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# 4

## Local autoantigen expression as essential gatekeeper of memory T-cell recruitment to islet grafts in diabetic hosts

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## ABSTRACT

It is generally believed that inflammatory cues can attract non-cognate, 'bystander' T-cell specificities to sites of inflammation. We have shown that recruitment of naïve and *in vitro*-activated autoreactive CD8+ T-cells into endogenous islets requires local autoantigen expression. Here we demonstrate that absence of an autoantigen in syngeneic extra-pancreatic islet grafts in diabetic hosts renders the grafts 'invisible' to cognate memory (and naïve) T-cells. We monitored the recruitment of IGRP<sub>206-214</sub>-reactive CD8+ T-cells into IGRP<sub>206-214</sub>-competent and IGRP<sub>206-214</sub>-deficient islet grafts in diabetic wild type or IGRP<sub>206-214</sub><sup>-/-</sup> nonobese diabetic (NOD) hosts (harboring either naïve and memory, or only naïve IGRP<sub>206-214</sub>-specific T-cells, respectively). All four host-donor combinations developed recurrent diabetes within two weeks. Wild type hosts recruited IGRP<sub>206-214</sub>-specific T-cells into IGRP<sub>206-214</sub><sup>+/+</sup> but not IGRP<sub>206-214</sub><sup>-/-</sup> grafts. In IGRP<sub>206-214</sub><sup>-/-</sup> hosts, there was no recruitment of IGRP<sub>206-214</sub>-specific T-cells, regardless of donor type. Graft-derived IGRP<sub>206-214</sub> activated naïve IGRP<sub>206-214</sub>-specific T-cells, but graft destruction invariably predated their recruitment. These results indicate that recurrent diabetes is driven exclusively by autoreactive T-cells primed during the primary autoimmune response, and demonstrate that local antigen expression is a *sine-qua-non* requirement for accumulation of memory T-cells into islet grafts. These findings underscore the importance of tackling autoreactive T-cell memory after beta-cell replacement therapy.

## INTRODUCTION

Nonobese diabetic (NOD) mice develop a form of T1D that results from destruction of  $\beta$ -cells by CD4<sup>+</sup> and CD8<sup>+</sup> T-cells recognizing many autoantigenic peptides (1). A significant fraction of islet-associated CD8<sup>+</sup> cells recognize the mimotope NRP-V7 in the context of the MHC molecule K<sup>d</sup> (2). These cells are a significant component of the earliest NOD islet CD8<sup>+</sup> infiltrates (2; 3), are diabetogenic (4; 5) and target residues 206-214 of islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) (6). The peripheral IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cell pool is sizeable (7) and, upon recruitment into islets, undergoes a local avidity maturation process that contributes to disease progression (8).

Studies in infection and autoimmune disease models have suggested that recruitment of T-cells into sites of extra-lymphoid inflammation does not require local expression of cognate peptide-MHC (pMHC) (9-11). We have recently shown, however, that cues emanating from pancreatic islets undergoing spontaneous autoimmune inflammation in NOD mice cannot recruit naïve or newly activated bystander T-cell specificities. This was established by monitoring the recruitment of naïve or *in vitro*-activated IGRP<sub>206-214</sub>-specific CD8<sup>+</sup> T-cells in gene-targeted NOD mice expressing a T-cell 'invisible' IGRP<sub>206-214</sub> sequence. These mice developed diabetes with normal incidence, but their insulinitic lesions could not recruit either cell type. These results indicated that recruitment of naïve T-cells or effector CTL to a site of autoimmune inflammation results from an active process that is strictly dependent on local display of cognate pMHC (12).

Here, we asked whether this revised paradigm also applies to: (i) recruitment of memory (autoantigen-experienced) autoreactive T-cells; and/or (ii) recruitment of naïve and memory T-cells to syngeneic islet grafts. We reasoned that the 'non-physiological' lymphatic and vascular anatomy of islets grafts transplanted under the kidney capsule (13-15), coupled to a high rate of graft cell death (16), should allow recruitment of 'graft-irrelevant' (i.e. non-autoreactive) memory T-cells to the site in response to local inflammatory cues, including those caused by grafting. We demonstrate that recruitment of CD8<sup>+</sup> T-cells to islet grafts during disease recurrence *exclusively* involves autoantigen-specific T-cells from the memory pool, excluding a role for bystander T-cell specificities or graft antigen-activated autoreactive T-cells.

## RESEARCH DESIGN AND METHODS

**Mice.** NOD.IGRP<sub>K209A/F213A</sub><sup>KI/KI</sup> mice, encoding an immunologically silent IGRP<sub>206-214</sub> epitope have been described (12). These studies were approved by the local Animal Care Committee.

**Diabetes.** Diabetes was monitored twice a week by measuring urine glucose levels and confirmed by tail vein blood glucose measurements. All recipient mice had at least two successive blood glucose measurements >22.2 mmol/l and were transplanted within 1-2 weeks of diabetes onset.

**Peptides and Tetramers.** The peptides IGRP<sub>206-214</sub>, NRP-V7, TUM and the corresponding tetramers (PE-labeled) were prepared as described (17).

**Flow Cytometry.** Cell suspensions were stained with pMHC tetramers and FITC- or PerCP-conjugated anti-CD8 $\alpha$  and anti-CD4 mAbs (BD Pharmingen) for 60 min at 4 °C, fixed in 1% Paraformaldehyde/PBS, and analyzed by Fluorescence-Activated Cell Sorting (FACS).

**Islet Isolation.** Pancreatic islets were isolated by hand-picking after collagenase P digestion of the pancreas and cultured overnight at 37°C, 5% CO<sub>2</sub>.

**Islet transplantation and graft harvest.** Five hundred islets were transplanted under the left kidney capsule. Successful engraftment was defined as restoration of glycemic control for >48h. Graft failure was defined as non-fasting blood glucose >15 mmol/l.

**Specificity of islet-associated CD8<sup>+</sup> T-cells.** The grafts of recurrent-diabetic hosts were cut into ~2 mm<sup>3</sup> fragments and cultured for 1 week in 0.5 units/ml rIL-2. T-cells were analyzed by FACS as described. Measurements of IFN $\gamma$  secretion by graft-associated T-cells (2 x 10<sup>4</sup>/well) in response to peptide-pulsed irradiated NOD splenocytes (10<sup>5</sup>/well) were determined by ELISA (R&D Systems) and normalized to values obtained with TUM.

**Adoptive Transfer.** Purified splenic CD8<sup>+</sup> T-cells were labeled with CFSE (2.5 mM), and injected i.v. (5x10<sup>6</sup>) 24h after transplantation. Mice were killed 7days later and the grafted and non-grafted kidney-draining Lymph Nodes (LNs), spleens, Pancreatic Lymph Nodes (PLNs) and Mesenteric Lymph Nodes (MLNs) examined for dilution of CFSE in the CFSE+CD8<sup>+</sup> gate.

**Statistical Analyses.** Data were compared by Mann-Whitney *U* or  $\chi^2$  Logrank tests. Statistical significance was assumed at P<0.05.

**Supplementary Materials.** Provides supporting data on the recruitment of IGRP<sub>206-214</sub>- or Insuli-B<sub>15-23</sub> reactive CD8<sup>+</sup> T-cells to islet grafts, as well as representative FACS profiles.

## RESULTS

NOD.IGRP<sub>K209A/F213A</sub><sup>KI/KI</sup> mice (referred to as IGRP<sub>206-214</sub><sup>-/-</sup> or 'epitope-deficient' mice) develop diabetes with the same incidence and kinetics as wild-type NOD ('epitope-competent') mice, but cannot trigger the activation or recruitment of naïve or *in vitro*-activated IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells (12). Here, we investigate if the naïve IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells of epitope-deficient hosts and/or their memory counterparts arising in epitope-expressing hosts are recruited into epitope-competent or epitope-deficient islet grafts (from NOD.scid and NOD.rag2<sup>-/-</sup>.IGRP<sub>K209A/F213A</sub><sup>KI/KI</sup> donors, respectively).

We first tracked the recruitment of IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells from diabetic IGRP<sub>206-214</sub><sup>+</sup> hosts (harboring both naïve and memory IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells) into IGRP<sub>206-214</sub><sup>+</sup> or IGRP<sub>206-214</sub><sup>-/-</sup> grafts. The presence of IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells recruited into the graft was analyzed by flow cytometry, using pMHC tetramers. We also measured the amount of IFN $\gamma$  that graft-infiltrating T-cells secreted in response to peptide-pulsed irradiated splenocytes, as an additional read-out of T-cell recruitment.

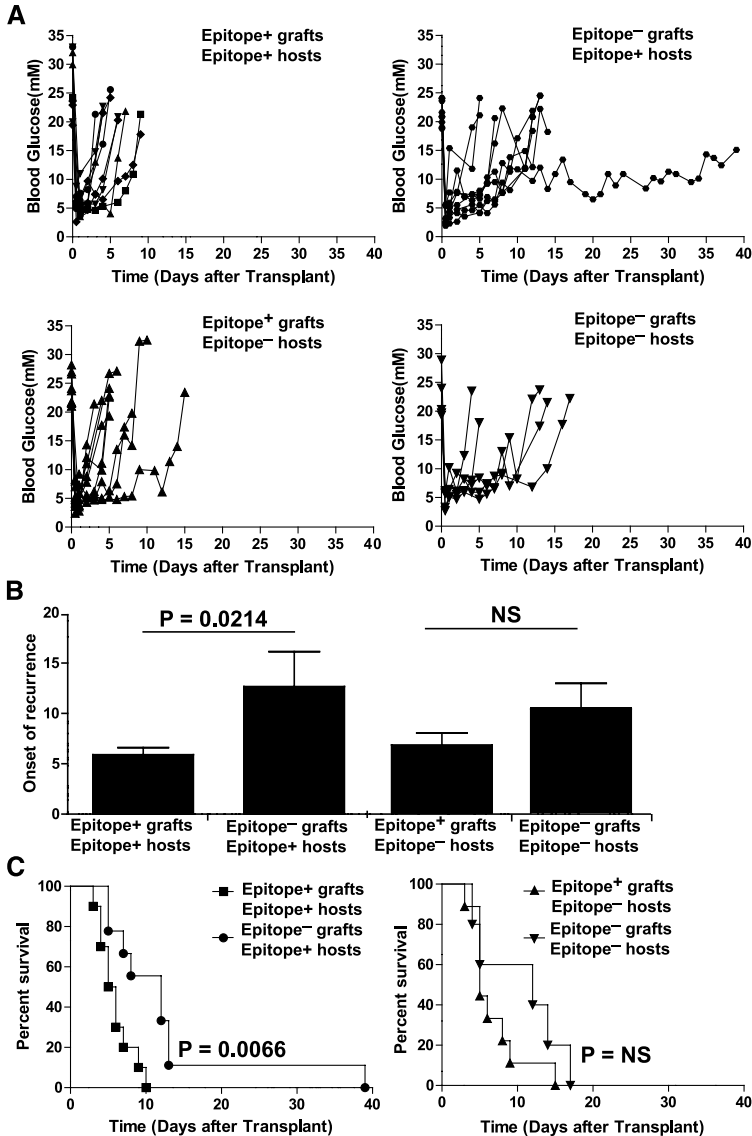
Diabetic IGRP<sub>206-214</sub><sup>+</sup> hosts receiving IGRP<sub>206-214</sub><sup>-/-</sup> islets developed recurrence of disease, but did so a few days later than those receiving IGRP<sub>206-214</sub><sup>+</sup> islets (12.7 $\pm$ 3.5 vs. 5.9 $\pm$ 0.7 days; Figs. 1A top, 1B and 1C left). This indicated that recruitment of naïve and/or pre-activated IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells contributes to, but is dispensable for graft destruction in diabetic IGRP<sub>206-214</sub><sup>+</sup> hosts. Importantly, however, whereas IGRP<sub>206-214</sub>-reactive T-cells accounted for a significant fraction of IGRP<sub>206-214</sub><sup>+</sup> graft-associated CD8<sup>+</sup> T-cells (18.2 $\pm$ 4.7%), they were undetectable in IGRP<sub>206-214</sub><sup>-/-</sup> grafts (Fig. 2A-2C and Supplementary Fig. 1A). Furthermore, the lymph nodes draining the grafted (left) kidney in mice receiving IGRP<sub>206-214</sub>-expressing islet grafts harbored more IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells than those draining the contralateral (non-grafted) kidney, and this was not seen in diabetic hosts grafted with IGRP<sub>206-214</sub><sup>-/-</sup> islets (Fig. 3A). In addition, the pancreatic lymph nodes (PLNs) and the spleen, and to a lesser extent the mesenteric lymph nodes (MLNs), of mice grafted with IGRP<sub>206-214</sub><sup>+</sup> islets contained more IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells than those from mice grafted with IGRP<sub>206-214</sub><sup>-/-</sup> islets (Figs. 3B-3C). This suggests that graft-derived IGRP<sub>206-214</sub><sup>+</sup> induces the activation and retention of host naïve and/or pre-activated IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells in graft-proximal lymphoid organs.

We next investigated whether the IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells recruited to the epitope-expressing grafts include naïve T-cells primed by graft-derived IGRP<sub>206-214</sub>. We followed the fate of IGRP<sub>206-214</sub><sup>+</sup> and IGRP<sub>206-214</sub><sup>-/-</sup> islet grafts and IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells in diabetic IGRP<sub>206-214</sub><sup>-/-</sup> hosts, which are unable to generate antigen-experienced IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells from an otherwise normal pool of naïve T-cell precursors. IGRP<sub>206-214</sub><sup>-/-</sup> hosts rejected IGRP<sub>206-214</sub><sup>+</sup> and IGRP<sub>206-214</sub><sup>-/-</sup> islets with kinetics similar to those seen in NOD mice (Figs. 1A-1C). Yet, IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells were barely detectable in IGRP<sub>206-214</sub><sup>+</sup> and IGRP<sub>206-214</sub><sup>-/-</sup> grafts implanted into IGRP<sub>206-214</sub><sup>-/-</sup> hosts (Figs. 2A and 2B), indicating that the grafts do not recruit newly primed IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells, at least within the first two weeks after transplantation. Interestingly, IGRP<sub>206-214</sub><sup>+</sup> grafts in

IGRP<sub>206-214</sub><sup>-/-</sup> hosts recruited slightly more InsB<sub>15-23</sub>-reactive CD8<sup>+</sup> T-cells than in IGRP<sub>206-214</sub><sup>+</sup> hosts (Supplementary Figs. 1B and 1C). Although these differences were not statistically significant, they suggest that in these mice the IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cell niche is occupied by other memory T-cell specificities. In addition, the islet graft-associated CD8<sup>+</sup> T-cells express markers of memory (i.e. are CD44<sup>high</sup>, CD62L<sup>-</sup> and CD127<sup>+</sup>; data not shown).

In agreement with the above data, the proximal lymphoid organs (graft-draining LN, PLN and spleen) and blood of IGRP<sub>206-214</sub><sup>-/-</sup> hosts transplanted with antigen-expressing islets contained fewer IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells than their IGRP<sub>206-214</sub><sup>+</sup> host counterparts, suggesting that graft-derived antigen does not induce a detectable peripheral expansion of naïve autoreactive T-cells (Fig. 3D). In addition, since the percentages of IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells in the graft-draining LNs, PLNs and spleen of IGRP<sub>206-214</sub><sup>+</sup> hosts grafted with IGRP<sub>206-214</sub>-deficient islets were also low (Fig. 3D, right panels), we conclude that the peripheral expansion of IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells seen in IGRP<sub>206-214</sub><sup>+</sup> islet-grafted IGRP<sub>206-214</sub><sup>+</sup> hosts (Figs. 3A-C) largely, if not exclusively involves antigen-experienced T-cells. Interestingly, NOD hosts grafted with IGRP<sub>206-214</sub><sup>-/-</sup> islets accumulated IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells in the bloodstream (Fig. 3D) suggesting that, in the absence of antigen in the graft and graft-draining lymphoid organs, pre-activated IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells are ‘trapped’ in the bloodstream.

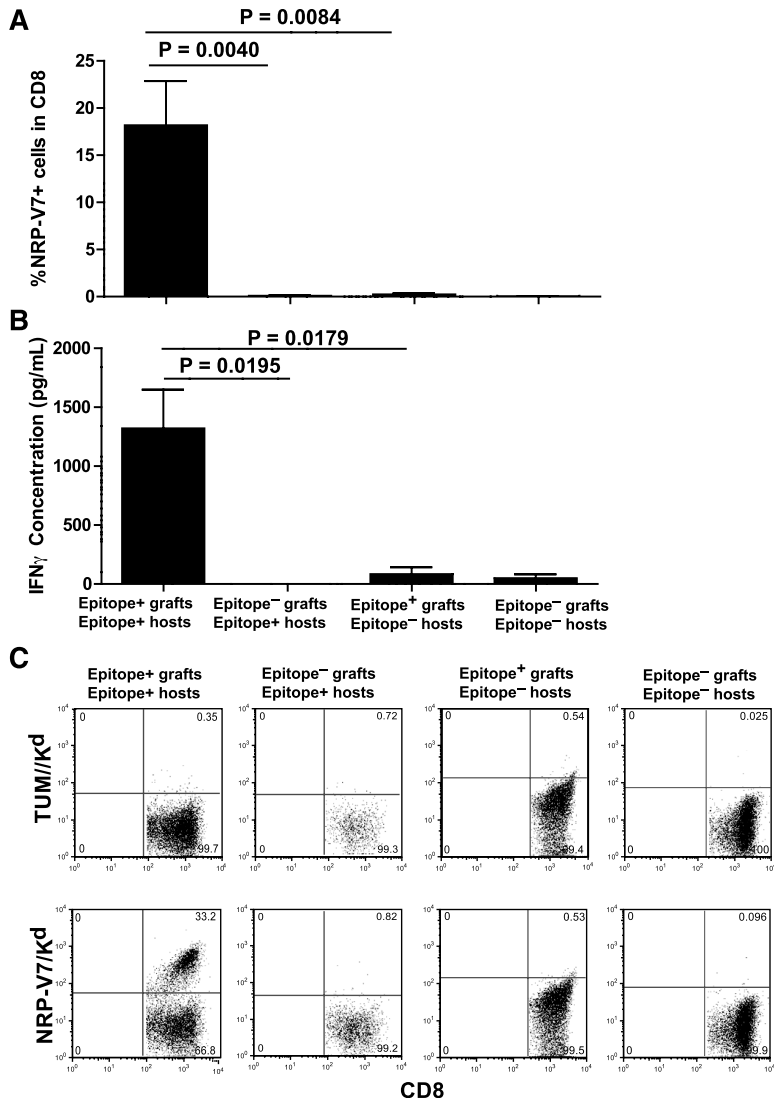
Finally, we asked whether absence of graft-antigen-primed naïve IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells in the epitope-expressing grafts of IGRP<sub>206-214</sub><sup>-/-</sup> hosts was caused by inability of graft-derived IGRP<sub>206-214</sub> to activate cognate naïve CD8<sup>+</sup> T-cells, or to protracted recruitment and/or accumulation of these T-cells into the graft. This was done by tracking the proliferation of naïve splenic CFSE-labeled IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T-cells from 8.3-TCR-transgenic mice in NOD hosts grafted with IGRP<sub>206-214</sub><sup>+</sup> or IGRP<sub>206-214</sub><sup>-/-</sup> islets the previous day. Various host lymphoid organs were examined for dilution of CFSE in the CFSE+CD8<sup>+</sup> gate 7 days after T-cell transfer. Naïve 8.3-CD8<sup>+</sup> T-cells proliferated vigorously in the LNs draining IGRP<sub>206-214</sub><sup>+</sup> (but not IGRP<sub>206-214</sub><sup>-/-</sup>) grafts and, to a lesser extent, the PLN and spleen, where some of the proliferation appears to be induced by host-derived (residual) IGRP<sub>206-214</sub> (Fig. 4A and B). There were very few donor 8.3-CD8<sup>+</sup> T-cells in IGRP<sub>206-214</sub><sup>+</sup> or IGRP<sub>206-214</sub><sup>-/-</sup> grafts (0.06 + 0.03% vs. 0.06 + 0.016% of CD8<sup>+</sup> cells, respectively) (Fig. 4C). These observations indicate that destruction of IGRP<sub>206-214</sub><sup>+</sup> and IGRP<sub>206-214</sub><sup>-/-</sup> grafts in IGRP<sub>206-214</sub><sup>+</sup> hosts (Fig. 1A) predates recruitment of newly activated T-cells.



**Figure 1 | Survival of islet grafts from IGRP<sub>206-214</sub>-competent or -deficient donors in spontaneously diabetic IGRP<sub>206-214</sub>-competent or -deficient NOD hosts.**

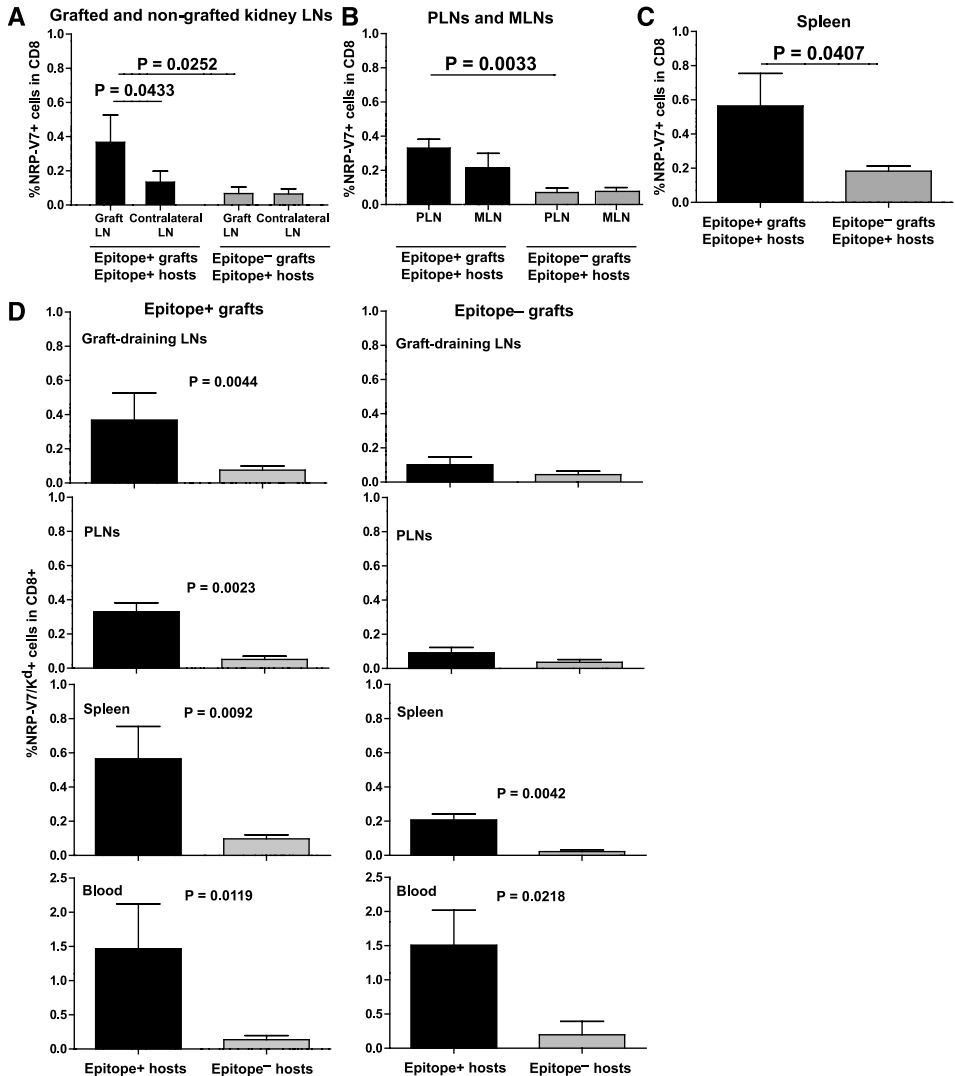
(A) Individual blood glucose curves of diabetic NOD hosts receiving NOD.*scid* (n=10) or NOD.*rag2*<sup>-/-</sup>.IGRP<sub>K209A/F213A</sub><sup>KI/KI</sup> islets (n=9), and diabetic NOD.IGRP<sub>K209A/F213A</sub><sup>KI/KI</sup> hosts receiving NOD.*scid* (n=9) or NOD.*rag2*<sup>-/-</sup>.IGRP<sub>K209A/F213A</sub><sup>KI/KI</sup> islets (n=5). (B) Average onset of disease recurrence after transplantation (in days) in the four different donor/host type combinations. P values were obtained by Mann-Whitney U. (C) Survival curves of grafts in diabetic NOD (left) or NOD.IGRP<sub>K209A/F213A</sub><sup>KI/KI</sup> hosts (right). P values were calculated via Log Rank test. For B and C, differences between epitope+ and epitope- grafts in epitope+ hosts remained statistically significant upon exclusion of the epitope-graft that survived out to 40 days (P=0.0395 in B; and P=0.0353 in C).





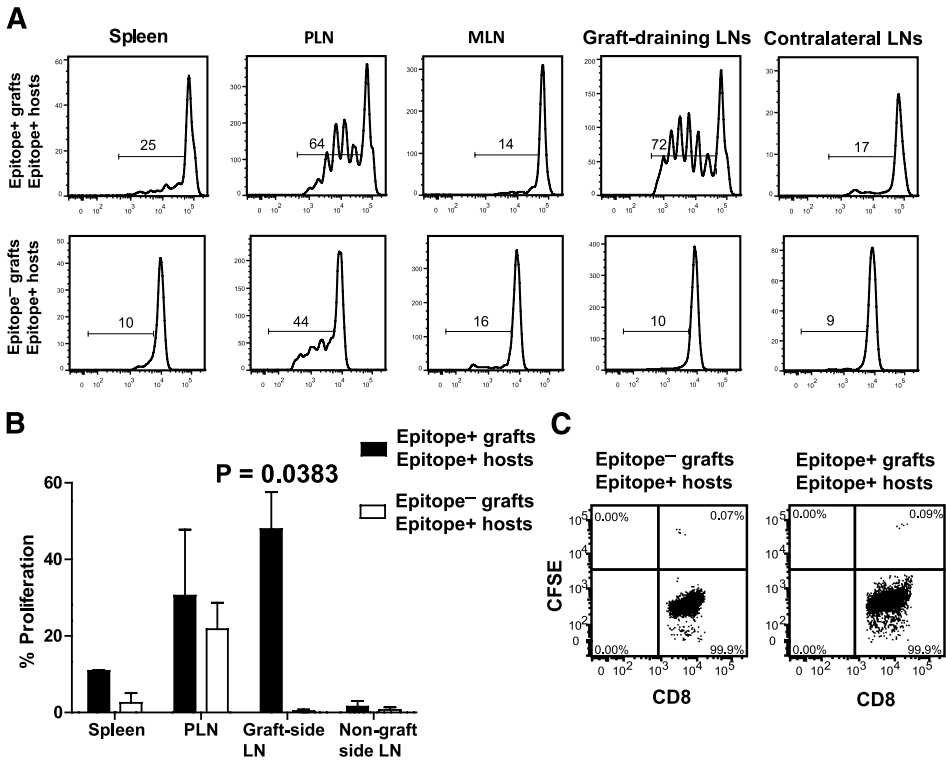
**Figure 2 | Recruitment of IGRP<sup>206-214</sup>-reactive CD8+ T-cells from diabetic IGRP<sup>206-214</sup>-competent or -deficient NOD hosts into islet grafts from IGRP<sup>206-214</sup>-competent or -deficient donors or NOD. rag2<sup>-/-</sup>.IGRP<sup>K209A/F213A</sup><sup>Kl/Kl</sup> donors.**

(A) Percentages of NRP-V7/K<sup>d</sup> tetramer+ in islet-graft-associated CD8+ T-cells. Data (average + SEM) correspond, from left to right, to 8, 4, 4 and 3 grafts per group, respectively. (B) IFN $\gamma$  secretion by islet graft-associated CD8+ T-cells in response to NRP-V7 peptide-pulsed NOD DCs. Data correspond, from left to right, to 5, 4, 4 and 3 grafts per group, respectively. (C) Representative FACS staining profiles of CD8+ T-cells isolated from islet grafts in the four different donor/host type combinations. TUM/K<sup>d</sup> was used as a negative control tetramer. P values in A and B were obtained with Mann-Whitney U. Grafts were harvested immediately after the last blood glucose measurement in Fig. 1.



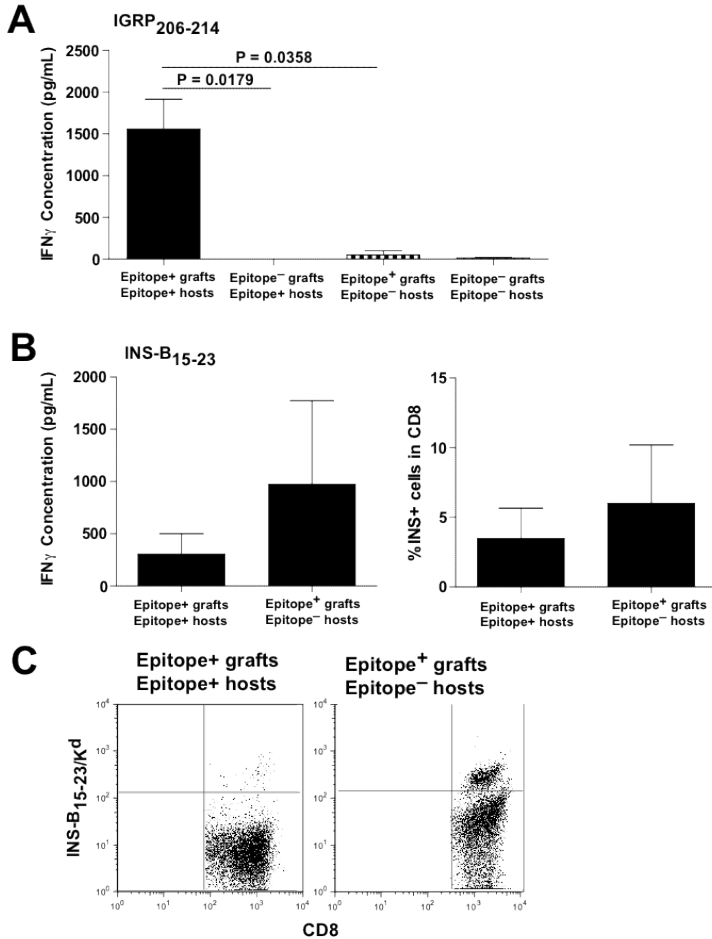
**Figure 3 |** Frequencies of IGRP<sub>206-214</sub>-reactive CD8+ T-cells in lymphoid organs and blood of diabetic IGRP<sub>206-214</sub>-competent or -deficient hosts grafted with IGRP<sub>206-214</sub>-competent or -deficient islets.

(A-C) Percentages of NRP-V7/K<sup>d</sup> tetramer+ cells in CD8+ T-cells from lymph nodes draining the grafted vs. contralateral kidneys (A), the PLN vs. MLNs (B) and the spleen (C). Data (average + SEM) correspond to 6 and 8 mice, respectively. (D) Frequencies of IGRP<sub>206-214</sub>-reactive CD8+ T-cells in lymphoid organs and blood of IGRP<sub>206-214</sub>-competent vs. IGRP<sub>206-214</sub>-deficient hosts grafted with IGRP<sub>206-214</sub>-competent (left) vs. IGRP<sub>206-214</sub>-deficient islets (right). Data (average + SEM) correspond to 6, 6, 8 and 5 mice per group, respectively. Background staining with the negative control tetramer TUM/K<sup>d</sup> was subtracted. P values were obtained with Mann-Whitney U.



**Figure 4 | Proliferation of naive CFSE-labelled 8.3-CD8<sup>+</sup> T-cells in lymphoid organs of IGRP<sub>206-214</sub><sup>-</sup> competent hosts grafted with IGRP<sub>206-214</sub><sup>-</sup> competent vs. IGRP<sub>206-214</sub><sup>-</sup> deficient islets.**

(A) Representative CFSE dilution profiles. (B) Average + SEM of the percentage of proliferated cells. (C) Representative flow profiles of graft-associated CD8<sup>+</sup> T-cells of these mice. Data in A-C correspond to 3 mice per host/donor combination. P values were obtained with Mann-Whitney U. (LN: Lymph node; MLN: mesenteric lymph node; PLN: pancreatic lymph node).



**Supplementary Figure 1 | Recruitment of IGRP<sub>206-214</sub>- and Insulin B<sub>15-23</sub>-reactive CD8<sup>+</sup> T-cells from diabetic IGRP<sub>206-214</sub>-competent or -deficient NOD hosts into islet grafts from IGRP<sub>206-214</sub>-competent or -deficient donors or NOD.rag2<sup>-/-</sup>.IGRP<sub>K209A/F213A</sub><sup>K1/K1</sup> donors.**

(A) IFN<sub>γ</sub> secretion by islet graft-associated CD8<sup>+</sup> T-cells in response to IGRP<sub>206-214</sub> peptide-pulsed NOD DCs. Data correspond, from left to right, to 5, 4, 3 and 3 grafts per group, respectively. (B) IFN<sub>γ</sub> secretion by islet graft-associated CD8<sup>+</sup> T-cells in response to InsB<sub>15-23</sub> peptide-pulsed NOD DCs. Data correspond, from left to right, to 5, 3, 4 and 3 grafts per group, respectively. (C) Examples of FACS staining profiles of graft-associated CD8<sup>+</sup> T-cells containing low versus high percentages of InsB<sub>15-23</sub>/K<sup>d</sup> tetramer-reactive cells.

## DISCUSSION

The data presented herein challenge a current paradigm stating that non-antigen-specific inflammatory cues can attract and retain non-cognate, 'bystander' T-cell specificities to sites of inflammation, including syngeneic islet transplants in diabetic mice. We demonstrate that absence of the cognate autoantigen in a syngeneic extra-pancreatic islet graft in a diabetic host renders the graft 'invisible' to cognate memory (and naïve) T-cells. Local antigen expression (in addition to MHC class I expression (18)) is thus a *sine-qua-non* requirement for accumulation of autoreactive CD8<sup>+</sup> T-cells into islet grafts.

The absolute need for local autoantigen expression is highlighted by two important considerations. First, IGRP<sub>206-214</sub>/K<sup>d</sup> (NRP-V7)-reactive CD8<sup>+</sup> T-cells are among the most prevalent in NOD islet infiltration (8). Second, the vascular beds irrigating islet grafts, including the subcapsular kidney space have a porous, fenestrated architecture (13-15) that could conceivably render them permeable to bystander T-cells. It is therefore remarkable that autoantigen-experienced (i.e. memory) IGRP<sub>206-214</sub>-reactive T-cells, despite their prevalence in the periphery, do not accumulate into IGRP<sub>206-214</sub>-deficient grafts. Recruitment of memory CD8<sup>+</sup> T-cells to islet grafts thus follows the same rules that we have described for the recruitment of naïve and *in vitro*-activated CD8<sup>+</sup> T-cells into endogenous islets (i.e. requiring a cognate pMHC interaction *in situ*). A recent report demonstrates a striking similarity in human insulinitis: all the CD8<sup>+</sup> T-cells found in the inflamed islets of type 1 diabetic patients bound self-pMHC complexes (19).

Our findings further imply that individual autoantigenic specificities, even when prevalent, play relatively minor roles in the anamnestic autoimmune response contributing to graft destruction in autoimmune disease-affected hosts. Our results also clearly indicate that destruction of syngeneic islet grafts in diabetic NOD mice is largely, if not exclusively effected by autoantigen-experienced T-cells primed during the primary autoimmune response. Although graft antigen-loaded APCs residing in the graft-draining lymph nodes can readily induce the activation of naïve autoreactive CD8<sup>+</sup> T-cells, graft destruction precedes recruitment of these T-cells into the graft. The high physical and functional pMHC-binding avidities of antigen-experienced T-cells coupled to their ability to mount rapid recall responses to limiting amounts of antigen (20) likely affords them a competitive advantage, particularly during the first two weeks after transplantation. Differences in the bio-distribution of memory vs. naïve T-cells may be another contributing factor. These considerations, however, do not exclude the likely involvement of graft antigen-primed naïve autoreactive T-cells in chronic loss of graft function, such as for example in the context of partially matched islet allografts.

Recurrent autoimmunity in allogeneic islet cell transplantation has become a topic of growing interest. For example, the pre-transplant peripheral frequencies of autoreactive T-cells in diabetic recipients are predictive of islet allograft fate, and post-transplant increases are associated with loss of graft function (21-24), suggesting that recurrent autoimmunity may contribute to allograft destruction. Although clinical islet

transplantation is a more complex situation, our model has allowed us to dissect the specific roles of bystander immunity vs. anamnestic and naive autoimmunity to islet graft rejection. Our observations emphasize the importance of developing therapies capable of preventing: (i) priming of naïve alloreactive T-cells causing allograft rejection; (ii) recruitment of memory autoreactive T-cells causing anamnestic autoimmunity in the immediate post-transplant period; and (iii) and the priming of naïve autoreactive T-cells causing chronic loss of graft function, paying special attention to the pre-transplant autoreactivity status of the diabetic host.

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G.A., X.C.-C, S.T. and J.W. researched data and contributed to the discussion and editing of the manuscript. J.Y. and B.X. assisted with experiments. J.W. and B.R. contributed to the discussion and reviewed and edited the manuscript. P.S. designed and supervised the study. P.S. is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of the data and the accuracy of data analysis. The authors have no conflicts of interest to declare.

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