Development and Function of Diabetogenic T-cells in B-cell–Deficient Nonobese Diabetic Mice

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Insulin-dependent diabetes (type 1 diabetes) in the NOD mouse is a T-cell–mediated autoimmune disease. However, B-cells may also play a critical role in disease pathogenesis, as genetically B-cell–deficient NOD mice (NOD.μMT) have been shown to be protected from type 1 diabetes and to display reduced responses to certain islet autoantigens. To examine the requirements for B-cells in the development of type 1 diabetes, we generated a B-cell–naive T-cell repertoire by transplantation of NOD fetal thymuses (FTs) into NOD.*scid* recipients. Surprisingly, these FT-derived NOD T-cells were diabetogenic in 36% of NOD.*scid* recipients, despite the absence of B-cells. In addition, T-cells isolated from NOD.μMT mice were diabetogenic in 22% of NOD *.scid* recipients. Together, these results indicate that B-cells are not an absolute requirement for the generation or effector function of an islet-reactive T-cell repertoire in NOD mice. We suggest that conditions favoring rapid lymphocyte expansion can reveal autoreactive T-cell activity and precipitate disease in genetically susceptible individuals. *Diabetes* 50:763–770, 2001

Type 1 diabetes in the NOD mouse is characterized by the infiltration of T-cells and antigen (Ag)-presenting cells (APCs) into the pancreatic islets of Langerhan (insulitis), resulting in the eventual destruction of insulin-producing pancreatic β-isle T-cells (1). Diabetes pathogenesis in NOD mice is T-cell–dependent; adoptive transfer of splenic T-cells from NOD donors into irradiated NOD or NOD mice congenic for the *scid* mutation (NOD.*scid*) causes type 1 diabetes in the recipients (2,3). It has been shown that B-cells are not required at the effector stage of NOD type 1 diabetes, as T-cells from diabetic NOD donors transfer type 1 diabetes to B-cell–depleted NOD neonates (4). However, recent evidence suggests that B-cells play an important role in the development of autoreactivity in NOD type 1 diabetes; NOD mice rendered B-cell deficient by antibody treatment are protected from the development of insulitis and diabetes (5–8). Using a genetic approach, several groups have shown that NOD mice homozygous for a germline mutation that disables production of membrane-bound Igμ (μMT), resulting in maturational arrest of B-cell development, affords protection against type 1 diabetes (6,8). However, the mechanism, or mechanisms, by which B-cells affect type 1 diabetes pathogenesis is unclear. B-cells could influence autoimmune disease through autoantibody production or as APCs involved in the selection or activation of autoreactive T-cells. High titers of anti-insulin and anti-GAD autoantibodies are found in prediabetic NOD mice and diabetic humans (9–11), but in contrast to T-cells, autoantibodies fail to transfer disease in NOD mouse models. When activated, B-cells become competent APCs (12) that may capture and present isle T-cell Ags to T-cells (13,14). In addition, Ag presentation by B-cells in the thymus could affect selection of the T-cell repertoire (15). Thus, there are several mechanisms by which B-cells could affect susceptibility to and onset of type 1 diabetes.

We present an independent approach to analyze the requirement(s) for B-cells in the development and function of an islet-reactive T-cell repertoire in NOD mice. To study the function of an NOD T-cell repertoire in the absence of B-cells, we transplanted NOD.NON-*Thy1*^{a} (NOD.*Thy1*^{a}) fetal thymuses (FTs) into NOD.*scid* mice, resulting in reconstitution of the recipients with NOD.derived *Thy1*^{a}-marked T-cells. Surprisingly, NOD.*scid* mice reconstituted with NOD FT-derived T-cells developed type 1 diabetes. Parallel experiments using donor T-cells from genetically B-cell–deficient NOD.μMT mice showed that T-cells from these mice were also able to transfer diabetes to a low frequency of NOD.*scid* recipients. Rather, our results indicate that B-cells are not absolutely required for the development of an autoimmune repertoire or for the effector function against isle T-cells. These results indicate that autoreactive T-cells generated in B-cell–deficient NOD animals can become pathogenic under conditions of T-cell reconstitution and expansion in lymphoid-deficient hosts.

RESEARCH DESIGN AND METHODS

Mice used in these studies were maintained in a pathogen-free facility at the Hospital for Sick Children, Toronto, Ontario, Canada, in which the incidence of diabetes was 80–85% for NOD females and 10–15% for NOD males at 30 weeks of age. NOD.*Thy1*^{a} congenic mice were obtained from Jackson Laboratories (Bar Harbor, ME) and bred in our facility. These mice were maintained by brother-sister mating at N8 backcross to NOD. The incidence of diabetes in our NOD.*Thy1*^{a} colony was 60–75% for females and 5–10% for males at 30 weeks of age. B-cell–deficient NOD.μMT mice were generated as described (8) and used at the N10 backcross to NOD. NOD.*scid* mice (Jackson Laboratories) were at the N10 backcross to NOD.

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Received for publication 28 August 2000 and accepted in revised form 22 December 2000.

Ab, antibody; Ag, antigen; APC, Ag-presenting cell; BSA, bovine serum albumin; Cμ2A, Ig Cμ 3′-primer; DC; dendritic cell; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; FT, fetal thymus; Igh, Ig heavy chain; KLH, keyhole limpet hemocyanin; LN, lymph node; mAb, monoclonal antibody; MHC, major histocompatibility complex; μMT, Igμ constant transmembrane region; PBL, peripheral blood lymphocyte; PBS, phosphate-buffered saline; PI, propidium iodide; RT-PCR, reverse transcriptase–polymerase chain reaction; VHALL, Ig Vh 5′-primer.

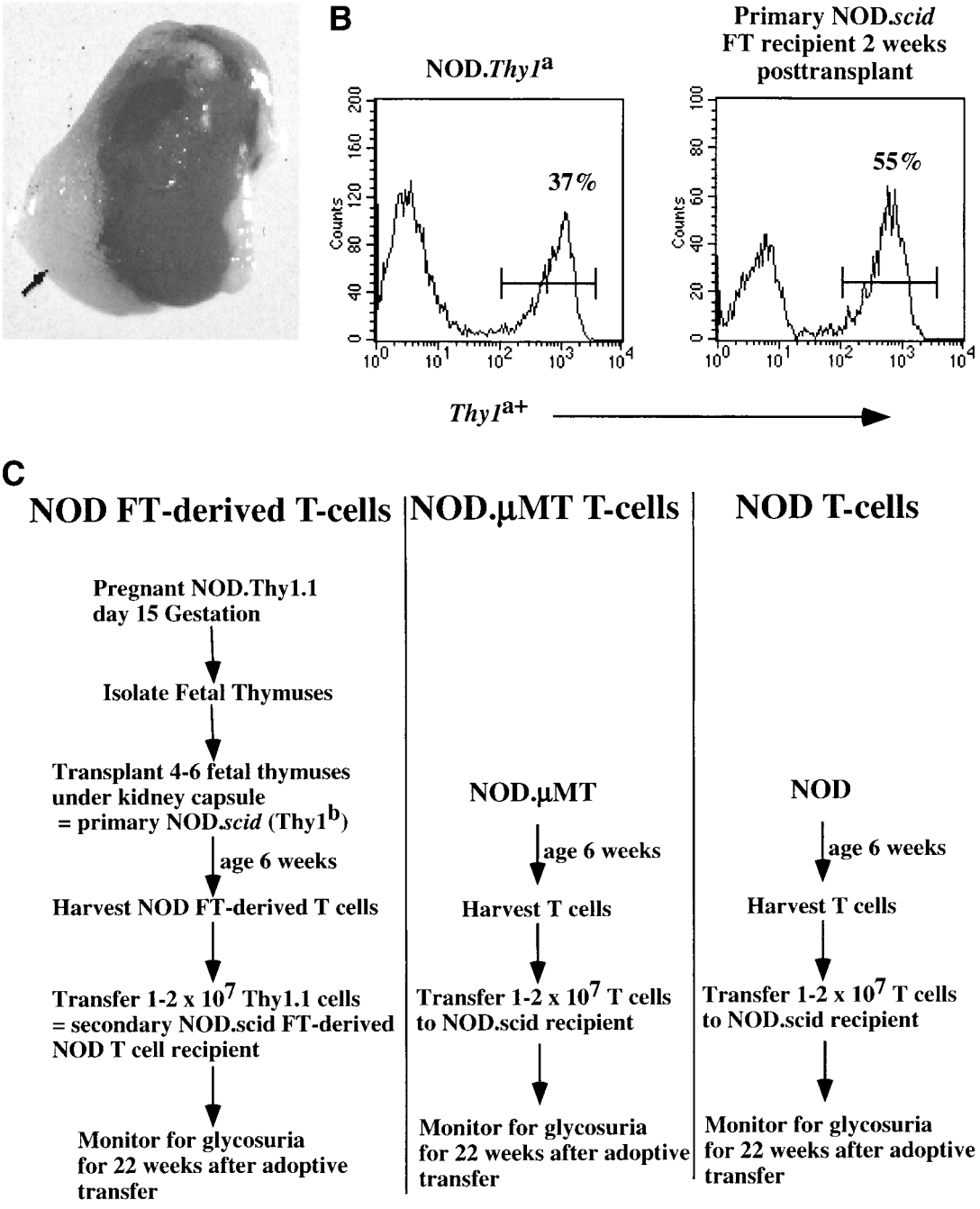

FIG. 1. Reconstitution of NOD.*scid* mice with *Thy1*$^{a+}$ FT-derived T-cells. T-cells from the NOD.*Thy1*^{a} NOD congenic strain express the *Thy1*^{a} allotype, whereas NOD.*scid* T-cell precursors express *Thy1*^{b}. NOD.*Thy1*^{a} females were bred with plug date designated as day 1. Four-week-old NOD.*scid* recipients were transplanted with 4–6 lobes of day-15 NOD.*Thy1*^{a} FTs under the left kidney capsule (RESEARCH DESIGN AND METHODS). Two weeks posttransplant, the NOD.*scid* FT recipients were examined for FT-derived T-cell reconstitution in the peripheral blood by flow cytometry using FITC-conjugated anti-*Thy1*^{a} mAb (19XE5). Dead cells were excluded by PI staining. *A*: A kidney from a transplanted NOD.*scid* recipient 4 weeks posttransplant, showing the NOD.*Thy1*^{a} thymus tissue under the kidney (↗). *B*: A representative FACS experiment showing the percentage of T-cells (*Thy1*$^{a+}$) in the PBL of a 4-week-old NOD.*Thy1*^{a} mouse and a NOD.*scid* recipient of NOD.*Thy1*^{a} FT. Compared with the PBL of NOD.*Thy1*^{a} control, the NOD.*scid* FT recipient was successfully reconstituted with *Thy1*^{a} FT-derived T-cells. *C*: Generation of NOD FT-derived B-cell–naive T-cells in NOD.*scid* recipients. The experimental protocol for transplantation of NOD.*Thy1*$^{a+}$ FT into primary NOD.*scid* recipients followed by adoptive transfer of FT-derived T-cells into secondary NOD.*scid* recipients can be found in RESEARCH DESIGN AND METHODS.

FT transplant. Embryonic day 15 (coitus plug = day 1) NOD.*Thy1*^{a} fetal thymuses were isolated, and four to six fetal lobes were transplanted under the left kidney capsule of 3- to 4-week-old NOD.*scid* recipients (Fig. 1*A*). Reconstitution of peripheral blood lymphocytes (PBL) with *Thy1*$^{a+}$ FT-derived T-cells (FT–T-cells) was assessed 2 weeks postoperatively. After red cell lysis, PBLs were stained with fluorescein isothiocyanate (FITC)-conjugated anti-*Thy1*^{a} monoclonal antibody (mAb) (19XE5) and anti-*Thy1*^{b} mAb (53-2.1) for analysis on the FACSCalibur (Becton Dickinson, Mountain View, CA). Dead cells were excluded by propidium iodide (PI) staining. Fluorescence-activated cell sorter (FACS) analysis of 5×10^3 cells was performed to assess reconstitution by the FT-derived T-cells (Fig. 1*B*). Data analyses were performed using CellQuest software (Becton Dickinson).

Adoptive transfer of *Thy1*$^{a+}$, FT-derived, and NOD.µMT T-cells. FT recipients were killed 7 weeks posttransplant, and peripheral lymph nodes (LNs) and spleens were isolated. LN and spleen cell suspensions were prepared and immunodepleted with anti–class II major histocompatibility complex (MHC) (10-2.16), anti-B220 (RA3-6B2), and anti-Mac-1 (M1/70) mAb, and magnetic beads (PerSeptive Biosystems, Framingham, MA). NOD.*scid*

immature T-cell precursors were depleted using anti-*Thy1*^{b} (53-2.1) mAb. Ten to twenty million *Thy1*$^{a+}$ FT–T-cells were injected intraperitoneally into each NOD.*scid* recipient (secondary recipients). Twenty million splenic T-cells from 6-week-old NOD.μMT mice were purified by immunodepletion with M1/70 and 10-2.16 and transferred into NOD.*scid* recipients. Similarly, 2×10^7 T-cells from 6-week-old NOD.*Thy1*^{a} mice, enriched by immunodepletion using M1/70, RA3-6B2, 10-2.16, and magnetic beads, were transferred into NOD.*scid* recipients as positive controls. NOD.*scid* recipients injected with phosphate-buffered saline (PBS) served as negative controls. All NOD.*scid* recipients were monitored for glycosuria weekly using Tes-Tape (Eli Lilly, Toronto) for 22 weeks postadoptive transfer. Recipients were scored as diabetic if they were positive for glycosuria by Tes-Tape on 2 consecutive days. Recipients that remained euglycemic at 22 weeks postadoptive transfer were killed, and their pancreata were isolated for histological examination.

Analysis of FT–T-cell recipients for the presence of mature B-cells. NOD.*scid* recipients of FT–T-cells were assessed for the presence of B-cells using flow cytometry and reverse transcriptase–polymerase chain reaction (RT-PCR). For flow cytometry, 1×10^6 LNs and spleen cells were stained with FITC-conjugated anti-B220 mAb (RA3-6B2) and biotinylated anti-CD19 mAb (1C3; Pharmingen). Stained cells were analyzed on the FACSCalibur. Ten thousand live (PI-excluding) cells were collected for each recipient. For RT-PCR analysis, RNA was prepared from spleen cells using Trizol (Gibco BRL, Burlington, ON, Canada). RNA isolated from 1×10^6 cells were used for the synthesis of cDNA using Superscript II reverse transcriptase (Life Technologies, Burlington ON, Canada), and NOD spleen RNA was used in mock (minus RT) control reactions. A PCR strategy was used to detect V(D)J recombination at the Ig heavy chain (Igh) locus (16). A degenerate Ig Vh 5′-primer (VHALL) and an Ig Cμ 3′-primer (Cμ2A) were used to amplify the cDNA as previously described (16). PCR products of 700 bp reflected transcription of the rearranged Igh locus. PCR products were separated on 0.8% agarose gels and transferred to nylon membranes (ZetaProbe; BioRad Laboratories, Hercules, CA). Southern blot hybridization analysis was performed using an Igμ constant region probe (17). Images were collected on a phosphorscreen (Molecular Dynamics, Sunnyvale, CA) and analyzed by ImageQuant software (Molecular Dynamics). β-actin PCR controls were performed simultaneously for each cDNA sample. Assay sensitivity was determined by titrations of cDNA from 10×10^6 to 1×10^6 NOD spleen cells.

Histological examination of pancreata of adoptive transfer recipients. Pancreata from all recipients were evaluated by histological examination of sections as described (18). Insulitis was scored on a scale of 0–4, where 0 represents the absence of insulitis and 4 represents severe invasive insulitis obscuring the islet. Immunohistochemistry of 8-μm serial sections was used to detect B220$^+$ B-cells and *Thy1*$^{a+}$ or *Thy1*$^{b+}$ T-cells. Pancreatic sections were fixed with ethanol and blocked with 50 mg/ml bovine serum albumin (BSA). Pancreatic sections were incubated in 1% peroxide to inhibit endogenous peroxidase activity. The slides were washed with PBS + 1% BSA (PBS + BSA) before staining with biotinylated anti-B220 (RA3-6B2) mAb (Pharmingen), anti-*Thy1*^{a} (19XE5) mAb, and anti-*Thy1*^{b} (53-2.1) mAb (Pharmingen). The sections were rinsed with PBS + BSA before incubation with streptavidin-conjugated peroxidase followed by chromagen stain (Sigma Chemicals, St. Louis, MO). The slides were counterstained with hematoxylin and rinsed in PBS + BSA.

RESULTS

Generation of B-cell–deficient NOD mice by FT transplantation. FT transplantation was used to generate a B-cell–naive T-cell repertoire of NOD origin (19). Thymuses were removed from embryonic day 15 NOD.*Thy1*^{a} fetuses and transplanted under the kidney capsule of 3- to 4-week-old NOD.*scid* recipients (primary recipients) (Fig. 1*A*). The procedure resulted in good reconstitution of NOD.*scid* recipients with *Thy1*$^{a+}$ FT-derived T-cells as early as 2 weeks posttransplant (Fig. 1*B*). By 7 weeks posttransplant, these primary NOD.*scid* hosts were fully reconstituted with FT-derived T-cells. *Thy1*$^{a+}$ FT–T-cells were recovered from the primary recipients and transferred into secondary NOD.*scid* hosts (Fig. 1*C*). Parallel experiments were performed to compare the diabetogenic potential of the NOD FT–T-cells with age-matched T-cells from unmanipulated NOD donors. Mice homozygous for a targeted mutation disrupting the Igμ constant transmembrane region (μMT) are genetically B-cell deficient (20). On the NOD background, this mutation (NOD.μMT) was previously reported to significantly reduce diabetes frequency (8). Here we performed parallel experiments to compare the diabetogenic potential of NOD.μMT T-cells with NOD FT-derived T-cells transferred into NOD.*scid* recipients (Fig. 1*C*). This approach enabled direct comparison of the diabetogenic potential of normal, with two different sources of B-cell–naive NOD T-cells. In both of the experimental groups, the NOD FT-derived and NOD.μMT T-cell repertoire developed in the absence of B-cells and was assessed for autoimmune effector function in a B-cell–deficient environment.

B-cell deficiency in NOD.*scid* FT–T-cell recipients. Interpretation of these experiments depended on verification of B-cell deficiency in NOD.*scid* recipients of NOD FT-derived and NOD.*scid* (21) T-cells. Rare B-cell precursors are present in day-15 FT, but their development has been found to require addition of exogenous cytokines (22). In addition, rare Ig$^+$ B-cells have been observed in aged CB-17.*scid* (23–25) and NOD.*scid* (21) mice, providing another potential source of B-cell contamination in the NOD FT-derived T-cell–reconstituted animals. We used two approaches to test for B-cells in FT-derived T-cell–reconstituted primary and secondary NOD.*scid* recipients (Fig. 1*C*). Two-color flow cytometric analysis of primary and secondary NOD.*scid* recipients of FT-derived T-cells was performed using antibodies to the B-cell markers B220 and CD19. B220$^+$CD19$^+$ cells were undetectable in the LNs and the spleens of both primary and secondary NOD.*scid* FT-T recipients. Indeed, these profiles were indistinguishable from age-matched unmanipulated NOD.*scid* mice (Fig. 2*A*). As an independent assessment, we used an RT-PCR approach followed by Southern blot hybridization to detect Igμ mRNA transcripts resulting from VDJμ recombination in the splenocytes of secondary NOD.*scid* FT-derived T-cell recipients (16). Titration experiments showed that this technique could detect Igμ transcripts in as few as 10 spleen cell equivalents from normal NOD animals (Fig. 2*B*, panel i). Under these experimental conditions, we could not detect full-length Ig VDJμ-containing transcripts in the splenocytes of NOD.*scid* FT–T-cell recipients (Fig. 2*B*, panel ii). Occasionally, we observed Igμ amplicons in NOD.*scid* mice (Fig. 2*B*, panel ii), which is consistent with reports that V(D)J recombination occurs with low frequency in *scid* mice (25,26). Together, the flow cytometry and PCR analyses strongly suggest that both primary and secondary NOD.*scid* mice harboring NOD FT-derived T-cells were indeed B-cell deficient.

Incidence of insulitis and diabetes in NOD.*scid* T-cell recipients. NOD.*scid* recipients of normal NOD, NOD FT-derived, and NOD.μMT T-cells were followed for 22 weeks postadoptive transfer to assess diabetes onset. A total of 66% of NOD.*scid* recipients of 6-week-old NOD T-cells became diabetic within this observation period (Table 1, Fig. 3). Unexpectedly, 40% of the recipients of NOD FT-derived T-cells and 22% of the recipients of NOD.μMT T-cells became diabetic during the observation period (Table 1, Fig. 3). These results suggest that B-cell–naive NOD T-cells created either by FT transplantation into NOD.*scid* recipients or by introgression of the μMT mutation onto the NOD background have the potential to cause diabetes in NOD.*scid* hosts.

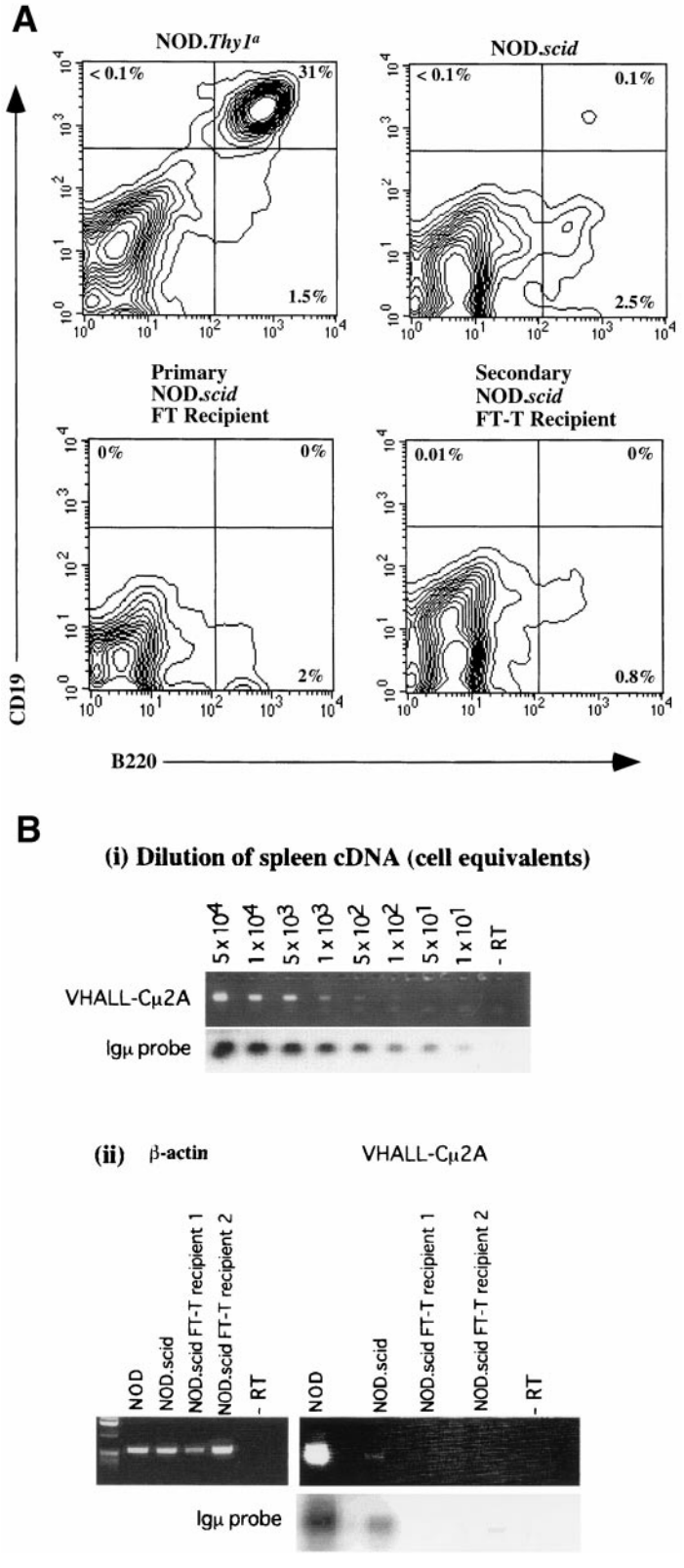

FIG. 2. The absence of B-cells in NOD.*scid* recipients of FT or FT-derived T-cells. *A*: LNs from 20-week-old NOD.*Thy1*^{a}, NOD.*scid*, primary NOD.*scid* FT recipients, and secondary NOD.*scid* FT-derived T-cell recipients were prepared into cell suspensions and stained with FITC-conjugated anti-B220 (RA3-6B2) and biotinylated anti-CD19 (1C3). Dead cells were excluded by PI staining. Diabetic secondary NOD.*scid* FT-derived recipients were also analyzed for the presence of B-cells by flow cytometry. In the NOD.*scid* mouse, B220$^+$CD19$^+$ cells constituted <0.1% of the LN population. In NOD.*scid* recipients of primary NOD FT and secondary NOD FT-derived T-cells, B220$^+$CD19$^+$ cells were not detectable by flow cytometry. *B*: RNA was isolated from 1×10^6 splenocytes from age-matched NOD, NOD.*scid*, and diabetic secondary NOD.*scid* FT-T recipients and reverse transcribed into cDNA. To detect Ig μ gene transcriptional product, a degenerate VHALL 5′ primer and a 3′ primer, Cμ2A, in the constant region of the Igh were used to amplify the cDNA. PCR products of 700 bp represent mRNA resulting from VDJ gene rearrangement at the Igh locus. β-actin controls were performed simultaneously for each cDNA sample. Southern blot analysis of the VHALL-Cμ2A PCR products was performed using ^{32}P-labeled Igμ probe. *B*, panel *i*: Sensitivity of the RT-PCR assay as indicated by the dilution of cDNA ranging from 5×10^4 to 10 spleen cell equivalents. *B*, panel *ii*: Assay of Igμ mRNA from 5×10^4 cell equivalents of NOD, NOD.*scid*, and secondary NOD.*scid* FT-T recipients.

TABLE 1
Diabetes incidence in NOD.*scid* recipients of NOD, NOD.μMT, and NOD FT-derived T-cells

T-cell donor	Incidence of diabetes	Time to diabetes average (days)
6-week-old NOD	67 (6 of 9)	114 (96–149)
6-week-old FT-derived NOD.*Thy1*^{a}	36 (8 of 22)	115 (94–182)
6-week-old NOD-μMT	22 (2 of 9)	130 (126–134)
PBS-control	0 (0 of 6)	

Data are % (*n*) or means (range).

Histological examination was performed on the pancreata of all NOD.*scid* T-cell recipients either at the time of diabetes onset or at 22 weeks post–T-cell transfer, whichever came first. Diabetic recipients of either NOD FT-derived or NOD.μMT T-cells displayed severe invasive insulitis (data not shown). Using immunohistochemical staining, the islet-infiltrating cells in these animals were of donor origin (*Thy1*$^{a+}$), and neither *Thy1*$^{b+}$ nor B220$^+$ cells were detected in the lesions (data not shown). NOD.*scid* recipients of NOD FT-derived or NOD.μMT T-cells that remained euglycemic 22 weeks after adoptive transfer were also examined for pancreatic infiltration. Among these mice, 2 of 7 recipients of NOD.μMT T-cells and 5 of 14 recipients of NOD FT-derived T-cells had mean insulitis scores >1.5; this is suggestive of progression toward invasive insulitis (Fig. 4). Some of these animals might have become diabetic over a longer observation period after T-cell transfer. Altogether, 13 of 22 (59%) recipients of NOD FT-derived T-cells and 4 of 9 (44%) recipients of NOD.μMT T-cells demonstrated invasive insulitis and/or diabetes 22 weeks after adoptive transfer, despite the absence of B-cells.

DISCUSSION

Several groups have reported that B-cells are critical for the development of NOD insulitis and type 1 diabetes (6–8). A suggested mechanism of this effect is that NOD B-cells are uniquely capable of capturing and presenting islet Ags associated with NOD type 1 diabetes in the context of the MHC class II molecules (13,14,27). Although NOD.μMT mice are protected from type 1 diabetes, some animals became diabetic with the same kinetics as B-cell–sufficient NOD littermates, albeit with a much lower incidence (28). In this report, we present evidence that insulitis and diabetes can develop in the absence of NOD B-cells, and we suggest that under some conditions macrophages (Mϕs) and dendritic cells (DCs) are sufficient to select an autoreactive T-cell repertoire and to precipitate autoimmune disease in NOD mice.

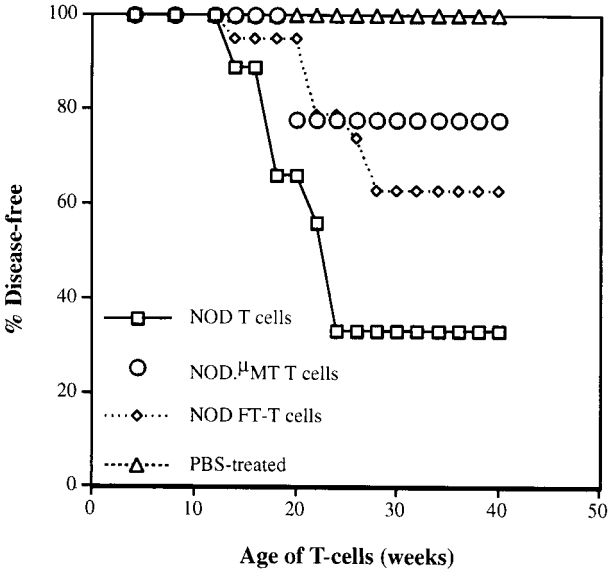

FIG. 3. Diabetes incidence in recipients of NOD, NOD FT-T, and NOD.μMT T-cells. NOD.*scid* recipients of T-cells from 6-week-old NOD (n = 9) (□), NOD.*Thy1*^{a} FT-T (n = 22) (◇), NOD.μMT (n = 9) (○) donors, or PBS treatment (n = 6) (△) were monitored for glycosuria for 22weeks postadoptive transfer.

There are multiple potential explanations for the difference observed in the NOD FT-derived and NOD.μMT T-cell transfer models compared with previously published results in NOD.μMT and anti–Igμ-treated NOD mice. Previously, gene-targeted disruption of the membrane-bound form of the IgM (μMT) B-cell receptor (6,8,28) or anti-Igμ treatment (5,7) was used to generate B-cell–deficient NOD mice. The μMT mutation was bred from a mixed 129/Sv × C57BL/6 background onto the NOD background. As a result, protective alleles at insulin-dependent diabetes loci (*Idd*) could have been transferred along with the μMT mutation, decreasing disease susceptibility in NOD.μMT mice. We do not favor this explanation, because the diabetes protection in NOD.μMT mice was demonstrated after the sixth backcross (N7) generation, and these mice were typed for microsatellite markers linked to 15 of the 19 known *Idd* susceptibility loci (8). The NOD.μMT animals used in the current study were products of three additional back-crosses (N10) and were typed for additional markers: D3Mit100 near the *Idd*10, 17, and 18 region and D10Mit87 near the *IFNγR* gene (data not shown) recently reported to be linked to a diabetes protection locus in 129/Sv mice (29). Diabetes incidence in NOD.μMT mice housed at the Jackson Laboratories from 1996 to the present is low, but detectable (3–5%) (D.V. Serreze, unpublished data); this is consistent with the idea that this mutation provides substantive but not absolute protection from diabetes. In independent studies, in vivo treatment with anti-Igμ Abs has suggested that removal of B-cells in the neonatal period protects NOD mice against type 1 diabetes. Furthermore, treatment of neonatal NOD mice with natural Ig alters diabetes development, although the mechanism of protection is unclear (30). In both instances, in vivo Ab treatment may provoke immunological modulations in addition to clearance or alteration of B-cell function. In contrast, our approach using NOD FT-derived T-cells did not require manipulation of the NOD genome or in vivo Ab treatments and thereby avoided effects independent of B-cell depletion.

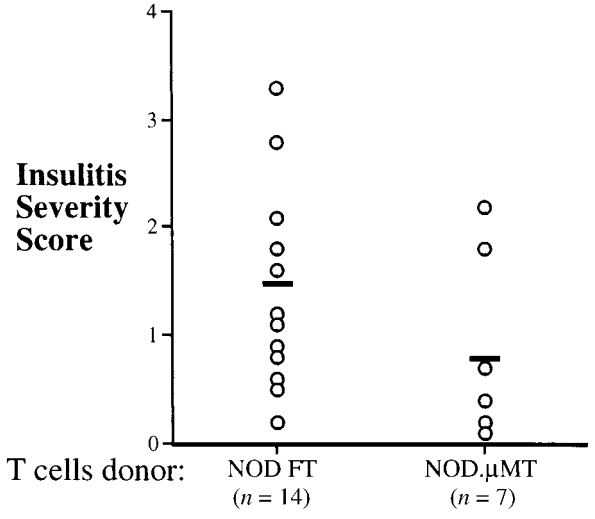

FIG. 4. Histological analysis of insulitis in euglycemic NOD.*scid* recipients of NOD, NOD FT-derived, and NOD.μMT-derived T-cells. Pancreatic sections of NOD.*scid* recipients that remained euglycemic 22 weeks after adoptive transfer were examined for insulitis (RESEARCH DESIGN AND METHODS). Data are shown for NOD.*scid* recipients of NOD FT-derived (n = 14) and NOD.μMT T-cells (n = 7). A total of 8-μ serial sections were made at 100-μ intervals, and 30 islets were examined in each recipient. For each islet, a score from 0 to 4 was given to indicate insulitis severity (0 = absence of insulitis and 4 = severe and complete invasive insulitis). An average insulitis score was calculated for each recipient. The bar represents the mean insulitis score from the average scores of each cohort. Note that the histogram plot does not resolve animals in the same group that had very similar insulitis scores, as shown by the individual circles, so the designated number of animals per group exceeds the number of circles.

T-cell responses to a variety of foreign Ags are poor in NOD.μMT homozygous mice compared with NOD.μMT/$^+$ and NOD controls, suggesting that the mutation causes global rather than autoantigen-specific effects on cellular immunity. For example, C57BL/6-μMT mice have reported defects in delayed-type hypersensitivity responses to in vivo challenges with pathogens such as *Chlamydia* (31), *Mycobacteria* (32), and *Listeria* (33). Furthermore, priming of NOD.μMT mice with keyhole limpet hemocyanin (KLH) generated lower recall responses compared with NOD T-cells (27), although T-cell responses to KLH were readily observed in C57BL/6-μMT mice (34). These data suggest that NOD.μMT T-cell responses to a broad array of antigens may be significantly different from the NOD parental strain. If this is indeed the case, the diabetes protection observed in NOD.μMT mice may reflect a general impairment in T-cell responses in addition to a specific requirement for B-cells in generation and function of diabetogenic T-cells.

A fundamental feature of autoimmune diabetes susceptibility may be the extent and the kinetics of self-reactive T-cell proliferation and apoptosis in peripheral tissues. Indeed, NOD T-cells demonstrate enhanced proliferation (35) and resistance to apoptosis (36–39) compared with T-cells from nondiabetes-prone strains. Based on these findings, our adoptive transfer approach into NOD.*scid* recipients may favor the expansion of islet-reactive NOD FT-derived and NOD.μMT T-cells precipitating the development of type 1 diabetes. NOD.*scid* mice are excellent adoptive transfer recipients because of the absence of

mature lymphocytes, depressed natural killer cell cytolytic activity, and the absence of circulating complement (21). The paucity of mature lymphocytes in NOD.*scid* mice permits rapid expansion of donor T-cells. We have shown that FT-derived T-cells mature and disseminate in the lymphoid organs of primary and secondary NOD.*scid* recipients. Perhaps the T-cell expansion caused by repopulation of the NOD.*scid* host is permissive for expansion and activation of autoreactive T-cells. Consistent with this idea, we show that the autoreactive T-cells in NOD.μMT mice that rarely cause diabetes in situ have heightened pathogenic potential when transferred into the lymphopenic NOD.*scid* microenvironment.

Multiple independent lines of evidence support this reasoning in the context of autoimmune diabetes. The first comes from analyses of the Diabetes-Prone BioBreeding (DP-BB) rat and the related DR-BB strain. In DP-BB rats, a locus (*iddm1)* conferring severe T-cell lymphopenia (*lyp)* is required for spontaneous diabetes, but the *lyp* gene has not yet been identified (40,41). Interestingly, reconstitution of DP-BB animals with histocompatible T-cells from normal rats affords partial protection from diabetes, highlighting the direct contribution of T-cell lymphopenia to diabetes development (42,43). Although the nonlymphopenic DR-BB strain is diabetes resistant under normal conditions, interventions that produce lymphopenia, such as ionizing radiation and cyclophosphamide, induce type 1 diabetes in this strain (44,45). Recent studies demonstrate that the diabetogenic potential of the DR-BB T-cell repertoire is revealed after adoptive transfer of DR-BB T-cells into thymectomized and irradiated DP-BB recipients (46). Collectively, these studies demonstrate that the pathogenic potential of autoreactive T-cells in the BB rat is enabled when these cells are placed in a lymphopenic environment. Similar to the contribution of *lyp* to diabetes in the BB rat, diabetes development in NOD mice is dramatically accelerated after recovery from cyclophosphamide-induced lymphopenia (47,48). A particularly revealing example of the cyclophosphamide effect was reported in lethally irradiated NOD mice reconstituted with 50:50 mixed bone marrow isolated from MHC class II–deficient (NOD.I-Aβ$^{-/-}$) and B-cell–deficient (NOD.μMT) mice (13). The resultant bone marrow chimeras were not spontaneously diabetic, a finding the authors argue reflects the absence of MHC class II–expressing B-cells. However, these mice have insulitis, and cyclophosphamide treatment caused rapid diabetes onset. Thus, the absence of MHC class II–expressing B-cells does not preclude the production and destructive function of an islet-reactive T-cell repertoire. Rather, these T-cells are generated in the absence of MHC class II–expressing B-cells and are pathogenic when conditions favor their expansion. We also report a high frequency of diabetes in 35-week-old NOD.μMT mice after cyclophosphamide treatment, underscoring the pathogenic potential of NOD T-cells that developed in the absence of B-cells. Considering the evidence from the BB rat and the cyclophosphamide-treated NOD mice, together with the data provided here, we suggest that conditions that favor extensive lymphocyte expansion can reveal autoreactive T-cells and precipitate disease in genetically susceptible individuals. This idea has important implications for broad T-cell–depleting therapies, which warn that the rebound effect of these agents may aggravate rather than alleviate autoimmune reactivity.

Previous reports suggest that NOD B-cells are uniquely capable of capturing and presenting specific islet Ags to islet-reactive NOD T-cells (13,14,27). However, we observed the development of type 1 diabetes in 36 and 22% of NOD.*scid* recipients of NOD FT-derived or NOD.μMT T-cells, respectively. Although these diabetes incidences are reduced compared with NOD.*scid* recipients of normal NOD T-cells, our results suggest that myeloid APCs, such as Mϕs and DCs, are sufficient to select, activate, and sustain autoreactive T-cells. In our study, the absence of lymphocytes in the NOD.*scid* hosts may have enhanced the density of myeloid APCs and increased their effectiveness as stimulators of autoreactive T-cells. Mϕs and mature DCs express high levels of costimulatory molecules (49,50) and are clearly implicated in type 1 diabetes pathogenesis. For example, the depletion of Mϕs from NOD mice transgenic for a diabetogenic T-cell receptor protected ectopic islet grafts from destruction and blocked the capacity of the T-cell receptor transgenic T-cells to transfer disease into NOD.*scid* recipients (51). Mϕs and DCs infiltrate NOD and NOD.*scid* islets before and independent of T- and B-cells (18,52–54) and produce proinflammatory mediators tumor necrosis factor-α and γ-interferon and nitric oxide that contribute to type 1 diabetes (55). Conversely, depletion of NOD Mϕs prevents the onset of type 1 diabetes (51,52,56,57), and in other rodent models of type 1 diabetes, DCs and Mϕs are necessary and sufficient to cause diabetes (58,59). Together, these data indicate that Mϕs and DCs are also critical APCs for diabetes development and can suffice to select and activate an islet-reactive T-cell repertoire. However, the presence of B-cells likely contributes to a greater efficiency of T-cell activation and expansion, potentiating diabetes development. For these reasons, therapeutic strategies for the prevention of type 1 diabetes in susceptible individuals will likely be more efficacious when directed toward multiple types rather than B-cells alone.

ACKNOWLEDGMENTS

This work was supported by grants from the Canadian Diabetes Association in honor of Madelen Francis Armstrong, the Juvenile Diabetes Foundation International, and the Medical Research Council of Canada. J.S.D. is a Research Scientist of the National Cancer Institute of Canada. P.P.L.C. is the recipient of a Juvenile Diabetes Foundation International Postdoctoral Fellowship.

We thank Philippe Poussier, Gillian Wu, Joan Wither, and Cynthia Guidos for their technical assistance and comments on this manuscript.

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