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L-Selectin Is Not Required for T Cell-Mediated Autoimmune Diabetes

Randall H. Friedline,* Carmen P. Wong,† Douglas A. Steeber,† Thomas F. Tedder,† and Roland Tisch2*

Administration of anti-L-selectin (CD62L) mAb to neonatal nonobese diabetic (NOD) mice mediates long term protection against the development of insulitis and overt diabetes. These results suggested that CD62L has a key role in the general function of β cell-specific T cells. To further examine the role of CD62L in the development of type 1 diabetes, NOD mice lacking CD62L were established. The onset and frequency of overt diabetes were equivalent among CD62L+/-, CD62L-/-, and CD62L-/- NOD littermates. Furthermore, patterns of T cell activation, migration, and β cell-specific reactivity were similar in NOD mice of all three genotypes. Adoptive transfer experiments with CD62L-/- CD4+ T cells prepared from BDC2.5 TCR transgenic mice revealed no apparent defects in migration to pancreatic lymph nodes, proliferation in response to β cell Ag, or induction of diabetes in NOD.scid recipients. In conclusion, CD62L expression is not essential for the development of type 1 diabetes in NOD mice.


Type 1 diabetes (T1D) is characterized by the T cell-mediated destruction of the insulin-producing β cells of the pancreas. In nonobese diabetic (NOD) mice, a model of spontaneous autoimmune diabetes, disease development proceeds in a regulated and progressive manner (1–6). Initial islet infiltration (insulitis) involves the recruitment of APCs including macrophage and dendritic cells (7). These APCs are believed to take up β cell Ags, traffic to the pancreatic lymph nodes, and present autoantigenic peptides to T cells (2, 8). Activation of islet-reactive T cells results in their homing to the pancreas and the initiation of β cell destruction. Infiltration of the pancreas is first detectable at 3–4 wk of age and progresses over several weeks. When ∼90% of β cells have been destroyed, normoglycemic regulation is lost, resulting in overt diabetes.

Diabetogenesis depends on the effective recruitment of lymphocytes into pancreatic tissue. In general, lymphocyte trafficking involves a cascade of adhesive contacts mediated by membrane-bound adhesion molecules and soluble chemotactic factors that induce lymphocyte rolling along the endothelium, firm adhesion to the endothelial wall, and extravasation into tissues (9). The selectin family, consisting of L-, E-, and P-selectin, induces rolling along high endothelial venules of secondary lymphoid organs and inflamed tissue, thus allowing critical secondary interactions mediated by chemokine signaling and integrins to stop cell rolling and promote diapedesis (10, 11). The disruption of L-selectin (CD62L), in particular, has profound effects in vivo, including the severe reduction of lymphocyte migration to peripheral lymph nodes, impaired recruitment of lymphocytes to sites of inflammation, and delayed primary T cell responses (12–15).

Several reports support a critical role for CD62L in organ-specific autoimmune diseases. For example, treatment of NOD mice with mAb against CD62L, or one of its ligands, peripheral lymph node addressin (PNAd), blocks the development of Sjögren’s syndrome by inhibiting infiltration of the lacrimal and salivary glands (16). Additionally, in a transgenic model of experimental autoimmune encephalomyelitis (EAE), mice deficient in CD62L (CD62L−/−) are refractory to demyelination and protected from EAE (17).

During the establishment and progression of T1D, PNAd and mucosal addressin cell adhesion molecule-1 (MadCAM-1) expression are up-regulated in the pancreas. Because both can serve as ligands for CD62L, it suggested that CD62L-mediated interactions may be involved in lymphocyte trafficking to the pancreas (18–20). Indeed, we and others have shown previously that administration of anti-CD62L mAb to NOD mice can protect against the development of insulitis and diabetes (21, 22). This protection was highly effective when administered to neonatal NOD mice but less so in adults, suggesting that CD62L interactions are important during the establishment of insulitis and β cell-specific T cell responses.

In this study, we established NOD mice deficient in CD62L expression to further examine the importance of this selectin in β cell-specific T cell reactivity and the development of T1D. Surprisingly, we found that onset and frequency of overt diabetes were equivalent among CD62L+/-, CD62L−/−, and CD62L−/- NOD littermates. Furthermore, T cell activation, migration, and β cell-specific reactivity were similar among all three genotypes. Finally, the ability of T cells from BDC2.5-transgenic (Tg) TCR mice to migrate into pancreatic lymph nodes, proliferate in response to β cell Ags, and initiate diabetes did not require CD62L expression.
Materials and Methods

Mice

All mice described herein were bred and maintained in a specific pathogen-free animal facility at the University of North Carolina (Chapel Hill, NC). NOD/LtJ and NOD.scid mice were originally obtained from The Jackson Laboratory (Bar Harbor, ME). In our NOD colony, onset of diabetes begins at ~12 wk of age, reaching an incidence of 80% in females and 20% in males by 30 wk of age. C57BL/6 mice bearing a disrupted CD2L gene (fifth generation backcross) were backcrossed for 10 generations onto the NOD genetic background. Backcrossing was facilitated by the MapPai microsatellite screening system (Research Diagnostics, Flanders, NJ) to select for pups with known NOD intervals containing idd loci (23). All CD2L−/− NOD mice used in these experiments were at the tenth backcross. At the tenth backcross, a careful analysis of chromosome 1 was performed to determine the region of C57BL/6 genetic material still remaining. C57BL/6 genetic material is contained within the markers D1 Mit36 and D1 Mit159, a 10.7-cM region containing the CD62L locus. This region is ~40 cM distant from the idd5.2 susceptibility locus (24). TCR Tg BDC2.5 NOD mice, originally obtained from Drs. C. Benoist and D. Matthis (Harvard University, MA), were bred in our colony and represent the 20th NOD backcross generation. Approximately 25% of BDC2.5 Tg mice develop overt diabetes by 25 wk of age in our colony. BDC2.5 Tg mice deficient in CD2L were generated by breeding CD2L−/− NOD mice (ninth generation backcross) with CD2L−/− NOD mice homozygous for the BDC2.5 clonotypic TCR.

Diabetes and insulinis assessment

Diabetes was determined by measuring urine glucose levels with Diastix strips (Bayer, Elkhart, IN). Insulitis was assessed by histology. Pancreases were fixed in 10% neutral buffered formalin, paraffin embedded, sectioned, and stained with H&E. Five sections 90 μm apart were cut from each block, and slides were viewed by light microscopy. Insulitis severity was scored as either peri-insulitis (a few lymphocytes surrounding the islets) or intrainsulitis (lymphocytic infiltration of the islets).

Antigens

The cloning and preparation of the murine β cell autoantigens glutamic acid decarboxylase (GAD65) and heat shock protein 60 (HSP60) have been previously described (25). Briefly, the cDNAs were engineered to encode six histidine residues at the C terminus of each protein. Recombinant GAD65 was expressed in a baculovirus expression system and purified using a Ni2+/agarose column. Recombinant GAD65 was expressed in E. coli (QIAGEN, Chatsworth, CA). HSP60 was produced in an Escherichia coli expression system and similarly purified. Each recombinant protein was further purified by preparative SDS-PAGE, electroeluted, and dialyzed extensively against PBS.

T cell proliferation and measurement of IFN-γ secretion

Assays to determine T cell proliferation and IFN-γ secretion in response to a panel of β cell autoantigens have been described previously (26). Briefly, splenocytes were isolated with Becton-Dickinson lysis buffer (10 μM EDTA, 1.0 mM KHCO3, 0.1 mM EDTA, pH 7.3) for 1 min, washed and resuspended in RPMI 1640 supplemented with 2% Nutridoma-SP (Boehringer Mannheim, Indianapolis, IN), 0.1 mM nonessential amino acids, 1.0 mM L-glutamine, 1.0 mM sodium pyruvate, 5 × 10−3 M 2-ME, and 100 U/ml penicillin-streptomycin. Cells were plated in a flat-bottom 96-well plate at 5 × 103 cells/well in triplicate for each Ag tested (separate 96-well plates were prepared for measuring proliferation and IFN-γ secretion). Ag was added at a final concentration of 10 μg/ml, and cultures were incubated for 3 days in a humidified 37°C incubator. For the measurement of T cell proliferation, cultures were pulsed with [3H]thymidine (1 μCi/well) during the last 18 h of incubation and harvested. Proliferation is expressed as a stimulation index (mean cpm of response to Ag divided by the mean cpm with media only). For the measurement of IFN-γ secretion, a capture ELISA was performed on 0.1 ml of supernatant from 72-h cultures using purified anti-IFN-γ (1’), biotinylated anti-IFN-γ (2’), and streptavidin-alkaline phosphatase (all from BD Pharmingen, San Diego, CA) and p-nitrophenyl phosphate (Life Technologies, Gaithersburg, MD), as the substrate. A standard curve was used to determine IFN-γ concentrations using linear regression analysis.

CFSE labeling and adoptive transfer of BDC2.5 Tg splenocytes

Donor splenocyte cell suspensions were prepared, and BCCs were lysed as described above. For diabetes induction experiments, splenocytes were negatively selected via the MACS system to enrich for CD4+ T cells using anti-CD8, -B220, -CD19, and -class II mAb (Miltenyi Biotec, Auburn, CA). CD4+ cells were enriched to ~60% according to flow cytometric analysis, the remaining cells being predominantly macrophages (not shown). Cells were adjusted to 6 × 105 cells/ml in PBS and injected i.v. (0.2 ml/mouse). Mice were monitored daily for diabetes. For CFSE labeling, freshly isolated splenocytes were resuspended in PBS and mixed with an equal volume of 2× CFSE (Molecular Probes, Eugene, OR) diluted in PBS. Cells were incubated in the dark at room temperature for 10 min and washed once with PBS and twice with PBS. Cells were adjusted to 7.5 × 106 cells/ml in PBS and injected i.v. (0.2 ml/mouse). Mice were sacrificed at the indicated time points and assessed for T cell activation and migration as described below.

Flow cytometric analysis of T cell migration and activation

Spleen, pancreatic lymph nodes, inguinal lymph nodes, and mesenteric lymph nodes were harvested from recipient NOD mice. Cell suspensions were prepared in FACS buffer (1× HBSS supplemented with 1.5% FBS, Ca2+ and Mg2+, 0.05% NaN3) and the total number of cells counted. The following conjugated Abs were purchased from PharMingen and used to identify T cell populations and assess activation: CD3-FITC, CD4-FITC, CD4-PerCP, CD8-PE, CD25-biotin, CD62L-biotin, Vβ4-PE (BDC2.5). After staining, cells were fixed in 2% paraformaldehyde and analyzed within 24 h on a FACScan (BD Biosciences, Franklin Lakes, NJ) with Summit software (Cytomation, Ft. Collins, CO) used for both acquisition and analysis.

Pancreatic islet isolation

Pancreatic islets were isolated as previously described (27). Briefly, pancreases from groups of two mice were perfused with 1.75 mg/ml collagenase P (Roche, Indianapolis, IN) and digested for 20 min at 37°C. Islets were purified from digested tissue using a Ficoll gradient and then hand picked. Purified islets were dissociated into a single-cell suspension using enzyme-free cell dissociation solution (Sigma, St. Louis, MO), washed, and analyzed via flow cytometry.

Results

CD2L−/− NOD mice do not exhibit defects in T cell migration to pancreatic lymph nodes

As first reported (15), the absence of CD2L defects in long term impaired migration of lymphocytes to peripheral lymph nodes. Gross analysis of CD2L−/− female NOD mice revealed substantially smaller peripheral lymph nodes than those of control mice and enlarged spleens as described previously (15, 28). Pancreatic lymph nodes, however, appeared normal in that overall cellularity was not substantially different from that of CD2L+/+ mice. Consistent with these observations, the total number of T cells detected in 5- and 13-wk-old CD2L−/− compared to CD2L+/+ NOD female mice was ~2-fold increased in the spleen, slightly reduced in the mesenteric lymph nodes, and reduced >10-fold in the inguinal lymph nodes, respectively (Table I). The total number of T cells found in the pancreatic lymph nodes was comparable between CD2L−/− and CD2L+/+ female NOD mice at either 5 or 13 wk of age (Table I). The distribution and activation profile of CD4+ and CD8+ T cells were examined in the pancreatic lymph nodes from 5- and 13-wk-old NOD mice. At 4–5 wk of age, β cell-specific T cell reactivity and infiltration of the pancreas are initially detected in NOD mice. At 13 wk of age, NOD mice are typically euglycemic yet exhibit maximal β cell autoimmunity and extensive insulitis. T cell frequencies in the pancreatic lymph nodes of 5- and 13-wk-old CD2L−/− and CD2L+/+ mice were not significantly different (Table II). Expression of the activation markers CD25, CD44, CD69, and 3G11 on pancreatic lymph node T cells revealed similar profiles between CD2L−/− and CD2L+/+ animals. At 5 wk of age, a trend toward higher frequencies of CD69+ cells in both the CD4+ (26 vs 18%) and CD8+ (16 vs 9%) T cell compartments of CD2L−/− vs CD2L+/+ mice was seen (Fig. 1, A and B, top panels), although the average number of CD69+ cells was not significantly different between CD2L−/− and CD2L+/+ animals (Table I). This trend was maintained in the CD4+ (32% vs

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β cell-specific T cell responses are similar between CD62L<sup>+/+</sup> and CD62L<sup>−/−</sup> NOD mice

GAD65 is an early β cell Ag targeted by T cells in NOD mice followed later by responses to HSP60 and other autoantigens (25, 29). We hypothesized that the absence of CD62L may interfere with lymphocyte migration required for the efficient generation of splenic T cell responses against β cell autoantigens. To determine whether the lack of CD62L affected the early generation of β cell-specific T cell responses, spleen cell cultures were prepared from 5-wk-old NOD mice, and proliferative responses specific for GAD65 and HSP60 were assessed (Fig. 2A). GAD65-specific T cell reactivity was similar among the three genotypes. HSP60 responses, although similar, were only slightly above background and not statistically significant. In addition, IFN-γ secretion in response to GAD65 and HSP60 was not significantly different between splenocytes that were prepared from CD62L<sup>+/+</sup>, CD62L<sup>−/−</sup>, and CD62L<sup>−/−</sup> mice (Fig. 2A).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Tissue</th>
<th>5 (wk)</th>
<th>13 (wk)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>35.0</td>
</tr>
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<td>1.6</td>
<td></td>
</tr>
<tr>
<td>MesLN</td>
<td>9.1</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>IngLN</td>
<td>5.6</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
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<td>79.0</td>
</tr>
<tr>
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<td>1.0</td>
<td></td>
</tr>
<tr>
<td>MesLN</td>
<td>6.4</td>
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<td></td>
</tr>
<tr>
<td>IngLN</td>
<td>0.3</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Groups of three to five female NOD mice.

<sup>b</sup> Average total number of CD3<sup>+</sup> T cells detected via flow cytometry in the spleen, pancreatic (PanLN), mesenteric (MesLN), and inguinal (IngLN) lymph nodes of individual mice. SEM for a given group <20%.

The absence of CD62L does not inhibit BDC2.5 Tg T cell migration and proliferation in NOD.scid recipients

Next, we further evaluated the importance of CD62L in the recruitment and activation of CD4<sup>+</sup> T cells. CD62L<sup>+/+</sup> or CD62L<sup>−/−</sup> BDC2.5 Tg NOD mice expressing a Va2/Nβ4 TCR specific for an unknown β cell autoantigen (30–35) were used as T cell donors for a series of adoptive transfer experiments. Flow cytometric analysis of CD62L<sup>+/+</sup> and CD62L<sup>−/−</sup> CD4<sup>+</sup> Vβ4<sup>+</sup> donor T cells before transfer revealed that only 5–10% of Tg T cells were CD25<sup>+</sup> or CD69<sup>+</sup>, indicating that most cells were not activated. Donor splenocytes were labeled with CFSE to track cell division and proliferation in vivo and then adoptively transferred into NOD.scid recipients. Two days after transfer, both CD62L<sup>+/+</sup> and CD62L<sup>−/−</sup> BDC2.5 Tg T cells were identified in the spleen, mesenteric lymph nodes, and pancreatic lymph nodes (Fig. 4A, top panels). As expected, CD62L<sup>−/−</sup> Tg T cells were unable to efficiently migrate to the inguinal lymph nodes, in contrast to CD62L<sup>+/+</sup> Tg T cells. CFSE labeling was not decreased in T cells detected in any of the lymphoid organs examined at 2 days after transfer (Fig. 4A).

By day 3, proliferation of the adoptively transferred T cells was detected in the pancreatic lymph nodes (Fig. 4A, bottom panels) but not in the inguinal or mesenteric lymph nodes. Interestingly, T cells detected in the inguinal and mesenteric lymph nodes showed no dilution in CFSE expression despite containing a high frequency of activated cells as indicated by CD69 staining (Fig. 4B). T cells were first detected in islets isolated from recipient animals 4 days post-transfer (data not shown). CD62L<sup>+/+</sup>/BDC2.5 Tg T cell donors were also used in these studies, and results were identical to those using CD62L<sup>+/+</sup> BDC2.5 Tg donor T cells. Importantly, these data demonstrate that migration to the pancreatic lymph nodes and proliferation in response to a β cell Ag were not significantly affected by the absence of CD62L.

The development of insulitis and overt diabetes is unaltered in CD62L<sup>−/−</sup> mice

To address whether the lack of CD62L expression had a direct effect on the migration of T cells to the pancreas and the establishment of insulitis, pancreases from 5- and 13-wk-old CD62L<sup>+/+</sup> and CD62L<sup>−/−</sup> female NOD littermates were examined for signs of insulitis (Table III).Irrespective of genotype, pancreases from 5-wk-old animals exhibited limited infiltration, consisting primarily of peri-insulitis. By contrast, pancreases from 13-wk-old CD62L<sup>−/−</sup> NOD mice exhibited a high frequency of intrainsulitis, identical with that seen in CD62L<sup>−/−</sup> animals. Finally, diabetes progression was monitored in groups of CD62L<sup>+/+</sup>, CD62L<sup>−/−</sup>, and CD62L<sup>−/−</sup> female NOD littermates for up to 25 wk of age. No significant difference in either the onset or frequency of diabetes was seen between the three genotypes of NOD female littermates (Fig. 3).
The absence of CD62L does not inhibit BDC2.5 Tg T cells from mediating diabetes in NOD.scid recipients

To address the importance of CD62L for the pathogenicity of BDC2.5 Tg T cells, CD4\(^+\) H11001 T cells prepared from the spleen of CD62L\(^{-/-}\) or CD62L\(^{-/-}\) BDC2.5 Tg mice were compared for the capacity to adoptively transfer overt diabetes to NOD.scid recipients. As shown in Table IV, CD62L\(^{-/-}\) T cells caused disease as efficiently, if not more so than CD62L\(^{+/+}\) Tg T cells. Within 8 days after transfer, all recipients of CD62L\(^{-/-}\) Tg T cells had become diabetic, vs 14 days for five of six mice receiving CD62L\(^{+/+}\) Tg T cells. Therefore, the absence of CD62L did not inhibit the diabetogenic potential of BDC2.5 Tg T cells, and may have actually accelerated the process.

Discussion
Administration of anti-CD62L mAb to young NOD mice prevents diabetes (19, 21, 36). This effect correlated with inhibition of lymphocyte migration to the pancreas and prevention of insulitis, supporting reports demonstrating that CD62L is critical for the early recruitment of lymphocytes to sites of chronic inflammation (12, 37). To further examine the influence of CD62L on \(\beta\) cell-specific T cell reactivity and migration, we established CD62L\(^{-/-}\) NOD mice. Surprisingly, \(\beta\) cell autoimmunity and the onset of diabetes were essentially unaltered in CD62L\(^{-/-}\) mice. Specifically, no significant difference was detected between CD62L\(^{+/+}\) and CD62L\(^{-/-}\) NOD mice with regard to: 1) the total number of T cells and the frequency and activation status of CD4\(^+\) and CD8\(^+\) T cells found in pancreatic lymph nodes (Fig. 1 and Tables I and II); 2) CD4\(^+\) T cell reactivity specific for the \(\beta\) cell autoantigens GAD65 and HSP60 (Fig. 2); 3) cellular infiltration of the pancreas (Table III); and 4) the onset and frequency of overt diabetes (Fig. 3). These results were confirmed by adoptive transfer experiments using BDC2.5 Tg T cells. Both CD62L\(^{+/+}\) and CD62L\(^{-/-}\) BDC2.5 Tg T cells efficiently migrated to the pancreatic lymph nodes, became activated and proliferated similarly in response to \(\beta\) cell Ag (Fig. 4), and effectively mediated overt diabetes in NOD.scid.
play similar splenic T cell responses specific for β cell autoantigens. Individual splenocyte cultures were established from groups of four CD62L⁺/⁺ or CD62L⁻/⁻ female NOD mice either 5 (A) or 13 (B)-wk-old and pulsed with 10 μg/ml GAD65, HSP60, or OVA as described in Materials and Methods. Proliferation is displayed as a stimulation index; ~3000 cpm was detected in media-only cultures. The range of cpm detected in cultures was: GAD65, 5-wk-old (8,710–17,577), 13-wk-old (10,073–21,376)/H11001 CD62L⁺/⁺; HSP60, 5-wk-old (3,825–9,449), 13-wk-old (5,119–8,497); OVA, 5-wk-old (2,643–4,975), 13-wk-old (2,015–3,630) ELISA measurements of IFN-γ were made in duplicate. Results represent the mean value of mice within a given group. ANOVA was used for statistical analysis. *, p = 0.003 vs CD62L⁺/+ or CD62L⁻/⁻ mice. †, p = 0.006 vs CD62L⁺/+ or CD62L⁻/⁻ mice. ‡, p = 0.01 vs CD62L⁺/+ or CD62L⁻/⁻ mice.

The latter observation is consistent with recent work by Dobbs and Haskins (38) which demonstrated that BDC2.5 Tg T cells continued to mediate overt diabetes despite pretreatment with anti-CD62L mAb and subsequent injection of the mAb into NOD.scid recipients (Table IV). The latter observation is consistent with recent work by Dobbs and Haskins (38) which demonstrated that BDC2.5 Tg T cells continued to mediate overt diabetes despite pretreatment with anti-CD62L mAb and subsequent injection of the mAb into NOD.scid recipients. On the basis of these data, the authors concluded that additional mechanisms independent of CD62L can mediate B2C.5 Tg T cell migration to the pancreas. Nevertheless, defects in T cell migration defined earlier in CD26L⁻/⁻/⁻ C56BL/6 mice (15) were also evident in CD62L⁻/⁻ NOD mice. For example, mucosal and pancreatic lymph nodes were well developed in CD62L⁻/⁻ NOD mice, whereas the cellularity of inguinal lymph nodes was substantially reduced (Table I). This migration profile was also observed for CD62L⁻/⁻/⁻ BDC2.5 Tg T cells injected into NOD.scid mice (Fig. 4). In addition, the spleens of CD62L⁻/⁻/⁻ NOD mice were somewhat larger than those of CD62L⁺/⁺ NOD mice containing ~2-fold more T cells (Table I), but the ratios of CD4⁺ and CD8⁺ T cells were similar in agreement with previous reports (R. H. Friedline and R. Tisch, unpublished results; Refs. 14 and 15). These data demonstrate that CD62L can indeed influence aspects of T cell migration but that the observed defects in CD62L⁻/⁻/⁻ NOD mice have no significant effect on the regulation of β cell-specific T effector cell function and subsequent development of T1D.

A number of possibilities may explain why the absence of CD62L expression had no obvious effect on the diabetogenic process. First, as suggested by the work of Dobbs and Haskins (38), redundancy in pancreatic migration mechanisms may permit the recruitment of T cells in the absence of CD62L-mediated interactions. During development, pancreatic tissue derives partially from the mesoderm as does the intestine (18, 39) and consequently shares adhesion properties with the mucosa. The αβ integrin ligand MadCAM-1 is constitutively expressed in the exocrine pancreas (18) and is detected in the islets during inflammation (20). Because pancreatic lymph node cellularity was not significantly affected by the absence of CD62L (Table I and Fig. 1), it is probable that recruitment relied on αβ-MadCAM-1 interactions in agreement with the report by Baron et al. (40). Indeed, administration of mAbs specific for MadCAM-1 or the additional MadCAM-1 receptors, αβ integrin, and αβ integrin effectively inhibits the progression of insulitis and the development of overt diabetes in NOD mice (19, 36, 41). A comparison of NOD mice lacking β integrins, CD62L, or both could be used to further disentangle the relative contribution of these adhesion molecules in T1D (42, 43).

A second nonmutually exclusive possibility is that the absence of CD62L does in fact reduce the efficiency of lymphocyte migration into the pancreatic lymph nodes and islets. However, the inability of CD62L⁻/⁻ T cells to migrate to peripheral lymph nodes

![Figure 2](image)

**Figure 2.** Five- and 13-wk-old CD62L⁺/⁺ and CD62L⁻/⁻ mice display similar splenic T cell responses specific for β cell autoantigens. Individual splenocyte cultures were established from groups of four CD62L⁺/⁺ or CD62L⁻/⁻ female NOD mice either 5 (A) or 13 (B)-wk-old and pulsed with 10 μg/ml GAD65, HSP60, or OVA as described in Materials and Methods. Proliferation is displayed as a stimulation index; ~3000 cpm was detected in media-only cultures. The range of cpm detected in cultures was: GAD65, 5-wk-old (8,710–17,577), 13-wk-old (10,073–21,376)/H11001 CD62L⁺/⁺; HSP60, 5-wk-old (3,825–9,449), 13-wk-old (5,119–8,497); OVA, 5-wk-old (2,643–4,975), 13-wk-old (2,015–3,630)

![Figure 3](image)

**Figure 3.** Cumulative incidence of diabetes is similar in CD62L⁺/⁺, CD62L⁺/⁻, and CD62L⁻/⁻ NOD mice. Tenth generation backcrossed CD62L⁻/⁻ mice plus CD62L⁺/⁺ and CD62L⁻/⁻ littermates were expanded and monitored for diabetes by testing for glycosuria. Animals were considered diabetic after two sequential positive tests for urine glucose.

Table III. Frequency of insulitis in 5- and 13-wk-old NOD mice

<table>
<thead>
<tr>
<th>Age (wk)</th>
<th>Genotype</th>
<th>No Infiltration</th>
<th>Peri-insulitis</th>
<th>Infiltration</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;50%</td>
</tr>
<tr>
<td>5</td>
<td>CD62L⁺/⁺</td>
<td>292 (90.7)</td>
<td>20 (6.2)</td>
<td>6 (1.9)</td>
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<td>15 (3.7)</td>
<td>8 (1.9)</td>
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<tr>
<td>13</td>
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<td>39 (16.6)</td>
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<td>CD62L⁻/⁻</td>
<td>96 (26.2)</td>
<td>60 (16.3)</td>
<td>53 (14.4)</td>
</tr>
</tbody>
</table>

*Insulitis was assessed by quantifying numbers of islets with leukocytic infiltrates from 5- and 13-wk-old CD62L⁺/⁺ or CD62L⁻/⁻ female NOD mice. Groups of five mice were examined. Insulitis was scored as either no infiltration, peri-insulitis characterized by leukocytes restricted to the islet periphery, or intransilits with either ≤50% or >50% of the islet filled with leukocytes.

| Number of islets counted per group with indicated level of infiltration. | Percent of total islets counted per group. |

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may promote increased circulation of lymphocytes through other tissues (44) such as the pancreatic lymph nodes and islets. An increase in available T cells could then compensate for the lack of CD62L and inefficient entry into the pancreatic lymph nodes and islets. Although an increased number of T cells was found in the spleen of CD62L−/−/− NOD mice (Table I), equivalent numbers of CD4+ and CD8+ T cells were detected in peripheral blood prepared from CD62L−/+/-/− and CD62L−/−/-/− NOD female mice (R. H. Friedline, C. P. Wong, and R. Tisch unpublished data). The latter observation suggests that if a pool of excess T cells indeed exists in circulation, the size of this pool is relatively small.

Although unlikely, another possible explanation for the apparent normal progression of diabetes in CD62L−/−/− NOD mice is the carryover of an unidentified genetic susceptibility locus from the C57BL/6 and/or 129 background that cosegregates with the disrupted CD62L gene. This putative susceptibility locus could negate any potential diabetes resistance contributed by the lack of CD62L, thus resulting in a phenotype indistinguishable from that of CD62L−/−/− NOD mice. A search for C57BL/6 susceptibility loci in BDC2.5 Tg mice by Gonzalez et al. (45) identified a locus on chromosome 1 located 50 cM away from the CD62L gene. Microsatellite mapping of linked C57BL/6 genetic loci in our CD62L−/−/− NOD mice revealed a region contained within 11 cM surrounding the disrupted CD62L gene, at least 40 cM distant from this identified susceptibility locus. Additional differences between the NOD and C57BL/6 strains have been identified in close distal proximity to the CD62L gene. Expression of FcγRII is reduced in macrophages prepared from NOD relative to C57BL/6 mice (46). At this point in time, we cannot conclusively rule out the possibility that disease progression is influenced independent of the CD62L mutation by potential genetic differences found in the ~11-cM C57BL/6 and 129 chromosomal segment(s) encompassing the CD62L gene. Nevertheless, the largely comparable phenotype (Table II and Fig. 1) and function (Tables I and IV and Fig.

Table IV. Diabetic potential of BDC2.5 Tg T cells is not impaired by the absence of CD62L−/−

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<tr>
<th>Donor</th>
<th>Diabetes Incidence</th>
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<tr>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>CD62L+/-+</td>
<td>1/6</td>
</tr>
<tr>
<td>CD62L−/−</td>
<td>3/6</td>
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a On day 0, groups of six 8-wk-old female NOD.scid mice were injected with 1.5 × 105 CD4+ T cells prepared from the spleen of wild-type (CD62L+/-+) or deficient (CD62L−/−) BDC2.5 TCR Tg NOD mice. Urine glucose levels were monitored daily.
4) of T cells, and equivalent APC function of macrophages (R. Tisch, unpublished data) seen in the respective genotypes would argue against such an effect.

The current results contrast with earlier findings that administration of anti-CD62L (MEL-14) mAb to young female NOD mice imparts long term protection against the development of insulitis and overt diabetes. Because treatment with MEL-14 mAb does not deplete lymphocytes in vivo or suppress T cell responses against β cell autoantigens (18, 20), it is possible that Ab treatment during the first 4 wk of life induces a form of immunoregulation preventing the establishment of insulitis (18). This regulation may involve the activation and/or expansion of a recently identified subset of regulatory T cells bearing a CD4+CD25+CD62L[bright] phenotype and capable of preventing the adoptive transfer of diabetes (47, 48). CD62L functions as a signal transduction molecule. As such, CD62L engagement by MEL-14 mAb generates signals that result in immediate intercellular adhesion by lymphocytes and neutrophils (49). The in vivo consequences of these signaling events may influence activation and/or effector function of lymphocyte subsets. Indeed, immune responses in mice treated with the MEL-14 mAb are significantly more reduced than those occurring in CD62L[bright]− mice (28, 50).

The results presented here are interesting in light of a recent study by Grewal et al. (17) demonstrating that CD62L expression is critical for the development of EAE in a TCR Tg mouse model. Peripheral T cell responses specific for autoantigens and the recruitment of perivascular infiltrates in the brain and spinal cord were not prevented in CD62L[bright]− mice. However, the transition from perivascular to parenchymal infiltration and the development of EAE was blocked. Parenchymal infiltration depended on the presence of CD62L[bright] macrophages. In NOD mice, comparable peripheral T cell responses against β cell Ags between CD62L[bright] and CD62L[bright]− mice were also observed (Figs. 2 and 4), supporting the concept that CD62L expression has minimal functional impact on T effector cell activation and expansion. However, in contrast to the findings made in the EAE model, the progression of TID was not inhibited by the absence of CD62L on macrophages or any other cell type (Table II and Fig. 3). Therefore, CD62L may play different roles in T cell-mediated autoimmunity depending on the organ-system affected, and the molecular processes that induce or propagate the disease.

Collectively, these studies demonstrate that the generation of β cell-specific T cell responses, establishment of insulitis, and the development of diabetes in NOD mice are not depend on the expression of CD62L. Our results and those reported for the previously discussed EAE model also establish that the pathogenic properties of T cells do not rely on CD62L in these two models of autoimmune disease. It is likely that the relevance of CD62L interactions in other autoimmune disorders will vary depending on their etiology and target tissues.

References


tion of islet cell antigens in the pancreatic lymph nodes. J. Exp. Med. 189:151.


