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Myung-Shik Lee
Scripps Research Institute

Lise Wogensen
Scripps Research Institute

Judith Shizuru
Scripps Research Institute

Michael B.A. Oldstone
Stanford University

Nora Sarvetnick
University of Nebraska Medical Center, noras@unmc.edu

Pancreatic Islet Production of Murine Interleukin-10 Does Not Inhibit Immune-mediated Tissue Destruction

Myung-Shik Lee, Lise Wogensen, Judith Shizuru,* Michael B. A. Oldstone, and Nora Sarvetnick
*Department of Neuropharmacology, The Scripps Research Institute, La Jolla, California 92037; and *Department of Medicine, Division of Immunology & Rheumatology, Stanford University School of Medicine, Stanford, California 94305*

Abstract

IL-10 inhibits macrophage-dependent antigen presentation, cytokine production, and generation of allospecific cells in vitro. These findings have lead to the widespread expectation that IL-10 may be a useful immunosuppressive agent to inhibit allograft rejection or autoimmunity in vivo. We used two experimental paradigms to study effects of murine IL-10 on in vivo immune responses. First, fetal pancreata or adult pancreatic islets from transgenic mice expressing IL-10 in pancreatic β cells (Ins-IL-10 mice) were grafted across the MHC barrier to examine if IL-10 could inhibit allograft rejection. Second, Ins-IL-10 mice were crossed with transgenic mice expressing lymphocytic choriomeningitis virus (LCMV) antigens in pancreatic β cells. These mice were infected with LCMV to elicit autoimmune diabetes, allowing us to ask if IL-10 protects islets from autoimmune destruction. We observed that allografts from IL-10–transgenic donors were rejected with comparable kinetics to the rejection of control nontransgenic allografts, indicating that IL-10 does not inhibit allograft rejection. After LCMV infection, IL-10 and LCMV antigen double transgenic mice developed diabetes earlier than LCMV antigen single transgenic littermates, suggesting that IL-10 does not inhibit islet antigen presentation or recognition. Our results contrast to in vitro observations and suggest that IL-10 cannot overcome immune-mediated tissue destruction within the pancreas. (*J. Clin. Invest.* 1994. 93:1332–1338.) Key words: transgenic mice • allograft • viral infection • autoimmunity • diabetes mellitus

Introduction

IL-10 inhibits alloantigen-induced T cell proliferation and allospecific cytotoxic T cell generation (1). IL-10 also inhibits macrophage-dependent, antigen-specific T cell proliferation and macrophage-dependent production of cytokines by T cells (2, 3). These findings suggest a role for IL-10 as a potential agent for prolongation of allograft survival and also for treatment of T cell–mediated autoimmune diseases, such as type I diabetes and multiple sclerosis (4). The in vivo effects of IL-10 have been studied indirectly in mice with anti–IL-10 antibody administration that resulted in increased levels of IFN-γ and depletion of peritoneal Ly-1 B cells (5). Furthermore, systemic administration of IL-10 inhibits TNF-α release in experimental endotoxemia (6, 7). However, the immunosuppressive effects of IL-10 in vivo have not been addressed directly despite broad interests in the clinical use of this cytokine, partly because of lack of suitable animal models.

In this report, we investigated the in vivo effects of murine IL-10 on immunological responses using transgenic mice expressing IL-10 in pancreatic β cells (Ins-IL-10 mice).[1] IL-10-transgenic mice have inflammatory lesions surrounding pancreatic islets; however, the islets remain intact and the mice are normoglycemic, indicating that the inflammatory cells are not destructive to the islets (8). This result is not inconsistent with the immunosuppressive effects of IL-10 observed in vitro, leading us to perform functional studies in vivo. To address the potential of IL-10 to inhibit or delay allograft rejection, we grafted fetal pancreata or adult islets from Ins-IL-10 mice into MHC-incompatible mice. To test the ability of IL-10 to modify the autoimmune response, we crossed Ins-IL-10 mice with transgenic mice expressing lymphocytic choriomeningitis virus (LCMV) glycoprotein (GP) or nucleoprotein (NP) antigen in pancreatic β cells (RIP-LCMV-GP and RIP-LCMV-NP) (9). RIP-LCMV-GP and RIP-LCMV-NP mice have normal phenotypes but develop autoimmune diabetes after LCMV infection. Double transgenic mice were infected with LCMV, and the prevalence and onset of diabetes were compared to those in single transgenic littermates.

We report that IL-10 did not substantially inhibit allograft rejection or development of autoimmune diabetes in our systems.

Methods

Engraftment of fetal pancreata. Ins-IL-10 lines were maintained by repeated crossing to BALB/c mice. Fetal pancreata were dissected from embryonic day 18 (E18) embryos. The presence of the transgene

Address correspondence to Nora Sarvetnick, Department of Neuropharmacology, CVN 10, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037.

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1. *Abbreviations used in this paper:* APC, antigen-presenting cells; E18, embryonic day 18; GP, glycoprotein; IL-10/GP, transgenic mice expressing both IL-10 and LCMV GP in pancreatic β cells; IL-10/NP, transgenic mice expressing both IL-10 and LCMV NP in pancreatic β cells; Ins-IL-10 mice, transgenic mice expressing IL-10 in pancreatic β cells; LCMV, lymphocytic choriomeningitis virus; NP, nucleoprotein; RIP-LCMV-GP, transgenic mice expressing LCMV GP in pancreatic β cells; RIP-LCMV-NP, transgenic mice expressing LCMV NP in pancreatic β cells; -/GP, single transgenic littermates expressing LCMV GP only; -/NP, single transgenic littermates expressing LCMV NP only.

in Ins-IL-10 mice embryos was tested with PCR amplification of genomic DNA. The pancreata from nontransgenic littermate embryos were used for control grafts. Fetal pancreata were transplanted under the kidney capsule of male allogeneic C57BL/6 mice (H-2^b) and control BALB/c mice (H-2^d) (Fig. 1 *A*).

Isolation of islets. Pancreatic islets were isolated from 2–6-mo-old Ins-IL-10 mice. We used two transgenic lines as islet donors, each having similar expression levels and histopathology. The pancreata were digested with collagenase P (Boehringer Mannheim Corp., Indianapolis, IN) as described (10). All islets were cultured for 3–7 d in RPMI 1640 supplemented with 20 mM Hepes, 300 μg/ml L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS. The isolated cultured islets appeared circumscribed and free of any detectable associated lymphocyte aggregates. 200 islets were grafted under the kidney capsule of male C57BL/6 mice, BALB/c mice, and congenic BALB.H-2^k mice (H-2^k). Islets from BALB/c mice served as controls.

In situ hybridization. Grafted tissue was dissected, fixed in zinc formalin, and embedded in paraffin. E18 fetal pancreata and adult pancreata were also embedded. Sections were deparaffinized and prehybridized for 4 h at 42°C in a buffer comprising 50% formamide, 0.3 M NaCl, 20 mM Tris, pH 8.0, 5 mM EDTA, 1× Denhardt's solution, 10% dextran sulfate, and 10 mM dithiothreitol. Hybridization was done for 16 h at 42°C in a humidified chamber, followed by washing, dehydration, and drying. Antisense and sense RNA probes were prepared by in vitro transcription of a linearized plasmid containing IL-10 cDNA, using ^{35}S-labeled UTP. Sections were covered with emulsion (NTB2; Kodak, Rochester, NY) and developed after 1–2 wk.

Production of IL-10 by transgenic islets. Pancreatic islets were isolated from two transgenic mice and two nontransgenic mice as described above. 75 islets from each mouse were cultured in a single well of 48-well plate in RPMI 1640 supplemented with 20 mM Hepes, 300 μg/ml L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% FCS. The IL-10 production between day 1 and day 5 of culture was measured. The glucose level in the culture medium was 207, 211, and 202 mg/dl on day 0, day 1, and day 5 (Glucometer 3; Miles Inc., Elkhart, IN). The IL-10 level in the culture supernatant was assayed using an ELISA. In brief, 96-well plates were coated with 2 μg/ml rat antimurine IL-10 (PharMigen, San Diego, CA) and blocked with 1% BSA in PBS, culture supernatant was added, and incubation was done overnight at 4°C. After washing, the plates were incubated with 2 μg/ml biotinylated antimurine IL-10 (PharMigen). Peroxidase-streptavidin (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) and chromogen were sequentially added, and the color reaction was stopped with 1% SDS in PBS. The standard curve was obtained using murine IL-10 COS cell supernatant.

Immunohistochemical staining. Deparaffinized sections of graft tissue were stained with an immunoperoxidase method using a polyclonal antibody to porcine insulin (Dako Corp., Carpinteria, CA) and avidin-biotin-complex (Vector Laboratories, Burlingame, CA). The sections were counterstained in hematoxylin solution and observed after dehydration in graded alcohol. Fresh frozen sections of the pancreatic tissue from Ins-IL-10 mice were stained using a monoclonal antibody to murine IL-10 (Genzyme Corp., Cambridge, MA) and the same immunoperoxidase technique.

Double transgenic mice. Ins-IL-10 mice were intercrossed to RIP-LCMV-GP or RIP-LCMV-NP mice to derive lines positive for both transgenes (IL-10/GP and IL-10/NP). RIP-LCMV-GP and RIP-LCMV-NP breeding mice were selected among those maintained on repeated crossing to C57BL/6. F1 mice were screened for the presence of both transgenes by PCR amplification of genomic DNA. 3–4-mo-old double transgenic mice and their littermates were infected with 2 × 10^5 plaque-forming units of LCMV Armstrong strain. Mice were eyebled monthly for the determination of glucose level using Glucometer 3. Mice showing blood glucose levels > 300 mg/dl were killed for islet histology (Fig. 1 *B*).

Statistical analysis. Gehan's test for survival (11) was used to compare development of diabetes after virus infection in double transgenic mice with that in single transgenic littermates.

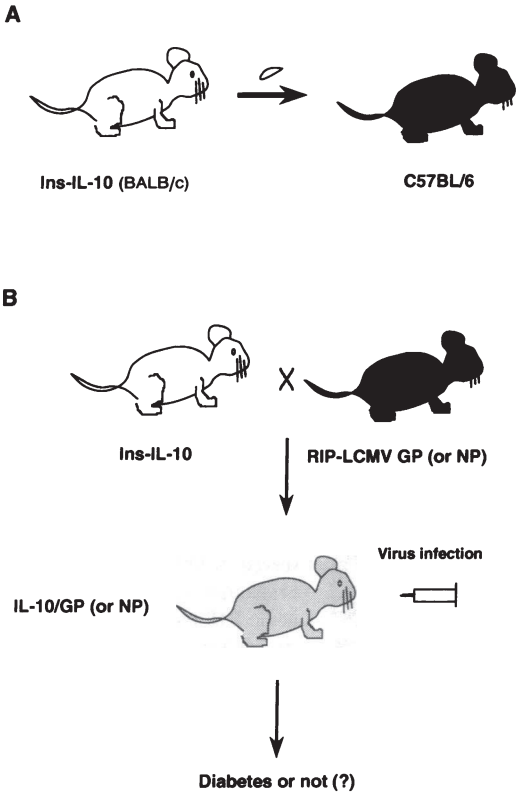

Figure 1. Two experimental models to study the in vivo effects of IL-10. (*A*) IL-10–transgenic fetal pancreata or adult islets were allografted under the kidney capsule to address if IL-10 could inhibit allograft rejection. (*B*) Double transgenic mice expressing both IL-10 and LCMV GP (or NP) antigen were produced and infected with LCMV to observe if IL-10 could inhibit LCMV-induced autoimmune diabetes.

Results

Rejection of fetal pancreata or adult islets from Ins-IL-10 mice by MHC-incompatible recipients. To study the potential of IL-10 to modify the islet allograft response, we initially performed grafts of fetal pancreatic tissue. Previous studies have demonstrated that after engraftment of fetal pancreatic tissue, the exocrine tissue atrophies, but the endocrine cells mature into islets indistinguishable from adult islets in ~ 3 wk (12). IL-10–transgenic and nontransgenic fetal pancreata from six litters were transplanted under the kidney capsule of allogeneic C57BL/6 and syngeneic BALB/c mice. 1 wk after transplantation of transgenic fetal pancreata to C57BL/6 mice (n = 6), a mononuclear cell infiltrate was observed with formation of glandular structures. However, complete necrosis of the allograft was observed 2 wk after transplantation (n = 6) (Fig. 2 *A*). Nontransgenic fetal pancreatic allografts were rejected with similar kinetics (both n = 4). Differentiation of islets and atrophy of exocrine tissue were observed in both transgenic and nontransgenic fetal pancreatic isografts to BALB/c mice 2 wk after transplantation (both n = 2). Interestingly, there were mononuclear cells accumulating near newly formed islets in the IL-10 isografts with a similar appearance to the mononuclear cell infiltration observed in the exocrine pancreas of Ins-

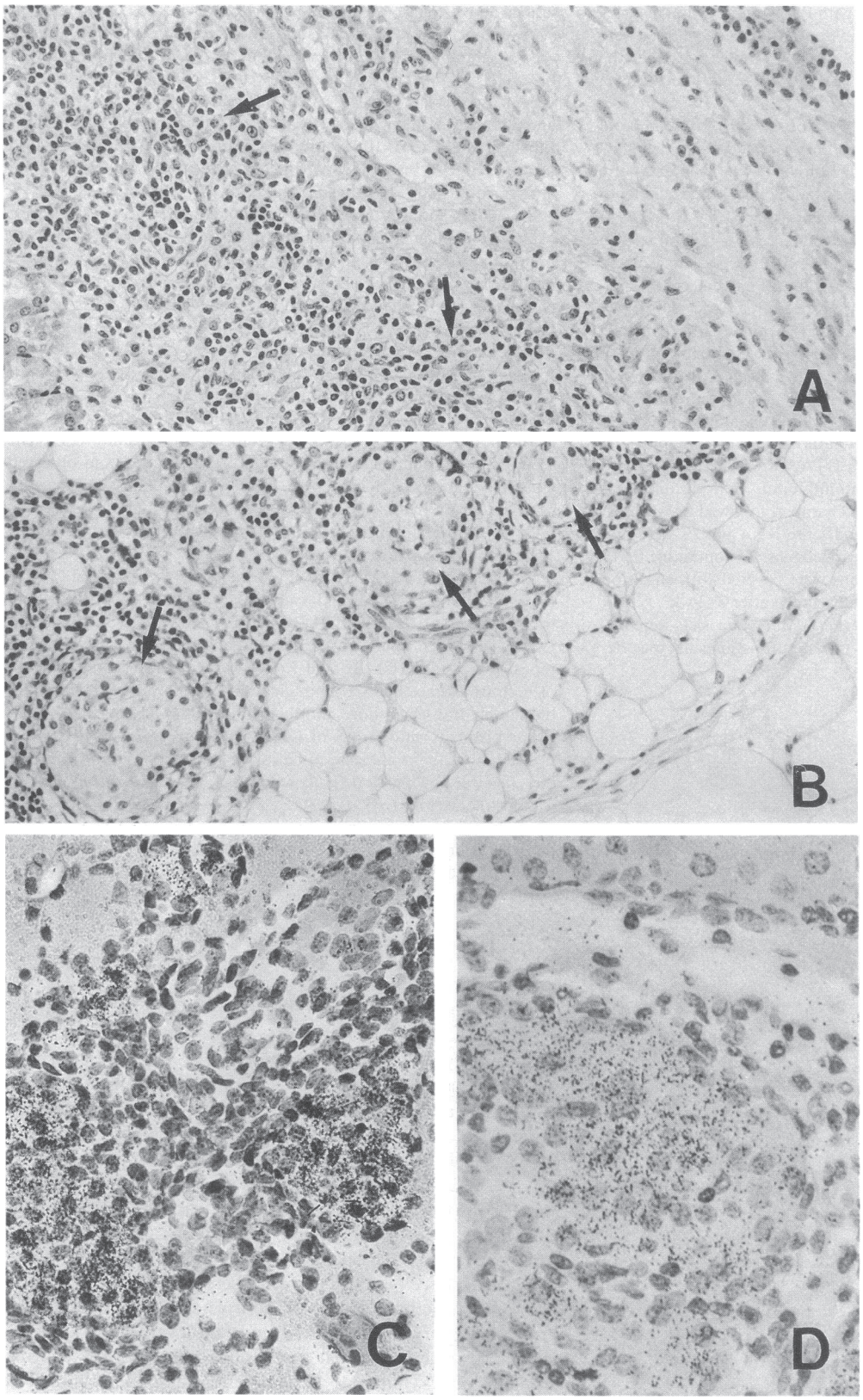

Figure 2. Graft of an IL-10–transgenic fetal pancreas. (*A*) H & E staining of IL-10–transgenic allograft 2 wk after transplantation under the kidney capsule. Necrosis of graft with inflammatory cell infiltration was observed (*arrows*). ×200. (*B*) H & E staining of IL-10–transgenic isograft 2 wk after transplantation. Differentiation into mature islets (*arrows*) with exocrine atrophy was observed under the kidney capsule. Surrounding mononuclear cell infiltration characteristic of IL-10–transgenic pancreas was also observed. ×200. (*C*) In situ hybridization of an IL-10–transgenic E18 pancreas. The signal was detected on fetal islets with IL-10 antisense probe. ×400. (*D*) In situ hybridization of IL-10–transgenic isograft 2 wk after transplantation. The signal was detected in developing islets with IL-10 antisense probe. ×400.

IL-10 mice (8) (Fig. 2 *B*). As judged by immunohistochemistry, insulin-containing cells were present 2 wk after the isograft of transgenic or nontransgenic fetal pancreata; however, they were absent in the transgenic and nontransgenic allografts. Although expression of the IL-10 transgene in the fetal pancreas could be expected from previous studies (13), we performed in situ hybridization to document the transgene expression. These studies demonstrated the expression of IL-10 in E18 transgenic islets, as well as that in the fetal pancreatic isografts 2 wk after transplantation (Fig. 2, *C* and *D*).

The fetal pancreas is strongly immunogenic in comparison with other fetal tissues (14). Additionally, it is possible that the fetal pancreas produces lower levels of IL-10 than adult pancreatic β cells. These factors could contribute to the observed lack of IL-10–mediated immunosuppression. Therefore, we decided to study the effect of IL-10 on the response to adult islet allografts. We isolated islets from Ins-IL-10 mice and transplanted them to C57BL/6 and BALB/c mice. Both IL-10–transgenic ($n = 2$) and nontransgenic adult pancreatic islets ($n = 2$) were rejected 2 wk after engraftment to C57BL/6 mice (Fig. 3 *A*). Both were accepted by BALB/c mice (both $n = 1$). As in the fetal transgenic pancreas grafts, there was a mononuclear cell infiltrate around, but not within transplanted IL-10–transgenic islets (Fig. 3 *B*). Furthermore, in situ hybridization studies demonstrated that IL-10 was expressed in isografts 2 wk after transplantation at levels comparable to that observed in pancreatic islets of Ins-IL-10 mice (Fig. 3 *C*).

Since C57BL/6 mice respond quite vigorously to BALB/c tissue, we tested the ability of IL-10 to modify the islet allograft response to a strain combination that was more closely related genetically and antigenically. We chose BALB.H-2^k congenic mice as recipients, since these mice share the same minor antigens with the BALB/c donor and would thus be more similar. We transplanted islets isolated from Ins-IL-10 mice into BALB.H-2^k mice under the kidney capsule. Both IL-10–transgenic ($n = 2$) and nontransgenic adult islets ($n = 2$) were rejected 2 wk after transplantation with similar histopathology as those grafted to C57BL/6 mice.

Development of diabetes in LCMV-infected IL-10/GP and IL-10/NP mice. We assessed the ability of IL-10 to protect pancreatic islets from destruction in a transgenic model where immune sensitization to islets occurs as a cross-reaction with viral antigens after LCMV infection (9). We crossed IL-10–transgenic mice with RIP-LCMV-GP and RIP-LCMV-NP

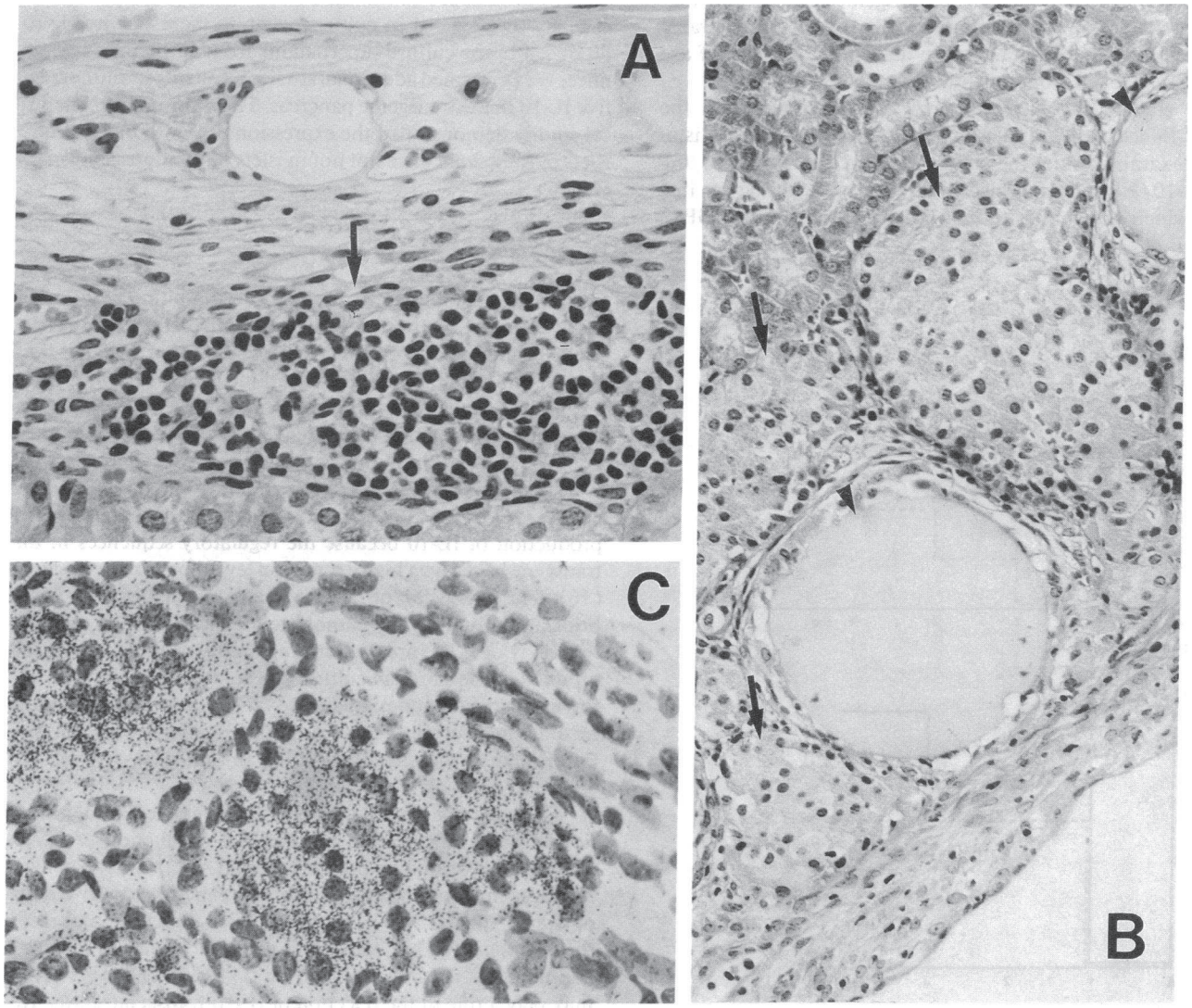

Figure 3. Graft of IL-10–transgenic adult islets. (*A*) H & E staining of an IL-10–transgenic islet allograft 2 wk after transplantation. Islet grafts were rejected like IL-10–transgenic fetal pancreata (*arrow*). ×400. (*B*) H & E staining of IL-10–transgenic islet isograft 2 wk after transplantation. Intact islets with surrounding mononuclear cell infiltration characteristic of IL-10–transgenic mice were observed (*arrows*). Colloid droplets are CM Affi-Gel Blue Gel (Bio-Rad Laboratories, Hercules, CA) used as a geographic marker (*arrowheads*). ×200. (*C*) In situ hybridization of IL-10–transgenic islet isograft 2 wk after transplantation showing positive signal. ×400.

mice to derive offspring segregating both, one, or no transgenes. These entire litters were monitored for diabetes for 3–4 mo and were then inoculated with LCMV. At regular intervals, we tested the blood glucose of infected double transgenic mice and their littermates to determine if IL-10 had any effect on the development of diabetes. The cumulative prevalence of diabetes after LCMV infection of IL-10/GP and IL-10/NP mice, as well as single transgenic littermates (-/GP and -/NP) is illustrated in Fig. 4. 4 mo after infection, six out of eight IL-10/GP mice (75%) developed diabetes, whereas two of five -/GP (40%) had diabetes. All of eight IL-10/NP mice (100%) developed diabetes 4 mo after infection compared to four of seven -/NP mice (57.1%). Development of diabetes was accelerated rather than delayed in IL-10/GP mice compared to -/GP mice as judged by Gehan's test ($P < 0.05$). IL-10/NP mice also appeared to have accelerated rather than delayed development of diabetes compared to -/NP mice; however, the difference was not significant ($P > 0.05$). Our blood glucose analysis of the uninfected mice indicated that none of the IL-10/NP double transgenic mice or any single transgenic mice became diabetic; however, 2 of the 10 uninfected IL-10/GP double transgenic mice became spontaneously diabetic at 3–4 mo of age and were excluded from our infection studies.

We studied the histopathology of the pancreas in the LCMV-infected and uninfected double transgenic mice. Histological examination of the pancreata from infected IL-10/GP and IL-10/NP mice revealed infiltration of lymphocytes into islets, which was similar to that observed in infected -/GP or -/NP mice (Fig. 5 *A*). This infiltration into the islets is in striking contrast to unmanipulated IL-10–transgenic mice or uninfected IL-10/GP (or NP) mice showing substantial periislet inflammation but no intra-islet lesions (Fig. 5 *B*). There was additionally profound infiltration of mononuclear cells in the exocrine pancreata of infected double transgenic mice, which is characteristic of Ins-IL-10 mice (8). We also studied the pancreas from one of the uninfected spontaneously diabetic IL-10/GP transgenic mice. The tissue section appeared to have a paucity of islets (data not shown).

Production of IL-10 by transgenic islets. Our results demonstrating that local IL-10 production did not inhibit islet allograft rejection or autoimmune destruction of islet tissue were quite contrary to our expectations. One possible explanation of our results was that no IL-10 was actually released from the transgenic islets. Therefore, we studied the production of IL-10 by isolated transgenic and nontransgenic islets by measuring the levels of IL-10 accumulating in the supernatants of cultured islets from individual mouse. We found that 75 isolated islets from each of two transgenic mice produced 14.2 and 6.7 U of IL-10 after 4 d in culture, whereas there was no IL-10 produced in the culture supernatants from two nontransgenic mice. We performed additional studies localizing immunoreactive IL-10 in the transgenic pancreas. The immunohistochemical studies demonstrated the expression of IL-10 in pancreatic islets of transgenic mice but not in islets of nontransgenic mice (Fig. 6).

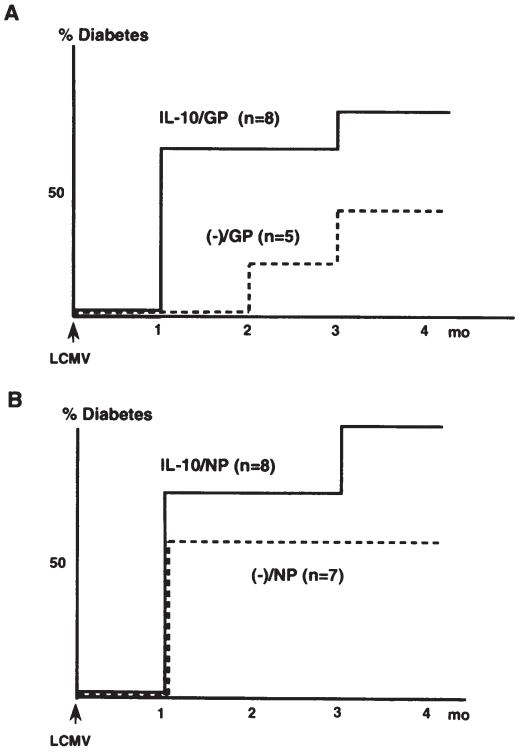

Figure 4. Cumulative prevalence of diabetes after LCMV infection of IL-10/GP (*A*) and IL-10/NP (*B*) double transgenic mice. Development of diabetes appeared to be accelerated rather than delayed in double transgenic mice compared to -/GP or -/NP single transgenic littermates.

Discussion

Our allograft experiments indicated no inhibition or delay in the rejection of IL-10–transgenic fetal pancreata or adult islets. These results are in contrast to a large amount of evidence that IL-10 is an immunoinhibitory cytokine. Thus, IL-10 may not be an obvious therapeutic agent to prevent islet allograft rejection, although this had been predicted on the basis of in vitro findings, such as inhibition of allospecific T cells generation (1) and suppression of a subset of lymphokines and monokines (2, 3, 15). Our observations are not likely to be explained by low production of IL-10 because the regulatory sequences in the transgene have given high expression levels for other transgenes (16). The expression of IL-10 was also verified by in situ hybridization analysis, immunohistochemical staining, and direct assay of IL-10 production from cultured islets. The IL-10 production measured in culture was comparable to levels used for in vitro functional studies (3). The glucose level in the medium for islet culture was in the upper portion of the physiological range, suggesting that similar amount of IL-10 would be produced in vivo.

There may be several factors influencing the lack of inhibition of allograft rejection by localized production of IL-10. First, the role of antigen-presenting cells (APC) other than macrophages must be considered. In vitro experiments have demonstrated that IL-10 suppresses Th1 function indirectly through the APC, but APC other than macrophages, such as B lymphocytes, are not inhibited by IL-10 (3, 17). These APC may play a role in the rejection of IL-10-transgenic grafts. Recently, Macatonia et al. (18) reported that murine IL-10 inhibited dendritic cell–induced IFN-γ production by T cells, but did not inhibit dendritic cell–induced T cell proliferation in primary mixed lymphocyte reaction. These data suggest that IL-10 is not likely to inhibit clonal expansion of alloreactive

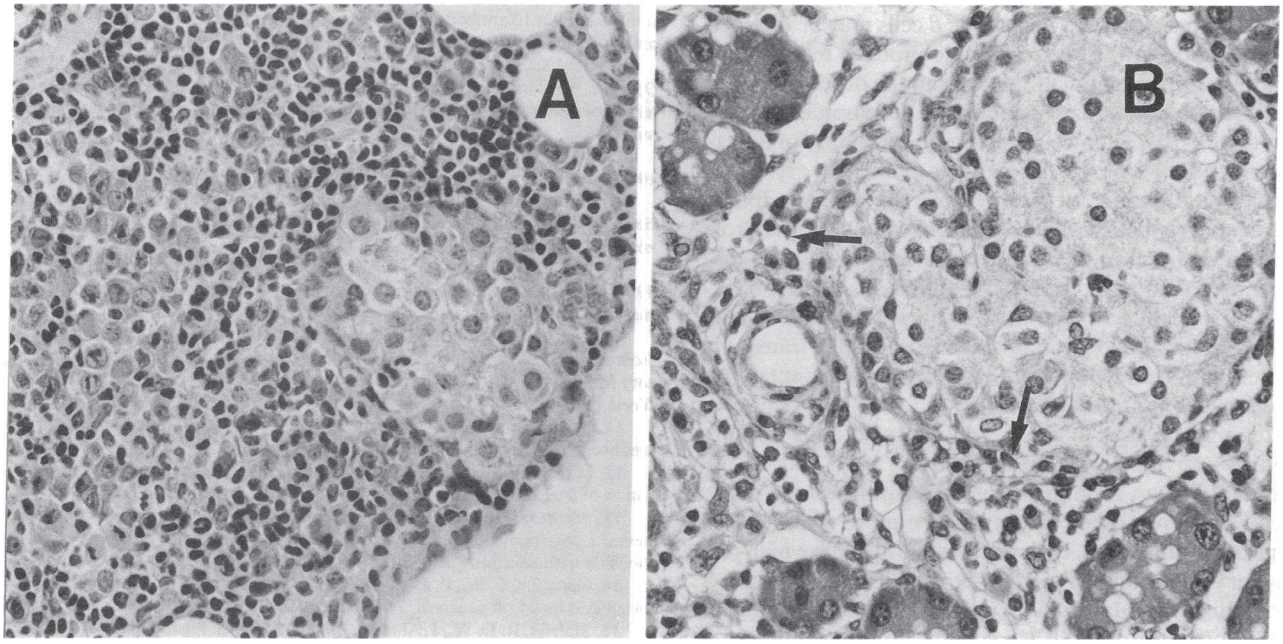

Figure 5. Histopathology of IL-10/NP mice 1 mo after LCMV infection (*A*) and before infection (*B*). ×400. Before infection, only peri-islet infiltration of inflammatory cells characteristic of Ins-IL-10 mice was observed (*arrows*). After infection, massive mononuclear cell infiltration into islets with fragmentation was observed.

cells in vivo because dendritic cells would be the main APC involved in the initiation of allograft rejection (19, 20). Second, immunostimulatory activities of IL-10 might be involved. IL-10 has some immunostimulatory activities including enhanced expression of class II MHC antigens on small dense B lymphocytes and induction of cytotoxic T cell differentiation; both events might be related to allograft rejection (21, 22). Third, allosensitization at locations other than the actual graft site could be occurring. The exact location of allosensitization and the types of cells involved in allograft rejection are not fully elucidated (23, 24). Donor and/or recipient APC may present allograft-specific antigens in locations other than the graft area that are devoid of the influence of the pancreatic IL-10. Finally, it is possible that the immune system of the host develops way(s) to circumvent or overcome prolonged suppression by IL-10 in vivo; e.g., by production of counter-regulatory cytokine(s).

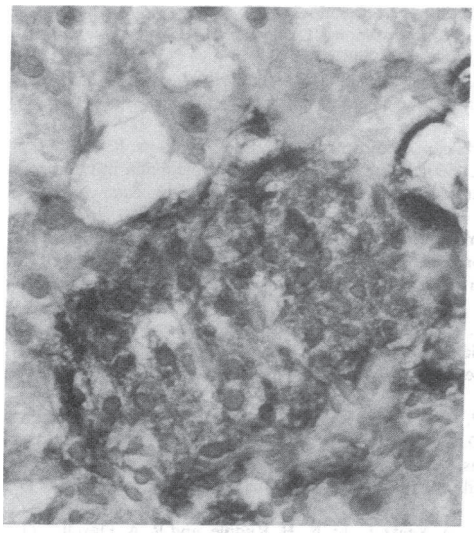

Figure 6. Immunohistochemical staining of a pancreatic section from a Ins-IL-10 mouse. IL-10 immunoreactivity was observed in pancreatic islets indicating the production of IL-10 by transgenic islets at the protein level. ×400.

RIP-LCMV-GP and RIP-LCMV-NP mice have defined viral antigens expressed in pancreatic β cells. They do not develop spontaneous diabetes; however, they develop diabetes after systemic administration of LCMV (9). Local production of IL-10 in the pancreas is not expected to inhibit systemic presentation of viral antigens to the host immune system. This is substantiated by a strong secondary cytotoxic T lymphocyte response of splenocytes from infected double transgenic mice to viral antigens (data not shown). However, we thought that IL-10 produced by pancreatic β cells might inhibit continued antigen presentation that would be necessary for perpetuation of the immune reaction to the virus antigens in pancreatic β cells. Additionally, the documented inhibition of cytokine production by Th1 cells after exposure to IL-10 could greatly dampen the anti-islet inflammatory response. Our results that transgenic production of IL-10 did not inhibit diabetes after virus infection suggest that APC other than macrophages in the pancreas are sufficient for the observed response or that continued antigen presentation is not necessary for the immune response toward virus antigens on the pancreatic β cells. However, it is also possible that the properties of this cytokine studied in vitro do not extrapolate well to in vivo situations because of counter-regulatory effects acting within the organism.

The apparent acceleration of diabetes in double transgenic mice compared to single transgenic littermates may be a result of several effects acting separately or together in synergy. One explanation is a storage disease effect that has been observed in other transgenic mouse models (25, 26). A substantial portion

of the cellular machinery in pancreatic β cells may be directed to the production of the two transgenic proteins that renders them more susceptible to malfunction and the development of diabetes. Our observation of spontaneous development of diabetes in two IL-10/GP mice supports the possibility of a storage disease effect. However, the presence of a massive lymphocytic infiltration within islets of all infected diabetic double transgenic mice with islet destruction indicates a primary role of immune destruction of β cells in the development of diabetes in infected animals. Immunostimulatory activities of IL-10, such as induction of cytotoxic T cell differentiation, may play a role in the acceleration of diabetes in double transgenic mice after LCMV infection, leading to an enhancement of immune destruction of islets (21, 22).

Taken together, our in vivo results contrast to the immunoinhibitory activities of IL-10 observed in vitro and do not support IL-10 as an immunosuppressive agent at least in the pancreas. However, other tissues may be more responsive to this type of manipulation. Pancreatic tissue may have high propensity for infiltration and inflammatory cell attack because of relatively high vascularity. Our observations also do not exclude the possibility of IL-10 as a systemic immunosuppressive agent against autoimmune disorders or allograft rejection. The effects of systemic administration of IL-10 may be different from those of localized transgenic long-term production. This was exemplified by the contrasting effects of systemic administration (27) and local production of TNF-α (28, 29), which resulted in islet protection in diabetes-prone mice and islet inflammation in transgenic mice, respectively.

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