Original Article

Transgenic Insulin (B:9-23) T-Cell Receptor Mice Develop Autoimmune Diabetes Dependent Upon RAG Genotype, H-2^{g7} Homozygosity, and Insulin 2 Gene Knockout

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A series of recent studies in humans and the NOD mouse model have highlighted the central role that autoimmunity directed against insulin, in particular the insulin B chain 9-23 peptide, may play in the pathogenesis of type 1 diabetes. Both pathogenic and protective T-cell clones recognizing the B:9-23 peptide have been produced. This report describes the successful creation of BDC12-4.1 T-cell receptor (TCR) transgenic mice with spontaneous insulitis in F1 mice (FVB × NOD) and spontaneous diabetes in NOD.RAG$^{-/-}$ (backcross 1 generation). Disease progression is heterogeneous and is modified by a series of genetic factors including heterozygosity (H-2^{g7}/H-2^{q}) versus homozygosity for H-2^{g7}, the presence of additional T-/B-cell receptor–rearranged genes (RAG^{+} versus RAG$^{-/-}$), and the insulin 2 gene knockout (the insulin gene expressed in the NOD thymus). Despite lymphopenia, 40% of H-2$^{g7/g7}$ BDC12-4.1 TCR^{+} RAG$^{-/-}$ Ins2$^{-/-}$ mice are diabetic by 10 weeks of age. As few as 13,500 transgenic T-cells from a diabetic TCR^{+} RAG$^{-/-}$ mouse can transfer diabetes to an NOD.scid mouse. The current study demonstrates that the BDC12-4.1 TCR is sufficient to cause diabetes at NOD backcross 1, bypassing polygenic inhibition of insulitis and diabetogenesis. *Diabetes* 55: 1978–1984, 2006

In the NOD mouse model of type 1A diabetes, a series of islet molecules are targeted by humoral and/or cellular adaptive immune responses (1). We have been particularly interested in immune responses directed against insulin (2–5) with the hypothesis that amino acids 9-23 from the insulin B chain (B:9-23) may be a primary target of NOD anti-islet autoimmunity (6–9). Wegmann et al. (10) and Daniel et al. (11) cloned T-cells directly from islets of pre-diabetic NOD mice and reported that the majority of isolated CD4 clones react with insulin. More than 90% of such insulin-reactive clones specifically respond to insulin peptide B:9-23 (11). Furthermore, immunization with B:9-23 peptide or DNA sequences decreases diabetes in NOD mice (12,13), and the same peptide can induce insulin autoantibodies and diabetes in genetically engineered strains of mice (14,15). We have recent evidence that this I-A^{g7}– and I-A^{d}–restricted sequence (16,17) may be essential for the initiation of anti-islet autoimmunity of NOD mice with the observation that NOD mice with both proinsulin 1 and proinsulin 2 genes knocked out do not develop autoimmune diabetes (6). These Ins1/Ins2 knockout mice were rescued from metabolic diabetes with a proinsulin 2 transgene, in which the B:9-23 sequence was mutated at position B16 (Y→A), a change in the peptide that alters reactivity with the Wegmann and colleagues (6,18) autoreactive T-cell clones but preserves hormonal activity. Sequences within B:9-23 (i.e., B:15-23) are also immunogenic to NOD CD8 T-cells (19). Of note, the 2H6 CD4 T-cell clone and transgenic T-cell receptor (TCR) mouse of Peng et al. (20) also recognizes insulin peptide B:9-23 but prevents type 1 diabetes. The 2H6 clone has the same Jα region (TRAJ53) KLTFGKGT, a sequence characteristic of NOD B:9-23–reactive T-cells but differs from the BDC12-4.1 with TCR Vα sequence (TRAV21/DV12 versus TRAV5D-4).

Multiple transgenic and retrogenic CD4 TCR mice have been produced with sequences from T-cell clones reacting with the islet autoantigen GAD (21–23). Given that diabetes has been inhibited in all of these mice, it has been hypothesized that recognition of the GAD molecule is protective; however, there is some evidence that cryptic epitopes of GAD may be pathogenic (24). Other transgenic CD4 TCR mice specific for unknown islet autoantigens are pathogenic (25–27). We hypothesize that TCR transgenics recognizing the same peptide of insulin could be either pathogenic or protective, depending on the T-cell receptor sequence, and that the BDC12-4.1 TCR transgenic mouse would be pathogenic similar to the T-cell line from which the receptor was cloned. This article describes the creation of BDC12-4.1 TCR mice, directly testing this hypothesis.

RESEARCH DESIGN AND METHODS

Creation of TCR^{+} transgenic mice. The TCR α (TRAV5D-4*04/TRAJ53*01) and β (TRBV1*01/TRBJ2–7*01) chains (28), as described by Simone et al. (17), were cloned from the BDC12-4.1 clone with the following sequence differences noted: the N region of the α chain is GAN, and the nDn region of the β

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FITC, fluorescein isothiocyanate; IFN, interferon; TCR, T-cell receptor.

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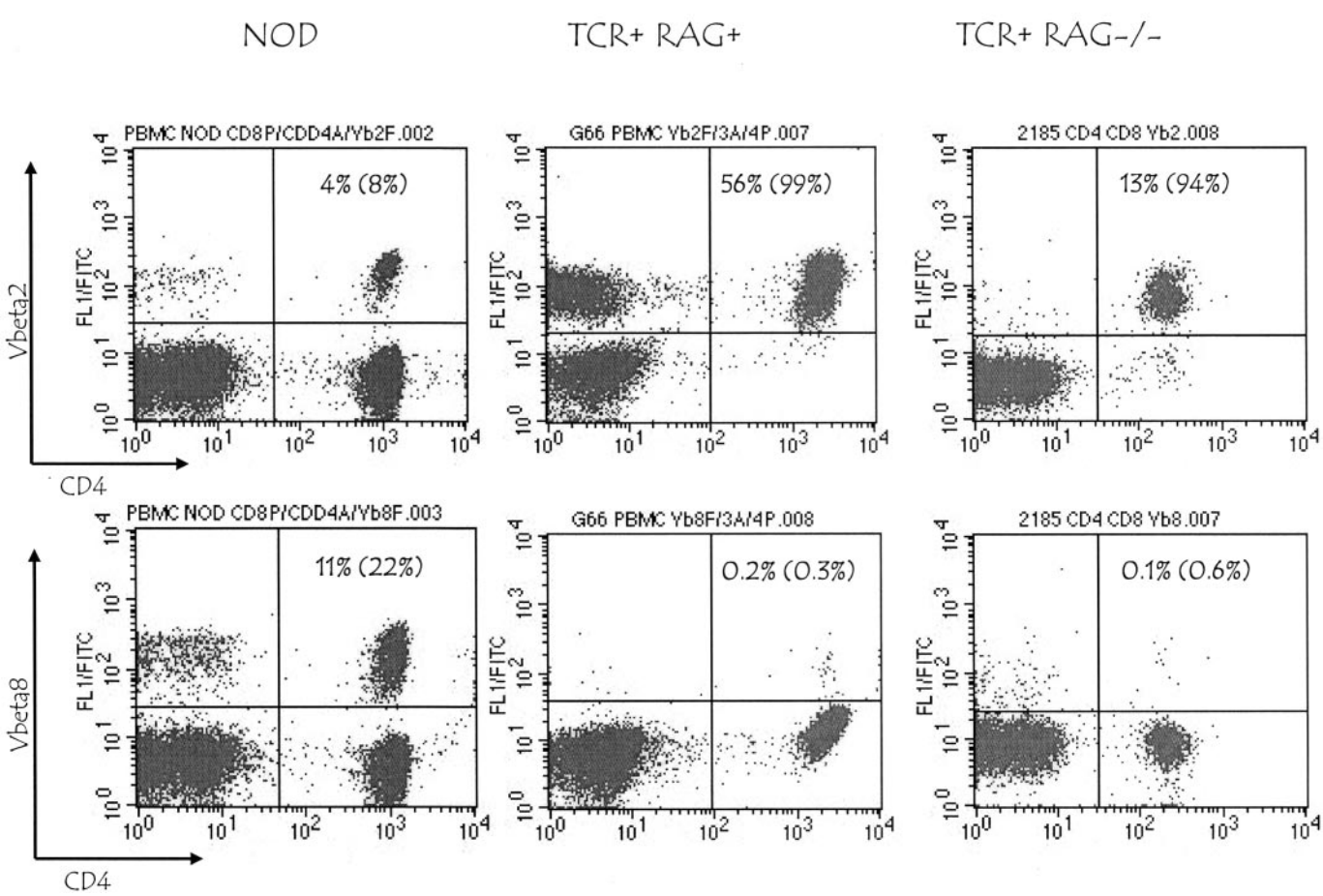

FIG. 1. Flow cytometry of peripheral blood mononuclear cells with FITC-conjugated Vβ2 and antigen-presenting cell–conjugated CD4 antibodies (*top panel*) and FITC-Vβ8 and antigen-presenting cell-CD4 (*bottom panel*). *Upper right quadrant* numbers represent percentage of Vβ (2 or 8)-expressing cells in the lymphocyte gate determined by forward and side scatter. Values in parentheses show the relative percentage of CD4$^+$ cells expressing the particular Vβ chain.

chain is PGLGN. The Genbank accession numbers (29) for the transgenic α and β TCR chains are DQ172905 and DQ180320, respectively. The α and β chains were subcloned into the TCR cassettes described and kindly provided by Mathis (30); the plasmids were microinjected separately into FVB embryos. Three α founders were produced by Myra Lipes, and one β founder was created at the University of Colorado Health Sciences Center. Expression of the transgenic α chain in the founders was confirmed by isolating mRNA from spleen cells from all three α lines, performing RT-PCR using primers (5′→3′) ATCCTCGGTCTCAGGACA and CAATGAAAACATATGCTCCTA and full-length sequencing. Expression of the β chain in the founder was confirmed by flow cytometry of lymphocytes stained with a fluorescein isothiocyanate (FITC)-conjugated Vβ2 antibody (553280) from BD Biosciences (Boston, MA).

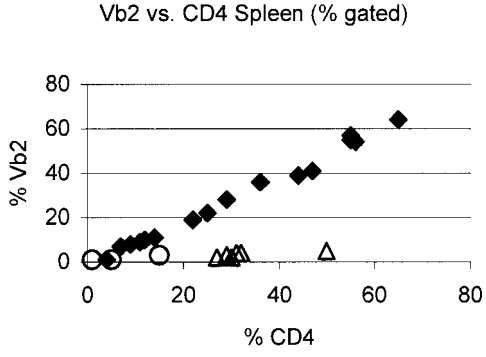

FIG. 2. Correlation between percent Vβ2 in splenocytes and percent CD4 T-cells in diabetic transgenic mice. ◆, diabetic TCR$^+$ RAG$^{-/-}$ H-2^{g7} homozygotes; △, nondiabetic wild-type NOD mice; ○, transgenic RAG$^{-/-}$ mice with a single TCR chain (α or β).

A FITC-conjugated Vβ8.1/8.2 antibody from BD Biosciences (553185) served as a control antibody to determine allelic exclusion of nontransgenic Vβ chains.

Offspring from the three α chain founder lines were bred with offspring from the β chain founder line to create three α$^+$β$^+$ TCR$^+$ transgenic FVB lines. Flow cytometry of peripheral blood mononuclear cells isolated from these three founder lines of α$^+$β$^+$ transgenic mice using the FITC-conjugated Vβ2 antibody confirmed the presence of transgenic T-cell receptors in only two of three lines: α10/β82 and α32/β82. For the current study, the α32/β82 FVB mice were crossed and backcrossed onto NOD RAG1$^{-/-}$ mice (strain 3729) obtained from The Jackson Laboratory (Bar Harbor, ME) to create backcross 1 (BC1) mice. (RAG is the recombinase activating gene.) BC1 mice were intercrossed to create TCR$^+$ RAG1$^{-/-}$ mice on a "mixed" background. No more than two backcrosses on the NOD (RAG$^{-/-}$) background occurred for the mice described in this manuscript. The α10/β82 FVB transgenic line is also being crossed with the NOD RAG1$^{-/-}$ mice in a similar manner, and to date, 8 of 34 mice of this founder strain have developed diabetes. Mice were bred and housed under specific pathogen-free conditions at the University of Colorado Health Sciences Center for Laboratory Animal Care in Denver, Colorado, and at the University of Colorado Health Sciences Center, Center for Comparative Medicine in Aurora, Colorado, following the institutional animal care and use committee guidelines for the use and care of laboratory animals.

Genotyping. The presence of TCR-α and TCR-β transgenes was determined at weaning by testing genomic DNA isolated from tail snips. PCR was performed using α primers (5′→3′) CAATGAAAACATATGCTCCTAC and CTGCCTCCACTGTTCGCGCC and β primers GTCTTGTTTCAGACCCCACAG and CTACCTTGTCCTGGCTTGCG. The α band appears at 360 bp and the β band at 550 bp. Homozygous RAG knockout status was originally determined at 6 weeks of age by testing for the presence of immunoglobulin in 5–10 μl of whole blood using a microouchterlony plate from The Binding Site (San Jose, CA) and goat anti-mouse polyvalent immunoglobulin (Sigma-Aldrich, St.

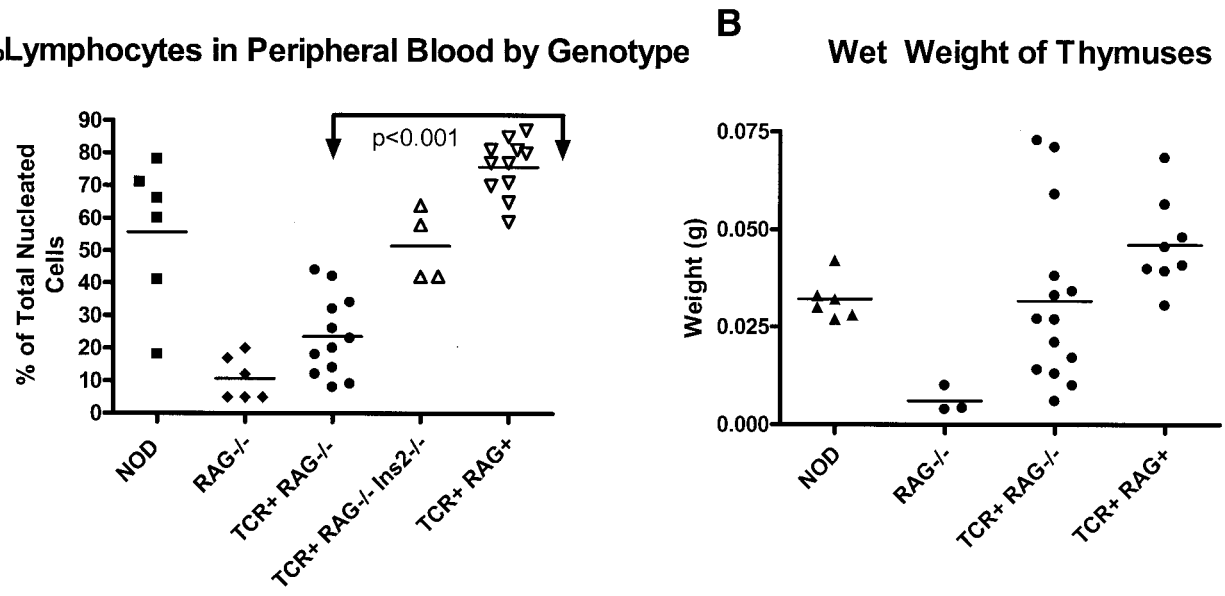

FIG. 3. TCR$^+$ RAG$^{-/-}$ mice are lymphopenic and have smaller thymuses. *A*: Results of complete blood counts and differentials performed on 250–300 μl of blood. TCR$^+$ RAG$^+$ mice show significantly more lymphocytes in the peripheral blood when compared with TCR$^+$ RAG$^{-/-}$ mice. *B*: Wet weights of thymuses obtained at necropsy. There was no difference in wet weights of thymuses between TCR$^+$ mice that were RAG$^+$ or RAG$^{-/-}$. However, thymic weights of TCR$^+$ RAG$^+$ mice were significantly larger than that of the NOD mouse.

Louis, MO). In later studies, RAG genotyping was determined by PCR of genomic DNA using primers and PCR conditions described by The Jackson Laboratories (31). H-2 genotyping was determined by PCR of genomic DNA with 6-carboxyfluorescein–conjugated D17Mit34 primers and the same PCR conditions used for α/β genotyping. The PCR product was either analyzed with an ABI 377 sequencer (Foster City, CA) or run on a 4% agarose gel. The H-2^q (FVB) genotype produces a peak (band) at 146 bp and the H-2^{g7} (NOD) at 124 bp.

Diabetes determination. Mice were followed for diabetes by biweekly testing of tail blood using a Freestyle glucometer and test strips from Abbott Laboratories (Abbott Park, IL). Mice whose glucose values were >250 mg/dl were tested the next day and were considered diabetic if the second reading was also >250 mg/dl.

White blood cell count/differential. Whole blood (250–300 μl) was collected into an EDTA-containing purple top microtainer tube (Becton Dickinson, Franklin Lakes, NJ) for complete blood count determination. Blood samples were analyzed by the Colorado State University Animal Hospital Diagnostic Laboratory (Fort Collins, CO) using an Advia 120 hematology analyzer. Results were confirmed in the diagnostic laboratory via a manual differential count.

Adoptive transfer. Spleens were extracted under aseptic conditions and homogenized. The red blood cells were lysed, and cells were then resuspended in 200 μl sterile PBS. Various amounts of primary unsorted splenocytes (150,000 to 8,000,000 cells) were transferred, injected either intravenously or intraperitoneally into 6- to 10-week-old NOD.scid recipients and the recipients followed for diabetes. Separate aliquots of splenocytes were doubly stained for Vβ2 and either CD3 or CD4 to count (via flow cytometry), and the approximate number of BDC12-4.1 transgenic cells was determined. Because 1–9% of all primary unsorted splenocytes stain doubly positive, the number of actual transferred transgenic T-cells ranged from 13,500 to 720,000.

Enzyme-linked immunosorbent spot analysis. Interferon (IFN)-γ and interleukin-4 ELISPOT (enzyme-linked immunosorbent spot analysis) kits (BD Biosciences, San Diego, CA) were used for splenocyte analysis. Spleen cells were prepared by homogenization in red blood cell lysis buffer and cultured at 2–4 × 10^5 cells/well in triplicate. Cells were cultured in Click's medium (Sigma-Aldrich) and 5% heat-inactivated mouse serum with 1 mol/l HEPES, pH 7.4 (Life Technologies, Carlsbad, CA) and L-glutamine (Cellgro, Herndon, VA) for 48 h at 37°C at 5% CO_2. Peptides used for stimulation were high-performance liquid chromatography purified (>90%) and dissolved in sterile lipopolysaccharide-free saline at a neutral pH (SynPep, Dublin, CA). 3-amino-9-ethylcarbazole substrate and Chromogen solution (BD Biosciences) were used as the detection method, and wells were counted and analyzed using the Immunospot reader and software version 3.

RESULTS

TCR expression. After creation of the transgenic lines in FVB mice and breeding onto the NOD.RAG background, there is excellent allelic exclusion of TCR β chains in CD4 T-cells in transgenic TCR$^+$ RAG$^+$ mice, with 99% of CD4$^+$ peripheral blood mononuclear cells expressing the transgenic Vβ2 chain and low expression of Vβ8, a nontransgenic control. In contrast, wild-type NOD mice express more Vβ8 than Vβ2 (Fig. 1).

Similar allelic exclusion is seen in splenocytes with an excellent correlation between percent Vβ2 and percent CD4 T-cells (R^2 = 0.98), as seen in Fig. 2 for TCR$^+$ RAG–deficient mice. The graph also shows the variability in the percentage of splenocytes expressing CD4 in these

TABLE 1
Subsets of thymocytes by genotype

Genotype/subset	CD4$^-$ CD8$^-$ (%)	CD4$^+$ CD8$^+$ (%)	CD4$^+$ CD8$^-$ (%)	CD4$^-$ CD8$^+$ (%)
NOD	4.8 ± 2.5	82 ± 3.5	12 ± 2.5	3.8 ± 0.9
TCR$^+$ RAG$^+$	3.6 ± 2.0	66 ± 4.2	17 ± 2.9	6.0 ± 1.2
TCR$^+$ RAG$^-$	3.6 ± 1.1	93 ± 1.4*	3 ± 0.5*	1 ± 0.0
TCR$^+$ RAG$^-$ Ins2$^-$	7.5 ± 4.3	88 ± 6.2	2.7 ± 1.3	1.4 ± 1.2

Data are means ± SE measured as percentages of gated cells. Thymic subsets vary according to genotype of transgenic mice. Significantly more double-positive thymocytes exist in TCR$^+$ RAG$^{-/-}$ than in TCR$^+$ RAG$^+$ ($P < 0.001$) mice. The TCR$^+$ RAG$^{-/-}$ mice show significantly fewer single positive CD4$^+$ T-cells compared with the TCR$^+$ RAG$^+$ mice ($P < 0.001$). *Statistically significant differences.

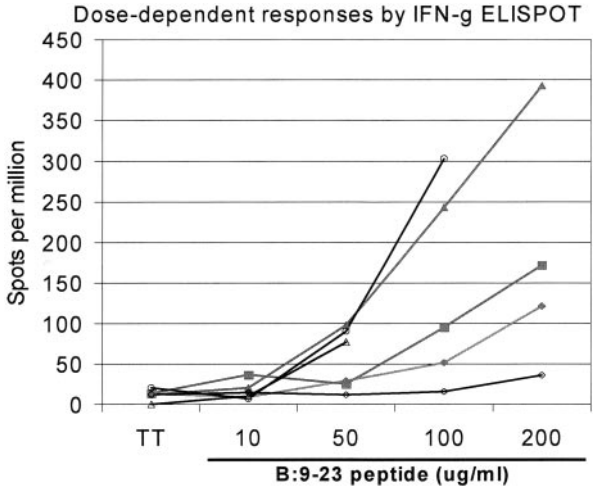

FIG. 4. IFN-γ dose response to B:9-23 peptide, expressed as spots per million by enzyme-linked immunosorbent spot analysis (ELISPOT) assay of splenocytes. Open symbols indicate BDC12-4.1 RAG$^+$, and closed symbols indicate BDC12-4.1 RAG$^{-/-}$ mouse. TT, tetanus toxin control peptide.

mice. As expected, the percentage of splenocytes expressing Vβ2 remains <5% at all ages for wild-type NOD mice (Fig. 2, △). No antibody exists to assess Vα allelic exclusion, but transgenic RAG$^{-/-}$ mice expressing a single TCR transgene (either α only or β only) demonstrate a lack of CD4$^+$-staining cells.

Lymphopenia. TCR$^+$ RAG$^{-/-}$ mice are lymphopenic compared with NOD and TCR$^+$ RAG$^+$ mice (Fig. 3). Not surprisingly, the percentage of lymphocytes in peripheral blood of TCR$^+$ RAG$^{-/-}$ mice is greater than in TCR$^-$ RAG$^{-/-}$ mice, with considerable heterogeneity within the NOD and transgenic TCR$^+$ RAG$^{-/-}$ groups. Despite expression of the transgenic TCR, the number of peripheral blood lymphocytes in TCR$^+$ RAG$^{-/-}$ mice was significantly lower ($P < 0.001$) than in TCR$^+$ RAG$^+$ mice in both percentage of total white blood cell count (Fig. 3*A*) and absolute lymphocyte count. The TCR$^+$ RAG$^+$ mice had a mean lymphocyte count of 4,400 cells/μl (means ± SE) (75 ± 2.6%); TCR$^+$ RAG$^{-/-}$ mice had a mean lymphocyte count of 883 cells/μl (23 ± 3.6%). As further evidence of lymphopenia, the percentage of CD4$^+$ T-cells in diabetic TCR$^+$ RAG$^{-/-}$ spleens, as determined by flow cytometric analysis, was 3.9 ± 0.8% compared with 19.2 ± 3.3% CD4$^+$ T-cells in wild-type NOD and FVB mice.

TCR$^-$ RAG$^{-/-}$ mice had extremely small thymuses as expected, but interestingly, thymic weight varied for TCR$^+$/RAG$^{-/-}$ mice, with some mice having normal thymic weights and others having very low weights. Wild-type RAG-deficient mice have small involuted thymuses. Introduction of transgenic TCR α and β chains restores cellularity and thymic mass of RAG-deficient mice almost to NOD levels (Fig. 3*B*). As seen in Table 1, the relative proportion of single-positive and double-positive thymic T-cells is modified by the presence of the BDC12-4.1 TCR. TCR$^+$ RAG$^+$ mice show more single-positive CD4$^+$ thymocytes compared with TCR$^+$ RAG$^{-/-}$ mice ($P < 0.001$). TCR$^+$ RAG$^{-/-}$ mice show the greatest percentage of T-cells in the double-positive stage (93%) but the lowest percentage of single-positive CD4$^+$ cells (3%), implying failure to progress to the single-positive stage (negative selection).

Despite severe lymphopenia, functional transgenic T-cells that recognize the insulin B:9-23 peptide could be demonstrated with splenocyte enzyme-linked immunosorbent spot analysis (Fig. 4). Even though only TCR$^+$ RAG$^{-/-}$ mice develop spontaneous diabetes, both TCR$^+$

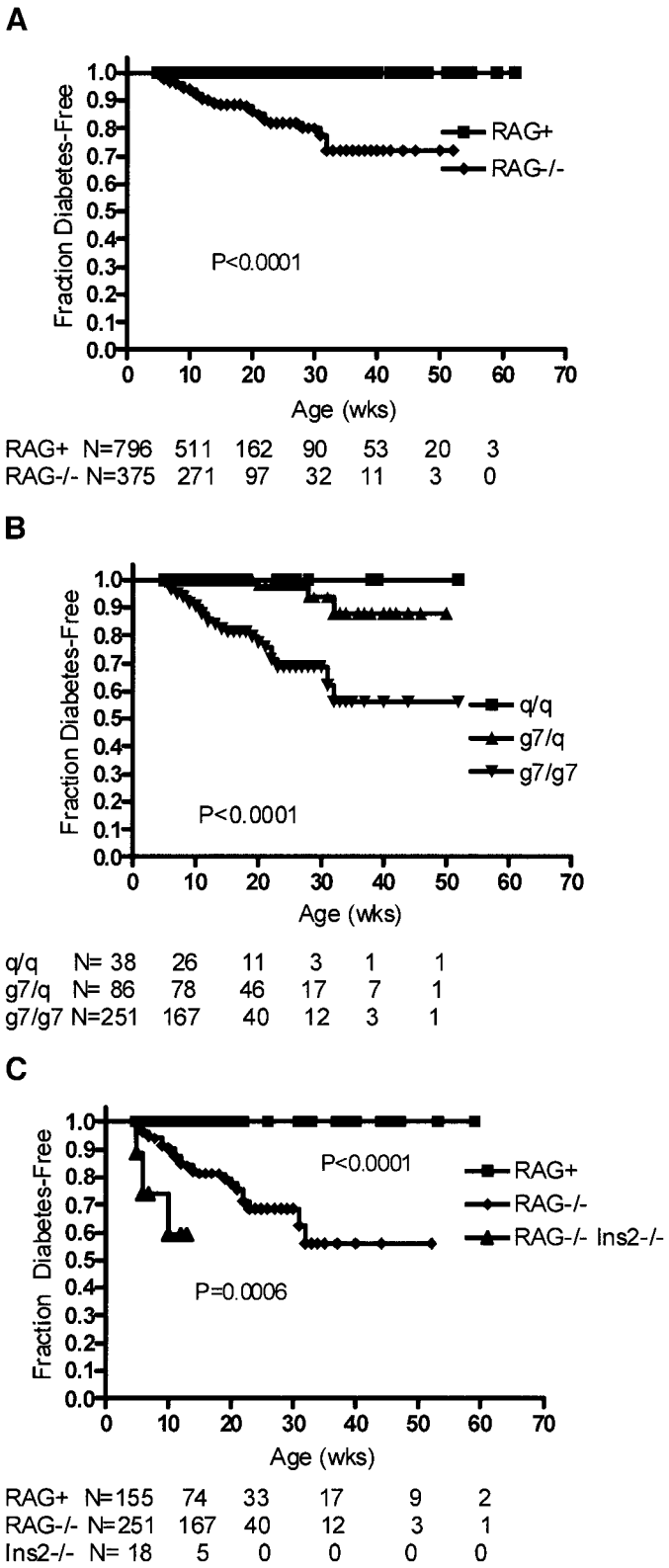

FIG. 5. Spontaneous diabetes develops in transgenic TCR$^+$ mice. Numbers below graphs indicate number of mice in each group at that age.

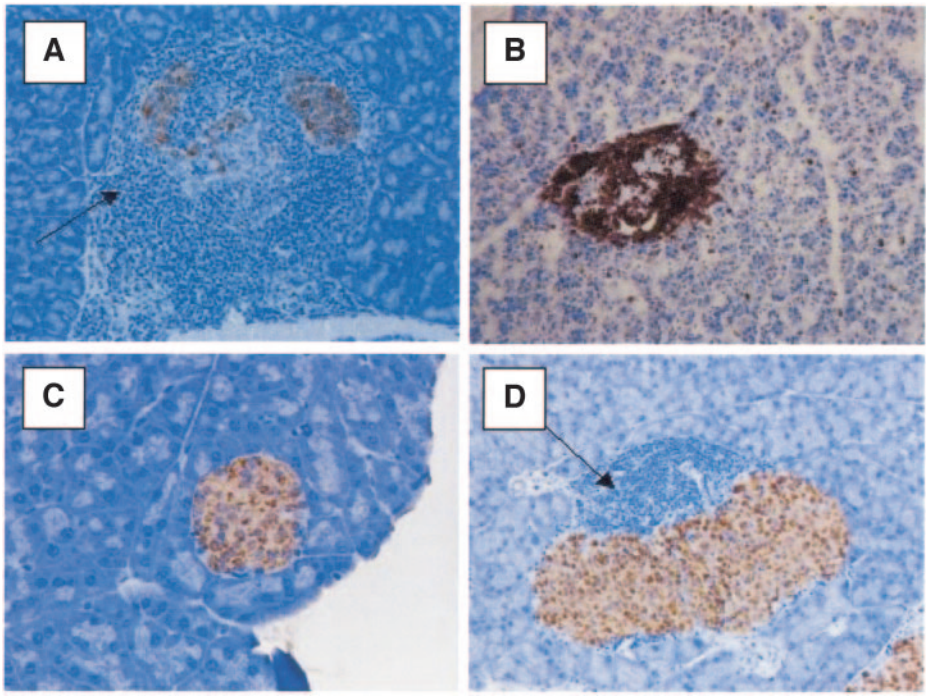

FIG. 6. Sections stained for insulin (*A*, *C*, and *D*) or CD4 (*B*). Insulitis with reduced insulin staining in a diabetic 11-week-old TCR$^+$ RAG$^{-/-}$ mouse (*A*) and in a CD4-stained frozen section taken from same mouse (*B*). Islets from a nondiabetic 10-week-old TCR$^+$ RAG$^{-/-}$ mouse that did not have insulitis (*C*) and in a 44-week-old nondiabetic TCR$^+$ RAG$^+$ mouse with peri-insulitis (*D*).

RAG$^+$ and TCR$^+$ RAG$^{-/-}$ splenocytes carrying the transgenic TCR produce IFN-γ when stimulated with B:9-23 peptide. In contrast, no IFN-γ production was detected in TCR$^+$ mice with the control tetanus toxin peptide. Although the responses are heterogeneous (35–390 spots per million splenocytes at the B:9-23 peptide concentration studied), responses are clearly dose dependent. In addition, interleukin-4–producing cells are also seen in all mice when stimulated with B:9-23 peptide (data not shown). This is consistent with the original T-cell clone producing both interleukin-4 and IFN-γ in response to stimulation by B:9-23 (11).

Disease progression. Unmanipulated transgenic mice were followed for development of type 1 diabetes. As seen in Fig. 5*A*, spontaneous diabetes occurs in untreated transgenic TCR$^+$ mice aged 4–32 weeks only on a RAG-deficient background. Furthermore, diabetes develops only in TCR$^+$ RAG$^{-/-}$ mice with at least one copy of H-2^{g7} (Fig. 5*B*) ($P < 0.001$). Fifty percent of H-2$^{g7/g7}$ mice, 15% of H-2$^{g7/q}$, and 0% of H-2$^{q/q}$ mice progressed to diabetes (Fig. 5*B*). Both sexes of transgenic BDC12-4.1 are equally affected ($P = 0.32$). We have created homozygous H-2^{g7} TCR$^+$ RAG$^{-/-}$ Ins2 knockout mice, and such Ins2$^{-/-}$ mice developed diabetes significantly earlier than homozygous H-2^{g7} TCR$^+$ RAG$^{-/-}$ Ins2$^+$ mice (Fig. 5*C*) ($P = 0.006$).

The majority of H-2$^{g7/g7}$ mice showed clear insulitis regardless of their RAG genotype. Diabetic TCR$^+$ RAG$^{-/-}$ mice show severe insulitis and/or complete (or near-complete) destruction of all insulin-producing cells (Fig. 6*A*). The infiltration consists of CD4 T-cells, with an absence of CD8 T-cells (Fig. 6*B*). Insulitis is heterogeneous, however, as seen in a nondiabetic 10-week-old TCR$^+$ RAG$^{-/-}$ H-2^{g7} mouse, all islets observed were free from insulitis (Fig. 6*C*). Even though TCR$^+$ RAG$^+$ H-2^{g7} mice do not develop diabetes, insulitis is still observed in some mice, as seen in a 44-week-old TCR$^+$ RAG$^+$ H-2^{g7} mouse (Fig. 6*D*). Figure 7 summarizes the expression of insulitis and/or ductal infiltrates in TCR$^+$ mice, both RAG$^{-/-}$ (*left panel*) and RAG$^+$ mice (*right panel*), subdivided by H-2^{g7} genotype. H-2$^{q/q}$ mice developed neither insulitis nor ductulitis (0 of 13 mice), as expected, since the initial T-cell clone was derived from NOD mice. Despite the presence of insulitis, no TCR$^+$ RAG$^+$ mouse has progressed to diabetes (Fig. 5*A*). No insulin autoantibodies could be detected in 15 TCR$^+$ RAG$^+$ mice at various ages tested (data not shown), even though most TCR$^+$ RAG$^+$ mice develop insulitis.

To test diabetogenicity of the transgenic T-cells, splenocytes from diabetic TCR$^+$ RAG–deficient mice were transferred to unprimed wild-type NOD.scid recipients (Fig. 8). Within 12 weeks of transfer, ~50% of the NOD.scid recipients developed diabetes. As few as 13,500 primary BDC12-4.1 CD4$^+$ T-cells can transfer diabetes to NOD.scid mice.

DISCUSSION

To understand disease development, a series of TCR NOD mice has been generated over the years with dramatically different phenotypes and variation in thymic deletion. This communication describes the first pathogenic anti-insulin

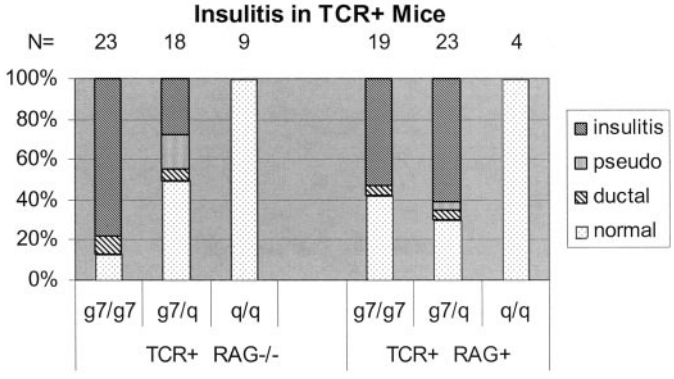

FIG. 7. Histology is heterogeneous in TCR$^+$ mice. *Left panel* shows percentage of transgenic (TCR$^+$) RAG$^{-/-}$ mice scored in each category of insulitis. *Right panel* shows distribution of histology findings in TCR$^+$ RAG$^+$ mice. Pseudo was scored when no lymphocytic infiltration was found but pseudoatrophic islets (small, glucagon-only–positive islets) were detected.

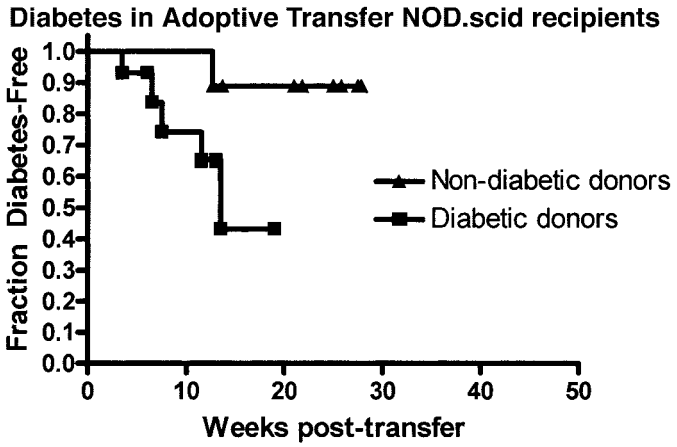

FIG. 8. Splenocytes from diabetic TCR$^+$ RAG$^{-/-}$ mice transfer disease into NOD.scid recipients (■). Nondiabetic donors (▲) were either 4- to 8-week-old nondiabetic NOD mice or nondiabetic TCR$^+$ RAG$^{-/-}$ mice.

CD4 TCR transgenic (derived from BDC 12-4.1 T-cell clone) where the target molecule is a well-defined natural autoantigen (insulin B:9-23 peptide) and modification of that specific target (insulin peptide B:9-23) influences disease pathogenesis (6). Similar to a number of other transgenic models and T-cell lines (32,33), spontaneous progression to diabetes is enhanced by homozygosity for H-2^{g7}, consistent with a major histocompatibility complex gene-dose effect in these mice (32,34). Because spontaneous diabetes occurs in unmanipulated BDC12-4.1 transgenic RAG$^{-/-}$ mice in spite of severe lymphopenia and a lack of CD8 and B-cells, these transgenic CD4$^+$ T-cells specific for B:9-23 are sufficient for disease in NOD mice (without in vitro activation). Because sialitis is not present in BDC-12-4.1 transgenic mice, as it is in wild-type NOD mice and in insulin knockout mice (6), the inflammatory potential of the BDC12-4.1 TCR is likely tissue specific. In addition, disease (insulitis and diabetes) occurs in backcross 1 mice, thus bypassing the usual polygenic inhibition of disease in noncongenic NOD mice.

It is noteworthy that there is variation in the age of diabetes onset and that a subset of homozygous H-2^{g7} TCR$^+$ RAG$^{-/-}$ mice does not develop insulitis. The "mixed" genetic background of the animals might explain these findings. Congenic NOD mice strains are currently being created for each of the founder lines. Disease incidence may increase and become more homogeneous when the transgene is introduced on a pure NOD background, although this did not happen with the BDC2.5 TCR transgenic mouse, possibly due to the regulatory nature induced by endogenously expressed α chains as a result of a lack of α chain allelic exclusion (35).

No BDC12-4.1 RAG$^+$ mouse develops diabetes even though B:9-23–reactive, IFN-γ–producing TCR transgenic T-cells are present, and the majority of homozygous H-2^{g7} mice develop insulitis. Of note, despite the presence of insulitis, these TCR$^+$ RAG$^+$ mice do not develop insulin autoantibodies, a phenotype tightly associated with insulitis (36). Like earlier transgenic mice on a RAG$^+$ background (25,37), these BDC12-4.1 TCR transgenic mice show excellent allelic exclusion of the Vβ chain in splenocytes and peripheral blood. Unlike other TCR transgenic mice (27,37,38), the BDC12.4-1 transgenic mice fail to develop diabetes on a RAG$^+$ background. One possible explanation is that the lack of allelic exclusion of the TCR α chain in RAG$^+$ mice allows multiple TCRs to be expressed on the same T-cells, which may impair pathogenicity. We overcame this obstacle by breeding the transgenic mice onto the RAG-deficient background. Another possibility is that T-cells or NK-T–cells carrying endogenous TCR chains may have, or may induce, regulatory cells as proposed in BDC2.5 mice (39,40).

Wegmann and colleagues (18) CD4 T-cell clones isolated from islets of young pre-diabetic NOD mice demonstrated dramatic α chain restriction (TRAV5D-4 coupled with either TRAJ53 or TRAJ42) without either Vβ or N region restriction. We therefore hypothesize that specific Vα and Jα gene segments alone (without nDn region restriction) (17) contribute to the development of type 1 diabetes by providing a large pool of anti–B:9-23 CD4 T-cell precursors. To investigate these questions, BDC12-4.1 transgenic mice are being bred with NOD Cα knockout mice. We speculate that exclusive expression of the 12-4.1 TCR α chain will allow diabetes to develop even in transgenic BDC12-4.1 RAG$^+$ mice.

For the BDC12-4.1 TCR transgenic mouse, the TCR epitope specificity is known. Furthermore, the antigen is naturally expressed in the animals, which allows for in vivo manipulation of either TCR or epitope. We have exploited this phenomenon by creating TCR$^+$ RAG$^{-/-}$ Ins2$^{-/-}$ mice. Pathogenicity of the BDC12-4.1 TCR is enhanced with elimination of the Ins2 gene (only Ins2 is expressed in the thymus, but both Ins1 and Ins2 are expressed in the islets). We are expanding our colony repertoire and will explore central and peripheral tolerance mechanisms that may be involved in breaking tolerance in the NOD mouse model, the best animal model for studying type 1 diabetes in humans.

Despite the genetic heterogeneity that exists in the model at this point, the BDC12-4.1 transgenic model confirms the sufficiency and pathogenicity of CD4 T-cells recognizing B:9-23 in the NOD mouse with multiple levels of genetic regulation and provides a platform for further study of the pathogenesis of insulin autoimmunity and diabetes in both mice and humans. Furthermore, if there exists in humans such primacy of autoantigens, and if pathogenic TCR sequences are conserved, as in the NOD mouse, then one might be able to someday develop therapies that target specific α or β chains to prevent disease.

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