Prevention of Type 1 Diabetes in the Rat With an Allele-Specific Anti–T-Cell Receptor Antibody Vβ13 as a Therapeutic Target and Biomarker

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In earlier studies of the Iddm14 diabetes susceptibility locus in the rat, we identified an allele of the T-cell receptor (TCR) β-chain, Tcrb-V13S1A1, as a candidate gene. To establish its importance, we treated susceptible rats with a depleting anti-rat Vβ13 monoclonal antibody and then exposed them to either polyinosinic-polyctydyllic acid or a diabetogenic virus to induce diabetes. The overall frequency of diabetes in the controls was 74% (n = 50), compared with 17% (n = 30) in the anti-Vβ13-treated animals, with minimal islet pathology in non-diabetic treated animals. T cells isolated from islets on day 5 after starting induction showed a greater proportion of Vβ13+ T cells than did peripheral lymph node T cells. Vβ13 transcripts recovered from day 5 islets revealed focused β3 usage and less CDR3 diversity than did transcripts from peripheral Vβ13+ T cells. CDR3 usage was not skewed in control Vβ16 CDR3 transcripts. Anti-rat Vβ13 antibody also prevented spontaneous diabetes in BBDDP rats. The Iddm14 gene is likely to be Tcrb-V13, indicating that TCR β-chain usage is a determinant of susceptibility to autoimmune diabetes in rats. It may be possible to prevent autoimmune diabetes by targeting a limited element of the T-cell repertoire. Diabetes 61:1160–1168, 2012

Type 1 diabetes is a T-cell–mediated autoimmune disorder, the fundamental cause of which is unknown (1). Most strategies to prevent, arrest, or reverse it target T cells, either directly by altering number or function or indirectly via tolerizing antigens. To date, some interventions seem to preserve some β-cells after onset (2,3), but none safely and effectively prevent or reverse the disease.

A major need is for new, disease-specific modalities that avoid broad-spectrum targeting of immune-system components. This is difficult because multiple antigenic specificities participate in the diabetogenic response, and multiple alleles of immune-system genes contribute to the process. In addition, diverse antigenic specificities may be recognized by individuals with different major histocompatibility complex (MHC) haplotypes and may be presented in an atypical fashion (4). Treatments that target the whole T-cell population or cytokine receptors have the potential to increase the risk of infection and other complications.

To address these problems, we study rat models of the disease. Type 1–like autoimmune diabetes, both spontaneous and inducible, is relatively common among inbred rat strains that, like humans, express a high-risk class II MHC haplotype; in rats, it is designated RT1B/Du (5–7). We previously reported that Iddm14 (formerly designated Iddm4) is a dominant non-MHC susceptibility locus important for both spontaneous and induced autoimmune diabetes in multiple rat strains (8–14).

Studies of Iddm14 in eight RT1B/Du rat strains led to the identification of a susceptibility haplotype in the Tcrb-V locus (10). Single nucleotide polymorphism (SNP) haplotype mapping of this region of chromosome 4 encompassed Tcrb-V1–4, 5.1–5.2, 6, 7, 8.1–8.4, 9–13, and 15–20. These comprise the majority of the 24 Tcrb-V family members identified in previous studies and by our own bioinformatics (10). Our SNP haplotype mapping revealed that six rat strains susceptible to diabetes (KDP, BBDR, BBDD, LEW.1WR1, LEW.1AR1-iddm, and PFG-RT1u) all share an allele of β-chain variable region gene Tcrb-V13, designated Tcrb-V13S1A1 (15). Three rat strains that are resistant to, or confer resistance to, diabetes in genetic studies all express different alleles, either Tcrb-V13S1A2 (BN and WF rats) or Tcrb-V13S1A3P (F344 rats) (15). These polymorphisms are of interest because preferential usage of the Tcrb-V13S1A1 gene product, designated Vβ13a, by CD4+ but not CD8+ cells has been reported (15). Here, we report prevention of autoimmune diabetes by selective depletion of Vβ13a+ T cells in LEW.1WR1 and BBDDP rats.
for 40 days or until diabetes diagnosis. In one experiment, diabetes was induced by exposure to poly IC (1 μg/g i.p.) on three consecutive days, followed 1 day later by injection of Kilham rat virus (KRV) (10^8 pfu), as described (17). Typically, 80–100% of animals became diabetic within 15–25 days. Poly IC alone at this dose is not diabetogenic; KRV alone induces diabetes in ~40% of LEW.1WR1 rats (17). Animals were tested for glycosuria for 40 days or until the diagnosis of diabetes, defined as a plasma glucose >250 mg/dl (OneTouch; Johnson & Johnson, Milpitas, CA).

**Diabetes prevention studies.** The hybridoma producing the 17D5 mouse anti-rat Vb13 monoclonal antibody (mAb; IgG2a) recognizes the product of the Tcrb-V13/D16 gene (18). The hybridoma producing the His42 mouse anti-rat Vb16 (IgG2b) mAb (19) was a gift from Dr. Thomas Hünig. Both antibodies were prepared as ascites and purified by affinity chromatography. Mouse OKT3 anti-human CD3 mAb (IgG2a) was obtained from the American Type Culture Collection. In previous studies, each mAb was administered intraperitoneally at a dose of 0.1 mg per rat in a volume of 0.5 mL. In studies in the LEW.1WR1 rat, mAb was injected three times weekly, and the first mAb injection was given 48 h before the first injection of poly IC. BBP rats were injected with mAb once weekly beginning at 45 days of age. TIMing and total number of doses in each experiment is described in the **RESULTS**.

**Measurement of T-cell depletion.** We quantified the effect of 17D5 and His42 on peripheral T-cell populations by measuring Vb4, Vb13, Vb15, and Vb16 mRNA transcriptions by quantitative RT-PCR. This method was used because we lacked anti-Vb13 and anti-Vb16 antibodies against a second epitope to allow us to distinguish depleted or only masked. Total RNA was isolated from spleens, mesenteric lymph nodes, and cervical lymph nodes (CLNs) at the onset of diabetes or at the end of the experiment. In brief, tissues were harvested and stored in RNA Later (Qiagen, Valencia, CA). RNA was prepared using Ultraspec (Biotech, Houston, TX) and treated with Turbo DNA-free (Applied Biosystems, Carlsbad, CA) to prevent genomic contamination. cDNA was synthesized from 2 μg total RNA using the High Capacity RNA-to-cDNA Kit (Applied Biosystems). The primers used for quantitative RT-PCR (qRT-PCR) were designed using Primer Express 3 (http://frod.wi.mitt.edu/primer3) and T-cell receptor (TCR)-Vb gene sequences. Primers were selected to be of optimal size for real-time PCR, with the 5′ primer located in the leader sequence and the 3′ primer in a region of the gene that did not contain SNPs. All primers were synthesized by Integrated DNA Technologies (Coralville, IA). Primer sequences are given in the Supplementary Data. Real-time PCR was performed with the 7500HT PCR System (ABI) using SYBR Green PCR Mix (Applied Biosystems). Amplification data were collected and analyzed using software from ABI-SDS2.2.

**Recovery and expansion of islet-infiltrating T cells.** To phenotype early islet-infiltrating T cells, we adapted the expansion method of Jarchum et al. (20). For each experiment, eight LEW.1WR1 rats were treated with poly IC, as described above. Animals were killed 48 h after one dose of poly IC (day 3 of diabetes pathogenesis) or 48 h after the second dose of poly IC (day 5 of pathogenesis). Pancreatic islets were isolated as described (21,22). Handpicked isolated islets were cultured for 7 days in 24-well tissue culture plates at a density of 50 islets/mL/well, as described (20), to expand infiltrating T-cell populations. Culture medium consisted of RPMI-1640 supplemented with 10% FBS (HyClone, Logan, UT), 1 mM Na pyruvate, nonessential amino acids, 28 μg/mL L-ascorbic acid-2-phosphate, 5 μg/mL transferrin, and 50 μM β-mercaptoethanol. Islets were cultured in 750μL of medium in normal islet medium (PeproTech, Rocky Hill, NJ). Cells were cultured in 5% CO2, 95% air at 37°C. On day 7, islets and infiltrating cells were collected and passed through a 40-micron strainer to retain the islets. Infiltrating cells were analyzed by flow cytometry.

**Flow cytometry.** Antibodies to the αβ TCR (clone RT3), CD25 (clone OX-39), CD4 (clone OX-35), CD69a chain (clone OX-8), and Vb13 TCR (clone 1B8) were from BD Pharmingen, and FoxP3 antibody (clone FJK-16A) was from eBiosciences. Isotype control antibodies (mouse IgG1, IgG2a, and IgG2b) and phycoerythrin- or allophycocyanin-conjugated streptavidin were from Pharmingen (San Diego, CA). Antibodies either were directly conjugated with fluorochromes (fluorescein isothiocyanate, peridinin-chlorophyll-protein complex (PerCP), allophycocyanin, or Pacific Blue) or were used as biotin conjugates followed by streptavidin. For analyses of FoxP3, cells were permeabilized using the FoxP3 Staining Buffer Set (eBiosciences), per manufacturer’s instructions. Data were acquired using an LSR II instrument (BD Biosciences) and analyzed with FlowJo software. A minimum of 100,000 viable cells in each sample were analyzed. The lymphocyte fraction was gated according to forward and side scatter.

**Analyses of Tcrb-V13 transcripts.** To analyze combinatorial variants in the CDR3 region of Vb13 T cells in islets early in the course of diabetes, three cohorts of BBP rats were treated with intraperitoneal poly IC, 1 μg/g on days 0 and +3, and killed on day +5, when spleens and pancreata were harvested. Islets were isolated as described (21,22), and hand-picked islets were immediately immersed in RNA Later (Applied Systems-Ambion, Austin, TX) or Trizol (Invitrogen, Carlsbad, CA). Total RNA was isolated according to the manufacturer’s protocols and reverse transcribed into cDNA (High Capacity cDNA Reverse Transcription Kit; Applied Biosystems). Tcrb-Vb13 transcripts were amplified with a Tcrb-Vb13-specific forward primer and a Cβ1/Cβ2 reverse primer (Supplementary Materials) using platinum high-fidelity Taq DNA polymerase (Invitrogen). Transcripts were cloned directly into the pcRII-TOPO vector with the TOPO TA cloning kit (Invitrogen). Transformants were plated on LB-agar-ampicillin plates. Single colonies in LB-ampicillin overnight, and plasmids were isolated with the QIAprep spin mini-prep kit (Qiagen, Valencia, CA). Tcrb-VJ3S1A1 plasmid inserts were sequenced by GeneWiz (Plainfield, NJ) with the T3 primer. Sequences were aligned and compared with the Multiple Sequence Alignment program by Clustal (http://www.genome.jp/tools/clustalw/). To exclude artifacts of PCR amplification or PCR bias, a cloning procedure was chosen for the relative abundance of Jb isotypes, as determined by sequencing versus relative abundance of Jb isotypes quantified by qRT-PCR (see below).

**Analysis of Jb relative abundance.** To analyze diversity and Jb frequency in T cells, we developed a qRT-PCR assay for TCR-Jb region abundance. The assay was applied to Tcrb-V13 and Tcrb-V16 transcripts from islets and spleens of poly IC LEW.1WR1 rats and untreated LEW.1WR1 rats. Forward primers were designed for Tcrb-V13 and Tcrb-V16, and PCR was performed with reverse primers in each of 12 TCR-Jb regions (Supplementary Data). The relative proportions of Jb isotypes were calculated after normalization to total Tcrb-V13 and Tcrb-V16 transcript abundance, respectively, using the comparative Ct method for quantification of results, as described (http://www.mlb Amelia.com/techlib/basics/rtpcr/index.html).

**Histology.** Pancreata were removed at diabetes onset or on day 40 and fixed in 10% buffered formalin. Paraffin-embedded sections were sectioned at 4-μm intervals and stained with hematoxylin and eosin. Intensity of insulin was scored as follows: 0, no inflammatory mononuclear cell (MNC) infiltration; 1+, small numbers of infiltrating MNCs with preservation of islet architecture; 2+, moderate MNC infiltration with preservation of islet architecture; 3+, many MNCs with loss of islet architecture; 4+, complete destruction of islets.

**Statistics.** Diabetes-free survival was analyzed using Kaplan-Meier methodology; equality of survival distributions was tested by log-rank statistic (23). Parametric data are given as arithmetic means ± 1 SD or ± SE, as indicated in the figure legends. Fisher exact statistic was used for analyzing 2 × 2 tables and the χ² test for larger tables. Comparisons of three of more means used one-way and two-way ANOVAs and the either the Bonferroni or least significant differences procedure for a posteriori contrasts (23). Two-tailed P values <0.05 were considered statistically significant.

**RESULTS**

**Depletion of Vb13+ T cells prevents poly IC-triggered autoimmune diabetes.** We first determined that a single injection of 17D5 mAb was associated with a reduction of ~60% in the number of Vb13+ T cells in the spleens of LEW.1WR1 rats (data not shown). We then determined the protective effect of anti-Vb13 mAb treatment on diabetes penetrance in LEW.1WR1 rats treated with poly IC three times weekly.

Trial 1 compared diabetes frequency in rats treated with either 17D5 anti-Vb13 mAb or mouse anti-human OKT8 as a control. Trial 2 compared diabetes frequency in rats treated with 17D5 anti-Vb13 mAb, His42 anti-Vb16 mAb, or vehicle. The combined results of the two trials are shown in Fig. 1. Diabetes frequency in rats given poly IC and 17D5 mAb was 10% (2 of 20). In contrast, diabetes frequency in controls averaged 80% (4 of 40; P < 0.001).

As shown in Fig. 2, treatment with 17D5 mAb was associated with a specific reduction in Tcrb-V13 mRNA transcripts, having no significant effect on Tcrb-V4, -15, or -16 transcripts; His42 mAb treatment was associated with a reduction of similar magnitude only in Tcrb-V16 transcripts. Depletion of Vb13+ T cells prevents insulin and preserves islet architecture. Pancreata from animals in trial 2 were analyzed histologically. As shown in Table 1,
there was significantly less insulitis in animals treated with anti-Vβ13 mAb than in rats in either control group; 9 of 10 of these animals were nondiabetic. In one Vβ13 mAb–treated rat that became diabetic, there was extensive insulitis (not shown). Likewise, there was significantly more insulin detectable in the anti-Vβ13 mAb–treated group. In contrast, glucagon immunohistochemistry revealed no detectable differences among groups. Fig. 3 shows histological samples from a diabetic rat that had received anti-Vβ16 mAb and a nondiabetic rat treated with anti-Vβ13 mAb. The diabetic animal had intense insulitis (Fig. 3D), no detectable insulin (Fig. 3E), and glucagon that was readily detectable but suggestive of collapse of the central core of β-cells (Fig. 3F). In contrast, the anti-Vβ13–treated rat displays little or no insulitis (Fig. 3A), abundant insulin staining (Fig. 3B), and glucagon staining in a normal annular pattern (Fig. 3C).

Depletion of Vβ13+ T cells prevents virus-triggered autoimmune diabetes. We also tested a second, possibly more physiologic model of induced diabetes, one triggered by virus. Trial 3 examined the protective efficacy of 17D5 anti-Vβ13 mAb in rats exposed to a small priming dose of poly I:C followed by infection with KRV. The priming dose of poly I:C is by itself nondiabetogenic but increases the penetrance of virus-triggered diabetes (from ~40 to ~100%) (17). As shown in Fig. 4, diabetes frequency in 17D5-treated rats was 30% (3 of 10) compared with 80% (8 of 10; P = 0.03) in both His42 mAb–treated animals and otherwise-un-treated controls.

CD4+ Vβ13+ T cells are abundant in islets early in the course of diabetes onset. Because the above data indicate that cells expressing the gene product of Tcrb–V13S1A1 are important in diabetes pathogenesis, we hypothesized that, as a fraction of total T cells, the percentage of Vβ13a+ T cells in the islets of susceptible rats early in pathogenesis should be greater than the percentage in peripheral lymphoid tissues. Islets were isolated 48 h after one or two injections of poly I:C (i.e., on day 3 or day 5 of diabetes progression) and cultured for 7 days; the infiltrating T-cell phenotype was analyzed as described (20).

We first analyzed pooled islets harvested from eight individual rats on day 3 after injection with poly I:C on day 0. The percentage of TCR+Vβ13+ T cells recovered from isolated islets was 4.1%, which was indistinguishable from the percentage (5.3%) recovered from pooled fresh CLNs from eight untreated animals.

In two additional experiments, we analyzed islets harvested on day 5 after injection with poly I:C on days 0 and +3. The percentages of TCR+Vβ13+ T cells recovered from isolated islets were higher than from day 3 islets (5.2 and 9.6%), whereas the percentages in T cells from fresh CLNs were about the same (3.5 and 3.3%). In these two trials, the percentage of CD4+ islet–derived T cells that were Vβ13+ averaged 19.5%, whereas in CLNs the percentage of CD4+Vβ13+ T cells averaged 3.5%. In contrast, there were no apparent differences in the percentage of CD8+ cells that were Vβ13+ recovered from day 5 islets (average 2.5%) compared with CLN T cells (2.0%). Finally, we observed that only a minority of CD4+CD25–Vβ13+ recovered islet T cells were FoxP3+ (9.6%). The percentage of TCR+CD4+CD25 FoxP3+ T cells that were Vβ13+ was 5.9% in the islets and 3.8% in CLNs.

Vβ13/Jβ mRNA transcripts in prediabetic islets are skewed. The gene products of Tcrb–V13S1A1 and Tcrb–V13S1A2 encode different amino acid sequences for both the CDR1 and CDR2 regions of the β-chain (15). This polymorphism distinguishes WF and other type 1 diabetes–resistant strains from BBDR, BBDP, KDP, and LEW.1WR1 type 1 diabetes–susceptible strains, all of which share the same class II MHC (10). CDR1 and CDR2 sequences are encoded within each Tcrb–V allele and are not altered by the combinatorial processes that create the CDR3 regions of the TCR.

**FIG. 1.** Frequency of diabetes in LEW.1WR1 rats treated with poly I:C given three times weekly (shaded bar). Compared with controls given an irrelevant mouse anti-human mAb (group 2) or the His42-depleting anti-rat Vβ16 mouse mAb (group 4) or no treatment (group 3), only anti-Vβ13 (group 1) protected against type 1 diabetes. Antibody injections (0.1 mg/dose) are indicated by arrows (overall log-rank statistic = 23.89; df = 3; P < 0.001). Diabetes frequency in groups 2, 3, and 4 was statistically similar (overall log-rank statistic = 0.30; df = 2; P = NS). The ratio of males to females in all treatment groups averaged 1:1.
To investigate the molecular mechanism by which Tcrb-V13S1A1 might confer susceptibility to autoimmunity, we performed a comparative analysis of Vβ13 mRNA transcripts from islets versus peripheral lymphoid tissues. These were cloned as cDNAs into plasmid vectors and sequenced. T cells were obtained from islets or spleens from rats on the fifth day of progression to diabetes or from the spleens of age-matched untreated control rats. The day 5 point was selected based on the flow cytometry data above.

The number of times that an individual CDR3 sequence was present in Vβ13 transcripts was quantified. As shown in Fig. 5, relatively few CDR3 sequences (13%) were found only once in the pools of islet mRNA transcripts; more (25%) were detected two to four times, and nearly two-thirds (62%) were detected more than four times (n = 157). In contrast, relatively more CDR3 sequences (27%) were found only once in the pools of splenocyte mRNA transcripts; 31% were detected two to four times, and fewer than one-half (43%) were detected more than four times (n = 107; χ²=11.3; df = 2; P < 0.005 vs. islet-derived transcripts).

Among spleen cells from untreated rats, the effect was more pronounced; 42% of CDR3 sequences were found only once in the pools of islet mRNA transcripts, 37% were found two to four times, and fewer than one-half (43%) were detected more than four times (n = 76; χ²=38.7; df = 2; P < 0.001 vs. islet-derived transcripts). The correlational analysis of the relative abundance of Jβ isotypes, as determined by sequencing versus relative abundance of Jβ isotypes (see RESEARCH DESIGN AND METHODS), showed significant positive correlation and demonstrates that uniqueness/diversity represented by CDR3 direct sequencing is an accurate measure of TCR-Vβ13 CDR3 diversity.

These data suggested that antigen-specific expansion of Vβ13+ T cells has occurred in the islets of prediabetic rats, but the possibility of unequal amplification of subsets of

![Chart](chart.png)

**FIG. 2.** To determine the extent and specificity of T-cell depletion resulting from treatment with the 17D5 and His42 mAbs, we measured relative transcript abundance for four Tcrb-V isotypes in T cells isolated from the spleens of rats that were either untreated, treated with 17D5 anti-rat Vβ13 mAb, or treated with His42 anti-rat Vβ16 mAb, as described in RESEARCH DESIGN AND METHODS. The control reference isotypes (Vβ4 and Vβ15) for this experiment were selected at random. The data show that Vβ13+ T cells are statistically significantly depleted by the 17D5 reagent and that Vβ16+ T cells are statistically significantly depleted by the His42 reagent; no other paired comparisons were statistically significant. Expression of each β-chain variable region transcript was adjusted for relative Cβ transcript abundance and is presented as the ratio of abundance in treated animals relative to untreated controls. Each data point represents the means ± SE of relative transcript abundance from 9 or 10 individual rats.

To investigate the molecular mechanism by which Tcrb-Vβ13S1A1 might confer susceptibility to autoimmunity, we performed a comparative analysis of Vβ13 mRNA transcripts from islets versus peripheral lymphoid tissues. These were cloned as cDNAs into plasmid vectors and sequenced. T cells were obtained from islets or spleens from rats on the fifth day of progression to diabetes or from the spleens of age-matched untreated control rats. The day 5 point was selected based on the flow cytometry data above.

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**TABLE 1**

Quantitative comparison of islet histology in treated and untreated rats

| Group | Treatment       | n  | n diabetic | Insulitis score* | Insulin score† | Glucagon score‡ |
|-------|-----------------|----|------------|------------------|----------------|-----------------|
| 1     | Poly IC alone   | 10 | 9          | 3.1 ± 1.6        | 0.5 ± 0.8      | 1.8 ± 0.2       |
| 2     | Anti-Vβ13 mAb   | 10 | 1          | 1.5 ± 0.9§       | 1.7 ± 0.6¶     | 1.8 ± 0.4‖      |
| 3     | Anti-Vβ16 mAb   | 10 | 10         | 3.4 ± 0.5        | 0.5 ± 0.5      | 1.9 ± 0.3       |

Samples of pancreatic tissue from prevention trial 2 were scored for intensity of insulitis (0–4), insulin staining (0–2), and glucagon staining (0–2), as described in RESEARCH DESIGN AND METHODS. Overall significance: *F2,27 = 8.263, P = 0.002. †F2,27 = 11.825, P < 0.001. ‡F2,27 = 0.587, P = NS. Paired comparisons: §P < 0.005 vs. groups 1 and 3. ¶P < 0.001 vs. groups 1 and 3. ‖No paired comparisons are statistically significant with respect to glucagon score.
targets, artificially skewing the observed diversity, cannot be entirely excluded. To address this issue and validate further our inference regarding the relative skewing of islet versus peripheral Tcrb-V13 transcripts, we developed a qRT-PCR assay to quantify relative utilization of the 12 Tcrb-J regions expressed by Vβ13+ (diabetes-associated) versus Vβ16+ (control) T cells. We observed differential expression of Tcrb-J regions between islet-homing versus peripheral T cells in the Vβ13+ samples that, as expected, significantly correlated with Jβ abundance determined by direct sequencing (Supplementary Fig. 1), As shown in Fig. 6A, we observed skewed Tcrb-J usage in islet-infiltrating Vβ13+ T cells with overrepresentation of Jβ1.3 and underrepresentation of Jβ2.1. Spleen Vβ13+ T cells from poly I:C–treated and untreated rats did not differ with respect to the frequency of individual Jβ usage. In contrast, the representation of different Jβ segments in Vβ16+ T-cell transcripts was not skewed in the islets in comparison with peripheral Vβ16 transcripts. (Fig. 6B), further supporting a role for Vβ13+ T cells in the early recognition of antigen in islets.

Depletion of Vβ13+ T cells prevents spontaneous autoimmune diabetes. In a final experiment, we sought to exclude the possibility that the protective effect of anti-Vβ13 mAb was restricted to the LEW.1WR1 strain and/or autoimmune diabetes that had been induced by a trigger. To do so, we treated cohorts of spontaneously diabetic BBDP rats with vehicle, anti-Vβ13 mAb, or anti-Vβ16 mAb. As shown in Fig. 7, treatment with anti-Vβ13 mAb through 115 days of age completely prevented diabetes, whereas...
diabetes occurred in 40%, or vehicle-injected, and 70%, or anti-Vβ16 mAb–treated, rats (P, 0.01). Among the rats that were nondiabetic at the end of the experiment, underlying insulitis was more severe among vehicle-treated controls (mean score 2.5 ± 0.8; n = 6) than among rats treated with anti-Vβ16 mAb (1.0 ± 1.7; n = 3) or anti-Vβ13 mAb (1.3 ± 0.7; n = 10; F2,16 = 4.01; P, 0.05).

DISCUSSION

These results demonstrate that depletion of T cells expressing a single β-chain variable region allele, Vβ13a, can prevent autoimmune diabetes in multiple rat models. Depletion of Vβ16+ T cells had no protective effect. The treatment was effective in animals in which diabetes was triggered by innate immune activation (poly I:C), triggered by parvoviral infection, or spontaneous. These functional results confirm and extend our hypothesis that Tcrb-V13S1A1 confers a high degree of susceptibility to rat autoimmune diabetes and is the Iddm14 gene.

The flow cytometry results suggest involvement of Vβ13+ T cells during early stages of disease progression. The limited data available do not permit identification of an autoreactive effector phenotype, but they do suggest that CD4+CD25+Vβ13+FoxP3+ cells play a role in the day 5 inflammatory lesion. This is consistent with previous studies of diabetes in the LEW.1AR1-iddm rat (24) and the BB rat (25), showing that CD4+ T cells are capable of adoptively transferring diabetes. Of interest, we have reported a CD4 bias for rat T cells expressing the Vβ13S1A1 variant β-chain; this skews the TCR repertoire and substantially alters the CD4+:CD8+ ratio among Vβ13+ T cells (15). The importance of Vβ13+ early in disease is highlighted by our finding that weekly anti-Vβ13 mAb started immediately after onset does not reverse hyperglycemia (J.P.M., unpublished data).

Sequencing of Vβ13+ TCR β-chains present in islets on day 5 of disease progression showed that a relatively limited number of “focused” CDR3 specificities from Vβ13+ T cells are clonally expanded. These specificities may mediate islet-antigen recognition in the prediabetic LEW.1WR1 rat. Oligoclonality was not observed in control tissues or in T cells bearing Vβ16 TCR β-chains. Taken together, the genetic, prevention, and phenotyping datasets imply that an immunologic synapse comprising rat class II B/Du on antigen-presenting cells and Vβ13a on T cells confers the geometry required for an unknown antigenic determinant to initiate diabetes. We speculate that the interaction of CDR1 and CDR2 with peptide MHC (pMHC) could affect thymic selection (15) and that these differences account for the specificity of Tcrb-V13S1A1 in conferring susceptibility to diabetes in the rat. Unusual binding geometry of TCR with pMHC recently has been implicated in the generation of autoreactivity at the crystallographic level (26,26). Our data
are consistent with recent literature suggesting that allelic and isotypic TCR α- and β-chain usage, independent of CDR3 usage, contributes to differential immunoreactivity in vitro and in vivo (27,28). Finally, we note that a small number, typically 2–5%, of WF rats that do not express Vβ13a can become induced to become diabetic (13), suggesting that non-Vβ13a+ TCRs can mediate autoimmune diabetes but with much lower efficiency.
Our results suggest that, when present in the context of a high-risk MHC class II haplotype (RT1B/Du), Tcrb-V13S1A1 is a major determinant of disease probability in the rat. We recognize that, based on human and mouse data, it has been assumed that redundancy among cognate rat TCRs would preclude reliance on any one allele of TCR α- or β-chains for disease susceptibility. Our data and data of others strongly suggest that this assumption may be faulty.

In the NOD mouse, no TCR chain family has been linked genetically to type 1 diabetes, but a limited TCR β-repertoire could nonetheless be important. Several analyses of T-cell repertoire, especially in early prediabetic stages, implicate an oligoclonal T-cell response by NOD mice (29,30). NOD and C57BL/6 mice share alleles at most Tcrb loci (http://phenome.jax.org), and thus Idd-congenic strains based on differences between NOD and C57BL/6 would unlikely be to show linkage to the Tcrb region. The one linkage test of a role for Tcrb-V used a parental strain (SJL) that has a major deletion of Tcrb-V and proved only that murine Tcrb-V5-, V8-, V9-, V11-, and -V12, and -V13 are not required for insulitis or spontaneous diabetes (31). However, NOD8.3 TCR transgenic mice, in which the majority of T cells are skewed toward expression of an IGRP-specific Vβ8 TCR, develop disease with rapid onset (32,33). In addition, the prediabetic NOD mouse has an oligoclonal T-cell response (29,30,34–36), and preferential TCR usage has been observed. In one case, it is a dramatic TCR α-chain restriction (predominantly AV13S3) (37). In another, preferential TCR β-chain usage (TRBV-15) has been reported (38).

Human datasets that fail to implicate the TCR in autoimmune diabetes are based on either classical gene mapping or SNP haplotyping. Neither method is ideal for detecting recessive protective alleles because “resistant” individuals would have to be homozygous for the loss of Tcrb-V elements, as we propose is important in the rat. Furthermore, stratification by four-digit MHC haplotype may be required to detect TCR effects in human type 1 diabetes, something that has to date not been reported.

We recognize that studies of anti-Vβ reagents in NOD mice have given conflicting results. Empirical treatment with an anti-Vβ8 mAb reportedly prevented disease in NOD/Wehi mice with cyclophosphamide-accelerated diabetes (39) and in islet isografted diabetic NOD mice (40). However, genetic analyses showed that NOD mice that cannot express Vβ8 nonetheless become diabetic. We would point out, however, that the selection of Vβ8 as a target was not based on genetic susceptibility analyses, as in our studies of rat Vβ13, and NOD mouse diabetes is notoriously easy to prevent with many reagents, including more than 10 different mAbs, among them IgG2a isotype control (41).

In conclusion, TCR β-chain usage may contribute to the penetrance of both spontaneous and induced autoimmune diabetes in genetically susceptible rats of multiple strains. The presence of abundant Vβ13+- T cells in prediabetic islets suggests that Vβ13a is a marker of the disease. Rats prone to autoimmune diabetes may offer the opportunity to determine how Tcrb-V chain variation contributes to autoimmunity and in detail how pMHC/TCR interaction initiates the development of β-cell–specific autoimmunity. Studies to knock down rat Tcrb-V13 expression and to clone diabeticogenic Vβ13+ T cells are underway in our laboratories.

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