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_J Immunol_ 2006; 176:1637-1644; doi: 10.4049/jimmunol.176.3.1637
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Early Autoimmune Destruction of Islet Grafts Is Associated with a Restricted Repertoire of IGRP-Specific CD8⁺ T Cells in Diabetic Nonobese Diabetic Mice

Carmen P. Wong, Li Li, Jeffrey A. Frelinger, and Roland Tisch

β cell replacement via islet or pancreas transplantation is currently the only approach to cure type 1 diabetic patients. Recurrent β cell autoimmunity is a critical factor contributing to graft rejection along with alloreactivity. However, the specificity and dynamics of recurrent β cell autoimmunity remain largely undefined. Accordingly, we compared the repertoire of CD8⁺ T cells infiltrating grafted and endogenous islets in diabetic nonobese diabetic mice. In endogenous islets, CD8⁺ T cells specific for an islet-specific glucose-6-phosphatase catalytic subunit-related protein derived peptide (IGRP206–214) were the most prevalent T cells. Similar CD8⁺ T cells dominated the early graft infiltrate but were expanded 6-fold relative to endogenous islets. Single-cell analysis of the TCR α and β chains showed restricted variable gene usage by IGRP206–214-specific CD8⁺ T cells that was shared between the graft and endogenous islets of individual mice. However, as islet graft infiltration progressed, the number of IGRP206–214-specific CD8⁺ T cells decreased despite stable numbers of CD8⁺ T cells. These results demonstrate that recurrent β cell autoimmunity is characterized by recruitment to the grafts and expansion of already prevalent autoimmune T cell clonotypes residing in the endogenous islets. Furthermore, depletion of IGRP206–214-specific CD8⁺ T cells by peptide administration delayed islet graft survival, suggesting IGRP206–214-specific CD8⁺ T cells play a role early in islet graft rejection but are displaced with time by other specificities, perhaps by epitope spread. The Journal of Immunology, 2006, 176: 1637–1644.

Type 1 diabetes (T1D) is an organ-specific autoimmune disease characterized by the destruction of the insulin-producing pancreatic β cells. The nonobese diabetic (NOD) mouse spontaneously develops a T-cell-dependent β cell destruction (1–3). CD4⁺ T cells have an essential role in both regulating and mediating the diabetogenic response. It is also evident that autoreactive CD8⁺ T cells play an important role in β cell destruction (4). CD8⁺ T cell clones established from islet infiltrates of NOD mice mediate diabetes upon adoptive transfer, and diabetes is exacerbated in transgenic NOD mice expressing TCRs derived from pathogenic CD8⁺ T cell clones (5–7). In addition, NOD mice that lack CD8⁺ T cells, either by anti-CD8 Ab depletion (8) or a disrupted β₂-microglobulin gene (9–12), fail to develop diabetes. Finally, pancreatic infiltrates (insulitis) of diabetic patients have significant numbers of CD8⁺ T cells (13–16).

A concerted effort has been made to elucidate the β cell specificities of CD8⁺ T cells involved in the pathogenesis of T1D. Early work showed that the TCR α-chain expressed by a high frequency of CD8⁺ T cells infiltrating the islets of NOD mice was shared with the pathogenic 8.3 CD8⁺ T cell clone (17). 8.3-like CD8⁺ T cells are specific for an H2Kd-restricted epitope of islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP206–214) and are detected with H2Kd (Kd) tetramers complexed with NRP mimotopes such as the high avidity NRP-A7 and NRP-V7 analogues (18–20). Notably, selective expansion in peripheral blood and islets of high avidity/affinity NRP-A7- or NRP-V7-specific clonotypes coincides with the onset of overt diabetes in NOD mice (19, 20). Peptides derived from the insulin B chain (InsB15–23) (21) and dystrophia myotonica kinase (DMK138–146) (22) are also targeted in NOD mice by islet-infiltrating H2Kd- and H2Dα-restricted CD8⁺ T cells, respectively. However, IGRP206–214-specific clonotypes typically predominate in the islets relative to InsB- and DMK-specific CD8⁺ T cells, especially at later stages of disease progression.

Islet or pancreas transplantation offers a permanent treatment for diabetic individuals. Analogous to other transplants, genetic differences in HLA between donor and recipient promote islet and pancreas graft rejection. In addition, successful β cell engraftment in diabetic patients is further complicated by recurrent autoimmunity (23, 24). The importance of β cell-specific CD8⁺ T cells in recurrent autoimmunity is highlighted by studies demonstrating that MHC class I-deficient syngeneic islet grafts survive indefinitely in diabetic NOD mice (25, 26). However, the specificity of CD8⁺ T cells associated with autoimmune-mediated destruction of islet grafts is undefined. One possibility is that T cell clonotypes involved in the destruction of endogenous islets are also recruited to the islet graft. Alternatively, “new” β cell specificities may be targeted in the islet graft due to “exhaustion” of clonotypes driving endogenous β cell destruction. Distinguishing between these and other possible scenarios is important for understanding the mechanism of recurrent autoimmunity and the development of strategies for inducing islet graft tolerance. Accordingly, the current study...
was initiated to gain insight into the nature of β-cell-specific CD8\(^+\) T cell epitopes in autoimmune-mediated islet graft rejection.

**Materials and Methods**

**Mice**

NOD/LtJ, NOD.Cg-Tg(TcrTcrrb/Ny8.3)Pesa (8.3 TCR transgenic), and NOD.CB17.Prkdc<sup>scid</sup>/J (NOD.scid) mice were bred and housed under specific pathogen-free conditions. Diabetes was monitored weekly by measuring urine glucose levels with Diastix (Bayer). Mice were diagnosed as diabetic when the level of urine glucose exceeded 0.25% for two successive measurements according to manufacturer’s guidelines. A urine glucose level of 0.25% is equivalent to a blood glucose value of ≈250 mg/dl as determined by an Autokit Glucose CII assay (WAKO) (data not shown). BALB/c and FVB/J mice were bred and housed in filter-secured isolator cages. Animals were maintained at an American Association of Laboratory Animal Care-accredited animal facility. All procedures were reviewed and approved by the University of North Carolina Institutional Animal Care and Use Committee.

**Peptides**

MHC class I peptides NRP-V7 (KYKNANVFL), IGRP<sub>206–214</sub> (VYLKTVFVFL), GAD<sub>546–554</sub> (SYQPLGDKV), InsB<sub>15–23</sub> (LYLVCGERG), InsB-G<sub>9</sub>V peptide was modified from its native sequence to increase MHC class I stability (27).

**Tetramers, Abs, and flow cytometry**

H2K<sup>b</sup> tetramers were prepared as described (28). Briefly, peptide/MHC monomers were purified by HPLC and biotinylated using biotin-protein ligase (AvidiT). Tetramers were assembled by conjugating MHC monomers with streptavidin-PE (Molecular Probes). Fluorescent-conjugated anti-mouse Abs used for cell surface staining include anti-CD4 purchased from BD Pharmingen, and anti-CD3, anti-CD8, anti-CD2L, and anti-CD44 purchased from eBioscience.

**Results**

To gain insight into the mechanism of recurrent β cell autoimmunity, the specificity and frequency of CD8\(^+\) T cells that infiltrate grafted vs endogenous islets were measured. Initially, the predominance of CD8\(^+\) clone(s) residing in the endogenous pancreas was assessed in nonobese 20-wk-old NOD female mice, which represent a late preclinical stage of T1D. ELISPOT was used to measure the relative frequency of IFN-γ, IL-4, and IL-10 secreting CD8\(^+\) T cells specific for a panel of known β cell autoantigen epitopes. This included IGRP<sub>206–214</sub> and the corresponding NRP-V7 tetramer. Alternatively, peptide-treated diabetic NOD mice received islet grafts, and islet infiltrates were analyzed by I microscopy. Survival curves were compared using Kaplan Meier log-rank test.
peptides. IFN-γ-secreting CD8+ T cells were detected in response to IGRP206–214 and NRP-V7, but not InsB15–23, DMK138–146, ProInsB25–C34, GAD65465–554, or the control influenza NP peptide (Fig. 1A). No IL-4 or IL-10-secreting T cells were detected above background in response to any of the peptides tested. Similar results were obtained when lymphocyte infiltrates isolated from islets of individual 20 wk-old NOD female mice were examined (data not shown). Consistent with the ELISPOT data, H2Kd tetramers complexed with NRP-V7 (Kd-NRPV7) bound CD8+ T cells from islets prepared from four individual non-diabetic 20-wk-old NOD female mice (Fig. 1B). Kd-NRPV7 bound 7.9 ± 2.8% of islet-infiltrating CD8+ T cells, whereas only minimal binding was observed with Kd-InsB15–23 (0.7 ± 0.3%) or Kd-NP (0.4 ± 0.1%) (Fig. 1B). Kd-NRPV7+ CD8+ T cells were also detected in the pancreatic lymph nodes (PLN) (0.4 ± 0.1%) and spleen (0.5 ± 0.2%), albeit at lower frequencies than that seen in the islets (Fig. 1B). Because increased binding to CD8+ T cells prepared from 8.3 TCR NOD transgenic mice was detected for Kd-NRPV7 compared with Kd tetramer complexed with IGRP206–214 (Kd-IGRP) (data not shown), NRP-V7 tetramers were used in subsequent experiments to detect IGRP206–214-specific clonotypes ex vivo.

The aforementioned results indicated that IGRP206–214-specific CD8+ T cells were the most prevalent of the known MHC class I-restricted β cell-specificities in the islets; therefore, efforts initially focused on Kd-NRPV7 binding in syngeneic islet grafts. Recent onset diabetic NOD female mice were transplanted with islets prepared from NOD.scid donor mice. Recurrent diabetes was typically detected ~2 wk postimplantation. The infiltrates from grafted and endogenous islets were compared 7 days postimplantation within individual recipients. Strikingly, a marked increase in the frequency of Kd-NRPV7+ CD8+ T cells was detected in islet grafts (42.1%) (Fig. 2A) compared with the endogenous islets (8.9%) (Fig. 2B). Few Kd-NRPV7+ CD8+ T cells were detected in the draining renal lymph node (0.7%), PLN (0.9%), or spleen (1.4%) of islet graft recipients (Fig. 2, C–E). In 10 recipients analyzed, a >6-fold increase in the frequency of Kd-NRPV7+ CD8+ T cells was detected in grafted vs endogenous islets (p = 0.003) (Fig. 2F). Minimal staining (<0.6%) was observed using the control Kd-NP tetramer in all samples analyzed. Furthermore, no significant staining above background was detected with Kd-InsB (0.8 ± 0.3%) or Kd-ProIns (0.6 ± 0.2%) tetramers. Consistent with a role as effector cells, 98% of Kd-NRPV7+ CD8+ T cells infiltrating the islet graft were CD62LlowCD44high (data not shown).

The marked increase in Kd-NRPV7+ CD8+ T cells infiltrating the transplant was dependent on H2Kd expression by the islet graft. In diabetic NOD (H2DbKd) recipients of BALB/c (H2DdKd) islets, a 5-fold increase of Kd-NRPV7+ CD8+ T cells was detected in grafted (13.9 ± 0.9%) vs endogenous (2.8 ± 1.0%) islets (p = 0.003) (Fig. 3). In contrast, in NOD recipients of FVB (H2DqKq) islets, Kd-NRPV7+ CD8+ T cells were detected in the graft, but the frequency of tetramer binding CD8+ T cells was equivalent to that detected in the endogenous islets (2.7 ± 0.6% vs 3.3 ± 1.9%, respectively) (Fig. 3). These results demonstrate that IGRP206–214-specific CD8+ T cells dominate the early infiltrate of syngeneic islet grafts, and that the frequency of this set of clonotypes is significantly expanded in grafts compared with the endogenous islets.

The TCR repertoire of IGRP206–214-specific CD8+ T cells in grafted and endogenous islet infiltrates is restricted and shared

To determine the diversity of IGRP206–214-specific CD8+ T cells residing in grafted vs endogenous islets, the TCR repertoire of Kd-NRPV7+ CD8+ T cells was examined in four individual recipients 7 days postimplantation via single-cell sorting and RT-PCR. A total of 53 Vβ TCR sequences were analyzed from Kd-NRPV7+ CD8+ T cells isolated from grafted and endogenous islets, of which all used the Vα17-Jα42 segment (IMGT nomenclature, TRAV16-TRAJ42) characteristic of IGRP206–214-specific clonotypes with a conserved N junction. Analysis of the TCR β-chain revealed preferential usage of Vβ8.1 (TRBV13–3), and Jβ2.4 (TRBJ2–4) and Jβ2.7 (TRBJ2–7) (Fig. 4, A and B). Alignment of the CDR3β segments indicated a restricted number of T cell clones in each recipient, with one or two dominant clonotypes comprising up to 87% of Kd-NRPV7+ CD8+ T cells analyzed (Fig. 4C). Notably, these clonotypes were found to be dominant in both grafted and endogenous islets of individual recipients (Fig. 4C). However, when the TCR repertoires of Kd-NRPV7+ CD8+ T cells were compared among the recipients, different sets of clones were detected in each recipient (Fig. 4C). The identity of the dominant clones also differed among the four recipient mice analyzed. Indeed, only two clonotypes with the respective CDR3β usage of SDSQNTL and SDGTYEQ were repeatedly observed (Fig. 4C). Taken together, these results indicate that in diabetic NOD mice, the TCR repertoire of IGRP206–214-specific CD8+ T cells infiltrating grafted and endogenous islets is shared and limited to a few dominant clonotypes. Furthermore, clonotypic variation exists within IGRP206–214-specific CD8+ T cells among individual recipient mice.

**FIGURE 1.** IGRP206–214-specific CD8+ T cells are the prevalent β cell-specific clonotypes in the islets of 20-wk-old NOD female mice. A, Pooled islet T cell infiltrates from four 20-wk-old NOD female mice were expanded in IL-2-containing medium. ELISPOT was used to measure the frequency of IFN-γ-secreting T cells upon restimulation with a panel of MHC class I-restricted peptides (NP, NRP-V7, IGRP206–214, InsB15–23, GAD65465–554, ProInsB25–C34, and DMK138–146). IFN-γ-specific SFC per 10,000 islet-infiltrating lymphocytes is shown after subtraction of background (approximately six SFC) in medium-only wells. Data are representative of four separate experiments. B, The average percentage (±SEM) of Kd-NRPV7+ (●) and Kd-InsB15–23+ (□) CD8+ T cells isolated from islet infiltrates, PLN, and spleens of four individual 20-wk-old NOD female mice was determined. Kd-NP served as a negative control (□).
The specificity of CD8⁺ T cells infiltrating an islet graft varies in a temporal manner

Next, the frequency of Kd-NRPV7⁺ CD8⁺ T cells was examined shortly before graft failure. The percentage of Kd-NRPV7⁺ CD8⁺ T cells present in the grafted islets was significantly reduced by day 13 postimplantation (Fig. 5A). An average of 4.7 ± 1.1% of CD8⁺ T cells bound Kd-NRPV7 tetramers compared with 24.1 ± 4.3% in infiltrates of day 7 grafted islets (p < 0.001). The former was not significantly expanded compared with that detected in the endogenous islets (2.9 ± 1.6%). To determine whether this reduction was attributed to an influx of non-Kd-NRPV7⁺ CD8⁺ T cells, the number of CD4⁺, CD8⁺, and Kd-NRPV7⁺ CD8⁺ T cells present within the grafted and endogenous islets was analyzed. A 7-fold increase in CD4⁺ T cells was observed in the islet graft infiltrates between days 7 and 13 (p = 0.006) (Table I). In comparison, the number of CD8⁺ T cells increased only slightly (1.5-fold) during this period. Strikingly, there was a 3-fold decrease in the number of Kd-NRPV7⁺ CD8⁺ T cells detected between days 7 and 13 in the grafted islets (p = 0.001) despite a relatively constant number of CD8⁺ T cells in the islet graft. Furthermore, the number of Kd-NRPV7⁺ CD8⁺ T cells found in the grafted and endogenous islets at 13 days postimplantation was equivalent (Table I). In contrast, at day 7 postimplantation, the number of Kd-NRPV7⁺ CD8⁺ T cells was increased >5-fold compared with the endogenous islets (Table I). No significant change in T cell numbers was observed in the endogenous islet infiltrates of the recipient mice between the two time points (Table I). The reduction of Kd-NRPV7⁺ CD8⁺ T cells in grafted islets could not be attributed to the influx of InsB-specific or ProIns-specific CD8⁺ T cells, as staining with Kd-InsB (0.7 ± 0.1%) and Kd-ProIns (0.4 ± 0.3%) tetramers, respectively, was not significantly above that detected with Kd-NP tetramers (0.4 ± 0.04%).

Similar to results observed at 7 days postimplantation, there was a preferential usage of Vβ8.1 (TRBV13–13) with Jβ2.4 (TRBJ2–4) or Jβ2.7 (TRBJ2–7) among Kd-NRPV7 binding CD8⁺ T cells detected in the grafted and endogenous islets (Fig. 5, B and C). The TCR clonotypes of Kd-NRPV7 binding CD8⁺ T cells detected in the grafted and endogenous islets were represented at similar frequencies (Fig. 5D), and the identity of the dominant clonotype(s) varied among the recipient mice. Collectively, these results demonstrate that the TCR repertoire of IGRP206–214-specific CD8⁺ T cells remains constant as islet graft destruction progresses, but that the number of these CD8⁺ T cells declines.

Depletion of IGRP206–214-specific CD8⁺ T cells delays islet graft rejection

Because IGRP206–214-specific CD8⁺ T cells dominated the early pool of graft-infiltrating CD8⁺ T cells, whether survival of the
were injected i.v. three times with soluble IGRP206–214 in PBS on/H9252
digenous islets. The TCR ensure continued depletion. Circulating levels of Kd-NRPV7munizations were given at 5 and 12 days postislet implantation to 2, 4, and 6 days before islet implantation. Two more peptide im-

FIGURE 3. Kd-NRPV7+ CD8+ T cells are detected in islet allografts. Diabetic NOD female mice were transplanted with partially mismatched BALB/c islets (H2DdKb) or fully mismatched FVB islets (H2DqKq). The frequencies of Kd-NRPV7+ CD8+ T cells in endogenous (□) and grafted (■) islets were analyzed 7 days postimplantation. Numbers indicate the average percentage (±SEM) of Kd-NRPV7+ CD8+ T cells from three recipients per group. The percentage of staining using control Kd-NP was <0.6%. *p = 0.0013, grafted BALB/c islets vs endogenous islets, Student’s t test.

transplanted islets could be enhanced by depleting these T cells was investigated. For this purpose, high doses of soluble peptide were administered. Injections of soluble IGRP206–214 or NRP-V7 peptides were equally effective in near complete depletion of Kd-NRPV7+ CD8+ T cells (data not shown). Diabetic NOD mice were injected i.v. three times with soluble IGRP206–214 in PBS on 2, 4, and 6 days before islet implantation. Two more peptide im-

FIGURE 4. A restricted TCR repertoire of Kd-NRPV7+ CD8+ T cells is detected in grafted and endogenous islets. The TCR β-chain repertoire of Kd-NRPV7+ CD8+ T cells present in the endogenous and grafted islets in individual transplant recipients were compared using single-cell RT-PCR. Kd-NRPV7+ CD8+ T cells in endogenous (□) and grafted islets (■) were analyzed 7 days postimplantation for Vβ (A) and Jβ (B) gene usage. Vβ2, Vβ6, Vβ8.1, Vβ10, and Vβ16 correspond to IMGT nomenclature of TRBV1, TRBV19, TRBV13–3, TRBV4, and TRBV3, respectively. Data represent averaged percentages derived from four islet recipients. C. Comparison of CDR3β usage of Kd-NRPV7+ CD8+ T cells in the endogenous islets and grafted islets from four individual transplant recipients. A total of 27, 14, 23, and 50 CDR3β sequences were analyzed from the endogenous islets of recipients 1–4, respectively. TCR sequences were compared with a total of 25, 14, 23, and 15 CDR3β sequences derived from the grafted islets of the respective recipients.

significantly reduced after IGRP206–214 (p = 0.002) but not HA peptide immunization (Table II). The frequency of Kd-NRPV7+ CD8+ T cells was also markedly reduced (<0.3%) in graft infiltrates of IGRP206–214-treated recipient mice examined 7 days post-

Discussion

Established autoimmunity in diabetic islet (or pancreas) transplant recipients is an important factor contributing to the failure of sub-

Comparison of CDR3β usage of Kd-NRPV7+ CD8+ T cells in islet allografts. Data represent averaged percentages derived from four islet recipients. C. Comparison of CDR3β usage of Kd-NRPV7+ CD8+ T cells in the endogenous islets and grafted islets from four individual transplant recipients. A total of 27, 14, 23, and 50 CDR3β sequences were analyzed from the endogenous islets of recipients 1–4, respectively. TCR sequences were compared with a total of 25, 14, 23, and 15 CDR3β sequences derived from the grafted islets of the respective recipients.

Comparison of CDR3β usage of Kd-NRPV7+ CD8+ T cells in islet allografts. Data represent averaged percentages derived from four islet recipients. C. Comparison of CDR3β usage of Kd-NRPV7+ CD8+ T cells in the endogenous islets and grafted islets from four individual transplant recipients. A total of 27, 14, 23, and 50 CDR3β sequences were analyzed from the endogenous islets of recipients 1–4, respectively. TCR sequences were compared with a total of 25, 14, 23, and 15 CDR3β sequences derived from the grafted islets of the respective recipients.
been reported to mediate autoimmune destruction of both alloge-
nic and syngeneic islet grafts (25, 26, 39–41). To develop effec-
tive strategies to induce and monitor islet transplantation tolerance
in the clinic, knowledge of the beta cell epitopes targeted by T cells
and the dynamics of autoimmune-mediated destruction of an islet
graft is needed. In the current study, these issues were examined by
comparing the repertoire of beta cell-specific CD8
T cells found
 infiltrating grafted and endogenous islets in diabetic NOD recipient mice.

A key observation made in this study is that autoimmune de-
struction of islet grafts is mediated by a restricted repertoire of
IGRP206–214-specific CD8
T cells predominated in
graft infiltrates 7 days posttransplantation with up to 42% of infil-
trating CD8
T cells binding Kd-NRPV7 tetramer (Fig. 2). At-
tempts to assess graft infiltrates at earlier posttransplantation times
were unsuccessful due to insufficient T cell numbers. Detection of
IGRP206–214-specific CD8
T cells in the islet grafts is consistent
with reports demonstrating the importance of this set of clonotypes
in mediating the progression of beta cell destruction in endogenous
islets (19, 20). The frequency of Kd-NRPV7 CD8
T cells at 7 days
posttransplantation represented an ~6-fold increase in grafted vs endogenous islets (Fig. 2). Expansion of IGRP206–214-specific
CD8
T cells was dependent on H2Kd expression by the trans-
planted islets. For example, a significant increase in Kd-NRPV7
CD8
T cells compared with endogenous islets was detected in
BALB/c (H2Kd) but not FVB (H2Kq) islets (Fig. 3).

Table I. CD4
, CD8
, and Kd-NRPV7 CD8
T cells present in islet grafts and endogenous islets at 7 and 13 days posttransplantation

<table>
<thead>
<tr>
<th>Days Posttransplantation</th>
<th>Islet Graft (absolute number per 10,000 gated events)</th>
<th>Endogenous Islets (absolute number per 10,000 gated events)</th>
</tr>
</thead>
</table>
|                         | CD4
 | CD8
 | CD4
, Kd-NRPV7 | CD4
 | CD8
 | CD8
, Kd-NRPV7 |
| Day 7                   | 655 (±241) | 826 (±207) | 153 (±29) | 3679 (±690) | 1390 (±340) | 31 (±8) |
| Day 13                  | 4731 (±707) | 1275 (±130) | 45 (±3) | 3410 (±808) | 1390 (±259) | 38 (±7) |

* Data represents averaged events (±SEM) from three recipient mice at each time point. A total of 10,000 events were analyzed within the lymphocyte gate based on forward and side scatter, and subsequently were gated on CD4
, CD8
 and CD8
 Kd-NRPV7
T cells.
presentation of the IGRP206–214 epitope by H2Kd expressing donor β cells and APC residing in the graft, respectively. Albeit reduced relative to NOD and BALB/c islets, a significant frequency of Kd-NRPV7+ CD8+ T cells was also detected in infiltrates of MHC mismatched FVB islets (Fig. 3). This result suggests that, in fully MHC mismatched islet grafts, autoimmune-mediated destruction occurs via cross-presentation and -priming by recipient APC. Notably, the frequency and number of Kd-NRPV7+ T cells varied in a temporal manner despite a relatively constant number of CD8+ T cells during infiltration and destruction of syngeneic islet grafts. For instance, a >3-fold reduction in the number of Kd-NRPV7+ CD8+ T cells was detected in NOD islet grafts 13 vs 7 days postimplantation (Table I). The progressive loss of Kd-NRPV7+ CD8+ T cells suggests that IGRP206–214-specific CD8+ T cells are recruited to the islet graft from a finite pool, and undergo expansion and subsequent contraction. A similar profile of expansion and contraction was detected in islet grafts after adoptive transfer of CD8+ T cells isolated from 8.3 TCR NOD transgenic mice (C. P. Wong and R. Tisch, unpublished results). The above findings also suggest that inter- (and intra-) molecular epitope spread occurs in an ordered progression during islet graft destruction. By 13 days postimplantation, IGRP206–214-specific CD8+ T cells are displaced as a major set of clonotypes in the islet graft by other CD8+ T cells that, however, do not include either InsB15–23- and ProInsB25–C34-specific CD8+ T cells. The specificity and diversity of these additional clonotypes are of obvious interest, and need to be defined. These results suggest a scenario in which IGRP206–214-specific CD8 T cells promote early autoimmune destruction of islet grafts and subsequent epitope spread. Indeed, a delay (albeit short-lived) was detected in the onset of recurrent diabetes in islet graft recipient mice treated with high doses of soluble peptide (Fig. 6) and depleted of IGRP206–214-specific CD8+ T cells (Table II), This delay in islet graft rejection may reflect the recruitment and/or differentiation of sufficient numbers of other pathogenic effectors. These results also indicate that islet graft rejection can be mediated in the absence of IGRP206–214-specific CD8+ T cells.

Single-cell analysis of TCR Vα and Vβ gene usage by Kd-NRPV7+ CD8+ T cells demonstrated that the immunodominant clonotypes mediating β cell destruction in the endogenous islets were also recruited to the islet grafts. All of the sorted Kd-NRPV7+ CD8+ T cells expressed the canonical Vα17-Jε42 element characteristic of IGRP206–214-specific clonotypes (17, 36). However, as determined by CDR3β sequences, up to two dominant clonotypes were detected in the endogenous islets that, in turn, were also found to dominate the islet graft of an individual recipient (Figs. 4 and 5). The diversity of these immunodominant clonotypes may in fact be greater based on recent findings by Santamaria and colleagues (42) showing that three different Vα17 elements are used by IGRP206–214-specific clonotypes. Due to the positioning of primers used in our study, the sequence spanning CDR1α that contains the respective substitutions in the Vα17 elements could not be determined. These findings indicate that IGRP206–214-specific CD8 T cells driving early islet graft infiltration are recruited from an already established pool of effector and/or memory T cells as opposed to naive precursors. Immunodominance within the islet graft is likely to be established by clonotypes found at a relatively high frequency and/or exhibiting increased avidity/affinity. Indeed, progression toward overt diabetes in NOD mice corresponds with the expansion of IGRP206–214-specific CD8+ T cells having increased avidity/affinity (20). However, whether recruitment of other β cell-specific clonotypes to the islet graft follow the same “rules” as IGRP206–214-specific CD8+ T cells remains to be determined.

In summary, autoimmune destruction of islet grafts is characterized by a restricted repertoire of β cell-specific CD8+ T cells, and an apparent ordered progression of epitopes that are targeted. Early infiltrates are dominated by established effector and/or memory IGRP206–214-specific CD8+ T cells that are needed for efficient islet graft rejection. Finally, tolerogenic strategies targeting graft-infiltrating β cell-specific CD8+ T cells may prove to be of significant clinical value in preventing recurrent autoimmunity in islet transplantation.

Acknowledgments
We thank Dr. P. Santamaria for providing 8.3-NOD mice used in this study, and Carrie Barnes for technical assistance.