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*J Immunol* 2003; 171:4040-4047; doi: 10.4049/jimmunol.171.8.4040

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Dynamics of Pathogenic and Suppressor T Cells in Autoimmune Diabetes Development

Silvia Gregori, Nadia Giarratana, Simona Smiroldo, and Luciano Adorini

In the nonobese diabetic (NOD) mouse, pathogenic and suppressor CD4+ T cells can be distinguished by the constitutive expression of CD25. In this study, we demonstrate that the progression of autoimmune diabetes in NOD mice reflects modifications in both CD4+T cell subsets. CD4+CD25+ suppressor T cells from 8-wk-old NOD mice delayed the onset of diabetes transferred by 16-wk-old CD25-depleted spleen cells. In contrast, in sera from 6-wk-old NOD mice, anti-CD54 mAb protected against diabetes, consistent with the role of CD54 in diabetes development. Administration of anti-CD54 mAb protects from disease (21, 22), and a CD54-dependent pathway has been implicated in the pathogenesis of IL-10-mediated diabetes (23). In addition, NOD mice with a disrupted CD54 gene are completely protected from T1D (24), demonstrating the dominant and nonredundant role of this molecule in diabetes development. The second checkpoint relies on several nonexclusive mechanisms, including APCs; cytokines; Th1/Th2 balance; modulation of surface molecules such as CD152, CD25, and NK-like receptors; recruitment of pathogenic cells; and number and function of suppressor cells (17). However, the relative contribution of these factors to the balance of pathogenic and protective T cells during T1D development is still poorly understood.

The progression of T1D in NOD mice is highly regulated, and different phases are characterized by specific features. Insulitis starts to appear at 3–4 wk and is well established by 6–8 wk of age. Progression to overt diabetes occurs in ~80% of females between 12 and 30 wk of age. Two major checkpoints have been defined in the pathogenesis of T1D in the NOD mouse. The first regulates the onset of insulitis and is active before 3 wk of age, while the second exerts its activity at ~8–12 wk of age by controlling the switch from nondestructive to destructive insulitis and the development of overt disease (17). The first checkpoint is controlled by mechanisms such as the composition of APC populations (18, 19) and the expression of integrins and adhesion molecules (20). Among the adhesion molecules involved in leukocyte trafficking into inflammatory sites and in the delivery of costimulatory signals during Ag presentation to T cells, CD54 appears to be critically important in the development of T1D. Administration of anti-CD54 mAb protects from disease (21, 22), and a CD54-dependent pathway has been implicated in the pathogenesis of IL-10-mediated diabetes (23). In addition, NOD mice with a disrupted CD54 gene are completely protected from T1D (24), demonstrating the dominant and nonredundant role of this molecule in diabetes development. The second checkpoint relies on several nonexclusive mechanisms, including APCs; cytokines; Th1/Th2 balance; modulation of surface molecules such as CD152, CD25, and NK-like receptors; recruitment of pathogenic cells; and number and function of suppressor cells (17). However, the relative contribution of these factors to the balance of pathogenic and protective T cells during T1D development is still poorly understood.

Results in this study indicate a dynamic interplay between CD4+CD25+ suppressor T cells and CD4+CD25− pathogenic T cells that correlates with diabetes progression in the NOD mouse. T1D development reflects both an age-dependent decrease in the capacity of CD4+CD25+ T cells to exert suppressive activity and...

Materials and Methods

Mice

NOD/Lt and NOD-SCID mice from The Jackson Laboratory (Bar Harbor, ME), as well as BALB/c and C57BL/6 (B6) mice were obtained from Charles River Laboratories (Calco, Italy). All mice were kept under specific pathogen-free conditions. Glucose levels in the tail venous blood were quantified using a EUROFlash (Lifsacan, Issy les Moulineaux, France). A diagnosis of diabetes was made after two sequential glucose measurements higher than 200 mg/dl.

Cell transfer

Spleen cells, depleted of red cells, were incubated with anti-CD25 mAb (ATCC 7D4; American Type Culture Collection, Manassas, VA), and cells expressing CD25 were negatively selected, after incubation with streptavidin beads, using miniMACS columns (Miltenyi Biotec, Bergisch Gladbach, Germany). CD4^+^CD25^-^ and CD4^+^CD25^+^ spleen cells were sorted with MultiSort Kit (Miltenyi Biotec). The purity of CD4^+^CD25^+^ cells was routinely >98%. Purified cells were mixed as indicated and adoptively transferred by i.v. injection into NOD-SCID mice. Glucose levels in the tail venous blood were quantified at the indicated time points.

Cell cultures

CD4^+^CD25^-^ and CD4^+^CD25^+^ spleen cells were sorted with MultiSort Kit (Miltenyi Biotec). Purified CD4^+^CD25^-^ cells (2 × 10^6^/well) were cultured for 5 days in round-bottom 96-well plates (Costar, Cambridge, MA) with 100 ml synthetic HL1 medium (17A2; BD Pharmingen, San Diego, CA). To measure cell proliferation, cultures were pulsed 8 h before harvesting with 1 μCi ^[3]H[TdR (40 μCi/mmol; Radiochemical Center, Amersham, U.K.). Incorporation of ^[3]HTdR was measured by liquid scintillation spectrometry. For quantification of secreted cytokines, purified CD4^+^CD25^-^ and CD4^+^CD25^+^ spleen cells were cultured for 24, 48, or 96 h in round-bottom 96-well plates (Costar) precoated with 10 μg/ml purified anti-CD3 mAb (17A2; BD Pharmingen) with 5 μg/ml anti-CD28 mAb. For proliferation assays, purified CD4^+^CD25^-^ spleen cells were cultured for 48 h in round-bottom 96-well plates (Costar) precoated with 0.01 μg/ml purified anti-CD3 mAb (17A2; BD Pharmingen) and 10 μg/ml purified anti-CD25 mAb (3E2; BD Pharmingen), or 10 μg/ml purified anti-CD28 mAb or 10 μg/ml purified isotype control mAb. Cultures were performed in synthetic IL-1 medium (Venetec Laboratories, Portland, ME) supplemented with 2 mM l-glutamine and 50 μg/ml gentamicin (Sigma-Aldrich). Cell proliferation was measured, as described above.

Quantification of secreted cytokines

Secreted IFN-γ, IL-4, IL-2, and IL-10 were quantified by two-site ELISA, as described (25, 26). For IFN-γ, polystyrene microtiter plates (Falcon 3012) were coated with 100 μg/ml AN-18.17.24 in carbonate buffer. Samples were titrated in test solution (PBS containing 5% FCS and 1 g/l phenol) and incubated overnight at 4°C. To detect bound cytokines, plates were then incubated with biotinylated goat anti-mouse XMGl2.12a mAb in test solution. After washing, the bound biotinylated Abs were revealed by an additional 30-min incubation with alkaline phosphatase-conjugated streptavidin (Jackson ImmunoResearch Laboratories, Avondale, PA) diluted 1/5000. The plates were washed again and incubated with the developing substrate p-nitrophenylphosphate disodium (Sigma-Aldrich) in diethanolamine buffer (pH 9.6; 100 μl/well). The reaction was stopped by adding 50 μl/well NaOH 3N, and absorbance was read at 405 nm. Cytokines were quantified from two to three titration points using standard curves generated by purified recombinant mouse cytokines, and results were expressed as cytokine concentration in pg/ml. The detection limit for all cytokines was 15 pg/ml.

Isolation of pancreas-infiltrating T cells

After removal of all visible pancreatic lymph nodes, pancreata were individually digested in 3 ml HBSS containing 1 mg/ml collagenase IV (Sigma-Aldrich), by shaking (200 rpm) at 37°C for 15 min. Single cell suspensions were collected after diluting the enzyme with ice-cold HBSS containing 5% FCS and removal of the aggregates by settling for 2 min on ice. Aggregates were further digested with collagenase IV at 0.5 mg/ml for 10 min, and at 0.25 mg/ml for 6 min. Single cell suspensions were washed three times, and passed over a MACS Preperation filter (Miltenyi Biotec) to remove cell aggregates and clumps. Single cell suspensions were incubated with anti-CD4 mAb-coated Microbeads and applied onto MiniMACS columns (Miltenyi Biotec). This procedure yielded a positive fraction containing CD4^+^ cells. Immediately after purification, cells were resuspended with PBS and iomomycin in complete medium, fixed, and stained the next day for intracellular production of cytokines.

Intracellular staining for cytokine production

Cells were stained for IFN-γ and IL-4, as previously described (25, 26). Reagents for intracytoplasmic staining contained 1% FCS, 0.5% saponin (Sigma-Aldrich), and 0.1% sodium azide. All incubations were performed at room temperature. Cells were washed, preincubated for 10 min with PBS containing saponin, and then incubated with FITC-labeled rat anti-mouse IFN-γ (XMG1.2) and PE-labeled rat anti-mouse IL-4 (11B11). Isotype controls were FITC- and PE-labeled rat IgG1 (R3-34). After 30 min, cells were washed twice with PBS/FCS/saponin and then with PBS containing 5% of FCS without saponin to allow membrane closure. The cell surface was then stained with CyChrome-labeled anti-CD4 (L3T4) for 15 min at room temperature. Analysis was performed with a FACScan flow cytometer (BD Biosciences, Mountain View, CA) equipped with CellQuest software and ~10,000 events were acquired.

Flow cytometric analysis

Stainings were performed in the presence of 100 μg/ml mouse IgG using the following mAbs, all from BD Pharmingen: CyChrome-labeled streptavidin, FITC-labeled anti-CD4 (L3T4), PE-labeled anti-CD4 (L3T4), FITC-labeled anti-CD69 (H1.2F3), PE-labeled anti-CD25 (JES5-2A5), PE-labeled anti-CD62L (MEL-14), PE-labeled anti-ICAM-1 (3E2), and biotinylated anti-CD25 (7D4). For CD132 stained the next day for intracellular production of cytokines.

Results

CD4^+^CD25^-^ suppressor T cells from 8-, but not from 16-wk-old NOD mice inhibit diabetes development

To evaluate the role of NOD CD4^+^CD25^-^ suppressor T cells in controlling T1D development, we cotransferred them, together with splenocytes depleted of CD4^+^CD25^+^ T cells, from early diabetic NOD mice, ~16–18 wk of age, into NOD-SCID recipients. As shown in Fig. 1A, transfer of 10^7^ CD25^-^-depleted spleen cells from prediabetic NOD mice induced a significantly accelerated diabetes compared with 10^7^ total spleen cells, indicating an enrichment in diabeticotect cells. Cotransfer of 9.5 × 10^6^ CD25^-^-depleted spleen cells and 5 × 10^5^ CD4^+^CD25^-^ spleen cells isolated from 8-wk-old NOD mice induced diabetes with kinetic comparable to total spleen cells, but cotransfer with 10^6^ CD4^+^CD25^-^ T cells significantly delayed diabetes onset, indicating that a sufficient number of CD4^+^CD25^-^ regulatory T cells is required to control T1D development. Interestingly, CD4^+^CD25^-^ spleen cells could transfer diabetes to NOD-SCID recipients, although with a significant delay, indicating that CD4^+^CD25^-^ spleen cells contain not only regulatory, but also pathogenic T cells. In contrast to CD4^+^CD25^-^ cells from 8-wk-old NOD mice, the same cell population isolated from 16-wk-old NOD mice could
not inhibit diabetes development and could not transfer disease either (Fig. 1B). The inability of CD4+CD25+ T cells from 16-wk-old NOD mice to transfer diabetes in NOD-SCID recipients could be explained by the heterogeneity of CD4+CD25+ T cells (27) and by the lower frequency of pathogenic T cells contained in this population. These results indicate an age-dependent decrease in the capacity of NOD CD4+CD25+ T cells to control diabetes development. In addition, a decrease in the pathogenic potential of CD4+CD25+ T cells from 16-wk-old NOD mice is also apparent.

**CD4+CD25- T cells isolated from 16-wk-old NOD mice are less susceptible to regulation by CD4+CD25+ cells**

We next compared the suppressive activity of CD4+CD25+ T cells and the sensitivity to suppression of CD4+CD25- T cells from 8- and 16-wk-old NOD mice. No differences were observed in the frequency and in the total number of CD4+CD25+ or CD4+CD25- cells in either spleen or pancreatic lymph nodes (data not shown). To determine the regulatory properties of CD4+CD25+ cells, we tested their ability to suppress the proliferative response of CD4+CD25- cells to alloantigens. The proliferation of CD4+CD25- cells from 8-wk-old NOD mice induced by C57BL/6 splenocytes was inhibited equally well by CD4+CD25+ cells from 8- or 16-wk-old NOD mice (Fig. 2). In contrast, CD4+CD25- cells from 16-wk-old NOD mice were less sensitive to suppression, and only CD4+CD25- cells from 8-wk-old NOD mice could inhibit their proliferation, albeit less efficiently compared with the inhibition exerted on CD4+CD25- cells from 8-wk-old NOD mice (Fig. 2). Even a higher ratio of CD4+CD25+ cells (3 x 10^5/well) from 8-wk-old mice was not able to inhibit the proliferation of 2 x 10^5 CD4+CD25- cells/well from 16-wk-old NOD mice (16,225 ± 477 vs 15,446 ± 1,302 cpm obtained from CD4+CD25- cells cultured alone). These results confirm the age-dependent impairment in the regulatory activity of CD4+CD25+ cells observed in vivo. In addition, they indicate that CD4+CD25- T cells become progressively less sensitive to the control exerted by the CD4+CD25+ T regulatory cells, suggesting an intrinsic increase in their pathogenic potential.

**Modulation of cytokine production by CD4+CD25+ and CD25- cells during diabetes development**

To determine whether the age-dependent modification in the function of CD4+CD25+ and CD25- cells could reflect a modification of their cytokine profile, we analyzed the ability to proliferate and the cytokine production of splenic CD4+CD25+ and CD4+
CD25− T cells in response to immobilized anti-CD3 and soluble anti-CD28 stimulation. CD4+CD25+ regulatory T cells either from 8- or 16-wk-old mice proliferate, compared with CD4+CD25− cells, less in response to polyclonal stimulation (Fig. 3A). In addition, CD4+CD25− regulatory T cells from either 8- or 16-wk-old mice produced, compared with CD4+CD25− cells, lower levels of IFN-γ and IL-4, but higher levels of IL-10 (Fig. 3B). Age-dependent effects in cytokine production were clearly observed in both T cell subsets. CD4+CD25+ T cells from 16-wk-old mice displayed a significant reduction in IL-10 production compared with cells from 8-wk-old mice. In contrast, the relatively low levels of IL-10−16-wk-old constant in CD4+CD25− cells. However, this T cell subset displayed a striking age-dependent increase in IFN-γ production (4719 ± 175 pg/ml at 16 wk vs 1387 ± 239 pg/ml at 8 wk, p = 0.0005) and significantly lower levels of IL-4. To determine the role of IL-10 in the suppression mediated by CD4+CD25+ T cells from 8-wk-old mice, we tested their ability to suppress the alloantigen-induced proliferative response of CD4+CD25− T cells from 8- or 16-wk-old NOD mice in the presence or absence of a neutralizing anti-IL-10 mAb (28). The addition of anti-IL-10 mAb did not affect the suppression mediated by CD4+CD25+ T cells (Fig. 4). Thus, although CD4+CD25− cells from 8-wk-old NOD mice produce higher levels of IL-10 compared with CD4+CD25+ cells from 16-wk-old NOD mice, their suppressive effect does not appear to be mediated by IL-10. These results support the observation that CD4+CD25+ T regulatory cells exert their suppressive activity through cell-cell contact rather than cytokine secretion (29).

Therefore, these data, demonstrating enhanced production of IFN-γ by CD4+CD25+ cells from 16- compared with 8-wk-old NOD mice, are consistent with the enhanced pathogenic activity of CD4+CD25+ cells during T1D progression. To determine whether the enhanced IFN-γ production by splenic CD4+CD25+ cells was paralleled by an age-dependent increase of Th1 cells in the target organ, pancreas-infiltrating CD4+ cells were analyzed for intracytoplasmic production of IFN-γ and IL-4. Results in Fig. 5 demonstrate that the percentage of Th1 cells infiltrating the pancreas was significantly higher in 16- compared with 8-wk-old NOD mice.

**Age-dependent up-regulation of CD54 expression by CD4+CD25+ cells in NOD mice**

To identify phenotypic modifications of CD4+ spleen cells, we investigated the expression of surface markers on cells isolated from 4-, 8-, and 16-wk-old and diabetic NOD mice as well as from 4-, 8-, and 16-wk-old BALB/c mice (Table I). The expression of CD69, CD62L, and CD132 was comparable on CD4−, 8-, and 16-wk-old NOD spleen cells from mice at different ages in both NOD and BALB/c strains. The percentage of CD4+CD25− cells expressing CD62L was significantly lower in NOD compared with BALB/c mice, as expected from the lower expression of CD62L in memory compared with naïve T cells (30). The percentage of CD28+ cells was
significantly increased in diabetic compared with prediabetic NOD mice, suggesting an involvement of this costimulatory pathway in the effector phase of T1D development. In addition, the percentage of CD4+CD25− expressing CD54+ was significantly higher in NOD at 16 wk of age \( (p = 0.035) \) and in diabetic NOD mice \( (p = 0.048) \) compared with 4- and 8-wk-old NOD mice. Conversely, the frequency of CD4+CD25+ cells expressing CD54 was comparable between NOD and BALB/c mice, but was considerably higher compared with CD4+CD25− cells, consistent with the higher expression of CD54 on CD4+CD25+ regulatory T cells (31).

Results in Fig. 6, A and C, confirm the significantly higher percentage of CD25+CD54− cells in 16-wk-old and diabetic NOD mice compared with 4- and 8-wk-old NOD mice. In contrast, the percentage of CD25+CD54− T cells was similar in NOD mice of different ages. Conversely, the percentage of both CD25+CD54+ and CD25−CD54+ cells was comparable among cells isolated from BALB/c mice of different ages (Fig. 6, B and D). These results suggest a possible role of CD54 in the pathogenicity of CD4+CD25− cells in NOD mice.

\textbf{CD54 blockade inhibits the proliferation of CD4+CD25− T cells}

Ligation of CD54 expressed on naive T cells delivers a costimulatory signal leading to T cell proliferation (32). Following costimulation with anti-CD3 and anti-CD54, a significantly higher proliferation was observed in CD4+CD25− cells from 16- compared with 8-wk-old NOD mice, in agreement with their higher frequency of CD54-expressing cells (Fig. 7). Conversely, as predicted by the similar percentage of CD28+ T cells, no difference was observed in the T cell proliferation induced by costimulation with anti-CD3 and anti-CD28 (Fig. 7). To evaluate the role of CD54 expressed by CD4+CD25− T cells, we analyzed their proliferative response to alloantigen in the presence of blocking anti-

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**Table 1. Surface marker expression by splenic CD4+CD25− and CD4+CD25+ T cells**

<table>
<thead>
<tr>
<th>Surface Markers</th>
<th>NOD</th>
<th>BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>% of positive cells in CD4+CD25− T splenocytes at the indicated ages (wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD69</td>
<td>1.7 ± 0.6</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>CD28</td>
<td>7 ± 1.6</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>CD62L</td>
<td>42 ± 10</td>
<td>41 ± 9</td>
</tr>
<tr>
<td>CD54</td>
<td>13 ± 1</td>
<td>23 ± 5</td>
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<tr>
<td>CD132</td>
<td>69 ± 15</td>
<td>73 ± 13</td>
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<tr>
<td></td>
<td>5* ± 8.0</td>
<td>6.5 ± 9 **</td>
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Spleen cells were stained with mAbs specific for the indicated surface markers and CD25, and analyzed by flow cytometry. Acquisition was performed on CD4+ T cells. Results are mean ± SE from 12 individual NOD mice at each age and from 6 individual BALB/c mice at each age. The p values were calculated by two-tailed Student’s t test between 4- and 16-wk-old and diabetic NOD mice vs 8-wk-old NOD mice \( (*, p < 0.05; **, p < 0.005) \), or between BALB/c vs NOD mice of the corresponding age \( (†, p < 0.05; ‡, p < 0.005) \).

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**FIGURE 6.** Selective age-dependent increase in the percentage of splenic CD4+CD25−CD54+ cells in NOD mice. Spleen cells isolated from 4-, 8-, and 16-wk-old or diabetic NOD mice (A), or from 4-, 8-, and 16-wk-old BALB/c mice (B) were stained with mAbs specific for CD45 and CD25 and analyzed by flow cytometry. Acquisition was performed on CD4+ T cells. Results are mean ± SE from 12 individual mice at each age. Values of \( p \) were calculated by two-tailed Student’s t test \( (*, p < 0.05) \).

Lower panels, Show the results of a representative experiment performed with NOD (C) or BALB/c (D) spleen cells stained with anti-CD54 mAb. Acquisition was performed on CD4+ T cells.
CD4+CD25+ T cell proliferation by costimulation via CD54. CD4+CD25+ T cells from 8- or 16-wk-old NOD mice (2 × 10^5/well) were stimulated by plate-bound anti-CD3 (0.01 μg/ml) in the presence of plate-bound anti-CD54, anti-CD28, or isotype control, all coated at 10 μg/ml. Bars represent mean cpm ± SE from triplicate cultures from six individual mice/group from two separate experiments. Values of p were calculated by Mann-Whitney U test (*, p ≤ 0.05).

**FIGURE 7.** Increased CD4+CD25+ T cell proliferation by costimulation via CD54. CD4+CD25+ T cells from 8- or 16-wk-old NOD mice (2 × 10^5/well) were stimulated by plate-bound anti-CD3 (0.01 μg/ml) in the presence of plate-bound anti-CD54, anti-CD28, or isotype control, all coated at 10 μg/ml. Bars represent mean cpm ± SE from triplicate cultures from six individual mice/group from two separate experiments. Values of p were calculated by Mann-Whitney U test (*, p ≤ 0.05). A representative experiment of three performed is shown.

**FIGURE 8.** Inhibition of CD4+CD25+ T cell proliferation by anti-CD54 mAb. CD4+CD25+ T cells from 8 (open bars)- and 16-wk-old (filled bars) and diabetic (striped bars) NOD mice (2 × 10^5/well) were incubated with T cell-depleted B6 cells (10^5/well) in the presence of 10 μg/ml of soluble anti-CD54 mAb or an isotype control. Bars represent mean of cpm ± SE from triplicate cultures. Backgound proliferation of CD4+CD25+ T cells from 8- or 16-wk-old, and diabetic NOD mice was 554 ± 173, 908 ± 107, and 1460 ± 184 cpm, respectively. Values of p were calculated by Mann-Whitney U test (*, p ≤ 0.05; **, p ≤ 0.005). A representative experiment of three performed is shown.
increased pathogenicity of CD4+CD25− T cells during T1D progression, as evidenced by the increased frequency, selectively in NOD mice, of CD4+CD25− T cells expressing this adhesion/costimulatory molecule. CD54 plays a role in T cell proliferation (44), and induces the development of Th1 cells characterized by enhanced secretion of IL-2 and IFN-γ (46).

Transgenic expression of pancreatic IL-10 has been shown to accelerate NOD diabetes via a CD54-dependent pathway (23), and several other studies have also suggested an important role of CD54 in the pathogenesis of T1D. Administration of anti-CD54 mAbs reduces insulitis and protects from diabetes (21). In addition, autoreactive T cell responses to β cell Ag are completely blocked by anti-CD54 mAbs (22). The key role of CD54 in T1D development has been conclusively shown by the complete production associated with T1D progression. Compared with CD4+ T cells in NOD mice, of CD4+ T cells expressing IL-2 receptor α-chains (CD25): breakdown of a simple mechanism of self-tolerance causes various autoimmune diseases. J. Immunol. 155:1513.

As evidenced above, the CD11a/CD18-CD54 receptor pair has been mostly studied focusing on the role of CD54 expressed by APCs interacting with CD11a+ T cells. However, a signaling role has been identified for CD54 expressed by T cells (50), and triggering CD4+ CD11a+ APCs has been shown to deliver a potent costimulatory signal leading to T cell activation, cytokine production, and proliferation comparable to costimulation via CD11a or CD28 (32). Our findings, showing that a significantly higher percentage of pathogenic T cells expresses CD54 during T1D development, support the important role of CD54 in disease pathogenesis, which could contribute to T cell interaction with APCs and T cell trafficking to the target tissue. Thus, the higher frequency of CD4+CD25− CD54+ NOD T cells can explain the increased Th1 infiltration into the pancreatic islets and the enhanced IFN-γ production associated with T1D progression. Compared with CD4+ CD25− cells, the percentage of CD4+CD25− cells expressing CD54 is 2- to 4-fold higher both in NOD and in BALB/c mice, in agreement with recent data documenting the higher expression of CD54 in regulatory compared with nonregulatory T cells (31). Interestingly, the frequency of CD4+CD25− cells expressing CD54 does not change during T1D development, suggesting a role for this adhesion/costimulatory molecule selectively in CD4+ CD25− cells.

In conclusion, we have demonstrated that progression of diabetes in NOD mice depends on both reduced suppressor activity of CD4+CD25− T cells and increased pathogenicity of CD4+CD25− T cells. The CD4+CD25− T cells are characterized by a progressive reduction in the capacity to inhibit the proliferation of CD4+CD25− T cells, associated with a progressively lower IL-10 secretion. Conversely, CD4+CD25+ pathogenic T cells progressively accumulate into the pancreas and produce higher levels of IFN-γ associated with enhanced frequency of CD54 expression. Therefore, these correlative data indicate that T1D in the NOD mouse is the result of a complex, progressive remodelling of suppressor and pathogenic T cells that underlies the disease process. Rebalancing these two cell subsets might be the key to successful immunomodulation in autoimmune diabetes.

References


