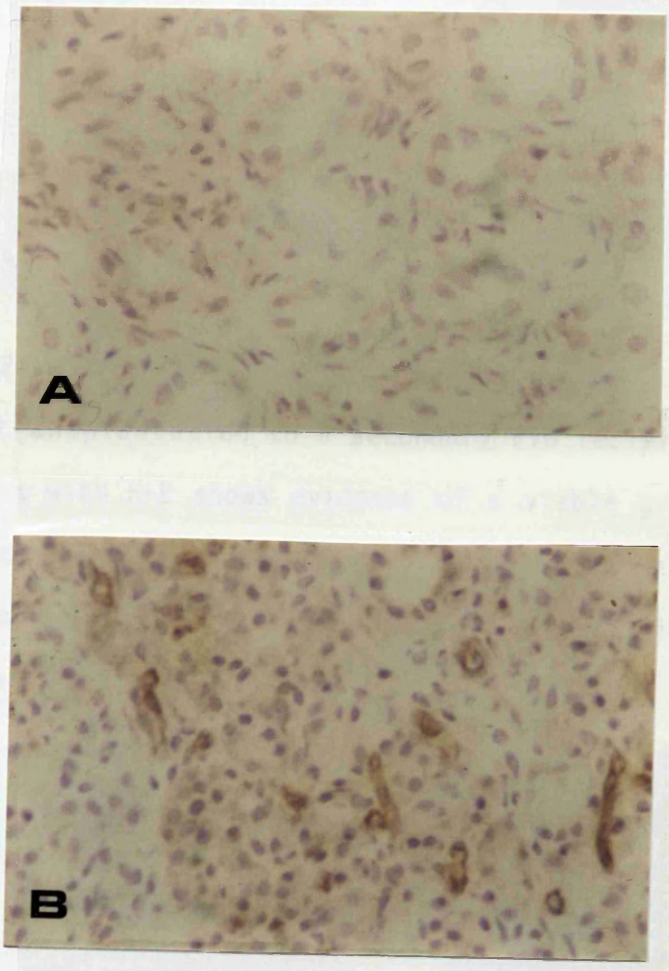

PLATE 6.1. Cryostat sections of DA foetal rat pancreas 35 days after transplantation to PVG recipients (Cyclosporin A treated [Group 1] or congenitally athymic (rnu/rnu) [Group 2]) (x1400). Sections have been labelled with F17-23-2 which labels class II antigen of DA (donor) origin only. Note neither section contains any remaining class II positive interstitial cells of donor origin. In grafts transplanted to congenitally athymic PVG rats (Plate 6.1B) there is evidence of class II expression on small tubular structures which is not seen in grafts from Cyclosporin A treated recipients (Plate 6.1A).

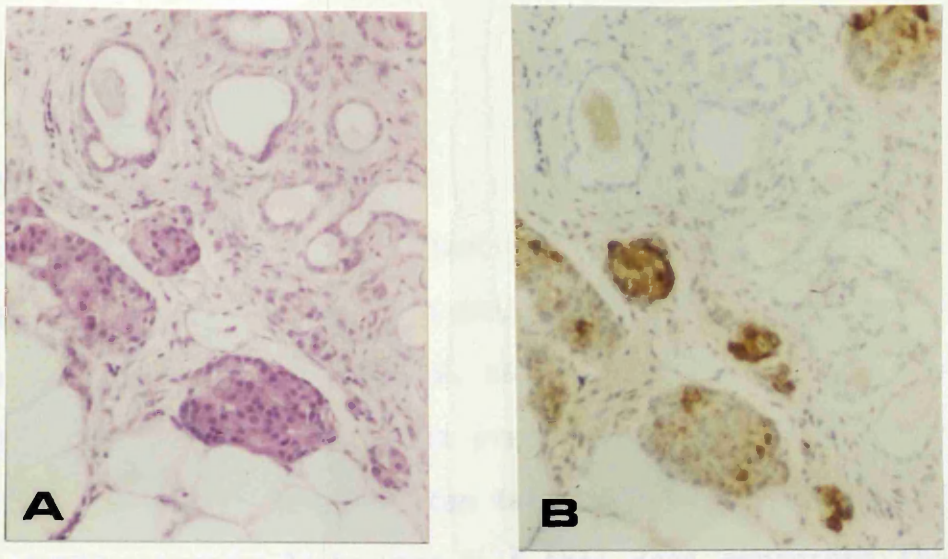

PLATE 6.2. Paraffin sections of PVG foetal pancreas 14 days after retransplantation to a secondary PVG recipient (Group 3) A) staining with H+E shows evidence of a viable graft with well preserved areas of endocrine tissue. B) labelling of a consecutive section with a polyclonal insulin antibody confirms the presence of viable beta cells (x900).

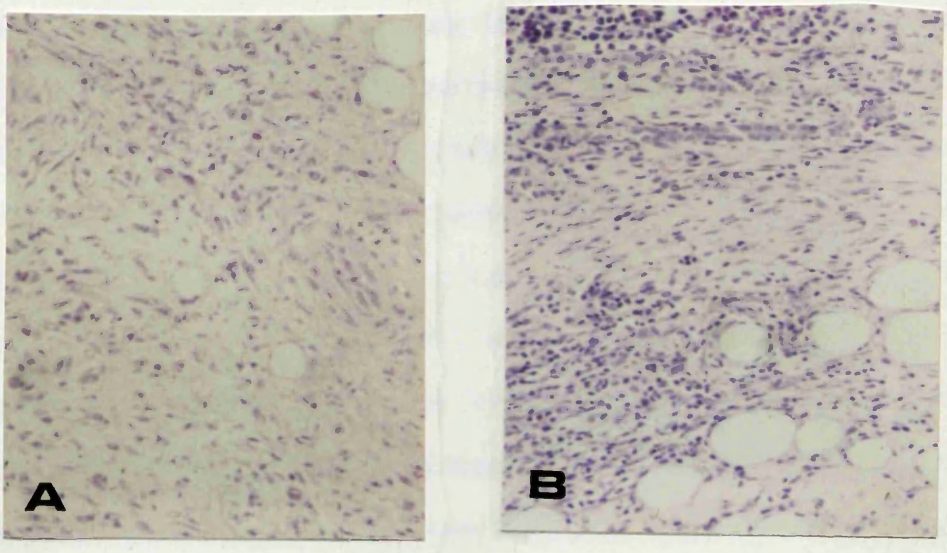

PLATE 6.3. H+E stained paraffin sections of DA foetal pancreas 14 days after retransplantation to a secondary PVG recipient. A) No evidence of any surviving pancreatic tissue in a graft retransplanted from a Cyclosporin A treated primary recipient (Group 4). B) similar appearance in a graft retransplanted from a congenitally athymic primary recipient (Group 5) (x900).

DISCUSSION

The occurence of rapid rejection in retransplanted foetal pancreas grafts, which have been depleted of donor-strain class II positive interstitial cells, suggests that, at least in certain strains of rats, antigen presentation and ultimately rejection of foetal pancreas can take place in the absence of donor-strain passenger leukocytes. If this does represent the true situation, then attempts to reduce graft immunogenicity by in vitro manipulations prior to transplantation would not be expected to prove to be successful in allowing prolonged graft survival without recipient immunosuppression.

However, although monoclonal antibody labelling of DA foetal pancreas after 35 days residence in a PVG host failed to reveal any evidence of surviving interstitial cells of donor origin, the possibility remains that sufficient class II positive cells to initiate a rejection reaction were present, but not detectable by the techniques used. Alternatively, it is possible that the transplanted tissue retained the capacity for class II induction under conditions of, for example, a non-specific inflammatory response resulting from the trauma of surgery, and that alloantigen presentation might be effected by cells other than interstitial dendritic cells. A wide variety of non-bone marrow derived cells have been shown to be capable of presenting antigen under certain circumstances (Fontana et al 1984, Malissen et al 1984, Bland & Watson 1986, Umetsu et al 1986, Rubinstein et al 1987). However antigens in most cases have been simple and have usually involved memory responses.

Evidence for presentation of transplantation antigens by non-bone marrow derived cells has been restricted to observations of recipient sensitisation by purified endothelial cells (Pober et al 1983, Hayry et al 1986) which appear to possess additional features, other than the capacity for class II MHC expression, to account for their enhanced immunogenicity. However, examination of longstanding foetal pancreas allografts in animals treated with Cyclosporin A reveals that endothelium is of recipient origin making antigen presentation by endothelial cells less likely. This still leaves the possibility that duct epithelial cells, with induced class II MHC expression can act as antigen presenting cells. If this was the case, an alternative explanation for the increased immunogenicity of foetal pancreas preparations compared with isolated islets (Simeonovic et al 1984) and proislets (Simeonovic & Lafferty 1987) would be antigen presentation by duct epithelium. A more likely explanation for the rejection of foetal pancreas allografts which have been depleted of donor-strain passenger leukocytes is the presentation of antigen by recipient class II positive cells. It is likely that antigen presentation by this alternative pathway would be more efficient in some strain combinations than in others reflecting varying degrees of similarlity in antigen configuration (Sherwood et al 1983). Whether or not passenger leukocyte depletion would successfully reduce immunogenicity would appear to depend on how effective alternative pathways of antigen presentation are in the particular strain combination or species being studied. The

results of a series of experiments carried out by Mullen et al (1987) using pigs suggest that in larger animals 'alloimmune reactions' can occur in the absence of donor class II positive cells. If this is true for human tissue transplantation then the likelihood of transplanting isolated islets without immunosuppression would appear to be remote. Depleting tissues of passenger leukocytes prior to transplantation would, however, at least remove one pathway of allosensitisation, leaving only antigen presentation by host cells which may be a less efficient route of sensitisation (Sherwood et al 1986). Therefore techniques of passenger leukocyte depletion although perhaps not effective on their own, may still prove to be synergistic with other techniques of preventing graft rejection (Miura et al 1987) and may allow lower doses of recipient immunosuppression to be used.

SUMMARY

1) Following 35 days residence in an interim host, no class II positive interstitial cells of donor strain were detectable in foetal pancreas allografts.

2) Despite this, retransplantation to an unmodified recipient (syngeneic with the interim host) resulted in rapid rejection of foetal pancreas allografts.

CHAPTER 7

CLASS II POSITIVE INTERSTITIAL CELLS WITHIN ISOLATED RAT ISLETS - THE EFFECTS OF TISSUE CULTURE AND ULTRAVIOLET IRRADIATION

Successful in vitro immunomodulation of endocrine grafts, including isolated islets and foetal pancreas, is generally considered to result from a reduction in the numbers of class II positive passenger leukocytes. The resulting diminished antigen presenting ability of the graft removes one potential source of alloantigen sensitisation, leaving only antigen presentation by host cells as an indirect pathway for the induction of rejection responses. Evidence that the in vitro techniques used do in fact reduce the passenger leukocyte population has for the most part been indirect and has depended on the demonstration of rejection when donor-strain leukocytes are restored (Talmage et al 1976, Lacy et al 1981, Moreland & Mullbacher 1987).

With the availability of specific anti-class II monoclonal antibodies it is now possible to count the number of class II positive cells in individual islets using indirect immunofluorescent staining of isolated islets (Gebel et al 1983, Forsen et al 1984, Gores et al 1986). By using these techniques a greater understanding can be obtained of the contribution that passenger leukocyte depletion makes to the known immunomodulating effect of such techniques as tissue culture and ultraviolet irradiation.

A prerequisite for such experiments is the isolation of adequate numbers of viable islets which have been altered as little as possible during the isolation procedure. Most techniques of islet isolation currently in use are based on the original observation by Moskalewski (1965) that digestion of the rodent pancreas with collagenase cleaves intact islets from the exocrine tissue. Distension of the pancreas by retrograde perfusion of the pancreatic duct with salt solution (Lacy & Kostianovsky 1967) and especially with collagenase (Gotoh et al 1985, Sutton et al 1986), improves the degree of separation achieved. Purification of the final islet preparation from the crude digestate usually involves a density gradient. Originally the sucrose polymer ficoll was used (Ballinger & Lacy 1972) but more recently, increased yields have been obtained using a bovine serum albumin density gradient (Lake et al 1987). An alternative approach to the purification step involves staining islets by perfusing the pancreas via the aorta with neutral red dye prior to removal of the gland. It has long been thought that islets preferentially take up this dye (Bensley 1911, Bretzel et al 1980, Downing et al 1980) and this has been confirmed by the careful experiment of Gray et al (1983). By using neutral red perfusion and collagenase digestion followed by hand picking of the islets with a micropipette under a dissecting microscope, large numbers of viable islets can be obtained (Dibelius et al 1986).

In the following experiments the effects of tissue culture and ultraviolet irradiation on the numbers of interstitial class

II positive cells within islets will be assessed. As hand picking of islets results in an extremely pure islet preparation and the omission of the density gradient step avoids the possibility of the gradient medium affecting antigen expression or being selectively toxic to a particular cellular component of the islet, an islet isolation technique combining neutral red perfusion and ductal injection of collagenase with hand picking of islets has been used.

MATERIALS AND METHODS

Islet isolation

Islets were isolated from male DA rats (body weight 200-300g). The pancreas was first stained uniformly pink by perfusion with neutral red solution via the aorta. Collagenase solution was then injected via the common bile duct to distend the pancreas prior to its removal from the donor. The distended pancreas was then chopped and digested with a further solution of collagenase. After washing, islets were hand picked under a dissecting microscope using a micropipette. (Full details are provided in Section 2).

Viability testing

Electron micrographs of freshly isolated and 21 day cultured islets were examined to allow determination of the true nature of the putative islets and to identify any ultrastructural evidence of cellular damage.

In addition, supravital staining of fresh, cultured and

ultraviolet irradiated islets was carried out using fluorescein diacetate (FDA) and ethidium bromide (EB). Fluorescein diacetate staining of live cells results in green fluorescence of the whole cell when viewed under ultraviolet (UV) light, the process being dependent on the cell having an intact membrane and active intracellular esterase enzymes (Rotman & Papermaster 1966). Ethidium bromide binds to the nuclear DNA of dead cells which then appear as red dots under UV light (Edidin 1970). Islet viability was scored from zero to four with a score of four representing an islet containing no dead cells and a score of zero representing an islet with no viable cells remaining. Scores of one, two and three represented islets with 25%, 50% and 75% of fluorescent cells viable (green).

Antibody labelling of isolated islets

Labelling with the anti-class II antibody MRC-OX6 was carried out in test-tubes with incubation at 4°C. Fluorescein conjugated rabbit anti-mouse antibody was used as a second antibody and MRC-OX21 served as a negative control.

Counting of class II positive cells

Wet preparations of MRC-OX6 labelled islets and MRC-OX21 negative controls were examined under UV light at magnifications of 250x and 400x. For each experimental group a minimum of 70 islets coming from at least three separate isolations and treatments were counted. No attempt was made to differentiate class II positive cells into subgroups depending on morphology.

Tissue culture

Groups of 50 islets were cultured in 95% air and 5% CO_2 in RPMI 1640 with 10% foetal calf serum and L-glutamine with streptomycin and penicillin added. Culture medium was renewed every two days.

Ultraviolet irradiation

Islets were held in culture for 24 hours to allow recovery from the isolation procedure. Islets suspended in culture medium in an open Petri dish with constant stirring were given a total dose of 900J/m^2 of UVB using Westinghouse FS-20 sunlamps. Following irradiation, islets were returned to culture for a further 24 hours prior to antibody labelling and counting

Class II positive cells in freshly isolated and cultured islets

Freshly isolated islets and islets cultured for 48 hours, 14 days and 21 days were labelled with MRC OX6 monoclonal antibody and numbers of positive cells compared.

Class II positive cells in ultraviolet treated islets

Irradiated islets were labelled with the MRC OX6 monoclonal antibody and the number of positive cells compared with non-irradiated controls cultured for 48 hours.

Statistical analysis

Student's unpaired t test was used in all comparisons.

RESULTS

Islet isolation

Islets were isolated from a total of 27 DA rats. Between 113 and 886 islets (mean 373.3 ± 33.0 SEM) per donor remained when hand picking had been repeated at least twice leaving an extremely clean preparation with purity assessed at greater than 99%. Islets with adherent exocrine tissue were discarded.

Viability testing

Electron microscopy confirmed the endocrine nature of the isolated islets with well maintained ultrastructural integrity in both the fresh and cultured islet preparations (Plates 7.1 and 7.2).

Supravital staining of more than 300 freshly isolated islets from 10 separate isolations demonstrated little evidence of cell death as a result of the isolation procedure with a mean viability score of 3.54 (maximum 4) (Plate 7.3). After 21 days in culture all islets recovered consisted of uniformly viable cells (scoring 4). Individual dead cells were also seen suspended in culture medium.

Islets irradiated with 900J/m^2 UVB and returned to culture for a further 24 hours had a mean viability score of 3.62, which was not significantly different from the score attained by freshly isolated islets.

Class II positive cells in freshly isolated and cultured islets

When labelled islets were examined under UV light at

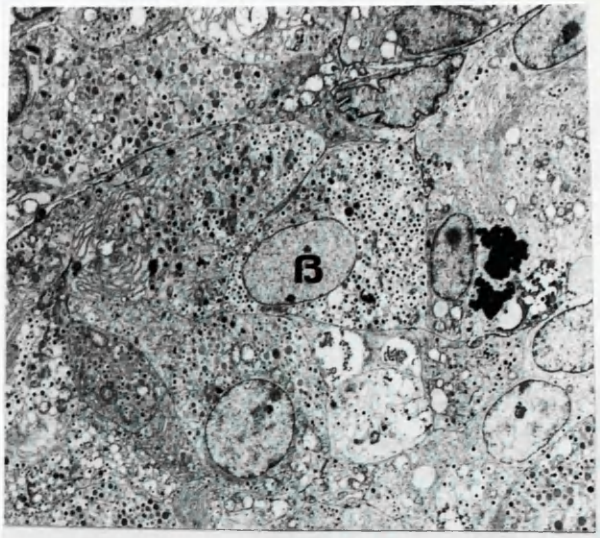

PLATE 7.1. Electron micrograph of freshly isolated adult rat islet. Note well preserved ultrastructural features with beta-cells (B) recognisable by their characteristic secretory granules (x2825).

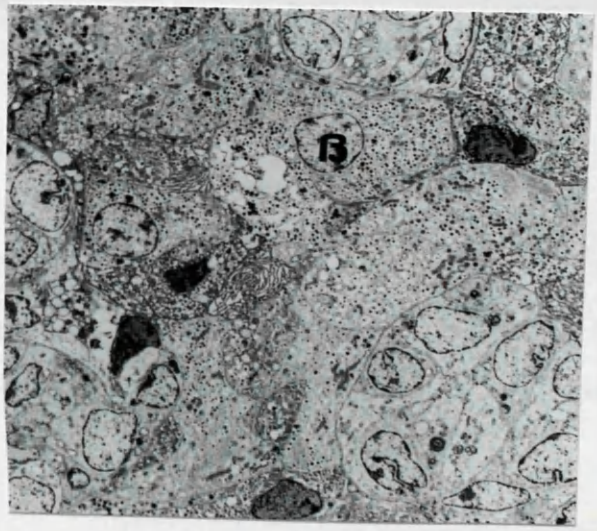

PLATE 7.2. Electron micrograph of 21 day cultured adult rat islet. No evidence of ultrastructural damage is apparent. Beta-cells (B) still contain many secretory granules (x2205).

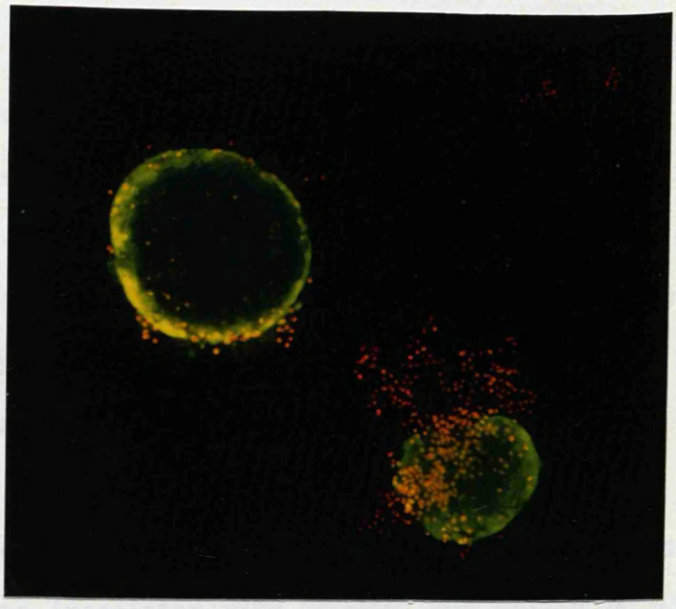

PLATE 7.3. Two viable freshly isolated islets stained with fluorescein diacetate and ethidium bromide and photographed using a fluorescent microscope (x100). A few scattered dead (red) exocrine cells are also shown.

magnifications of 250x and 400x discrete cellular staining was readily identifiable. By focussing up and down, positive cells within the depths of the islets could be identified and counted in addition to surface cells. A progressive decrease was noticed in the number of class II positive cells per islet with increasing duration of the culture period (Table 7.1). After 14 days of culture in 95% air and 5% CO_2 the number of class II positive cells per islet fell significantly to approximately one third of the number contained in freshly isolated islets ($p<0.001$). By the twenty first day of tissue culture only very occasional islets contained one or two class II positive interstitial cells ($p<0.001$).

Class II positive cells in ultraviolet treated islets

Islets irradiated with $900J/m^2$ UVB showed no alteration in the number of class II positive cells when islets were labelled and examined 24 hours later and compared with control islets cultured for 48 hours (10.45 ± 0.79 v 9.73 ± 0.66 (mean $\pm$ SEM).

DISCUSSION

The use of neutral red perfusion and collagenase digestion of the pancreas followed by hand picking of islets allowed isolation of a very pure preparation of viable islets in sufficient numbers to enable in vitro experiments to be carried out. Although there was a wide variation in the number of islets obtained from one donor, the mean number isolated was significantly lower than that described by Dibelius et al (1986)

TABLE 7.1

CLASS II POSITIVE CELLS IN FRESHLY ISOLATED AND CULTURED ISLETS

Duration of tissue culture	Number of islets counted	Class II positive cells per islet (Mean ± SEM)
Freshly isolated islets	92	11.02 ± (0.7)
48 hours	74	9.73 ± (0.66)
14 days	101	3.52* ± (0.36)
21 days	104	0.13* ± (0.01)

*Numbers of class II positive cells in 14 day and 21 day cultured islets were significantly lower than in freshly isolated islets ($p<0.001$).

using a similar isolation technique. It is likely that by using a prolonged period of static collagenase digestion, islets released early in the digestion process would be further digested becoming no longer identifiable as discrete units. However, as the aim of the isolation procedure in this instance was to obtain adequate numbers of pure undamaged islets, no attempt was made to improve islet yields.

The finding of 11.02 ± 0.7 (mean ± SEM) class II positive cells per DA rat islet is in broad agreement with some of the previously reported figures for rat islets (Ulrichs et al 1985, Gores et al 1986b) although much higher and somewhat lower numbers have also been described when different strains and antibodies have been used (Forsen et al 1984, Ulrichs et al 1985). Class II positive cells therefore would appear to be more numerous in rat islets than in mouse (Wright et al 1986) dog (Gebel et al 1983) and human islets (Nocera et al 1986).

The finding of a progressive reduction in the number of class II positive cells with increasing duration of tissue culture again is in agreement with previous reports (Rabinovitch et al 1982, Gebel et al 1983, Forsen et al 1984, Ulrichs et al 1985). There is however lack of agreement as to the length of time required to deplete class II positive cells to insignificant numbers. Rabinovitch et al (1982) and Forsen et al (1984) could find no class II positive cells remaining in Wistar-Furth and Lewis strain rat islets after culture at 37°C for seven and 12 days respectively, while Ulrichs et al (1985) found that some class II positive cells persisted even after 15

days of culture. The results described in this chapter would also suggest that a more prolonged period of culture was required in order to totally deplete islets of class II positive cells, and in this respect it was reassuring to note the excellent viability and ultrastructural integrity of 21 day cultured islets. The apparent improvement in viability scores of cultured islets presumably reflects some form of selection process operating with some of the lower scoring fresh islets doomed to death and disintegration with only the healthier islets remaining intact after 21 days in culture.

Exposure of islets to UVB irradiation in a dose of 900J/m^2 has been shown to reduce islet immunogenicity sufficiently to allow successful allografting without recipient immunosuppression in certain rat strain combinations (Lau et al 1984a). An interesting observation from the same group of researchers was the discovery that unless UVB irradiated islets were then rested in tissue culture for 24 hours prior to transplantation, rejection would proceed in an unmodified manner (Hardy et al 1984). Therefore although UVB irradiation had been shown to have no effect on class II expression on peripheral blood and dendritic cells when this was assessed immediately after irradiation (Lau et al 1983, Aprile and Deeg 1986, Gruner et al 1986) there remained the possibility that the effect of UVB on antigen expression took several hours to occur. Support for a delayed effect of UVB on antigen expression seemed to be provided by measurements of HLA-DR antigen expression on peripheral blood monocytes at various time intervals after

irradiation (Gruner et al 1984) and this might have explained the importance of the 24 hour recovery period described by Hardy et al, although these results are as yet unconfirmed.

The perceived lack of effect of UVB irradiation on class II expression described in this chapter is in keeping with the findings from skin where the immunomodulating effect of UV irradiation appears to be independent of changes in class II expression (Czernielewski et al 1984) or depletion of Langerhan's cells (the main class II positive cell in skin) (Odling et al 1987). It is more likely therefore, that if UVB irradiation affects antigen presentation its immunomodulating effect on islets is due to some other effect on the dendritic cell, which reduces its ability to present antigen independent of antigen expression.

As each freshly isolated islet contains on average only 11 class II positive cells, in 1500 pure islets (a number generally agreed to be sufficient to reverse diabetes in rats) there would only be 1.65×10^4 class II positive cells. This number is probably just adequate to induce a rejection response (Lechler & Batchelor 1982), therefore in strain combinations where antigen presentation is mainly by donor strain cells, purity of preparation is extremely important and only a relatively modest decrease in dendritic cell numbers or activity should be effective in prolonging survival. On the otherhand, in those strain combinations where even 21 days tissue culture fails to prolong the survival of purified islets, by which time less than 200 class II positive cells per 1500 islets would remain,

antigen presentation by host cells is likely to be of major importance.

SUMMARY

1) Neutral red perfusion and collagenase digestion of the adult rat pancreas followed by hand picking of islets allows isolation of a very pure preparation of viable islets, in sufficient numbers to enable in vitro experiments to be carried out.

2) Culture of isolated islets in standard medium results in a progressive decrease in the number of class II positive cells.

3) After 21 days of tissue culture, islets contain only very occasional class II positive cells.

4) Irradiation with $900J/m^2$ UVB does not result in a reduction in the number of class II positive cells found within islets.

CHAPTER 8

FOETAL PANCREAS TRANSPLANTATION IN DIABETIC RATS - PROBLEMS WITH THE EXPERIMENTAL MODEL

Foetal pancreas isograft and allograft survival is usually evaluated by monitoring the diabetic control of the recipient. For this to prove a reliable means of assessment there are several prerequisites for a proposed experimental model. Firstly, it must be possible to establish a stable diabetic state in a group of potential recipient animals. Secondly, successful transplantation should be accompanied by a consistent improvement in glucose homeostasis. Finally, following the subsequent removal of the transplanted tissue, the animal should return to its diabetic state.

Experimental model of diabetes mellitus

As discussed in Section 1 the classical model for the production of diabetes is total pancreatectomy (Minkowski 1892, Banting and Best 1922). In the rat, technical difficulties associated with the blood supply and size of the pancreas have led researchers to develop an alternative model in which diabetes is induced by the administration of a chemical, selectively toxic to the beta cells of the endocrine pancreas. The original agent used was Alloxan (reviewed by Okamoto 1970) but this has for the most part been superceded by streptozotocin, a drug with fewer side effects. When given as a

single intravenous dose of 55-65mg/kg, streptozotocin reliably induces a stable diabetic state by virtue of its toxic effect on pancreatic beta cells (Junod et al, 1967).

In a series of experiments streptozotocin was injected into the dorsal vein of the penis in 20 DA and 17 PVG rats in a single dose of 65mg/kg. As streptozotocin is rapidly inactivated at neutral pH, it was prepared immediately before administration and the pH adjusted to 4.5. Blood glucose was measured before and 48 hours and two weeks after streptozotocin injection and the results are summarised in Table 8.1.

Random blood glucose estimations in DA and PVG rats prior to streptozotocin treatment fell within a fairly narrow range with no rat having a blood glucose higher than 14mmol/litre. All rats showed a diabetic response to streptozotocin although there was a wide range of blood glucose levels at both 48 hours and two weeks after injection. No rat had a blood glucose measurement lower than 21mmol/l at any time after streptozotocin injection. In a group of 10 DA and six PVG rats followed for a further 10 weeks, blood glucose results in DA rats remained stable while PVG rats showed a slight tendency to worsening of hyperglycaemia.

It would therefore appear that a single injection of 65mg/kg of streptozotocin reliably induces a diabetic state in the group of DA and PVG rats under study. However, the somewhat unpredictable severity of the diabetes could lead to problems with the standardisation of post-operative insulin regimens.

TABLE 8.1

BLOOD GLUCOSE LEVELS IN DA AND PVG RATS BEFORE AND AFTER INTRAVENOUS STREPTOZOTOCIN (65mg/Kg)

Strain	Number	Random Blood Glucose (mean ± SE)		
		Before	48 hrs after	2 weeks after
DA	20	10.75±0.26 (range 8.2-12.5)	30.91±1.29 (range 21.4-47.4)	34.44±1.32 (range 24.1-47.6)
PVG	17	11.25±0.32 (range 8.7-13.8)	39.1±1.14 (range 31.8-48.2)	35.3±1.78 (range 26.5-51.6)

* Values are expressed in mmol/litre

Transplantation of foetal pancreas

Transplantation of foetal rat pancreas at an appropriate stage of development to the renal subcapsular site has been shown to successfully reverse chemically-induced diabetes (Brown et al 1974). However, results are very dependent on the exact gestational age of the transplanted tissue, on the careful control of hyperglycaemia in the first few days post-transplant and on the volume of tissue transplanted (Brown et al 1974, Garvey et al 1980b). The particular importance of the avoidance of extremes of hyperglycaemia while the transplanted foetal pancreas is maturing, is emphasised by the observation that a single foetal pancreas is sufficient to fully reverse diabetes in rats if it is first of all allowed to mature and differentiate for three weeks in a normoglycaemic intermediate host prior to transplantation to the diabetic recipient (Mullen et al 1977).

Isografts

Foetal pancreas isografts were carried out on 16 DA and three PVG recipients which had been rendered diabetic by a single streptozotocin injection given at least two weeks prior to transplantation. Seven or eight foetal pancreas glands (17-18 days gestation) were transplanted beneath the capsule of each recipient's left kidney. In four of the DA isograft experiments foetal pancreases had been kept in tissue culture for 21 days prior to transplantation (see Chapter 1). All recipient animals received eight daily injections of protamine zinc insulin at a

dose of 3-6 units per day depending on daily blood glucose estimations.

Only four of 12 recipients of uncultured foetal pancreas isografts had complete reversal of diabetes. This occurred immediately after cessation of insulin in two animals and after five and seven weeks in the other two recipients. All four of these successfully transplanted animals reverted to the diabetic state when their left kidney, bearing the pancreas graft, was later removed.

All three PVG animals receiving foetal pancreas isografts became normoglycaemic within two weeks of transplantation. However, when nephrectomies were carried out a further four weeks later, one animal died immediately afterwards and the other two remained normoglycaemic.

The four DA rats receiving isografts of 21 day cultured foetal pancreas all eventually became normoglycaemic. Two had normal blood glucoses two weeks after transplantation and the others became normoglycaemic after three and 13 weeks. However, in one of the animals with normoglycaemia at two weeks, blood glucose levels were again elevated one week later. The other animal with normoglycaemia at two weeks post-transplant remained normoglycaemic despite removal of the kidney bearing the pancreas graft four weeks after reversal of the diabetic state, while the other two recipients restored to normoglycaemia, again became diabetic following removal of their grafts.

Allografts

Seven diabetic PVG rats were transplanted with DA foetal pancreas in a similar manner to that described for the isograft experiments. In addition to eight daily insulin injections, recipients of allografts were given 15mg/kg of Cyclosporin A by gavage on a continual daily basis. Four allograft recipients died within one month of transplantation (six days, six days, four weeks, and four weeks). One further animal survived for 12 weeks after transplantation, but remained hyperglycaemic. Two of the seven PVG recipients became normoglycaemic, in one case immediately after the cessation of insulin and in another a further one week later. However when these two animals had their grafts removed four weeks after the restoration of normoglycaemia, only one became hyperglycaemic again, the other dying on the first post-opertive day.

Problems with the experimental model

Intravenous streptozotocin administration (65mg/kg) is a reliable method of inducing a stable diabetic state in DA and PVG rats. As the severity of the resultant hyperglycaemia varies through a wide range it is impractical to administer a standard daily dose of insulin when treating recipients in the first eight days after transplantation. Instead, a sliding scale regimen was derived and subsequently modified. The dose of subcutaneous protamine zinc insulin (PZI) injected daily varied from three units (when blood glucose was between 12 and 15mmol/litre) to six units (when blood glucose was greater than

24mmol/litre). On the first day that the blood glucose fell below 12mmol/litre, three units of PZI were given unless the blood glucose was less than 5mmol/litre. On subsequent days no insulin would be given when the blood glucose was less than 12mmol/litre.

Although it is generally recommended that transplantation is only carried out after the recipient has been diabetic for at least two weeks, lest there is a spontaneous return to normoglycaemia, there was no evidence from this current study to support the necessity for this practice.

The reason or reasons for the relatively poor rate of success of foetal pancreas isografts in this study are difficult to determine. Reversal of diabetes with return to the hyperglycaemic state following graft removal confirmed successful transplantation in only 33% of uncultured DA isografts. In the three PVG isograft recipients, although all three animals became normoglycaemic following transplantation, the death of one animal and the continued normoglycaemia following graft removal in the other two animals makes it impossible to exclude the possibility that reversal of diabetes was due to spontaneous recovery of native beta cell function. Although no untransplanted rat spontaneously returned to normoglycaemia, temporary function of transplanted tissue or the affects of exogenous insulin administration may allow damaged but viable native beta cells an opportunity to repair and recover.

Even if the PVG isografts are regarded as 'successes' this still only gives a total response rate of 58% which is poorer than the results obtained by earlier investigators (Brown et al 1974, Garvey et al 1980b). Possible reasons for inconsistent results include the difficulty of maintaining diabetic control with exogenous insulin during the critical period of graft maturation, and perhaps problems related to the handling of the foetal pancreas tissue and its placement on the kidney surface. Whatever the reasons it is clear that in our hands assessing graft survival by using functional criteria alone would produce misleading results.

It is interesting to note that in two of the successful uncultured isografts and in one of the successful cultured isografts, reversal of hyperglycaemia occurred several weeks after transplantation. This delayed reversal has previously been described (Garvey et al 1980b, Mullen and Shintaku 1980) and is thought to be due to the immature pancreatic tissue requiring a period of maturation and growth before being able to respond to a hyperglycaemic challenge. During this time graft survival can not be determined by measuring blood glucose levels.

Culturing foetal pancreas for three weeks prior to transplantation did not appear to adversely affect graft function, and all four recipients of cultured tissue became normoglycaemic. However, the spontaneous recurrence of hyperglycaemia in one recipient and continued normoglycaemia following graft removal in another prevents any conclusion being

drawn regarding the benefits of culturing to foetal pancreas isograft function.

Of seven allograft recipients, four died while still hyperglycaemic and a further animal died following nephrectomy. This high mortality is probably related to the combined effects of prolonged hyperglycaemia with accompanying weight loss and dehydration and to the oral administration of Cyclosporin A which, as discussed in Chapter 3, even in normoglycaemic animals, results in an increased mortality (Rynasiewicz et al 1980).

In view of these problems with the experimental model, particularly with regard to the Cyclosporin A treated animals, a decision not to use diabetic animals in graft survival experiments seems justified. This would also avoid any immunosuppressive effect of the diabetic state influencing the rejection response. To avoid the difficulties described above, in the interpretation of the results of foetal pancreas transplant experiments, the use of gross inspection and histological examination, perhaps coupled with the measurement of graft insulin content, would allow a clearer picture of graft survival to be obtained.

SUMMARY

1) Intravenous administration of a single dose of Streptozotocin (65mg/kg) is a reliable way of inducing diabetes mellitus in DA and PVG rats, although the severity of the resulting hyperglycaemia is somewhat unpredictable.

2) The functional results of foetal pancreas isografts can be inconsistent. Delayed reversal of hyperglycaemic and spontaneous recovery of native beta cell function may confuse the interpretation of results.

3) There is a high mortality in diabetic recipients of foetal pancreas allografts receiving oral Cyclosporin A.

4. CONCLUDING REMARKS

There is at least suggestive evidence indicating that close control of a diabetic patient's glucose metabolism is necessary if long-term complications are to be minimised. It is unlikely that mechanical systems of insulin delivery will ever reach the required level of sophistication, and even if they did, the diabetic patient would be destined to a life of permanent attachment to a mechanical device. What is required is a method of beta cell replacement which could be instituted soon after the diagnosis of diabetes had been established but before complications had developed. As only a proportion of diabetics develop long-term complications, any treatment would have to be associated with a lower level of morbidity and inconvenience than occurs as a consequence of regular exogenous insulin administration, although if a subgroup of patients at increased risk could be identified at an early stage, a slightly higher level of morbidity might be acceptable.

Of the alternatives for beta cell replacement although the results of whole organ and segmental pancreas transplantation are steadily improving as technical difficulties are gradually overcome and immunosuppressive regimens and the diagnosis of rejection become more sophisticated, the hope for the future is that transplantation of isolated islets or foetal pancreas will become a clinical reality.

The two major obstacles preventing the widespread introduction of this form of beta cell replacement are firstly the need to develop an effective method of islet isolation from a suitable donor source and secondly, in common with other types

of tissue transplantation, the barrier of rejection needs to be overcome. Recent advances in islet isolation technology (Gray et al 1986, Lake et al 1987) have gone part of the way to solving the first problem but donor supply remains a major concern.

Regarding the problems of rejection, it would appear that the early impression of increased inherent immunogenicity of isolated islets (Nash et al 1977) and foetal pancreas (Garvey et al 1979, Spence et al 1979, Mullen & Shintaku 1980) was likely to have been related, at least in part, to contamination with significant quantities of lymphoid tissue and highly purified preparations of islets (Gotoh et al 1986) of foetal proislets (Prowse et al 1983) were later shown to fare better

With the realisation of the central importance of particular MHC class II positive cell types in the antigen presenting phase of the afferent limb of the rejection response, an exciting new area of transplantation research has opened up. The possibility of being able to transplant tissue without the need for immunosuppression, or at least with lower doses, has been suggested by the results of experiments designed to reduce graft immunogenicity by reducing the numbers of these passenger class II positive cells. The abilities of both isolated islets and foetal pancreas to remain viable in culture medium make these tissues uniquely appropriate for this line of transplantation research, and the need to develop a system of beta cell replacement which doesn't require high levels of immunosuppression provides further impetus to these research

initiatives.

Foetal pancreas would seem to have some advantages over isolated islets for clinical transplantation. Firstly, the problems of isolation associated with islets don't exist and secondly foetal pancreas has a greater potential for growth and development (Maitland et al 1980). If an appropriate gestational age is chosen, a period in tissue culture allows for the selective survival of the endocrine component (Mandel et al 1982). The possible additional advantage of a lower immunogenicity has been tentatively suggested by some clinical results in non-immunosuppressed recipients where there appeared to be moderately long-term survival of transplanted human foetal pancreas (Chastan et al 1980).

Based on earlier experience with foetal skin grafts (Billingham & Silvers 1964, Wachtel & Silvers 1972, Heslop et al 1973) it was initially hoped that the immaturity of the foetal pancreas tissue would confer advantages in terms of a lower inherent immunogenicity. With the realisation of the importance of MHC antigen expression in the rejection response, the low levels of MHC antigen expression found in foetal tissue in general (Ozato et al 1985, Sewell et al 1986) seemed to support this prospect. However, transplantation of foetal pancreas without immunosuppression was invariably followed by rapid rejection (Spence et al 1979, Garvey et al 1979, Mullen & Shintaku 1980), a finding seemingly at odds with the prevailing theories regarding immunogenicity and usually explained as being a consequence of the transplantation of closely-associated

lymphoid tissue.

To help resolve some of these uncertainties surrounding the transplantation of foetal pancreatic tissue, it seemed desirable to first establish some basic data regarding the MHC antigen expression of rat foetal pancreas in both the unstimulated and the stimulated state.

The labelling of rat foetal pancreas with appropriate anti-class I and anti-class II monoclonal antibodies clearly established the very low levels of MHC antigen expression found in foetal pancreas compared to adult rat pancreas, findings which were in broad agreement with what had been described in various species for other foetal organs (Ozato et al 1985, Sewell et al 1986). However, despite this seeming lack of target antigen for the efferent limb of the immune response and despite the apparent scarcity of class II positive cells to take part in antigen presentation, unmodified allografts were nevertheless rapidly rejected.

By allowing the foetal pancreas to develop in culture for seven days before assessing antigen expression it became clear that the necessary intracellular mechanisms for the synthesis and expression of MHC antigens were present within the supposedly immature tissue. The development of a pattern of antigen expression more closely resembling that of the adult animal than the chronologically-equivalent neonatal animal, seemed to point to the existence within the foetal environment of an inhibiting factor (perhaps of importance in the tolerance of the mother to the partly foreign tissue of the foetus) or the

lack of a stimulating factor which was present in standard unmodified culture medium. That foetal pancreas was indeed capable of expressing high levels of MHC antigen was confirmed by adding interferon-gamma to the culture medium and observing de novo expression of class II antigen and increased levels of class I antigen expression.

Careful examination of monoclonal antibody-labelled cryostat sections failed to find any evidence of class II antigen expression on the endocrine cells of the pancreas from any of the strains of rats tested, regardless of maturity, even in the presence of interferon-gamma stimulation, confirming the findings of the majority of earlier reports (Faustman et al 1980, Parr et al 1980, Baekkeskov et al 1981, Hart & Fabre 1981, Alejandro et al 1982, Rabinovitch et al 1982, Hart et al 1983, Gebel et al 1983, Steiniger et al 1984). However, within individual adult islets and also scattered throughout the rest of the pancreas, a separate population of interstitial class II positive dendritic cells was observed suggesting that even a completely pure preparation of isolated islets would contain cells potentially capable of presenting antigen.

Changes in antigen expression in response to transplantation have been well described in other tissues and other species (see Introduction and Historical Review). Following the observed changes in foetal rat pancreas antigen expression in culture and with interferon-gamma it seemed likely that this tissue would also be able to increase the levels of antigen expression in response to transplantation.

By choosing the DA to PVG rat strain combination for the transplantation of 17 day gestation foetal pancreas it was possible to separately identify, donor and recipient strain MHC antigen expression on cryostat sections using available monoclonal antibodies specific for polymorphic determinants differing in the two strains. Direct examination confirmed the initial growth and vascularisation of both isografts and allografts with signs of rejection becoming obvious in allografts by the sixth day. Serial examination of antigen expression again confirmed that foetal pancreas was indeed capable of expressing antigen. Even in the absence of a rejection response, foetal pancreas transplanted into the adult environment as an isograft rapidly developed levels of antigen expression similar to the adult pattern, again providing suggestive evidence that the low levels of antigen expression seen in freshly isolated foetal pancreas were a consequence of the foetal environment.

The changes in antigen expression following allografting of foetal pancreas were similar to that seen with vascularised adult rat pancreas allografts (Steiniger et al 1985). As MHC class II antigen expression appears to be particularly important in contributing to the overall immunogenicity of a tissue, the finding of increased levels of MHC class II antigen expression on duct epithelium and exocrine cells of allografted foetal pancreas may be significant and may even suggest that foetal pancreas would be likely to be more immunogenic than isolated islets or proislets. If these non-bone marrow derived class II

positive cells were able to present antigen in a manner relevant to the transplantation response, this would also provide an explanation for the described relative resistance to immunomodulation of foetal pancreas compared with isolated islets (Simeonovic et al 1980, Garvey et al 1980a).

As no form of immunomodulation has been completely effective for foetal pancreas it is likely that some form of immunosuppression would be required to enable successful allografting. Currently, Cyclosporin A has generally proved to be the most useful immunosuppressive agent with activity in a wide range of clinical and experimental organ transplants. However, early results with isolated islets and foetal pancreas were disappointing (Morris et al 1980, Garvey et al 1980b) although more recently when highly purified islets were used and when blood levels of Cyclosporin A were maintained for longer, results have been more encouraging (Dibelius et al (1986).

Previous studies of the effect of Cyclosporin A on foetal pancreas allograft survival had used the reversal of diabetes as the main indicator of graft survival. An investigation of the reliability of the streptozotocin-diabetic rat as an experimental model in foetal pancreas transplantation revealed several potential problems. Firstly, the severity of the induced diabetes was very variable making the standardisation of maintenance insulin regiments difficult. Secondly, the functional response to transplantation was unreliable as although all Cyclosporin A treated allograft recipients had histological evidence of surviving foetal pancreas tissue,

several animals did not become normoglycaemic at any stage. In addition, in those animals showing a good functional response, the time to reversal of diabetes was very variable and on occasions reflected recovering native beta cell function rather than allograft survival. Also Cyclosporin A is toxic to the beta cells and might be expected to further compromise graft function making the assessment of graft survival even more difficult. Therefore, in order to investigate whether Cyclosporin A was of value in prolonging foetal pancreas allograft survival in two strain combinations, serial histological evaluation was used rather than functional studies. This also allowed the use of non-diabetic recipients thereby avoiding the high death rate seen in diabetic rats given Cyclosporin A by gavage.

The finding that Cyclosporin A does indeed allow prolonged survival of foetal pancreas grafts was in disagreement with earlier reports (Garvey et al 1980b) and indicated that the pessimism regarding the likelihood of Cyclosporin A being of value in islet and foetal pancreatic transplants may have been unwarranted.

Possible mechanisms by which Cyclosporin A exerts its immunosuppressive effect in foetal pancreas allografts were suggested by measuring MHC antigen expression and determining the phenotype of infiltrating cells. In agreement with the results of Cyclosporin A treatment in other transplanted organs (Milton et al 1986), antigen expression was found to be reduced to a level similar to that of isografts, while changes in the

magnitude and phenotype of the cellular infiltrate suggested that there were also effects on the efferent phase of the rejection response. In particular there were much smaller numbers of NK cells and $T_{(cytotoxic/suppressor)}$ cells and very few IL-2 receptor positive T cells (signifying activity) within the foetal pancreas in Cyclosporin A treated recipients.

The presence of large numbers of MRC OX8 positive/MRC OX19 negative cells in the infiltrate found in unmodified rejecting allografts would be consistent with theories of rejection implicating T cells as the initiators of the rejection response with non-specific effector cells recruited into the area producing the tissue damage. However, the alternative explantation that activated T cells actually cause most of the graft damage with non-specific cells infiltrating the area as a secondary respone to injury, cannot be excluded.

Although the changes in MHC antigen expression on transplanted foetal pancreas allografts were, to an extent, predictable, the increased antigen expression on the recipient's kidney tubules adjacent to the graft was unexpected. The tubular antigen was confirmed to be recipient antigen and the pattern of induction was consistent with the diffusion of a soluble mediator from effector cells within, or close to, the graft. Although there was evidence from transplants between F1 and parent-strain animals, that a mild graft versus host reaction did occur on occasions, it was felt that the MHC antigen induction as a consequence of this was unlikely to contribute significantly to the immunogenicity of foetal

pancreas grafts.

The altered pattern of antigen expression in allografted pancreas was very similar to that observed in foetal pancreas glands cultured with interferon-gamma, a lymphokine thought to be of particular importance in antigen induction following the transplantation of other organs in other species (Skoskiewicz et al 1985, Leszczynski et al 1986, Hayry et al 1986). In order to establish whether the changes in antigen expression seen following vascularised pancreas allografts in rats (Steiniger et al 1985) could be explained as being wholly as a consequence of the release of interferon-gamma, this lymphokine was administered intraperitoneally to groups of rats and its effects assessed using sequential analysis of cryostat sections labelled with appropriate monoclonal antibodies. These experiments confirmed that interferon-gamma on its own, could produce profound alterations in both the distribution and intensity of MHC class I and class II antigen expression and is therefore worthy of consideration as an antigen-inducing agent in pancreas allografting and as such may represent a target for immumnosuppressive strategies. In contrast to the marked increase in class I antigen expression on pancreatic endocrine and exocrine cells following interferon-gamma administration, the absence of class II expression on both these cell types suggests that the induction of class II antigen expression on exocrine cells following allografting (Steiniger et al 1985) requires the participation of an additional factor (probably a lymphokine), or higher concentrations of interferon-gamma.

Although no definite conclusions can be drawn regarding the cell lineage of the class II positive cells which increased in a dose dependent manner following interferon-gamma administration, one possibility is that class II negative cells already present within the pancreas are converted to class II positive cells in situ, in a similar manner to that reported for class II negative macrophages exposed to interferon-gamma in vitro (Steeg et al 1982). If this was the case there would be clear implications for immunomodulation techniques used in islet transplantation which involve treatment with anti-class II antibody and complement and might explain the relative ineffectiveness of this method of immunomodulation compared with others in certain strain combinations (Terasaka et al 1986). It may therefore be necessary to ensure maximal expression of class II antigens by treatment with interferon-gamma prior to immunomodulation treatment with class II antibody.

Most experimental methods of immunomodulation appear to achieve their effect by removing or inactivating donor-strain class II positive cells prior to transplantation. It is, however, becoming increasingly well recognised that host class II positive cells can, in certain circumstances, provide an alternative route for antigen presentation (Sherwood et al 1986).

The relative importance of host and donor cells in antigen presentation would appear to depend on the tissue transplanted and the particular strain-combination being considered. In an attempt to ascertain the relative importance of the two routes

of antigen expression in foetal pancreas allografts in the DA to PVG strain combination, foetal pancreas grafts were depleted of donor-strain class II positive cells by two systems of interim hosting. The availability of a congenitally athymic PVG rat made this strain combination particularly convenient to study.

The finding of rapid rejection of retransplanted DA foetal pancreas grafts despite the apparent absence of donor-strain passenger leukocytes suggests that in this strain-combination antigen presentation by host cells is probably effective in initiating a rejection response. Therefore in at least some rat strain combinations immunomodulation of foetal pancreas prior to transplantation would not be expected to prevent subsequent rejection and at least some degree of recipient immunosuppression would be required. Whether or not antigen presentation by recipient cells would be of significance in clinical foetal pancreas transplantation cannot be predicted from these results. However, immunomodulation, by removing one pathway of antigen presentation, may allow a reduction in the immunosuppression required, which would be of particular importance if the beta-cell-toxic agent, Cyclosporin A was being used.

That the immunomodulating effect of culturing islets is due to a reduction in the number of class II positive cells, is based mainly on indirect evidence. By labelling isolated rat islets with appropriate anti-class II antibodies before and after various periods in tissue culture, it was established that in agreement with previous reports (Rabinovitch et al 1982,

Gebel et al 1983, Forsen et al 1984, Ulrichs et al 1985), class II positive cells did indeed diminish rapidly being almost completely undetectable after 21 days. This may not, of course, be the only immunomodulating effect of tissue culture, but labelling of class II positive cells may be of value in in vitro studies designed to ascertain the optimal length of culture for isolated islets or foetal pancreas prior to clinical transplantation. In contrast to extended periods of tissue culture, UVB in doses shown to reduce islet immunogenicity (Lau et al 1984a), does not result in a reduction in the total number of class II positive cells, again confirming previous reports which studied irradiated skin (Czernielewski et al 1984, Odling et al 1987) and suggests that at least some forms of immunomodulation act independently of changes in antigen expression but may still inhibit antigen presentation.

Counting the numbers of class II positive cells in freshly isolated islets revealed that the total number in a mass of purified islets sufficient to reverse diabetes in rats was just above the number regarded as being sufficient to induce a rejection response (Lechler & Batchelor 1982). Therefore in strain combinations where presentation of antigen by donor-strain cells is the dominant mechanism, only a relatively modest decrease in dendritic cell numbers would be required to prolong graft survival. In addition, as only very small numbers of class II positive cells exist after 21 days, rejection of purified cultured islets would imply that antigen presentation was by the alternative pathway involving recipient cells.

Taken together, direct labelling of class II positive cells may provide a useful means by which the relative importance of host and donor antigen presenting cells in graft rejection could be ascertained for various tissues in different species. As a result, immunomodulation of donor tissue or recipient immunosuppression could be used where appropriate.

In summary, although rat foetal pancreas normally expresses very low levels of MHC antigen, following various forms of stimulation such as allografting or interferon-gamma treatment, both class I and class II antigen expression increase. By studying transplanted foetal pancreas grafts using serial histological examination of antibody-labelled cryostat sections, changes in antigen expression and the immunosuppressive effect of Cyclosporin A were demonstrated. Finally measurements of MHC antigen expression may be useful in assessing the effectiveness of immunomodulation techniques and may provide some help in enabling the determination of the relative importance of host and donor antigen-presenting cells in the rejection of different tissues.

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PUBLICATIONS ARISING FROM THE WORK DESCRIBED IN THIS THESIS

Brown, M.W., Bolton, E.M., More, I.A.R. & Bradley, J.A. (1988) Immunohistological observations on rat foetal pancreas allografts transplanted into unmodified and cyclosporine treated recipients. Transplantation, **46**, 800-806.

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PRESENTATIONS TO LEARNED SOCIETIES

British Transplantation Society - Belfast 1987. Prolongation of rat foetal allograft survival by Cyclosporin: An immunohistological study of antigen expression.

First International Congress on Pancreatic and Islet Transplantation - Stockholm 1988. An immunohistological re-evaluation of the ability of Cyclosporin A (CyA) to prolong rat foetal pancreas allograft survival.

British Transplantation Society - Cardiff 1988. Rejection of rat foetal pancreas allografts despite elimination of interstitial Ia^+ cells.

Surgical Research Society - Bristol 1988. The contribution of interstitial Ia^+ cells to the immunogenicity of rat foetal pancreas allografts.

International Congress of the Transplantation Society - Sydney 1988. Immunogenicity of rat foetal pancreas allografts: the role of interstitial Ia positive cells.

CO-PRESENTATION

Joint Meeting of Societe Francaise Immunologie and British Society of Immunology - Paris 1988. Contribution of Ia positive interstitial cells to the immunogenicity of rat foetal pancreas allografts.