Protection From Type 1 Diabetes in the Face of High Levels of Activated Autoaggressive Lymphocytes in a Viral Transgenic Mouse Model Crossed to the SV129 Strain

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In comparing the incidence of virally induced type 1 diabetes in F1 crosses of RIP-LCMV mice to three different mouse strains identical at the major histocompatibility complex H-2Db locus, we surprisingly found that disease development was reduced by 80% in F1 crosses to the SV129 genetic background and by 60% after eight backcrosses to the original C57BL/6 RIP-LCMV mice. In this model, diabetes is strongly dependent on a virally induced H-2Db–restricted cytotoxic T-cell (CTL) response. Importantly, numbers and effector functions of autoaggressive CD4 and CD8 lymphocytes were not decreased in the protected mice, and CTLs were still able to kill syngeneic islet cells in vitro with equal efficacy compared with CTLs from the original RIP-LCMV strain. Furthermore, CTLs were able to extravasate into islets in vivo, and no evidence for induction of regulatory cells was observed. However, regeneration of β-cells in islets under “attack” occurred only in the protected SV129-crossed animals, whereas it was not evident at any time in any mice that developed diabetes. Thus, genetic factors can “override” the diabetogenic potential of high numbers of autoaggressive lymphocytes through, for example, increased islet regeneration. This finding has important implications for interpreting numbers and pathogenicity of autoreactive lymphocytes in prediabetic patients of genetically diverse backgrounds. Diabetes 50:2700–2708, 2001

Numbers of autoaggressive CD4 and CD8 lymphocytes are thought to directly correlate with the incidence and severity of type 1 diabetes and other autoimmune disorders (1–10). Indeed, increased numbers are usually present in mice with more severe disease (11–13), but the situation in humans is still unclear, and lymphocytes with specificity for islet antigens are not always consistently detectable in peripheral blood of prediabetic or diabetic individuals (unpublished, 4th International Diabetes Workshop, November 1999, Fiuggi, Rome). Thus, it appears possible that genetic, potentially non–major histocompatibility complex (MHC)-linked factors could “override” the aggressive potential of autoreactive lymphocytes. We sought to further investigate this issue in a model of antigen—specifically, (virally) induced diabetes, using varied backcrosses to the SV129 strain (14). This mouse strain is of particular importance because most targeted gene knockout mice are generated on this background, and extensive crossing to different diabetes models is usually required to obtain a particular knockout in a given model (15–18). The “danger” of carrying over diabetes-protective alleles using this strategy is a concern, especially if the final goal is prevention of disease. The notion that this can be the case emerged from earlier experimentation by our laboratory in which lower diabetes incidence occurred in control groups after crossing transgenic or NOD mice to several SV129 knockout mice (19).

The RIP-LCMV model for virally induced diabetes that we used for experiments reported here was developed in 1990 by the laboratories of Zinkernagel and Oldstone (20,21). These mice have the main advantage over the NOD model of spontaneous diabetes because autoreactive lymphocytes are easily traceable, and the time-point for induction of autoreactivity can be experimentally chosen. In RIP-LCMV mice, the self-antigen is a glycoprotein (GP) derived from lymphocytic choriomeningitis virus (LCMV) and is costimulatively expressed under the control of the rat insulin promoter (RIP) in pancreatic β-cells. Diabetes only develops after autoreactive (LCMV-specific) and CD8 lymphocytes have been activated systemically and reach the islets, where they have to be further driven by local antigen-presenting cells (APCs) that take up β-cell antigens and provide costimulation to cause sufficient β-cell destruction (22,23). Local activation of APCs in the pancreas, which occurs through systemic LCMV infection before autoreactive (anti-LCMV) lymphocytes enter the islets, is required for diabetes development (23). It is important to emphasize that the pancreatic autoimmune process begins after most LCMV has been cleared systemically and only starts when the systemic anti-LCMV response reaches its peak (23). Thus, LCMV infection precipitates and initiates the pancreatic autoimmune pro-
process. However, continuation of the autoimmune response is independent from the presence of LCMV and can be viewed as a true “hit-and-run” event (22–24). Thus, insulitis in RIP-LCMV mice is an independent, localized (in islets and pancreatic lymph nodes), and self-perpetuating true autoimmune reaction that does not rely on external influx of LCMV-primed lymphocytes (19).

Previous studies have shown that CD8 T-cells, perforin, as well as interferon-γ (IFN-γ) contribute in a multifactorial fashion to islet destruction (22,24,25). Whereas IFN-γ and CD8 cells are absolutely essential, diabetes can occur in the absence of perforin (22). Most dominant and subdominant CD8 and CD4 epitopes have been mapped for the recognition of LCMV antigens on the H-2D^b (D^F-K^A^a) MHC background (26,27). Under these premises, one would assume that the incidence of RIP-LCMV diabetes would be similar on any H-2D^b background because autoreactive cytotoxic T-cell (CTL) are likely generated at sufficient quantities. Our earlier studies have demonstrated that >1 in 3,000 CTL precursors are required for autoimmune diabetes in RIP-LCMV mice (6) and that H-2D^b mice generate on average 1 in 100 LCMV-specific CTL precursors 7 days after infection (28). Therefore, the reduction of disease in crosses to the SV129 background reported in this article came as a surprise to us, because we did not expect non-MHC-linked factors to overcome the “power” of autogenerative CTL in this model. As a possible explanation, we provide evidence that attack of islets from SV129 mice induces increased β-cell regeneration, an observation that is not true for other H-2D^b RIP-LCMV mouse strains in the prediabetic stage. This finding has important implications when using autoreactive lymphocytes as an indicator for ongoing disease severity, because correlation between their frequencies and disease might possibly be low in an outbred population, where the effect of other genes might be able to overcome their autoimmune potential.

**RESEARCH DESIGN AND METHODS**

**Islet isolation.** Islets of Langerhans were isolated from mice as previously described (22,29,30). In brief, the common bile duct was cannulated, and the pancreas was dissected with 3 ml of Dulbecco’s modified Eagle’s medium containing 1.5 units/ml collagenase P (Boehringer Mannheim, Indianapolis, IN). Pooled pancreases were digested at 37°C for 20 min and were then disrupted by shaking. Islets were purified on Histopaque-1077 density gradients (Sigma, St. Louis, MO). The gradient was centrifuged with gradually increasing speed from 25 to 800g for 4 min then at 800g for 10 min. Islets were aspirated from the media/gradient interface, washed, and hand-picked if necessary. The islets were dispersed into single cells with 0.2% trypsin (Calbiochem, La Jolla, CA) and 10 mmol/l EDTA in Hank’s balanced salt solution (HBBS), and then they were allowed to recover in complete medium (CMRL, 7% fetal calf serum [FCS], 1% penicillin/streptomycin, and 1% glu-tamine) for 1 h before staining with monoclonal antibodies (mAbs). To ensure the purity of the β-cell preparation, β-cells sorted with a fluorescence-activated cell sorter (FACS) were fixed on immunohistochemistry slides and stained for intracellular insulin as previously described (31). The antibodies used were a polyclonal guinea pig anti-insulin antibody (Iako, Carpinteria, CA) as primary antibody and a biotinylated goat anti–guinea pig IgG antibody (Vector Laboratories, Burlingame, CA) as secondary antibody. Of the isolated islet cells, 56% were β-cells on average, as evidenced by positive insulin stain, and >88% of the cells in the final-gated autofluorescence population were β-cells.

**Detection of MHC class I on β-cells.** Whole islets were trypanized, and islet cells were counted. To stain class I MHCs, we used 28-14-8 (Pharmingen, La Jolla, CA) and biotinylated mouse IgG2a mAb directed against murine H-2D^b that only cross-reacts with H-2L^d and H-2D^d. Secondary antibodies were as follows: phycoerythrin-conjugated goat F(ab’)^2 anti-rat IgG (H+L) (Caltag Labs, Burlingame, CA) and phycoerythrin-conjugated streptavidin (Caltag Labs). Islet cell or spleen cell suspensions were incubated for 20 min on ice with mAb diluted in Dulbecco’s phosphate-buffered saline (DPBS) containing 1% FCS. They were then washed and incubated for 15 min with a secondary antibody as necessary. Cells were finally washed and resuspended in DPBS with 1% FCS and 2.5 µg/ml propidium iodide to stain dead cells. Analysis was performed on a FACSort or FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) using Cell Quest software (Becton Dickinson). β-Cells were sorted on a FACSort plus (Becton Dickinson) based on flavin adenine dinucleotide autofluorescence according to the method previously described (32). Background levels were determined by staining MHC class I–deficient islets from β2m−/− mice.

**Cytotoxicity assays.** CTL activity was measured in a 5- to 6-h in vitro 51Cr release assay (33,34). Briefly, to determine the amount of MHC-restricted CTL lysis, syngeneic or allogeneic target cells were either infected with LCMV-strain ARMSTRONG (ARM) (multiplicity of infection = 1), or uninfected cells were coated with LCMV peptides GP 33-41 (KAVYNFATC), GP amino acid 276-286 (SGVEPVNGYCL), and nucleoprotein (NP) 396-404 (FQPQNGQFQ), all of which were H-2D^d-restricted (27). Assays used splenic lymphocytes harvested 7 days after primary LCMV infection (10^5 pfu i.p.) at effector-to-target ratios of 50:1, 25:1, and 12:1 or CTL clones and secondary CTL lines at ratios of 10:1 and 5:1. To determine CTL activity after secondary stimulation, spleen cells harvested from mice 30–180 days after primary inoculation with 10^6 × 10^6 pfu LCMV i.p. were incubated with MHC-matched, irradiated, LCMV-primed target cells (γ irradiated) at presence of T-cell growth factor (supernatant from concanavalin A–stimulated splenocytes) containing interleukin-2 (IL-2) and irradiated syngeneic spleen feeder cells for 5–12 days (34). The MC37 (H-2K^d^D^b^) and BALB/c (H-2^k^) cells used as CTL targets were grown as previously reported (34). When islet cells were used as targets, whole islets were isolated from collagenase-digested pancreatas of at least three mice and labeled for 1 h at 37°C in the presence of 1 mCi of 50Cr. After washing three times in glucose-free HBSS (Gibco, Gaithersburg, MD), islets were dispersed by trypsinization, washed again three times, and then plated at 1–2 × 10^5 cells/well in 96-well flat-bottom plates for the cytotoxicity assay. In some assays, cytokines (IFN-γ, tumor necrosis factor-α [TNF-α], and IL-1β) were added directly to the target cells at 1 µg/ml (22). Where indicated, cytotoxicity assays were carried out over an extended time period of 20 h. Precursor frequencies of LCMV-specific CTL were determined as described (28).

**Transgenic mice.** Generation and characterization of RIP-LCMV transgenic mice that develop type 1 diabetes after LCMV infection has been previously described (21,34). For this report, we used only RIP-GP 34-20 (H-2^D^b^) transgenic mice, which express viral GP from the LCMV strain ARMSTRONG only in the β-cells of their islets and not in any other organs (34). SV129 and BALB/c mice were obtained from Jackson Laboratories (Bar Harbor, ME). NOD mice were obtained from the rodent breeding colony at the Scripps Research Institute. F1 crosses were obtained in a single mating of RIP-GP mice with SV129, BALB/c, or NOD mice. Backcrosses to the C57BL/6 up to N8 were performed on a FACSort or FACScalibur (Becton Dickinson) flow cytometer (BDU) (Fig. 5) was performed by first staining freshly thawed pancreatic sections for insulin using a primary guinea pig anti insulin antibody (DAKO) and biotinylated anti guinea pig secondary antibody combined with an avidin-alkaline-phosphatase reaction.
conjugate and Vector-red staining kit (both from Vector Laboratories). Therefore, staining for BrDU was performed as described using the Pharmingen BrDU staining kit, using rat anti-BrDU as primary antibody to avoid cross-reactivity with the insulin staining and a peroxidase-coupled secondary antibody with 3,3′-diaminobenzidine tetrahydrochloride as a color substrate (brown).

**Flow cytometry.** Staining of cell surface antigen and intracellular antigens was performed as described. Virus-specific stimulation was provided by the addition of 1 μg/μl of class I and 2 μg/μl of class II LCMV-CD4 or -CD8 peptides (27,37–39) in the presence of 50 units/ml recombinant human IL-2. All stimulation cultures contained 1 μg/ml brefeldin A (BFA B7651; Sigma) to block protein transport into post-Golgi compartments and to allow cytokines to accumulate within cells. Negative controls were stained with cytokine-specific phycoerythrin-conjugated antibodies preincubated for 30 min at 4°C with an excess of recombinant cytokine. Cells were acquired and analyzed on a FACSOr or FACSCalibur flow cytometer (Beckton Dickinson, San Jose, CA) using Cell Quest software (Beckton Dickinson).

**Low-dose streptozotozin treatment.** For the studies displayed in Fig. 5, groups of 10 C57BL/6 or SV129 H-2b males were injected with 60 mg/kg body wt i.p. streptozotozin three times, which was dissolved immediately before injection in 100 mmol/l citrate buffer at pH 4.2. BrDU was provided in the drinking water at 0.8 mg/ml continuously.

**RESULTS**

Incidence of virally induced autoimmune diabetes is only reduced in H-2Dd RIP-LCMV transgenic mice when crossed to the SV129 genetic background. Because SV129 embryonic stem cells are preferably used to generate targeted gene knockout mice, our intention was to evaluate whether the genetic background of this strain could influence the development of autoimmune disease per se. For the first experiment, RIP-LCMV-GP mice that develop diabetes dependent on H-2Dd-restricted cytotoxic CD8 lymphocytes but independent from CD4 cells (34) were crossed to various H-2Db mouse strains, and the incidence of hyperglycemia was tracked after LCMV infection. As shown in Fig. 1, only the SV129 genetic constellation appeared to prevent disease development over up to 10 backcrosses to the original RIP-LCMV-GP C57BL/6 background (data shown for N8 but not N10). In contrast, a high degree of diabetes was observed in crosses to BALB/b and NOD H-2db mice. This finding was surprising because all of these mice express the H-2Db MHC class I allele, to which the vast majority of the H-2Db LCMV (anti-self) CTL response is restricted (27,40). In addition, because all F1 offspring will still express 50% of the original C57BL/6 H-2db MHC molecules (and in N8 backcrosses to the original C57BL/6 will express even more), the lower diabetes incidence in SV129 F1 crosses is likely not caused by MHC-linked factors. We further investigated this issue by precisely determining autoreactive (LCMV-specific) CD8 and CD4 effector functions.

**Autoreactive CTL activities are not reduced in RIP-LCMV-GP × SV129 N8 offspring.** The anti-LCMV CD8 (and CD4) responses can be considered as true autoreactive lymphocytes in RIP-LCMV mice because the transgene is expressed as a pancreatic self-antigen, and mice are tolerant unless systemic LCMV infection is initiated (34). Therefore, the enumeration of these populations according to their effector functions is a very close and accurate measurement of the initial autoreactive islet-specific response in such mice, if lymphocytes can actually enter the islets, which is equivalent between RIP-GP and RIP-GPX SV129 N8 mice (Table 1). As displayed in Fig. 2, perforin-mediated cytotoxic responses to the LCMV GP (islet antigen expressed transgenically) and NP were reduced 7 days after infection during the primary ex vivo response in SV129 mice compared with C57BL/6 RIP-LCMV mice. Interestingly, this defect was seen neither in the memory response (Fig. 2) nor in the N8 RIP-LCMV/SV129 mice that had been backcrossed to the original RIP-LCMV-GP

![FIG. 1. Protection from autoimmune diabetes in RIP-LCMV × SV129 F1 crosses.](image)
accomplished by days 8 
(LCMV) clearance occurred with similar kinetics and was 
ties in anti-LCMV CTL in B6- and SV129-crossed mice, viral 
compared with the original RIP-LCMV-GP mice (that de-
1), but the autoreactive CTL response was not impaired 
crosses to the original C57BL/6 genetic background (Fig.
Thus, because diabetes was still reduced after eight back-
shown).
were carried out in parallel using three mice per group. The means 
production (Fig. 3) was also re-
10 from major organs (data not 
percentages of noninfiltrated islets, infiltrated islets, and those with perinsulinitis are shown. Note that protected and diabetic mice only 
diverge immunopathologically after initiation of the local autoimmune process around day 10 post-LCMV infection, when systemic virus has 
already been cleared, and that the principal feature in SV129 crosses is that lymphocytes still enter into the islets, but insulinitis remains mild 
and never fills the whole islet. Values for RIP-LCMV-GP (C57BL/6) are shown in normal type. Values for RIP-LCMV-GP (SV129) are shown 
in bold. Insulinitis score: none = no lymphocytes in or around islets; peri = lymphocytes solely around the islets; intra = lymphocytes found 
in the islets but do not fill out the whole islet; full = whole islet appears filled with lymphocytes. –, no histological signal detectable; –/+,
8–10 positive cells per islet; +, 20–40 positive cells per islet; ++, too many cells to count.
C57BL/6 background for eight generations but were still 
largely protected from diabetes (Figs. 1 and 2). Similarly, 
the inflammatory autoreactive cytokine response reflected in 
LCMV-specific IFN-γ production (Fig. 3) was also 
reduced in SV129 mice but not in the N8 backcrosses (Fig. 3). 
Thus, because diabetes was still reduced after eight back-
crosses to the original C57BL/6 genetic background (Fig.
1), but the autoreactive CTL response was not impaired 
compared with the original RIP-LCMV-GP mice (that 
developed a high degree of diabetes) (Figs. 2 and 3), other 
genetic, likely non–MHC-linked, factors have to account 
for the protection from diabetes. Along with the similari-
ties in anti-LCMV CTL in B6- and SV129-crossed mice, viral 
(LCMV) clearance occurred with similar kinetics and was 
accomplished by days 8–10 from major organs (data not 
shown).

SV129 islets can be killed by autoreactive CTL in vitro. We next tested whether islets from SV129 mice might be resistant to destruction by autoreactive perforin-positive CTLs or by inflammatory cytokines in general. Previous studies have implicated both of these pathways in islet destruction (22). As shown in Fig. 4, this is not the case, and islets of SV129 mice are as susceptible as islets from 
C57BL/6 mice or RIP-LCMV×C57BL/6 N8 mice (not shown) to CTL killing and cytokine-mediated death. As expected, MHC class I levels on islets from both strains were upregulated to very similar levels after LCMV infection, an event that is essential in sensitizing β-cells to CTL 
recognition (data not shown) (22). This observation strengthens the notion that genetic factors other than 
MHC class I could be responsible for protecting SV129-
crossed RIP-LCMV mice from autoimmune diabetes. We further dissected the underlying mechanism of protection.

Islet cells from SV129 × RIP-LCMV-GP mice regenerate “under attack” during the prediabetic phase. Islets from the different inter- and backcrossed RIP-LCMV mice shown in Fig. 1 were evaluated histologically. Double-staining for insulin (red) and BrDU incorporation (brown) was performed during the prediabetic, diabetic, and nondiseased stages and is shown in Fig. 5. Interestingly, signs of β-cell regeneration, as evidenced by double-staining for BrDU as well as insulin, were only observed in the majority of SV129 crosses that had not developed autoimmune diabetes. In contrast, uninfected islets as well 
as islets from diabetic (Fig. 5) or nondiabetic C57BL/6 
controls do not exhibit this phenotype, and β-cell regen-
eration is not seen. On average, 5 of 9 β-cells double-stained 
for insulin and BrDU were typically found per islet from the nondiabetic RIP-LCMV × SV129 (C57BL/6 N8 
[Fig. 5B] or F1 [Fig. 5E]) mice, whereas no BrDU incorpo-
ration was observed in any of the diabetic or uninfected normoglycemic controls. Interestingly, low-dose streptozotocin treatment appeared to have a similar effect on 
islets of SV129 but not C57BL/6 mice, as shown in Fig. 5D. 
Thus, β-cells appear to generate only under attack in 
SV129 but not B6 mice, and increasing the islet cell mass 
by regeneration is likely one of the pathways preventing 
diabetes in mice crossed to the SV129 background. Indeed,
nondiabetic but LCMV-infected RIP-LCMV-GP \times SV129 mice had close to normal insulin content in the pancreas (data not shown).

Although autoreactive lymphocytes can enter islets of C57BL/6 and SV129 mice to a similar degree in vivo, fewer lymphocytes and activated APCs are found in islets of RIP-LCMV \times SV129 mice protected from diabetes. As shown in Fig. 6, islet infiltration in nondiabetic RIP-LCMV-GP mice crossed to the SV129 strain and back-crossed for eight generations to the C57BL/6 original genetic background is reduced, and fewer CD4 and CD8 lymphocytes are found at day 14 after the triggering LCMV infection (Table 1). It is difficult to distinguish precisely between cause and effect from such data, but the histological findings at earlier stages post-LCMV infection (Table 1) indicate that lymphocytes can still enter into the islet area of SV129-crossed transgenic mice but are present to a lesser extent inside islets in protected animals compared with diabetic non-SV129 controls (Table 1). A primary defect in lymphocytes trafficking therefore appears unlikely because similar numbers of CD4 and CD8 lymphocytes are able to reach the islets initially after LCMV infection in both mouse strains (Table 1). Furthermore, no change in the production of protective cytokines such as IL-4 or IL-10 was evident by Elispot analysis (data not shown) when comparing RIP-LCMV-GP C57BL/6 and RIP-LCMV-GP \times SV129(C57BL/6-F8) mice at day 14 post-LCMV infection, and immune deviation is consequently not the likely cause for the observed lack of diabetes in the SV129-crossed animals.

### DISCUSSION

In this study, we demonstrate that genes other than the MHC class I genes can overcome the autoaggressive...
potential of high numbers of self-reactive CD8 (and CD4) lymphocytes. Autoimmune diabetes development is drastically reduced in the RIP-LCMV transgenic mouse model when crossed once to SV129 mice and is even reduced after eight back-crosses to the original C57BL/6 background, whereas equally high levels of autoreactive CD8 and CD4 lymphocytes are generated systemically. Islets can be killed by such CTLs and their cytokines in vitro.

FIG. 5. Islets from SV129 × RIP-GP N8 (back-cross) mice regenerate under attack, whereas islets from RIP-GP C57BL/6 mice do not. To selectively label dividing cells, all mice were injected 24 h before euthanasia with BrDU as previously described (36). LCMV infection was established with 1 × 10⁵ pfu when indicated, and all mice were RIP-LCMV-GP transgenic and crossed for eight generations (N8) to the original C57BL/6 H-2D⁺ background. Six mice were analyzed for each group and at least 15 islets were surveyed per pancreas. As representatively shown in A, no BrDu incorporation was seen in any of the uninfiltrated or diabetic islets. As shown in B, 0–9 double-positive β-cells were observed in RIP-GPX SV129 N8 islets on average 5 double-positive β-cells. The insulitis score for diabetic and protected mice is displayed in Table 1. A: Islet from an uninjected RIP-LCMV-GP × SV129 N8 mouse. Insulin staining in red is evident, and no lymphocellular infiltration is seen. No incorporation of BrDU occurred. B: Islet from an LCMV-infected RIP-LCMV-GP × SV129 N8 mouse 2 months after infection without diabetes. Note the extensive remaining insulin staining and the incorporation of BrDU into nuclei of β-cells (arrows, double-positive staining) as well as infiltrating lymphocytes. Incorporation of BrDU into β-cells was never seen in RIP-LCMV mice crossed to other genetic backgrounds. However, it was also observed in RIP-GP × SV129 F1 intercrosses protected from diabetes (F). C: Islet from one of the few diabetic RIP-LCMV-GP × SV129 N8 mice 2 months after LCMV infection. Note that no incorporation of BrDU into nuclei of the remaining β-cells is seen. This type of histological finding is typical for RIP-LCMV mice that are crossed to genetic backgrounds other than SV129 and that are developing a high degree of diabetes. D: Islet of a nondiabetic SV129 (H-2b) mouse 6 days after low-dose streptozozadin treatment (see RESEARCH DESIGN AND METHODS) showing some β-cell proliferation. In comparison, an islet of a C57BL/6J (H-2b) mouse 6 days after low-dose streptozozadin treatment showed no β-cell proliferation and appeared similar to that seen in A. E: Islet from an LCMV-infected RIP-LCMV-GP × SV129 F1 mouse 1 month after infection without diabetes. Note the extensive remaining insulin staining and the incorporation of BrDU into the nuclei of β-cells, similar to the finding shown in B.
However, β-cells regenerate in vivo under attack in protected mice, a finding that we think accounts for at least some of the protection from diabetes in SV129 crosses that maintain sufficient insulin production. Of course, additional, still unknown factors might contribute to this protection as well. Our observation is important in illustrating that genetic factors can easily overcome the autoaggressive potential of islet antigen-specific CD8 and CD4 lymphocytes. As a consequence, one might expect that the correlation between autoreactive lymphocytes and diabetes development could possibly be low in human populations with diverse genetic backgrounds.

Previous studies using tetramers and other functional in vitro assays have shown that up to one in three lymphocytes becomes an activated CTL during acute LCMV infection in C57/BL/6 mice (39,41). This translates into a very high degree of autoreactivity in RIP-LCMV-GP mice that have a fully functional anti-LCMV T-cell repertoire and that do not delete or anergize LCMV CD8 or CD4 cells as a consequence of expressing the LCMV antigen on β-cells (34). In humans, such high frequencies of autoreactive cells are never present systemically, and it is unlikely and has never been shown that they might reach such numbers locally in the islets (4). Thus, the pathogenic potential of autoreactive lymphocytes, even if they were restricted exclusively to diabetes susceptibility MHC alleles, might be overestimated, and other factors can be at least equally as important for the pathogenesis of autoimmune diabetes. One of these factors, which is illustrated in this report, is the potential of β-cells to regenerate under attack, but others are likely also playing a role. For example, local inflammation involving the activation of APCs in the pancreas and islets might be important in providing a suitable environment for autoaggressive lymphocytes (23). We find fewer activated APCs in islets of protected RIP-GP/SV129 mice (Table 1); however, this is likely the consequence of the prevention of diabetes (Table 1). Further studies will be required to clarify this issue. Second, the susceptibility of islet cells to cytokines or CTL killing contribute to diabetes pathogenesis, but in the present study, no differences were noted between the SV129 and C57BL/6 mouse strains, ruling out this possibility as a contributing factor out (Fig. 4). Third, the degree of extravasation of lymphocytes into the pancreas and islets might differ between SV129 and C57BL/6 mouse strains. Again, in this study, it is experimentally difficult to distinguish between cause and effect, as seen with the APCs. Fewer lymphocytes are present in the islets of nondiabetic RIP-LCMV-GP × SV129 mice; however, after LCMV infection, initially the same numbers of CD4 and CD8 lymphocytes enter the islets of RIP-LCMV SV129 and C57BL/6 transgenics (Table 1). Therefore, the lower degree of infiltration in SV129 mice is more likely a consequence of maintaining sufficient islet cell mass as well as a slowly subsiding inflammatory process. Finally, because differences in cytokine profiles were not observed (Table 1), differences in effector function of autoreactive CD4 and CD8 lymphocytes are less likely. However, it cannot be
excluded that hence unknown effector functions other than cell number, CTL killing, and cytokine production determine the diabetogenicity of CD4 or CD8 lymphocytes, and that these functions differ between SV129 and C57BL/6 H-2Db mouse strains.

In summary, the degree of systemically activated autoreactive CD4 and CD8 lymphocytes does not predict the degree of autoimmune diabetes in RIP-LCMV mice, which diverges drastically between C57BL/6 and SV129-Nα genetic backgrounds. Islet cell regeneration is likely one of the factors accounting for this difference, but other non-MHC-linked genes have possible additional effects that still need to be discovered. It is important to consider that our findings might not be generally applicable to every diabetes model crossed to the SV129, as evidenced by good diabetic incidence found in other models after back-crossing to SV129 (42). However, our findings suggest being careful with the interpretation of protection from diabetes in SV129-crossed models and, most importantly, exercising caution toward using autoreactive T-cell activity as a sole correlate for disease activity in type 1 diabetes.

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