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Induction of Diabetes in Nonobese Diabetic Mice by Th2 T Cell Clones from a TCR Transgenic Mouse

Michelle Poulin and Kathryn Haskins

We have produced a panel of cloned T cell lines from the BDC-2.5 TCR transgenic (Tg) mouse that exhibit a Th2 cytokine phenotype in vitro but are highly diabetogenic in vivo. Unlike an earlier report in which T cells obtained from the Tg mouse were cultured for 1 wk under Th2-promoting conditions and were found to induce disease only in NOD.scid recipients, we found that long-term T cell clones with a fixed Th2 cytokine profile can transfer disease only to young nonobese diabetic (NOD) mice and never to NOD.scid recipients. Furthermore, the mechanism by which diabetes is transferred by a Tg Th2 T cell clone differs from that of the original CD4+ Th1 BDC-2.5 T cell clone made in this laboratory. Whereas the BDC-2.5 clone rapidly causes disease in NOD.scid recipients less than 2 wk old, the Tg Th2 T cell clones can do so only when cotransferred with other diabetogenic T cells, suggesting that the Th2 T cell requires the presence of host T cells for initiation of disease. The Journal of Immunology, 2000, 164: 3072–3078.

The nonobese diabetic (NOD) mouse has long been used as an animal model for insulin-dependent diabetes mellitus because of its many similarities to human disease (1, 2). As in human diabetes, the NOD mouse exhibits a T cell-mediated destruction of the islet β cells of the pancreas (3–11). To understand the role of T cells in diabetes in the NOD mouse, islet-specific T cell clones have been produced by several groups (1, 12–19). These cells have been primarily CD4+ and of the Th1 phenotype, and many have been shown to be diabetogenic in the young NOD mouse (18, 20–25).

The BDC-2.5 TCR transgenic (Tg) mouse (26) was made with the TCR α-chain (Var1) and β-chain (Vβ4) of the diabetogenic T cell clone BDC-2.5, which was produced in our laboratory (12). This NOD-derived T cell clone proliferates and makes Th1 cytokines in vitro in response to whole islet cells or membrane fractions obtained from β tumor cells (13, 27). In vivo, it rapidly and reproducibly transfers diabetes into young (<2-wk-old) NOD or NOD.scid recipients but not into adult mice. As previously described (28) and confirmed by studies in this lab (C. Dobbs and K. Haskins, manuscript in preparation), T cells from the BDC-2.5 TCR Tg mouse bred onto the NOD.scid background can rapidly transfer diabetes at very low cell numbers (≤10^6) into adult NOD.scid mice.

Evidence from several labs has indicated that there is a protective, regulatory role in autoimmune diabetes for the IL-4/IL-10-secreting subset of CD4+ T cells, which are generally referred to as Th2 T cells. Treatment of NOD mice with IL-4 gave rise to IL-4-secreting spleen cells and delayed diabetes onset (29) and, in subsequent studies, protection by IL-4-secreting splenic T cells was shown to be transferable (30). Inserting the IL-4 gene under the rat insulin promoter on the NOD background was also found to be protective (31). Similar protective results were seen after systemic treatment of NOD mice with IL-10 (32). On the other hand, it has been reported that IL-4-secreting T cells obtained from a 4-day culture of spleen cells from the BDC-2.5 TCR Tg mouse could not protect against disease transfer (33) and, in a subsequent study, short-term (7-day) cultures of CD4+ T cells with IL-4 were found to induce diabetes in NOD.scid recipients (34).

In an attempt to resolve the discrepancies in the literature with respect to protective vs diabetogenic properties of IL-4-secreting T cells and to determine whether protective Th2 T cell clones could in fact be obtained from a mouse bearing the TCR of a diabetogenic Th1 T cell clone, we have investigated the in vivo properties of long-term T cell lines and clones with stable Th2 cytokine-secreting profiles from the BDC-2.5 TCR Tg mouse. Our results indicate that IL-4/IL-10-secreting CD4+ T cell lines bearing the transgenic TCR, like the original clone BDC-2.5, can rapidly cause diabetes in young NOD recipients but cannot induce disease in NOD.scid mice of any age.

Materials and Methods

Mice

Breeding pairs of NOD and NOD.scid mice were obtained from either The Jackson Laboratory (Bar Harbor, ME) or the breeding colony at the Barbara Davis Center. BDC-2.5 TCR Tg breeding pairs were obtained from Dr. Jonathan Katz (Washington University, St. Louis, MO). Mice were bred and housed under specific pathogen-free conditions in the University of Colorado Health Sciences Center’s Center for Laboratory Animal Care.

Isolation and maintenance of Tg T cell lines and clones

To produce Th2 T cell lines from the BDC-2.5 TCR Tg mouse, splenic T cells (1 x 10^7/ml) from an untreated, nondiabetic female donor were incubated with islet cells or β cell membranes (prepared from islet cell tumors) in a primary culture for 5 days before being combined in a 20-ml secondary culture in high-glucose DMEM (Life Technologies, New Island, NY) (supplemented with 44 mM sodium bicarbonate, 0.55 mM L-arginine, 0.27 mM L-asparagine, 1.5 mM L-glutamine, 1 mM sodium pyruvate, 50 mg/L gentamicin sulfate, 50 μM 2-ME, 10 mM HEPES, and 10% FCS).
with islet Ag, APC (2.5 × 10^5 irradiated NOD spleen cells), 3.5 U/ml IL-2 (IL-2-containing EL4 supernatant), 5 ng/ml IL-4 (X63-IL-4 supernatant) (35), and anti-IFN-γ mAb (XMG1.2) at a concentration of 2.5% v/v. Islet Ag for these cultures was in the form of either fresh islet cells (5 × 10^5) or 12 μg of a membrane preparation made from β tumor cells (27). Restimulation cultures were incubated up to 24 h at 37°C and 10% CO₂. T cells were harvested, washed three times, resuspended in HBSS, and injected into young recipients.

Proliferation and cytokine assays

At the end of each 2-wk growth cycle, Tg T cell lines and clones were assayed for Ag specifictiy and cytokine production. To assess Ag specificity, T cells were plated in 96-well flat-bottom plates at 2 × 10^5 cells/well alone, with 2.5 × 10^5 syngeneic peritoneal cells as APC to determine nonspecific proliferation, or with 2.5 × 10^5 syngeneic peritoneal cells as APC plus 5000 irradiated NOD islet cells, or with 10 μg of β-membrane, to determine Ag-specific proliferation. Plates were incubated at 37°C and 10% CO₂ for 3 days. Treated thymidine was added at a concentration of 0.5 μCi/ml for the final 6 h of culture. Assays were harvested on a Packard (Meriden, CT) Filtermate 196 harvester and read on a Packard Topscatter.

Cytokine production was assessed by ELISA analysis of Con A-stimulated supernatants. Briefly, Tg T cells were plated in 96-well flat-bottom plates at 2 × 10^5 cells/well alone, with 2.5 × 10^5 syngeneic peritoneal cells as APC and Con A (Sigma, St. Louis, MO) at a final concentration of 2.5 μg/ml. Plates were incubated at 37°C and 10% CO₂, and supernatants were harvested by centrifugation. The cytokine production by Tg T cell lines and clones was tested at the end of a 2-wk growth cycle and from expansion flasks on the day of injection for adoptive transfer experiments. Cytokine levels were determined by specific sandwich ELISAs for the following cytokines: IL-2, IL-4, IL-6, IL-10, and IFN-γ. Cytokine ELISAs were performed using purified mAb (PharMingen, San Diego, CA) for capture and biotinylated mAb (PharMingen) for detection and then using streptavidin-HRP (Sigma) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) as the substrate. Assays were read on a TiterTec (Huntsville, AL) Multiskan Plus platereadet at 405 nm.

Confirmation of Tg TCR phenotype or genotype

Tg T cell lines were assayed for the expression of the BDC-2.5 TCR chain Vβ4 by flow cytometry using a Vβ4-specific mAb, KT4-10. The presence of the Vα1 and Vβ4 transgenes was confirmed by PCR analysis using transgene-specific primers.

In vivo transfer of Tg T cell lines and clones

Recipients of Tg T cell lines were young NOD or NOD.scid mice 3–14 days of age. Recipients were given two i.p. injections of 1 × 10^7 cells/wk apart. Recipients were monitored for diabetes by daily urine (Diastrip, Bayer, Elkhart, IN) or blood glucose (Glucotrend, Boehringer Mannheim, Indianapolis, IN) tests starting at 6 days postinjection until they became diabetic or the experiment was terminated. Overt diabetes was defined as a positive urine glucose (>1%) and then a positive blood glucose test of >250 mg/dl (14 mM). Recipients were sacrificed when blood glucose readings were 320 mg/dl (18 mM) or higher. At sacrifice, the pancreata were removed for histological analysis.

Histology

Histological sections were read visually by two independent scorers to determine the extent of islet infiltration and pancreatic destruction. Infiltration was scored as follows: 0, no infiltration; 1, mild peri-islet or polar infiltration; 2, moderate peri-islet or mild intraislet infiltration; and 3, severe intraislet infiltration. Granulation was also scored from 0 to 3 by the following criteria: 3, 75–100% granulated islets; 2, 50–75% granulated islets; 1, 20–50% granulated islets; and 0, complete degranulation.

Cotransfer of Tg Th2 (Tg/T2) line with diabeticogenic Th1 clones or diabetic spleen cells

For cotransfer experiments using diabetic spleen cells, recipient NOD.scid mice (9–14 days of age) were injected i.p. on day 0 with 2 × 10^7 diabetic spleen cells, 1 × 10^6 of the transgenic T cell line 2.5 Tg/T2-X, or spleen cells and 2.5 Tg/T2-X together. To obtain spleen cells, diabetic NOD female donors were sacrificed and their spleens were removed under sterile conditions. Spleens were ground into a single-cell suspension using a tissue homogenizer; the cells were washed twice with HBSS and counted using a hemacytometer. Splenocytes were resuspended in HBSS at a concentration of 4 × 10^7 cells/ml, and 2 × 10^7 cells were injected into mice in a volume of 50 μl. For cotransferring a CD4⁺ T cell clone, we used the T cell clone BDC-6.3, another Th1 diabeticogenic T cell clone from our original panel (13). This T cell clone is Vβ4 Vα3 in its TCR and proliferates to the same islet Ag preparations as BDC-2.5 but, unlike BDC-2.5, cannot transfer disease to NOD.scid mice of any age. Cotransfer experiments were performed by injecting NOD.scid recipients (7–14 days of age) i.p. on days 0 and 7 with 1 × 10^6 of BDC-6.3, 1 × 10^7 of 2.5 Tg/T2-X, or BDC-6.3 and 2.5 Tg/T2-X together. All T cell clones were cultured in expansion cultures as described previously. Recipients were monitored as described above. After diabetic animals were sacrificed, their pancreata were placed in formalin and histologically examined as described previously.

Statistical analysis

Statistical significance within experiments was determined using JMP analysis software (SAS Institute, Cary, NC). Survival analysis was done using the product-limit (Kaplan-Meier) method. The endpoint of the experiment was defined as diabetes. Data on animals that did not become diabetic by the end of the experiment were censored. The p values shown were determined by Log-Rank test.

Results

In vitro characterization of TCR Tg T cell lines and clones

T cell lines were generated from spleen cells obtained from non-diabetic female BDC-2.5 TCR transgenic mice and cultured in the presence of NOD islet Ag and IL-2. Lines were pushed toward a Th2 phenotype by the addition of IL-4 and anti-IFN-γ mAb. Tg/T2 lines were selected on the basis of specificity for islet cells and production of IL-4, and the absence of production of IFN-γ. Subsequently, the Tg/T2 lines were extensively analyzed for cytokine production, with testing for IL-2, IL-4, IL-6, IL-10, and IFN-γ. As these lines, coming from TCR transgenic animals, were comprised primarily of BDC-2.5 Tg-positive T cells, uncloned lines tended to become clonal with continued culture. However, some lines were cloned by limiting dilution shortly after being established. Each line or clone selected showed a reproducible response to islet cell Ag above background in routine T cell proliferation assays, although the magnitude of the proliferative response was somewhat less than that of the parent Th1 clone, BDC-2.5 (data not shown). All of the Tg/T2 lines and clones were shown to bear the BDC-2.5 TCR by flow cytometry or PCR analysis (data not shown).

As shown in Table II, two TCR Tg lines and one clone appeared to have a Th2 phenotype. Every Tg/T2 line or clone showed a distinct but consistent cytokine profile, producing different levels

<table>
<thead>
<tr>
<th>T Cell Clone</th>
<th>TCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDC-2.5</td>
<td>Vα1 Vβ4</td>
</tr>
<tr>
<td>BDC-6.3</td>
<td>Vα3 Vβ4</td>
</tr>
<tr>
<td>2.5 Tg/T2-1 D6</td>
<td>Vα1 Vβ4</td>
</tr>
<tr>
<td>2.5 Tg/T2-2</td>
<td>Vα1 Vβ4</td>
</tr>
<tr>
<td>2.5 Tg/T2-X</td>
<td>Vα1 Vβ4</td>
</tr>
<tr>
<td>2.5 Tg/T2-1 D6</td>
<td>Vα1 Vβ4</td>
</tr>
<tr>
<td>2.5 Tg/T2-2</td>
<td>Vα1 Vβ4</td>
</tr>
<tr>
<td>2.5 Tg/T2-X</td>
<td>Vα1 Vβ4</td>
</tr>
</tbody>
</table>

| 2.5 Tg/T2-1 D6 | Vα1 Vβ4 |
| 2.5 Tg/T2-2  | Vα1 Vβ4 |
| 2.5 Tg/T2-X  | Vα1 Vβ4 |
of the Th2 cytokines IL-4, IL-6, and IL-10. The T cell clone 2.5 Tg/T2-1.D6 made no IL-10 detectable by ELISA and, like BDC-2.5, produced small but variable amounts of IL-2 (data not shown), but as described below, the in vivo activity of this clone was the same as the lines 2.5 Tg/T2-2 and 2.5 Tg/T2-X.

Transfer of TCR Tg/T2 lines and clones into young NOD recipients

To characterize the in vivo properties of the Tg/T2 lines and clones, clone transfer experiments were conducted in young NOD recipients. Recipient mice less than 14 days old were injected i.p. with Tg T cell lines or clones (1 × 10^7 cells/injection). The diabetogenic Th1 T cell clone BDC-2.5 was used as a positive control. With the two lines and the clone 2.5 Tg/T2-1.D6, these transfers resulted in a rapid onset of diabetes in recipient mice, with hyperglycemia developing by 14 days posttransfer (Table III). The very efficient transfer of disease by the Tg/T2 line into young NOD mice suggested that they might be working through recruitment of host T cells. To test whether our transgenic Th2 lines and clones could transfer diabetes to NOD.scid mice, we performed cotransfers of the Tg/T2 line 2.5 Tg/T2-X with diabetic spleen cells. Our rationale for performing this experiment was to determine whether the 2.5 Tg/T2-X line, which is diabetogenic in the young NOD, would have an accelerating effect on the diabetic spleen cells. As shown in Fig. 3a, cotransfer of the Tg/T2 line Tg/T2-X with diabetic spleen cells did indeed lead to an acceleration of disease onset. After cotransfer, 50% of recipients were diabetic by day 13, whereas when diabetic spleen cells were transferred alone, only by day 18 were 50% of recipients diabetic. This is a statistically significant acceleration with a p value of 0.0005 by Log-Rank analysis. Cotransfers were also performed using reduced numbers of diabetic spleen cells. As shown in Fig. 3, b and c, disease was accelerated to a

Transfer of TCR Tg/T2 lines and clones into young NOD recipients

Table II. Cytokine production by the Th1 T cell clone BDC-2.5 and by 2.5 TCR Tg/T2 line/clone

<table>
<thead>
<tr>
<th>T Cell Line/Clone</th>
<th>IFN-γ (ng/ml)</th>
<th>IL-4 (ng/ml)</th>
<th>IL-6 (ng/ml)</th>
<th>IL-10 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDC-2.5</td>
<td>30.7 ± 4.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2.5 Tg/T2-1.D6</td>
<td>0</td>
<td>0.14 ± 0.04</td>
<td>8.0 ± 3.0</td>
<td>0</td>
</tr>
<tr>
<td>2.5 Tg/T2-2</td>
<td>0</td>
<td>3.1 ± 0.3</td>
<td>6.6 ± 1.0</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>2.5 Tg/T2-X</td>
<td>0</td>
<td>4.8 ± 0.9</td>
<td>14.5 ± 7.9</td>
<td>4.2 ± 0.35</td>
</tr>
</tbody>
</table>

*a* Supernatants from Con A-stimulated cultures were assessed for cytokine content by specific cytokine sandwich ELISAs. For the 2.5 Tg/T2 lines and clone, results from several assays were averaged and the SD are shown in parentheses. Results from a representative assay are shown for BDC-2.5.

Table III. Diabetogenicity of the Th1 clone BDC-2.5 and of 2.5 TCR Tg/T2 lines and clones in young NOD.scid recipients

<table>
<thead>
<tr>
<th>Disease Incidence</th>
<th>Infiltrate</th>
<th>Granulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDC 2.5</td>
<td>12/12</td>
<td>3</td>
</tr>
<tr>
<td>2.5 Tg/T2-1.D6</td>
<td>4/5</td>
<td>0</td>
</tr>
<tr>
<td>2.5 Tg/T2-2</td>
<td>4/6</td>
<td>2.75</td>
</tr>
<tr>
<td>2.5 Tg/T2-X</td>
<td>6/9</td>
<td>2</td>
</tr>
</tbody>
</table>

*a* NOD recipients less than 14 days of age received either one or two injections of 1 × 10^7 T cell clones i.p. 1 wk apart. Although the parent Th1 clone BDC-2.5 caused diabetes rapidly and reproducibly, none of the transgenic Th2 lines and clones tested caused diabetes in young NOD.scid recipients. Granulation and infiltrate were assessed as described in Materials and Methods.

Table IV. Diabetogenicity of the Th1 clone BDC-2.5 and 2.5 TCR Tg/T2 lines and clones in young NOD.scid recipients

<table>
<thead>
<tr>
<th>Disease Incidence</th>
<th>Infiltrate</th>
<th>Granulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDC 2.5</td>
<td>9/10</td>
<td>3</td>
</tr>
<tr>
<td>2.5 Tg/T2-1.D6</td>
<td>0/4</td>
<td>0</td>
</tr>
<tr>
<td>2.5 Tg/T2-2</td>
<td>0/5</td>
<td>0</td>
</tr>
<tr>
<td>2.5 Tg/T2-X</td>
<td>0/17</td>
<td>0</td>
</tr>
</tbody>
</table>

*a* NOD.scid recipients 5–14 days of age received two injections of 1 × 10^7 T cell clones i.p. 1 wk apart. Although the parent Th1 clone BDC-2.5 caused diabetes rapidly and reproducibly, none of the transgenic Th2 lines and clones tested caused diabetes in young NOD.scid recipients. Granulation and infiltrate were assessed as described in Materials and Methods.
greater extent with transfer of two to four times fewer spleen cells. Fig. 4A shows islet infiltrate after transfer of diabetic spleen cells plus 2.5 Tg/T2-X. Although cotransferred mice became diabetic significantly earlier than mice that received diabetic spleen cells alone, histological analysis did not demonstrate any apparent difference between the two groups.

**Cotransfer of a TCR Tg/T2 line with a CD4+ Th1 diabetogenic T cell clone**

The T cell clone BDC-6.3 is from our original panel of islet-specific Th1 diabetogenic T cell clones (13), and although it can cause diabetes in young NOD mice, it cannot induce diabetes in the NOD.scid, even when the recipients are very young. To determine whether a CD4+ T cell clone alone could reconstitute an environment leading to disease induction by a diabetogenic Tg/T2 line, we conducted cotransfer experiments with the Tg/T2 line, 2.5 Tg/T2-X, and the BDC-6.3 clone in young NOD.scid mice. As shown in Fig. 5, 7- to 12-day-old NOD.scid mice transferred with either BDC-6.3 or 2.5 Tg/T2-X alone did not become diabetic by day 28 postinjection. In contrast, seven of nine mice that received both 2.5 Tg/T2-X and BDC-6.3 became diabetic by day 19. To demonstrate that this induction of disease was not merely a result of greater cell numbers (2×10^7 cells in a cotransfer vs 1×10^7 cells when clones were administered separately), we performed the cotransfer with 5×10^6 cells each of BDC-6.3 and 2.5 Tg/T2-X, and recipients (two of five) became diabetic by day 12 (data not shown). It should be noted that the recipients in this group became diabetic with the same kinetics as mice transferred with 2×10^7 total cells. We also found that transfer of 1×10^7 BDC-6.3 on day 0 and then injection of 1×10^7 2.5 Tg/T2-X on day 7 led to rapid diabetes onset in three of three recipients (data not shown). Furthermore, we determined that animals receiving 1×10^7 cells of either BDC-6.3 or 2.5 Tg/T2-X alone remained normoglycemic for greater than 60 days posttransfer (data not shown). Histological analysis showed no significant infiltrate or degranulation of islets in mice that received 2.5 Tg/T2-X alone (see Fig. 2C), although it is apparent that this clone gets into the pancreas upon administration i.p. In some cases, incomplete granulation was seen, as illustrated in Fig. 2A, but this is typical in 3- to 6-wk-old NOD.scid mice in which some islets are not completely granulated. Pancreatic sections from mice transferred with BDC-6.3 alone indicated that this clone could also migrate to the pancreas in that some islets showed mild to moderate infiltrate and degranulation, although most were free of infiltrate and completely granulated (Fig. 4B). In contrast, in the cotransferred animals, there was complete degranulation of islets (Fig. 4C) accompanied by an extensive mononuclear infiltrate. These data show that the Tg/T2 line 2.5 Tg/T2-X is not inert.
on day 0 with 2.3 scid mice received 1 Fb cells. Each group in

In this study, our goal was to isolate and establish long-term, Th2 T cell clones from the 2.5 TCR Tg mouse. Because the Tg T cells in culture generally do not survive past a week or two, probably due to their already high state of activation out of the animal. We have produced a panel of islet-specific T cell lines and clones from the 2.5 TCR Tg mouse that, upon in vitro stimulation, were found to produce several Th2 cytokines including IL-4, IL-6, and IL-10 but not IFN-γ. Despite their apparent Th2 phenotype, these T cell lines caused rapid diabetes onset in young NOD recipients. Surprisingly, however, transfer of these cells did not lead to diabetes in young NOD.scid mice, and theoretically NOD.scid mice less than 2 wk of age should be the most susceptible recipients.

This study is not the first attempt to obtain T cells with a Th2 phenotype from the 2.5 TCR Tg mouse. Katz et al. (33) previously reported success in producing IL-4-secreting T cells after culture of Tg T cells for 4 days under Th2-promoting conditions. These cultures were not diabeticogenic in young NOD mice but also were not protective. A later publication by Pakala et al. (34) reported that TCR Tg T cells cultured under similar short-term conditions caused diabetes in adult NOD.scid mice but not in young NOD recipients. It is hard to evaluate the significance of this latter observation because diabetes is readily induced in young NOD.scid mice using Tg T cells subjected to no treatment whatsoever (our unpublished data). Furthermore, because a recent report by other investigators indicated that short-term Th2 cultures could not transfer diabetes to adult NOD.scid mice (36), the result of Pakala et al. may not be a consistent finding. Our original goal was to determine whether long-term Tg T cell lines with consistent cytokine production patterns would in fact prove to have protective properties. The establishment of long-term, stable Th2 T cell lines (especially from a TCR Tg mouse with a TCR from a Th1 T cell clone) is an important point because it is well documented in the literature that T cell cultures of less than 3 wk do not have a fixed cytokine production profile (37–39). As demonstrated previously by Murphy et al. (38), repeated restimulation of T cell cultures with specific Ag and Th1 and Th2 growth conditions can yield lines and clones with a “locked-in” phenotype, a feature we deemed critical to carrying out these studies. Furthermore, in the same report, it was found that low-level production of Th1 cytokines by short-term “Th2” lines was not detectable by ELISA and could only be detected by intracellular staining. Disease can be induced in NOD.scid recipients with as few as 1 × 10^7 spleen cells from a TCR Tg donor, so even a very small, undetectable population of Th1-like cells could account for the in vivo action of short-term Th2 cultures.

In contrast to the report of Pakala et al. (34) with short-term cultures, the work we have presented here has clearly demonstrated that cloned T cell lines with a Th2 phenotype can be diabeticogenic in young NOD but not NOD.scid recipients. Furthermore, we have established that transfer effected by the Tg/T2 T cell lines takes place by a different mechanism than that of disease induced by Th1 T cell clones. Because the Tg/T2 lines readily cause diabetes in young NOD recipients but not in the lymphocyte-deficient NOD.scid, it was logical to speculate that they do so by recruiting host effector cells. This theory was supported by the experiment in which the Tg/T2 T cell line 2.5 Tg/T2-X was shown to accelerate disease transfer by diabetic spleen cells into young NOD.scid recipients. We felt that a more stringent test would be achieved if we could cotransfer the Tg/T2 cell line with a defined T cell clone incapable of causing disease in a NOD.scid recipient.

in the NOD.scid and is in fact able to induce disease in the presence of other effector T cells, even though it does not have the ability to cause diabetes on its own.

Discussion

In this study, our goal was to isolate and establish long-term, cloned Th2 T cell lines from the BDC-2.5 TCR Tg mouse and to characterize their in vitro and in vivo properties. This is the first report of Th2 T cell clones (or of any T cell clones) isolated from the 2.5 TCR Tg mouse. The significance of this achievement may not be readily obvious because the T cell repertoire of the Tg mouse is highly skewed toward T cells with the BDC-2.5 receptor; however, Tg T cells in culture generally do not survive past a week or two, probably due to their already high state of activation out of the animal.

FIGURE 3. Diabetes in young NOD.scid recipients transferred with diabetic spleen cells alone or diabetic spleen cells with the 2.5 TCR Tg/T2 line, 2.5 Tg/T2-X. a, NOD.scid mice 9–14 days of age were injected i.p. on day 0 with 2 × 10^7 diabetic spleen cells (○; n = 7) or with 2 × 10^7 diabetic spleen cells plus 1 × 10^7 2.5 Tg/T2-X cells (●; n = 7). b, NOD.scid mice received 1 × 10^7 diabetic spleen cells alone (○) or cotransferred with 1 × 10^7 2.5 Tg/T2-X cells (●). c, NOD.scid mice received 5 × 10^7 diabetic spleen cells alone (○) or cotransferred with 1 × 10^7 2.5 Tg/T2-X cells (●). Each group in b and c contained three recipients. a represents data combined from two separate experiments.
The Th1 T cell clone BDC-6.3 is another CD4\(^+\) islet-specific clone from our original panel (13). This clone is very diabetogenic in young NOD mice but cannot by itself cause disease in the NOD-\(\text{scid}\). Therefore, it was an excellent candidate for examining whether the Tg/T2 clone worked by “recruiting” other effector cells, and indeed, we found that cotransfer with BDC-6.3 led to rapid onset of diabetes in young NOD-\(\text{scid}\) recipients. The host cells in the NOD recipient might well include CD8 T cell effectors, but as in other work we have published (20), it is clear that a CD4 Th1 T cell clone is all that is needed for disease induction to take place.

One interpretation of our results with the Th2 T cell clones from the TCR Tg mouse is that these clones become “Th1-like” in vivo. We think this is unlikely. The parent BDC-2.5 clone causes disease in both young NOD and NOD-\(\text{scid}\) recipients, and at least in our hands, spleen cells from the 2.5 TCR Tg/NOD mouse transfer diabetes to young (and in some cases adult) NOD-\(\text{scid}\) mice. On the other hand, the Th2 T cell clones from the 2.5 TCR Tg mouse can induce disease only in young NOD mice (unless other cell populations are provided).

It is tempting to speculate that because the Th2 T cell clones from the Tg mouse bear the receptor of what is obviously a very autoaggressive T cell, they are diabetogenic because of their Ag specificity. Regardless of the fact that they secrete Th2 cytokines, by virtue of their islet reactivity they can initiate an inflammatory reaction in the pancreas that attracts other Th1 effector T cells to the site. Because most of the Tg/T2 T cell clones we have isolated fall into this category of being diabetogenic in the young NOD, this is an attractive hypothesis. However, we have isolated one Tg/T2 T cell clone that is not diabetogenic in any circumstance we have tested, and this would suggest that the Ag specificity may not be the only explanation. This clone, like the others in the Tg/T2 panel, bears both the V\(\beta\)4 and the V\(\alpha\)1 transgene from BDC-2.5. However, it is possible that it also bears some endogenous TCR V\(\alpha\) that alters its functional reactivity.

There have been a variety of reports in the literature describing T cell lines and clones with protective properties (reviewed in Ref. 25). There has also been much indirect evidence for a protective role for T cells with the Th2 cytokine phenotype (i.e., IL-4/IL-10-secreting), including prevention of disease with recombinant IL-4 and IL-10 therapy (29, 32) and transfer of protection with spleen cells from NOD mice treated with IL-4 (30) or, as demonstrated more recently, with T cells from mice immunized with GAD-65 (40). Gallichan et al. (41) have also recently shown that islet-reactive Th2 cells are responsible for the protection from diabetes

**FIGURE 4.** Representative histology of pancreatic sections stained with A/F from young NOD-\(\text{scid}\) recipients transferred with one of the following: diabetic spleen cells and 2.5 Tg/T2-X (A), BDC-6.3 (B), or BDC-6.3 and 2.5 Tg/T2-X (C). Arrows indicate islet tissue. C lacks arrows because all islet tissue has been disrupted or destroyed. Magnification for all photos is \(\times100\). If histology was scored as in Tables III and IV, lesion severity in A and C would have been scored as a 3 for infiltration and a 0 for granulation.

**FIGURE 5.** Cotransfer of the 2.5 TCR Tg/T2 line 2.5 Tg/T2-X with a CD4\(^+\) Th1 diabetogenic T cell clone BDC-6.3. Young NOD-\(\text{scid}\) recipients 7–14 days of age were injected with \(1 \times 10^7\) of BDC-6.3 (\(\bigcirc\); \(n = 6\)), with \(1 \times 10^7\) of 2.5 Tg/T2-X (\(\triangle\); \(n = 17\)), or with \(1 \times 10^7\) of each (\(\bigtriangleup\); \(n = 9\)) on days 0 and 7 of the experiment. By day 19, seven of nine cotransferred recipients were diabetic. Recipients of 2.5 Tg/T2-X or BDC-6.3 alone did not become diabetic by the time the experiment was terminated at 28 days. The figure represents data combined from two separate experiments, except for the 2.5 Tg/T2-X-only group, which represents animals combined from six experiments done identically.
observed in transgenic NOD mice that bear the IL-4 gene under the rat insulin promoter. The fact remains, underscoring the importance of the work reported here, that there has been no clear demonstration with defined, islet-reactive Th2 T cell clones (i.e., IL-4, IL-10, and IFN-γ) that this type of T cell can delay, arrest, or prevent disease in the NOD mouse. We were surprised to find that cloned T cell lines with a reproducible pattern of Th2 cytokine production were not only not protective but were in fact diabetogenic. Two major questions that arise from our work are how a Th2 T cell clone can be diabetogenic and what the requirements are for a Th2 T cell clone to be protective. Whether the diabetogenic properties of our Tg/T2 cells are due to the specificity for Ag dictated by the BDC-2.5 TCR or to a lack of a necessary factor (e.g., TGF-β) is being addressed in studies underway. Work in progress with another panel of Th2 T cell clones derived from nontransgenic NOD mice and not yet demonstrated to be diabetogenic may help to answer these questions.

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References

10. Serreze, D. V., E. H. Leiter, S. M. Worthen, and L. D. Shultz. 1988. NOD marrow (e.g., TGF-β) is being addressed in studies underway. Work in progress with another panel of Th2 T cell clones derived from nontransgenic NOD mice and not yet demonstrated to be diabetogenic may help to answer these questions.