The Pathogenesis of Adoptive Murine Autoimmune Diabetes Requires an Interaction between α4-Integrins and Vascular Cell Adhesion Molecule-1

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Abstract

An adoptive transfer model of insulin-dependent diabetes mellitus (IDDM) in the nonobese diabetic mouse was used to examine the roles of α4-integrin, vascular cell adhesion molecule 1 (VCAM-1); and intercellular adhesion molecule 1 (ICAM-1) in the pathogenesis of autoimmune diabetes. Antibodies specific for both α4-integrin and one of its ligands, VCAM-1, were able to delay onset of diabetes and decrease the incidence of the disease in adoptive transfer studies. This blocking of disease was accompanied by a marked decrease in lymphocytic infiltration of the islets of Langerhans. Furthermore, these antibodies preferentially block entrance of CD4 T cells into the tissue. Antibodies specific for ICAM-1 had little effect on the onset or incidence of IDDM. Thus, we conclude that an α4-integrin–VCAM-1 interaction is important in T cell entry into the islets of Langerhans and in the pathogenesis of IDDM. In addition, the cascade of events leading to T cell transit across endothelium may be different for CD4 and CD8 cells, and may differ depending on the endothelium involved. Our results support the more general conclusion that an α4-integrin–VCAM-1 interaction may be crucial in allowing activated effector CD4 T cells to leave the blood and enter tissue to clear infection. (*J. Clin. Invest.* 1994. 93:1700–1708.) Key words: nonobese diabetic mouse • lymphocytes • cell adhesion • islets of Langerhans

Introduction

The nonobese diabetic (NOD)[1] mouse spontaneously develops autoimmune diabetes and is an animal model for human insulin-dependent diabetes mellitus (IDDM). IDDM is believed to be an autoimmune disease in which activated T lymphocytes destroy the insulin-producing β-cells of the pancreatic islets (1, 2). The islets of Langerhans in normal mice are almost completely devoid of lymphocytes, whereas NOD mice gradually develop insulitis, a mononuclear cell infiltration of the islets consisting mostly of T lymphocytes, before they develop IDDM (3–5). IDDM can be adoptively transferred by splenic T cells from diabetic donors and prevented by in vivo T cell depletion (6, 7). Adoptive transfer experiments using antibody depletion, cell sorting, and transfer of islet-specific cloned T cell lines have demonstrated that both CD4 and CD8 T cells can contribute to the transfer of IDDM (7–10).

It has been suggested that macrophages might also be involved in the assault on β cells (11, 12). In fact, adoptive transfer of diabetes in the NOD mouse can be blocked by antibodies against an adhesion-promoting type 3 complement receptor on macrophages which is involved in the migration of macrophages into inflammatory sites (13). The administration of this antibody prevents intraislet infiltration by both macrophages and T cells. It has been suggested that the receptor on macrophages is involved in allowing these cells to enter the islets, and that blocking entry of the macrophages also prevents the entry of T cells.

The mechanism by which the T cells enter islets is still unclear. Recently, our laboratory has shown that surface expression of α4-integrin is required for the entry of activated effector CD4 T cells that transfer experimental allergic encephalomyelitis (EAE) into brain parenchyma (14). α4-Integrin-high, myelin basic protein–specific cloned CD4 T cells can enter the brain parenchyma, whereas α4-integrin–low variants of these clones are unable to induce disease or enter brain parenchyma. Furthermore, antibodies to α4-integrin, to one of its ligands, vascular cell adhesion molecule 1 (VCAM-1), and to intercellular adhesion molecule 1 (ICAM-1) each delayed onset of EAE transferred with these cloned T cells or with polyclonal T cells. On the basis of these studies, we proposed that the elevated level of α4-integrin, or very late antigen 4 (VLA-4), expressed on effector T cells facilitates interaction with VCAM-1, which can be induced on endothelium, allowing activated T cells to leave blood and enter peripheral tissues (14).

To test the generality of this result, and to examine the means by which islet-specific T cells enter the islets of Langerhans to cause IDDM, we have here examined the roles of α4-integrin, VCAM-1, and ICAM-1 in the pathogenesis of IDDM in the NOD mouse. Antibodies against α4-integrin and VCAM-1 can significantly delay onset of diabetes in adoptive transfer studies, and this blocking is accompanied by a marked decrease in insulitis. Antibodies against ICAM-1 do not appear to have the same dramatic effects in blocking IDDM as they do in blocking EAE. In addition, immunohistochemical analysis of pancreatic sections from diabetic animals demonstrates a strong induction of VCAM-1 on blood vessels only within islets. α4-Integrin on the invading CD4 and CD8 T cells appears to be modulated, as was also observed in T cell invasion into the brain. These changes were not seen in animals that did not develop diabetes. Thus, these studies provide evidence of a role for a VLA-4–VCAM-1 interaction in the pathogenesis of IDDM, and support the hypothesis that a VLA-4–VCAM-1 interaction is involved in allowing activated effector T cells to

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1. *Abbreviations used in this paper:* EAE, experimental allergic encephalomyelitis; ICAM-1, intercellular adhesion molecule 1; IDDM, insulin-dependent diabetes mellitus; LFA-1, lymphocyte function-associated antigen 1; NOD, nonobese diabetic; VCAM-1, vascular cell adhesion molecule 1; VLA-4, very late antigen 4.

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leave blood and enter the islets of Langerhans. This is proposed to reflect a general mechanism for moving activated CD4 T cells from blood to tissues.

Methods

Mice. A breeding nucleus of inbred NOD mice was kindly provided by Dr. Masakazu Hattori (Joslin Clinic, Boston, MA). Our NOD/Caj colony has been maintained under sterile pathogen-free conditions by brother-sister mating at the Section of Immunobiology, Yale University School of Medicine. The clinical onset of diabetes in the NOD/Caj colony was determined by urinary glucose (Diastix, Ames, Elkhart, IN). Animals showing positive urine values were tail clipped and analyzed for blood glucose with a glucometer, and values $\geq$ 250 mg/dl were classified as overtly diabetic. Diabetes is first observed in NOD/Caj mice at 3 mo of age, and incidence is 95% in the females and 65% in the males at 6 mo of age.

Monoclonal antibodies. LPAM-1 (anti-α4-integrin) (15, 16) was provided by Dr. Irving Weissman (Stanford, CA). Anti-VCAM-1 was purchased from Pharmingen (San Diego, CA). Other antibodies used were YCD3-1 (anti-CD3) (17), M1/89.18.7.HK (anti-CD45) (18), M1/9.3.HL.2 (anti-CD45) (18, 19), YN/1 (anti-ICAM-1) (20), GK1.5 (anti-CD4) (21), 53-6.72 (anti-CD8) (22), and N418 (anti-mouse CD11c) (23).

In vivo blocking of adoptive transfer of diabetes. NOD/Caj mice (7–8 wk old, females) were irradiated (730 rad) and then randomly divided into treatment groups. Splenocytes isolated from newly diabetic female NOD mice were incubated with saline or mAbs for 30 min at 37°C. Irradiated recipient animals received intravenous injections of 2×10^7 splenocytes or splenocytes mixed with antibody, while negative control mice were treated intravenously with saline. Antibody concentrations were standardized for protein concentration by spectrophotometer (280 nm). Ascites fluid contained 12 mg/ml of protein whereas the purified antibody (anti-VCAM-1) contained 250 μg per treatment. Ascites fluid containing isotype matched control anti-CD45 antibodies were standardized to treatment antibodies by dilution in PBS, pH 7.4, 150 mM to obtain similar plateau-level flourescent staining on purified T cells from NOD mice. Animals that received multiple doses of antibodies were injected intravenously with antibody every 3 d. Mice were tested for the onset of diabetes by urine analysis and at the time of sacrifice blood glucose was determined. On the day when the first animal showed overt diabetes, mice were randomly selected from each treatment group and killed. Their spleen and pancreas were removed and prepared for light microscopy. The endpoint of the study was when all adoptively transferred mice in the control groups had developed diabetes.

FACS analysis and sorting. Splenocytes from newly diabetic female NOD mice were stained by indirect immunofluorescence. One million cells were incubated with the primary mAbs for 30 min at 37°C. After washing, the cells were incubated with appropriate secondary antibody conjugated to fluorescein (goat anti-rat IgG, Hyclone Laboratories Inc., Logan, UT). Cells were washed and fixed with 1% paraformaldehyde. Immunofluorescence analysis was performed on a FACStar Plus (Becton Dickinson & Co., Mountain View, CA) equipped with logarithmic amplifiers.

NOD splenic T cells used in cell sorting experiments were isolated on an anti-mouse Ig column. The purified T cells were then stained according to a standard protocol using the LPAM-1 antibody (anti-α4 integrin) diluted in cell culture medium plus FCS. The secondary antibody was a goat anti-rat IgG conjugated to fluorescein (Hyclone Laboratories Inc.), which was dialyzed in PBS to remove azide. Cells were sorted according to fluorescence intensity. Cells collected were the top and bottom 10% of the intensity curve.

Proliferation studies. Splenic T cells from newly diabetic NOD female mice (1×10^5) and the α4-integrin-high and -low sorted populations of T cells were cultured with 10^5 mitomycin-C-treated NOD spleen cells or 25 NOD islets (10) in 0.2 ml of Click's medium with 5% FCS in 96-well round-bottom microtiter plates. After 3 d, 1 μCi/well [^{3}H]thymidine (6.7 Ci mmol^{-1}; ICN, Biomedicals Inc., Costa Mesa, CA) was added to the culture for 16 h and the mean counts per minute for thymidine incorporation were calculated from duplicate cultures.

Immunohistochemistry. Unless specified otherwise, two mice from each treatment group were killed at the time when diabetes (blood glucose > 250 mg/dl) was first observed in positive control mice receiving only spleen cells. Specimens from both pancreas and spleen were obtained and prepared for light microscopy and immunohistochemistry. For light microscopy, the tissue was fixed in 10% buffered formalin, paraffin embedded, and stained with hematoxylin-eosin. For immunohistochemistry, the tissues were fixed with periodate-lysine-paraformaldehyde, washed, and then infused with increasing concentrations of sucrose in phosphate buffer (final concentration 30%). The tissue was snap frozen in tissue-tek compound in 2-methyl butane and 7-μm sections were cut. The sections were incubated with 3% BSA and Triton X-100 as preincubation blockers. Primary antibodies (biotinylated anti-CD4 [GK1.5]; biotinylated anti-CD8 [53-6.72]; anti-VCAM-1; and anti-α4 integrin [LPAM-1] were diluted in 1% BSA/Triton X and added to the sections for 90 min. When necessary, sections were incubated with a biotinylated second antibody (goat anti-rat IgG, Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD), and streptavidin-conjugated horseradish peroxidase (Zymed Laboratories, Inc., South San Francisco, CA) was used to label bound biotinylated reagents. Color was developed with diaminobenzidine-nickel ammonium sulfate. The counterstain is Mayer's hemalum.

Results

T cells isolated from newly diabetic NOD mice express significant levels of α4-integrin and ICAM-1. Activated T cells generally express elevated levels of α4-integrin which may be important in their migration into peripheral tissues as well as their interaction with target cells. To assess levels of α4-integrin, ICAM-1, and VCAM-1 on the splenic T cells of newly diabetic NOD mice, T cells were purified on an anti-mouse Ig column and stained by indirect immunofluorescence with antibodies against α4-integrin (LPAM), ICAM-1 (YN-1), and VCAM-1 (Pharmingen). Fluorescence intensity was measured by FACS. The T cells from newly diabetic NOD mouse spleen express significant levels of both α4-integrin and ICAM-1, but, as would be expected, they do not express VCAM-1 (Fig. 1).

The ability of spleen cells to transfer diabetes can be inhibited with antibodies specific for α4-integrin. To determine whether the α4-integrin seen on the T cells of newly diabetic NOD mice plays a role in the induction of diabetes in vivo, we asked whether antibodies to α4-integrin or to VCAM-1 could influence the adoptive transfer of diabetes in the NOD mouse. Spleen cells were isolated from newly diabetic female NOD mice. One portion of these spleen cells was passed over an anti-mouse Ig column to separate the T cells. These purified T cells were then stained with saturating concentrations of antibodies specific for α4-integrin, ICAM-1, and VCAM-1, as well as isotype-matched control antibodies directed against the mouse CD45 molecule (Fig. 1). These anti-CD45 antibodies were used in in vivo experiments to control for depletion of T cells by antibody-mediated mechanisms. The amount of anti-CD45 used stained the NOD T cells $\sim$ 50 times more intensely than either the anti-α4-integrin antibody or the anti-ICAM-1 antibody (Fig. 1). Thus, control antibodies were used in excess. A suitable endothelial cell-specific antibody to serve as a control for anti-VCAM-1 was not available, so no control antibody was used for experiments with anti-VCAM-1. However, inasmuch as anti-ICAM-1, like anti-VCAM-1, is known to

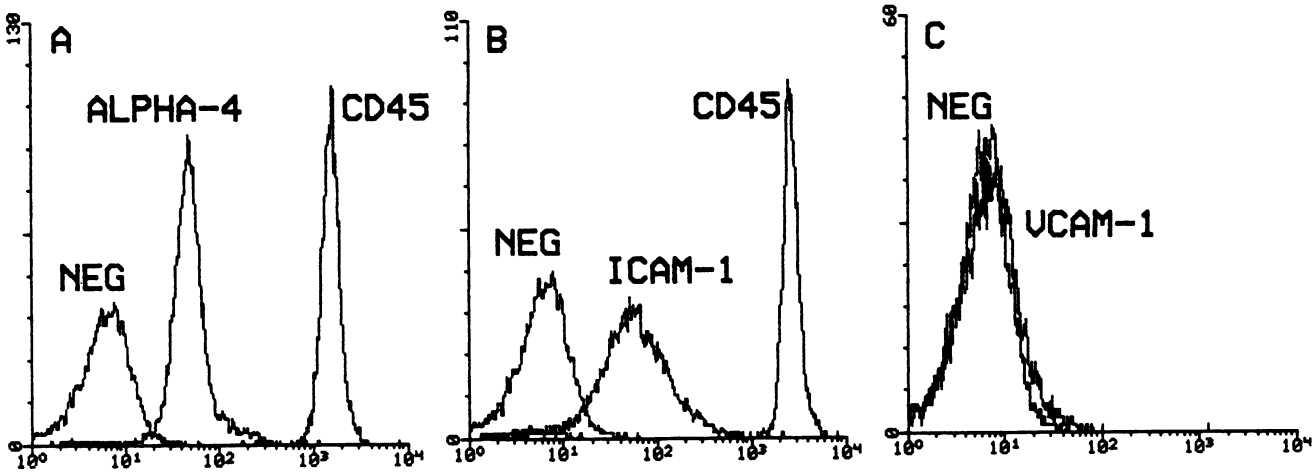


Figure 1. Splenic T cells from newly diabetic NOD mice express significant levels of α4-integrin (LPAM-1), ICAM-1 (YN-1), and CD45 (M1/89.18.17.HK and M1/9.3.HL.2). T cells were purified on an anti-mouse Ig column and stained by indirect immunofluorescence with the various antibodies. Cells were analyzed by FACS.

bind activated endothelial cells and has no effect in these studies, it can serve as a control.

The remaining spleen cells from the newly diabetic NOD mice were used for an in vivo adoptive transfer experiment. The various antibodies were mixed with the spleen cells and incubated at 37°C for 30 min. 2×10^7 spleen cells, together with the antibody, were then injected into 7–8-wk-old, irradiated female NOD mice.

Treatment of the cells used in adoptive transfer of diabetes with the antibody against α4-integrin significantly delayed the onset of diabetes and significantly decreased the incidence of disease (Fig. 2). When antibody to α4-integrin was given once at the time of injection of spleen cells, the onset of IDDM was delayed by 5 d and the incidence of disease was decreased by 25% as compared to mice that received either spleen cells alone or spleen cells plus the isotype-matched control anti-CD45 antibody (Fig. 2 *A*). If the animals were injected three additional times with anti-α4-integrin antibody, the onset of diabetes was delayed by 17 d and the incidence of disease was decreased by 90% as compared to the animals given either PBS or four injections of the isotype-matched control anti-CD45 antibody (Fig. 2 *B*).

The ability of spleen cells to transfer diabetes can also be inhibited with antibodies specific for VCAM-1. As VCAM-1 is the endothelial cell ligand for α4:β1 integrin (VLA-4), we next tested the ability of an antibody to VCAM-1 to inhibit the pathogenesis of diabetes. Again, 2×10^7 spleen cells from diabetic NOD mice were injected into 7–8-wk-old irradiated female NOD mice with and without antibody. Antibody directed against VCAM-1 given once at the time of injection of spleen cells delayed onset of diabetes by 4 d (Fig. 3) equivalent to the delay achieved with one dose of anti-α4-integrin. These results suggest that an interaction between VLA-4 on the T cell and VCAM-1 on endothelial cells and/or antigen-presenting cells is involved in the pathogenesis of IDDM in the NOD mouse.

Antibodies specific for ICAM-1 do not significantly inhibit the ability of spleen cells to transfer diabetes. When antibodies against ICAM-1 were given at the time of injection of spleen cells, there was little effect on either the onset or the incidence of IDDM as compared to the animals that received either spleen cells alone or spleen cells with the isotype-matched control anti-CD45 antibody (Fig. 4 *A*). This failure to inhibit transfer of IDDM was seen even when the animals were injected three more times with antibody (Fig. 4 *B*). Thus, ICAM-1 is not detectably involved in this process. The same antibody, given once, profoundly blocked transfer of EAE (14). Table I shows a compilation of the incidence of diabetes on day 20 in three experiments using antibodies to α4-integrin and ICAM-1.

Both α4-integrin–high and α4-integrin–low–expressing T cells isolated from newly diabetic NOD mice proliferate in response to NOD islets. In earlier studies of EAE, we could show no role for α4-integrin in T cell activation (14). However, we did observe that only α4-integrin–high T cells would respond to antigen in the EAE system. In order to control for the possibility that α4-integrin is required for the activation of islet-specific T cells in vivo, we isolated α4-integrin–high and –low–expressing populations of T cells from diabetic animals and compared their responses to NOD islets. Spleen cells were obtained from newly diabetic NOD mice, and their T cells were purified over an anti-mouse Ig column. T cells were then stained with antibody against α4-integrin and sorted according to fluorescence intensity by FACS. Cells collected were the top and bottom 10% of the fluorescence intensity curve. These cells proliferate in response to NOD islets but not NOD spleen cells, and both the α4-integrin–high and –low populations of T cells proliferate similarly (Fig. 5). Thus, expression of high levels of α4-integrin is not required for T cell activation by islet cells.

Antibodies specific for α4-integrin and VCAM-1 affect the entry of T cells into the islets of Langerhans. Because we have previously shown that a VLA-4–VCAM-1 interaction is crucial for the entry of T cells into brain parenchyma, and because antibodies to α4-integrin and to VCAM-1 affect the pathogenicity of T cells that cause IDDM, we were interested to determine whether these treatments of T cells with antibodies to α4-integrin and VCAM-1 would influence their entry into the islets of Langerhans. Two mice from each of the various treatment groups in the foregoing experiments were killed on the first day that the control animals who received only spleens cells developed disease. Pancreas and spleen were removed from these animals and processed to obtain histologic sections (Fig. 6). Mice given spleen cells along with anti-α4-integrin one or four times showed almost no insulitis (Fig. 6, *H* and *J*), as com-

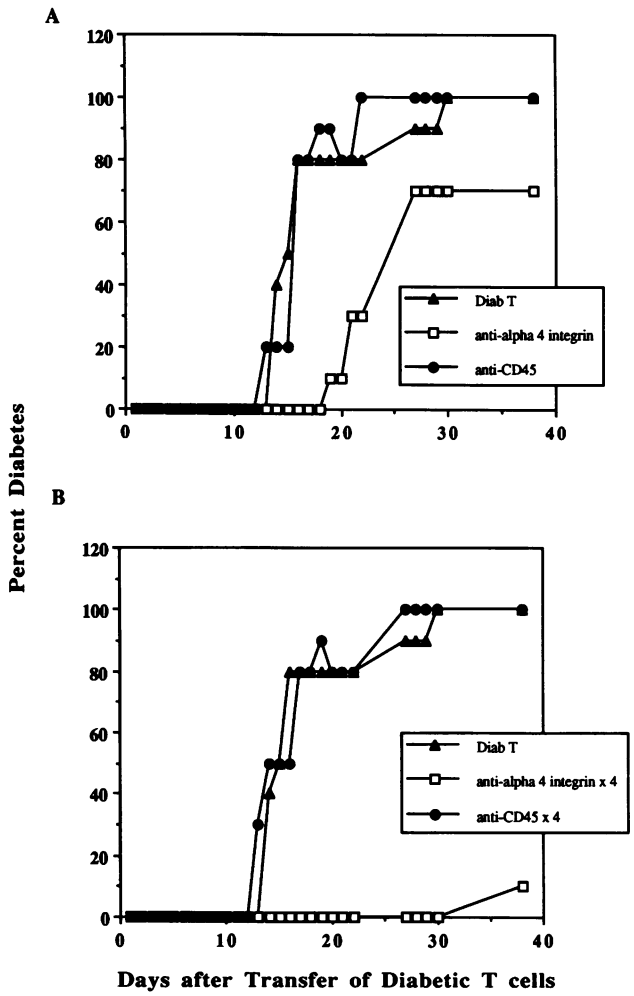


Figure 2. Adoptive transfer of diabetes can be inhibited by mAb specific for α4-integrin. 2×10^7 spleen cells from newly diabetic NOD mice were incubated in PBS or the specified antibody and injected into irradiated NOD female mice (7–8 wk old). All groups had at least 10 animals. (*A*) T cells treated with PBS (▲), anti-α4-integrin (LPAM-1, IgG2b [□]), and the isotype matched control anti-CD45 (M1/89.18.7.HK, IgG2b [●]). (*B*) T cells treated on transfer and recipients treated on days 3, 6, and 9 after transfer with PBS (▲), anti-α4-integrin (LPAM-1, IgG2b [□]), and the isotype matched control anti-CD45 (M1/89.18.7.HK, IgG2b [●]).

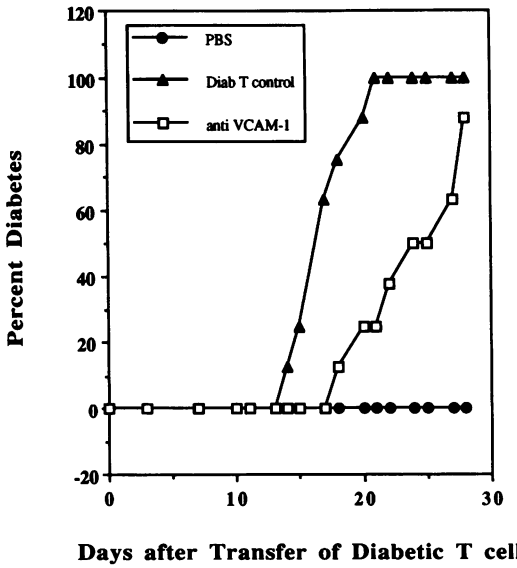


Figure 3. Adoptive transfer of diabetes can be inhibited by mAb specific for VCAM-1. 2×10^7 spleen cells from newly diabetic NOD mice were incubated in PBS or antibody to VCAM-1. Irradiated female 7–8-wk-old NOD mice were injected with PBS only (●), T cells incubated with PBS (▲), or T cells incubated with anti-VCAM-1 (□). All groups had at least seven animals.

pared to mice given spleen cells only (Fig. 6, *B* and *E*), whereas mice given spleen cells in combination with isotype matched control antibodies directed against CD45 or antibodies specific for ICAM-1 showed significant insulitis (Fig. 6, *C, D, G,* and *I*). Mice given spleen cells along with antibody specific for VCAM-1 showed slightly less insulitis than mice in the control groups, but there was still significant lymphocytic infiltrate in these mice (Fig. 6 *F*). No lymphocytes were seen in the islets of control irradiated mice given no spleen cells (Fig. 6 *A*). These studies show that anti-α4-integrin antibodies prevent the development of insulitis; this strongly suggests that α4-integrin on the activated T cells that produce this lesion is required for their entry into the islet.

Islets of Langerhans of diabetic mice are infiltrated by CD4, CD8, and α4-integrin–positive cells and express VCAM-1 on endothelium. Pancreatic sections from irradiated NOD mice that received spleen cells or PBS were stained with antibodies to CD4, CD8, α4-integrin, and VCAM-1. Tissue sections from control animals that received only PBS and did not develop diabetes showed no evidence of insulitis (Fig. 6 *A*), and thus were negative for expression of CD4, CD8, and α4-integrin (data not shown). In addition, the islets and surrounding pancreatic tissue in such mice showed no evidence of VCAM-1 expression (data not shown). In contrast, pancreatic sections from animals that received spleen cells and developed diabetes showed infiltration by CD4 T cells, CD8 T cells, and α4-integrin–positive cells, and expressed VCAM-1 on endothelial cells (Fig. 7). The majority of lymphocytes in the islet appear to be CD8 positive (Fig. 7 *B*), but there are definitely CD4-positive T cells as well (Fig. 7 *A*). As was seen in brain sections from animals with EAE (14), expression of α4-integrin appears to be limited to lymphocytes surrounding small blood vessels, and those located at the outer edges of the islets (Fig. 7 *C*). Expression of VCAM-1 is only seen in the islets, and it appears to be present on endothelium that has been exposed to the lymphocytic infiltrate (Fig. 7 *D*). However, VCAM-1 may also be induced on other cells within the islets, such as dendritic cells. Islets contain numerous dendritic cells, as detected by staining with anti-mouse CD11c, but the staining pattern seen with anti-VCAM-1 is clearly distinct from the pattern seen with the antidendritic cell antibody (data not shown).

Antibody to α4-integrin or VCAM-1 affects CD4 and CD8 T cell distribution in infiltrated islets. Two animals from each experimental group were also killed at the time that the first animal in that group developed diabetes. These sections were stained with antibodies specific for CD4 and CD8. Fig. 7, *A* and *B,* shows sections from an animal that was given spleen cells alone. The islets of Langerhans from these animals show extensive lymphocytic infiltration throughout the islets by both CD4 and CD8 T cells. Fig. 7, *E* and *F,* are sections from animals that received spleen cells plus one dose of anti-α4-integrin. The islets of Langerhans show evidence of decreased CD4 and CD8

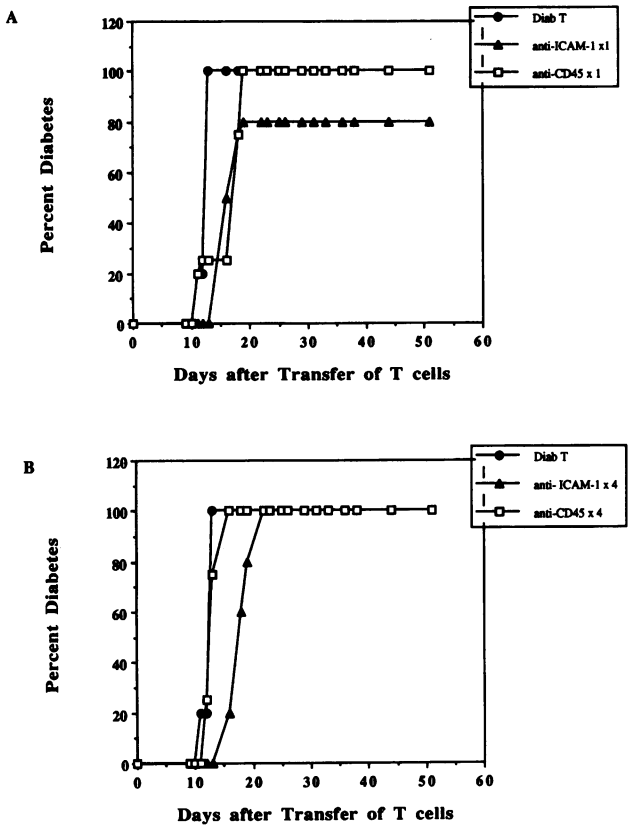


Figure 4. Adoptive transfer of diabetes is minimally inhibited by mAb specific for ICAM-1. 2×10^7 spleen cells from newly diabetic NOD mice were incubated in PBS or antibody against ICAM-1 and injected into irradiated NOD female mice (7–8 wk old). All groups had at least five animals. (*A*) Animals were given spleen cells plus PBS or antibody once at the time of injection of cells. (*B*) Animals were given spleen cells plus PBS or antibody once at the time of injection of cells, followed by three more doses of antibody on days 3, 6, and 9. T cells treated with PBS (●), anti-ICAM-1 (YN-1, IgG2a [▲]), or the isotype matched control anti-CD45 (M1/93.HL.2, IgG2a [□]).

cell infiltration. The CD4 cells remain at the outer edges of the islet, while CD8 cells have penetrated into the islet. This pattern was seen more clearly in animals treated with anti-VCAM-1. The CD4 cells remain in a ring on the outer edges of the islet, while CD8 cells have extensively infiltrated the entire islet (Fig. 7, *G* and *H*). These patterns were observed for all the animals in each group that were killed at the same time (data not shown).

Discussion

The focus of the present experiments was to examine the roles of α4-integrin, VCAM-1, and ICAM-1 in the pathogenesis of IDDM in the NOD mouse. Because other groups have shown that these molecules are involved in the entry of T cells into sites of inflammation (24–28), and because we have previously shown involvement of these molecules in T cell entry into brain parenchyma (14), these experiments were undertaken to test the generality of the hypothesis that elevated levels of α4-integrin expressed on effector T cells interacting with VCAM-1 on endothelium is involved in allowing activated T cells to leave the blood and enter normal tissue. In addition, we thought that these experiments might give some insight into

Table I. Incidence of IDDM on Day 20 in Three Experiments

Antibody treatment	Incidence	Percent diabetes
PBS	19/20	95
Anti-CD45 (M1/89.18.7.HK)	19/20	95
Anti-α4-integrin (LPAM-1)	6/20	30
Anti-CD45 × 4 (M1/89.18.7.HK)*	18/20	90
Anti-α4-integrin × 4 (LPAM-1)*	0/20	0
Anti-CD45 (M1/9.3.HL.2)	7/10	70
Anti-ICAM-1 (YN-1)	6/10	60
Anti-CD45 × 4 (M1/9.3.HL.2)*	7/10	70
anti-ICAM-1 × 4 (YN-1)*	7/10	70

The incidence of adoptive transfer of IDDM was significantly decreased by the in vivo treatment with antibodies specific for α4-integrin. Antibodies specific for ICAM-1 or isotype-matched control antibodies do not significantly affect the incidence of disease.
* All animals received 2×10^7 diabetogenic NOD spleen cells. Animals were given a total of four antibody treatment.

the means by which islet-specific T cells enter the islets of Langerhans to cause IDDM.

In order for autoreactive T cells to cause disease, the cells must be able to leave the blood and enter into the target tissue. In the T cell mediated autoimmune disease EAE, we have shown that the entry of an encephalitogenic CD4 T cell clone into brain parenchyma requires cell surface VLA-4 binding to endothelial cell VCAM-1, and probably lymphocyte function-associated antigen 1 (LFA-1) binding to ICAM-1. The current studies demonstrate a similar role for a VLA-4–VCAM-1 interaction in the pathogenesis of IDDM in the NOD mouse; and this role also appears to be in allowing entry of CD4 T cells into

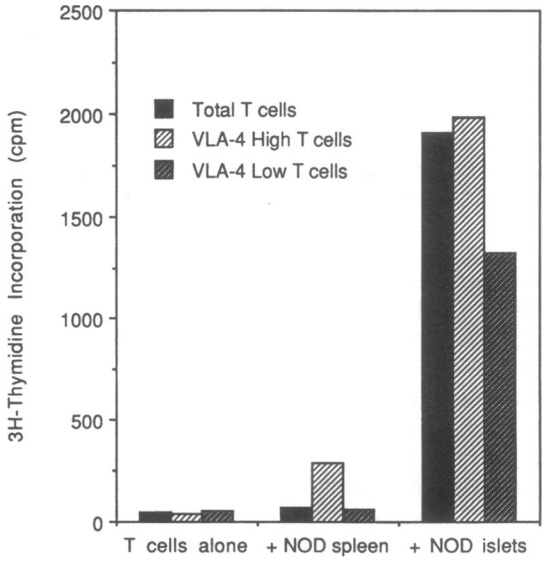


Figure 5. Proliferation of splenic T cells from newly diabetic NOD mice, sorted according to α4-integrin expression, in response to NOD islets of Langerhans or NOD spleen cells. Sorted T cells were plated in triplicate either alone, with mitomycin-C–treated NOD spleen cells, or with NOD islets. Plates were pulsed with [^{3}H]thymidine after 48 h and harvested 15 h later. Data are expressed as counts per minute. This experiment was repeated several times with similar results.

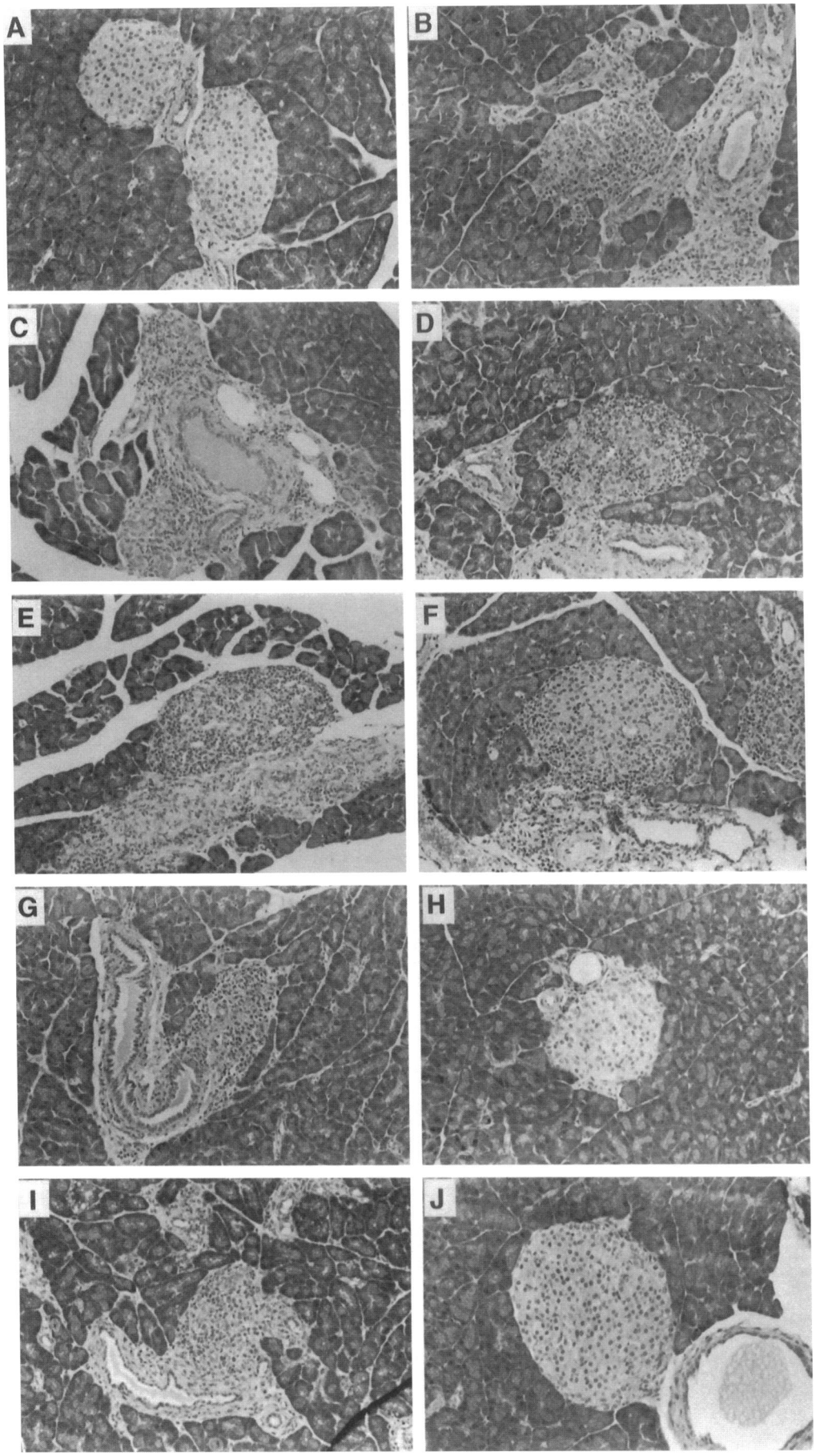
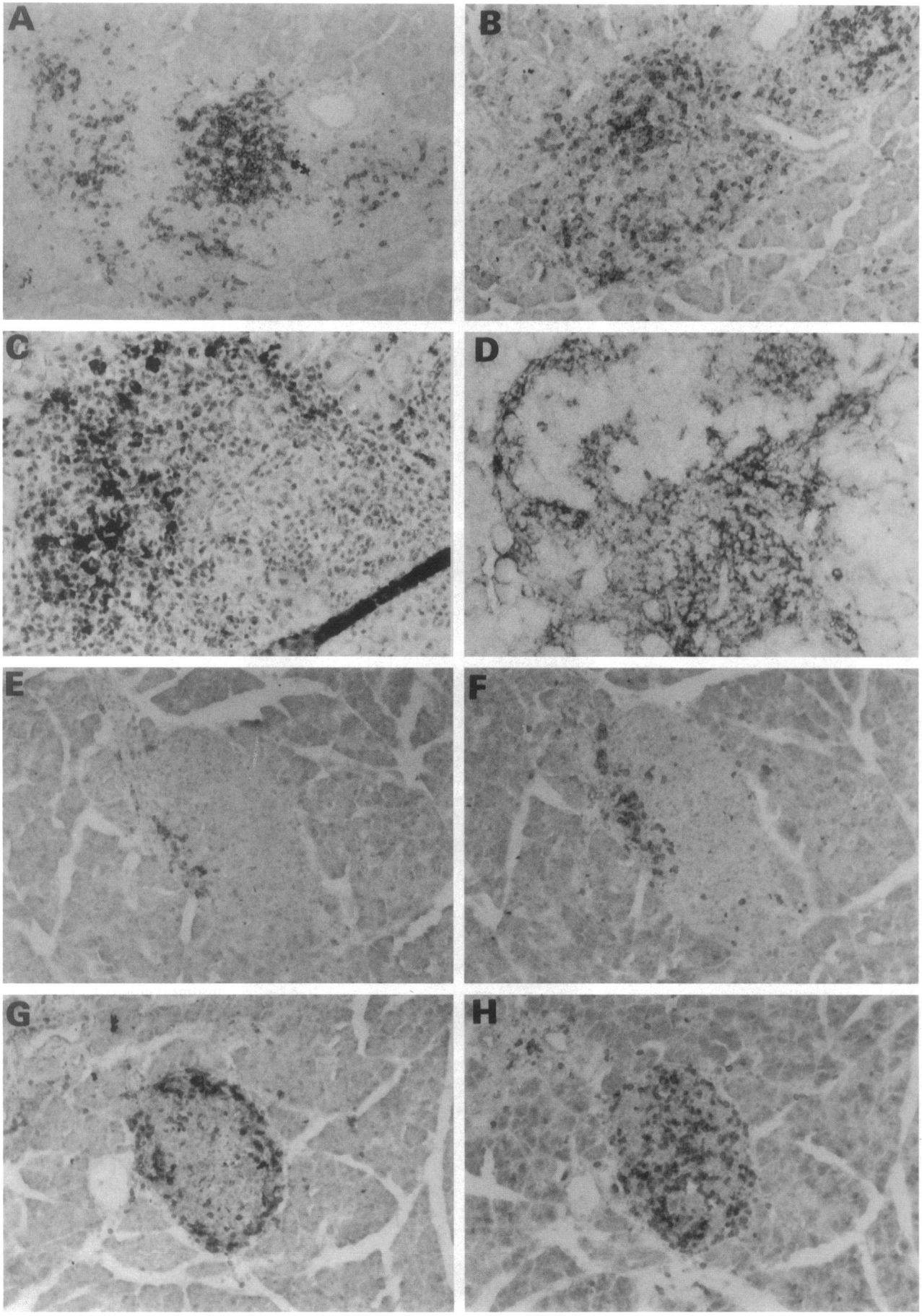


Figure 6. Pancreatic sections stained with hematoxylin-eosin from the animals in the various treatment groups. Each irradiated female NOD mouse received: (*A*) PBS, (*B*) 2×10^7 spleen cells, (*C*) 2×10^7 spleen cells plus anti–CD45 (IgG2a), (*D*) 2×10^7 spleen cells plus anti–ICAM-1 (IgG2a), (*E*) 2×10^7 spleen cells, (*F*) 2×10^7 spleen cells plus anti–VCAM-1, (*G*) 2×10^7 spleen cells plus anti–CD45 ×1 (IgG2b), (*H*) 2×10^7 spleen cells plus anti–α4 integrin ×1 (IgG2b), (*I*) 2×10^7 spleen cells plus anti–CD45 ×4 (IgG2b), (*J*) 2×10^7 spleen cells plus anti–α4-integrin ×4 (IgG2b).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the islets of Langerhans. This is evidenced by the fact that antibodies directed against both α4-integrin on the spleen cells and VCAM-1 on the endothelial cells can delay the onset of diabetes and significantly decrease the incidence of diabetes in adoptive transfer experiments. Furthermore, this blocking of disease is accompanied by a marked decrease in lymphocytic infiltration in the islets of Langerhans. Immunohistochemistry of pancreatic sections from diabetic and nondiabetic animals shows that only the diabetic animals develop infiltration by CD4, CD8, and VLA-4–positive T-cells in the islets, and these islets demonstrate strong induction of VCAM-1 on blood vessels. Furthermore, these antibodies appear to preferentially block entry of CD4 T cells into islets, as islets of Langerhans from animals treated with anti–α4-integrin and anti–VCAM-1 demonstrate the presence of CD8 T cells throughout the islet, while CD4 T cells are confined to the periphery of the islet. Antibody against ICAM-1 has no effect on the transfer of IDDM, in contrast to its potent effect in blocking EAE. The reason for this difference is unknown.

One possible explanation for the effect of anti–α4-integrin antibodies is that they cause CD4 T cells to aggregate by activating α4-integrin binding. We have never observed such effects, and attempts to demonstrate homotypic adhesion induced by anti–α4-integrin antibodies failed. In addition, VCAM-1 has been suggested to be able to co-stimulate T cell growth, based on studies in which either VCAM-1 or anti–α4-integrin co-immobilized in plastic surfaces with anti–CD3 synergizes for T cell activation. However, anti–α4-integrin does not inhibit murine CD4 T cell responses (14) or human CD4 T cell responses to antigen, and α4-integrin–low NOD T cells respond as well to islets as α4-integrin–high T cells. Thus, we believe these alternative explanations for our results are unlikely.

The process of T cell transit across endothelium is believed to involve a cascade of events (29–31). Many molecular pathways have been shown to participate in this process, and these pathways vary dramatically with the activation state of the interacting cells and the differentiation state of the T cell (32). In addition, the various components of the cascade may differ depending on the particular type of endothelium involved. From our studies on lymphocyte entry into the central nervous system (CNS) we believe the process is initiated when activated CNS antigen-specific T-cells bind to endothelium. It has been proposed that this binding is mediated by an LFA-1–ICAM-1 interaction (33, 34), but the initial tethering could involve selectin binding or both selectin and integrin molecules. Once the initial binding to endothelium has taken place, we believe the activated cells upregulate VCAM-1 on the endothelium by virtue of their cytokine production. The activated T lymphocyte must have surface expression of VLA-4 to bind to the newly induced VCAM-1 and enter into the CNS.

The conclusion that was suggested by our earlier studies was that a VLA-4–VCAM-1 interaction is involved in the immune surveillance of tissues by activated T cells, including the CNS. We believe that α4-integrins play a role in the emigration of recently activated lymphocytes from blood into tissue. Once in the tissue, if CD4 T cells encounter specific antigen they are activated to produce cytokines that activate local endothelium to recruit further effector cells. However, it is not yet clear whether the α4-integrin–VCAM-1 interaction is required for the emigration of these first T cells, which we refer to as pioneer cells, from the blood into all tissues, or whether the main role for α4-integrin–VCAM-1 interaction is in the recruitment of further cells, which we refer to as settler cells, by cytokines released by these pioneer lymphocytes.

Our current studies involving T cell entry into the islets of Langerhans support this general hypothesis, but suggest that the cascade of events leading to T cell transit across endothelium may be different for CD4 and CD8 T cells, and may be different depending on the type of endothelium involved. The observation that CD4 T cells are found at the outer edges of the islet in animals treated with antibodies specific for α4-integrin and VCAM-1, while the CD8 cells have extensively invaded the entire islet, suggests that these antibodies preferentially block entry of CD4 T cells into tissues. What molecules regulate the migration of CD8 T cells is not clear. Although these data might be interpreted as suggesting that only CD4 T cells are crucial for β cell destruction, they could equally support the notion that CD8 T cells require signals from proximal CD4 T cells to mediate disease.

The fact that antibodies against ICAM-1 do not appear to have the same dramatic effects in blocking IDDM as they do in blocking EAE supports the idea that the cascade of events leading to T cell transit across endothelium into the islets may be different from the cascade involved in T cell transit across brain endothelium. Alternatively, ICAM-1 may be acting on a population of cells in the brain which is not necessary for onset of diabetes. In addition, an LFA-1–ICAM-1 interaction may be involved in the process of T cell binding to antigen-presenting cells, and thus be involved in T cell activation. It is possible that such an interaction is necessary for antigen presentation and T cell activation in the CNS, but is not necessary in the islets of Langerhans.

Finally, in that we have previously observed spontaneous loss of α4 integrin on encephalitogenic, myelin basic protein–specific T cells in vitro without loss of antigen specificity or lymphokine production (14), and inasmuch as we have also observed modulation of α4-integrin expression in vivo when cloned T cells migrate from perivascular cuffs into brain parenchyma, it is worth noting that expression of α4-integrin on T cells in the islets of Langerhans of diabetic animals appears to be limited to lymphocytes surrounding vessels, and at the outer edges of the islets. These results suggest that the regulated expression of this and other integrins may be important in effector T cell function or in migration from sites of egress from the blood to sites of action within the tissues themselves. The analysis of these and many other issues is crucial to understand how T cells, upon activation in the lymphoid tissues, seek out cells

Figure 7. Frozen sections of the pancreas from animals that received spleen cells with and without antibodies specific for α4-integrin or VCAM-1 are stained for expression of CD4, CD8, α4-integrin, or VCAM-1. Representative sections are from animals treated with: (*A* and *B*) spleen cells alone and stained for CD4 (*A*) and CD8 (*B*); (*C* and *D*) spleen cells alone stained for α4-integrin (*C*) and VCAM-1 (*D*); *C* and *D* are a higher magnification of the same islet seen in *A* and *B*; (*E* and *F*) spleen cells plus anti–α4-integrin given once at the time of injection of cells, stained for CD4 (*E*) and CD8 (*F*); (*G* and *H*) spleen cells plus anti–VCAM-1 given once at the time of injection of cells, stained for CD4 (*G*) and CD8 (*H*).

expressing antigens in peripheral tissues during infection or autoimmune disease.

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