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A Mechanism for IL-10-Mediated Diabetes in the Nonobese Diabetic (NOD) Mouse: ICAM-1 Deficiency Blocks Accelerated Diabetes

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Neonatal islet-specific expression of IL-10 in nonobese diabetic (NOD) mice accelerates the onset of diabetes, whereas systemic treatment of young NOD mice with IL-10 prevents diabetes. The mechanism for acceleration of diabetes in IL-10-NOD mice is not known. Here we show, by adoptive transfers, that prediabetic or diabetic NOD splenocytes upon encountering IL-10 in the pancreatic islets readily promoted diabetes. This outcome suggests that the compartment of exposure, not the timing, confers proinflammatory effects on this molecule. Moreover, injection of IL-10-deficient NOD splenocytes into transgenic IL-10-NOD.scid/ scid mice elicited accelerated disease, demonstrating that pancreatic IL-10 but not endogenous IL-10 is sufficient for the acceleration of diabetes. Immunohistochemical analysis revealed hyperexpression of ICAM-1 on the vascular endothelium of IL-10-NOD mice. The finding suggests that IL-10 may promote diabetes via an ICAM-1-dependent pathway. We found that introduction of ICAM-1 deficiency into IL-10-NOD mice as well as into NOD mice prevented accelerated insulitis and diabetes. Failure to develop insulitis and diabetes was preceded by the absence of GAD65-specific T cell responses. The data suggest that ICAM-1 plays a role in the formation of the “immunological synapse”, thereby affecting the generation and/or expansion of islet-specific T cells. In addition, ICAM-1 also played a role in the effector phase of autoimmune diabetes because adoptive transfer of diabetogenic BDC2.5 T cells failed to elicit clinical disease in ICAM-1-deficient IL-10-NOD and NOD mice. These findings provide evidence that pancreatic IL-10 is sufficient to drive pathogenic autoimmune responses and accelerates diabetes via an ICAM-1-dependent pathway.


Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease caused by T cell-mediated destruction of insulin-producing β cells of the pancreatic islets of Langerhans. The nonobese diabetic (NOD) mouse spontaneously develops IDDM and has been used as an animal model for human IDDM. The NOD mouse develops insulitis beginning at 3–4 wk of age and develops diabetes by 14 wk of age. The islet infiltrate consists of CD4 and CD8 T lymphocytes, B cells, macrophages, and dendritic cells.

Previous experimental findings have shown that treatment of young NOD mice with anti-IL-10 mAb prevents insulitis (3) and that the expression of IL-10 in pancreatic β cells precedes insulitis (4). These findings implicate that IL-10 as an immunostimulatory factor in IDDM of NOD mice. BALB/c mice expressing an IL-10 transgene in their insulin-producing β cells (IL-10-BALB/c mice) did not develop diabetes (3), but their offspring (IL-10-NOD mice) from backcrosses (N2-N3) to NOD mice became diabetic at an accelerated rate (5). Similarly, NOD mice expressing IL-10 in the glucagon-producing α cells of the pancreas experienced an acceleration in the onset of diabetes (4). Our recent studies have demonstrated that promotion of diabetes by IL-10 in NOD mice requires autoreactive T cells but not B cells (6). Depending upon the circumstances, pancreatic IL-10 supported autoimmune diabetes either via CD4 or CD8 T cell pathway (6, 7). Additionally, pancreatic IL-10 promoted insulitis and diabetes in NOD mice independently of several death-inducing or -signaling molecules such as Fas, perforin, TNFR-1, and TNFR-2 (8). Taken together, these results demonstrate that pancreatic expression of IL-10, as opposed to its expression in the periphery by systemic administration, enhances pancreatic islet inflammation and loss of peripheral tolerance leading to accelerated diabetes of the NOD mouse. In that context, we also found that susceptibility to cyclophosphamide-induced autoimmune diabetes correlates with pancreatic islet inflammation (8). That is, Fas-deficient, insulitis-free NOD.Fas/lpr mice completely resisted cyclophosphamide-induced diabetes, whereas their littermate NOD.Fas/lpr mice readily succumbed to autoimmune diabetes.

Yet the mechanism(s) by which IL-10 promotes autoimmune insulitis and diabetes remains unknown. Therefore, in this study, we have examined the mechanism(s) of IL-10-accelerated diabetes in NOD mice. We examined here, in an adoptive transfer model, whether T cell exposure to IL-10 in the islets in the effector phase of diabetes would counterregulate their diabetogenic potential. Using the IL-10-deficient NOD splenocytes, we also examined...
whether pancreatic IL-10 alone, without IL-10 produced by auto-
reactive lymphocytes, is sufficient to drive accelerated autoim-
mune diabetes. Finally, we tested the role of the adhesion/costimu-
lation (ICAM-1/LFA-1) pathway in IL-10-mediated diabetes of the
NOD mouse.

Our results demonstrated, first, that exposure to IL-10 during the
effector phase in the islet environment does not inhibit the diabe-
togenic susceptibility of the T cells because adoptive transfer of
splenocytes from diabetic NOD mice into IL-10-NOD.scid/scid mice readily promoted diabetes. Second, pancreatic IL-10 acceler-
ates the transfer of diabetes by splenocytes from prediabetic
NOD mice, demonstrating that the compartment of exposure, but
not the timing, confers proinflammatory effects on these mole-
cules. Third, adoptively transferring IL-10-deficient NOD sple-
ocytes into IL-10-NOD.scid/scid mice revealed that pancreatic IL-
10, but not endogenous IL-10 produced by lymphocytes, is suffi-
cient to promote the diabetes of NOD mice. Fourth, pancreatic IL-
10 hyperinduced ICAM-1 expression on vascular endothelium.

Fifth, when the ICAM-1 deficiency was introduced into IL-10-
NOD and NOD mice, accelerated as well as spontaneous insulitis
and diabetes were prevented. Overall, the findings presented here
demonstrate that ICAM-1 is required for spontaneous insulitis and
diabetes of NOD mice and for the accelerated diabetes of IL-
10-NOD mice.

Materials and Methods

Mice

NOD/shi mice were part of the rodent breeding colony at The Scripps Research Institute (La Jolla, CA). IL-10-BALB/c mice, which express the IL-10 transgene in their islets under the control of human insulin promoter (5), were backcrossed to NOD/shi mice for 10-11 generations. The N4F1 intercross at the N2 backcross level. At N4 backcross, mice were intercrossed to generate homozygous (N4F1) N4-N5 backcross generation mice that were backcrossed to NOD mice for 9 generations (by Drs. Diane Mathis and Christophe Benoist (Institut National de la Santé et de la Recherche Médici-
cale, France) (9).

Generation of IL-10-deficient NOD mice

ICAM-1 deficient C57BL/6 mice (10) were purchased from The Jackson Laboratory (Bar Harbor, ME). These mice were backcrossed onto NOD mice and were four generations. The mice were selected for NOD MHC at the N2 backcross level. At N4 backcross, mice were intercrossed to obtain wild-type, heterozygous, and knockout mice. The N4F1 intercross was used to introduce ICAM-1 deficiency into IL-10-NOD mice. Heterozy-
gous mice were typed by neo PCR (www.Jax.org). Then the knockout mice were typed by flow cytometry using anti-ICAM-1 mAb (PharMingen, San Diego, CA).

Generation of IL-10-deficient NOD mice

IL-10-deficient (−/−) C57BL/6 mice (11) (provided by Dr. Ralph Kuhn and Werner Muller, Institute for Genetics, University of Cologne, Germany) that were backcrossed to NOD mice for 9 generations (by Drs. Jonathan Katz and Bo Wang, Cincinnati, OH) were further backcrossed to NOD mice until 10 to 12 generations at the Scripps Research Institute. Mice of N10 to N12 backcross generation mice were intercrossed and were used in the experiments described in this manuscript. The heterozygous mice were intercrossed to generate homozygous (−/−), heterozygous (+/−), and wild-type (++) mice (12). The primers used to type mice for the IL-10 gene disruption are as follows (www.jax.org): backward (otMR086): 5′-GTG GGT GCA ATT GTC TTC CGG C-3′ (1723-1700 in exon I), forward (otMR087): 5′-GCC TTC ATG ATA AAA GGG GGA CC-3′ (1523-1546) (in intron), and backward (otMR088): 5′-CTG TCC GTG TCA CCA TCT TG-3′ (neocassette). A 200-bp PCR product indicates the wild-type allele, and a 400-bp product indicates the mutant allele.

Generation of IL-10-NOD.scid/scid mice

IL-10-BALB/c mice, which express the IL-10 transgene in their islets under the control of human insulin promoter, were backcrossed to NOD.scid/ scid mice for 10- to 11 generations. The IL-10-NOD.scid/scid mice were selected for NOD MHC at N2 backcross level as well as for scid/scid mutation by flow cytometry. IL-10-NOD.scid/scid mice never develop insulitis or diabetes throughout their lifetime (B.B. and N.S., unpublished data).

MHC typing of mice

The presence of I-Abβ2 was determined on tail DNA by PCR using the following primer set: 5′-GAT ACA TCT ACA ACC GGG AGG AG-3′ (1148-1170) and 5′-CTG TTC CAC TAC TCG GGC TCT G-3′ (1229-
1250). PCR amplification for I-Abβ2 yielded a 103-bp product from BALB/c but not NOD mice. The presence of I-Eβ1 was tested in tail DNA by PCR using the following primer set: 5′-ATG AGC TCC CAG AAG TCA TGG G-3′ and 5′-GGA GAG ACA GCA GCT CTC AGC-3′. PCR amplification for I-Eα1 yielded a 277-bp product from BALB/c but not from NOD mice.

Lymphocyte proliferation assays

Splenocytes from indicated mice were cultured at 5 × 106 cells/well in 200 µl of serum-free HL-1 medium (BioWhittaker, Walkersville, MD) supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 × 10−5 M l-glutamine, and 3 × 10−3 M 2-ME. Cells were cultured in 96-well flat-bottom microculture plates (Becton Dickinson, Franklin Lakes, NJ) in the presence of indicated Ags for 5 days (13). The cultures were pulsed with 1 µCi of [3H]Tdr/well during the last 18 h of the assay and were later harvested. [3H]Tdr uptake was measured in a beta scintillation counter. The results were expressed as a stimulation index, i.e., (mean cpm with Ag)/mean cpm without Ag). OVA (Sigma, St. Louis, MO) was used as a control Ag. Stimulation index values >1 over the background values were considered significant. Background values correspond to cpm obtained with the splenocytes culture without GAD65. Recombinant GAD65 was prepared as described earlier (13).

Adoptive transfers

To demonstrate the role of pancreatic IL-10 in driving the accelerated autoimmune diabetes, we injected i.v. IL-10-deficient (−/−) and -sufficient (+/+ ) NOD splenocytes at 3 × 107/mouse into IL-10-NOD.scid/scid and NOD.scid/scid mice. Mice were monitored for diabetes by measuring blood glucose (BG) levels at weekly intervals.

To demonstrate the role of ICAM-1 molecule in the effector phase of autoimmunity, adoptive transfer experiments were performed. ICAM-1-deficient (−/−) or sufficient (+/+ ) N4-N5 backcross generation mice were irradiated (700 rad) and were injected with Con A-activated BDC2.5/NOD splenocytes at 5 × 108/mouse i.v. The mice were monitored for diabetes by measuring BG levels at weekly intervals. For activation of BDC2.5/NOD splenocytes, Con A was used at 5 µg/ml for 1 × 107 cells/ml of complete RPMI 1640 medium containing 10% FBS.

Assessment of diabetes

Starting at 4–5 wk of age, mice were tested for diabetes by weekly or bi-weekly measurements of BG levels using a one-step Bayer Glucometer Elite (Bayer, Elkhart, IN). Animals were considered diabetic when BG levels were >300 mg/dl. In most instances, BG levels exceeded 500 mg/dl.

Histological analysis

Lymphocytic infiltration of the islets was evaluated on hematoxylin and eosin (H&E)-stained paraffin sections of pancreas taken at several levels throughout the organ (13). Paraffin-embedded pancreas sections were stained with an immunoperoxidase method using polyclonal Abs to porcine insulin and synthetic glucagon, followed by a biotinylated secondary Ab and an avidin-biotin complex as described earlier (6). The ICAM-1 ex-
pression on vascular endothelium of frozen pancreatic tissue from age-
matched, sex-matched IL-10-NOD and nontransgenic (non-tg) NOD mice was determined using biotinylated hamster Ab against mouse ICAM-1 (PharMingen). As a control IgG, we used biotinylated anti-TNF (hamster IgG, group 1) (PharMingen).

Results

Late exposure of IL-10 in the pancreatic environment does not inhibit the diabeticogenic potential of autoreactive lymphocytes

Pancreatic expression of IL-10 accelerated diabetes (6), whereas systemic administration of IL-10 prevented diabetes (14, 15) of NOD mice, suggesting that the site of expression and/or timing of experience determined the pro- or anti-inflammatory effects of IL-
10. To address whether the site of expression determined the proin-
flammatory effects of IL-10, we used an adoptive transfer system using splenocytes from diabetic and prediabetic NOD mice. The results appear in Table I. Adoptive transfer of splenocytes from 20-wk-old diabetic NOD mice into IL-10-NOD.scid/scid mice
readily elicited clinical disease with faster kinetics (by a margin of 3 wk) than that observed in NOD.scid/scid mice. The findings show that exposure of diabetogenic splenocytes to IL-10 in the pancreatic islets did not prevent their disease-causing potential. Next, to confirm this conclusion, we adoptively transferred splenocytes from prediabetic NOD (8-wk-old) mice into IL-10-NOD.scid/scid mice and control NOD.scid/scid mice. We found that these splenocytes caused diabetes beginning at 4 wk of post transfer in IL-10-NOD.scid/scid recipients. The same sample of splenocytes caused diabetes in non-tg NOD.scid/scid mice only after 7 wk posttransfer. Our results suggest that exposure to IL-10 in the pancreatic environment by autoreactive T cells later in life precipitates disease pathogenesis rather than preventing disease. The findings also demonstrate that location of expression, not the timing of experience, plays a role in conferring a proinflammatory effect on IL-10 in promoting autoimmune diabetes.

Local expression of IL-10 is necessary for the induction of accelerated diabetes by splenocytes from diabetic IL-10-NOD mice

NOD mice spontaneously develop diabetes beginning at 14–15 wk of age, and their splenocytes transfer clinical disease into NOD.scid/scid mice. Because IL-10-NOD mice develop accelerated diabetes beginning at 4–5 wk of age, we tested whether their splenocytes transfer disease into NOD.scid/scid mice. The results appear in Table II. Transfer of splenocytes from 20-wk-old diabetic NOD mice readily caused disease beginning at 3 wk post transfer, and 82% of the recipient NOD.scid/scid mice succumbed to diabetes by 7 wk of age (n = 6). During the same period of time, transfer of splenocytes from 4- to 5-wk-old diabetic IL-10-NOD mice into NOD.scid/scid mice elicited disease beginning at 8 wk post transfer, and 71% of them became diabetic by 16 wk post transfer (n = 7). However, splenocytes from diabetic IL-10-NOD mice readily caused diabetes upon transfer into IL-10-NOD.scid/scid mice (n = 6) beginning at 3 wk post transfer. The findings 1) demonstrate that splenocytes from diabetic IL-10-NOD mice cause clinical disease with distinct kinetics from those splenocytes from diabetic NOD mice and 2) confirm that local expression of IL-10 in the islets is necessary for splenocytes from diabetic IL-10-NOD mice to cause accelerated disease in NOD.scid/scid mice.

Transgenic IL-10 produced in the pancreatic islets, independent of IL-10 secreted by autoreactive lymphocytes, can drive accelerated diabetes

To address whether tg IL-10 produced in the pancreatic islets is sufficient to induce acceleration of diabetes in IL-10-NOD mice, we performed adoptive transfer experiments using splenocytes from age-matched cohorts of nondiabetic IL-10-deficient (−/−) and IL-10-sufficient (+/+ ) NOD mice. As recipients, we used IL-10-NOD.scid/scid and NOD.scid/scid mice. The results appear in Table III. Adoptive transfer of splenocytes from 12-wk-old nondiabetic IL-10-deficient (−/−) NOD mice into IL-10-NOD.scid/scid mice readily provoked diabetes at 2 wk post transfer (3 of 7 mice; 43% incidence). Furthermore, 6 of 7 mice (86% incidence) became diabetic by 4 wk of transfer. During the same period of time, adoptive transfer of splenocytes from nondiabetic IL-10-deficient (−/−) NOD mice did not cause diabetes (n = 6; 0% incidence) in NOD.scid/scid recipients. We also found that transfer of splenocytes from IL-10-sufficient (+/+ ) NOD mice into IL-10-NOD.scid/scid mice caused diabetes beginning at 2 wk post transfer (2 of 6; 33%). By 4 wk post transfer, 5 of 6 IL-10-NOD.scid/scid recipient mice (83% incidence) developed diabetes. During the same period of time, none of the NOD.scid/scid mice that have received splenocytes from IL-10-sufficient (+/+ ) NOD mice developed diabetes (n = 6; 0% incidence). Thus, pancreatic IL-10, independent of IL-10 production by autoreactive lymphocytes, is sufficient to drive accelerated diabetes.

ICAM-1-deficient NOD mice resist IL-10 accelerated diabetes

Because the ICAM-1/LFA-1 pathway is important for extravasation of autoreactive lymphocytes to the inflamed sites (10), the acceleration of autoimmune diabetes in IL-10-NOD mice could result from hyperinduction of ICAM-1 on the vascular endothelium. Therefore, ICAM-1 expression was examined in the pancreatic tissue of age- and sex-matched IL-10-NOD mice and non-tg littermate controls. As shown in Fig. 1, ICAM-1 expression was

Table I. Late experience of IL-10 in the islet micro environment is not inhibitory for autoreactive T lymphocytes

<table>
<thead>
<tr>
<th>Donor Splenocytes</th>
<th>Recipients</th>
<th>Diabetes Onset (weeks after injection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prediabetic NOD</td>
<td>IL-10-NOD-scid/scid</td>
<td>0/5 0/5 2/5 2/5 5/5 7 8 9 10 11 12 13 100</td>
</tr>
<tr>
<td>Prediabetic NOD</td>
<td>NOD-scid/scid</td>
<td>0/7 0/7 0/7 0/7 0/7 2/7 2/7 2/7 3/7 4/7 4/7 5/7 71</td>
</tr>
<tr>
<td>Diabetic NOD</td>
<td>IL-10-NOD-scid/scid</td>
<td>0/5 0/5 2/5 2/5 2/5 2/6 2/6 4/6 6/6 100</td>
</tr>
<tr>
<td>Diabetic NOD</td>
<td>NOD-scid/scid</td>
<td>0/6 0/6 2/6 2/6 2/6 2/6 2/6 4/6 6/6 100</td>
</tr>
</tbody>
</table>

* Indicated recipient mice (10–14 wk old) were injected i.v. with 2 × 10⁶ splenocytes from 8-wk-old prediabetic NOD or 18– to 21-wk-old diabetic NOD mice. The recipients were monitored for diabetes beginning at 1 wk posttransfer. Mice were considered diabetic if the BG levels were >300 mg/dl.

Table II. Local expression of IL-10 in the pancreatic islets is necessary for induction of accelerated diabetes

<table>
<thead>
<tr>
<th>Donor Splenocytes</th>
<th>Recipients</th>
<th>Diabetes Onset (weeks after transfer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prediabetic NOD</td>
<td>IL-10-NOD-scid/scid</td>
<td>0/6 3/6 5/6 6/6</td>
</tr>
<tr>
<td>Prediabetic NOD</td>
<td>NOD-scid/scid</td>
<td>0/6 0/6 1/6 2/6 2/6 4/6 5/6 5/6 5/6 5/6 5/6 5/6</td>
</tr>
<tr>
<td>Diabetic NOD</td>
<td>IL-10-NOD-scid/scid</td>
<td>0/6 0/6 2/6 4/6 4/6 5/6 5/6 5/6 5/6 5/6 5/6 5/6</td>
</tr>
<tr>
<td>Diabetic NOD</td>
<td>NOD-scid/scid</td>
<td>0/7 0/7 0/7 0/7 0/7 1/7 0/7 2/7 2/7 3/7 5/7</td>
</tr>
</tbody>
</table>

* Splenocytes (2 × 10⁵) from recent diabetic 20-wk-old NOD or 5-wk-old IL-10-NOD mice were injected i.v. into 8- to 10-wk-old male or female NOD-scid/scid or IL-10-NOD-scid/scid mice. The recipient mice were monitored for diabetes at weekly intervals. Mice were considered diabetic if the BG levels were >300 mg/dl.
up-regulated on the vascular endothelium in the pancreata of 5-wk-old diabetic IL-10-NOD mice compared with that in the pancreata of age-matched non-tg NOD mice. This finding confirms previous data in IL-10-BALB/c mice, where pancreatic expression of IL-10 up-regulated the expression of ICAM-1 on vascular endothelium (5).

To assess the role of the ICAM-1 in IL-10-accelerated diabetes in NOD mice, we introduced ICAM-1 gene disruption into IL-10-NOD mice. IL-10-NOD mice that were wild-type (+/+), heterozygous (+/−), or knockout (−/−) for the ICAM-1 gene disruption were monitored for diabetes by measuring BG levels at weekly intervals beginning at 4 wk of age. As shown in Table IV, the IL-10-NOD mice that are wild type (+/+; n = 6) or heterozygous (+/−; n = 11) for ICAM-1 expression readily developed diabetes. A noteworthy observation was that ICAM-1 deficiency in IL-10-NOD mice dramatically affected the incidence of diabetes over a 12-wk period (0 of 4; 0% incidence) and a 16-wk period (0 of 5; 0% incidence). During this period of time, littermate non-tg ICAM-1-deficient-NOD mice (n = 7) also failed to develop diabetes. Similarly, a separate group of ICAM-1-deficient NOD mice (n = 7) also failed to develop diabetes over a 24-wk period (data not shown). These results demonstrate that ICAM-1 expression is required for spontaneous diabetes as well as for IL-10-accelerated diabetes.

ICAM-1-deficient IL-10-NOD mice develop peri-insulitis only

To understand whether ICAM-1 deficiency abrogated acceleration of diabetes in IL-10-NOD mice by blocking insulitis, we examined the lymphocytic infiltration in the pancreata of IL-10-NOD mice that are heterozygous (+/−) or deficient (−/−) for ICAM-1 molecule. The degree of insulitis was calculated and is shown in Table V. Representative micrographs of the pancreata from ICAM-1-deficient IL-10-NOD and NOD mice are shown in Fig. 2. These findings demonstrate that the absence of ICAM-1 resulted in a striking reduction of insulitis in IL-10-NOD mice. Yet, because the extent of peri-insulitis was not affected, apparently ICAM-1 expression is not required for lymphocytes to extravasate into the pancreas. However, this adhesion molecule is required for entry of lymphocytes into the islet compartment.

ICAM-1 deficiency affects the generation of GAD65-specific T cells in IL-10-NOD mice

To further understand whether the protection observed in ICAM-1-deficient IL-10-NOD mice is due to defective islet Ag-specific priming, we assessed the proliferation of splenocytes against graded doses of GAD65 in vitro. As shown in Fig. 3, splenocytes from female ICAM-1-sufficient NOD mice (n = 3) responded to GAD65 very well (mean stimulation index at 10 g/ml = 10.4). In contrast, splenocytes from ICAM-1-deficient NOD mice (n = 3) failed to respond to GAD65 in vitro (mean stimulation index at 10 g/ml = 2.0). Similarly, splenocytes from female ICAM-1-deficient IL-10-NOD mice (n = 3) also failed to respond to GAD65 in vitro (mean stimulation index at 10 g/ml = 2.0). Moreover, GAD65-specific T cells from ICAM-1-deficient (−/−) IL-10-NOD mice produced insignificant amounts of IL-2 and IFN-γ and the levels were below the limits of detection (data not shown).

**Table III.** Transgenic IL-10 produced by pancreatic tissue, but not lymphocytes, is sufficient to promote accelerated diabetes

<table>
<thead>
<tr>
<th>Donor NOD Mice</th>
<th>Age</th>
<th>Recipients</th>
<th>Cell Dose</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10-deficient (−/−)</td>
<td>5–6 wk</td>
<td>IL-10-NOD-scid/scid</td>
<td>3 × 10⁷</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>3/3</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NOD-scid/scid</td>
<td>3 × 10⁷</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>IL-10-deficient (−/−)</td>
<td>12 wk</td>
<td>IL-10-NOD-scid/scid</td>
<td>3 × 10⁷</td>
<td>0/7</td>
<td>0/7</td>
<td>3/7</td>
<td>3/7</td>
<td>6/7</td>
<td>6/7</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NOD-scid/scid</td>
<td>3 × 10⁷</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0</td>
</tr>
<tr>
<td>IL-10-sufficient (+/−)</td>
<td>12 wk</td>
<td>IL-10-NOD-scid/scid</td>
<td>3 × 10⁷</td>
<td>0/6</td>
<td>0/6</td>
<td>2/6</td>
<td>2/6</td>
<td>5/6</td>
<td>5/6</td>
<td>83</td>
</tr>
</tbody>
</table>

*Indicated groups of mice were injected with splenocytes from IL-10-deficient or -sufficient NOD mice into 8- to 10-wk-old NOD-scid/scid mice or IL-10-NOD-scid/scid mice. Mice were monitored for diabetes by measuring BG levels. Mice were considered diabetic if the BG levels were >300 mg/dl.
ICAM-1 deficiency blocks IL-10 accelerated autoimmune diabetes in NOD mice

Table IV. ICAM-1 deficiency blocks IL-10 accelerated autoimmune diabetes in NOD mice

<table>
<thead>
<tr>
<th>Mice</th>
<th>Incidence of Diabetes (age at weeks)</th>
<th>% Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10-NOD.ICAM-1 (+/+)</td>
<td>0/6 2/6 5/6 5/6 5/6 5/6 6/6 6/6</td>
<td>100</td>
</tr>
<tr>
<td>IL-10-NOD.ICAM-1 (+/−)</td>
<td>1/11 7/11 10/11 10/11 10/11 10/11 10/11 10/11</td>
<td>91</td>
</tr>
<tr>
<td>IL-10-NOD.ICAM-1 (−/−)</td>
<td>0/4 0/4 0/4 0/4 0/4 0/4 0/4 0/4**</td>
<td>0</td>
</tr>
<tr>
<td>NOD.ICAM-1 (+/−)</td>
<td>0/8 0/8 0/8 0/8 0/8 0/8 0/8 0/8**</td>
<td>0</td>
</tr>
<tr>
<td>IL-10-NOD.ICAM-1 (−/−)</td>
<td>0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5* 0/5</td>
<td>0</td>
</tr>
<tr>
<td>NOD.ICAM-1 (−/−)</td>
<td>0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5 0/5*</td>
<td>0</td>
</tr>
</tbody>
</table>

* Indicated mice of both sexes were bled at weekly intervals for their BG levels. Mice were considered diabetic if the BG levels were >300 mg/dl. Cohorts of mice of N4-N5 backcross generation were included in these experiments. **, Terminated at 12 wk of age; *, terminated at 16 wk of age.

Clearly, the ICAM-1-dependent pathway is required for the generation and/or expansion of GAD65-specific T cells in IL-10-NOD mice.

**ICAM-1 deficiency affects autoimmune diabetes even at the effector phase of autoimmunity**

Although ICAM-1 deficiency forestalled the GAD65-specific T cell priming in IL-10-NOD mice, ICAM-1-deficient IL-10-NOD mice developed peri-insulitis but not diabetes. We reasoned that in the absence of ICAM-1, autoreactive T cells in IL-10-deficient NOD mice may have used another pathway to home to the pancreatic tissue, but failed to penetrate the islets and accelerate the onset of diabetes. To learn whether ICAM-1 deficiency would have blocked diabetes at an effector phase of autoimmunity, we performed adoptive transfers by using preactivated islet-specific BDC2.5 T cells. For this purpose, we irradiated the recipient ICAM-1-deficient and -sufficient NOD mice. These recipient mice were injected i.v. with Con A-activated, 8-wk-old female BDC2.5 splenocytes (5 × 10⁴/mouse). Mice were monitored for diabetes at weekly intervals. As shown in Fig. 4, ICAM-1-deficient IL-10-NOD (n = 4) as well as ICAM-1-deficient NOD (n = 4) recipients of Con A-activated BDC2.5 splenocytes remained free from diabetes. Immunohistochemical analysis of frozen sections of these recipient mice showed that CD4/Vβ T cells are around the islets of Langerhans (peri-insulitis) (data not shown). In contrast, ICAM-1-deficient NOD (n = 4) recipients of Con A-activated BDC2.5 splenocytes became diabetic. Immunohistochemical analysis on frozen sections of these recipient mice showed that the CD4/Vβ T cells penetrated the islets of Langerhans (insulitis) (data not shown). These data suggest that ICAM-1/LFA-1 interaction also plays a role in the effector phase of spontaneous diabetes in NOD mice and of accelerated diabetes in IL-10-NOD mice.

**Discussion**

The data presented here demonstrate that pancreatic expression of IL-10 up-regulates ICAM-1 on the pancreatic vascular endothelium. ICAM-1 expression is required for accelerated insulitis and diabetes of IL-10-NOD mice because ICAM-1-deficient IL-10-NOD mice resisted developing accelerated disease. Interestingly enough, exposing autoreactive T cells to IL-10 in the pancreatic environment during the initial and effector phases of the autoimmune response did not block the diabetogenicity of cells. Additionally, IL-10 production by pancreatic tissue, but not by autoreactive lymphocytes, was sufficient for accelerated disease in NOD mice. Moreover, several lines of evidence indicate that the observed results in ICAM-1-deficient IL-10-NOD mice and NOD mice are representative of the effects of targeted deletion of ICAM-1 and not the flanking genomic segment.

Our findings have shown thattg expression of IL-10 in the pancreatic tissue is adequate to drive the pathogenic autoimmune response in vivo. These results emphasize the power of the tg approach to modify autoimmune responses via local production of cytokines. Furthermore, splenocytes from diabetic IL-10-NOD mice, in comparison to splenocytes from diabetic NOD mice, transferred clinical disease with slower kinetics into NOD.scid/scid mice, but accelerated the timing of diabetes upon injection into IL-10-NOD.scid/scid mice. The disparity in these outcomes may be related to the lower frequency of pathogenic T cells in splenocytes of diabetic IL-10-NOD mice. When frequency is limited, IL-10 could effectively promote accelerated diabetes. Alternatively, pancreatic IL-10 may prime β cells to undergo apoptosis exerted by islet-reactive T cells.

We and others have speculated upon the disparity of effects of IL-10 on IDDM in the NOD mouse. Indeed, early exposure to IL-10 in the pancreatic islets accelerates disease (4, 6, 16). However, systemic exposure to IL-10 during the later prediabetic phase inhibits disease (14, 15). This inhibition was attributed to the induction of a protective Th2 response (15). However, we have shown here that localized pancreatic IL-10 could not counterregulate the disease state induced by splenocytes from prediabetic and diabetic NOD mice when the autoreactive lymphocytes encountered IL-10 during the later part of life or in later stages of activation. This result suggests that

Table V. ICAM-1 deficient IL-10-NOD mice develop peri-insulitis only

<table>
<thead>
<tr>
<th>Mice</th>
<th>No. of Mice</th>
<th>Total No. of Islets</th>
<th>Insulitis Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10-NOD.ICAM-1 (+/+) wild type</td>
<td>5</td>
<td>122</td>
<td>0 0 0 0 122</td>
</tr>
<tr>
<td>IL-10-NOD.ICAM-1 (+/−) heterozygous</td>
<td>7</td>
<td>166</td>
<td>0 8 3 155</td>
</tr>
<tr>
<td>IL-10-NOD.ICAM-1 (−/−) knockout</td>
<td>6</td>
<td>353</td>
<td>25 328 0 0</td>
</tr>
<tr>
<td>NOD.ICAM-1 (−/−) knockout</td>
<td>5</td>
<td>406</td>
<td>0 406 0 0</td>
</tr>
</tbody>
</table>

* Paraffin-embedded pancreas sections from the indicated mice (both sexes) were scored for lymphocytic infiltration by hematoxylin and eosin staining. Mice for IL-10-NOD.ICAM-1 (+/+) and IL-10-NOD.ICAM-1 (−/−) groups are between 4 and 7 wk of age, whereas mice for IL-10-NOD.ICAM-1 (−/−) and NOD.ICAM-1 (−/−) mice are between 12 and 16 wk of age. The insulitis index was calculated as described earlier (14).
pancreatic IL-10 and systemic IL-10 may have distinct avenues of immunoregulatory functions. In fact, the critical target cell for the disparate effects of the cytokine may differ in each case. Systemic IL-10 may block disease through a more generalized mechanism such as suppression of APC function (17, 18), effector Th1 development, or the induction of T cell apoptosis (19). However, within the pancreas, IL-10 may only affect CD8 T cells that infiltrate early in the disease process (6) and/or prime the β cells for apoptosis via infiltrating autoreactive CD8 T cells. In this context, works from Groux et al. have shown that IL-10 could be a stimulatory factor for CD8 T cells (20, 21). It could be argued that the accelerated diabetes observed in IL-10-NOD mice may result from the direct toxic effect of IL-10 on β

FIGURE 2. ICAM deficiency blocks insulitis in the NOD mouse and in the IL-10-NOD mouse. Paraffin-embedded sections of the pancreata from 5-wk-old diabetic ICAM-1-sufficient (+/+; +/−) mice and 16-wk-old ICAM-deficient nondiabetic NOD and 16-wk-old ICAM-deficient IL-10-NOD mice were stained by hematoxylin and eosin. ICAM-1-deficient IL-10-NOD mice, as opposed to ICAM-1-deficient NOD mice, develop peri-insulitis but not insulitis (magnification ×400).

FIGURE 3. Splenocytes from ICAM-1-deficient NOD and IL-10-NOD mice do not respond to GAD65 in vitro. Splenocytes from indicated female mice (n = 3) of 8- to 10-wk old were cultured in the presence of GAD65 (10 μg/ml). The values were expressed as mean cpm ± SD. No responses were detected against the irrelevant Ag OVA (data not shown).
Mice were monitored for diabetes (BG levels and ICAM-1-deficient NOD mice and ICAM-1-deficient (IL-10-NOD mice (n = 4) NOD mice and ICAM-1-deficient IL-10-NOD mice (n = 4). Mice were monitored for diabetes (BG levels >300 mg/dl). Similar findings were observed upon transfer into neonate ICAM-1-deficient (IL-10-NOD mice and ICAM-1-deficient IL-10-NOD mice (data not shown).

To define more precisely the proinflammatory effects of pancreatic IL-10 on autoimmune diabetes with respect to the timing vs location of experience, it would be necessary to establish an inducible (e.g., tetracycline-regulated) gene transcription system where islet-specific expression of IL-10 could be turned on or off at will. In that regard, Green and coworkers have recently shown that duration of the inflammatory signal (TNF-α), in conjunction with the B7-1 molecule, is a major criterion in the breakdown of peripheral tolerance to islet Ags in C57BL/6 mice not genetically predisposed to developing autoimmunity (23). If the duration of the TNF-α signal is critical for promoting autoimmune diabetes, it is still unclear why pancreateically expressed TNF-α in two different NOD lines either accelerates or prevents autoimmune diabetes (22, 24, 25).

Despite its status as an immunosuppressive cytokine, IL-10 transgenically expressed in the islets of Langerhans has up-regulated the expression of ICAM-1 on vascular endothelium and accelerated the onset of diabetes. ICAM-1 is known to play a role in inflammatory responses by promoting the migration of leukocytes into the site of inflammation (10). In this context, as this study shows, ICAM-1 deficiency has effectively blocked diabetes in IL-10-NOD mice, confirming the potential role for ICAM-1 in spontaneous diabetes observed in anti-ICAM-1 mAb-treated NOD mice (26). The protection we observed was accompanied by the accumulation of lymphocytes outside islets but not within them, consequently limiting the accessibility of the islets to destruction by infiltrating lymphocytes. Clearly, from its expression pattern on endothelial cells, ICAM-1 must be a critical traffic signal for circulating lymphocytes, inducing recruitment into the islets. ICAM-1 is not only involved in the extravasation of lymphocytes to the inflamed sites, but also in the formation of the “immuno-logical synapse” (26) and in the effector stages of target cell killing (27). Therefore, ICAM-1 can play a role in the effector stages of autoimmune diabetes as adoptive transfer of activated BDC2.5 T cells failed to elicit diabetes in ICAM-1-deficient IL-10-NOD and NOD mice. The work presented here indicates that induction or expression of ICAM-1 is critical for accelerated diabetes because ICAM-1 deficiency precluded the transfer of disease by preactivated diabetogenic BDC2.5 T cells (9).

From the foregoing results, we demonstrate that ICAM-1 expression is pivotal for the acceleration of diabetes. Additionally, our data document that the location, but not the timing, of IL-10 expression determines its pro- or anti-inflammatory effects on autoimmune diabetes of NOD mice. These findings may provide avenues for immune intervention to halt spontaneous as well as accelerated diabetes.

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References


