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Leukocyte Extravasation into the Pancreatic Tissue in Transgenic Mice Expressing Interleukin 10 in the Islets of Langerhans

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Summary

Transgenic expression of interleukin 10 (IL-10) in the islets of Langerhans leads to a pronounced pancreatic inflammation, without inflammation of the islets of Langerhans and without diabetes. A scattered infiltration of macrophages ($M\phi$) precedes localized accumulations of $CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, and $M\phi$. This recruitment of inflammatory cells to the pancreas is somewhat surprising, since the biological activities of IL-10 *in vitro* indicate that IL-10 is a powerful immunosuppressive cytokine. Since endothelial cells play a major role in leukocyte extravasation, we examined if vascular changes and extralymphoid induction of peripheral and mucosal type vascular addressins contributed to IL-10-induced homing of mononuclear cells to the pancreas. The endothelium lining small vessels was highly activated in areas of inflammation, as the endothelial cells became cuboidal, and exhibited increased expression of major histocompatibility complex class II (Ia), intercellular adhesion molecule 1, and von Willebrand Factor. Furthermore, induction of vascular addressins simultaneously with accumulation of mononuclear cells around islets and vessels indicated that the endothelial cells take on the phenotype of differentiated endothelium specialized for leukocyte extravasation. In conclusion, pancreatic inflammation and vascular changes are prominent in IL-10 transgenic mice. We hypothesize that IL-10, in addition to its immuno-inhibitory properties, is a potent recruitment signal for leukocyte migration *in vivo*. These effects are relevant for *in vivo* therapeutic applications of IL-10.

IL-10 is produced from Th lymphocytes, $Ly1^+$ B lymphocytes, and macrophages ($M\phi$)¹ (1–3). IL-10 decreases $M\phi$ production of IL-1, IL-6, TNF- α , and nitrogen oxide, and inhibits IFN- γ and IL-3 secretion from Th1 lymphocytes (1, 4–6). IL-10 has not only antiinflammatory properties, but stimulates differentiation of mast cells and CTLs (1). Taken together, these *in vitro* studies indicate that IL-10 is a powerful immunosuppressor and, therefore, may be a potential immunosuppressive compound for treatment of autoimmune diseases and allograft rejections.

To correlate *in vitro* observations with *in vivo* effects, we created transgenic mice with localized expression of IL-10. Since transgenic expression of IL-10 in pancreatic β cells led to homing of inflammatory cells into the exocrine pancreatic tissue, the expression of endothelial cell (EC) antigens, antigens specific for ECs specialized for lymphocyte diapedesis, and the expression of adhesion molecules on infiltrating cells were examined.

Materials and Methods

Construction of the Transgene. A recombinant plasmid containing the human insulin promoter and a terminator sequence from the hepatitis B virus gene (7) was cleaved at its unique BamHI site, followed by blunt-ending of the site with the Klenow fragment of DNA polymerase I. The 594-bp SacI fragment encoding the murine IL-10 was excised from a cDNA clone (8), blunt-ended as above, and inserted into the BamHI site of the insulin promoter-containing plasmid. Isolation, purification, and injection of a restriction fragment of the hybrid DNA molecule were performed as described previously (7).

Screening of Transgenic Mice. For Southern blot analysis, 5–10 μ g of DNA, extracted from 2-cm-long tail segments, was digested with PstI overnight. The DNA was subjected to electrophoresis on 0.8% agarose gels and transferred to nitrocellulose membranes (NitroPure; Micron Separations Inc., Westboro, MA). Hybridization was performed with radiolabeled restriction fragments of the IL-10 cDNA (Prime-It™ Random Primer Labeling Kit; Stratagene, La Jolla, CA). Transgenic founders were bred with BALB/c mice and killed for histological characterization after transmission to one or two litters, as tested by PCR of tail DNA using transgene-specific primers. Mice were maintained in microisolator cages under pathogen-free conditions.

Expression Analysis by *In Situ* Hybridization. Two 3-mo-old IL-

¹ Abbreviations used in this paper: EC, endothelial cell; ICAM-1, intercellular adhesion molecule 1; $M\phi$, macrophages; vWF, von Willebrand Factor.

10 transgenic mice and one normal BALB/c mouse were killed. Excised pancreata were fixed in 4% paraformaldehyde in PBS for 10 min, washed in 10, 15, and 20% sucrose, and frozen in Tissue-Tek (Miles Laboratories Inc., Elkhart, IN). The RNA probe was prepared by *in vitro* transcription of a linearized plasmid containing IL-10 cDNA, using digoxigenin-modified UTP (Boehringer Mannheim Biochemicals, Indianapolis, IN). *In situ* hybridization using nonisotope-labeled probes was performed as described (9). After overnight incubation at 37°C with 50 μ l of probe-containing hybridization mixture, sections were sequentially reacted with sheep anti-digoxigenin antibody (1 μ g/ml) (Boehringer Mannheim Biochemicals), followed by incubation with biotin-labeled rabbit anti-sheep IgG (7.5 μ g/ml) (Vector Laboratories, Inc., Burlingame, CA), and horseradish peroxidase (HRP)-labeled avidin-biotin complex (ABC-kit; Vector Laboratories, Inc.). HRP was visualized using 3,3'-diaminobenzidine as chromogene.

Immunohistochemistry. 1- ($n = 4$), 4- ($n = 3$), and 6- ($n = 2$)-wk-old mice, and 2- ($n = 1$), 3.5- ($n = 1$), 5- ($n = 2$), and 6- ($n = 1$) mo-old mice were killed. Mice were obtained from two different lines of IL-10 transgenic mice. Normal BALB/c mice or negative littermates served as controls. Fresh frozen sections were dehydrated in acetone for 5 min at -20°C and blocked in 2.0% normal goat serum. Infiltrating inflammatory cells were demonstrated by reaction with anti-common leukocyte antigen T200 antibody (5 μ g/ml; Boehringer Mannheim Biochemicals). CD4⁺ Th lymphocytes, CD8⁺ CTL, and B lymphocytes were demonstrated by rat anti-mouse L3T4, rat anti-mouse Ly2, and rat anti-mouse Ly5 (CD45R/B220), respectively (all 5 μ g/ml; Pharmingen, San Diego, CA). M ϕ were identified by the F4/80 antibody (culture supernatant diluted 1:10; Serotec, Indianapolis, IN). Tissue specimens were reacted with antibodies against murine LFA-1 and Mac-1 (both 5 μ g/ml; Boehringer Mannheim Biochemicals), and against murine α -selectin and lymphocyte Peyer's patch high endothelial venule adhesion molecules 1 and 2 (LPAM-1 and -2) (R1-2) (7 μ g/ml; kindly provided by Dr. I. L. Weissman, Stanford University Medical Center, Stanford, CA). Antibody R1-2 reacts with the α_4 chain, and identifies both LPAM-1 ($\alpha_4\beta_7$) and LPAM-2 ($\alpha_4\beta_1$, VLA-4) (10). Expression of vascular addressins were demonstrated by the antibodies MECA 32, MECA 79, and MECA 367 (kindly provided by Dr. E. Butcher, Stanford University). Peripheral LNs and mucosal lymphoid tissue served as positive control organs for the antibodies MECA 79 and 367, respectively. Furthermore, sections were stained with antibodies against ICAM-1 (5 μ g/ml; Pharmingen), Ia (5 μ g/ml, Boehringer Mannheim Biochemicals), and vWF (18 μ g/ml; Dako Corp., Carpinteria, CA). After incubation with biotinylated secondary antibodies, immunostaining was performed as described above.

Permeability Studies. Two transgenic mice and one normal BALB/c mouse were killed 30 min after intravenous injection of 0.5 g BSA in 500 μ l 0.9% NaCl. The pancreas was isolated, and fresh frozen sections were reacted with a polyclonal antibody against bovine albumin (Sigma Chemical Co., St. Louis, MO) following the above protocol.

Results

Initial Characterization of IL-10 Transgenic Mice. Histological examination of the transgenic mice demonstrated substantial accumulations of inflammatory cells within the exocrine pancreatic tissue in five of nine independent lines of transgenic mice. The infiltrates surrounded the islets of Langerhans, ranging from a modest peri-islet infiltration in young mice (Fig. 1 A) to a profound inflammation of the exocrine tissue,

in which the acini were replaced by dense sheets of inflammatory cells, necrotic tissue, and areas of adipose tissue in older mice (Fig. 1 B). Although the islets of Langerhans were embedded in a sea of leukocytes and necrotic exocrine tissue, they remained circumscribed and entirely intact, and the mice never became diabetic. Furthermore, the organization of the endocrine cells showed the normal mantle of glucagon- and somatostatin-containing cells surrounding a core of insulin-containing β cells (data not shown). The islet-specific expression of the transgene was demonstrated by *in situ* hybridization (Fig. 2). No hybridization was found in the pancreas of control mice.

Investigation of the chronology of the pancreatic inflammation indicated that mononuclear cells began to infiltrate the pancreas of 1-wk-old transgenic mice. At this age the majority of the infiltrating cells were identified as M ϕ by the F4/80 antibody. Focal accumulations of mononuclear cells around vessels and islets were demonstrated at 4–6 wk of age, increasing at 2–3 mo of age. At 2 mo of age lymphocytes dominated the inflammation, whereas $\sim 30\%$ of the infiltrating cells were identified as macrophages. Of the infiltrating lymphocytes, ~ 40 and 30% were CD4⁺ and CD8⁺ T lymphocytes, respectively, and $\sim 30\%$ were B lymphocytes (Fig. 3, A–C). A subpopulation of the lymphocytes expressed the Ly1 antigen. The proportion of different lymphocyte subsets was similar at 6 mo of age.

As evaluated by bromodeoxyuridine incorporation *in vivo*, the mitotic activity of the extravasated lymphocytes was very low (D. Gu, unpublished observation), implying that IL-10-induced homing of leukocytes, rather than proliferation of inflammatory cells within the pancreas, was responsible for the observed lesions. Since the vessels were very prominent in the pancreatic tissue of IL-10 transgenic mice, and since leukocyte extravasation is controlled by a specific interaction between leukocyte adhesion molecules and the endothelium lining the vascular wall, we looked for the presence of endothelial changes, the possible expression of vascular addressins, and the expression of different adhesion molecules in the pancreatic inflammation.

Characterization of the Endothelium in IL-10 Transgenic Mice. The antibody MECA 32 recognizes an antigen present on all types of endothelium, and was therefore used for identification and structural characterization of ECs. In pancreata from 1-wk-old transgenic mice the MECA 32-positive vessels had a larger outer diameter than the MECA 32-reacting vessels in negative littermates, and they appeared more frequently (Fig. 4, A and B). In older transgenic mice (>6 –8 wk) structural changes in small vessels were demonstrated in areas of inflammation only (Fig. 4 C). ECs lining the small vessels were plump and cuboidal, and demonstrated increased staining intensity (Fig. 4 D) when compared with the ECs lining small vessels in noninflammatory areas of the same pancreas (Fig. 4 E) or with negative littermates.

Unstimulated endothelium has low expression of intercellular adhesion molecule 1 (ICAM-1) and MHC class II antigens (11). However, the expression of ICAM-1 and Ia increases in inflammatory foci. Furthermore, exposure to

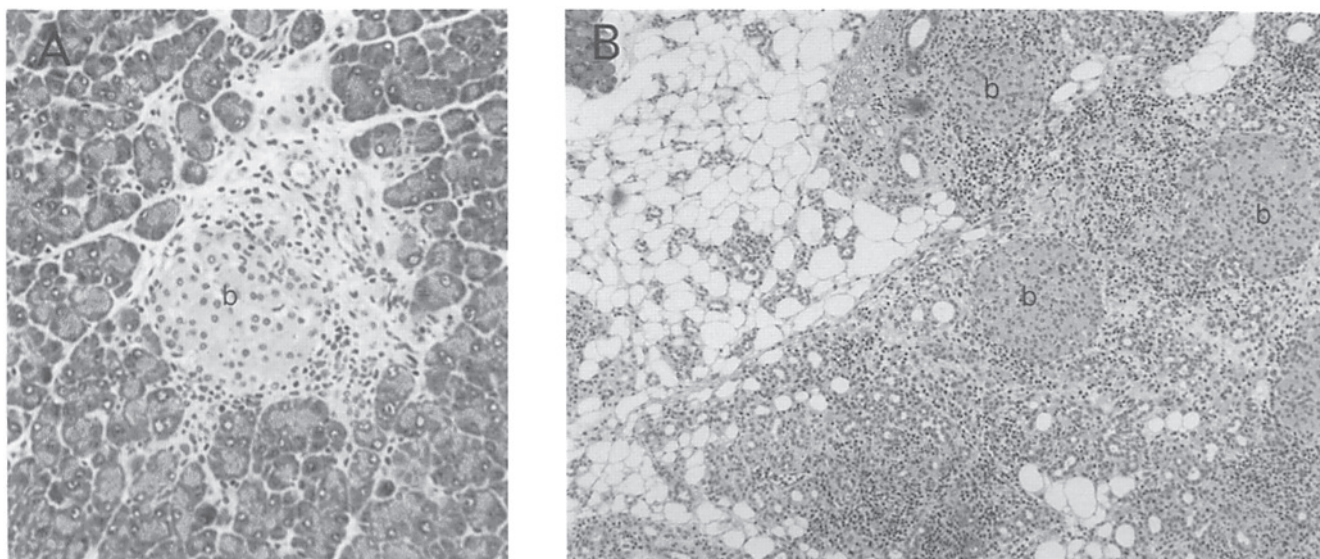


Figure 1. Hematoxylin and eosin staining of pancreatic tissue from mice expressing IL-10 in the pancreatic β cells. In early stages of inflammation infiltrating cells have a peri-vascular or peri-islet localization (A) ($\times 80$). In later stages a profound inflammation involves most of the pancreatic tissue, leaving the islets of Langerhans intact (B) ($\times 40$). (b) Islet of Langerhans.

cytokines potentiates histamine- and thrombin-stimulated secretion of von Willebrand Factor (vWF), and increased secretion and leakage of vWF into adjacent tissue is demonstrated in inflamed foci (12, 13). Pancreatic tissue from the transgenic mice was subjected to antibodies against ICAM-1, Ia, and vWF to determine if the pancreatic endothelium in the transgenic mice showed any functional changes. In pancreata of 1-wk-old transgenic mice, vessels expressing ICAM-1 and Ia appeared more frequently than in nontransgenic littermates (Fig. 5, A and B). Expression of ICAM-1 and Ia increased progressively on the surface of the ECs lining small arteries, venules, pancreatic ducts, and interacinar capillaries in mice from 4 wk to 2 mo of age (Fig. 5, C and D). Cells expressing ICAM-1 and Ia were also demonstrated in the islets of Langerhans. However, expression seemed to be localized to the capillaries and not to the endocrine cells. In pancreata from 1-wk-old normal mice only a few vessels showed expression of vWF (Fig. 6 A), whereas small vWF-expressing vessels appeared throughout the pancreas in age-matched transgenic mice (Fig. 6 B). In pancreatic tissue from normal adult mice, vWF staining had a granular appearance and was expressed on the luminal edge of scattered larger arteries and venules (Fig. 6 C). As described for rats (14), some islets of Langerhans could easily be identified by a characteristic expression of vWF (Fig. 6 C). Distant from inflammatory foci, the expression of vWF in pancreata from transgenic mice >4 wk of age was similar to normal controls, whereas small, vWF-positive vessels appeared in the infiltrates, including small vessels lined by high ECs (Fig. 6 D). Expression of vWF was induced on interacinar capillaries diverging out from inflammatory foci, and vWF was seen leaking out into the surrounding parenchyma. Pancreatic ducts embedded in inflammatory cells did not express vWF (Fig. 6 D).

During stimulation of ECs with cytokines the vascular permeability increases (15). Therefore, we tested the permeability for albumin of the pancreatic vessels. After injection of BSA, reaction of the pancreatic tissue with an albumin antibody demonstrated extravascular accumulations of albumin in inflammatory loci only (Fig. 6 E). No albumin deposits were detectable in the pancreatic tissue from untreated transgenic mice. We could not demonstrate sediments of albumin in the pancreas of a normal BALB/c mouse after injection of BSA.

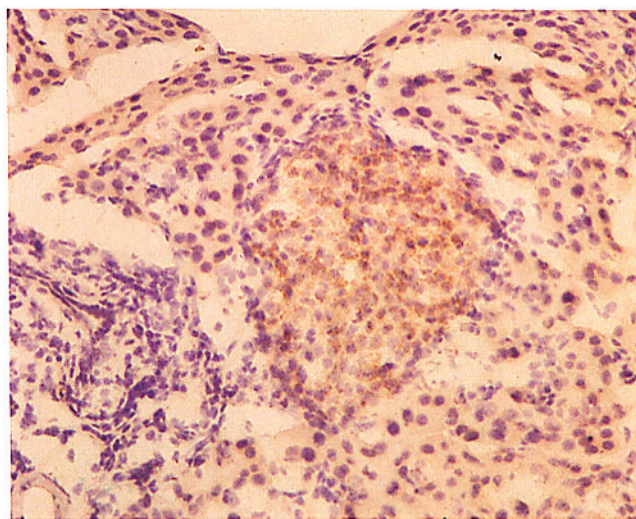


Figure 2. Demonstration of IL-10 expression in islets of Langerhans by in situ hybridization. Hybridization with digoxigenin-labeled probe shows islet-specific expression of IL-10.

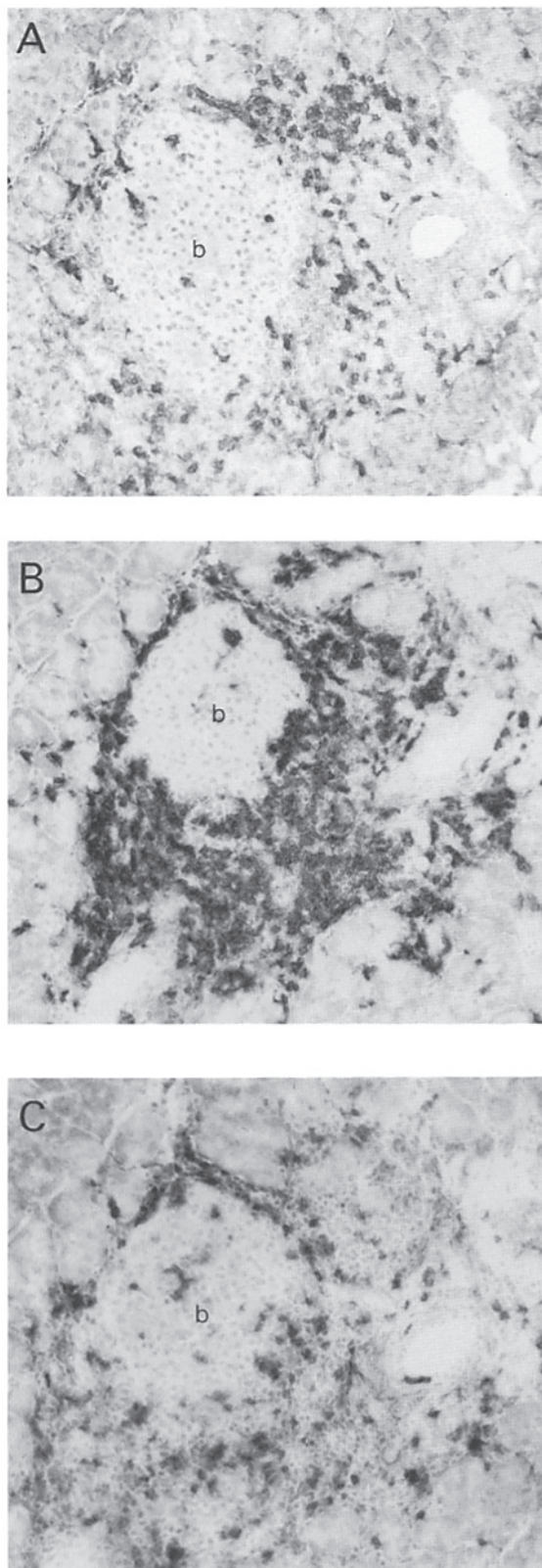


Figure 3. Lymphocyte subsets in a peri-islet infiltrate in IL-10 transgenic mice. To identify lymphocyte subsets in the peri-islet infiltrations, pancreatic sections from a 2-mo-old IL-10 transgenic mouse were reacted with Ly5 (CD45R/B220), L3T4, and Ly2 antibodies for detection of B lymphocytes (A), CD4⁺ Th lymphocytes (B), and CD8⁺ CTL (C), respectively (all $\times 72$). (b) Islet of Langerhans.

In summary, our observations indicate that ECs are highly activated, and have increased adhesion properties. Furthermore, the increased permeability may contribute to spreading of the inflammatory process.

Expression of Vascular Addressins in IL-10 Transgenic Mice. Leukocytes may not only pass through stimulated endothelium but also through tissue-specific postcapillary high endothelial venules (HEV), specialized for lymphocyte diapedesis (16). The two best characterized types of HEV are HEV in peripheral LN, identified by the mAb MECA 79 (17) recognizing the peripheral LN vascular addressin (PNAd) (18), and HEV in mucosal lymphoid tissue (Peyer's patches and appendix), defined by the mAb MECA 367 (19) recognizing the mucosal vascular addressin (MAd) (20). Expression of peripheral and mucosal HEV in nonlymphoid organs is described in areas of chronic inflammation, contributing to homing of inflammatory cells in peripheral tissues (21–23). To examine if induction of extralymphoid HEV could be a factor responsible for homing and extravasation of inflammatory cells to the pancreas in IL-10 transgenic mice, pancreatic tissue was reacted with the antibodies MECA 367 and MECA 79. The antigen specific for HEV in Peyer's Patches, defined by MECA 367, was expressed in a scattered manner both in 1-wk-old normal and transgenic mice. Small vessels reactive with the MECA 367 antibody were induced in areas of inflammation (Fig. 7 A), even when only very few inflammatory cells were accumulating around vessels and islets. The number of small vessels expressing this antigen increased with the severity of the surrounding inflammation. The antigen specific for HEV in peripheral lymph nodes, identified by MECA 79, was not expressed in pancreatic tissue from young or adult normal mice. This antigen was expressed in small vessels in the inflammatory infiltrates in transgenic mice >3.5 mo of age only (Fig. 7 B). The findings indicate that leukocyte extravasation in the present model of pancreatic inflammation may take place not only through activated ECs, but also through differentiated ECs with phenotypical characteristics of mucosal HEV and peripheral type HEV.

Expression of Leukocyte Adhesion Molecules in IL-10 Transgenic Mice. Since the ligand for L-selectin is expressed on peripheral LN HEV (24) and on activated endothelium (25), we tested the expression of this leukocyte adhesion molecule on the infiltrating cells. In mice >4 wk of age L-selectin-expressing mononuclear cells were demonstrated around vessels and islets of Langerhans (Fig. 8 A). The presence of L-selectin-expressing leukocytes suggests that an interaction between these cells and ligands on induced peripheral-type HEV may take place.

Because the integrin ICAM-1 is hyperexpressed in our transgenic mice, and the ECs express antigens characteristic for mucosal HEV, we tested if infiltrating cells expressed the respective integrin ligands, such as Mac-1/LFA-1 and LPAM-1/LPAM-2, respectively (10). At 1 and 4 wk of age, the diffusely infiltrating F4/80 cells predictably expressed Mac-1 and LFA-1, although the latter in smaller amounts (Fig. 8, B and C). Also, at 4–6 wk of age, when localized accumulation of F4/80 cells was evident, the cells expressed Mac-1 and LFA-1 (Fig. 8, D and E). Approximately 30–40% of

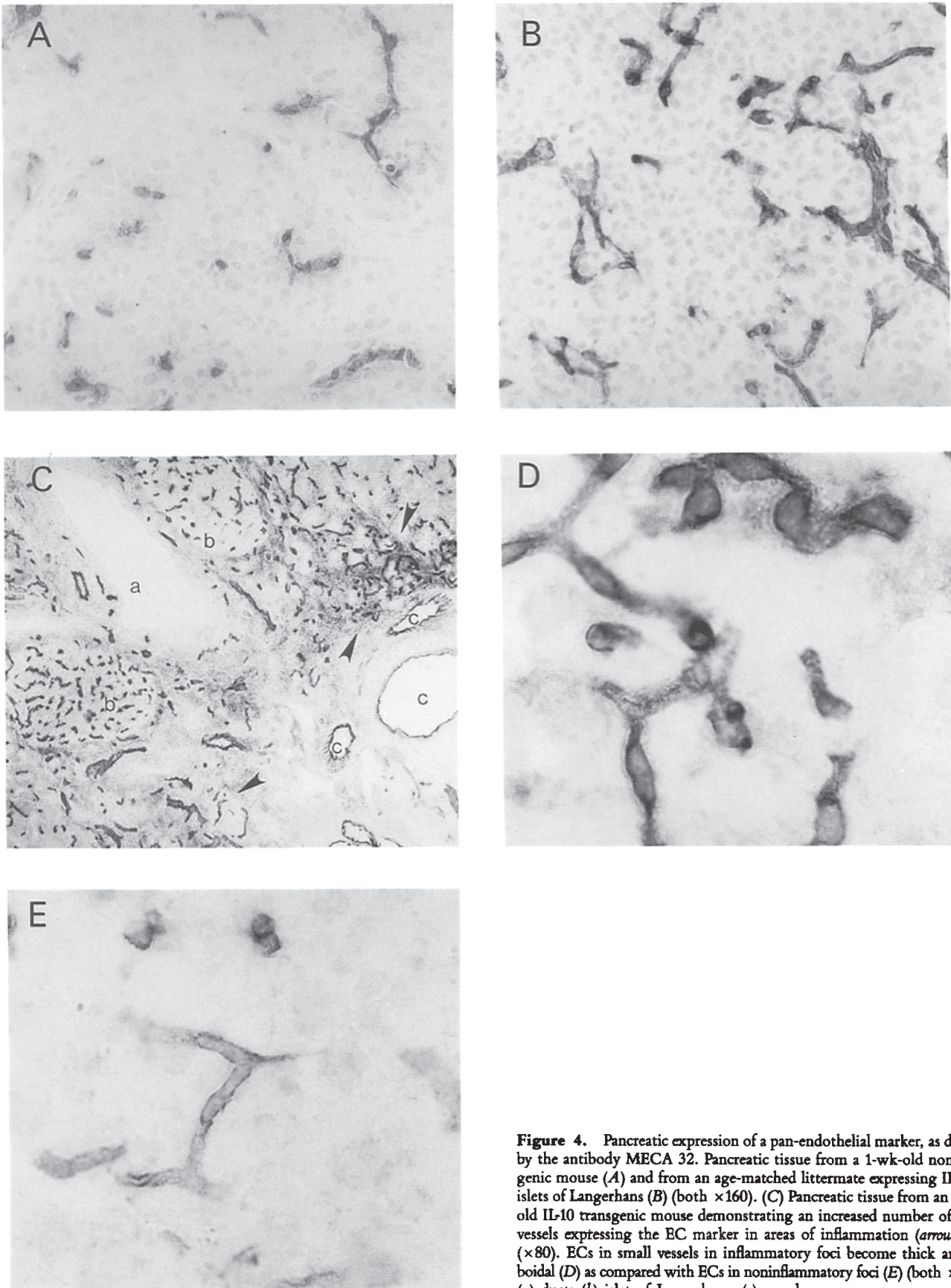


Figure 4. Pancreatic expression of a pan-endothelial marker, as defined by the antibody MECA 32. Pancreatic tissue from a 1-wk-old nontransgenic mouse (A) and from an age-matched littermate expressing IL-10 in islets of Langerhans (B) (both $\times 160$). (C) Pancreatic tissue from an 8-wk-old IL-10 transgenic mouse demonstrating an increased number of small vessels expressing the EC marker in areas of inflammation (arrowheads) ($\times 80$). ECs in small vessels in inflammatory foci become thick and cuboidal (D) as compared with ECs in noninflammatory foci (E) (both $\times 400$). (a) duct; (b) islet of Langerhans; (c) vessels.

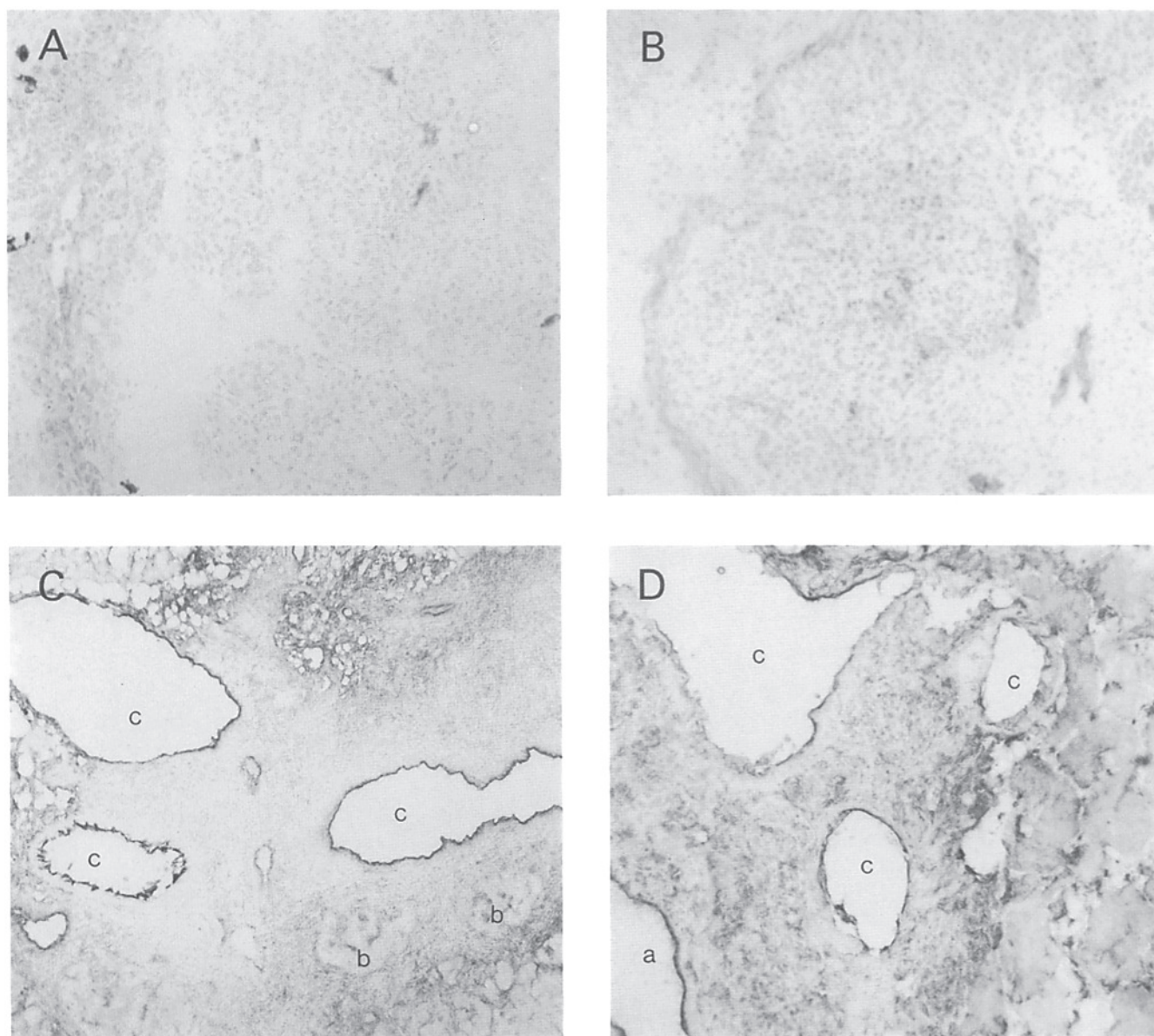


Figure 5. Pancreatic expression of ICAM-1 and Ia. Pancreatic tissue from a 1-wk-old nontransgenic mouse (A) and from an age-matched littermate expressing IL-10 in the islets of Langerhans (B) reacted with an anti-ICAM-1 antibody (both $\times 80$). ICAM-1 (C) ($\times 40$) and Ia (D) ($\times 80$) expression in pancreatic tissue from an 8-wk-old IL-10 transgenic mouse. (a) duct; (b) islet of Langerhans; (c) vessels.

the infiltrating cells expressed the α_4 integrin chain of LPAM-1 and -2 in all tested mice and followed the same pattern of infiltration as described above (data not shown). The observations support the notion that extravasation of inflammatory cells in the present model of pancreatic inflammation in part is mediated by interaction between upregulated endothelial adhesion molecules and vascular addressins, and their respective leukocytic ligands.

For the understanding of the pathogenetic mechanisms in IL-10-induced inflammation, it was interesting to test if the infiltrating leukocytes exhibited high levels of ICAM-1 expression. However, in the pancreata of 1-wk-old transgenic mice, only diffusely scattered cells were ICAM-1 positive (Fig.

5 A). In older mice some of the infiltrating cells, located in the periphery of the inflammatory foci, expressed ICAM-1 on the cell surface, although a rather large number of cells did not show detectable expression of this adhesion molecule (Fig. 5 C).

Discussion

Transgenic expression of IL-10 in the islets of Langerhans leads to a pronounced pancreatic inflammation dominated by $CD4^+$ and $CD8^+$ T and B lymphocytes. The islets of Langerhans remain entirely intact, and the transgenic mice do not develop diabetes. The observation contrasts, at least superficially, the reported in vitro activities of human and

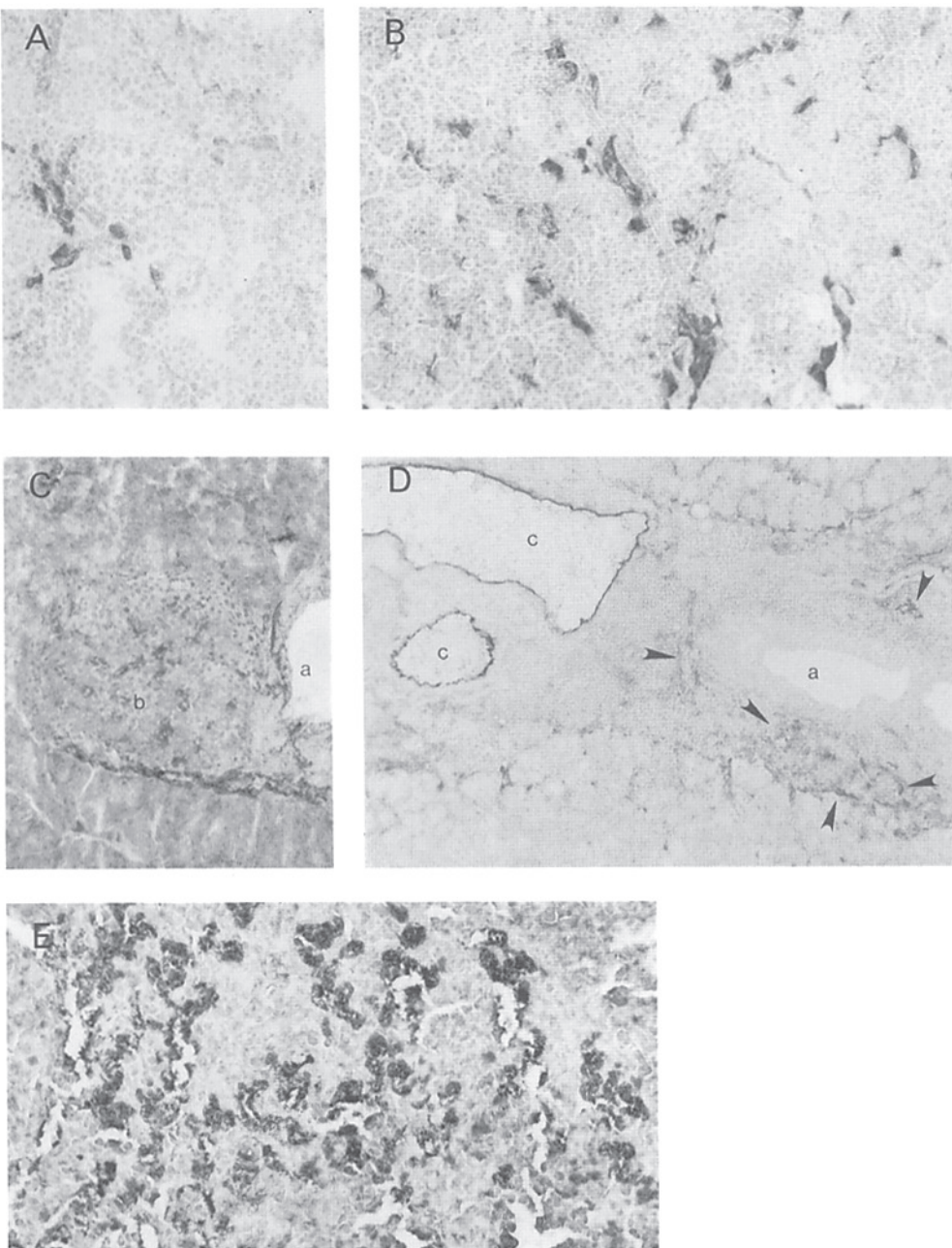


Figure 6. Pancreatic expression of vWF and demonstration of increased permeability. The expression pattern of vWF in pancreatic tissue from a 1-wk-old normal (A) and from a 1-wk-old IL-10 transgenic mouse (B) (both $\times 60$). The latter shows an increase in the number of small vessels expressing vWF. vWF expression in an islet of Langerhans in an adult normal mouse (C) ($\times 60$). Demonstration of pronounced vWF staining in larger vessels, and the appearance of smaller vessels expressing vWF in inflamed foci (D) (arrowheads) ($\times 30$). For the demonstration of increased vascular permeability transgenic mice were injected intravenously with 0.5 g BSA. After 30 min the mice were killed, and the pancreases were processed for immunohistochemistry. The presence of albumin in inflamed foci indicates an increased vascular permeability (E) ($\times 120$). (a) duct; (b) islet of Langerhans; (c) vessels.

murine IL-10, which indicate that IL-10 is a powerful immunosuppressor (1). The chronological appearance of leukocyte subsets and the pattern of migration in IL-10 transgenic mice is similar to what is demonstrated in several animal models of insulin dependent diabetes mellitus (IDDM) (26–28) and in transgenic mice expressing IFN- γ in the pancreatic β cells (M.-S. Lee, personal communication), although the rapidity of the process may differ. This implies the existence of a common migration pattern, irrespective of the initial lesion resulting in pancreatic infiltration. Very puzzling, and in contrast to animal models of IDDM- and IFN- γ -expressing mice (7), lymphocyte penetration into islets and subsequent β cell

destruction are entirely absent in IL-10 transgenic mice. The mechanism of β cell killing in IFN- γ transgenic mice is thought to occur by IFN- γ -induced activation of quiescent autoreactive lymphocytes in vivo (29). In contrast, the absence of β cell destruction in IL-10 transgenic mice may be explained by IL-10-induced inhibition of the antigen-presenting capacity of M ϕ , inhibition of antigen-specific T cell proliferation, and reduced cytokine production; consequently, the β cells are not exposed to cytotoxic cytokines and antigen sensitization is prevented. However, the lack of insulinitis in the IL-10 transgenic mice could reflect differences in the expression pattern of appropriate adhesion molecules

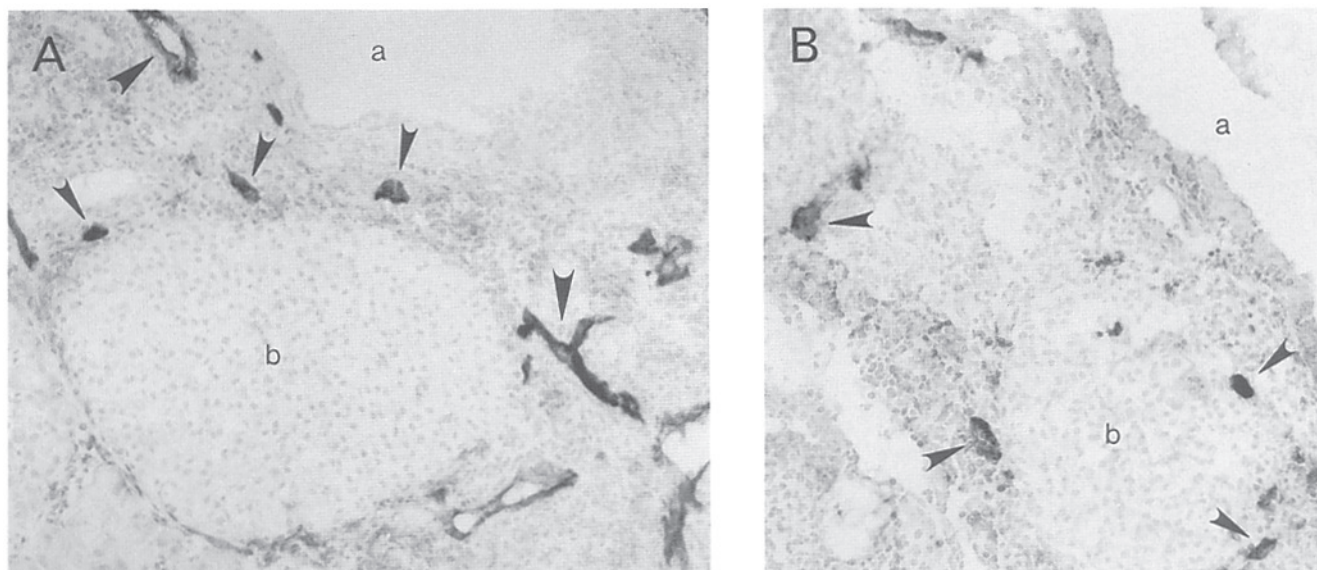


Figure 7. Pancreatic expression of vascular addressins. Expression of the mucosal (*arrowheads*) (A) and the peripheral LN (*arrowheads*) (B) vascular addressins in IL-10 transgenic mice, identified by the antibodies MECA 367 and MECA 79, respectively (both $\times 80$). (a) duct; (b) islet of Langerhans.

on the islet vasculature, inhibiting β cell exposure to the immune system.

Since murine IL-10 enhances the growth factor-induced proliferation of mature CD4⁺ and CD8⁺ T lymphocytes (30), and enhances the differentiation of cytotoxic T cell precursors into CD8⁺ cytotoxic T cells (31), local proliferation of lymphocytes could in part explain the accumulation of inflammatory cells in the pancreas. However, the mitotic activity of the extravasated lymphocytes was very low. To elucidate the role of the endothelial cells in IL-10-induced leukocyte extravasation, we examined the pancreas for vascular changes. The functional and structural changes of the endothelium, such as increased expression of ICAM-1, Ia, and vWF, increased permeability, neoexpression of vascular addressins, and appearance of small vessels with cuboidal ECs, indicate that the ECs are highly activated (11–13) and take on the phenotype of differentiated endothelium specialized for leukocyte extravasation (16, 32). The leukocyte ligands for ICAM-1 (Mac-1 and LFA-1), Mad (LPAM-1/2), and PNad (I-selectin) were expressed on a majority of the infiltrating cells mediating adhesion to the activated endothelium and neo-expressed vascular addressins. Therefore, our data suggest that IL-10 is a potent signal for leukocyte extravasation *in vivo*.

Expression of MHC class I and II, and ICAM-1 on ECs, are influenced by cytokines at sites of inflammation (33–38). Furthermore, cytokines, particularly IFN- γ , are decisive for the maintenance and “adhesiveness” of peripheral LN and

mucosal HEV (39–42). This indicates that normal “flat” endothelium may differentiate into endothelium with HEV characteristics by exposure to IFN- γ and other cytokines in inflammatory foci, e.g., in arthritis, experimental allergic encephalomyelitis (21, 23, 43), or as reported herein. Since we did not find any evidence of vasculogenesis, indicated by the absence of mitotic activity in small vessels, it is most likely that preexisting vessels develop phenotypic characteristics of HEV. In IL-10 transgenic mice the initial observation was stimulation of the ECs, indicated by increased expression of ICAM-1, Ia, and vWF, and homing of Mac-1-positive cells to the pancreas. Preliminary studies show that IL-10 induces ICAM-1 expression on ECs *in vitro*. Therefore, vascular changes *in vivo* may, at least partly, be mediated directly by IL-10, although the Th1 cell production of granulocyte-M ϕ /monocyte-CSF (GM-CSF), lymphotoxin (LT), and TNF- α , which is not downregulated by IL-10 (44), and thrombin and histamine, produced from the infiltrating cells, most likely plays a major role in the differentiation of the ECs.

In conclusion, transgenic expression of IL-10 in pancreatic β cell leads to a pronounced pancreatic inflammation. Homing of target cells to the location of IL-10 production may be important for the manifestation of the immunosuppressive function on otherwise distant, circulating leukocytes. We hypothesize that IL-10, in addition to its *in vitro* immunoinhibitory properties, can attract leukocytes *in vivo*. These results may have important consequences on the therapeutic use of IL-10.

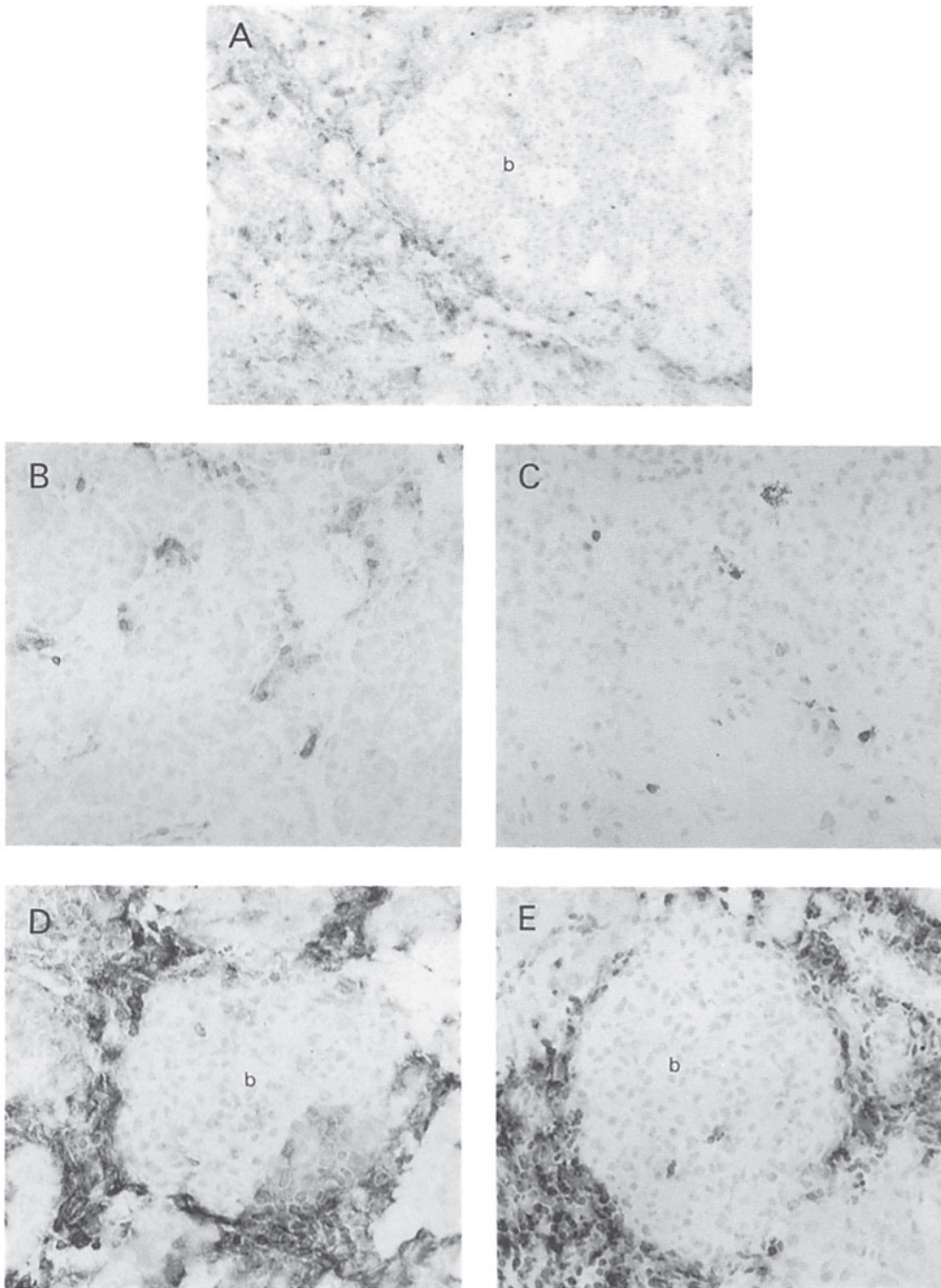


Figure 8. Expression of leukocyte adhesion molecules in IL-10 transgenic mice. To identify the presence of different leukocyte adhesion molecules, pancreatic tissue was reacted with antibodies recognizing l-selectin (A) ($\times 120$), Mac-1 (B and D), and LFA-1 (C and E). Mac-1-positive cells were scattered in 1-wk-old mice (B) ($\times 120$), but showed a localized distribution in older animals (D) ($\times 120$). A similar pattern was demonstrated for LFA-1 expression in young (C) ($\times 120$) and older mice (E) ($\times 120$). (b) Islet of Langerhans.

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References

- de Waal Malefyt, R., H. Yssel, M.-G. Roncarolo, H. Spits, and J.E. de Vries. 1992. Interleukin-10. *Curr. Opin. Immunol.* 4:314.
- Del Prete, G., M. De Carli, F. Almerigogna, M.G. Giudizi, R. Biagiotti, and S. Romagnani. 1993. Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. *J. Immunol.* 150:353.
- Yssel, H., R. de Waal Malefyt, M.-G. Roncarolo, J.S. Abrams, R. Lahesmaa, H. Spits, and J.E. de Vries. 1992. IL-10 is produced by subsets of human CD4⁺ T cell clones and peripheral blood T cells. *J. Immunol.* 149:2378.
- Fiorentino, D.F., A. Zlotnik, T.R. Mosmann, M. Howard, and A. O'Garra. 1991. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* 147:3815.
- Oswald, I.P., T.A. Wynn, A. Sher, and S.L. James. 1992. Interleukin 10 inhibits macrophage microbicidal activity by blocking the endogenous production of tumor necrosis factor a required as a costimulator factor for interferon γ -induced activation. *Proc. Natl. Acad. Sci. USA.* 89:8676.
- Gazzinelli, T.T., I.P. Oswald, S.L. James, and A. Sher. 1992. IL-10 inhibits parasite killing and nitrogen production by IFN γ -activated macrophages. *J. Immunol.* 148:1792.
- Sarvetnick, N., D. Liggitt, S.L. Pitts, S.E. Hansen, and T.A. Stewart. 1988. Insulin-dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon- γ . *Cell.* 52:773.
- Moore, K.W., P. Vieira, D.F. Fiorentino, M.L. Trounstein, T.A. Khan, and T.R. Mosmann. 1990. Homology of cytokine synthesis inhibitor factor (IL-10) to the Epstein-Barr virus gene BCRF1. *Science (Wash. DC).* 248:1230.
- Brigati, D.J., D. Myerson, J.J. Leary, B. Spalholz, S.Z. Travis, C.K.Y. Fong, G.D. Hsiung, and D.C. Ward. 1983. Detection of viral genomes in cultured cells and paraffin-embedded tissue sections using biotin-labeled hybridization probes. *Virology.* 126:32.
- Holzmann, B., and I.L. Weissman. 1989. Peyer's patch-specific lymphocyte homing receptors consist of a VLA-4-like α chain associated with either of two integrin β chains, one of which is novel. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1735.
- Hogg, N., P.A. Bates, and J. Harvey. 1991. Structure and function of intercellular adhesion molecule-1. In *Integrins and ICAM-1 in Immune Responses*. N. Hogg, editor. Karger, Basel. 98-115.
- Wagner, D.D., and R. Bonfanti. 1991. Von Willebrand Factor and the endothelium. *Mayo Clin. Proc.* 66:621.
- Paleolog, E.M., D.C. Crossman, J.H. McVey, and J.D. Pearson. 1990. Differential regulation by cytokines of constitutively and stimulated secretion of von Willebrand Factor from endothelial cells. *Blood.* 75:688.
- Dib, S.A., P. Vardi, S. Bonner-Weir, and G.S. Eisenbarth. 1988. Selective localization of Factor VIII antigenicity to islet endothelial cells and expression of class II antigens by normal human pancreatic ductal epithelium. *Diabetes.* 37:483.
- Royall, J.A., R.L. Berkow, J.S. Beckman, M.K. Cunningham, S. Matalon, and B.A. Freeman. 1989. Tumor necrosis factor and interleukin 1 α increase vascular endothelial permeability. *Am. J. Physiol.* 257:L399.
- Picker, L.J., and E.C. Butcher. 1992. Physiological and molecular mechanisms of lymphocyte homing. *Annu. Rev. Immunol.* 10:561.
- Streeter, P.R., B.T.N. Rouse, and E.C. Butcher. 1988. Immunohistologic and functional characterization of a vascular addressin involved in lymphocyte homing into peripheral lymph nodes. *J. Cell Biol.* 107:1853.
- Berg, E.L., M.K. Robinson, A. Warnock, and E.C. Butcher. 1991. The human peripheral lymph node vascular addressin is a ligand for LECAM-1, the peripheral lymph node homing receptor. *J. Cell Biol.* 114:343.
- Streeter, P.R., E.L. Berg, B.T.N. Rouse, R.F. Bargatze, and E.C. Butcher. 1988. A tissue-specific endothelial cell molecule involved in lymphocyte homing. *Nature (Lond.)* 331:41.
- Nakache, M., E.L. Berg, P.R. Streeter, and E.C. Butcher. 1989. The mucosal vascular addressin is a tissue-specific endothelial cell adhesion molecule for circulating lymphocytes. *Nature (Lond.)* 337:179.
- Freemont, A.J., C.J.P. Jones, M. Bromley, and P. Andrews. 1983. Changes in vascular endothelium related to lymphocyte collections in diseased synovia. *Arthritis Rheum.* 26:1427.
- Freemont, A.J., and W.L. Ford. 1985. Functional and morphological changes in post-capillary venules in relation to lymphocytic infiltration into BCG-induced granulomata in rat skin. *J. Pathol.* 147:1.
- O'Neill, J.K., C. Butter, D. Baker, S.E. Gschmeissner, G. Kraal, E.C. Butcher, and J.L. Turk. 1991. Expression of vascular addressins and ICAM-1 by endothelial cells in the spinal cord during chronic relapsing experimental allergic encephalomyelitis in the Biozzi AB/H mouse. *Immunology.* 72:520.
- Gallatin, W.M., I.L. Weissman, and E.C. Butcher. 1983. Cell-surface molecule involved in organ-specific homing of lymphocytes. *Nature (Lond.)* 304:30.
- Lewinsohn, D.M., R.F. Bargatze, and E.C. Butcher. 1987. Leukocyte-endothelial cell recognition: evidence of a common molecular mechanism shared by neutrophils, lymphocytes, and other leukocytes. *J. Immunol.* 138:4313.
- Signore, A., P. Pozzilli, E.A.M. Gale, D. Andreani, and P.C.L. Beverly. 1989. The natural history of lymphocyte subsets infiltrating the pancreas of NOD mice. *Diabetologia.* 32:282.
- Hananberg, H., V. Kolb-Bachofen, G. Kantwerk-Funke, and H. Kolb. 1989. Macrophage infiltration precedes and is a pre-

- requisite for lymphocytic insulinitis in pancreatic islets of pre-diabetic BB-rats. *Diabetologia*. 32:126.
28. O'Reilly, L.A., P.R. Hutchings, P.R. Crocker, E. Simpson, T. Lund, D. Kioussis, J. Baird, and A. Cooke. 1991. Characterization of pancreatic islet cell infiltrates in NOD mice: effect of cell transfer and transgene expression. *Eur. J. Immunol.* 21:1171.
 29. Sarvetnick, N., J. Shizuru, D. Liggitt, L. Martin, B. McIntyre, A. Gregory, T. Parslow, and T. Stewart. 1990. Loss of pancreatic islet tolerance induced by β -cell expression of interferon γ . *Nature (Lond.)*. 346:844.
 30. MacNeil, I.A., T. Suda, K.W. Moore, T.R. Mosmann, and A. Zlotnik. 1990. IL-10, a novel growth cofactor for mature and immature T cells. *J. Immunol.* 145:4167.
 31. Chen, W., and A. Zlotnik. 1991. IL-10: a novel cytotoxic T cell differentiation factor. *J. Immunol.* 147:528.
 32. Picker, L.J. 1992. Mechanism of lymphocyte homing. *Curr. Opin. Immunol.* 4:277.
 33. Issekutz, T.B. 1990. Effects of six different cytokines on lymphocyte adherence to microvascular endothelium and in vivo lymphocyte migration in the rat. *J. Immunol.* 144:2140.
 34. Cavender, D.E. 1990. Organ-specific and organ non-specific lymphocyte receptors for vascular endothelium. *J. Invest. Dermatol.* 94:41.
 35. Doukas, J., and J.S. Pober. 1990. IFN γ enhances endothelial activation induced by tumor necrosis factor but not IL-1. *J. Immunol.* 145:1727.
 36. Carlos, T.M., B.R. Schwartz, N.L. Kovach, E. Yee, M. Rosso, L. Osborn, G. Chi-Rosso, B. Newman, R. Lobb, and J.M. Harlan. 1990. Vascular cell adhesion molecule-1 mediates lymphocyte adherence to cytokine-activated cultured human endothelial cells. *Blood*. 76:965.
 37. Graber, N., T.V. Gopal, D. Wilson, L.D. Beall, T. Polte, and W. Newman. 1990. T cells bind to cytokine-activated endothelial cells via a novel, inducible sialoglycoprotein and endothelial leucocyte adhesion molecule-1. *J. Immunol.* 145:819.
 38. Leeuwenberg, J.F.M., E.J.U. vonAsmuth, T.M.A.A. Jeunhomme, and W.A. Buurman. 1990. IFN γ regulates the expression of the adhesion molecule ELAM-1 and IL-6 production by human endothelial cells in vitro. *J. Immunol.* 145:2110.
 39. Hendriks, H.R., and I.L. Eestermans. 1983. Disappearance and reappearance of high endothelial venules and immigrating lymphocytes in lymph nodes deprived of afferent lymphatic vessels: a possible regulatory role of macrophages in lymphocyte migration. *Eur. J. Immunol.* 13:663.
 40. Duijvestijn, A.M., A.B. Schreiber, and E.U. Butcher. 1986. Interferon γ regulates an antigen specific for endothelial cells involved in lymphocyte traffic. *Proc. Natl. Acad. Sci. USA.* 83:9114.
 41. Chin, J., J. Cai, and K. Johnson. 1990. Lymphocyte adhesion to cultured Peyer's Patch high endothelial venule cells is mediated by organ-specific homing receptors and can be regulated by cytokines. *J. Immunol.* 145:3669.
 42. Chin, Y.H., J. Cai, and X. Xu. 1991. Tissue-specific homing receptor mediates lymphocyte adhesion to cytokine-stimulated lymph node high endothelial venule cells. *Immunology*. 74:478.
 43. Jalkanen, S., A.C. Steere, R.I. Fox, and E.C. Butcher. 1986. A distinct endothelial cell recognition system that controls lymphocyte traffic into inflamed synovium. *Science (Wash. DC)*. 233:556.
 44. Fiorentino, D.F., A. Zlotnik, P. Vieira, T.R. Mosmann, M. Howard, K.W. Moore, and A. O'Garra. 1991. IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J. Immunol.* 146:3444.