Breakdown in Repression of IFN-γ mRNA Leads to Accumulation of Self-Reactive Effector CD8+ T Cells


*J Immunol* 2012; 189:701-710; Prepublished online 8 June 2012; doi: 10.4049/jimmunol.1102432
http://www.jimmunol.org/content/189/2/701

Supplementary Material

http://www.jimmunol.org/content/suppl/2012/06/08/jimmunol.1102432.DC1

References

This article cites 43 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/189/2/701.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Breakdown in Repression of IFN-γ mRNA Leads to Accumulation of Self-Reactive Effector CD8+ T Cells

Pheh-Ping Chang,* Sau K. Lee,* Xin Hu,* Gayle Davey,† Guowen Duan,* Jae-Ho Cho,‡ Guna Karupiah,§ Jonathan Sprent,‡ William R. Heath,‡ Edward M. Bertram,§ and Carola G. Vinuesa* 

Tight regulation of virus-induced cytotoxic effector CD8+ T cells is essential to prevent immunopathology. Naturally occurring effector CD8+ T cells, with a KLRG1hi CD62Llo phenotype typical of short-lived effector CD8+ T cells (SLECs), can be found in increased numbers in autoimmune-prone mice, most notably in mice homozygous for the san allele of Roquin. These SLEC-like cells were able to trigger autoimmune diabetes in a susceptible background. When Roquin is mutated (Roquin−/−), effector CD8+ T cells accumulate in a cell-autonomous manner, most prominently as SLEC-like effectors. Excessive IFN-γ promotes the accumulation of SLEC-like cells, increases their T-bet expression, and enhances their granzyme B production in vivo. We show that overexpression of IFN-γ was caused by failed posttranscriptional repression of Ifng mRNA. This study identifies a novel mechanism that prevents accumulation of self-reactive cytotoxic effectors, highlighting the importance of regulating Ifng mRNA stability to maintain CD8+ T cell homeostasis and prevent CD8-mediated autoimmunity. The Journal of Immunology, 2012, 189: 701–710.
In a separate study, an effector CD122<sup>hi</sup> and MHC class I-dependent CD8<sup>+</sup> population was also described in unimmunized mice (27). It is unclear whether KLRG1<sup>hi</sup> effector CD8<sup>+</sup> T cells found in unimmunized mice can mediate autoimmune disease.

Roquin is a RING-1/CCCH-type zinc finger protein that controls the expression of ICOS (Icos) mRNA posttranscriptionally. Loss of Roquin, either specifically on T cells or in the hematopoietic system, caused expansion on CD8<sup>+</sup> effector-like T cells but not autoimmunity (28). In contrast, Roquin bearing the san allele, which is a gain-of-function-allele (29, data not shown), causes follicular helper T (Tfh) cell-dependent lupus (30–33); it is achieved, at least in part, through posttranscriptional regulation of Icos mRNA. In this article, we show that sanroque mutation of Roquin causes the accumulation of naturally occurring KLRG1<sup>hi</sup> CD8<sup>+</sup> effector cells, which can trigger autoimmune diabetes. The sanroque mutation causes failure of the posttranscriptional control of I<sup>fng</sup> mRNA that leads to excessive IFN-γ signaling, and this is a major driver of SLEC-like cell accumulation with enhanced effector features.

Materials and Methods

Mice

The mice were housed in specific pathogen-free conditions at the Australian National University Bioscience Facility. Roquin<sup>pan/pan</sup> C57BL/6 mice were crossed to Th2<sup>Th2</sup>, OT-I, and I<sup>fng</sup>/I<sup>fng</sup> mice. These mice are all on a C57BL/6 background. Th2<sup>Th2</sup> mice carry a loss-of-function point mutation at the Fas gene that results in high titers of antinuclear Ab (ANA*) in the mice, as well as an accumulation of double-negative T cells in the periphery as the mice age; in these respects, they are very similar to the Fas<sup>lo</sup> mouse strain (L. Tze and C. Goodnow, unpublished observations). RIP-mOVA mice, which express membrane-bound OVA (mOVA) in their pancreatic β cells under the control of rat insulin promoter (RIP), were bred at the University of Melbourne, transferred to the Australian Bioscience Services for experiments, and allowed to acclimatize to this facility for 1 wk prior to injection. All animal procedures were approved by the Australian National University Ethics and Experimentation Committee.

Bone marrow chimeras

Recipient mice were sublethally irradiated with 900 cGy (450 cGy in two doses) and were reconstituted via i.v. injection with 2 × 10<sup>5</sup> donor bone marrow cells.

OT-I cell preparation for adoptive transfer

OT-I cells were purified from the spleens and pooled lymph nodes of OT-I mice (Roquin<sup>pan/pan</sup> or Roquin<sup>+/+</sup> mice) using the CD8<sup>+</sup> T cell Isolation kit II (MACS; Miltenyi Biotec). To transfer CD44<sup>hi</sup> OT-I T cells, OT-I T cells were sorted from the spleens and pooled lymph nodes using a FACSaria (BD Biosciences).

Adoptive-transfer experiments

Purified OT-I cells were spiked with fixed numbers of B cells prior to i.v. injection into nonirradiated RIP-mOVA recipients. The urine of recipient mice was collected to assess glucosuria by Uristix (Bayer). Mice were started to acclimatize at this facility for 1 wk prior to injection. All animal procedures were approved by the Australian National University Animal Ethics and Experimentation Committee.

Bone marrow chimeras

Recipient mice were sublethally irradiated with 900 cGy (450 cGy in two doses) and were reconstituted via i.v. injection with 2 × 10<sup>5</sup> donor bone marrow cells.

OT-I cell preparation for adoptive transfer

OT-I T cells were purified from the spleens and pooled lymph nodes of OT-I mice (Roquin<sup>pan/pan</sup> or Roquin<sup>+/+</sup>) using the CD8<sup>+</sup> T cell Isolation kit II (MACS; Miltenyi Biotec). To transfer CD44<sup>hi</sup> OT-I T cells, OT-I T cells were sorted from the spleens and pooled lymph nodes using a FACSaria (BD Biosciences).

Adoptive-transfer experiments

Purified OT-I cells were spiked with fixed numbers of B cells prior to i.v. injection into nonirradiated RIP-mOVA recipients. The urine of recipient mice was collected to assess glucosuria by Uristix (Bayer). Mice were started to acclimatize at this facility for 1 wk prior to injection. All animal procedures were approved by the Australian National University Animal Ethics and Experimentation Committee.

Abs

All FACS Abs were used from BD Pharmingen, with the exception of anti-mouse CD8α-PE–Cy7 (53-6.7; BioLegend), anti-mouse KLRG1-allophycocyanin (2F1; eBioscience), anti-mouse KLRG1–PE–Cy7 (2F1; eBioscience), anti-mouse CD127 (IL-7Rα)–PE (A7R34; BioLegend), anti-mouse CD62L (L-selectin)–PerCP–Cy5.5 (MEL-14; eBioscience), anti-mouse/human CD44-Pacific Blue (IM7; BioLegend), anti-human/mouse T-bet–PerCP/Cy5.5 (4B10; eBioscience), and mouse anti-human granulocyte B-PE (GB1; Invitrogen).

Flow cytometry

Spleen and lymph node cell suspensions were prepared by sieving and gentle pipetting. For surface staining, cells were maintained in the dark at 4°C throughout. Cells were incubated in FACS buffer with each Ab for 20 min and washed thoroughly with FACS buffer. For intracellular staining, cells were fixed and permeabilized using the Monocyte Regulatory T Cell Staining Kit (eBiosciences), following the manufacturer’s instructions. Flow cytometers (FACSCalibur and LSR II; BD) and software (CellQuest or FACSDiva, respectively; BD) were used for the acquisition of flow cytometric data, and FlowJo software (Tree Star) was used for analysis.

In vitro stimulation

Splenocytes from unimmunized mice were stimulated with 500 ng/ml ionomycin, 50 ng/ml PMA, and 1:250 GolgiStop for 4 h at 37°C before being subjected to IFN-γ staining.

Histology

Pancreases were fixed in 10% neutral-buffered formalin, embedded in paraffin wax, sectioned at 4 μm thickness, dewaxed, and stained with H&E. Gene-expression profiling

For gene-expression analysis, Roquin<sup>pan/pan</sup> and Roquin<sup>+/+</sup> CD44<sup>hi</sup> CD8<sup>+</sup> T cells were sorted, and RNA was isolated using TRizol reagent (Invitrogen), followed by a second isolation using RNeasy (QIAGEN). RNA was amplified twice and hybridized to GeneChip Mouse Gene 1.0 ST Array (Affymetrix) by the Australian Cancer Research Foundation Biomolecular Resource Facility (John Curtin School of Medical Research, UNSW). Data were analyzed using Partek software. The t test was used to identify significantly differentially expressed genes (p = 0.05) between Roquin<sup>pan/pan</sup> and Roquin<sup>+/+</sup> CD44<sup>hi</sup> CD8<sup>+</sup> T cells samples (n = 3/group).

All datasets have been deposited at National Center for Biotechnology Information/Gene Expression Omnibus under accession number GSE37319 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE37319).

In vitro cell activation

A total of 5 × 10<sup>5</sup> sorted CD44<sup>hi</sup> CD8<sup>+</sup> T cells was activated in the presence of 5 μg/ml anti-CD3 (BD Pharmingen), 2 μg/ml anti-CD28 (BD Pharmingen), and 10 ng/ml recombinant mouse IL-12/70 (eBioscience) for 16 h.

Actinomycin D treatment

The transcriptional inhibition reagent actinomycin D (10 μg/ml) was added to the media for resuspension of in vitro-activated CD8<sup>+</sup> T cells. Cells in triplicate wells were harvested at 0, 0.5, 1, or 3 h. Cells were then subjected to RNA extraction with TRizol reagent, followed by I<sup>fng</sup> real-time PCR.

Real-time PCR

RNA was isolated with TRizol reagent (Invitrogen). cDNA was synthesized using M-MLV Reverse Transcriptase (Invitrogen). Inventoried TaqMan Gene Expression Assays (Applied Biosystems) were used to amplify Th2<sup>Th2</sup> (Mm00450960_m1) and Gapdh (Mm99999915_g1) sequences. I<sup>fng</sup>, Roquin, Hprt, and β-actin cDNA were amplified using Power SYBR Green PCR Master Mix (Applied Biosystems). Primers for I<sup>fng</sup> were: 5′-ACAG-GAACGCGAAAAGATG-3′ and 5′-TGACCTATGTAAGTGG-3′; primers for Roquin were: 5′-CGTAAGAGTTGATGTATGTTG-3′ and 5′-AGCCATCTTCATGTGACT-3′; primers for Hprt were: 5′-TATGAAGTTCACGTGGAC-3′ and 5′-GTTGGAACGATAGCCTTCTT-3′; and primers for Hprt were: 5′-ACAGTACACCGCAAAAAAGT-3′ and 5′-AGAGTTTCTTCTTCACCAGCA-3′. Real-time PCR was quantified with an ABI 7900 Prism, and fold changes in expression were determined by the 2<sup>-ΔΔCT</sup> method, with the results normalized to Hprt for I<sup>fng</sup>, β-actin for Roquin, or Gapdh for Th2<sup>Th2</sup>.

Statistical analyses

Data were analyzed using the Mann–Whitney U test, with the exception of bone marrow chimera and I<sup>fng</sup> mRNA-expression experiments, for which the paired t test and unpaired t test was used, respectively. Unless otherwise stated, each symbol represents one mouse, and bars represent the median values for each group.

Results

SLEC-like CD8<sup>+</sup> T cells are increased in mice prone to autoimmune disease

Terminally differentiated cytotoxic KLRG1<sup>hi</sup> CD127<sup>lo</sup> SLEC CD8<sup>+</sup> T cells reacting to viral Ags are induced upon primary response to virus infection (12). The existence of small numbers of
KLRG1hi CD8+ T cells, possibly reacting to self-Ag, in unimmunized healthy C57BL/6 mice (26, 27), led us to hypothesize that their numbers would be higher in mouse strains prone to autoimmune disease. Given that these KLRG1hi CD8+ T cells resembled virus-induced SLECs—they expressed low levels of CD62L, CD127, and CD122; at least 40% were in cell cycle (Ki-67); and, unlike naive cells, died rapidly ex vivo in a manner comparable to virus-induced SLECs (Fig. 1A–C)—we refer to these cells hereafter as “SLEC-like” cells. We investigated SLEC-like cell numbers in five strains of mice on a C57BL/6 background that develop severe (Roquinsan/san) or mild (Aire2/2, Cbl-b2/2, FasThe/The) autoimmunity or fail to develop autoimmunity (B6.Yaa). Statistically significant increases in SLEC-like cells were found in the four strains that develop overt autoimmune symptoms (Fig. 1D) but not in B6.Yaa mice; the Yaa gene by itself is unable to induce significant autoimmune responses in mice without the BXSB/MpJ background (34). Among the mice prone to autoimmune disease, Roquinmice, which were previously shown to develop lupus and be more susceptible to autoimmune diabetes (32), had by far the highest proportion of SLEC-like CD8+ T cells, with an increase detected as early as 7 wk of age (Fig. 1D). Hence, we decided to use Roquinmice to investigate the regulation of SLEC-like cells.

Given the recently reported CD8+ effector-like T cell expansion in Roquin-deficient mice (28), we assessed Roquin mRNA expression in Roquinmice CD8+ T cells. Sorted CD44lo CD8+ T cells were activated in the presence of anti-CD3, anti-CD28, and IL-12 for 16 h. Real-time PCR revealed an ~4-fold reduction in the Roquin mRNA level in both Roquin+/+ and Roquinsan/san activated CD8+ T cells compared with naive CD8+ T cells. Roquin

**FIGURE 1.** KLRG1hi CD8+ T cells resemble virus-reactive SLECs and are increased in mice prone to autoimmune disease. (A) Representative flow cytometric contour plots showing KLRG1hiCD62Llo cells among CD8+ CD44hi spleen cells from unimmunized C57BL/6 mice (top left panel) and CD62L+/–/–specific KLRG1hiCD62Llo cells from C57BL/6 mice 8 d after a secondary challenge with influenza A/PR8/34 virus, which are serologically distinct from A/HKx31 (bottom left panel). Flow cytometric graphs show CD127 (IL-7Rα) (middle panels) and CD122 (IL-2/IL-15Rβ) (right panels) fluorescence intensity in KLRG1hiCD62Llo (filled histograms) and KLRG1loCD62Lhi (open histograms) CD8+ CD44hi spleen cells from unimmunized and immunized C57BL/6 mice. Numbers represent the percentage of cells in the indicated gates. (B) Proportion of proliferating Ki-67+ cells among CD44hiKLRG1hi and naive CD44lo T cells from unimmunized mice (left panel) and mice 7 d postprimary challenge with influenza virus (right panel). Numbers represent the percentage of cells in the indicated gates. (C) Representative contour plot showing the gating strategy to identify live (7AAD–Annexin V–) CD8+ T cells (left panel). Bar graphs show live cells among naive (CD44hi CD8+) and CD44hiKLRG1hi CD8+ T cells from splenic cells incubated at 37°C in culture media for 2 or 24 h after harvesting. (D) Bar graphs showing the proportion of SLEC-like cells (KLRG1hiCD62Llo) among CD44hi CD8+ T cells in the blood of mice. (E) Bar graph showing Roquin mRNA normalized to β-actin in sorted CD44hi CD8+ T cells of Roquin+/+(open bars) and Roquinmice (black bars) mice in the absence or the presence of α-CD3, α-CD28, and IL-12p70 after 16 h of in vitro culture. Each symbol represents one mouse (C, D) or the average values of technical triplicates of one mouse (E). In (C)–(E), bars represent the median values for each group. All data are representative of two independent experiments. *p < 0.05, **p < 0.005, ***p < 0.0005.
expression was comparable between Roquin<sup>+/+</sup> and Roquin<sup>san/san</sup> naive CD8<sup>+</sup> T cells. Activated Roquin<sup>san/san</sup> CD8<sup>+</sup> T cells expressed slightly more Roquin (Fig. 1E). Together, this indicates that the accumulation of SLEC-like CD8<sup>+</sup> T cells in Roquin<sup>san/san</sup> mice cannot be attributed to reduced Roquin expression.

Roquin<sup>san</sup> acts cell autonomously to promote accumulation of SLEC-like cells

Autoimmune-prone Roquin<sup>san/san</sup> mice have an accumulation of CD4<sup>+</sup> and CD8<sup>+</sup> activated/memory T cells (32). In-depth analysis revealed an ∼3-fold increase in Roquin<sup>san/san</sup> CD8<sup>+</sup> T cells in 7–8-wk-old Roquin<sup>san/san</sup> mice compared with Roquin<sup>+/+</sup> littermates (Fig. 2A). The increase in total CD8<sup>+</sup> T cells was due to accumulation of effector and/or memory CD44<sup>hi</sup> CD8<sup>+</sup> T cells (Fig. 2A). To determine which CD44<sup>hi</sup> CD8<sup>+</sup> subsets were dysregulated in Roquin<sup>san/san</sup> mice, expression of CD62L and KLRG1 was assessed. Effector T cells downregulate CD62L to circulate to inflamed tissues, whereas resting memory T cells maintain its expression; KLRG1 is a marker of terminally differentiated SLECs. The most prominent expansion of Roquin<sup>san/san</sup> effectors occurred among KLRG1<sup>hi</sup> CD62L<sup>lo</sup> SLEC-like cells, whose frequency was increased by 19-fold. There was a less-pronounced (2-fold) increase in the frequency of non–SLEC-like (KLRG1<sup>lo</sup> CD62L<sup>lo</sup>) effectors in Roquin<sup>san/san</sup> mice (Fig. 2B). Roquin<sup>san</sup> did not significantly increase the absolute numbers of resting memory phenotype (KLRG1<sup>lo</sup> CD62L<sup>hi</sup>) CD8<sup>+</sup> T cells (Fig. 2B, bottom panel).

To investigate whether Roquin<sup>san</sup> acts cell autonomously to induce the accumulation of SLEC-like cells, as well as whether non-hematopoietic cells contribute to this effect, two sets of mixed bone marrow chimeras were constructed. In the first set, Roquin<sup>san/san</sup>

---

**FIGURE 2.** Accumulation of KLRG1<sup>lo</sup> effector and SLEC-like CD8<sup>+</sup> T cells in Roquin<sup>san/san</sup> mice. (A) Representative flow cytometric contour plots showing CD44<sup>lo</sup> and CD44<sup>hi</sup> cells among CD8<sup>+</sup> spleen cells in 7–8-wk-old unimmunized Roquin<sup>+/+</sup> (top left panel) and Roquin<sup>san/san</sup> (bottom left panel) mice. Numbers represent the percentage of cells in the indicated gates. Right panels, Bar graphs enumerating total CD8<sup>+</sup>, CD44<sup>lo</sup>CD8<sup>+</sup>, and CD44<sup>hi</sup>CD8<sup>+</sup> T cells from 7–8-wk-old unimmunized Roquin<sup>+/+</sup> (open bars) and Roquin<sup>san/san</sup> (black bars) mice. (B) Representative flow cytometric contour plots showing CD62L<sup>lo</sup> KLRG1<sup>hi</sup> (SLEC-like), CD62L<sup>lo</sup>KLRG1<sup>lo</sup> (non–SLEC-like effector), and CD62L<sup>hi</sup>KLRG1<sup>lo</sup> (resting memory phenotype) cells among CD8<sup>+</sup>CD44<sup>hi</sup> spleen cells from Roquin<sup>+/+</sup> (top left panel) and Roquin<sup>san/san</sup> (bottom left panel) mice. Bar graphs (right panels) showing percentage (left column) and numbers (right column) of SLEC-like cells (top panels), CD62L<sup>hi</sup> KLRG1<sup>lo</sup> (middle panels), and CD62L<sup>lo</sup> KLRG1<sup>lo</sup> (bottom panels) cells among CD44<sup>hi</sup> CD8<sup>+</sup> spleen cells from Roquin<sup>+/+</sup> (open bars) and Roquin<sup>san/san</sup> (filled bars) mice. (C) Percentages of Ly5ab and Ly5a of SLEC-like cells of 9-wk 1:1 mixed bone marrow chimeras reconstituted with either Roquin<sup>+/+</sup> Ly5ab and Roquin<sup>san/san</sup> (sanroque) Ly5a bone marrow (left panels within bar graphs) or Roquin<sup>+/+</sup> Ly5ab and Roquin<sup>san/san</sup> Ly5a bone marrow (right panels within bar graphs); bone marrow recipients are sublethally irradiated Roquin<sup>san/san</sup> Ly5b mice (left panel) or Roquin<sup>+/+</sup> Ly5b mice (right panel). Statistical analysis comparing CD8<sup>+</sup> T cell subsets from the same chimeric mice was performed using the paired t test. Each symbol represents one mouse, and bars represent the median values for each group. Data are representative of five (A, B) experiments, three independent experiments (C, right panel), or one experiment (C, left panel). *p < 0.05, **p < 0.005.
Ly5b mice were sublethally irradiated and reconstituted with either a 1:1 mix of Roquin+/+ Ly5ab and Roquin+/+ Ly5a bone marrow or a 1:1 mix of Roquin−/− Ly5ab and Roquin−/− Ly5a bone marrow. In a second set of chimeras, recipient Roquin−/− Ly5b mice were sublethally irradiated and reconstituted with either a 1:1 mix of Roquin−/− Ly5ab and Roquin+/+ Ly5a bone marrow or a 1:1 mix of Roquin+/+ Ly5ab and Roquin+/+ Ly5a bone marrow. SLEC-like CD8+ T cells derived from Roquin+/+ Ly5a progenitors were increased by 5- and 3-fold compared with those derived from Roquin+/+ Ly5ab in Roquin+/+ hosts and Roquin+/+ hosts, respectively (Fig. 2C). Together, these indicate that the accumulation of Roquin+/+ SLEC-like cells is CD8+ T cell intrinsic, and the nonhematopoietic system in Roquin+/+ mice may enhance this effect.

Expansion of SLEC-like cells is associated with autoimmune diabetes

To investigate whether abnormal SLEC-like CD8+ T cell expansion in Roquin+/+ mice may contribute to autoimmune diabetes, we investigated SLEC-like cell accumulation and diabetes induction after adoptive transfer of Roquin+/+ OT-I cells into RIP-mOVA mice (35). In our study, injection >5 × 10^9 OT-I cells into nonirradiated RIP-mOVA recipients led to diabetes induction, whereas 2 × 10^6 OT-I cells rarely caused disease (data not shown). This is somewhat less efficient than originally reported (35). To examine the effect of the san mutation at conditions unlikely to cause diabetes with wild-type cells, we injected 1 × 10^9 OT-I cells from either Roquin+/+ or Roquin+/+ mice into nonirradiated RIP-mOVA recipients. The adoptively transferred OT-I cells were spiked with a fixed number of B cells to control for variations in the inoculum. The phenotype of OT-I donor cells from young Roquin+/+ and Roquin+/+ mice was investigated prior to transfer: only 0.5% KLRG1hi cells were present in the Roquin+/+ cell preparation; most of these were Vo2lo compared with 0.1% KLRG1hi cells in the Roquin+/+ cells (data not shown). It should be noted that SLEC-like cells were significantly reduced in sanroque OT-I mice compared with non–TCR-transgenic mice (data not shown).

By day 10 after transfer, all five mice that had received Roquin+/+ OT-I cells developed diabetes, with near complete islet cell destruction (Fig. 3A, 3B). In contrast, none of the RIP-mOVA mice receiving Roquin+/+ OT-I cells developed diabetes, and all had intact islets with little or no lymphocytic infiltrate (Fig. 3A, 3B). To exclude the possibility that the small difference in the starting population is an important contributor to the development of diabetes, we next sorted naive CD44lo Roquin+/+ OT-I or Roquin+/+ OT-I cells and transferred them into RIP-mOVA mice. Adoptive transfer of 2 × 10^6 sorted naive (CD44hi) OT-I cells from either Roquin+/+ or Roquin+/+ mice spiked with fixed numbers of B cells into nonirradiated RIP-mOVA recipients also caused autoimmune diabetes in RIP-mOVA mice receiving Roquin+/+ CD44lo OT-I 9 d later. None of the RIP-mOVA mice receiving Roquin+/+ OT-I cells developed diabetes except for one, which had significantly lower blood glucose than the levels seen in recipients of Roquin+/+ cells (data not shown).

To assess whether mice developing diabetes have increased numbers of SLECs, mice that became diabetic were sacrificed on day 10, and both total OT-I and OT-I SLECs were enumerated. Total numbers of OT-I cells were comparable in pancreatic lymph nodes, but there was a 10-fold increase in OT-I SLECs from Roquin+/+ donors compared with recipients of Roquin+/+ OT-I cells (Fig. 3C). These results demonstrate that Roquin+/+ CD8+ T cells readily differentiate into SLEC-like T cells and mediate autoimmune diabetes.

Enhanced effector properties in Roquin+/+ SLEC-like CD8+ T cells

Next, we investigated the effector properties of Roquin+/+ SLEC-like CD8+ T cells. IFN-γ and granzyme B are two important effector proteins for CD8+ T cell-mediated killing. Intracellular flow cytometric staining revealed an ∼6-fold increase in the proportion of IFN-γ-producing CD8+ T cells, as well as a 2-fold increase in IFN-γ expression on a per-cell basis among CD44hi CD8+ T cells in Roquin+/+ mice compared with wild-type littermates (Fig. 4A). KLRG1hi SLEC-like CD8+ T cells from Roquin+/+ mice also expressed ∼2-fold higher amounts of granzyme B than did their wild-type counterparts (Fig. 4B).

![FIGURE 3. SLEC-like cells can trigger autoimmune diabetes in susceptible background.](http://www.jimmunol.org/)

A. Representative photomicrographs of H&E-stained pancreas sections from RIP-mOVA recipients of Roquin+/+ (left panel) and Roquin+/+ (right panel) OT-I cells. Original magnification ×40. B. Urine glucose in RIP-mOVA recipients of MACS-purified Roquin+/+ OT-I cells (open bar) and Roquin+/+ OT-I cells (filled bar) 10 d after adoptive transfer. C. Left panels, Representative flow cytometric contour plots showing KLRG1hi SLEC-like cells gated on OT-I cells from pancreatic lymph node (pLN). Numbers represent the percentage of cells in the indicated gates. Right panels, Bar graphs showing the percentages of SLEC-like cells among total OT-I cells (left panel) and the percentages of OT-I cells among total lymphocytes (right panel). In (B) and (C), each symbol represents an individual mouse, and bars represent the median values for each group. All data are representative of two independent experiments. *p < 0.05.
FIGURE 4. Enhanced effector properties in Roquin<sup>san/san</sup> SLEC-like CD8<sup>+</sup> T cells. (A) Left panels, Flow cytometric graphs showing the proportion of IFN-γ-producing splenic CD8<sup>+</sup> T cells. Numbers represent the percentage of cells in the indicated gates. Right panels, Bar graphs enumerating IFN-γ-producing splenic CD8<sup>+</sup> T cells (left panel) and showing IFN-γ MFI (right panel) among CD44<sup>hi</sup>CD8<sup>+</sup> T cells from unimmunized Roquin<sup>+/+</sup> (open bar) and Roquin<sup>san/san</sup> (filled bars) mice. (B) Flow cytometric histograms showing granzyme B fluorescence intensity in SLEC-like cells from Roquin<sup>+/+</sup> (left panel) and Roquin<sup>san/san</sup> (middle panel) mice. Open histograms show the proportion of SLEC-like cells; shaded histograms show the proportion of CD44<sup>lo</sup> CD8<sup>+</sup> T cells. Bar graph (right panel) showing granzyme B-producing cells among SLEC-like splenic CD8<sup>+</sup> T cells. (C) Bar graphs showing proportion of Ki-67<sup>+</sup> cells among CD44<sup>hi</sup>CD8<sup>+</sup> T cells (left panel) and SLEC-like cells (right panel) in Roquin<sup>+/+</sup> (open bars) and Roquin<sup>san/san</sup> (filled bars) mice. (D) Microarray analysis of sorted naive CD8<sup>+</sup> T cells from Roquin<sup>san/san</sup> (<i>n</i> = 3) and Roquin<sup>+/+</sup> (<i>n</i> = 3) mice. Gene-expression values in naive Roquin<sup>san/san</sup> CD8<sup>+</sup> T cells versus naive Roquin<sup>+/+</sup> CD8<sup>+</sup> T cells analyzed with GeneChip Mouse Gene 1.0 ST Array. *<i>p</i> < 0.05, **<i>p</i> < 0.005.

To test whether accumulation of effector CD8<sup>+</sup> T cells in the presence of Roquin<sup>san</sup> is a consequence of increased proliferation, cycling cells were enumerated by flow cytometry using Ki-67 staining. There was a ~3-fold increase in the proportion of cycling SLEC-like CD8<sup>+</sup> T cells in Roquin<sup>san/san</sup> mice compared with Roquin<sup>+/+</sup> littermates; the naive CD44<sup>lo</sup> CD8<sup>+</sup> T cell compartment was unaffected (Fig. 4C).

Several transcription factors were shown to play critical roles in the differentiation and function of SLECs (10, 11, 13). To detect primary changes caused by the <i>san</i> mutation, we compared the gene-expression profiles of sorted naive Roquin<sup>san/san</sup> and Roquin<sup>+/+</sup> CD8<sup>+</sup> T cells. Naive cells were chosen to use in the comparative gene-expression profiling because KLRG1 was already upregulated in Roquin<sup>san/san</sup> naive CD8<sup>+</sup> T cells (Fig. 4D), and this approach had been successful in identifying Icos as one of the mRNAs responsible for Tfh cell accumulation within the CD4<sup>+</sup> compartment (33). Klrk1 mRNA was increased by 20-fold in naive Roquin<sup>san/san</sup> CD8<sup>+</sup> T cells. Of the transcription factors known to influence effector/memory T cell differentiation—Eomesodermin (<i>Eomes</i>), Blimp (<i>Prdm1</i>), B cell lymphoma 6 (<i>Bcl6</i>), and T-bet (<i>Tbx21</i>)—Tbx21, a transcription factor required for terminal effector CD8<sup>+</sup> T cell differentiation, was significantly upregulated (Fig. 4D). This increase in T-bet within naive CD44<sup>lo</sup> CD8<sup>+</sup> T cells was confirmed by real-time PCR and protein quantification by flow cytometry (Fig. 4D). Taken together, these data suggest that Roquin<sup>san</sup> promotes the expression of IFN-γ, granzyme B, and T-bet responsible for increased SLEC formation and function.

**T-bet reduction deviates the accumulation of activated CD8<sup>+</sup> T cells from SLEC-like cells to cells with a CD62L<sup>lo</sup>KLRG1<sup>lo</sup> phenotype**

T-bet was shown to cooperate with Eomes to regulate SLEC and memory CD8<sup>+</sup> T cells (12, 36), and overexpression of T-bet can enhance SLEC formation in response to viral infection (12). Therefore, we hypothesized that the increased expression of T-bet observed above may cause the accumulation of self-reactive SLEC-like cells. We first investigated whether decreasing the gene dose of T-bet would have an impact on SLEC-like cell numbers. To do this, we crossed <i>sanroque</i> mice with Duane mice. Duane mice are homozygous for a missense point mutation in Tbx21 (<i>Tbx21<sup>Tbx21<sup>Du/Du</sup></sup></i>) that causes a reduction in T-bet protein, and the residual protein found in these mice is defective (37). Decreasing the amount of T-bet did not decrease the accumulation of CD44<sup>hi</sup> CD8<sup>+</sup> T cells in Roquin<sup>san/san</sup> Tbx21<sup>Tbx21<sup>Du/Du</sup></sup> mice that virtually lacked detectable T-bet protein (Fig. 5A, 5B). Nonetheless, SLEC-like cells were reduced by 4-fold in Roquin<sup>san/san</sup> Tbx21<sup>Tbx21<sup>Du/Du</sup></sup> mice compared with Roquin<sup>san/san</sup> Tbx21<sup>Tbx21<sup>+/+</sup></sup> mice, with Roquin<sup>san/san</sup> Tbx21<sup>Tbx21<sup>Du/Du</sup></sup> mice
T-bet reduction delays the onset of diabetes

We next examined whether reducing the expression of T-bet in Roquin<sup>san/san</sup> OT-I mice would decrease their diabetogenic potential. We transferred 2 × 10<sup>6</sup> OT-I cells from either Roquin<sup>san/san</sup> Tbx21<sup>+/+</sup> or Roquin<sup>san/san</sup> Tbx21<sup>Du/+</sup> mice, spiked with fixed numbers of B cells, into nonirradiated RIP-mOVA recipients. On day 8, five of six mice receiving Roquin<sup>san/san</sup> Tbx21<sup>+/+</sup> OT-I cells, but none of the recipients of Roquin<sup>san/san</sup> Tbx21<sup>Du/+</sup> cells, were diabetic (Fig. 5D). Three of four recipients of Roquin<sup>san/san</sup> Tbx21<sup>Du/+</sup> OT-I cells and all recipients of Roquin<sup>san/san</sup> Tbx21<sup>+/+</sup> OT-I cells became diabetic 24 h later. Granzyme B and IFN-γ expression were still elevated in self-reactive Roquin<sup>san/san</sup> early effector cells, but their amounts were lower or comparable, respectively, to those of Roquin<sup>san/san</sup> SLEC-like cells (Fig. 5E, 5F). Together, these findings suggest that Roquin<sup>san/san</sup> SLEC-like cells play a key role in causing autoimmune diabetes in RIP-mOVA mice, and diabetes can be delayed by reducing T-bet expression.

Excessive IFN-γ signaling promotes the accumulation of Roquin<sup>san/san</sup> SLEC-like CD8<sup>+</sup> T cells

The fact that T-bet reduction could not reverse the accumulation of effecter CD8<sup>+</sup> T cells in Roquin<sup>san/san</sup> mice, prompted us to look into other causes. Roquin<sup>san/san</sup> CD4<sup>+</sup> T cells were shown to produce excessive amounts of two cytokines known to influence CD8<sup>+</sup> T cell homeostasis: IL-21 and IFN-γ (32). IL-21 is important for CD8<sup>+</sup> T cell survival and memory formation (38, 39), whereas IFN-γ promotes virus-specific CD8<sup>+</sup> T cell expansion (17, 40). Enumeration of SLEC-like cells in Roquin<sup>san/san</sup> mice deficient in IL-21 excluded dysregulation of this cytokine as the cause of SLEC-like cell accumulation in Roquin<sup>san/san</sup> mice (data not shown). Therefore, we investigated whether excessive IFN-γ production could explain the SLEC-phenotype and aberrant expansion of Roquin<sup>san/san</sup> effecter CD8<sup>+</sup> T cells.

Roquin<sup>san/san</sup> mice were crossed with mice lacking the IFN-γ ligand-binding chain (also known as the α-chain) of the IFN-γR (Ifngr1<sup>−/−</sup>). Loss of IFN-γ signaling almost completely reversed the accumulation of Roquin<sup>san/san</sup> SLEC-like cells (Fig. 6A) and reduced the absolute numbers of CD62L<sup>lo</sup>KLRG1<sup>lo</sup> effecter cells (Fig. 6B), which remained comparable between Roquin<sup>san/san</sup> and Roquin<sup>san/+</sup> mice (Supplemental Fig. 2). This result suggests that overproduction of IFN-γ, as seen in Roquin<sup>san/san</sup> mice, perturbs SLEC-like cell homeostasis. Together, these results suggest that IFN-γ is the predominant factor responsible for the expansion and accumulation of KLRG1<sup>lo</sup> SLEC-like cells in Roquin<sup>san/san</sup> mice, but additional factors act to disrupt this compartment (Fig. 6).

We also assessed the ability of Roquin<sup>san/san</sup> CD8<sup>+</sup> T cells deficient in IFN-γ production to induce autoimmune diabetes in susceptible RIP-mOVA mice by transferring 1 million sorted CD4<sup>+</sup> Roquin<sup>san/san</sup> Ifngr1<sup>−/−</sup> OT-I cells. Although none of the recipients became overtly diabetic, likely as a result of the smaller number of transferred cells, recipients of Roquin<sup>san/san</sup> Ifngr1<sup>−/−</sup> OT-I cells preserved a larger number of islets/pancreas unit area compared with recipients of Roquin<sup>san/san</sup> OT-I cells (Supplemental Fig. 2). This result suggests
that IFN-γ overproduction contributes to the increased ability of Roquin<sup>san/san</sup> CD8<sup>+</sup> T cells to induce autoimmunity in a susceptible host.

Excessive IFN-γ promotes the overexpression of granzyme B and T-bet, as well as the proliferation of Roquin<sup>san/san</sup> effector CD8<sup>+</sup> T cells

Having observed that Roquin<sup>san/san</sup> CD8<sup>+</sup> effector T cells are more potent producers of granzyme B than are their wild-type counterparts (Fig. 4B), we sought to determine whether this might be a consequence of excessive IFN-γ production. Indeed, loss of IFN-γ signaling completely normalized granzyme B production by SLEC-like cells to wild-type levels (Fig. 7A).

IFN-γ is required to upregulate T-bet in CD8<sup>+</sup> T cells in an indirect manner upon virus infection (12, 41). We wondered whether the increased expression of T-bet in Roquin<sup>san/san</sup> mice is a consequence of excessive IFN-γ. To assess this, we measured T-bet mean fluorescence intensity (MFI) in naive (CD44<sup>lo</sup>) CD8<sup>+</sup> T cells and SLEC-like cells from Roquin<sup>san/san</sup> mice deficient or not in IFN-γR1. In the absence of IFN-γR signaling, T-bet expression in Roquin<sup>san/san</sup> SLEC-like cells was comparable to that of Roquin<sup>san/+</sup> mice (Fig. 7B). A decrease in T-bet expression was also observed in the naive CD8<sup>+</sup> T cell compartment in Roquin<sup>san/san</sup>Ifngr1<sup>−/−</sup> mice, although T-bet amounts were still higher than were those of Roquin<sup>san/+</sup> mice (Fig. 7C), suggesting that other factors contribute to elevated T-bet expression in naive CD8<sup>+</sup> T cells.

IFN-γ was shown to promote Ag-specific CD8<sup>+</sup> T cell expansion during viral infections (17, 40), and we showed that Roquin<sup>san/san</sup> CD8<sup>+</sup> T cells are hyperproliferative (Fig. 2C). To formally test whether excessive IFN-γ drives CD8<sup>+</sup> effector T cell accumulation through enhancing their proliferation, cycling (Ki-67<sup>+</sup>) cells were enumerated. Proliferating CD8<sup>+</sup> T cells were reduced by half in the absence of IFN-γR1 (Fig. 7D). IFN-γR signaling acted on CD62L<sup>lo</sup>KLRG1<sup>lo</sup> effector CD8<sup>+</sup> T cells, whose proliferative rate was corrected with wild-type levels in mice lacking IFN-γR1, but it did not influence the proliferation of SLEC-like cells themselves (Fig. 7D).

Together, these data suggest that excessive IFN-γ predominantly acts by enhancing the cytotoxic potential and T-bet transcription of SLEC-like CD8<sup>+</sup> T cells, but it only affects the proliferative rate of CD62L<sup>lo</sup>KLRG1<sup>lo</sup> effector CD8<sup>+</sup> T cells.

Roquin<sup>san</sup> represses Ifng mRNA decay

Our results showed that IFN-γ dysregulation explains SLEC-like cell accumulation driven by Roquin<sup>san</sup>. To understand the mechanism by which Roquin<sup>san</sup> causes IFN-γ overproduction by CD8<sup>+</sup> T cells, we asked whether, as described for Icos (33), this accumulation occurred at the level of Ifng mRNA. Purified Roquin<sup>san/san</sup> and Roquin<sup>san/+</sup> CD44<sup>hi</sup> CD8<sup>+</sup> T cells were subjected to in vitro activation in the presence of anti-CD3, anti-CD28, and IL-12 for 16 h. Ifng mRNA was ~60-fold higher in activated Roquin<sup>san/san</sup> CD8<sup>+</sup> T cells compared with wild-type counterparts (Fig. 8A).

Given the reported enhancement of Icos mRNA stability by Roquin<sup>san</sup> (30, 33), we tested whether IFN-γ overproduction was due to the prolonged half-life of Ifng mRNA. Naive CD44<sup>hi</sup> CD8<sup>+</sup> T cells of Roquin<sup>san/san</sup> and Roquin<sup>san/+</sup> mice were treated with actinomycin D 16 h after activation to inhibit mRNA transcription. Ifng mRNA was measured at different intervals after treatment. Roquin<sup>san</sup> prolonged the half-life of Ifng mRNA by >18-fold (Fig. 8B). Thus, overproduction of IFN-γ by Roquin<sup>san/san</sup> CD8<sup>+</sup> T cells is a consequence of failed posttranscriptional repression of Ifng expression.

Discussion

The nature and regulation of KLRG1<sup>hi</sup> CD8<sup>+</sup> T cells that develop in the absence of immunization have been obscure. The data in this study reveal that these SLEC-like cells accumulate in mice prone to autoimmunity, in particular Roquin<sup>san/san</sup> mice, which were shown previously to develop lupus and to be more susceptible to autoimmune diabetes. Our work reveals that overproduction of IFN-γ is, in large part, responsible for the pathogenic accumulation of SLEC-like CD8<sup>+</sup> T cells.

A very recent report demonstrated that Roquin deletion in T cells caused a ~6.5-fold increase in CD62L<sup>lo</sup>CD44<sup>hi</sup> effector-like CD8<sup>+</sup> T cells, an increase in KLRG1<sup>hi</sup> CD8<sup>+</sup> T cells, and a decrease in naive and memory-like CD8<sup>+</sup> T cells (28). In contrast, Roquin<sup>san/san</sup> mice bearing a point mutation (Met<sup>399</sup> to Arg substitution) in the RNA-binding ROQ domain in Roquin display an increase in effector-like CD8<sup>+</sup> T cells (SLEC-like cells and
FIGURE 7. Loss of IFN-γ signaling alleviates the overexpressing effector phenotypes of Roquin<sup>san/san</sup> SLEC-like cells. (A) Flow cytometric histograms (three left panels) showing granzyme B fluorescence intensity in SLEC-like cells from Roquin<sup>+/+</sup> (left panel) and Roquin<sup>san/san</sup> (middle and right panels) mice, deficient or not in IFN-γR1. Open histograms show the proportion of SLEC-like cells; shaded histograms show the proportion of CD44<sup>hi</sup> CD8<sup>+</sup> T cells. Right panel, Bar graph showing granzyme B-producing cells among SLEC-like splenic CD8<sup>+</sup> T cells. Bar graphs showing T-bet MFI in SLEC-like cells (B) and naive CD44<sup>lo</sup> CD8<sup>+</sup> T cells (C) from unimmunized Roquin<sup>+/+</sup> (open bars), and Roquin<sup>san/san</sup> (filled bars) mice, deficient or not in IFN-γR1. (D) Bar graphs showing the proportion of Ki-67<sup>+</sup> cells among total CD8<sup>+</sup> (left panel), CD62L<sup>lo</sup> KLRG1<sup>lo</sup> CD44<sup>hi</sup> CD8<sup>+</sup> T cells. Unlike Roquin<sup>san/san</sup> mice, Roquin-null mice do not develop autoimmunity. These contrasting phenotypes can be partially explained by intact RING-mediated activity in Roquin<sup>−/−</sup> mice, which opposes the action of the RNA-binding ROQ and CCCH domains through a different mechanism (R. Ramiscal and C.G. Vinuesa, unpublished observations). Thus, it is likely that Roquin controls CD8<sup>+</sup> T cell homeostasis through several mechanisms. This work allows dissection of the contribution of Roquin-mediated RNA regulation to this effect.

Our work highlights the crucial role for posttranscriptional repression of Ifng mRNA in the control of naturally occurring CD8<sup>+</sup> T cell effector cells, including those harboring self-reactivities. Failure to repress Ifng mRNA caused by the san mutation in Roquin is a major contributor to the aberrant accumulation of SLEC-like CD8<sup>+</sup> T cell effectors. Posttranscriptional repression of IFN-γ emerges as a powerful mechanism to control the expansion of highly cytotoxic SLEC-like cells.

A role for IFN-γ as the principal effector cytokine of CD8<sup>+</sup> T cell-mediated immunity has long since been established (42). In the last decade, dual opposing roles for IFN-γ in the homeostatic control of T cells were also described (17, 25, 40): IFN-γ limits the expansion of T cells through stimulating the expression of caspases downstream of Fas (25), and, paradoxically, it can also enhance expansion of CD8<sup>+</sup> T cell effector clones during viral infection (17) through an unknown molecular mechanism. Our study establishes a new role for IFN-γ in the control of naturally occurring CD8<sup>+</sup> T cell effectors and highlights the importance of controlled posttranscriptional regulation of this cytokine: IFN-γ overproduction in the presence of Roquin<sup>−/−</sup> promotes the proliferation of KLRG1<sup>lo</sup> effectors and the formation of highly cytotoxic SLEC-like cells and increases MHC-I expression on APCs, which promotes CD8<sup>+</sup> T cell activation (Supplemental Fig. 3).

Roquin was reported to bind to and repress Icos mRNA (29, 30, 33); prolonged Icos mRNA stability in the presence of Roquin<sup>−/−</sup> contributes to aberrant accumulation of CD4<sup>+</sup> Tfh cells and lupus. The present work identifies IFN-γ as a novel target of Roquin<sup>−/−</sup>-mediated repression. IFN-γ is a cytokine known to be regulated posttranscriptionally: the RNA-binding protein tristetraprolin was reported to destabilize IFN-γ mRNA (43). Accumulation of Ifng mRNA causes excessive production of IFN-γ, which acts, at least in part, to promote the expansion and accumulation of KLRG1<sup>lo</sup> and terminally differentiated effector CD8<sup>+</sup> T cells in Roquin<sup>san/san</sup> mice.

In summary, we described a novel posttranscriptional mechanism operating to regulate SLEC-like cell homeostasis and prevent the accumulation of self-reactive CD8<sup>+</sup> effectors, as well as their cytotoxic potential. Aberrant accumulation of highly cytotoxic

CD62L<sup>hi</sup>KLRG1<sup>lo</sup>CD44<sup>hi</sup>CD8<sup>+</sup> T cells) but no changes in the absolute number of naive CD8<sup>+</sup> and resting memory-like CD8<sup>+</sup> T cells. Unlike Roquin<sup>san/san</sup> mice, Roquin-null mice do not develop autoimmunity. These contrasting phenotypes can be partially explained by intact RING-mediated activity in Roquin<sup>−/−</sup> mice, which opposes the action of the RNA-binding ROQ and CCCH domains through a different mechanism (R. Ramiscal and C.G. Vinuesa, unpublished observations). Thus, it is likely that Roquin controls CD8<sup>+</sup> T cell homeostasis through several mechanisms. This work allows dissection of the contribution of Roquin-mediated RNA regulation to this effect.

Our work highlights the crucial role for posttranscriptional repression of Ifng mRNA in the control of naturally occurring CD8<sup>+</sup> T cell effector cells, including those harboring self-reactivities. Failure to repress Ifng mRNA caused by the san mutation in Roquin is a major contributor to the aberrant accumulation of SLEC-like CD8<sup>+</sup> T cell effectors. Posttranscriptional repression of IFN-γ emerges as a powerful mechanism to control the expansion of highly cytotoxic SLEC-like cells.

A role for IFN-γ as the principal effector cytokine of CD8<sup>+</sup> T cell-mediated immunity has long since been established (42). In the last decade, dual opposing roles for IFN-γ in the homeostatic control of T cells were also described (17, 25, 40): IFN-γ limits the expansion of T cells through stimulating the expression of caspases downstream of Fas (25), and, paradoxically, it can also enhance expansion of CD8<sup>+</sup> T cell effector clones during viral infection (17) through an unknown molecular mechanism. Our study establishes a new role for IFN-γ in the control of naturally occurring CD8<sup>+</sup> T cell effectors and highlights the importance of controlled posttranscriptional regulation of this cytokine: IFN-γ overproduction in the presence of Roquin<sup>−/−</sup> promotes the proliferation of KLRG1<sup>lo</sup> effectors and the formation of highly cytotoxic SLEC-like cells and increases MHC-I expression on APCs, which promotes CD8<sup>+</sup> T cell activation (Supplemental Fig. 3).

Roquin was reported to bind to and repress Icos mRNA (29, 30, 33); prolonged Icos mRNA stability in the presence of Roquin<sup>−/−</sup> contributes to aberrant accumulation of CD4<sup>+</sup> Tfh cells and lupus. The present work identifies IFN-γ as a novel target of Roquin<sup>−/−</sup>-mediated repression. IFN-γ is a cytokine known to be regulated posttranscriptionally: the RNA-binding protein tristetraprolin was reported to destabilize IFN-γ mRNA (43). Accumulation of Ifng mRNA causes excessive production of IFN-γ, which acts, at least in part, to promote the expansion and accumulation of KLRG1<sup>lo</sup> and terminally differentiated effector CD8<sup>+</sup> T cells in Roquin<sup>san/san</sup> mice.

In summary, we described a novel posttranscriptional mechanism operating to regulate SLEC-like cell homeostasis and prevent the accumulation of self-reactive CD8<sup>+</sup> effectors, as well as their cytotoxic potential. Aberrant accumulation of highly cytotoxic
SLEC-like cells, despite their short lifespan, can pose a threat for autoimmune. Thus, manipulation of this axis to curtail the expansion and survival of self-reactive SLECs may be useful to dampen the pathological accumulation of these cells that is often seen in inflammatory diseases and that was shown in this study to contribute to autoimmune diabetes.

Acknowledgments

We thank Axel Kallies (Walter and Eliza Hall Institute of Medical Research) for critical reading of the manuscript, Kaiman Peng and Stephen Ohm (Australian Cancer Research Foundation Biomolecular Resource Facility, JCSMR) for help with microarray gene profiling and analysis, Anne Prins (Microscopy and Cytometry Resource Facility, JCSMR) for help for cell sorting, and Michael Devoy for processing H&E-stained pancreas section images prior to islet quantification.

Disclosures

The authors have no financial conflicts of interest.

References

Figure S1. Comparable Number of SLEC-Like and CD44hiCD8+ T Cells in Roquin+/+ Ifng−/− Mice
Bar graphs showing the absolute numbers of SLEC-like cells (a) and CD44hiCD8+ T cells (b) from spleens of mice with the indicated genotypes. Each symbol represents an individual mouse and bars represent the median values for each group. Data are representative of one experiment. 'ns', not statistically significant.
Figure S2. Higher Number of Islets per mm² in Susceptible Hosts with Transfer of Roquin<sup>can/ban</sup> CD8<sup>+</sup> T Cells Deficient in Producing IFN-γ

Bar graph showing the number of islet per mm² from H&E stained pancreas section of RIP-mOVA recipients after one month of transferring one million of sorted CD44<sup>b</sup> OT-I cells (with genotypes as indicated) spiked with wild-type B cells. Each symbol represents the average number of islet per mm² from two pancreas sections of individual mouse and bars represent the median values for each group. Quantification was performed with ImageJ 1.45s Java 1.6.0_20 (32-bit) free software. Data are from one experiment. *, P<0.05; 'ns', not statistically significant.
Figure S3. Elevated MHC-I Expression in Roquin<sup>san/san</sup> Antigen Presenting Cells

Bar graphs showing mean fluorescence intensity of H2Db and H2kb in CD8<sup>+</sup> dendritic cells (DC), CD8<sup>-</sup> DC, macrophage, monocytes and B cells in mice with the indicated genotypes.

Each symbol represents an individual mouse and bars represent the median values for each group. Data are representative of one experiment. *, P<0.05; **, P<0.005; 'ns', not statistically significant.