Development of Memory-Like Autoregulatory CD8⁺ T Cells Is CD4⁺ T Cell Dependent

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Progression of spontaneous autoimmune diabetes is associated with development of a disease-countering negative-feedback regulatory loop that involves differentiation of low-avidity autoreactive CD8+ cells into memory-like autoregulatory T cells. Such T cells blunt diabetes progression by suppressing the presentation of both cognate and noncognate Ags to pathogenic high-avidity autoreactive CD8+ T cells in the pancreas-draining lymph nodes. In this study, we show that development of autoregulatory CD8+ T cell memory is CD4+ T cell dependent. Transgenic (TG) NOD mice expressing a low-affinity autoreactive TCR were completely resistant to autoimmune diabetes, even after systemic treatment of the mice with agonistic anti-CD40 or anti−4-1BB mAbs or autoantigen-pulsed dendritic cells, strategies that dramatically accelerate diabetes development in TG NOD mice expressing a higher affinity TCR for the same autoantigenic specificity. Furthermore, whereas abrogation of RAG-2 expression, hence endogenous CD4+ T cell and B cell development, decelerated disease progression in high-affinity TCR-TG NOD mice, it converted the low-affinity TCR into a pathogenic one. In agreement with these data, polyclonal CD4+ T cells from prediabetic NOD mice responded against pancreatic β cells. Thus, in chronic autoimmune responses, CD4+ Th cells contribute to both promoting and suppressing pathogenic autoimmunity.

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Generation of productive CD8+ T cell responses requires the activation of APCs by CD4+ Th cells (1, 2) or pathogen-associated molecular patterns (3), a process referred to as APC licensing. Ligation of CD40 or TLRs on APCs by CD40L (CD154) on CD4+ Th cells or pathogen-associated molecular patterns, respectively, induces the upregulation of CD8+ T cell-cosimulator molecules and the secretion of proinflammatory cytokines (4–6). Most importantly, CD4+ Th cells play a critical role in the generation of functional memory CD8+ cells in response to a variety of foreign and self-Ags, such that unhelped memory CD8+ cells generated and maintained in the absence of CD4+ Th cells have significantly reduced long-term survival and effector function capabilities than their helped counterparts (7–11).

Type 1 diabetes (T1D) in both humans and NOD mice results from a chronic, CD4+ and CD8+ T cell-dependent autoimmune response against pancreatic β cells (12). We and others have shown that CD8+ cells play a critical role in initiation and progression of T1D (reviewed in Ref. 13). A significant fraction of islet-infiltrating CD8+ cells in NOD mice employ highly homologous TCRs rearrangements and recognize an epitope from islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP206–214 or its mimotopes NRP-A7 and NRP-V7) in the context of the MHC class I molecule Kd (14, 15). This T cell subset, however, is not homogeneously pathogenic and consists of clones engaging peptide–MHC (pMHC) over a wide range of avidities, which correlate with the clones’ pathogenic activity (16–18). In fact, whereas high-avidity IGRP206–214-reactive CD8+ cells differentiate into diabeticogenic CTL, their low-avidity counterparts differentiate into memory-like autoregulatory CD8+ cells with powerful antidiabetic properties (19).

We have previously shown that differentiation of high-avidity IGRP206–214-reactive CD8+ cells into diabeticogenic CTL is a CD4+ Th-dependent process (14). In addition to upregulating the costimulatory properties of autoantigen-loaded dendritic cells (DCs), ligation of CD40 on DCs by CD154 on CD4+ Th cells releases immature DCs from suppression by Foxp3+CD4+ regulatory T cells (Tregs) (20). Because the autoregulatory CD8+ cells that arise from naïve low-avidity precursors in response to chronic autoantigenic stimulation display a memory phenotype, we reasoned that their development might also require the licensing of autoantigen-loaded DCs (and their unlocking from Treg-mediated suppression) by CD4+ Th cells. In this study, we test this hypothesis by following the fate of high- and low-avidity IGRP206–214-reactive CD8+ cells in the presence or absence of CD4+ Th cells or surrogate Th-like DC-activation stimuli. Our data demonstrate that CD4+ Th cells promote the development of autoregulatory T cell memory.

Materials and Methods

Mice

NOD.Jltmice were from The Jackson Laboratory (Bar Harbor, ME), 17.4o.8.3B-NOD and 17.6o.8.3B-NOD mice have been described (14, 19). Tcrα−/− and Rag2−/− TCR-TG NOD mice were generated by backcrossing the TCR transgenes onto NOD.Tcrα−/− and NOD.Rag2−/− mice, respectively. All mice were housed and studied in specific pathogen-free facilities. These studies were approved by the University of Calgary.
Faculty of Medicine’s Animal Care Committee and followed the guidelines of Canadian Council on Animal Care.

**Diabetes**

Diabetes was monitored by measuring urine glucose level using Diastix (Bayer, Toronto, Ontario, Canada). Animals were considered diabetic after 2 consecutive d of glucosuria.

**Flow cytometry and Abs**

Processed thymi, spleens, and lymph nodes were stained at 4°C for 30 min in the dark. Stained samples were thoroughly washed and fixed with 1% paraformaldehyde until analysis. Three-color flow cytometry was performed using following mAbs: anti-CD8α–PerCP (53.6.7), anti-CD4–FITC (SK3, 1:5), anti-Vβ1.8/8.2–PE (MR5-2), anti-CD69–PerCP/Cy5.5 (H1.2F3), anti-CD62L–PE (MEL-14) (all from BD Biosciences, San Jose, CA), anti-CD44–FITC (IM7.8.1) (Caltag Laboratories, Burlingame, CA), and anti-CD122–PE (SH4) (eBioscience, San Diego, CA).

**In vitro suppression assays**

CD8+ cells were purified from the spleens and lymph nodes of transgenic (TG) mice and FACs-sorted into CD44loCD122+ or CD44hiCD122+ populations. Sorted cells (1.7–2.3 × 10^6) were cultured in U-bottom 96-well plates with NRP-A7–pulsed (1 μM) bone marrow-derived DCs (BM-DCs; 10^4). After 20 h of culture, purified 17.4a/8.3β–CD8+ cells (2 × 10^5) were labeled with CFSE (2.5 μM) and added to the wells. CFSE dilution was analyzed by FACs after 48 h. Percent suppression was calculated as the ratio of the decrease in proliferation between responders without suppressors and responders with suppressors to the percentage of proliferation of responder cells without suppressors.

**Adoptive transfer**

CD4+ T cells and B cells were purified from the spleens of 7–14 wk old NOD/LtJ mice using BD IMag anti-mouse CD4 particles-DM (BD Biosciences) and anti-B220–coated magnetic beads (Miltenyi Biotec, Bergisch-Gladbach, Germany), respectively, according to the manufacturers’ instructions. Thirty million purified cells were injected into 5–10-wk-old Rag2−/− mice, and followed for diabetes development for 150 d. Some 17.6α/8.3β-NOD.Rag2−/− mice were bled on days 0, 8, and 18 to assess the percentage of circulating CD44hi CD8+ cells. CD4+ T cells and B cells were purified from the spleens of 7–14 wk old transgenic (17.4a/8.3β-TCR-TG mice. Mice were followed for diabetes development for 200 d.

**Statistical analysis**

Data were compared using log-rank, Mann–Whitney U test, or Student’s t test. Statistical significance was assumed at p < 0.05.

**Results**

**Diabetes suppression by memory-like autoregulatory CD8+ T cells**

NOD mice expressing a low-affinity IGRF_{206/214}-reactive TCR (17.6α/8.3β-NOD) are almost completely resistant to T1D (19). T1D resistance in these mice is not mediated by Foxp3+ CD4+ Tregs, but rather by a memory-like subset of antidiabeticogenic CD8+ cells that arises from naive low-avidity precursors (19). This outcome was remarkably different from that seen in mice expressing a nearly identical TCR targeting the same pMHC complex with ~10-fold higher affinity (17.4α/8.3β-NOD), which develop accelerated T1D (14, 19). Phenotypic analyses indicated that both types of TCRs foster the development and selection of CD8+ cells as compared with non-TG NOD mice (Fig. 1A). As expected, the percentage of CD8+CD4− thymocytes was significantly higher in mice expressing the high-affinity TCR (17.4α/8.3β) than in mice expressing its low-affinity counterpart (17.6α/8.3β) (p = 0.03; Fig. 1A). However, unlike the former, the latter accumulated a sizeable population of CD44hiCD122+ CD8+ cells (Fig. 1B). This population primarily arose in the periphery, because thymic CD8+CD4− cells, unlike splenic CD8+ cells, did not harbor CD122+ cells (Fig. 1C). The increased frequency of CD44hiCD122+CD8+ T cells in the peripheral lymphoid organs of 17.6α/8.3β- versus 17.4α/8.3β-NOD mice could not be explained by selective pairing of the 17.6α-chain with an endogenous TCRβ-chain capable of driving the differentiation of the CD8+ T cells of these mice into memory-like CD8+ T cells because allelic exclusion of the endogenous TCRβ locus was very efficient in both TCR-TG strains. In fact, their spleen and pancreatic lymph nodes (PLNs) harbored very low percentages of CD8+ T cells expressing endogenous Vβ elements (0.63 ± 0 and 0.33 ± 0.01, respectively, for 17.4α/8.3β-NOD mice; 2.39 ± 0.31 and 0.28 ± 0.01, respectively, for 17.6α/8.3β-NOD mice). Furthermore, the percentages of CD8+ T cells expressing endogenous Vβ elements in 17.6α/8.3β-NOD mice were significantly lower than the percentage of memory-like autoregulatory CD8+ T cells (10.10 ± 0.66 and 5.02 ± 0.61 in spleen and PLNs, respectively). These memory-like CD8+ cells had regulatory properties, as they readily suppressed the activation of naive high-avidity autoreactive CD8+ cells by peptide-pulsed DCs in vitro (Fig. 1D).

We have previously demonstrated that CD44hiCD25− Treg-mediated suppression of T1D is also associated with suppression of autoantigen presentation by DCs in the PLNs (20, 21). Notably, ligation of CD40 on DCs by CD154 on CD4+ Th cells provides a signal that releases DCs from the suppressive activity of CD4+ Tregs (20). As a result, systemic activation of DCs with an agonistic anti-CD40 mAb, CpG DNA, or LPS was readily able to overcome suppression by CD4+CD25+ T cells, leading to T1D (20). To ascertain whether systemic activation of DCs or systemic delivery of activated Ag-pulsed DCs could also overcome tolerance mediated by memory-like autoregulatory CD8+ cells in 17.6α/8.3β-NOD mice, we treated these mice with an agonistic anti-CD40 mAb or with LPS-activated NRP-V7–pulsed DCs. We also asked if polyclonal activation of the CD8+ cells of these mice with an agonistic Ab against 4-1BB, a costimulatory molecule expressed primarily on effector CD8+ cells, could overcome their diabetes resistance [i.e., by promoting effector CD8+ cell survival and by increasing the IFN-γ secretory and cytolytic activities of these T cells, as described in other settings (22)]. Rat-IgG- and LPS-activated DCs pulsed with a 17D-irrelevant peptide (TUM) were used as negative controls. Additional controls involved the use of age- and sex-matched 17.4a/8.3β-NOD mice.

Administration of agonistic anti-CD40 or anti–4-1BB mAbs (but not rat-IgG) dramatically accelerated diabetes in 17.4a/8.3β-NOD mice (Fig. 2A). This was associated with upregulation of CD44 and CD69 and downregulation of CD62L on a significant fraction of splenic and lymph node CD8+ cells, as expected (Fig. 2B). Anti-CD40 and anti–4-1BB mAb treatment, however, failed to induce diabetes in 17.6α/8.3β-NOD mice (Fig. 2A). mAb treatment in these mice did not induce (in anti-CD40-mAb-treated mice), or did
so only weakly (in anti–4-1BB mAb-treated mice), the upregulation of CD44 and CD69 or the downregulation of CD62L on CD8+ cells (Fig. 2C). Likewise, whereas a single dose of NRP-V7–pulsed DCs upregulated CD44 in a significant fraction of CD8+ cells (Fig. 2D) and rapidly induced diabetes in 17.4a/8.3β-NOD mice (Fig. 2A), NRP-V7–pulsed DCs could neither upregulate CD44 on CD8+ cells (Fig. 2E) nor trigger disease in any 17.6a/8.3β-NOD mice (Fig. 2A), even after a second dose.

Taken together, these data indicate that the suppressive activity of the CD44hiCD122+CD8+ cells of 17.6a/8.3β-NOD mice cannot be overcome by strategies that mimic (hence bypass the need for) CD4+ T cell help, such as agonistic anti-CD40 mAb treatment and NRP-V7–DC transfer. The inability of anti–4-1BB mAb to activate CD8+ cells or trigger T1D in 17.6a/8.3β-NOD mice further indicates that these mice do not harbor preactivated diabetogenic CD8+ cells, suggesting that the memory-like

**FIGURE 1.** Low-avidity IGRP206–214-reactive CD8+ cells spontaneously differentiate into memory-like autoregulatory cells. A, Representative FACS profiles of thymic and splenic T cells of non-TG NOD, 17.6a/8.3β-NOD, and 17.4a/8.3β-NOD mice. Data (mean ± SEM) correspond to 5–13 TCR-TG mice (7–14 wk old) (p values: 1 versus 2, 0.0210; 1 versus 3, 0.0159; 2 versus 3, 0.0300; 4 versus 5, 0.0084; 4 versus 6, 0.0035; 5 versus 6, 0.0021). B, Representative CD44 and CD122 staining profiles of CD8+ cells developing in 17.6a/8.3β- and 17.4a/8.3β-NOD mice (mean ± SEM). Sample sizes for 17.6a/8.3β-NOD: spleen, n = 11; PLN, n = 8. Sample sizes for 17.4a/8.3β-NOD: spleen, n = 6; PLN, n = 4, p values: 1 versus 2, 0.0005; 3 versus 4, 0.0081. Mice were 8–14 wk old. C, Representative CD122 staining (left panel) and average percentage of CD122+ cells (right panel) in thymic CD8+CD4+ and splenic CD8+ T cell populations from 17.6a/8.3β-NOD mice (n = 4). D, Proliferation of CFSE-labeled 17.4a/8.3β-CD8+ cells in response to NRP-A7–pulsed BM-DCs in the presence of naive CD44loCD122– or memory-like CD44hiCD122+CD8+ cells from 17.6a/8.3β-NOD mice. Right panel represents percent suppression of proliferation averaged over four experiments.

The Journal of Immunology
FIGURE 2. 17.6a/8.3β-NOD mice are resistant to strategies that bypass the need for CD4+ T cell help or potentiate effector CTL responses. A, 17.4a/8.3β- and 17.6a/8.3β-NOD mice were treated with anti-CD40 mAb (n = 7 and 5, respectively), anti–4-1BB mAb (n = 7 and 6, respectively), or LPS-activated NRP-V7–pulsed DCs (n = 4 and 3, respectively). As controls, 17.4a/8.3β- and 17.6a/8.3β-NOD mice received rat-IgG (n = 6) or were left untreated (n = 9), respectively, or were treated with LPS-activated TUM-pulsed DCs (n = 6 and 4, respectively). All mice were followed for at least 40 d for development of diabetes. Representative CD44, CD69, and CD62L stainings of splenic, mesenteric lymph node (MLN), or PLN CD8+ T cells in 17.4a/8.3β-NOD (B, D) and 17.6a/8.3β-NOD (C, E) mice that received anti-CD40 mAb, anti–4-1BB mAb, or NRP-V7–pulsed DCs compared with the same staining in mice that received rat IgG or TUM-pulsed DCs.
FIGURE 3. CD4+ T cell help is required for development and/or maintenance of functional memory-like autoregulatory CD8+ cells. A and B, Representative FACS profiles of splenic T cells of 17.6a/8.3β- and 17.4a/8.3β-NOD.Rag2^-/- or NOD.Tcra^-/- mice. Data (mean ± SEM) correspond to five to nine mice per strain (7–14 wk old) (p values: 1 versus 3, 0.0022; 2 versus 6, 0.0002; 4 versus 8, 0.0022). C and D, Disease survival curves of 17.6a/8.3β-NOD.Rag2^-/- (n = 16) versus 17.4a/8.3β-NOD.Rag2^-/- (n = 106) and 17.6a/8.3β-NOD.Tcra^-/- (n = 14) versus 17.4a/8.3β-NOD.Tcra^-/- (n = 28). E, Representative CD44 and CD122 profiles of splenic and PLN CD8+ cells developing in 17.6a/8.3β-NOD.Tcra^-/- and 17.6a/8.3β-NOD.Rag2^-/- mice. Percentages and absolute numbers of splenic (F) and PLN (G) CD44hi CD122+ CD8+ cells in 17.6a/8.3β-NOD.Tcra^-/- and 17.6a/8.3β-NOD.Rag2^-/- mice. Sample sizes for left panel in F: n = 13 for NOD.Tcra^-/- and n = 8 for NOD.Rag2^-/- TCR-TG mice. Sample sizes for right panel in F: n = 7 for NOD.Tcra^-/- and n = 6 for NOD.Rag2^-/- TCR-TG mice. Sample sizes for left panel in G: n = 9 for NOD.Tcra^-/- and n = 7 for NOD.Rag2^-/- TCR-TG mice. Sample sizes for right panel in G: n = 3 for NOD.Tcra^-/- and n = 6 for NOD.Rag2^-/- TCR-TG mice. H, Left panel, Proliferation of CFSE-labeled 17.4a/8.3β-CD8+ cells to NRP-A7+ pulsed DCs in the presence of naive CD44loCD122+ or memory-like CD44hiCD122+ T cells.
autoregulatory CD8+ cells afford these mice a profound state of autoregulation.

In the absence of CD4+ T cells or B cells, naive low-avidity 17.6a/8.3β-CD8+ cells spontaneously differentiate into diabetogenic effectors

The above observations prompted us to consider the possibility that differentiation of naive 17.6a/8.3β-CD8+ cells into memory-like autoregulatory T cells might be a CD4+ Th cell-dependent/potentiated process and thus enhanced (rather than overcome) by the various Th-like stimuli employed above. This seemed a reasonable interpretation of the data given that CD4+ Th cells have been shown to play important roles both in the primary activation and differentiation of naive T cells into CTL and in the generation of functional CD8+ T cell memory against viral (8, 10), bacterial (9) and self-Ags (11).

We thus sought to investigate if development of the memory-like autoregulatory CD8+ cells of 17.6a/8.3β-NOD mice was a CD4+ T cell-dependent process. To this end, we followed the fate of the 17.6a/8.3β and 17.4a/8.3β TCR transgenes in TCR-TG NOD.Rag2−/− mice, which cannot express endogenous TCR or Ig rearrangements and therefore harbor monospecific CD8+ T cell repertoires devoid of CD8+ T cells and B cells. As expected, TCR expression of the low-affinity 17.6a/8.3β TCR led to positive selection of CD8+ cells, although to a lesser extent than its high-affinity 17.4a/8.3β TCR counterpart (Fig. 3A). Nearly all thymic and peripheral CD8+CD4− cells in both types of mice expressed the TCRβ-chain and bound NRP-V7/Kd tetramers (Fig. 3A and data not shown). Furthermore, both strains lacked CD4+CD8− thymocytes as well as CD8+ T cells and B cells in their peripheral lymphoid organs (Fig. 3A and data not shown). We compared the development of memory-like autoregulatory CD8+ cells in Rag2−/− TCR-TG mice because Tcrα−/− TCR-TG mice have a more restricted TCRαβ repertoire than their Tcrα-compotent counterparts, and therefore (as is also the case in Rag2−/− TCR-TG mice), most of their peripheral CD8+ cells bind NRP-V7/Kd tetramers (data not shown). In addition, and despite their inability to express endogenous TCRα-chains, Tcrα−/− 17.6a/8.3β- and 17.4a/8.3β-TCR-TG mice support the development of CD4+ cells expressing endogenous TCRs (Fig. 3B).

Unlike 17.6a/8.3β-NOD.Tcrα−/− mice, 17.6a/8.3β-NOD.Rag2−/− mice developed diabetes spontaneously (Fig. 3C, 3D). In fact, the incidence and average age at onset of disease in 17.6a/8.3β-NOD.Rag2−/− mice were indistinguishable from those seen in 17.4a/8.3β-NOD.Rag2−/− mice (Fig. 3C). Remarkably, the peripheral lymphoid organs of 17.6a/8.3β-NOD.Rag2−/− mice contained significantly reduced percentages and absolute numbers of memory-like autoregulatory CD8+ cells (CD8+CD44hiCD122+; Fig. 3E-G) as compared with their 17.6a/8.3β-NOD.Tcrα−/− counterparts. Furthermore, whereas the CD8+CD44hiCD122+ cells of 17.6a/8.3β-NOD.Tcrα−/− mice could efficiently suppress the proliferation of 17.4a/8.3β-CD8+ cells in response to peptide-pulsed DCs in vitro, the few CD8+CD44hiCD122+ cells arising in 17.6a/8.3β-NOD. Rag2−/− mice lacked such suppressive activity (Fig. 3H).

To confirm that the naive CD8+ cells of 17.6a/8.3β-NOD.Tcrα−/− mice had intrinsic diabeticogenic potential (rather than being irreversibly silenced), we compared the ability of CD8+CD122+ and CD8+CD122− cells sorted from 17.6a/8.3β-NOD.Tcrα−/− mice to transfer diabetes into NOD.scid recipients after in vitro activation with NRP-A7-pulsed DCs. In agreement with the ability of the CD8+CD122+ cells of 17.6a/8.3β-NOD mice to suppress diabetes transfer by splenocytes from prediabetic NOD donors into NOD.scid hosts (19), all NOD.scid mice that were transfused with in vitro-activated CD8+CD122+ cells remained diabetes free for at least 30 d after transfer. In contrast, all NOD.scid mice that received in vitro-activated CD8+CD122− cells from the same donors developed diabetes shortly after transfer (Fig. 3I).

Thus, in the presence (but not absence) of B cells and/or endogenous CD4+ T cells, naive 17.6a/8.3β-CD8+ cells differentiate into memory-like antidiabeticogenic autoregulatory T cells rather than diabeticogenic effectors. It should be noted that the diabetes resistance of 17.6a/8.3β-NOD.Tcrα−/− mice (versus 17.6a/8.3β-NOD.Rag2−/− mice) cannot be attributed to the presence of Foxp3+CD4+ T cells because, as shown previously (19), only CD8+ but not CD4+ T cells from 17.6a/8.3β-NOD.Tcrα−/− mice could inhibit the transfer of diabetes by NOD T cells into NOD.scid mice.

CD4+ T cell-assisted differentiation of high- versus low-avidity autoreactive CD8+ cells into diabetogenic effectors and antidiabeticogenic suppressors, respectively

Collectively, and in the context of our previous observations (14), the above experiments revealed that abrogation of CD4+ T cell (and B cell) development has opposite effects in 17.4a/8.3β-NOD.Rag2−/− versus 17.6a/8.3β-NOD.Rag2−/− mice: it decelerates disease in the former but fosters it in the latter. This prompted us to consider the possibility that CD4+ Th cells can actively promote both the differentiation of high-avidity autoreactive CD8+ cells into diabetogenic effectors and the differentiation of their low-avidity counterparts into memory-like anti-diabeticogenic suppressors.

To test this hypothesis, we compared the effects of purified polyclonal CD4+ cells from prediabetic NOD mice on the natural history of diabetes in 17.4a/8.3β-NOD.Rag2−/− and 17.6a/8.3β-NOD.Rag2−/− mice. Whereas a single transfusion of CD4+ cells promoted diabetes development in 17.4a/8.3β-NOD.Rag2−/− mice (Fig. 4A), it decelerated its progression in 17.6a/8.3β-NOD. Rag2−/− mice (Fig. 4B). In contrast, adoptive transfer of purified B cells from prediabetic NOD mice into 17.6a/8.3β-NOD.Rag2−/− mice had no significant effects on the natural history of disease in these mice (Fig. 4C). Notably, the CD4+ T cell-induced delay in diabetes development in 17.6a/8.3β-NOD.Rag2−/− mice was associated with increased frequency of CD8+CD44hiCD122+ cells in peripheral blood (Fig. 4D). Thus, CD4+ Th cells can simultaneously support the differentiation of high-avidity autoreactive CD8+ cells into diabetogenic effectors and the differentiation of their low-avidity counterparts into memory autoregulatory T cells.

Discussion

During the progression of T1D, low-avidity autoreactive CD8+ T cell clones are progressively replaced by their less prevalent but immunologically fit high-avidity counterparts (16). Rather than playing a passive role in the disease process, low-avidity autoreactive CD8+ cells function as a source of autoantigen-specific negative-feedback regulatory loops that aim to counter disease progression. They do so by differentiating into subsets of memory-like autoregulatory CD8+ T cells that acquire the ability to kill and suppress autoantigen-loaded APCs in the pancreas-draining lymphoid organs of 17.6a/8.3β-NOD.Tcrα−/− mice. Right panel, Percent suppression of proliferation averaged over 24 experiments using 17.6a/8.3β-NOD.Tcrα−/− CD8+ cells or five experiments using 17.6a/8.3β-NOD.Rag2−/− CD8+ cells. I, Survival curves of NOD.scid mice transferred with in vitro-activated CD44hiCD122+ (n = 4) and CD44hiCD122− (n = 4) CD8+ cells from 17.6a/8.3β-NOD.Tcrα−/− mice.
nodes, thus blunting the activation of both cognate and noncognate pathogenic effectors simultaneously (19). In this study, we have shown that genesis of these antidiabetic memory-like autoregulatory CD8+ cells from naive low-avidity precursors in vivo is a CD4+ Th-dependent process. Because differentiation of naive high-avidity autoreactive CD8+ cells into diabetogenic effectors is also a CD4+ Th cell-dependent process, we conclude that CD4+ Th cells contribute to both promoting and extinguishing diabetogenic autoimmunity.

The general requirement for CD4+ T cell help in the priming of CD8+ T cell responses against foreign Ags and autoantigens is well documented (23). For example, Rag2−/− RAG2−/− CD8+ T cells from prediabetic NOD mice were treated with either untreated (n = 98) or received CD4+ T cells from prediabetic NOD mice (n = 7). Survival curves of 17.6a/8.3β-NOD.Rag2−/− mice that were either untreated (n = 16) or received CD4+ T cells (B; n = 8) or B cells (C; n = 5) from prediabetic NOD mice. D. Fold increase in memory-like autoregulatory CD8+ cells in the peripheral blood of 17.6a/8.3β-NOD.Rag2−/− mice that were untreated (n = 5) or received CD4+ T cells (n = 7) or B cells (n = 4) from prediabetic NOD mice. The p values were obtained using two-way ANOVA test comparing the differences between the percentages of memory-like autoregulatory CD8+ cells from 17.6a/8.3β-NOD.Rag2−/− mice that were untreated versus the mice that received CD4+ T cells or B cells at both 8- and 18-d time points.

FIGURE 4. CD4+ cells promote the differentiation of high- and low-avidity autoreactive CD8+ cells into diabetogenic effectors and anti-diabetogenic suppressors, respectively. A. Disease survival curves of 17.4o/8.3β-NOD.Rag2−/− mice that were either untreated (n = 98) or received CD4+ T cells from prediabetic NOD mice (n = 7). Survival curves of 17.6a/8.3β-NOD.Rag2−/− mice that were either untreated (n = 16) or received CD4+ T cells (B; n = 8) or B cells (C; n = 5) from prediabetic NOD mice. D. Fold increase in memory-like autoregulatory CD8+ cells in the peripheral blood of 17.6a/8.3β-NOD.Rag2−/− mice that were untreated (n = 5) or received CD4+ T cells (n = 7) or B cells (n = 4) from prediabetic NOD mice. The p values were obtained using two-way ANOVA test comparing the differences between the percentages of memory-like autoregulatory CD8+ cells from 17.6a/8.3β-NOD.Rag2−/− mice that were untreated versus the mice that received CD4+ T cells or B cells at both 8- and 18-d time points.
individual autoantigenic specificities in non-TG mice are very small in size and phenotypically heterogeneous (containing naive, effector, and autoregulatory T cells over a spectrum of avidities). Furthermore, because NOD CD8+ T cells cannot cause β cell loss (hence autoantigen shedding) in the absence of CD4+ T cells, naive low-avidity CD8+ T cells would not be able to differentiate into memory-like autoregulatory cells in a CD4+ T cell-deficient environment, regardless of the role of CD4+ T cell help in autoregulatory CD8+ T cell development. Because of these limitations, studies on the role of CD4+ Th cells in the homeostasis of autoregulatory CD8+ T cells in non-TCR-TG systems are not currently feasible.

In sum, this study establishes a previously unrecognized, ambiguous role for CD4+ T cell help in chronic autoimmune responses: it fosters the diabetogenic potential of high-avidity autoreactive CD8+ cells while boosting the antidiabetogenic properties of their low-avidity counterparts by promoting the generation and/or survival and suppressive function of memory-like autoregulatory CD8+ cells. This discovery challenges the generally held view that CD4+ T cell help invariably promotes effector T cell responses.

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