Type 1 diabetes of both humans and NOD mice is characterized by selective destruction of β-cells within pancreatic islets (1–4). For the NOD mouse, multiple studies demonstrate that insulin is a primary autoantigen for triggering anti-islet autoimmunity (5,6). To date, with the exception of preproinsulin (7), deletion of several characterized islet target antigens does not alter progression to diabetes in the NOD mouse (8–10). Even the prominent CD8 targeting of the molecule islet glucose-6-phosphatase catalytic subunit-related protein indicate that the insulin B chain peptide, amino acids 9–23 (insulin B9–23) containing a native tyrosine at position 16, is essential for development of diabetes (13). NOD mice lacking native insulin but producing an insulin with a mutation of the B9–23 sequence (B:16A) do not develop diabetes (7), and elimination of insulin-reactive T cells results in the dramatic prevention of diabetes (14,15).

Most T-cell receptor (TCR) interactions with peptide-major histocompatibility complex (MHC) complexes occur through binding of six complementarity determining regions (CDR; three each for α- and β-chains). The CDR3 region is most often crucial for antigen recognition (16). In this region, which includes the N region for α-chains and nD region for β-chains, highly variable amino acid sequences are generated from gene rearrangements of V and J segments (plus D segment for β-chains) (16). The CDR1 and CDR2 regions are germline-encoded by the V segments and, for many TCRs, predominantly interact with the α helices of the MHC molecule (17,18). In NOD mice, TCRs targeting the insulin B9–23 peptide presented by the I-Aβ MHC class II molecule frequently use the α gene segment TRAV5D-4*04 (formerly termed α 13S3) rearranged to the Jα gene segments TRAJ53 and 42 (19,20). Among these TCRs, the N region sequences of the α-chains were highly variable, and no consistent TCR β-chain usage was apparent. Two anti-insulin B9–23 TCR α-chains (derived from T-cell clones 12–4.1 and 12–4.4) using the same Vα (TRAV5D-4*04) and Jα (TRAJ53) gene segments, but having unique N region sequences, were capable of inducing insulin autoimmunity in Ca knockout NOD mice (21). In this article, we show that the sequences underlying such induction of insulin autoimmunity are relatively simple. Namely, the germline-encoded sequences of Vα TRAV5D-4 CDR1 and CDR2 combined with many CDR3 sequences and diverse Jα elements are sufficient to induce anti-insulin autoimmunity.

RESEARCH DESIGN AND METHODS

Mice. NOD.scid mice (NOD.CB17-Prkdcscid/J, 001303) and Ca knockout NOD mice (NOD.129P2(Cr)-Tcrαm13S3/J) (12) were purchased from the Jackson Laboratory (Bar Harbor, ME). B16A double insulin-knockout NOD scid mice were generated in the Eisenbarth laboratory (13). All three strains, NOD/SCID, mice, and retrogenic mice were maintained in a pathogen-free animal colony at the Barbara Davis Center satellite animal facility and the Center for Comparative Medicine. All animal experiments were approved by the Animal Care and Use Committee of the University of Colorado Denver.

Generation of α-chain retrogenic mice. Retrogenic mice were generated using the modified version of the protocol described previously (22,23). TCR α-chain constructs were either generated by PCR using cDNA from original T-cell clones (12–4.4, 12–4.1, BDC-6.9, BDC-10.1, BDC2.5, 14H4, 5P, and 6C5) or assembled based on sequences. For the NY4.1 α-chain, a sequence published in the National Center for Biotechnology Information was used (accession number U80816). The 2H6 sequence was kindly provided from Dr. Li Wen (Yale University, New Haven, CT). TCR α-chain constructs encoding all α-chains detected by the 454 high-throughput sequencing and chimeric human Vα TRAV13-1 α-chains were also assembled by PCR with overlapping primers. TCR α-chain constructs were cloned into mouse stem cell virus (MSCV)-based retroviral vectors carrying green fluorescent protein (GFP) (pMiG3) (22). Phoenix cells were cotransfected with the pMiG plasmids and the pCL-Eco packaging vector using Lipofectamine 2000 (Life Technologies/Invitrogen) to produce replication-incompetent retroviruses encoding TCR α-chain sequences. Bone marrow cells were prepared from Ca knockout NOD mice treated with 5-fluorouracil (Sigma-Aldrich) and spin-infected with the retroviral supernatant daily for 4 consecutive days. The bone marrow cells...
were cultured in complete DMEM containing 20% heat-inactivated fetal bovine serum, 20 ng/mL IL-3, 50 ng/mL IL-6, and 50 ng/mL stem cell factor (Life Technologies/Invitrogen). NOD.scid mice or B6.A double insulin-knockout NOD.scid mice received 210 rad of radiation from the IBL 457 50Cs irradiator (CIS Bio International) or 225 rad from the RS2000 X-ray irradiator (Rad Source Technologies, Suwanee, GA) and were injected with the bone marrow cells (2 × 10⁵ cells) intravenously. For all experiments, cultured bone marrow cells were assessed for GFP expression by flow cytometry prior to the injection; ~50–70% of bone marrow cells were positive for GFP. Peripheral blood mononuclear cells (PBMC) from all recipient mice were assessed for T-cell gene knock-in (a TCR receptor was expressed) generally, 5–25% of PBMC expressed GFP and CD4. PBMC were stained with anti-CD4, anti-CD8, and anti-TCRβ antibodies (eBioscience) and analyzed on a FACSCalibur (BD Biosciences, San Jose, CA).

Measurement of micro-insulin autoantibody assay. All retrogene mice were bled at 4, 5, 8, 10, 12, 16 weeks after bone marrow transfer. Female NOD mice were bled at 4, 5, 8, 10, 12, 16 weeks of age for comparison. Insulin autoantibody (IAA) levels were measured with the 96-well filtration plate micro-IAA assay previously described and expressed as an index (24). A value >0.01 is defined as positive.

Assessment of diabetes incidence. The blood glucose levels were measured weekly. Mice were considered diabetic after two consecutive blood glucose values >250 mg/dL.

Assessment of insulin. The pancreata were obtained when killed (8–20 weeks after bone marrow transplant) and were fixed in 10% formalin. Paraffin-embedded tissue sections were stained with hematoxylin and eosin, and sections from islet grafts were also stained with polycolonal guinea pig anti-insulin antibodies (Linco Research Immunology, St. Charles, MO), followed by incubation with a peroxidase-labeled anti-guinea pig IgG antibody (Kirkegaard and Perry laboratories). More than 10 pancreatic islets from an individual mouse were randomly selected and evaluated for lymphocytic infiltration (no insulin, peri-inslet insulitis, intraislet insulitis) by the same reader (M.N.) blinded to the group of mice.

IFN-γ enzyme-linked immunospot assay. IFN-γ enzyme-linked immunospot (ELISPOT) assay was performed according to the manufacturer’s instructions (BD Biosciences). Spleen cells, harvested from retrogenic mice (7 × 10⁵ cells/well), were incubated in the presence or absence of 100 ng/mL of antigens (insulin B:9–23: SHLVEALALVCGERG; hen egg lysozyme [HEL]:11–25: AMKRHRLNYGYSLL; Genemed Synthesis) or 100 pancreatic islets harvested from NOD.scid mice along with spleen cells from young NOD mice (1 × 10⁵ cells/well) overnight. IL-2 secretion in supernatants was measured by enzyme-linked immunospot assay according to manufacturer’s instructions (BD Biosciences).

RESULTS

Retrogenic NOD mice expressing Vα TRAV5D-4 α-chains with multiple CDR3α sequences develop anti-insulin autoimmunity. To investigate which α-chain TCR sequences are sufficient to induce insulin autoantibodies, we first created a series of retrogenic mice expressing TCR α-chain sequences derived from NOD CD4 T-cell clones into TCR α knockout NOD mice (Supplementary Table 1). In these mice, individual T cells express the introduced TCR receptor α-chain that associates with endogenously selected β-chain sequences produced in vivo. The α-chain TCR sequences used are derived from clones that react to the insulin B:9–23 peptide, unknown islet antigens, or completely unrelated antigens. Despite different N regions and βα sequences, six out of seven α-chain retrogenic strains expressing the TRAV5D-4 sequence developed high levels of insulin autoantibodies (Fig. 1A–G and Supplementary Table 1). Of note, α-chains originally derived from diabetic T-cell clones that react with unknown islet antigens [i.e., NY.1 described by Santamaria et al. (26) and BDC-6.9 established by Haskins et al. (27)], or even a nondiabetogenic clone reactive with an HEL peptide [1H4 established by Vignali et al. (28)], could induce insulin autoantibodies. Three strains expressing α-chains originally reacting with the insulin B:9–23 peptide developed relatively the higher level of insulin autoantibodies compared with female wild-type NOD mice (Fig. 1A–C and N; P = 0.03 [12–4.4], 0.04 [12–4.1], and <0.01 [8–11] versus wild-type NOD). In contrast, only one of the retrogenic strains expressing TCR α-chains other than Vα TRAV5D-4 developed insulin autoantibodies (Fig. 1H–M; P < 0.02 versus strains that express α-chains with TRAV5D-4). Retrogene mice expressing TCR α-chains derived from clones known to be highly diabetic, but that do not use Vα TRAV5D-4 [BDC-2.5 and BDC-10.1 established by Haskins et al. (27)], and those expressing TCR α-chains from clones that respond to the insulin B:9–23 peptide [2H6 established by Wen et al. (29) and 12–2.35 established by Wegmann et al. (30)] did not lead to the production of
insulin autoantibodies. The only non–TRAV5D-4 α-chain that induced insulin autoantibodies is derived from a diabetogenic insulin B:9–23-reactive T-cell clone [12–1.19 established by Wegmann et al. (30)]. It is notable that none of the three additional retrogenic mouse strains expressing α-chains containing this Vα segment (TRAV13–1) used by this 12–1.19 α-chain developed insulin autoantibodies (see below; Fig. 4D). Although TRAV5D-4 sequences induced insulin autoantibodies, only a subset of the Vα TRAV5D-4 retrogenic mice developed overt diabetes (Supplementary Fig. 1), and the severity of lymphocytic infiltration in the islets was consistent with the diabetes incidence (Fig. 2). Of note, although α-chain retrogenic mice are lymphopenic compared with wild-type mice in general, and peripheral CD4 and CD8 T-cell populations have broad variations even in the same strain (Supplementary Fig. 2), the number of T cells in the periphery did not correlate with the development of insulin autoantibodies, insulitis, and diabetes, and the percentages of CD4 and CD8 T cells do not statistically differ by strains by one-way ANOVA.

Expression of the insulin B:9–23 sequence is required for the development of anti-insulin autoimmunity. We next asked whether development of insulin autoimmunity in TRAV5D-4 α-chain retrogenic mice is associated with the recognition of the insulin B:9–23 peptide. Spleen cells from strains expressing TRAV5D-4 α-chains that produced high levels of insulin autoantibodies (from clones 12–4.1, 12–4.4, 8–1.1, and BDC-6.9) responded to insulin B:9–23, but did not respond to either the insulin B:9–23 peptide with B16:A mutation or a control tetanus toxin 830–843 peptide (Fig. 3A). Responses to the insulin B:9–23 peptide by spleen cells from 14H4, 5F2, and NY4.1 α-chain retrogenic mice were undetectable. The levels of insulin autoantibodies in these strains are not as high as those of other TRAV5D-4 α-chain strains showing reactivity to the insulin B:9–23 peptide, which may suggest the low probability of

FIG. 1. Ca knockout NOD mice retrogenic for α-chains containing TRAV5D-4 but not non–TRAV5D-4 develop anti-insulin autoimmunity. Levels of IAA in mice retrogenic for TCR α-chains with TRAV5D-4 (A–G) or with non–TRAV5D-4 (H–M) and in female NOD/Bdc mice (N). The α-chain sequences are derived from NOD CD4 T-cell clones (A: 12–4.4 [n = 8]; B: 12–4.1 [n = 6]; C: 8–1.1 [n = 5]; D: BDC-6.9 [n = 4]; E: NY4.1 [n = 9]; F: 14H4 [n = 5]; G: 5F2 [n = 5]; H: 2H6 [n = 7]; I: 12–2.35 [n = 7]; J: 12–1.19 [n = 8]; K: BDC-10.1 [n = 7]; L: BDC-2.5 [n = 7]; M: 6C5 [n = 4]). Symbols represent individual mice, and each panel represents a different retrogenic strain with a unique N and TRAJ region. IAA index ≥0.01 is defined as positive.
B:9–23-reactive T cells in the strains without the response to B:9–23. In the retrogenic mice that express an α-chain other than TRAV5D-4, spleen cells from only the 12–1.19 strain, which developed insulin autoantibodies, responded to insulin B:9–23 (Fig. 3B; \( P < 0.05 \) versus strains that express α-chains with TRAV5D-4).

To further examine how frequently T cells infiltrating pancreatic islets of TRAV5D-4 α-chain retrogenic mice recognize the insulin B:9–23 peptide, we reconstituted β-chains detected in the islets along with the original α-chain on the TCR-null 5KC CD4 + T cells (a T-cell hybridoma without T-cell receptor α- and β-chain genes) (31). We analyzed 146 β-chain sequences amplified by 5’ rapid amplification of cDNA ends PCR from the islets of retrogenic mice expressing either the 12–1.1 or 8–1.1 α-chain and found 90 unique junction sequences (Supplementary Table 2). We randomly chose 30 unique β-chain sequences and tested 5KC cell lines expressing those β-chain sequences for response to islets and the insulin B:9–23 peptide. As shown in Fig. 3C, 17 out of 30 5KC CD4 + T-cell lines responded to islets isolated from NOD.scid mice, and 14 of the 17 islet-responding lines (82%) were insulin B:9–23-reactive. Thus, the majority of TCRs of CD4 T cells infiltrating and responding to pancreatic islets of TRAV5D-4 α-chain retrogenic mice reacted with the insulin B:9–23 peptide.

We then asked whether the expression of native insulin B:9–23 sequence is required for TRAV5D-4 α-chain retrogenic mice to develop anti-insulin autoimmunity in vivo. To test this, we used B16:A double insulin-knockout NOD.scid mice (both native ins 1 and ins 2 genes knocked out) as bone marrow recipients (13). With the knockout of native insulin genes (Ins1 and Ins2) and a preproinsulin transgene producing insulin in islets with alanine rather than tyrosine at position B16, retrogenic mice do not express native insulin in their islets and lymphoid epithelial cells. The lack of native insulin B:9–23 expression in the recipient mice abrogated development of insulin autoantibodies (Fig. 3E; \( P < 0.01 \)), diabetes, and lymphocyte reactivity to the insulin B:9–23 (Fig. 3D; \( P < 0.02 \)). Thus, development of anti-insulin autoimmunity of TRAV5D-4 α-chain retrogenic mice is dependent upon the expression of the native insulin B:9–23 sequence.

TRAV5D-4 α-chains with variable CDR3 sequences induce anti-insulin autoimmunity. To characterize the utilization of the Vα TRAV5D-4 in NOD pancreatic islets, we PCR-amplified TCRs from cDNA of 20-week-old NOD islets and sequenced these amplicons using high-throughput sequencing. Within ~100 islets extracted from two mice, we found >1,000 unique in-frame TRAV5D-4 sequences with relatively few expanded identical CDR3 sequences. Individual mice had notably different TRAV5D-4 repertoires and TCR variant frequencies in islets (Fig. 4A); for example, we detected approximately 1,400 counts of the same TRAV5D-4 sequence in one mouse (x-axis), whereas that sequence was not detected at all in the second mouse (y-axis). To assess whether multiple TRAV5D-4 α-chain sequences observed in these islets would induce insulin autoantibodies in retrogenic mice, we produced five TRAV5D-4 α-chain retrogenic strains using sequences obtained from the 454 sequencing (Supplementary Table 3), and four out of five strains developed insulin autoantibodies (Fig. 4B). Of the sequences chosen, two were observed at high frequency and three were singletons (observed once) in the high-throughput sequencing experiments. Thus,
multiple intraislet α-chains containing Vα TRAV5D-4 with different CDR3 sequences are capable of inducing anti-insulin autoimmunity. As controls, we created retrogens with TRAV6 or TRAV13–1 α-chain sequences that were also observed at high frequency in islets based on 454 sequencing (Supplementary Table 3). None of the strains with these control Vα segments developed insulin autoantibodies (Fig. 3C and D).

Essential amino acid sequences in the TRAV5D-4 CDR1 and CDR2 for the recognition of insulin B:9–23. Evidence that TRAV5D-4 α-chains with diverse CDR3α sequences are capable of inducing anti-insulin autoimmunity (in response to the insulin B9–23 peptide) led to the hypothesis that the TRAV5D-4 CDR1 and/or CDR2 sequences might be crucial to the recognition of the B9–23/I-A^d complex. To assess which specific amino acids in CDR1 and CDR2 are important for response to the insulin B9–23, we performed an in vitro alanine scan of the α-chain CDR1 and CDR2 regions in the 8–1.1 TRAV5D-4 αβ anti-insulin B9–23 TCR. For this experiment, we used 5KC CD4+ T cells (31) as recipients of TCR reconstitution, and the complete set of alanine mutations at each position of CDR1α and CDR2α were tested for response to insulin B9–23 peptides in 5KC T-cell lines. As shown in Fig. 5A, mutations of asparagine in CDR1α (position 5) and arginine in CDR2α (position 2) abrogated the response to the insulin B9–23 peptide. Notably, reactivity of other insulin B9–23-reactive TCRs (12–4.1 and 12–4.4) also depended on these two amino acid sequences at positions CDR1α-5 and CDR2α-2 (Fig. 5B). In contrast, the two anti-HEL:11–25-reactive TCRs that also contain Vα TRAV5D-4 (14H4 and 5F2) responded to their peptide target presented by I-A^d, despite replacing either CDR1α-5 or CDR2α-2 with alanine (Fig. 5C). Importantly, retrogenic mice with these TRAV5D-4 chains containing alanine mutations at CDR1α-5 and CDR2α-2 positions did not develop insulin autoimmunity in response to the insulin B9–23 peptide (Fig. 5D).
develop any anti-insulin antibodies (Fig. 5D; for each position, \( P < 0.01 \) versus no mutated TCR), and T-cell responsiveness to insulin B:9–23 in these mice, as measured by IFN-\( \gamma \) ELISPOT, was completely abrogated (Fig. 5E; for each position, \( P < 0.02 \) versus no mutated TCR). This lack of anti-insulin autoimmunity was not due to a lack of T cells in the periphery as both CD4 and CD8 T cells were present at levels comparable to the nonmutated \( 8–1.1 \) \( \alpha \)-chain retrogenic mice.

**Chimeric \( \alpha \)-chains with the human ortholog of murine TRAV5D-4 are capable of inducing anti-insulin autoimmunity.** To explore the potential relevance of our findings to human type 1 diabetes, we produced retrogenic NOD mice with a chimeric human \( V\alpha \) TRAV13–1 \( \alpha \)-chain. TRAV13–1 is the human ortholog of murine TRAV5D-4 (70% amino acid identity) with identical amino acids at the key positions of CDR1\( \alpha \)-5 and CDR2\( \alpha \)-2 (asparagine and arginine) as the murine TRAV5D-4 (Supplementary Fig. 3). In these retrogenic experiments, we engineered mice to express chimeric \( \alpha \)-chains composed of the \( V\alpha \) sequences from human TRAV13–1, but with N, J\( \alpha \), and C\( \alpha \) regions from mice, specifically 8–1.1 or 12–4.1 \( \alpha \)-chain CDR3 sequence. These chimeric \( \alpha \)-chain retrogenic mice developed insulin autoantibodies (Fig. 6A) and diabetes (Fig. 6B).

**DISCUSSION**

It is generally assumed that for typical immune responses, CDR3 sequences of both \( \alpha \)- and \( \beta \)-chains dominate the specificity to peptide recognition. In our experiments, different levels of insulin autoantibodies were induced in retrogenic mice by TRAV5D-4 \( \alpha \)-chains with diverse CDR3 sequences (Fig. 1). The quantitative differences between retrogenic strains expressing TRAV5D-4 \( \alpha \)-chains are likely determined by influence of varying CDR3 and J\( \alpha \) sequences contributing to recognition of the insulin B:9–23-I-A\(^{b7} \) complex. Our study provides evidence that amino acids asparagine and arginine in CDR1 and CDR2 (respectively) in the TRAV5D-4 gene segment are essential for anti-insulin reactivity. Mutating a single amino acid of CDR1 and CDR2 in vitro and in vivo abrogated targeting of insulin B:9–23. This suggests that the CDR1 and CDR2 regions of the specific germline-encoded \( V\alpha \) sequence predispose TCRs to target the insulin B:9–23 peptide presented by I-A\(^{b7} \) and are usually sufficient for the induction of anti-insulin autoimmunity in NOD mice. Such a simple TCR motif may relate to the ease and frequency of activating islet autoimmunity when other diabetes-promoting genetic and/or environmental factors exist.

It was notable that retrogenic strains expressing TRAV5D-4 \( \alpha \)-chains developed peri-islet insulitis or even intraislet insulitis, which indicates that even T cells expressing islet-unrelated TRAV5D-4 \( \alpha \)-chains are capable of recognizing islet antigens. TRAV5D-4 \( \alpha \)-chain retrogenic mice had greater lymphocytic infiltration than non-TRAV5D-4 \( \alpha \)-chain strains. TRAV5D-4 \( \alpha \)-chains, even ones derived from islet-unrelated antigens, paired with appropriate \( \beta \)-chains may have a higher chance to recognize islet antigens.
The evidence that TRAV5D-4 α-chains induce insulin autoantibodies may suggest that such TCRs recognize the insulin B:9–23 peptide and initiate islet infiltration. Indeed, NOD mice transgenic or retrogenic for the 12–4.1 and 12–4.4 TRAV5D-4 α-chains have the greater number of T cells reacting with the insulin B:9–23 peptide (32).

There are other examples of dominant V gene segment usage in autoimmune diseases. Tisch and colleagues (33) have found a preponderance of a specific TCR Vβ usage among T cells responding to the anti-islet BDC-2.5 mimotope. In the BB rat model, a specific Vβ sequence (Tcrβ-V13) that is polymorphic among rat strains contributes to disease.
The current study indicates that despite dramatic diversity in N-region and Jα sequences, producing retrogenic mice having a common Vα sequence, TRAV5D-4, results in insulin autoimmunity. In the presence of the appropriate class II MHC alleles, such a Vα may set the stage for generation of insulin-specific autoimmunity. Even the related human sequence (TRAV13-1) as a chimeric retrogenic with murine CDR3 and Cα region induces insulin autoantibodies. In conclusion, by use of an α-chain retrogenic mouse model, we identified a pathogenic α-chain sequence that elicits insulin autoimmunity via the recognition of insulin B9–23 peptides. It is notable that the Vα TRAV5D-4 sequence that is encoded in the germline with fixed CDR1 and CDR2, but a diversity of CDR3 sequences, recognizes one essential peptide to provoke autoimmunity. Targeting of the interaction of pivotal germline Vα or Vβ sequences with cognate peptide–MHC complexes may provide a disease-specific immunotherapeutic strategy for prevention of autoimmunity.

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REFERENCES

1. Di Lorenzo TP, Peakman M, Roep BO. Translational mini-review series on type 1 diabetes: Systematic analysis of T cell epitopes in autoimmune diabetes. Curr Exp Immunol 2007;148:1–16.

2. Atkinson MA, Gianani R. The pancreas in human type 1 diabetes: providing new answers to age-old questions. Curr Opin Endocrinol Diabetes Obes 2009;16:279–285.

3. Santamaría P. The long and winding road to understanding and conquering type 1 diabetes. Immunity 2010;32:437–445.

4. Bluestone JA, Herold K, Eisenbarth GS. Genetics, pathogenesis and clinical reagents and T-cell clones. M.N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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LG., and G.S.E. analyzed data. T.C. and D.P. conducted biocomputational analyses. M.N., T.C., TS., KH., D.A.A.V., LG., D.P., and G.S.E. wrote and edited the manuscript. D.A.A.V. provided training in and advice on the generation of retrogenic mice. K.H. and D.A.A.V. provided essential reagents and T-cell clones. M.N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

L.G., D.P., and G.S.E. wrote and edited the manuscript. T.C. and D.P. conducted biocomputational analyses. M.N., T.C., T.S., K.H., D.A.A.V., L.G., and G.S.E. analyzed data. T.C. and D.P. conducted biocomputational analyses. M.N., T.C., T.S., K.H., D.A.A.V., L.G., and G.S.E. analyzed data.

20. Abiru N, Wegmann D, Kawasako E, Gottlieb P, Simone E, Eisenbarth GS. Dual overlapping peptides recognized by insulin peptide B29-33 T cell receptor V133S T cell clones of the NOD mouse. J Autoimmun 2000;14:231–237.

21. Kobayashi M, Jasinski J, Liu E, et al. Conserved T cell receptor alpha-chain induces insulin autoantibodies. Proc Natl Acad Sci USA 2008;105:10090–10094.

22. Hoist J, Szymczak-Workman AL, Vignali KM, Burton AR, Workman CJ, Vignali DA. Generation of T-cell receptor retrogenic mice. Nat Protoc 2006;1:406–417.

23. Scott-Browne JP, White J, Kappler JW, Gapin L, Marrack P. Germline-encoded amino acids in the alpha-beta T-cell receptor control thymic selection. Nature 2009;458:1043–1046.

24. Yu L, Robles DT, Abiru N, et al. Early expression of antisilin insulin autoantibodies of the human NOD mouse: evidence for early determination of subsequent diabetes. Proc Natl Acad Sci USA 2000;97:1701–1706.

25. Nicolas MR, Coulombe M, Beilke J, Gelhaus HC, Gill RG. CD4-dependent generation of dominant transplantation tolerance induced by simultaneous perturbation of CD154 and LFA-1 pathways. J Immunol 2002;169:4831–4839.

26. Verdaguer J, Schmidt D, Amrani A, Anderson B, Averill N, Santamaría P. Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. J Exp Med 1997;186:1663–1676.

27. Tsuchi K, Portas M, Bergmann B, Lafferty K, Bradley B. Pancreatic islet-specific T-cell clones from nonobese diabetic mice. Proc Natl Acad Sci USA 1989;86:8000–8004.

28. Burton AR, Vincent E, Arnold PY, et al. On the pathogenesis of autoantigen-specific T-cell receptors. Diabetes 2008;57:1321–1330.

29. Du W, Song FS, Li MO, et al. TGF-beta signaling is required for the function of insulin-reactive T regulatory cells. J Clin Invest 2006;116:1366–1370.

30. Daniel D, Wegmann DR. Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide B(29-33). Proc Natl Acad Sci USA 1996;93:956–960.

31. White J, Pullen A, Choi K, Marrack P, Kappler J. Antigen recognition properties of mutant V beta 3+ T cell receptors are consistent with an immunoglobulin-like structure for the receptor. J Exp Med 1999;187:119–125.

32. Zhang L, Jasinski JM, Kobayashi M, et al. Analysis of T cell receptor beta chains that combine with dominant conserved TRAV5D-4’04 anti-insulin B9-23 alpha chains. J Autoimmun 2009;33:42–49.

33. Li L, He Q, Garland A, et al. beta-cell-specific CD4+ T cell clones in peripheral blood and the pancreatic islets are distinct. J Immunol 2009;183:7785–7790.

34. Mordes JP, Cort I, Norwosi E, et al. Analysis of the rat Iddm14 diabetes susceptibility locus in multiple rat strains: identification of a susceptibility haplotype in the Tcrb-V locus. Mamm Genome 2009;20:162–169.

35. Nakayama M, Abiru N, Moriyama H, et al. Prime role for an insulin epitope in peptide-MHC recognition by self-reactive T cell receptors. Curr Opin Immunol 2005;17:290–294.

36. Zhang L, Jasinski JM, Kobayashi M, et al. Analysis of T cell receptor alpha chains from nonobese diabetic mice. Proc Natl Acad Sci USA 1999;96:10354–10359.