

A Comparison of Organ Cultured Fetal Pancreas Allo-, Iso-, and Xenografts (Pig) in Non-Immunosuppressed Non-Obese Diabetic Mice

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The response of non-immunosuppressed non-obese diabetic/Lt mice to an isograft (H-2^{g7}), major histocompatibility complex-mismatched allografts (CBA, H-2^k; BALB/c, H-2^d), and xenograft of fetal pig pancreas was assessed by light microscopy. In non-obese diabetic mice, isografts were rapidly invaded by lymphoid cells, and the graft pathology was similar to that in the host pancreas. In prediabetic mice the graft site was invaded by small mononuclear cells (CD4 and CD8+ve T cells) and macrophages, and in diabetic mice specific β -cell destruction was found. The allografts were invaded and destroyed within 10 to 14 days by mononuclear cells that included many blast cells. In the allograft sites the infiltrating cells soon disappeared, and within 3 weeks only a scar remained. The xenografts, in contrast, were invaded by macrophages and eosinophils with some neutrophils and mast cells and multinucleated giant cells. Xenograft destruction also occurred over 8 to 10 days, but the site remained large and swollen with a central necrotic zone and massive fibrosis forming a large granuloma, and the infiltrate persisted for many weeks. Thus, there are marked differences in the host response to a challenge with tissue that is prone to cell-specific autoimmune disease, to a graft of immunogenic allogeneic tissue, and to a transplant of discordant xenogeneic islets. Because of the differences in the host response to these grafts different immunosuppressive strategies may be needed to cope with their destruction. (Am J Pathol 1995, 147:834–844)

The use of cross-species donors may be the solution to the severe shortage of organs in clinical transplantation, but formidable problems in the control of rejection of such tissues still need to be solved.¹ Some slight success has been achieved in clinical organ replacement using closely related (“concordant”) animal donors,^{2–6} ie, apes and Old World monkeys such as baboons, but major problems with this approach include ethical issues related to the use of species closely related to humans as well as a shortage of such animals and the risk of transmission of disease. In contrast, if domestic species such as pigs, already widely utilized for human needs, could be used some of these problems would be less significant. Indeed, fetal pig islets have been transplanted into a few patients with type I diabetes mellitus recently with at least some biochemical and histological evidence of graft survival.⁷

However, the use of such “discordant”⁸ donors raises another major issue in xenotransplantation, the rapid rejection of the grafts due to the presence in the recipient of cross-reactive natural antibodies that produce hyperacute rejection (HAR). HAR is at present essentially untreatable and is believed to be due to the reaction of the cross-reactive natural antibodies with the donor endothelial cells in the graft.⁹ This results in endothelial cell activation, capillary damage, intravascular thrombosis, and graft infarction. Free grafts of cells could be used, eg, in islet cell transplantation in insulin-dependent diabetes or in central nervous system tissue transplantation in Parkinson’s disease. These grafts become vascular-

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ized by an ingrowth of host vessels that should not be targets for cross-reactive natural antibodies. In the absence of HAR in such situations, however, rejection is still seen in non-immunosuppressed recipients, and even with immunosuppression it is difficult or even impossible to control.¹ It has been assumed that if HAR could be avoided the cellular response mounted by the recipient would be qualitatively similar to that generated against allogeneic tissues¹ and could, in principle, be controlled by the sorts of immunosuppression that are usually effective in allotransplantation; however, in practice this is not so.

In this study we describe the natural history of islet transplants in non-obese diabetic (NOD) mice, a strain that develops spontaneous autoimmune diabetes.¹⁰ We show that there are striking differences in the response of a host against three types of organ cultured fetal pancreas; an autoimmune recurrence of disease in isografts that is T-cell mediated and β -cell specific, a strong alloresponse that is predominantly mononuclear, and a qualitatively quite different response against the xenografts that is dominated by eosinophils and macrophages and may require different forms of immunosuppression for its control. However, HAR is not a problem in this model of xenotransplantation. When immunosuppression is used that does not prevent infiltration of the isograft site or prevent rejection of an allograft, the xenograft is still temporarily protected.¹¹

Materials and Methods

This paper describes the natural history of the rejection of allo- and xenografts and of recurrent autoimmune destruction of isografts in NOD mice from data collated from experiments in which control (ie, non-immunosuppressed) animals were examined as a part of other studies, eg, where the effects of immunosuppression on graft survival were tested or where graft sites were taken for an analysis of cytokine profiles. The data from studies on immunosuppressed mice will be reported separately. A total of 244 individual grafts taken from days 1 to 30 post-transplantation were examined in non-immunosuppressed NOD mice: 54 isografts, 67 major histocompatibility complex- (MHC)-mismatched allografts, and 123 xenografts, as shown in Table 1.

Recipients were adult male and female NOD mice, either diabetic or prediabetic, of two inbred sublines; a low diabetes incidence NOD/Wehi line and a high diabetes incidence NOD/Lt line.¹² The majority of the grafts were in NOD/Lt female mice. The mice were

Table 1. *Number and Type of Graft Examined at Various Times Post-transplantation in Non-immunosuppressed NOD Mice*

| Days after transplantation | Number of specimens examined | | |
|----------------------------|------------------------------|------------|------------|
| | Isografts | Allografts | Xenografts |
| 1 | 3 | 3 | 3 |
| 2 | — | — | 5 |
| 3 | 3 | 6 | 6 |
| 4 | — | — | 5 |
| 5 | 3 | 3 | 3 |
| 7 | — | 3 | 8 |
| 9–11 | 6 | 9 | 14 |
| 13–15 | 8 | 8 | 13 |
| 20–24 | 17 | 22 | 23 |
| 27–30 | 14 | 13 | 43 |

Animals were untreated controls from a range of experiments where various types of immunosuppression were tested. In most cases prediabetic mice were used. The graft site was serially sectioned and alternate slides stained with hematoxylin and eosin or aldehyde fuchsin, or left unstained for immunocytochemistry to detect islet endocrine cells in cases when aldehyde fuchsin was negative, to detect α - and δ -cells, or to identify residual surviving endocrine cells in advanced rejection.

bred at the Walter and Eliza Hall Institute animal house under specific pathogen-free (SPF) conditions and were transferred to a clean but non-SPF holding facility at age 4 to 6 weeks and maintained there until use. They were given free access to mouse chow (Barastoc pellets, Victoria, Australia) and acidified drinking water. Mice were observed for cage wetting and weight loss, and when this was noted random blood glucose measurements were made using a Beckman Astra 4 multichannel analyzer (Beckman Instruments, Porterville, CA). Mice with a consistent random blood glucose >15 mmol/L were treated with daily insulin injections (Ultralente Novo Nordisk, Denmark, 1 to 4 U/day) before and after transplantation. Non-diabetic mice were not given exogenous insulin. Donor tissues for iso- and allografts were from 16- to 18-day fetal mice from age dated pregnancies, and for xenografts from fetal pigs aged between 60 and 100 days.

Tissue for transplantation was removed aseptically from the fetal donors and placed in organ culture as previously described.¹³ The fetal mouse pancreas was kept *in vitro* for 7 to 14 days, and the fetal pig pancreas for 4 to 7 days. The precise duration and mode of culture varied between experiments, but this had no effect on the host response and survival of the grafts in non-immunosuppressed recipients. The fetal pig tissue was routinely maintained in 90% O₂/10% CO₂ for 2 to 3 days and was then transferred to 10% CO₂/90% air for the remainder of the culture time. Fetal mouse pancreas was maintained in 10% CO₂/90% air.

Transplantation was usually of single pieces of tissue placed under the renal capsule. Similar amounts of tissue were used for all types of grafts. In some cases grafts were placed under the capsule of both kidneys. In most experiments only xenografts were used, but in two experiments each mouse was grafted with isogeneic, allogeneic, and xenogeneic tissue, and in one experiment allo- and isografts but not a xenograft were used in the same animal. The methods used have been described elsewhere.¹⁴ Tissue that was transplanted was also taken for histological examination before transplantation to confirm that it was viable, and in many experiments athymic (nude) BALB/c or CBA mice were also transplanted so that the development of the tissue in a recipient unable to reject the graft could also be assessed.

Grafts were removed from non-immunosuppressed mice at intervals from day 1 post-transplantation by removing the entire kidney, which was either fixed in Bouin's fluid or frozen in isopentane in liquid N₂ for phenotyping of the infiltrating cells. The Bouin's-fixed kidneys were placed in 70% ethanol for 24 hours, and the graft sites identified and cut out for routine processing for light microscopic examination. The specimens were coded, and paraffin-blocked specimens were serially sectioned at 4 μ m. Alternate slides were stained with hematoxylin and eosin for a general examination of the graft site, and with aldehyde fuchsin (AF) to detect β -cells, mast cells, and connective tissue elements (elastin and collagen); unstained slides were processed for anti-hormone immunocytochemistry to detect insulin (β -cells), glucagon (α -cells), and somatostatin (δ -cells), but this was not done routinely. For immunocytochemistry of infiltrating cells frozen sections were used. These specimens were placed in Tissue-Tec and rapidly frozen in isopentane in liquid N₂. Biotinylated anti-rat primary antibodies were used to detect CD4 and CD8 (GK1.5 and 53-6.7, respectively), F4/80 to detect macrophages and rat anti-mouse immunoglobulin (Ig) or B220 to detect B cells. Avidin-peroxidase was used to label these. The reactions were detected with aminobenzidine.

The graft sites were scored for graft survival and for the type of infiltrate present. All specimens were coded and in each experiment were examined without knowledge of the treatment, if any, that the mouse had received. In addition, the pancreas of each recipient was also taken for histological evaluation by scoring the severity of insulinitis in all islets seen in three longitudinal sections of each pancreas.¹⁵ Grafts were scored by assessing the extent of graft survival and the degree of infiltration. In graft

scores 4+ represented a graft showing well developed islets and ducts, while a score of 0 indicated the absence of any evidence of a graft. Intermediate scores represent variations in graft destruction with 3+ representing an essentially intact graft with some perigraft infiltration, 2+ a graft with obvious persisting endocrine cells, and 1+ a graft showing the presence of a few remaining endocrine cells. Similarly, infiltrate scores ranged from 0, where there is no infiltrate, to 4+, where there is an extensive infiltrate and no remaining graft. Insulinitis scores in the pancreas were assessed by scoring each islet seen in each of three longitudinal sections from 0 to 4+, where 0 is a normal islet with no evidence of infiltration, 1+ indicates a focal peri-islet or periductal infiltrate where less than 25% of the islet periphery is affected, 2+ represents peri-islet infiltration where 25 to 75% of the islet periphery is affected, 3+ indicates intra-islet invasion with disruption of the endocrine cells and loss of β -cells, and 4+ represents severe damage with total loss of β -cells. All individual islet scores were then summed and converted to a percentage of the maximum possible score that would have been found had all islets been scored 4+.

Results

Pretransplant Morphology and Development in Nude Mice

Examination of the fetal mouse pancreas after 7 to 14 days *in vitro* showed that there was excellent survival of islets and ducts but total loss of exocrine tissue. The fetal pig pancreas after 3 to 5 days *in vitro* was similar, except that immunoperoxidase cytochemistry revealed less obvious development of islets, and the tissue consisted of ducts, small clusters of islet cells that contained insulin, glucagon, or somatostatin; however, β -cells were negative by aldehyde fuchsin staining. Although exocrine-like cells were prominent preculture, no viable acinar tissue remained after a few days *in vitro*. Thus, in these experiments no obvious exocrine tissue was transplanted, and the grafts consisted of islet cells and ducts with variable amounts of connective tissue. The detailed development of fetal pig pancreas *in vitro* will be described separately (J. Kovarik, M. Koulmanda, and T. E. Mandel, submitted for publication). To confirm that the fetal pancreas could develop and differentiate into islets after transplantation, nude mice that cannot reject allo- or xenogeneic grafts were examined. These confirmed that the

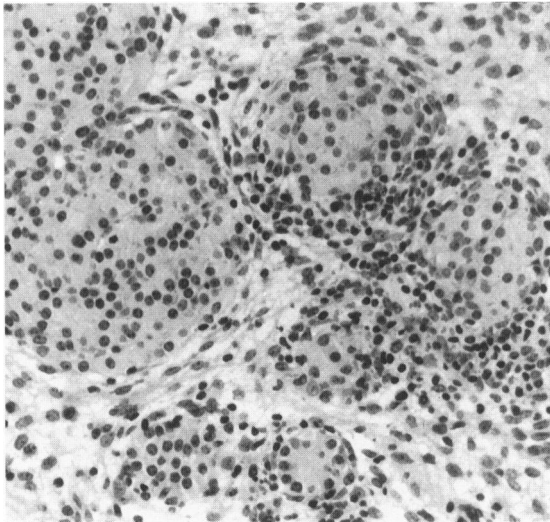


Figure 1. Infiltrating cells in the isograft site of a prediabetic NOD mouse 11 days after transplantation showing a peri-islet collection of predominantly small MNC. Well developed islets are present and stain positive for β -cells with the AF stain. ($\times 250$).

grafts were viable and that post-transplant differentiation could occur with excellent development of islets and strong staining of β -cells by the AF method, as previously reported.¹⁶

Isografts in Prediabetic Recipients

Prediabetic recipients remained well and maintained random blood glucose levels within normal limits (7 to 10 mmol/L). One day after transplantation the isograft sites were edematous but there was only a minimal cellular response consisting of a few neutrophils and macrophage-like cells. The transplanted tissue was obvious and resembled closely the pre-transplant appearance of the tissue that was used for that graft. The graft sites began to be invaded by mononuclear cells (MNC) by about the third day. The infiltrating cells were generally small with the appearance of typical lymphocytes. The infiltrate was sparse or even absent and in prediabetic recipients was always peri-islet and spared the islets (Figure 1). Indeed, when the recipients were sacrificed and the severity of the insulinitis in the pancreas scored there was a good correlation between the intensity of the insulinitis around the islets and the severity of the infiltrate in the graft site after 14 days post-transplantation (Figure 2). Well stained β -cells were present in the graft within days of transplantation (Figure 3). Immunocytochemistry showed that all major lymphocyte subsets were present but B cells (slg or B220+ve) were relatively sparse while typical T cells (CD3/Thy1+ve) and the two major T-cell subsets

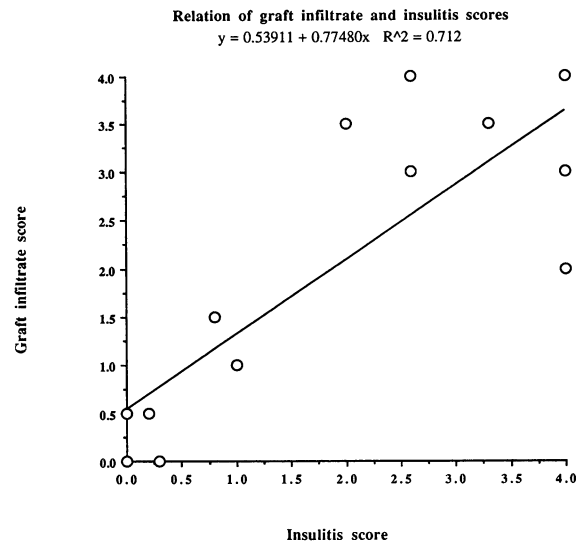


Figure 2. Correlation between insulinitis severity (horizontal axis) and infiltration of isografts (vertical axis) in prediabetic and diabetic mice. The grafts were assessed 14 or 30 days post-transplantation in a mixed population of prediabetic and diabetic recipients.

(CD4+ve and CD8+ve) were common, with usually more CD4+ve than CD8+ve cells. Macrophages (F4/80+ve) were common and were scattered throughout the graft site. Granulocytes were usually absent or at most infrequent. Hormone immunocytochemistry showed that the three major endocrine cells (α , β , and δ) were well preserved.

Isografts in Diabetic Recipients

Diabetic recipients (blood glucose levels >15 mmol/L) were treated with daily insulin injections,

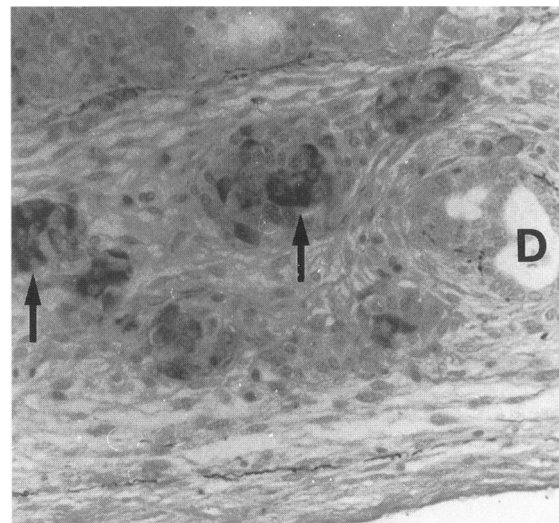


Figure 3. An isograft 5 days after transplantation into a prediabetic recipient showing some well differentiated AF+ve β -cells (arrows) and AF-ve ducts (D). There is only a minimal infiltrate in the graft site ($\times 300$, AF stain).

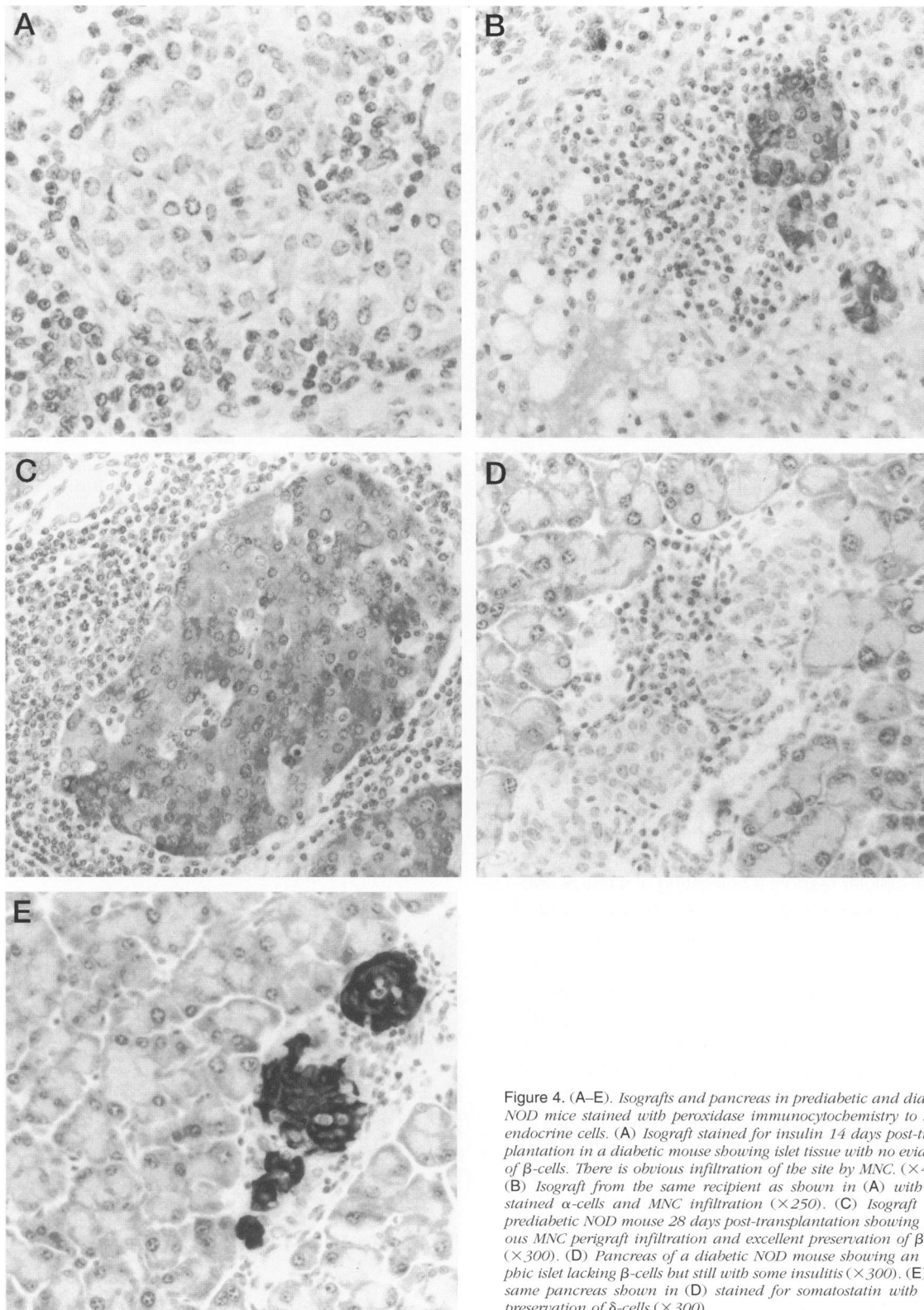


Figure 4. (A–E). Isografts and pancreas in prediabetic and diabetic NOD mice stained with peroxidase immunocytochemistry to show endocrine cells. (A) Isograft stained for insulin 14 days post-transplantation in a diabetic mouse showing islet tissue with no evidence of β -cells. There is obvious infiltration of the site by MNC. ($\times 400$). (B) Isograft from the same recipient as shown in (A) with well stained α -cells and MNC infiltration ($\times 250$). (C) Isograft in a prediabetic NOD mouse 28 days post-transplantation showing obvious MNC perigraft infiltration and excellent preservation of β -cells ($\times 300$). (D) Pancreas of a diabetic NOD mouse showing an atrophic islet lacking β -cells but still with some insulinitis ($\times 300$). (E) The same pancreas shown in (D) stained for somatostatin with good preservation of δ -cells ($\times 300$).

and although this did not maintain their blood glucose levels within normal limits, the mice remained relatively healthy with a good maintenance of their body weight in contrast to untreated diabetic recipients that lost weight rapidly and died within weeks. In contrast to the appearance of isografts in prediabetic recipients, the graft sites in non-immunosuppressed diabetic mice were rapidly invaded by MNC, but in this instance the invading cells entered the islets and quickly destroyed most of the endocrine cells. By 14 days post-transplantation there was generally complete destruction of β -cells, and immunocytochemistry showed that whereas β -cells were absent, α - and δ -cells remained (Figure 4, A and B). This was also quite apparent in AF-stained specimens where obvious islets and well developed ducts were present, but no AF-positive cells remained. This was in marked contrast to isografts in prediabetic animals that had well stained β -cells despite often marked peri-islet infiltration (Figure 4 C). The appearance of the grafts resembled that of the islets in the recipient's pancreas, which also showed a selective loss of β -cells with retention of α - and δ -cells (Figure 4, D and E). That β -cell damage or degranulation was not due to diabetes per se was established from experiments where spontaneously diabetic mice were immunosuppressed, and even in the presence of quite severe hyperglycemia some granulated β -cells remained in the grafts when they survived for a sufficiently long period (T. E. Mandel and M. Koulmanda, unpublished observation).

Allografts

On the day after transplantation, the allograft was essentially identical to the appearance of an isograft. The response against MHC-mismatched allogeneic islets occurred rapidly, and a slight but obvious cellular response was present in the graft site by 2 days. At that time there was a sparse infiltrate of mainly small MNC that were frequently still within the lumen of small vessels. The severity of infiltration was greater by the third day, and by four to five days there was a large infiltrate consisting almost solely of MNC, some of which were large and blast-like and others of which had the appearance of macrophages. Immunocytochemistry showed that CD4+ve, CD8+ve, and F4/80+ve cells were all present in large numbers and these cells were starting to invade the grafted tissue, but there were few or no granulocytes. By days 5 and 7 there was some damage to the graft, but some islets and ducts were generally still well preserved despite the presence of a marked MNC infiltrate (Figure 5). By 9 to 12 days

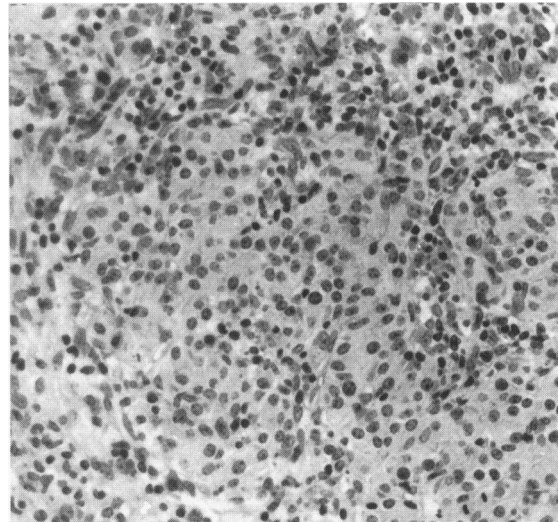


Figure 5. Infiltration of an allograft with MNC on day 5. The transplanted tissue is still intact and consists of well developed islets ($\times 250$, H&E).

there was little or no evidence of viable grafted islets, but there was a heavy infiltrate of predominantly MNC, many of which were large and blast-like (Figure 6). Small nests of hormone-positive cells were sometimes still detectable by immunocytochemistry, as were ducts embedded in the infiltrate. By 14 to 15 days there was no evidence of a graft, but a moderately heavy MNC infiltrate was still present. After this time the infiltrate started to diminish and collagen in the graft site increased so that the site was converted to a mildly cellular scar. By 21 days the resolution of the inflammatory response was well ad-

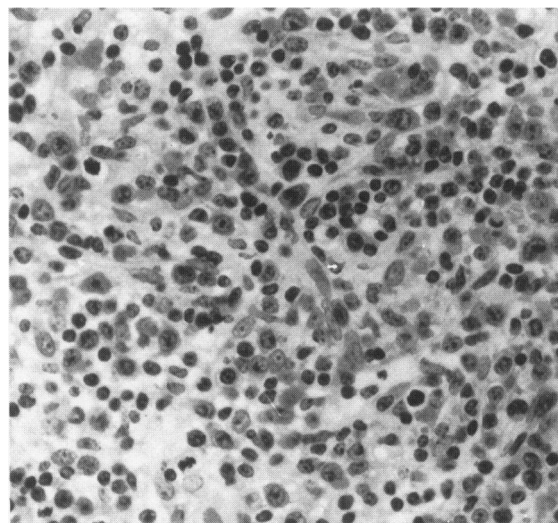


Figure 6. Infiltrate in an allograft site at 11 to 13 days showing mostly MNC, many of which are blasts. There is no evidence of any surviving graft ($\times 400$, H&E).

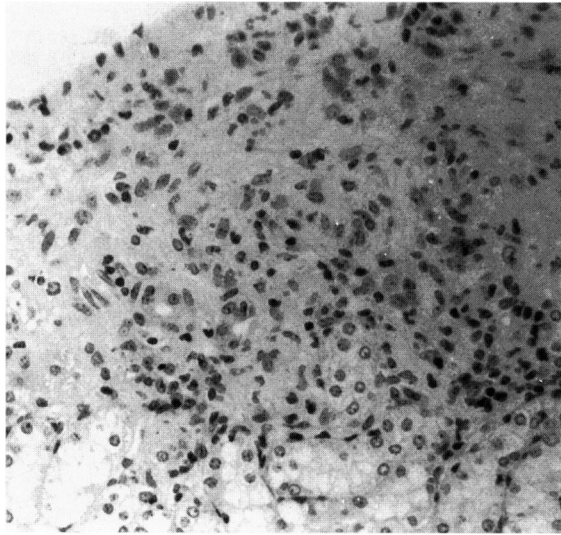


Figure 7. Resolving allograft site with a scar and a residual infiltrate of predominantly small MNC that are mainly in the superficial renal cortical zone 28 days after transplantation ($\times 250$, H&E).

vanced, and by 28 days there was only a thin relatively acellular scar left (Figure 7).

Xenografts

The xenograft site resembled the iso- and allograft sites on day 1 with edema and a minimal cellular reaction but with large amounts of graft tissue present. The site began to become infiltrated only after day 3 when a few MNC were seen usually within the lumina of small vessels. By day 4 to 5 there was a variable but often slight perigraft infiltrate of MNC

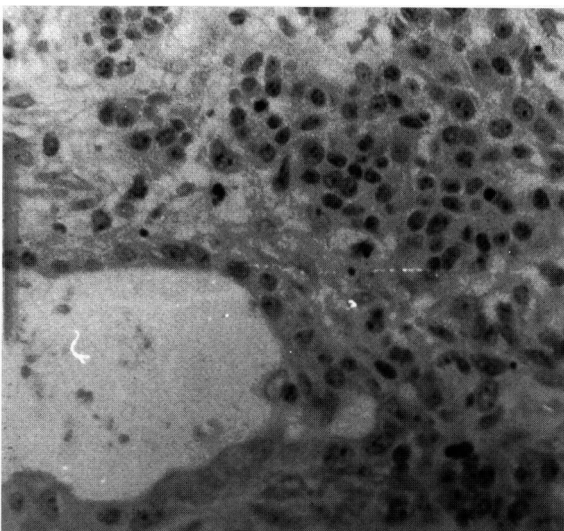


Figure 8. Xenograft at 3 days showing well a preserved graft consisting of islets and ducts with only a few mainly small MNC ($\times 400$, H&E).

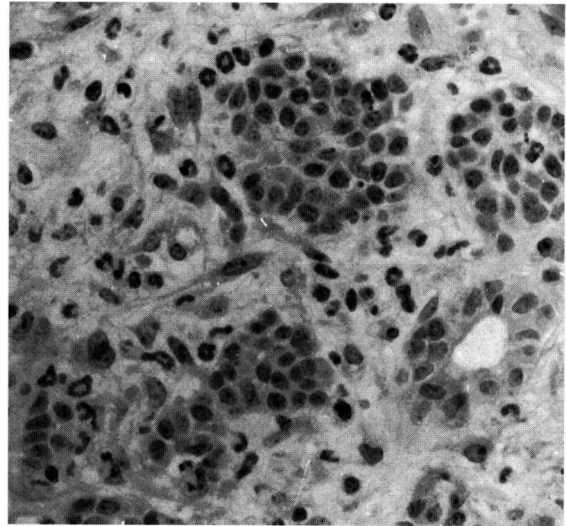


Figure 9. Xenograft at 7 days showing good survival of the graft consisting of islets and ducts but with only a sparse infiltrate consisting of eosinophils and some MNC of varying size ($\times 400$, H&E).

(Figure 8); however, by day 7 the infiltrate was often large and was clearly destroying the graft but was quite variable in severity in different specimens. Nonetheless, in contrast to the appearance of the allograft site at this time, the xenograft infiltrate was dominated by granulocytes, mainly eosinophils with few neutrophils (Figure 9). Some mast cells, particularly around the edge of the site, and many plasma cells, macrophages, and multinucleate giant cells were seen. The phenotype of the MNC showed that T cells, both CD4+ve and CD8+ve, were present as well as many F4/80+ve macrophages. Ductal and islet cells were still evident at 7 to 9 days in most specimens, and even by 11 days islet cells and ducts were sometimes present.

In contrast to the allograft site, the xenograft site remained large and infiltrated for a prolonged period so that at 28 days when the allograft sites had resolved into a thin scar the xenograft site was still large and swollen and readily recognizable macroscopically even 6 to 8 weeks after transplantation. When examined microscopically, the xenograft site showed a large central acellular necrotic zone where there were often ghosts of cells present surrounded by a dense infiltrate of mixed MNC and granulocytes (Figure 10). In addition, there was much fibrosis with large amounts of collagen and elastin detected by the aldehyde fuchsin stain. The infiltrate persisted for many weeks after all trace of a graft had disappeared, and the residual infiltrating cells included many eosinophils and some conspicuous multinucleate giant cells (Figure 11).

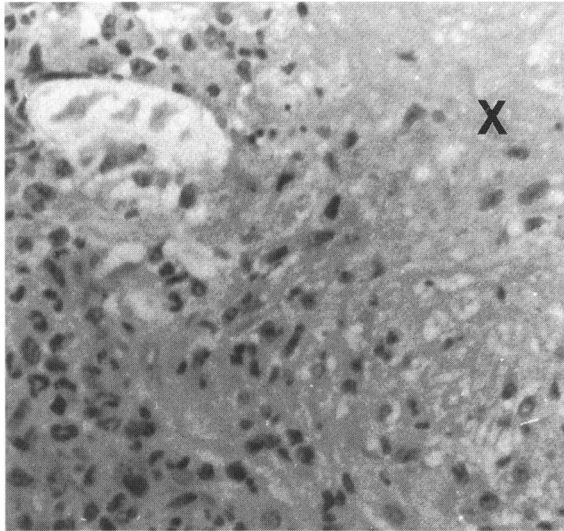


Figure 10. Xenograft site at 15 days showing a large central necrotic zone (X) surrounded by an extensive infiltrate ($\times 250$, H&E).

Discussion

There are obvious differences between the appearance of the inflammatory reactions in the three transplant situations. Recurrent autoimmune disease occurs in isografts in diabetic and prediabetic recipients with a severity that resembles that seen in the recipient pancreas. In non-immunosuppressed diabetic mice there is rapid specific β -cell destruction, whereas in prediabetic mice there is peri-islet infiltration without islet invasion and with good survival of β -cells. Rejection of MHC-mismatched allografts occurs rapidly with a predominantly MNC infiltrate of many activated cells, but apparently via-

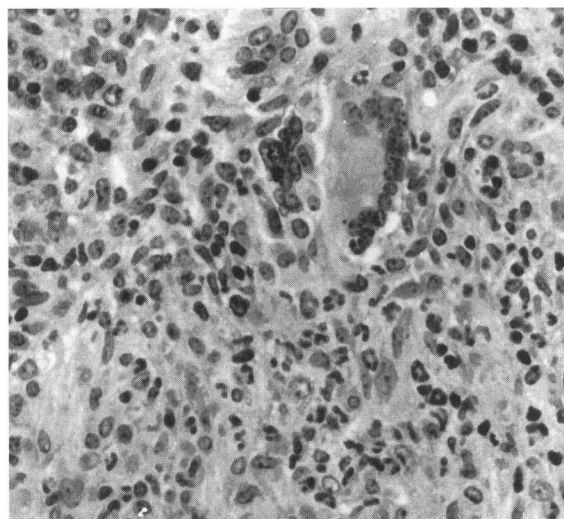


Figure 11. Extensive fibrosis of a xenograft site 28 days post-transplantation with a moderately large residual infiltrate of MNC, granulocytes and multinucleate giant cells. ($\times 400$, H&E).

ble graft tissue is still present for 8 to 10 days. Once rejection has occurred there is rapid resolution of the infiltrate. In contrast, rejection of the xenograft is quite different from the allograft response in that xenograft rejection is slightly slower with a predominance of eosinophils, a relative paucity of lymphoid cells, and prolonged persistence of the reaction. Thus, in the three instances there was a marked cellular response that differed qualitatively and quantitatively.

In recurrent autoimmunity the infiltrate lacked granulocytes and consisted of mainly T cells and macrophages. The mechanism of β -cell damage is still controversial, but the consensus seems to be that initial macrophage invasion results in the recognition and processing of autoantigenic epitopes, presentation of these to CD4+ve T cells with subsequent activation of CD8+ve effector cells. We have previously shown that progressive damage to islets in NOD mice can be prevented by interfering with each of these steps^{15,17} with agents that act selectively against macrophages (eg, silica), or with monoclonal antibodies against either CD4 or CD8+ve T cells. Many studies have reported the prevention of autoimmune β -cell damage, but it is still unclear what the precise effect of the various putative effector cells is. Similar graft pathology has been reported in humans with pancreas grafts from an identical twin non-diabetic donor and also in immunosuppressed recipients of an HLA-matched sibling pancreas graft,¹⁸⁻²⁰ and the major infiltrating cells appear to be CD8+ve T cells.

In an MHC-mismatched allograft there was rapid invasion of the site by MNC but in contrast to isografts, in the allograft all grafted cells were destroyed, including finally the ducts that probably contain the precursors of the differentiated endocrine cells.²¹ It is not clear why ducts are spared for longer than the differentiated cells. However, the inflammatory infiltrate rapidly cleared when the graft had been destroyed with rapid resolution of the inflammation and the formation of a thin relatively acellular scar.

In the xenografts of discordant donor tissue, the appearance of the graft site was quite different and the predominant infiltrating cells were mostly eosinophils with a few neutrophils and some mast cells, and large numbers of macrophages including multinucleate giant cells. The inflammatory cells persisted, and a large granuloma with a central necrotic zone developed. Thus the xenografts were rapidly but not hyperacutely rejected, and indeed there was persistence of apparently viable grafted tissue for at least as long as in the MHC-mismatched allografts.

The striking feature of the xenograft response was the presence of large numbers of eosinophils. Eosinophils are not usually a major feature of allograft rejection, but have been described in a variety of settings particularly when severe rejection was occurring.²²⁻²⁸ However, in this study the xenogeneic tissue survived for at least as long as did the allografts and, indeed, the appearance of a response to the xenograft was even slightly delayed. Thus, initial severity of rejection per se seems not to be the sole reason for the presence of these cells.

The precise role of eosinophils in graft destruction is poorly understood, but it is known that they can be potent cytotoxic cells via the production of a number of defined secreted products, ie, major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin,²⁹ which can cause cell damage in transplantation^{27,30} as well as in many other better known situations such as asthma and parasitic infestations.³¹⁻³³ The many functions of eosinophils in a range of disease states have recently been comprehensively reviewed^{32,33} but in neither review was xenotransplantation mentioned.

Eosinophils have also been reported to activate platelets,³⁴ and this too may exacerbate graft damage via platelet-derived factors. The persistence of the infiltrate and the formation of a large granuloma with evidence of central necrosis and extensive fibrosis suggests that perhaps a quite different form of rejection was occurring in the xenografts. Treatment strategies different from those used to control allotransplantation may be needed to counter the effects of eosinophils. Even when hyperacute rejection is avoided by using recipient endothelium to line the graft vessels, rejection will still be a serious problem. Eosinophils have also been associated with inflammatory fibrosis as a consequence of the release of their granules,³⁵ and this may be a reason for the extensive fibrosis of the xenograft sites.

Other studies with this and similar models have shown that treatment with anti-T cell monoclonal antibodies against the CD3 and/or CD4 epitopes is effective in preventing acute xenograft destruction,^{11,36} and when rejection does occur it corresponds to the appearance of MNC and eosinophils. The dependence of eosinophils on a prior CD4 T cell-mediated response is well documented in other models³⁷ and interleukin (IL)-5³⁸ as well as other cytokines³⁹⁻⁴¹ are also implicated in eosinophil recruitment. The important role of T cells in an anti-xenograft response is not being challenged by these data. It is well known that T cells are necessary for xenograft rejection because such grafts survive well

in T cell-depleted nude mice.¹⁶ What we stress is that T cells may act via a different final effector mechanism. Thus, it may be necessary to develop immunosuppressive protocols that are different from those that are generally effective against allograft rejection. These may include the use of agents that act against eosinophils (eg, anti-IL-5), drugs that are anti-macrophage in their action (eg, deoxyspergualin) or interfere with the products of these cells such as NO (eg, NG-monomethyl-L-arginine), as well as more potent anti-B cell reagents (eg, cyclophosphamide, rapamycin), since antibody-mediated effects may also be important in xenograft rejection.^{42,43}

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