

THE SPECIFICITY OF REJECTION AND THE ABSENCE
OF SUSCEPTIBILITY OF PANCREATIC ISLET β CELLS
TO NONSPECIFIC IMMUNE DESTRUCTION IN
MIXED STRAIN ISLETS GRAFTED BENEATH
THE RENAL CAPSULE IN THE RAT

BY ROBERT SUTTON, DEREK W. R. GRAY, PHILIP MCSHANE,
MARGARET J. DALLMAN, AND PETER J. MORRIS

*From the Nuffield Department of Surgery, University of Oxford,
John Radcliffe Hospital, Headington, Oxford OX3 9DU, United Kingdom*

β cells of the endocrine pancreas have been shown to be sensitive to several lymphokines in vitro, notably the macrophage product IL-1, which can impair β cell insulin secretion (1-3). Combinations of IL-1 and either TNF, lymphotoxin or IFN- γ are cytotoxic to rat islet cell monolayer cultures, as shown by accelerated ^{51}Cr release and phase contrast microscopy (4). These data suggest that lymphokines may be important mediators of the autoimmune destruction of β cells during the development of insulin-dependent diabetes (5).

Allogeneic and even syngeneic macrophages have been shown to lyse cultured mouse islets (6). In vivo, the macrophage toxin silica has been shown to reduce the incidence of autoimmune diabetes in both NOD mice (7) and BB rats (8), and to prevent islet allograft rejection (9, 10). These findings might suggest that macrophage products are important in β cell destruction, both in autoimmune diabetes, and in islet allograft rejection.

Macrophages are known to produce lymphokines, notably IL-1 (11, 12), and there is evidence that other lymphokines such as IFN- γ and TNF are released during allograft rejection (13). If islet cells are particularly sensitive to lymphokines, the effects of such lymphokine release might explain why isolated islets appear especially susceptible to allograft rejection. Islets transplanted into the portal vein across a major histocompatibility barrier in rats are usually rejected within 3 or 4 d, whereas kidney, heart, liver, and skin allografts are rejected in ~ 7 or 8 d (14, 15). Similarly transplantation of the whole pancreas as a vascularized organ results in islet rejection around 7 to 8 d (16, 17). In addition, isolated islet rejection is difficult to prevent using protocols that are effective in preventing the rejection of vascularized organ allografts (18-20). It would seem therefore that the effector mechanisms of rejection

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of isolated islet allografts might be different in some way from that for other tissues and organs.

Nevertheless, the rejection of isolated islet allografts displays features that are typical of the rejection of vascularized organ and other tissue allografts, including genetic control (21–23), a latent period before allograft destruction (24, 25), occurrence in all vascularized regions of the body (20), an inverse relationship between graft size and graft survival (26, 27), memory (28, 29), and T cell dependency (30, 31). One feature of islet allograft rejection that has not previously been examined and that might be different from that for other tissues and organs is specificity. Skin allograft rejection was shown to be highly specific using adult allophenic mouse donors produced by fusing two H-2-disparate embryonic blastocysts (32, 33). Similar results have been reported more recently (34). If the effector mechanisms of islet allograft rejection are in some way different, and if this difference is attributable to the destructive effects of lymphokine release on islet tissue, then it might be expected that islet allograft rejection would be relatively nonspecific. Once local lymphokine release has been induced, there might be nonspecific bystander lysis of β cells, regardless of histocompatibility.

In the experiments described below we examined the specificity of islet allograft rejection amongst mixed strain rat islets grafted to the renal subcapsular site.

Materials and Methods

For each experiment islets were isolated by ductal collagenase injection as previously described (35) from three adult DA (RT-1^a) and either three adult Lewis (LEW, RT-1^b) or three adult PVG (RT-1^c) rats to obtain at least 1,000 islets from each strain. Allogeneic islets from a single donor strain were used throughout each experiment. Islets from each strain were kept separately in 60-mm petri dishes, and viewed through a dissecting microscope against a black background with side illumination.

200 syngeneic DA islets were handpicked with a siliconized glass micropipette and pediatric aspirator, then placed into each of five numbered receivers that were kept on ice. The receivers were constructed by cutting off the lowermost portion of a universal container with a heated scalpel, producing a receptacle with the shape of an inverted cone. 200 allogeneic (LEW or PVG) islets were similarly placed into each of five other labeled receivers. To ensure that a similar quantity of islet tissue was placed into each container, groups of similarly sized islets were hand picked 20 at a time, and placed into each receiver in rotation. The receivers containing DA and allogeneic (LEW or PVG) islets were then randomized separately, using random number tables.

In each experiment islets were transplanted into three normal DA recipients beneath each renal capsule using a miniature blood clot technique as previously described (36). In the first recipient, 200 syngeneic islets were placed under the light renal capsule, and 400 syngeneic islets (two aliquots of 200, mixed together gently but thoroughly with microdissecting forceps before transplantation) under the left renal capsule. In the second recipient, 200 syngeneic islets were placed under the upper pole of the right renal capsule, 200 allogeneic islets (LEW or PVG) under the lower pole of the right renal capsule, and a mixture of 200 syngeneic and 200 allogeneic islets (mixed together before transplantation) under the left renal capsule. In the third recipient, 400 allogeneic islets (2 aliquots of 200, mixed together before transplantation) were placed under the right renal capsule, and 200 allogeneic islets under the left renal capsule.

Kidneys bearing islet grafts were excised 21 d after islet transplantation for acid alcohol extraction and radioimmunoassay of insulin content (36, 37), or for histological examination. Paraffin-embedded sections were stained with hematoxylin and eosin as well as by a two-layer immunoperoxidase technique for insulin and glucagon. Fresh frozen sections for immunohistology were stained by a two-layer immunoperoxidase technique using a panel

of mAbs directed at either DA class I (MN4), LEW class I (NDS 60), PVG class I (OX 27), or rat leukocyte common (OX 1 with OX 30) antigens, with antiinsulin and negative (OX 21) controls.

Statistical analysis of the results of insulin assay was carried out with the paired *t* test and by analysis of variance; confidence intervals were calculated from standard errors of the means.

Results

Table I displays the results of insulin extraction assays from six experiments with LEW allogeneic islets and six experiments with PVG allogeneic islets, as well as the results with data pooled from both experimental groups. The results of insulin extraction from all kidneys grafted with only allogeneic islets are not listed, as none contained >1 mU of insulin. The paired *t* test and analysis of variance showed no significant differences between the insulin extracted from kidneys grafted with only 200 syngeneic islets, the insulin extracted from kidneys grafted with 200 syngeneic and 200 allogeneic islets under separate poles of the kidney capsule, and the insulin extracted from kidneys grafted with a mixture of 200 syngeneic and 200 allogeneic islets. The quantity of insulin extracted from kidneys grafted with 400 syngeneic islets was significantly greater than and approximately twice that extracted from the other kidneys listed in Table I.

When examined by paraffin histology all grafts that were originally composed of only syngeneic sites showed intact morphology with plentiful insulin containing beta cells (see Fig. 1 *a*); there was also preservation of glucagon-containing α cells (not shown). No surviving islet tissue could be demonstrated at the site of any previous

TABLE I
Results of Insulin Extraction and Radioimmunoassay

Islets	Number of experiments	Insulin (mU per kidney) from kidneys bearing islets implanted beneath the renal capsule 21 d before removal*			
		200 Syngeneic	400 Syngeneic	200 Syngeneic 200 Allogeneic (separate)	200 Syngeneic 200 Allogeneic (mixed)
DA and LEW to DA:	6				
Mean (SD)		72.3 (26.7)	119.0 (71.0)	66.0 (25.4)	82.5 (44.6)
95% CIs		44.4-100.3	44.5-193.5	39.3-92.7	35.7-129.3
DA and PVG to DA:	6				
Mean (SD)		40.8 (27.9)	127.0 (41.6)	63.7 (37.4)	63.8 (26.0)
95% CIs		11.9-70.1	83.3-170.7	24.4-102.9	36.5-91.2
Combined data:	12				
Mean (SD)		56.5 (31.8)	123.0 (55.4)	64.8 (30.5)	73.2 (36.2)
95% CIs		36.4-76.8	87.8-158.2	45.5-84.2	50.2-96.1

The results from kidneys in which either 200 or 400 allogeneic islets only were implanted are not given as in no case was more than 1 mU insulin extracted.

* The power of these experiments to detect a difference of 20 mU of insulin was 0.45 (assuming an SD of 34.8, i.e., the SD of the pooled difference between the insulin extractions of columns three and four, with *L* at 0.05); to detect a difference of α 30 mU, 0.75; to detect a difference of 40 mU, 0.95.

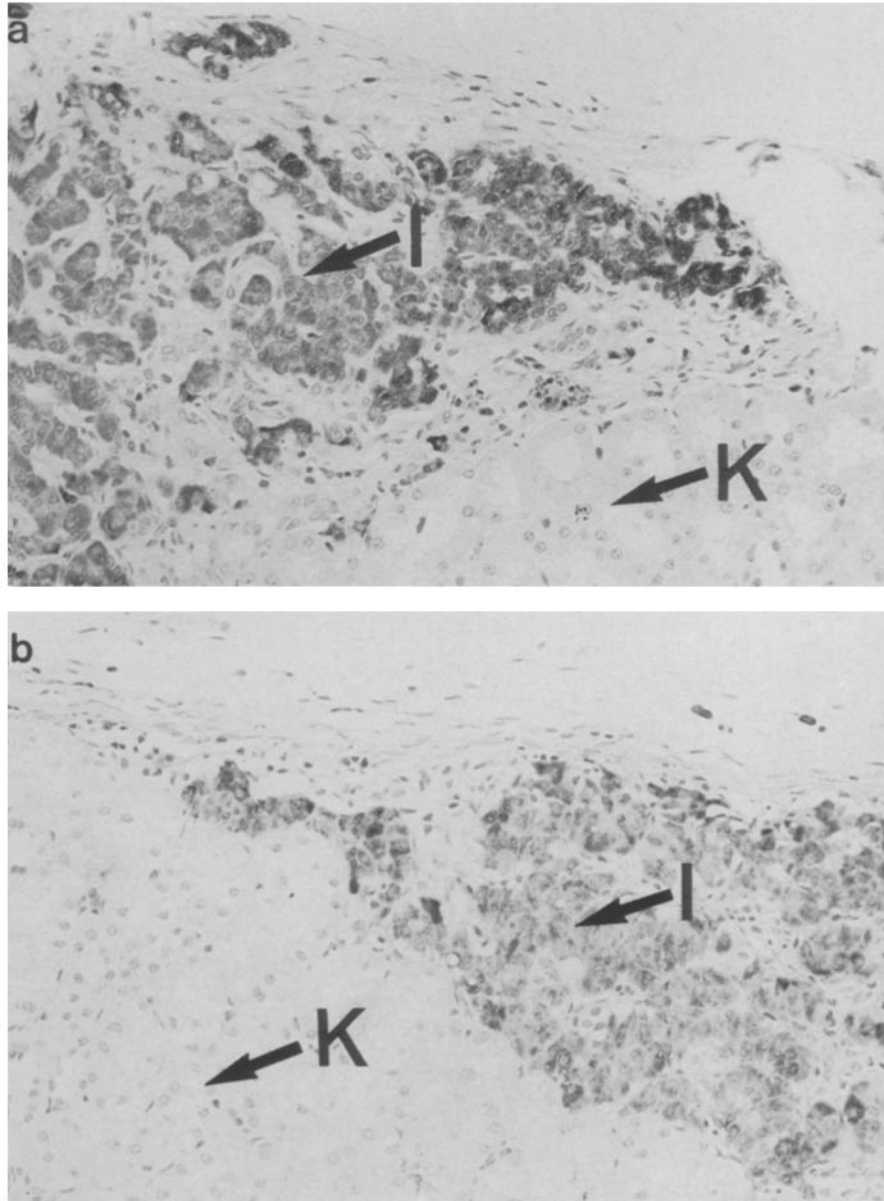


FIGURE 1. Immunoperoxidase-stained paraffin sections of kidneys (*K*) bearing subcapsular islet grafts (*I*) originally composed of syngeneic islets only or mixed syngeneic and allogeneic islets: (*a*) syngeneic only, stained for insulin (polyclonal guinea pig anti insulin, $\times 250$); (*b*) mixed syngeneic and allogeneic, stained for insulin (polyclonal guinea pig antiinsulin, $\times 250$), showing similar pattern of staining to that in *a*).

graft originally composed of allogeneic islets, whether or not syngeneic islet tissue had been grafted under the renal capsule of the same kidney. However, grafts originally composed of mixed syngeneic and allogeneic islets displayed intact morphology with evidence of both plentiful surviving β cells (see Fig. 1 *b*), and obvious preservation of α cells (not shown), similar to the appearance of grafts of syngeneic islets alone.

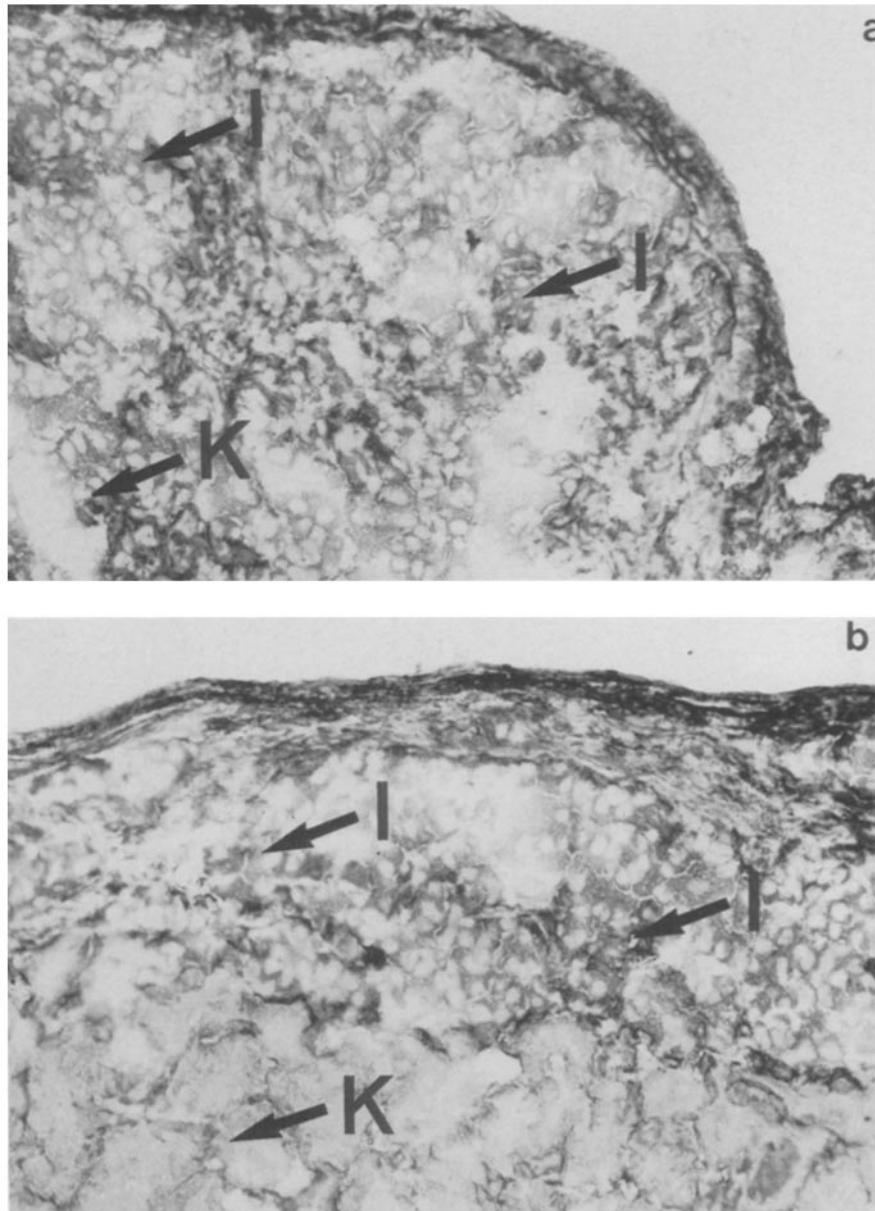


FIGURE 2. Immunoperoxidase stained fresh frozen sections of kidneys (*K*) bearing subcapsular islet grafts (*I*) originally composed of syngeneic islets only or mixed syngeneic and allogeneic islets: (*a*) DA islet graft to DA recipient stained for DA class I antigens (MN4, $\times 300$), with graft staining more pronounced than recipient kidney; (*b*) mixed DA and LEW islet graft to DA recipient stained for DA class I antigens (MN4, $\times 300$), showing similar pattern of staining to that in *a*.

Immunohistology of fresh frozen tissue demonstrated uniform expression of DA class I antigens in recipient kidneys, and throughout the substance of all grafts with syngeneic DA islets, whether or not allogeneic islets had originally contributed to their composition (see Fig. 2 *a* and *b*). The expression of DA class I antigens was

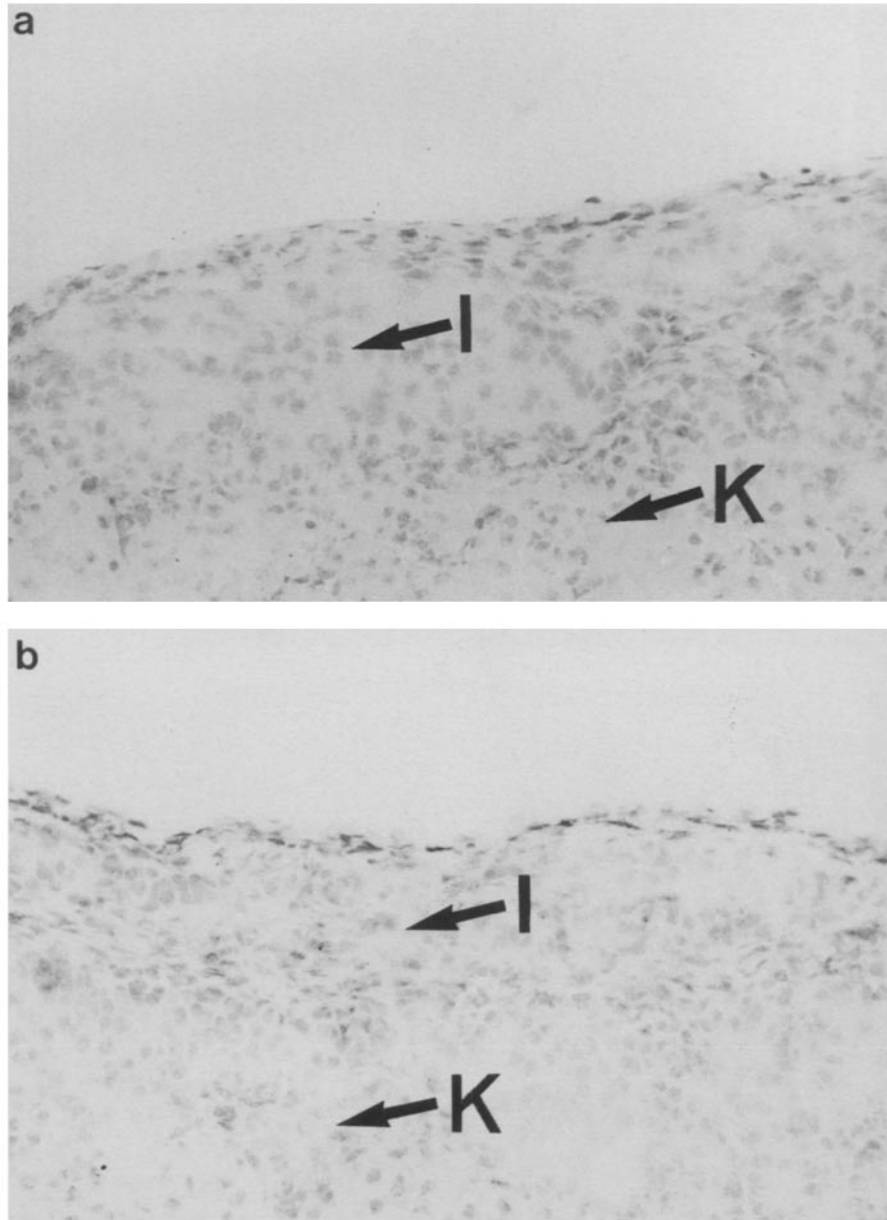


FIGURE 3. Immunoperoxidase-stained fresh frozen sections of kidneys (*K*) bearing subcapsular islet grafts (*I*) originally composed of mixed syngeneic and allogeneic islets: (a) mixed DA and Lew islet graft to DA recipient stained for LEW class I antigens (NDS 60, $\times 300$), showing no uptake of stain; (b) mixed DA and PVG islet graft to DA recipient stained for PVG class I antigens (Ox27, $\times 300$), again showing no uptake of stain.

more intense in all grafts than in recipient kidneys, again whether or not allogeneic islets had originally contributed to their composition. No surviving allogeneic tissue, either LEW or PVG, was demonstrable within any graft originally containing allogeneic islets, using the appropriate mAbs directed at class I antigens (see Fig. 3,

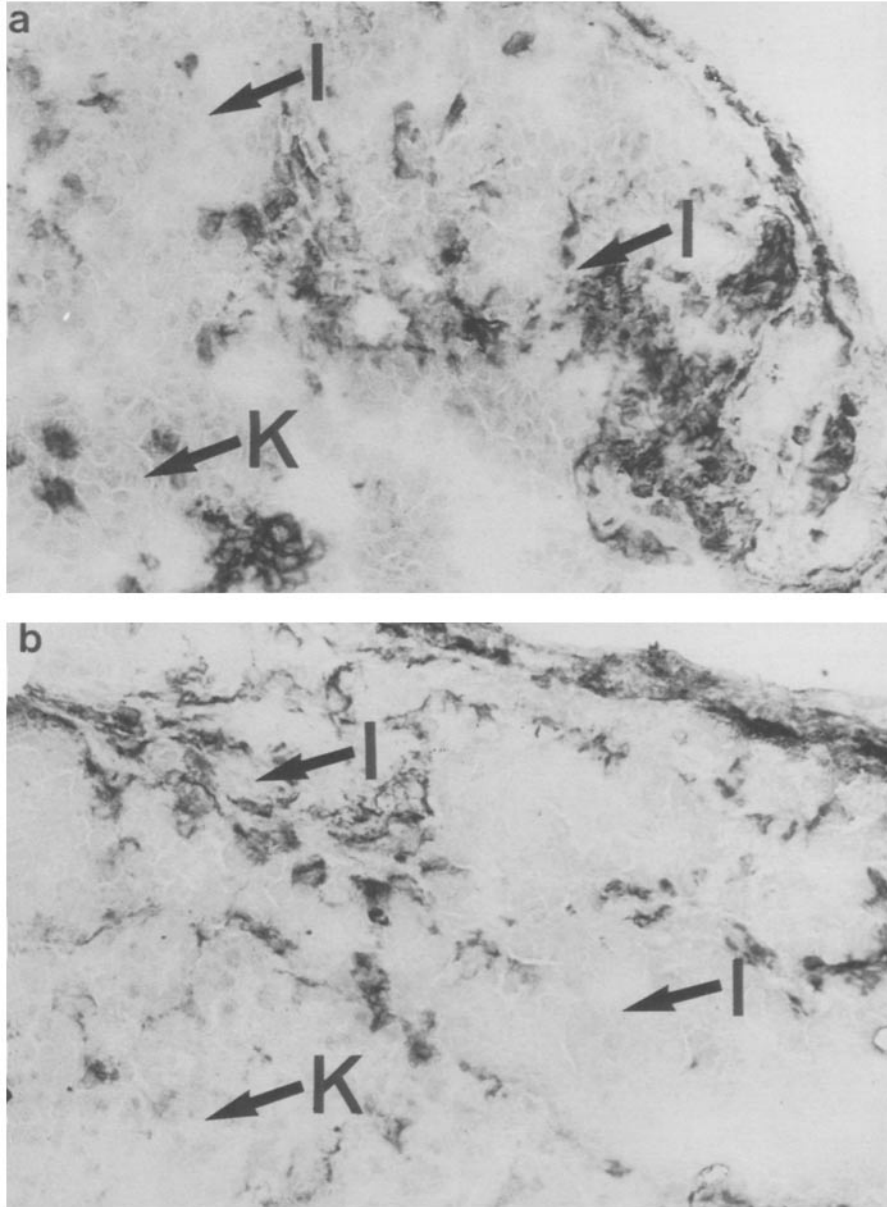


FIGURE 4. Immunoperoxidase-stained fresh frozen sections of kidneys (*K*) bearing supcapsular islet grafts (*I*) originally composed of syngeneic islets only or mixed syngeneic and allogeneic islets: (*a*) DA islet graft to DA recipient stained for rat leukocyte common antigens (OX 1 with OX 30, \times 300); (*b*) mixed DA and LEW islet graft to DA recipient stained for rat leukocyte common antigens (OX 1 with OX 30, \times 300), showing similar pattern of staining to that in *a*.

a and *b*). The leucocyte infiltrates present within eight grafts originally composed of only syngeneic islets and that present within four grafts originally composed of mixed syngeneic and allogeneic islets were not noticeably different (see Fig. 4, *a* and *b*).

Discussion

These experiments were designed to detect quantitative differences between the survival of syngeneic islets alone, allogeneic islets alone, and syngeneic islets mixed with allogeneic islets, and then to confirm the survival of either syngeneic or allogeneic islets by immunohistology. The results of insulin extraction from kidneys bearing grafts originally composed of only syngeneic islets substantiate the quantitative method of assessment, as kidneys bearing grafts originally composed of 400 syngeneic islets alone contained approximately twice as much insulin as kidneys bearing grafts originally composed of 200 syngeneic islets alone (see Table I). The pooled results of all 12 insulin extraction experiments demonstrate this relationship most clearly, presumably because of the effects of islet aliquot randomization, as well as because of a decrease in the standard error of the mean with increasing numbers of experiments.

The failure to extract insulin from any of the kidneys grafted originally with either 200 or 400 allogeneic islets alone is compatible with the destruction of all allografted tissue within 21 d of transplantation. Previously published metabolic and histological data have demonstrated that both LEW and PVG islets grafted into untreated DA recipients are destroyed within several days of intraportal transplantation (20, 38, 39), and within ~ 1 wk of renal subcapsular transplantation (20, 25). This rapid destruction of freshly isolated islets after transplantation across a major histocompatibility barrier is in keeping with the results of others who have used different strains (14, 17). In this study, the results of insulin extraction were confirmed and extended by immunohistology, where neither LEW nor PVG class I MHC-bearing tissue could be demonstrated beneath the capsule of DA kidneys, regardless of whether allogeneic islets were implanted alone or as a component of mixed syngeneic and allogeneic islet grafts.

The finding that the insulin content of kidneys grafted originally with both 200 syngeneic and 200 allogeneic islets was not significantly different from kidneys grafted with 200 syngeneic islets alone suggests that the rejection of allogeneic islets is extremely specific. The careful mixing of syngeneic and allogeneic islets before transplantation should have resulted in close approximation of syngeneic and allogeneic islet tissue. Despite this close approximation of syngeneic and allogeneic islets the effector mechanisms resulting in the destruction of the allogeneic islets had not apparently damaged the syngeneic islets.

The specificity of skin allograft rejection has previously been examined using adult allophenic mouse donors originally produced by fusing two H-2-disparate embryonic blastocysts and observing the survival of graft melanoblasts and hair follicle cells on parental strain recipients (32, 33); similar experiments have been carried out more recently by others (34). These experiments demonstrated that skin allograft rejection was exquisitely specific for allogeneic cells, but their design did not permit accurate quantification of the survival of donor cells that were syngeneic with the parental recipients. Thus, although the specificity of the effector arm of the immune response to target allogeneic cells was shown, it was not possible to exclude the possibility of simultaneous nonspecific immune destruction of some syngeneic cells adjacent to allogeneic cells undergoing destruction.

However, the experiments reported here demonstrate the exquisite specificity of islet allograft rejection *in vivo*, as there was no biochemical or histological evidence that syngeneic islets were destroyed as a result of contiguous allograft rejection. If

nonspecific immune destruction does occur at all during islet allograft rejection, the results of this experiment suggest that the extent to which such destruction might occur is likely to be insignificant. This finding is not compatible with rejection being a delayed-type hypersensitivity reaction mediated by T helper cells, but is compatible with an effector response mediated by T cytotoxic cells (40). If so, then the rejection of isolated allogeneic islets occurs by a mechanism not different to that of other tissues and organs. This is particularly relevant since islets, and especially β cells, appear to be peculiarly vulnerable to the effector arm of both autoimmune (41) and alloimmune responses (14, 16, 19, 20). The vulnerability of islets to alloimmune attack would therefore seem to be attributable to the manner in which isolated islets are transplanted, including their small size, exposed surface, and intravascular location, rather than to intrinsic differences between the effector mechanisms of the rejection of islets and other tissues and organs. This explanation is supported by experiments that have shown that isolated islet allografts transplanted within a vascularized organ allograft, either as a composite islet/kidney (39) or islet/liver (15) graft or within the whole pancreas (16, 17) undergo rejection at a slower rate, commensurate with rejection of the organ bearing the islets.

The lack of damage to bystander syngeneic islets, despite vigorous and complete rejection of allogeneic islets, raises questions about the effect of lymphokines on islet cells, and more specifically, β cells. IL-1, TNF, IFN- γ , and other lymphokines have been shown to be produced locally during allograft rejection (13). Pronounced class I expression was seen in grafts with syngeneic islets, both in grafts originally composed of syngeneic islets alone, and in grafts of mixed syngeneic and allogeneic islets. Whether syngeneic β cells in grafts originally composed of mixed syngeneic and allogeneic islets upregulated their MHC products in response to lymphokines specifically produced during contiguous allograft rejection remains undetermined. Thus, the lack of bystander damage observed in the present experiments suggests that either the deleterious effects of these lymphokines previously noted during *in vitro* studies (1, 2, 4) are not relevant to *in vivo* events, or the production and subsequent activity of lymphokines is highly localized and directed, and so syngeneic islet damage does not occur. The latter possibility might usefully be investigated by repeating the present experiments using mixed single cell preparations made from syngeneic and allogeneic islets.

Summary

The specificity of rejection of isolated pancreatic islets was examined in the rat using a quantitative model in which syngeneic (DA) or a mixture of syngeneic and allogeneic (DA and LEW or PVG) islets were implanted beneath the capsule of the kidney of nondiabetic normal rats (DA). 3 wk after transplantation total insulin extraction assays of the kidney with its islet implant together with immunohistological examination of the site of transplantation for evidence of syngeneic or allogeneic tissue demonstrated the total destruction of allogeneic islets without any evidence of damage to syngeneic islets either distant or in immediate proximity to allogeneic islets. Pancreatic islets, and especially β cells, appear to be particularly vulnerable to the effector arm of both autoimmune and alloimmune responses, a vulnerability that has been attributed to the cytotoxic effects of lymphokines, notably IL-1, released in both autoimmune and alloimmune responses. The experiments reported here

demonstrate not only the exquisite specificity of the allograft reaction but are not compatible with a hypothesis that B cells within an intact islet are nonspecifically susceptible to destruction by lymphokines.

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