A predominant role of integrin α_4 in the spontaneous development of autoimmune diabetes in nonobese diabetic mice

(lymphocyte/L-selectin/monoclonal antibody)

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ABSTRACT To elucidate the role of cell adhesion molecules in the pathogenesis of insulin-dependent diabetes mellitus and to determine the predominant lymphocytic homing pathway(s) involved in the selective lymphocytic infiltration of pancreatic islets (insulinitis), nonobese diabetic mice were treated with monoclonal antibodies specific for the L-selectin and integrin α_4 lymphocyte adhesion molecules. Treatment of neonatal mice with either anti-L-selectin or anti-integrin α_4 monoclonal antibodies for the first 4 weeks of life led to a significant and long-term protection against spontaneous occurrence of insulinitis and diabetes. The same treatment failed to inhibit lymphocytic infiltration of the salivary glands (sialadenitis). This tissue-specific inhibition of inflammation may be attributed to differences between the pancreas and salivary gland in their expression of endothelial ligands for L-selectin (peripheral vascular addressin) and for integrin α_4 (mucosal addressin cell adhesion molecule 1 and vascular cell adhesion molecule 1). Mucosal addressin cell adhesion molecule 1 is highly expressed by vessels within the inflamed islets but was not detected in the salivary glands. In contrast, peripheral vascular addressin- and vascular cell adhesion molecule 1-expressing vessels can be found in almost every area of inflammation within the salivary glands but are seen in only 40–50% of inflamed islets. Anti-L-selectin and anti-integrin α_4 treatment had no demonstrable effect on anti- β -cell autoimmunity or on the immune responses to foreign antigens. Therapeutic treatment with anti-L-selectin after the onset of insulinitis from 10 to 14 weeks of age delayed the onset but failed to prevent spontaneous insulin-dependent diabetes mellitus, whereas anti-integrin α_4 treatment resulted in a significant and long-lasting suppression of the disease. These data strongly suggest that integrin α_4 plays a prominent role in the spontaneous development of insulinitis and diabetes in nonobese diabetic mice.

Insulin-dependent diabetes mellitus (IDDM) is the result of organ-specific autoimmune destruction of the insulin-secreting β cells in the pancreatic islets of Langerhans. It has become evident that IDDM is a multifactorial disease mediated by T cells in which both CD4⁺ and CD8⁺ T cells are required for β -cell destruction (1). A great deal of effort has focused on the determination of genetic and environmental factors that trigger the diabetogenic response and the precise role autoreactive T cells play in mediating the diabetogenic process. An issue that has only very recently been addressed is the mechanism by which autoreactive and effector lymphocytes migrate to and enter target organs.

Adherence of lymphocytes and other inflammatory cells to vascular endothelium, followed by transendothelial migration into the target organ, is essential for eventual tissue

damage and destruction in organ-specific autoimmune disease. For IDDM, lymphocytic infiltration of the islets (insulinitis) is a prerequisite for the development of diabetes. Thus, a better understanding of lymphocyte trafficking to the islets may be helpful in gaining insight into the events linked to disease pathogenesis and, more importantly, to develop an anti-cell adhesion molecule (CAM)-based therapy for treatment of IDDM in patients.

We have demonstrated (2) that blocking L-selectin or integrin α_4 lymphocyte adhesion molecules in nonobese diabetic (NOD) mice leads to partial or complete protection, respectively, against the development of diabetes in an adoptive transfer model. In contrast, anti-integrin β_2 treatment does not prevent the onset of disease (3). This suggests that L-selectin and integrin α_4 may be involved in lymphocyte homing to the islets. This notion has been recently confirmed by other investigators (4, 5). Nevertheless, with regard to the spontaneous development of IDDM, it is critical to determine (i) which adhesion pathway(s) is essential for spontaneous disease progression, (ii) whether blockade of L-selectin or integrin α_4 is sufficient to stop the disease after its onset, and (iii) the duration and the time at which the treatment should be applied to achieve a significant therapeutic effect. In the present study, we have addressed these issues by administering anti-L-selectin and anti-integrin α_4 monoclonal antibodies (mAbs) at early or late stages of the spontaneous disease process and by determining the expression of the endothelial CAMs in the islets and other lymphoid and extralymphoid organs.

MATERIALS AND METHODS

Mice. NOD/McD mice were bred and maintained at the Stanford University Animal Facility under barrier isolation conditions. In our NOD colony, the frequency of overt diabetes reaches 70–80% in females and 10–20% in males by the age of 30 weeks.

Treatment Regimen with Anti-Lymphocyte Adhesion Molecule mAbs. Hybridomas that produce mAbs utilized in this study were purchased from American Type Culture Collection. mAbs Mel-14 (anti-L-selectin), R1-2 (anti-integrin α_4), and immunoglobulin-isotype-matched controls M1/9.3.17 and M1/89.18.7 (anti-leukocyte common antigen) were prepared using protein G affinity chromatography (Pharmacia) as described (2). NOD mice from separate litters were randomized before treatment. Two regimens were applied: (i)

Abbreviations: IDDM, insulin-dependent diabetes mellitus; NOD, nonobese diabetic; mAb, monoclonal antibody; PNAd, peripheral vascular addressin; CAM, cell adhesion molecule; MAdCAM-1, mucosal addressin CAM 1; VCAM-1, vascular CAM 1; OVA, ovalbumin; CPH, carboxypeptidase H; GAD65, 65-kDa isoform of glutamic acid decarboxylase; HSP60, 60-kDa heat shock protein; LN, lymph node; ICAM-1, intercellular CAM 1.

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Newborn mice were treated i.p. with 30 μ l of PBS only or with Mel-14, R1-2, and control mAbs (M1/9.3.17 and M1/89.18.7) [each at 80 μ g/g (body weight)] every other day from the first day after birth for 4 weeks. (ii) Ten-week-old female mice were injected i.p. with Mel-14, R1-2, or control mAbs (each at 1 mg) every other day for 4 weeks.

Histopathology. Histopathology for examination and evaluation of insulinitis and sialadenitis was performed as described (6).

ELISA for Determination of Anti- β -Cell Autoantibody. An antigen-specific ELISA for determining autoantibody response to a panel of murine β -islet-cell antigens including carboxypeptidase H (CPH), the 65-kDa isoform of glutamic acid decarboxylase (GAD65), 60-kDa murine heat shock protein (HSP60), and peripherin was carried out as described (7).

Determination of the Immune Response to Ovalbumin (OVA). Immune response to OVA was examined as described (6).

Immunohistology. Pancreata, salivary glands, spleens, peripheral and mesenteric lymph nodes (LNs), and Peyer's patches were frozen, cut, and stained with a panel of mAbs using a three-step immunoperoxidase technique as described (8). mAbs included Mel-14, R1-2, MECA-79 [anti-peripheral vascular addressin (PNAd)], MECA-367 [anti-mucosal addressin CAM 1 (MAdCAM-1)], MK-2.7 [anti-vascular CAM 1 (VCAM-1)], 53-2.1 (anti-Thy1.2), 53-6.7 (anti-CD8), GK1.5 (anti-CD4), and RA3-6B2 (anti-CD45R/B220). MECA-79 and MECA-367 were generously provided by E. C. Butcher (Stanford University). MK2.7 and RA3-6B2 were kindly provided by P. Kincade (University of Oklahoma) and by R. Coffman (DNAX), respectively.

RESULTS

Treatment of Neonatal NOD Mice with Mel-14 or R1-2 Prevents Development of Spontaneous IDDM. Insulinitis can be seen histologically in the pancreata of most NOD mice by 4–5 weeks of age (9), suggesting that the initial few weeks of life are critical for the development of lymphocytic infiltration of the islets. This initial inflammation in turn triggers a subsequent cascade of immunopathologic events leading to β -cell destruction. To verify this notion, newborn NOD mice were treated i.p. with PBS, Mel-14, R1-2, M1/9.3.17, and M1/89.18.7 from the first day after birth every other day for 4 weeks. The mice were then followed for the spontaneous onset of diabetes. Fig. 1 illustrates that all female mice treated with R1-2 remained completely free of diabetes. In addition anti-L-selectin treatment led to a significant and long-lasting protection from IDDM (30 vs. 85% diabetes in control group at 42 weeks of age). In contrast, mice treated with control mAbs developed IDDM with an incidence similar to that seen for PBS-treated animals, suggesting that the protective effect observed with the Mel-14 and R1-2 mAbs is specific. To determine whether administering Mel-14 and R1-2 mAbs results in the depletion of L-selectin- or integrin α_4 -expressing cells, respectively, mononuclear cells were analyzed by immunofluorescence staining and flow cytometry and by immunohistological examination of frozen sections of spleen, LNs, and Peyer's patches. No significant deletion was observed in animals treated with Mel-14, R1-2, or control mAbs (data not shown).

Anti-L-Selectin- or Anti-Integrin α_4 -Mediated Protection May Result from Selective Inhibition of Lymphocytic Infiltration into the Pancreatic Islets. To determine whether anti-L-selectin and anti-integrin α_4 treatment is indeed inhibiting lymphocyte accumulation in the islets, we examined histologic sections of pancreata from 42-week-old NOD mice that had been treated with mAbs from birth to 4 weeks of age. As demonstrated in Table 1, insulinitis was seen in all PBS-treated

FIG. 1. Prevention of spontaneous development of IDDM in NOD mice by anti-L-selectin or anti-integrin α_4 treatment. Newborn female NOD mice were treated i.p. with PBS ($\circ$, $n = 10$), Mel-14 ($\blacktriangle$, $n = 12$), R1-2 ($\blacksquare$, $n = 16$), M1/9.3.4 ($\triangle$, $n = 5$), or M1/89.18.7 ($\square$, $n = 5$) for 4 weeks. Diabetes was determined by measuring glycosuria biweekly and presented as diabetes incidence: number of diabetic mice/total number of mice within the same treatment group.

mice, but in only 60% of Mel-14-treated and 57% of R1-2-treated mice. Furthermore, in animals with insulinitis, the extent of inflammation in the islets of Mel-14- and R1-2-treated mice was markedly less than that seen in PBS-treated mice. Interestingly, the incidence of sialadenitis did not appear to be significantly affected by Mel-14 or R1-2 treatment in the same mice (Table 1), suggesting that these antibodies affect lymphocyte homing to sites of inflammation in a tissue-selective manner.

To further study the tissue-specific effect of anti-L-selectin and anti-integrin α_4 treatment, we examined the expression of various endothelial adhesion molecules in pancreata, submandibular salivary glands, and other lymphoid tissues. These molecules include PNAd (a ligand for L-selectin) (10, 11), MAdCAM-1 (a ligand for integrin $\alpha_4\beta_7$) (12, 13), and VCAM-1 (a ligand for integrin $\alpha_4\beta_1$) (14). As illustrated in Fig. 2, MAdCAM-1 expression was found on vascular endothelium within or adjacent to every infiltrated islet (100%). In contrast, no MAdCAM-1 expression was seen in the inflamed salivary glands (0%). PNAd and VCAM-1 were expressed by vascular endothelium in areas of inflammation in both pancreas and salivary gland. In the salivary gland, almost every area of inflammation contained several vessels expressing these two adhesion molecules ($\approx 100\%$), whereas only 40–50% of inflamed islets contained PNAd- or VCAM-1-expressing vessels. No observable difference in the expression of MAdCAM-1, PNAd, and VCAM-1 by vascular endothelium in LNs and Peyer's patches was detected in PBS-, Mel-14-, or R1-2-treated mice (data not shown). Therefore, our data suggest that autoreactive lymphocytes may primarily utilize an $\alpha_4\beta_7$ -MAdCAM-1 adhesion pathway to enter the islets whereas sialadenitis is mediated by specific interactions of $\alpha_4\beta_1$ -VCAM-1 and/or L-selectin-PNAd and that the tissue specificity of the homing process may be influenced by distinct patterns of expression of endothelial adhesion molecules in different organs.

Baron *et al.* (4) have shown that integrin α_4 preferentially mediates homing of CD4⁺ effector cells to the islets, whereas anti-integrin α_4 treatment has no effect on the entry of CD8⁺ T cells. To further address this issue, we analyzed the proportions of infiltrating CD4⁺ and CD8⁺ T cells and B cells in the islets and salivary glands. In mice that were treated with Mel-14 or R1-2 and displayed insulinitis and sialadenitis, no significant skewing toward a particular subset of lymphocytes was found *in situ* (data not shown).

Table 1. Treatment with Mel-14 (anti-L-selectin) or R1-2 (anti-integrin α_4) inhibits development of insulinitis but not sialadenitis

Treatment	Insulinitis incidence, no. of mice afflicted/total no. of mice (%)	Insulinitis severity, no. of islets afflicted/total no. of islets (%)				Sialadenitis severity, no. of mice afflicted/total no. of mice				
		-	+	++	+++	-	+	++	+++	+++
PBS	3/3 (100)	26/57 (45.6)	6/57 (10)	7/57 (12.3)	18/57 (31.6)	0/3	0/3	3/3	0/3	0/3
Mel-14	3/5 (60)	112/139 (80.8)	7/139 (5.0)	10/139 (7.1)	10/139 (7.1)	0/5	4/5	1/5	0/5	0/5
R1-2	4/7 (57)	169/184 (92.0)	11/184 (5.9)	4/184 (2.1)	0/184 (0)	0/8	4/8	4/8	0/8	0/8

Newborn NOD mice were treated i.p. with PBS, Mel-14, or R1-2 for 4 weeks. The diabetes-protected animals were sacrificed at 42 weeks of age and the histopathology of the islets and salivary glands was analyzed. Insulinitis was scored by incidence and severity. A minimum of 13 islets per individual was examined. Insulinitis was graded as described (2): -, no lymphocytic infiltration; +, few lymphocytes surrounding the islets; ++, many surrounding lymphocytes; +++, intraislet lymphocyte infiltration and accumulation. Severity of sialadenitis was scored as follows: -, no inflammation; +, small foci of perivascular inflammation; ++, small foci of periductular/periacinar inflammation; +++, large foci periductular/periacinar inflammation.

Treatment with Anti-L-Selectin or Anti-Integrin α_4 mAbs Does Not Block the Immune Responses to Self or Foreign Antigens. One of the major concerns of using anti-CAM mAb as a therapy is the depletion of immune competent cells and the induction of immune suppression leading to potential infections and tumor development. To address this issue, we carried out two sets of experiments. In the first, 15-week-old mice that had been treated with PBS, Mel-14, or R1-2 from birth for 4 weeks were examined for both *in vivo* autoantibody responses and *in vitro* T-cell proliferative responses to a panel of β -islet-cell antigens. We have demonstrated (7) that NOD mice develop spontaneous and age-dependent autoimmune responses to the panel of β -islet-cell antigens and that the response to GAD65 appears to be correlated with the onset of insulinitis. The data in Fig. 3 indicate that the autoantibodies specific for CPH, GAD65, HSP60, and peripherin were detected in all PBS-, Mel-14-, and R1-2-treated mice and the extent of the responses was comparable among the treatment groups. In parallel to the antibody response, the *in vitro* T-cell proliferative responses to these antigens were also unaffected by Mel-14 or R1-2 treatment in the first 4 weeks of life (data not shown).

Secondly, to assess the immune responses to foreign antigens, adult NOD mice that had been treated i.p. with PBS or 1 mg of Mel-14 or R1-2 every other day from 10 to 14 weeks of age were immunized with chicken OVA. Ten days later, draining LNs and spleens were removed and lymphocytes

were enumerated and assayed *in vitro* for proliferative responses to OVA. Relative to PBS-treated animals, the numbers of lymphocytes found in the draining LNs of Mel-14- or R1-2-treated mice were markedly reduced by 87 and 72%, respectively (data not shown). This suggests that both L-selectin and integrin α_4 may be involved in the process of lymphocyte accumulation in local peripheral LNs *in vivo* during an immune response. To further study whether these mice were immunocompetent, lymphocytes from draining LNs and spleens were tested for their ability to respond to OVA. Fig. 4 illustrates that T cells from PBS-, Mel-14-, or R1-2-treated mice responded equally well to OVA, indicating that both local and systemic immune responses to a foreign antigen remain unaffected by anti-L-selectin or anti-integrin α_4 treatment. The fact that priming of an immune response was not altered by the blockade of lymphocyte trafficking and that a proliferative response could be detected in the splenic cell cultures suggests that naive T cells may become sensitized in other lymphoid tissues rather than in the draining LNs in Mel-14- or R1-2-treated mice.

As an additional test to determine whether anti-L-selectin or anti-integrin α_4 treatment can induce a general immunosuppressive effect, the ability of mice pretreated with Mel-14, R1-2, or PBS to mount an anti-viral response was assessed. Mice were inoculated i.p. with a sublethal dose of murine cytomegalovirus (3×10^6 plaque-forming units) (15). All of the PBS-, anti-L-selectin-, or anti-integrin α_4 -treated animals were resistant to cytomegalovirus-induced death, and the viral titers in the pancreata, salivary glands, and other organs were comparable (data not shown), suggesting that the ability

FIG. 2. Expression of endothelial adhesion molecules in inflamed pancreatic islets and salivary glands. Frozen sections of pancreata and submandibular salivary glands from PBS-treated mice at 42 weeks of age were stained with mAb against PNAd (MECA-79), MAdCAM-1 (MECA-367), and VCAM-1 (MK2.7). Each focus of lymphocytic inflammation was evaluated microscopically for presence of vessels expressing these endothelial adhesion molecules ($n = 6$ mice; data are the mean $\pm$ SD).

FIG. 3. Autoantibody responses to a panel of pancreatic β -cell antigens. Sera from mice treated with PBS, Mel-14, or R1-2 were examined for autoantibody responses to a panel of β -cell antigens including CPH, GAD65, HSP60, and peripherin by using an antigen-specific ELISA. Data are presented as the mean $\pm$ SD of the OD₄₀₅ values in the ELISA.

FIG. 4. Anti-L-selectin or anti-integrin α_4 treatment failed to inhibit the immune response to OVA. Mice treated with PBS, Mel-14, or R1-2 were immunized with OVA. Ten days after immunization, the mononuclear cells (MNC) from spleens or from the draining LNs were tested for their ability to respond to OVA *in vitro*. The proliferative response was assayed by measuring the [3 H]thymidine uptake (cpm) and is presented as stimulation index (mean cpm in the presence of antigen/mean cpm with medium only).

to clear the virus was preserved in the Mel-14- and R1-2-treated mice.

Therapeutic Potential for Treatment of Ongoing Anti- β -Cell Autoimmunity in NOD Mice. To assess the potential application of anti-L-selectin or anti-integrin α_4 treatment to an ongoing diabetogenic response, NOD mice at 10 weeks of age, which exhibit extensive insulinitis, were treated i.p. with Mel-14, R1-2, or control antibodies for 4 weeks. As illustrated in Fig. 5, Mel-14 treatment delayed the onset of disease marginally and failed to protect mice from spontaneous diabetes. In contrast, anti-integrin α_4 treatment significantly reduced the incidence of overt diabetes (30 vs. 84% of control mice at 42 weeks of age), further suggesting that integrin α_4 mediated lymphocyte adhesion plays an important role during the spontaneous development of IDDM at early and late stages of the disease process.

DISCUSSION

In the present study, we have attempted to determine which lymphocyte homing pathway(s) is essential for autoreactive and diabetogenic lymphocytes to migrate to the pancreatic islets. Two approaches were taken. The first was to

FIG. 5. Therapeutic treatment with Mel-14 or R1-2. Female NOD mice at age of 10 weeks were treated i.p. with PBS (○, $n = 7$), Mel-14 (▲, $n = 6$), R1-2 (■, $n = 6$), M1/9.3.4 (△, $n = 5$), or M1/89.18.7 (□, $n = 5$) every other day for 4 weeks. Mice were monitored weekly for the onset of diabetes.

treat mice with mAbs specific for adhesion molecules expressed on lymphocytes and to determine their effect on the disease process. Depending on the age of the animal or the extent of disease progression, both anti-L-selectin and anti-integrin α_4 can partially or completely prevent and treat spontaneous disease. This is consistent with our previous finding using an adoptive transfer model of diabetes (2). In both cases, anti-integrin α_4 treatment consistently provided a more efficient protective effect than did blocking the L-selectin receptor. Similar data has been shown in the model of experimental allergic encephalomyelitis (16). The fact that neonatal treatment with Mel-14 was able to prevent diabetes while treatment during 10–14 weeks of age only delayed the onset could be due to decreased levels of expression of L-selectin on memory and/or activated autoreactive T cells in adult animals. Unlike the selectin family, which initiates the adhesion cascade and is functionally overlapping and redundant, integrins are generally thought to be involved in the later steps of the adhesion process and are responsible for mediating strong adhesion to vascular endothelium and extracellular matrix components such as fibronectin (17, 18). Therefore, integrin α_4 mediated adhesion may be essential for both naive and memory/activated T cells to enter the islets. Alternatively, the differential effect between Mel-14 or R1-2 may also be due to the different affinities of the antibody and/or the epitopes they recognize. In the previous study (3), we have shown that anti-integrin β_2 treatment failed to prevent IDDM. This has been confirmed and extended by Baron *et al.* (4) who demonstrated that blocking the ligand of integrin β_2 , intercellular cell adhesion molecule 1 (ICAM-1), had no protective effect in an adoptive transfer model of diabetes. Thus, these data suggest that during the late effector stages of the diabetogenic process, the integrin β_2 pathway may not have as significant a role as does the integrin α_4 pathway.

Although the signaling functions of selectins and integrins are not fully understood, it has been shown that integrin β_2 and ICAM-1 can function as accessory molecules to induce signals leading to T-cell activation *in vitro* (19) and that interruption with this interaction induces tolerance *in vivo* (20). However, it is still unknown whether Mel-14 or R1-2 transduces a signal resulting in a functional change in autoreactive lymphocytes. What is clear is that both Mel-14 and R1-2 are able to block neutrophil and lymphocyte migration *in vivo* without depleting L-selectin- and integrin α_4 expressing cells (21, 22) and that Mel-14 or R1-2 treatment fails to induce immune tolerance to self islet antigens, to mediate immunosuppression to foreign antigens, and to interrupt T-cell responses *in vitro* (2, 22). Moreover, the fact that sialadenitis continues to develop in Mel-14- or R1-2-treated NOD mice provides further evidence that administering these mAbs does not jeopardize immune or other inflammatory responses. Thus, these data indicate that the effect of anti-L-selectin or anti-integrin α_4 treatment is not achieved by interfering with the ability of immune competent cells to respond to antigen. This in turn suggests that anti-L-selectin or particularly anti-integrin α_4 treatment may be a preferential choice for the development of an anti-CAM-based immunotherapy.

The second approach was to analyze the expression of the endothelial ligands for the various lymphocyte adhesion molecules in the islets, salivary glands, and other tissues to gain insight into the mechanism of tissue-specific lymphocyte homing in autoimmunity. It is of particular interest that many MAdCAM-1-expressing vessels are seen in the pancreas; these vessels are prominently associated with inflamed islets but are also scattered throughout the exocrine pancreas. In marked contrast, there is no MAdCAM-1 expression in NOD salivary glands even on the vessels surrounding infiltrated areas. Furthermore, PNA- and VCAM-1-expressing vessels

are detected in only some (40–50%) inflamed islets but in essentially all inflamed areas in the NOD salivary glands (Fig. 2). Consistent with our finding, Hanninen *et al.* (8) and Faveeuw *et al.* (23) have recently demonstrated that (i) MAdCAM-1 is primarily expressed on endothelium in the islets while VCAM-1 is constitutively expressed on dendritic cells found in inflamed islets and on vessels in the exocrine pancreas of NOD and control nonautoimmune-prone mice. Insulinitis is associated with a marked increase in islet-associated vascular expression of MAdCAM-1 but not VCAM-1 (8). In addition, ICAM-1 expression was detected on vessels found in the exocrine pancreas and on the endothelial and dendritic cells within inflamed islets (23). (ii) The islet-infiltrating lymphocytes predominantly expressed integrin $\alpha_4\beta_7$ as early as 4 weeks of age when insulinitis is first detectable, whereas L-selectin was only expressed at very low levels up until 12 weeks of age (8). The majority of the infiltrating T cells found in inflamed islets and salivary glands have the memory/activated phenotype: L-selectin^{low} and CD44(Pgp-1)^{high} (23). (iii) *In vitro* blockade of L-selectin or integrin $\alpha_4\beta_7$, or its ligand MAdCAM-1 inhibits lymphocyte attachment to the inflamed vessels in NOD islets (8). Furthermore, anti-ICAM-1 and anti-integrin β_2 treatment were unable to block the transfer of diabetes in recipient mice (3, 4). Thus, these data suggest that although multiple families of adhesion molecules are involved in lymphocyte trafficking to extranodal inflammatory sites in the NOD mouse, the integrin $\alpha_4\beta_7$ –MAdCAM-1 interaction may play a predominant role in mediating tissue-specific migration of diabetogenic cells into pancreatic islets. In contrast, interactions between L-selectin and PNA^d and between $\alpha_4\beta_1$ and VCAM-1 may be primarily involved in lymphocyte trafficking to the salivary glands that causes sialadenitis. If this is correct, in addition to the antigen-specific T-cell receptor, organ-specific T cells may also express defined homing receptors facilitating selective entry into their target tissues.

The long-term protective effect mediated by neonatal treatment may be due to the fact that the first few weeks after birth is an essential period for lymphocyte trafficking and migration to the islets (6, 9). Therefore, inhibiting the adhesion process during this time may prevent autoreactive lymphocytes entering the islets and thus prevent subsequent events leading to eventual β -islet-cell destruction. This is supported by the finding that treatment with Mel-14 and R1-2, in addition to preventing overt diabetes, markedly reduced the incidence and severity of insulinitis (Table 1). An alternative explanation is that Mel-14 or R1-2 may influence lymphocyte development particularly in early life since both L-selectin and integrin α_4 have been implicated in thymic development (24). In light of our finding (6) that neutralization of tumor necrosis factor α activity within the first 3 weeks of life leads to complete and long-term prevention of spontaneous IDDM in NOD mice (6), interrupting an inflammatory response at an early phase either by blocking proinflammatory cytokines or by inhibiting inflammatory cell infiltration into the target organs may prove to be an effective means to prevent autoimmune and inflammatory diseases. Furthermore, the fact that blockade of integrin α_4 was effective in treating an ongoing diabetogenic process suggests that the progression of autoimmune and inflammatory destruction of the β islet cells may require a continuous recruitment of lymphocytes and/or

inflammatory cells from the circulation. If this is indeed the case, an anti-CAM approach may have great potential to treat the ongoing autoimmune or inflammatory diseases in patients (25).

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