

## CD8<sup>+</sup> T cells in Autoimmunity

S. Tsai, A. Shameli, P. Santamaria

*Julia McFarlane Diabetes Research Centre (JMDRC), Department of Microbiology and Infectious Diseases, and Institute of Infection, Inflammation and Immunity, Faculty of Medicine, The University of Calgary, Calgary, Alberta, Canada.*

### **LAS CÉLULAS T CD8<sup>+</sup> EN LA AUTOINMUNIDAD**

*Recibido: 17 Enero 2008*

*Aceptado: 8 Febrero 2008*

#### RESUMEN

Las células T autoreactivas CD8<sup>+</sup> se están empezando a reconocer como contribuidores importantes en distintos modelos de autoinmunidad; el papel que estas células juegan en enfermedades autoinmunes humanas tan sólo se está empezando a comprender. Estas células pueden actuar bien como ejecutoras de daño tisular, bien como reguladoras y, por lo tanto, ejercer un efecto anti-patogénico. Recientes avances en este campo incluyen una mejor comprensión de los mecanismos que controlan su activación, de sus mecanismos de acción, de su regulación negativa, y de sus especificidades antigénicas. Una contribución de especial importancia es el desarrollo de métodos analíticos que permiten la detección de estas especificidades antigénicas en pacientes humanos e individuos con riesgo genético a padecer enfermedades autoinmunes. Colectivamente, estos avances ofrecen esperanzas de cara al desarrollo de nuevas modalidades terapéuticas capaces de atacar selectivamente el compartimento celular CD8<sup>+</sup> en los procesos autoinmunes.

**PALABRAS CLAVE:** Autoinmunidad / Linfocitos T CD8<sup>+</sup> / Linfocitos T CD8<sup>+</sup> supresores.

#### ABSTRACT

Autoreactive CD8<sup>+</sup> T cells are emerging as important players in several animal models of autoimmunity; their roles in the human autoimmune diseases are only beginning to be understood. Autoreactive CD8<sup>+</sup> T cells can act as pathogenic effector cells and mediate tissue damage, while other autoreactive CD8<sup>+</sup> T cells may have regulatory properties and serve to protect self against autoimmune attacks. Recent advances made in the field include a better understanding of their activation, mechanism of action, negative regulation, and antigenic specificities. Importantly, advances have been made in the development of strategies that detect these antigenic specificities in human patients and at risk individuals. Together, these findings foster hope for the development of novel antigen-specific therapeutic strategies that target the CD8<sup>+</sup> T cell compartment of autoimmune processes.

**KEY WORDS:** Autoimmunity / CD8<sup>+</sup> T-lymphocytes / CD8<sup>+</sup> suppressor T-lymphocytes.

## INTRODUCTION

The design of the immune system incorporates a series of checkpoints, both centrally and in the periphery, that aim to preclude self-reactivity. In the thymus, positively selected thymocytes whose T cell receptors (TCRs) recognize self peptide/Major Histocompatibility Complexes (MHCs) with high avidity are deleted<sup>(1)</sup>. However, thymocytes expressing lower-affinity TCRs or TCRs whose cognate antigens are not presented in the thymus escape central tolerance and populate the peripheral lymphoid organs<sup>(2)</sup>. There, these autoreactive T cells will have to overcome additional checkpoints, such as anergy, activation-induced cell death, and regulatory T cell (Treg)-mediated dominant inhibition, to be able to cause tissue damage<sup>(3, 4)</sup>. In addition, there are built-in mechanisms within the T cells themselves that negatively regulate T cell activation. These are achieved through receptor-ligand interactions between T cells and professional antigen presenting cells (APCs) or antigen-expressing non-hematopoietic cell types. In autoimmunity, these checkpoints are inadequate or defective.

In the case of T-cell mediated autoimmune disorders, such as Multiple Sclerosis (MS) and Type 1 Diabetes (T1D), CD4<sup>+</sup> T cells were traditionally thought to be the key effectors of tissue damage. This was due, in part, to the fact that disease susceptibility and/or resistance are strongly associated with certain MHC class II alleles<sup>(5-7)</sup>. More recently, it has become evident that cytotoxic CD8<sup>+</sup> T cells also play major roles as effectors of autoimmunity. This is not surprising because, unlike most CD4<sup>+</sup> T cell specificities, CD8<sup>+</sup> T cells can directly recognize and kill antigen-expressing cell types. Activated CD8<sup>+</sup> T cells can kill target cells via the Fas/FasL pathway, or by releasing cytolytic granules at the effector/target cell junction. Activated cytotoxic T lymphocytes (CTLs) can also cause tissue damage by secreting high levels of pro-inflammatory cytokines, such as TNF $\alpha$  and IFN $\gamma$ .

CD8<sup>+</sup> T cells have been shown to contribute to the pathogenesis of several animal models of autoimmunity, notably type 1 diabetes (T1D) in the nonobese diabetic (NOD) mouse, and experimental autoimmune encephalomyelitis (EAE), a model of demyelinating central nervous system (CNS) diseases. Interestingly, and in addition to their role as pathogenic effectors, subsets of CD8<sup>+</sup> T cells have also been identified as negative regulators of autoimmune responses in several models. Here, we discuss these recent findings on the roles of autoreactive CD8<sup>+</sup> T cells in autoimmunity, with a focus on these two types of organ-specific autoimmune disorders.

## PATHOGENIC ROLE OF AUTOREACTIVE CD8<sup>+</sup> T CELLS

**CD8<sup>+</sup> T cells in T1D.** T1D is a prototypic T cell-dependent autoimmune disease characterized by a CD4<sup>+</sup> and CD8<sup>+</sup> T cell-dependent autoimmune process that specifically targets the pancreatic beta cell. Epidemiological studies have shown that inheritance of certain human MHC class I alleles, such as HLA-A\*0201, increases the genetic susceptibility for T1D when expressed in the context of certain MHC class II alleles<sup>(7-10)</sup>. Importantly, significant CD8<sup>+</sup> T cell infiltration was observed within the pancreas of recently-diagnosed diabetic patients, as well as in diabetic patients transplanted with pancreatic grafts from healthy monozygotic twins or HLA-identical siblings<sup>(11-13)</sup>. There is also extensive evidence from studies using murine models of T1D that CD8<sup>+</sup> T cells play crucial roles in the pathogenesis of T1D<sup>(14,15)</sup>. NOD mice lacking the  $\beta$ 2-microglobulin or the CD8 $\alpha$  genes are T1D-resistant<sup>(16-21)</sup>. Furthermore, transfer of diabetes from young female NOD mice into NOD.scid recipients using splenic lymphocyte subsets is most efficient when CD4<sup>+</sup> and CD8<sup>+</sup> T cells are transferred together<sup>(22)</sup>. Studies of beta cell-specific TCR-transgenic mice have demonstrated that autoreactive CD8<sup>+</sup> T cells have pathogenic activity.

In NOD mice, CD8<sup>+</sup> T cells are found in the earliest lymphocyte infiltrates of pancreatic islets. Wong et al. reported the presence of a population of CD8<sup>+</sup> T cells recognizing a insulin-derived epitope in the islets of 3-4 week-old NOD mice<sup>(23)</sup>. These cells wane with age and are progressively replaced by another population of CD8<sup>+</sup> T cells that recognize an epitope consisting of residues 206-214 of the beta cell antigen islet-associated glucose-6-phosphatase catalytic subunit-related protein (IGRP<sub>206-214</sub>)<sup>(24-26)</sup>. IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T cells are highly diabetogenic<sup>(27)</sup>, are prevalent in the islets and circulation of pre-diabetic NOD mice and undergo cyclic expansion and contraction prior to the onset of overt diabetes<sup>(28)</sup>. Another unique feature of the IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T cell subset is that it undergoes a process of avidity maturation during disease progression, whereby low-avidity clonotypes are progressively replaced by their higher-avidity, more pathogenic counterparts<sup>(29)</sup>. This process is developmentally controlled<sup>(30)</sup>, such that during early stages of the disease, the high-avidity clonotypes (unlike their lower avidity counterparts) are kept in check by both central and peripheral tolerance. At the level of the target organ, on the other hand, pancreatic islet inflammation shelters these clonotypes from peripheral tolerance and fuels their local expansion, allowing them to undertake the diabetogenic process.

Our recent data suggest that low-avidity autoreactive CD8<sup>+</sup> T cells are not pathogenic, and that they may actually possess immune regulatory properties. We have shown that

selective deletion of high-avidity IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T cells through systemic administration of IGRP<sub>206-214</sub> mimotopes frees up a “niche” within pancreatic islets that fosters the recruitment, expansion and local retention of protective (anti-diabetogenic) low-avidity CD8<sup>+</sup> T cells<sup>(31)</sup>. Notably, deletion of both high and low-avidity IGRP<sub>206-214</sub>-reactive clonotypes enhanced the recruitment of subdominant IGRP epitope specificities to islets and did not hinder diabetes development. The hypothetical anti-diabetogenic properties of these low-avidity CD8<sup>+</sup> T cells have been documented in a strain of NOD mice that expresses a transgenic low-affinity IGRP<sub>206-214</sub>-reactive TCR (also referred to as 17.6-NOD mice). These mice, which do not develop diabetes, export a population of non-pathogenic, low-avidity autoreactive CD8<sup>+</sup> T cells (expressing low levels of the low affinity transgenic TCR along with endogenous TCR chains) that suppresses the pathogenic activity of CD8<sup>+</sup> T cells expressing high levels of the transgenic TCR (lacking expression of endogenous TCR chains). The latter engage target peptide-MHC complexes with high avidity and possess pathogenic activity. In fact, selective removal of the low-avidity, non-pathogenic CD8<sup>+</sup> T cells of these mice by introduction of a *rag2* gene deficiency (which does not impair the development of the pathogenic high-avidity subset) resulted in the loss of protection from diabetes (our unpublished observations).

**Antigenic epitopes recognized by diabetogenic CD8<sup>+</sup> T cells.** Identification of auto-reactive T cell clones present in the peripheral blood of diabetic patients provides important information that may aid in the monitoring of disease progression or clinical assessment of the efficacy of therapeutic interventions aimed at curbing autoimmunity. Recent years have seen advances in this area. Using an ELISPOT approach, peripheral blood mononuclear cells (PBMCs) are isolated and tested for reactivity against a panel of peptides. Candidate peptides can be selected based on their predicted or characterized binding to specific HLA class I molecules<sup>(32, 33)</sup> or from products of natural antigen processing via proteasome digestion<sup>(34)</sup>. Using this approach, researchers have identified several diabetes-associated antigenic epitopes, including peptides derived from prepro-islet amyloid polypeptide (ppIAPP), IGRP and glial fibrillary acidic protein (GFAP)<sup>(32)</sup>, as well as several epitopes in the pro-insulin polypeptide<sup>(33-35)</sup>.

A number of recent reports described the identification of HLA-A\*0201-restricted CD8<sup>+</sup> T cell epitopes for T1D using “humanized” HLA-transgenic mice. These mice, designated NOD.β2m<sup>null</sup>.HHD, lack murine MHC class I molecules but express a chimeric MHC class I molecule that carries the murine H-2D<sup>b</sup> cytoplasmic, transmembrane and

α3 domains, and the HLA-A\*0201 α1 and α2 (peptide-binding) domains covalently linked to the human β2m chain<sup>(36)</sup>. Since these mice lack murine MHC class I molecules, they can only export HLA-A2-restricted CD8<sup>+</sup> T cells into the peripheral immune system. As a result, islet-derived CD8<sup>+</sup> T cells from NOD.β2m<sup>null</sup>.HHD mice can be used as probes to identify potential HLA-A\*0201-restricted antigenic epitopes<sup>(36)</sup>. Using this approach, CD8<sup>+</sup> T cell responses to three HLA-A2-restricted, murine IGRP-derived peptides, IGRP<sub>228-236</sub>, IGRP<sub>265-273</sub>, and IGRP<sub>337-345</sub> were described<sup>(37)</sup>. Of these, the IGRP<sub>265-273</sub> epitope was found to be shared by human and mouse IGRP, and human IGRP<sub>228-236</sub> differed from the murine sequence at two amino acid residues<sup>(37)</sup>. Another group of investigators immunized NOD.β2m<sup>null</sup>.HHD mice with antigen-encoding plasmid vectors and screened the mice for vaccination-elicited CD8<sup>+</sup> T cell responses. This approach led to the identification of several candidate epitopes within the 65 KD glutamic acid decarboxylase (GAD65) autoantigen, including GAD<sub>114-123</sub> and GAD<sub>536-545</sub>, as well as an epitope in the insulinoma-associated protein (IA-2), IA-2<sub>805-813</sub>, all of which were validated in humans, using PBMCs from newly-diagnosed diabetic patients<sup>(38)</sup>.

**CD8<sup>+</sup> T cells in MS.** Multiple sclerosis is a chronic inflammatory demyelinating disease of the central nervous system (CNS) characterized by lesions infiltrated by CD4<sup>+</sup> and CD8<sup>+</sup> T cells. EAE has been extensively used as an animal model of MS. EAE is induced by active immunization of genetically susceptible strains with defined components of the myelin sheath, together with complete Freund’s adjuvant. Animals induced to develop EAE display demyelinated, lymphocyte-infiltrated CNS lesions and progressive ascending paralysis.

The role of CD4<sup>+</sup> T cells in EAE has been studied extensively. They were thought to have a Th1 phenotype, until recent data suggested that they produce IL-17 and represent a novel Th subset (Th17)<sup>(39)</sup>. Because myelin-reactive CD4<sup>+</sup> T cells can transfer disease, EAE (and by extension, MS) was widely considered to be a CD4<sup>+</sup> T cell-mediated autoimmune disease. A major role for CD4<sup>+</sup> T cells in MS is consistent with the fact that HLA-DQ and HLA-DR polymorphisms, especially at the DRB1 locus, are strongly associated with disease susceptibility and resistance<sup>(40)</sup>. However, increasing evidence supports a pathogenic role for CD8<sup>+</sup> T cells in both EAE and MS. Transgenic expression of CD86 on microglial cells can induce a spontaneous, T cell-mediated demyelinating disease in the absence of CD4<sup>+</sup> T cells<sup>(41)</sup>. Furthermore, CD8<sup>+</sup> T cells specific for components of myelin, including myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) can transfer EAE to healthy recipients<sup>(42-44)</sup>.

MS is clearly associated with MHC class I gene polymorphisms. The HLA-A\*0301 allele, for example, appears to increase the risk of MS independently of DRB1, while certain HLA-A and HLA-C alleles are negatively associated with MS, suggesting a protective effect<sup>(45, 46)</sup>. There is also clinical evidence to support the contention that the pathological features of MS are not exclusively mediated by CD4<sup>+</sup> T cells. For example, treatment of MS patients with CD4<sup>+</sup> T cell-depleting monoclonal antibodies did not ameliorate disease<sup>(47)</sup>. Furthermore, analyses of the mononuclear cell infiltrates in CNS lesions of MS patients have documented an enrichment for CD8<sup>+</sup> T cells<sup>(48-51)</sup>. There is some evidence suggesting that these CD8<sup>+</sup> T cells undergo clonal expansion *in situ*<sup>(51, 52)</sup>, that they interact directly with demyelinated axons, and that they are capable of lysing neurons, at least *in vitro*, in a MHC class I-restricted manner<sup>(53, 54)</sup>. In addition, it has been shown that CD8<sup>+</sup> T cells specific for different myelin-derived antigenic epitopes are present in both healthy individuals and in MS patients<sup>(54-56)</sup>. Although the use of HLA-A\*0201-transgenic mice<sup>(57)</sup> has enabled the characterization of some of these CD8<sup>+</sup> T cell specificities<sup>(58)</sup>, the mechanisms through which encephalitogenic CD8<sup>+</sup> T cells are activated and contribute to disease progression in MS and EAE remain largely unknown.

### ACTIVATION OF CD8<sup>+</sup> T CELLS

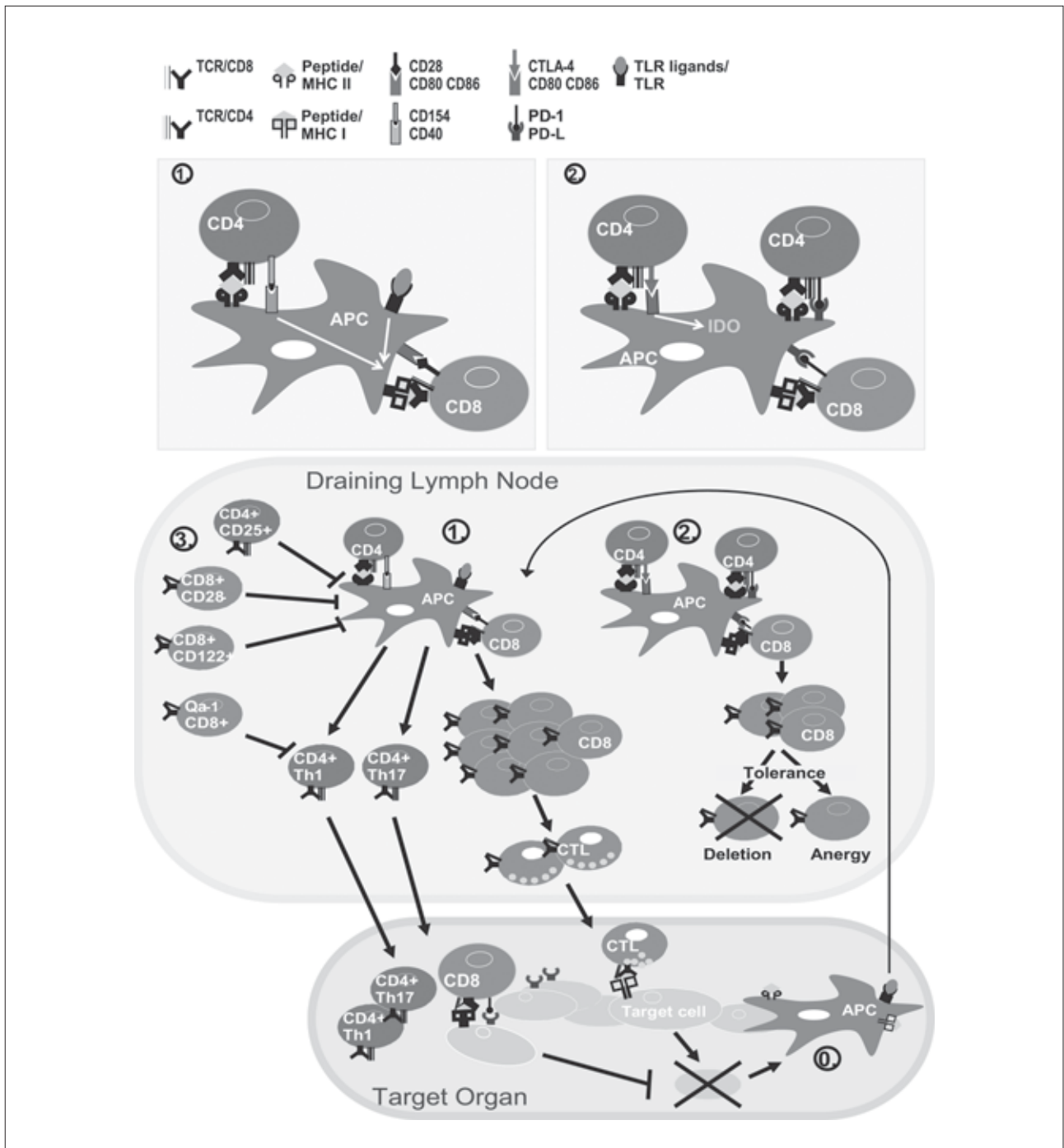
The activation of autoreactive CD8<sup>+</sup> T cells requires recognition of cognate antigenic epitopes presented by MHC class I molecules on professional APCs. Because most target cells in organ-specific autoimmune diseases lack professional APC properties (i.e. they do not express essential immune co-stimulatory molecules), the target tissue/organ alone is unlikely to trigger an autoimmune response all by itself. A recent study using transgenic NOD mice in which most APCs were MHC class I-deficient showed that beta cells cannot directly prime CTLs in the absence of MHC class I-expressing APCs<sup>(59)</sup>. For T1D, this initial priming event may occur in the pancreatic lymph nodes (PLN), because surgical removal of the PLNs in three-week-old NOD mice protected them from the development of insulinitis and diabetes<sup>(60)</sup>. Also, upon adoptive transfer into NOD hosts, naïve CD8<sup>+</sup> T cells specific for IGRP<sub>206-214</sub> proliferate specifically in the PLNs, demonstrating that the PLNs harbour autoantigen-loaded APC types that are capable of inducing the activation of autoreactive CD8<sup>+</sup> T cells<sup>(64)</sup>.

The mechanisms through which beta cell autoantigens access APCs in the regional lymph nodes remain unclear. Abrogation of MHC class I expression on beta cells of NOD mice *in vivo* does not impair the activation of diabetogenic

CD8<sup>+</sup> T cells in the PLNs (an event referred to as “cross-priming”), suggesting that beta cell autoantigens access the cross-presentation pathway in a T cell-independent manner (that is, without the need of a preceding CD8<sup>+</sup> T cell attack on beta cells)<sup>(62)</sup>. It has been proposed that antigen shedding results from a wave of beta cell apoptosis, peaking at 2 weeks of age, that is associated with neonatal remodelling of pancreatic islets<sup>(63)</sup>. This physiologic wave of beta cell apoptosis may result in shedding of beta cell antigens followed by capture and “cross-presentation” by professional APCs<sup>(61, 63)</sup>. In support of this model, it has been shown that dendritic cells (DCs) capture apoptotic beta cells<sup>(61)</sup> and ferry apoptotic beta cell debris to the PLNs<sup>(64)</sup>. Furthermore, induction of beta cell apoptosis with low doses of streptozotocin in two-week old NOD mice enhanced the priming of adoptively transferred IGRP<sub>206-214</sub>-reactive CD8<sup>+</sup> T cells<sup>(64)</sup>.

Multiple factors affect the efficiency of cross-presentation and activation of autoreactive CD8<sup>+</sup> T cells, such as, for example, the antigen dose, the presence of immune-complex forming antibodies, or the frequency of effector cells being presented to<sup>(65)</sup>. A recent study using the RIP-mOVA model of autoimmune diabetes showed that anti-OVA IgG antibodies enhanced OVA-specific CD8<sup>+</sup> T cell priming through the formation of Fcγ receptor-binding immune complexes<sup>(66)</sup>. Target cell death itself fosters cross-presentation. One of the consequences of cell death is the release of so-called endogenous adjuvants, such as heat shock proteins and uric acid. These molecules promote DC activation and thus can fuel the inflammatory process<sup>(67)</sup>. Indeed, the inflammatory milieu of the target tissue has a significant impact on the outcome of cross-presentation. For example, inflammation in the target organ, such as the pancreas, is associated with increased chemokine production and MHC class I expression, leading to enhanced T cell recruitment and accumulation<sup>(68)</sup>. Along these lines, it has been shown that activation of Toll-like receptor molecules can augment autoimmunity<sup>(69, 70)</sup>.

Another important factor to consider in the activation of autoreactive CD8<sup>+</sup> T cells is the requirement for CD4<sup>+</sup> T cell help. In the absence of CD4<sup>+</sup> T cells, naïve IGRP<sub>206-214</sub>-reactive T cells cannot efficiently accumulate in pancreatic islets despite undergoing activation in the PLNs. This was demonstrated in studies of mice expressing a transgenic IGRP<sub>206-214</sub>-reactive TCR in a *rag2*-deficient background. 8.3-NOD.*rag2*<sup>-/-</sup> mice, which export autoreactive CD8<sup>+</sup> T cells in a CD4<sup>+</sup> T cell-deficient environment, develop diabetes at a significantly slower rate and decreased frequency than their *rag2*-competent counterparts<sup>(27)</sup>. This observation is consistent with the finding that CD8<sup>+</sup> splenocytes from



**Figure 1.** Dual role of autoreactive CD8<sup>+</sup> T cells. (0.) Professional APCs ferry self antigens from the target organ to the regional lymph nodes, where they prime autoreactive CD4<sup>+</sup> and CD8<sup>+</sup> T cells. (1.) CD8<sup>+</sup> T cells are activated by engaging peptide/MHC class I via their TCRs and costimulatory molecules CD80/CD86 via CD28, a process that is enhanced by pro-inflammatory signals, such as those provided by CD4<sup>+</sup> T helper cells (CD40/CD154 interaction), and by occupation of the TLR with TLR ligands. These CD8<sup>+</sup> T cells undergo clonal expansion and differentiation into CTLs, and they mediate cell damage in the target organ through various means (cytolytic granules, Fas/FasL, secretion of IFN $\gamma$  and TNF $\alpha$ ) (2.) A different outcome of cross-priming is the induction of tolerance (also referred to as cross-tolerance). In the absence of co-stimulatory signals, naïve CD8<sup>+</sup> T cells become tolerized via apoptotic deletion or induction of anergy. Ligation of negative costimulatory molecules PD-1 and CTLA-4 by their respective ligands (PD-L and CD80, CD86) on the dendritic cells or target cells also abrogates T cell responses. In some cases, subsets of DCs are rendered tolerogenic through the up-regulation of IDO, an enzyme involved in tryptophan catabolism. (3.) Subsets of autoreactive CD8<sup>+</sup> T cells, in addition to the classical CD4<sup>+</sup>CD25<sup>+</sup> Tregs, are shown to down-regulate autoimmune responses, presumably by acting on APCs or directly on the pathogenic T cells.

diabetic donors cannot transfer diabetes in NOD.*scid* mice in the absence of CD4<sup>+</sup> T cell co-transfer<sup>(22)</sup>. However, not all autoreactive CD8<sup>+</sup> T cell specificities require CD4<sup>+</sup> T cell help to trigger diabetes<sup>(71)</sup>.

### CROSS-TOLERANCE AND AUTOREACTIVE CD8<sup>+</sup> T CELLS

Cross-presentation of self-antigens to CD8<sup>+</sup> T cells may lead to another outcome: T cell tolerance (also referred to as "cross-tolerance")<sup>(72)</sup>. Cross-tolerance may occur in the thymus, or in the periphery. Negative co-stimulatory molecules of the CD28 family have been shown to be critical for peripheral cross-tolerance<sup>(73)</sup>. One well-studied example is the CTL-associated antigen 4 (CTLA-4). Ligation of CTLA-4 on activated T cells by CD80 and CD86 on DCs serves to downregulate/terminate T cell responses<sup>(74)</sup>. Loss of CTLA-4 results in lymphoproliferative disease and multi-organ autoimmunity<sup>(75, 76)</sup>, and *in vivo* blockade of CTLA-4 augments autoimmune responses in EAE<sup>(77)</sup>. Programmed death-1 (PD-1) is another inhibitory receptor that is up-regulated on activated T cells, B cells, and myeloid cells. The interaction of PD-1 with its ligands, PD-L1 and PD-L2, on multiple cell types, including DCs, macrophages, and lymphocytes, and parenchymal cells such as pancreatic beta cells, results in a negative signal that aims to terminate T cell immune responses. Recent studies have shown that the interaction of PD-1 on CD8<sup>+</sup> T cells and PD-L1 on DCs and pancreatic beta cells is important for inducing and maintaining peripheral CD8<sup>+</sup> T cell tolerance to self antigens<sup>(78, 79)</sup>. Keir and coworkers found that loss of PD-1 lowers the threshold of activation of PD-1-deficient OT-1 CD8<sup>+</sup> T cells, allowing these T cells to efficiently transfer diabetes to RIP-OVA hosts (unlike their PD-1-wild type counterparts). It was also shown that abrogation of the PD-1/PD-L1 interaction fostered CD8<sup>+</sup> T cell-mediated autoimmunity, including type 1 diabetes in NOD mice<sup>(78, 80-84)</sup>. There is some evidence suggesting that whereas PD-L1 expression in PLN DCs negatively regulates the priming of diabetogenic T cells early on in the disease process, PD-L1 expression in islet cells inhibits islet destruction at a later phase in diabetes progression<sup>(85)</sup>. Of note, similar to CTLA-4, the PD1/PD-L1 pathway could potentially be exploited to treat autoimmune diseases. Fife and coworkers have shown that reversal of diabetes upon systemic administration of an anti-CD3 mAb or antigen-coupled APCs is a PD-1 and PD-L1-dependent phenomenon<sup>(86)</sup>. Furthermore, stimulation of PD-1 with a dimeric PD-L1 Ig fusion protein, in conjunction with CD154 co-stimulation blockade, prolonged the survival of islet allografts<sup>(87)</sup>.

### CD8<sup>+</sup> T CELLS AS NEGATIVE MODULATORS OF AUTOIMMUNE DISEASES

CD8<sup>+</sup> T cells with immune regulatory activities have also been identified. These cells, which naturally co-exist with their pathogenic counterparts, can be activated and/or expanded by specific immuno-therapeutic strategies.

**Qa-1 (HLA-E)-restricted CD8<sup>+</sup> T cells.** Qa-1 (the murine counterpart of HLA-E) is a non-classical MHC class Ib molecule that forms a heterodimer with  $\beta_2$ -microglobulin ( $\beta_2m$ ) and can present peptides from both self- and foreign antigens<sup>(88)</sup>. Qa-1-deficient mice develop exaggerated CD4<sup>+</sup> T cell responses upon viral infection and immunization with self peptides, and display increased susceptibility to EAE induction<sup>(89)</sup>. The regulatory role of the Qa-1/CD8 TCR interaction was first demonstrated in studies involving vaccination with autoantigen-reactive CD4<sup>+</sup>V $\beta$ 8.2<sup>+</sup> T cells (TCV), which protected mice from subsequent induction of EAE, through a Qa-1-restricted, CD8<sup>+</sup> T cell-dependent mechanism<sup>(90, 91)</sup>. It was found that TCV-induced CD8<sup>+</sup> T cells selectively killed activated CD4<sup>+</sup> T cells in a V $\beta$ -specific manner<sup>(92)</sup>. One way this could occur was through the recognition of a V $\beta$ 8.2-derived peptide presented in the context of Qa-1 molecules on the surface of activated CD4<sup>+</sup> T cells<sup>(93, 94)</sup>. Jiang and coworkers provided evidence that Qa-1-restricted CD8<sup>+</sup> T cells selectively delete the high-affinity CD4<sup>+</sup> T cell clonotypes, sparing their lower-affinity counterparts<sup>(95)</sup>. Of note, a similar population of CD8<sup>+</sup> regulatory T cells was documented in patients treated with glatiramer acetate (GA), an approved therapy for MS<sup>(96-98)</sup>. Untreated MS patients were found to have defective CD8<sup>+</sup> T cell-mediated immune suppression, as compared to healthy untreated controls<sup>(96)</sup>. Treatment with GA might induce HLA-E-dependent, GA-specific cytotoxic responses with suppressive properties.

**CD8<sup>+</sup> CD122<sup>+</sup> T cells** - Suzuki and co-workers observed that IL-2/IL-15 receptor  $\beta$ -chain (CD122)-deficient mice spontaneously accumulate activated T cells, resulting in the abnormal and exhaustive differentiation of B cells into antibody-secreting plasma cells<sup>(99)</sup>. This phenotype of abnormal T and B cell activation could be reversed by the transfer of purified CD8<sup>+</sup>CD122<sup>+</sup> T cells, or higher numbers of CD4<sup>+</sup>CD25<sup>+</sup> T cells<sup>(100)</sup>. In the same study, CD8<sup>+</sup>CD122<sup>+</sup> T cells were shown to play a crucial role in the regulation of CD8<sup>+</sup>CD122<sup>-</sup> T cells, in an APC-independent manner. A subsequent study found that CD8<sup>+</sup>CD122<sup>+</sup> T cells could suppress the proliferation of their CD122<sup>-</sup> counterparts by secreting IL-10 into the culture medium<sup>(101)</sup>. The protective role of CD8<sup>+</sup>CD122<sup>+</sup> T cells has been documented in other autoimmune disorders. In a model of Graves' disease, a B cell-mediated, T cell-dependent autoimmune disease of the thyroid gland, *in vivo* depletion of CD122<sup>+</sup> cells via the systemic administration of monoclonal anti-CD122 antibodies resulted in an elevated incidence of

hyperthyroidism<sup>(102)</sup>. A recent study using an EAE model showed that depletion of CD8<sup>+</sup>CD122<sup>+</sup> T cells resulted in an increase in CNS-infiltrating T cells and cytokine production, while transfer of CD8<sup>+</sup>CD122<sup>+</sup> regulatory T cells resulted in a decrease in disease symptoms, particularly in the recovery phase of EAE<sup>(103)</sup>. The mechanism(s) through which CD8<sup>+</sup>CD122<sup>+</sup> T cells regulate autoimmunity remain(s) to be determined.

**CD8<sup>+</sup>CD28<sup>-</sup> T cells.** CD8<sup>+</sup>CD28<sup>-</sup> T cells isolated from humans were shown to suppress alloreactive CD4<sup>+</sup> T cell responses *in vitro*<sup>(104,105)</sup>, suggesting a regulatory role for this particular CD8<sup>+</sup> T cell subset. Najafian and coworkers<sup>(106)</sup> showed that depletion of CD8<sup>+</sup> T cells prior to EAE induction with MOG led to heightened disease progression, similar to what has been described in CD8-deficient mice. The adoptive transfer of CD8<sup>+</sup>CD28<sup>-</sup> T cells into CD8-deficient hosts reversed this phenotype and significantly decreased disease severity. At the cellular level, CD8<sup>+</sup>CD28<sup>-</sup> T cells suppressed the proliferation of MOG-specific CD4<sup>+</sup> T cells both *in vitro* and *in vivo*. It was shown that suppression was mediated through a MHC class I-restricted, contact-dependent mechanism that required the presence of APCs. The CD8<sup>+</sup>CD28<sup>-</sup> T cell/APC interaction led to downregulation of several co-stimulatory molecules, including CD80, CD86 and CD40, on APCs. In another study, CD8<sup>+</sup>CD28<sup>-</sup> T cells were shown to exert regulatory activity in an animal model of myasthenia gravis (MG), an autoimmune disease characterized by antibody-mediated blockade of acetylcholine receptors (AChR) at neuromuscular junctions. Ben-David and coworkers showed that treatment with an APL consisting of two tandemly-fused myasthenogenic peptide analogs suppressed MG-associated autoimmunity through the induction of a group of CD8<sup>+</sup>CD28<sup>-</sup> cells that also express FoxP3. These CD8<sup>+</sup>CD28<sup>-</sup>FoxP3<sup>+</sup> T cells were shown to produce immunosuppressive cytokines such as IL-10 and TGF- $\beta$ , and could transfer protection into MG-prone animals<sup>(107,108)</sup>. Another study showed that antigen-primed CD8<sup>+</sup>CD28<sup>-</sup> T regulatory cells could suppress up-regulation of the inhibitory receptors immunoglobulin-like transcript 3 (ILT3) and ILT4 on APCs, rendering them tolerogenic<sup>(109)</sup>. A recent report suggests that suppression of ILT3/ILT4 upregulation on endothelial cells may enhance heart allograft survival<sup>(110)</sup>.

Additional types of regulatory CD8<sup>+</sup> T cells have also been described. Regulatory CD8<sup>+</sup> T cells induced by a tolerogenic anti-DNA IgG-derived peptide in murine systemic lupus are found in both the CD28<sup>+</sup> and CD28<sup>-</sup> subsets and suppress autoimmune antibody responses in a Foxp3-dependent and TGF $\beta$ -dependent manner<sup>(111)</sup>. Another study documented the induction of regulatory CD8<sup>+</sup> T cells in lupus-prone animals treated with a nucleosomal histone peptide<sup>(112)</sup>. The induced CD8<sup>+</sup> T cell population expressed

glucocorticoid-induced tumor necrosis factor receptor (GITR) and TGF $\beta$ , and suppressed autoantibody production by secreting TGF, in a contact-independent manner<sup>(112)</sup>. Another subset of CD8<sup>+</sup> (and CD11c<sup>+</sup>) T cells with regulatory properties has been described in an animal model of collagen-induced arthritis<sup>(113)</sup>. This subset of T cells was shown to produce large amounts of IFN $\gamma$ , which induced indoleamine 2,3-deoxygenase activity in DCs and monocytes, resulting in the suppression of antigen-specific CD4<sup>+</sup> T cell responses.

## CONCLUSIONS

There is now no doubt that, in autoimmunity, CD8<sup>+</sup> T cells play important roles as both effectors of tissue damage and immunoregulators. Pathogenic CD8<sup>+</sup> T cells mediate tissue damage through their cytotoxic activity, as well as through the secretion of cytokines that further fuel inflammation. However, when the suppressive activity of autoreactive T cells targets pathogenic immune cell types, it suppresses autoimmunity. Thus, advances in our understanding of the biology of autoreactive CD8<sup>+</sup> T cells, coupled to antigenic epitope discovery and characterization, may accelerate the discovery of novel antigen-specific therapeutic strategies to prevent and/or blunt autoimmune responses.

## ACKNOWLEDGEMENTS

Some of the work discussed in this review is supported by grants from the Canadian Institutes of Health Research, the Juvenile Diabetes Research Foundation and the Canadian Diabetes Association. S.T. and A.S. are supported by studentships from the Alberta Heritage Foundation for Medical Research (AHFMR). P.S. is a Scientist of the AHFMR. The JMDRC is supported by the Diabetes Association (Foothills).

## DISCLOSURES

The authors declare no financial conflicts of interest.

### CORRESPONDENCE TO:

Pere Santamaria  
Julia McFarlane Diabetes Research Centre (JMDRC), Department of Microbiology and Infectious Diseases, and Institute of Infection, Inflammation and Immunity  
Faculty of Medicine,  
The University of Calgary  
3330 Hospital Drive NW Calgary  
Alberta, T2N 4N1 Canada  
Phone: (403) 220-8735. Fax: (403) 270-8520.  
E-mail: psantama@ucalgary.ca

## BIBLIOGRAFÍA

1. Palmer E. Negative selection-clearing out the bad apples from the T-cell repertoire. *Nat Rev Immunol* 2003; 3: 383-391.
2. Zehn D, Bevan MJ. T cells with low avidity for a tissue-restricted antigen routinely evade central and peripheral tolerance and cause autoimmunity. *Immunity* 2006; 25: 261-270.
3. Van Parijs L, Abbas AK. Homeostasis and self-tolerance in the immune system: turning lymphocytes off. *Science* 1998; 280: 243-248.
4. Redmond WL, Sherman LA. Peripheral tolerance of CD8 T lymphocytes. *Immunity* 2005; 22: 275-284.
5. Dyment DA, Sadovnick AD, Willer CJ, Armstrong H, Cader ZM, Wiltshire S, et al. An extended genome scan in 442 Canadian multiple sclerosis-affected sibships: A report from the Canadian Collaborative Study Group. *Hum Mol Genet* 2004; 13: 1005-1015.
6. Lincoln MR, Montpetit A, Cader MZ, Saarela J, Dyment DA, Tiislar M, et al. A predominant role for the HLA class II region in the association of the MHC region with multiple sclerosis. *Nat Genet* 2005; 37: 1108-1112.
7. Nepom GT, Kwok WW. Molecular basis for HLA-DQ associations with IDDM. *Diabetes* 1998; 47: 1177-1184.
8. Langholz B, Tuomilehto-Wolf E, Thomas D, Pitkaniemi J, Tuomilehto J. Variation in HLA-associated risks of childhood insulin-dependent diabetes in the Finnish population: I. Allele effects at A, B, and DR loci. DiMe Study Group. *Childhood Diabetes in Finland. Genet Epidemiol* 1995; 12: 441-453.
9. Noble JA, Valdes AM, Bugawan TL, Apple RJ, Thomson G, Erlich HA. The HLA class I A locus affects susceptibility to type 1 diabetes. *Hum Immunol* 2002; 63: 657-664.
10. Tait BD, Colman PG, Morahan G, Marchinovska L, Dore E, Gellert S, et al. HLA genes associated with autoimmunity and progression to disease in type 1 diabetes. *Tissue Antigens* 2003; 61: 146-153.
11. Itoh N, Hanafusa T, Miyazaki A, Miyagawa J, Yamagata K, Yamamoto K, et al. Mononuclear cell infiltration and its relation to the expression of major histocompatibility complex antigens and adhesion molecules in pancreas biopsy specimens from newly diagnosed insulin-dependent diabetes mellitus patients. *J Clin Invest* 1993; 92: 2313-2322.
12. Santamaria P, Nakhleh RE, Sutherland DE, Barbosa JJ. Characterization of T lymphocytes infiltrating human pancreas allograft affected by isletitis and recurrent diabetes. *Diabetes* 1992; 41: 53-61.
13. Somoza N, Vargas F, Roura-Mir C, Vives-Pi M, Fernandez-Figueras MT, Ariza A, et al. Pancreas in recent onset insulin-dependent diabetes mellitus. Changes in HLA, adhesion molecules and autoantigens, restricted T cell receptor V beta usage, and cytokine profile. *J Immunol* 1994; 153: 1360-1377.
14. Vizler C, Bercovici N, Cornet A, Cambouris C, Liblau RS. Role of autoreactive CD8<sup>+</sup> T cells in organ-specific autoimmune diseases: insight from transgenic mouse models. *Immunol Rev* 1999; 169: 81-92.
15. Nagata M, Santamaria P, Kawamura T, Utsugi T, Yoon JW. Evidence for the role of CD8<sup>+</sup> cytotoxic T cells in the destruction of pancreatic beta-cells in nonobese diabetic mice. *J Immunol* 1994; 152: 2042-2050.
16. Serreze DV, Chapman HD, Varnum DS, Gerling I, Leiter EH, Shultz LD. Initiation of autoimmune diabetes in NOD/Lt mice is MHC class I-dependent. *J Immunol* 1995 158: 3978-3986.
17. Serreze DV, Leiter EH, Christianson GJ, Greiner D, Roopenian, DC. Major histocompatibility complex class I-deficient NOD-B2mnull mice are diabetes and insulinitis resistant. *Diabetes* 1994; 43: 505-509.
18. Wang B, Gonzalez A, Benoist C, Mathis D. The role of CD8<sup>+</sup> T cells in the initiation of insulin-dependent diabetes mellitus. *Eur J Immunol* 1996; 26: 1762-1769.
19. DiLorenzo TP, Graser RT, Ono T, Christianson GJ, Chapman HD, Roopenian DC, et al. Major histocompatibility complex class I-restricted T cells are required for all but the end stages of diabetes development in nonobese diabetic mice and use a prevalent T cell receptor alpha chain gene rearrangement. *Proc Natl Acad Sci USA* 1998; 95: 12538-12543.
20. Sumida T, Furukawa M, Sakamoto A, Namekawa T, Maeda T, Zijlstra M, et al. Prevention of insulinitis and diabetes in beta 2-microglobulin-deficient non-obese diabetic mice. *Int Immunol* 1994; 6: 1445-1449.
21. Pearson T, Markees TG, Serreze DV, Pierce MA, Marron MP, Wicker LS, et al. Genetic disassociation of autoimmunity and resistance to costimulation blockade-induced transplantation tolerance in nonobese diabetic mice. *J Immunol* 2003; 171: 185-195.
22. Christianson SW, Shultz LD, Leiter EH. Adoptive transfer of diabetes into immunodeficient NOD-scid/scid mice. Relative contributions of CD4<sup>+</sup> and CD8<sup>+</sup> T-cells from diabetic versus prediabetic NOD.NON-Thy-1a donors. *Diabetes* 1993; 42: 44-55.
23. Wong FS, Karttunen J, Dumont C, Wen L, Visintin I, Pilip IM, et al. Identification of an MHC class I-restricted autoantigen in type 1 diabetes by screening an organ-specific cDNA library. *Nat Med* 1999; 5: 1026-1031.
24. Santamaria P, Utsugi T, Park BJ, Averill N, Kawazu S, Yoon JW. Beta-cell-cytotoxic CD8<sup>+</sup> T cells from nonobese diabetic mice use highly homologous T cell receptor alpha-chain CDR3 sequences. *J Immunol* 1995; 154: 2494-2503.
25. Verdaguer J, Yoon JW, Anderson B, Averill N, Utsugi T, Park BJ, Santamaria P. Acceleration of spontaneous diabetes in TCR-beta-transgenic nonobese diabetic mice by beta-cell cytotoxic CD8<sup>+</sup> T cells expressing identical endogenous TCR-alpha chains. *J Immunol* 1996; 157: 4726-4735.
26. Lieberman SM, Evans AM, Han B, Takaki T, Vinnitskaya Y, Caldwell JA, et al. Identification of the beta cell antigen targeted by a prevalent population of pathogenic CD8<sup>+</sup> T cells in autoimmune diabetes. *Proc Natl Acad Sci USA* 2003; 100: 8384-8388.
27. Verdaguer J, Schmidt D, Amrani A, Anderson B, Averill N, Santamaria P. Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. *J Exp Med* 1997; 186: 1663-1676.
28. Trudeau JD, Kelly-Smith C, Verchere CB, Elliott JF, Dutz JP, Finegood DT, et al. Prediction of spontaneous autoimmune diabetes in NOD mice by quantification of autoreactive T cells in peripheral blood. *J Clin Invest* 2003; 111: 217-223.
29. Amrani A, Verdaguer J, Serra P, Tafuro S, Tan R, Santamaria P. Progression of autoimmune diabetes driven by avidity maturation of a T-cell population. *Nature* 2000; 406: 739-742.
30. Han B, Serra P, Yamanouchi J, Amrani A, Elliott JF, Dickie P, et al. Developmental control of CD8 T cell-avidity maturation in autoimmune diabetes. *J Clin Invest* 2005; 115: 1879-1887.
31. Han B, Serra P, Amrani A, Yamanouchi J, Maree AF, Edelstein-Keshet L, Santamaria P. Prevention of diabetes by manipulation



- of anti-IGRP autoimmunity: high efficiency of a low-affinity peptide. *Nat Med* 2005; 11: 645-652.
32. Standifer NE, Ouyang Q, Panagiotopoulos C, Verchere CB, Tan R, Greenbaum CJ, et al. Identification of Novel HLA-A\*0201-restricted epitopes in recent-onset type 1 diabetic subjects and antibody-positive relatives. *Diabetes* 2006; 55: 3061-3067.
  33. Hassainya Y, Garcia-Pons F, Kratzer R, Lindo V, Greer F, Lemonnier FA, et al. Identification of naturally processed HLA-A2--restricted proinsulin epitopes by reverse immunology. *Diabetes* 2005; 54: 2053-2059.
  34. Toma A, Haddouk S, Briand JP, Camoin L, Gahery H, Connan F, et al. Recognition of a subregion of human proinsulin by class I-restricted T cells in type 1 diabetic patients. *Proc Natl Acad Sci USA* 2005; 102: 10581-10586.
  35. Pinkse GG, Boitard C, Tree TI, Peakman M, Roep BO. HLA class I epitope discovery in type 1 diabetes: Independent and reproducible identification of proinsulin epitopes of CD8 T cells--report of the IDS T Cell Workshop Committee. *Ann NY Acad Sci* 2006; 1079: 19-23.
  36. Serreze DV, Marron MP, Dileo TP. "Humanized" HLA transgenic NOD mice to identify pancreatic beta cell autoantigens of potential clinical relevance to type 1 diabetes. *Ann NY Acad Sci* 2007; 1103: 103-111.
  37. Takaki T, Marron MP, Mathews CE, Guttmann ST, Bottino R, Trucco M, et al. HLA-A\*0201-restricted T cells from humanized NOD mice recognize autoantigens of potential clinical relevance to type 1 diabetes. *J Immunol* 2006; 176:3257-3265.
  38. Blancou P, Mallone R, Martinuzzi E, Severe S, Pogu S, Novelli G, et al. Immunization of HLA class I transgenic mice identifies autoantigenic epitopes eliciting dominant responses in type 1 diabetes patients. *J Immunol* 2007; 178: 7458-7466.
  39. Furuzawa-Carballeda J, Vargas-Rojas MI, Cabral RA. Autoimmune inflammation from the Th17 perspective. *Autoimmun Rev* 2007; 6:169-175.
  40. Sospedra M, Martin R. Immunology of multiple sclerosis. *Annu Rev Immunol* 2005; 23: 683-747.
  41. Huseby ES, Ohlen C, Goverman J. Cutting edge: myelin basic protein-specific cytotoxic T cell tolerance is maintained in vivo by a single dominant epitope in H-2k mice. *J Immunol* 1999; 163: 1115-1118.
  42. Sun D, Whitaker JN, Huang Z, Liu D, Coleclough C, Wekerle H, Raine CS. Myelin antigen-specific CD8<sup>+</sup> T cells are encephalitogenic and produce severe disease in C57BL/6 mice. *J Immunol* 2001; 166: 7579-7587.
  43. Huseby ES, Liggitt D, Brabb T, Schnabel B, Ohlen C, Goverman J. A pathogenic role for myelin-specific CD8<sup>+</sup> T cells in a model for multiple sclerosis. *J Exp Med* 2001; 194: 669-676.
  44. Ford ML, Evavold BD. Specificity, magnitude, and kinetics of MOG-specific CD8<sup>+</sup> T cell responses during experimental autoimmune encephalomyelitis. *Eur J Immunol* 2005; 35: 76-85.
  45. Fogdell-Hahn A, Ligens A, Gronning M, Hillert J, Olerup O. Multiple sclerosis: A modifying influence of HLA class I genes in an HLA class II associated autoimmune disease. *Tissue Antigens* 2000; 55: 140-148.
  46. Yeo TW, De Jager PL, Gregory SG, Barcellos LF, Walton A, Goris A, et al. A second major histocompatibility complex susceptibility locus for multiple sclerosis. *Ann Neurol* 2007; 61: 228-236.
  47. van Oosten BW, Lai M, Hodgkinson S, Barkhof F, Miller DH, Moseley IF, et al. Treatment of multiple sclerosis with the monoclonal anti-CD4 antibody cM-T412: results of a randomized, double-blind, placebo-controlled, MR-monitored phase II trial. *Neurology* 1997; 49: 351-357.
  48. Hauser SL, Bhan AK, Gilles F, Kemp M, Kerr C, Weiner HL. Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann Neurol* 1986; 19: 578-587.
  49. Grenier Y, Ruijs TC, Robitaille Y, Olivier A, Antel JP. Immunohistochemical studies of adult human glial cells. *J Neuroimmunol* 1989; 21: 103-115.
  50. Booss J, Esiri MM, Tourtellotte WW, Mason DY. Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. *J Neurol Sci* 1983; 62: 219-232.
  51. Jacobsen M, Cepok S, Quak E, Happel M, Gaber R, Ziegler A, et al. Oligoclonal expansion of memory CD8<sup>+</sup> T cells in cerebrospinal fluid from multiple sclerosis patients. *Brain* 2002; 125: 538-550.
  52. Babbe H, Roers A, Waisman A, Lassmann H, Goebels N, Hohlfeld R, et al. Clonal expansions of CD8<sup>+</sup> T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction. *J Exp Med* 2000; 192: 393-404.
  53. Neumann H, Medana IM, Bauer J, Lassmann H. Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases. *Trends Neurosci* 2002; 25: 313-319.
  54. Jurewicz A, Biddison WE, Antel JP. MHC class I-restricted lysis of human oligodendrocytes by myelin basic protein peptide-specific CD8 T lymphocytes. *J Immunol* 1998; 160: 3056-3059.
  55. Tsuchida T, Parker KC, Turner RV, McFarland HF, Coligan JE, Biddison WE. Autoreactive CD8<sup>+</sup> T-cell responses to human myelin protein-derived peptides. *Proc Natl Acad Sci USA* 1994; 91: 10859-10863.
  56. Dressel A, Chin JL, Sette A, Gausling R, Hollsbach P, Hafler DA. Autoantigen recognition by human CD8 T cell clones: Enhanced agonist response induced by altered peptide ligands. *J Immunol* 1997; 159: 4943-4951.
  57. Pascolo S, Bervas N, Ure JM, Smith AG, Lemonnier FA, Perarnau B. HLA-A2.1-restricted education and cytolytic activity of CD8<sup>+</sup> T lymphocytes from beta2 microglobulin (2m) HLA-A2.1 monochain transgenic H-2Db ,2m double knockout mice. *J Exp Med* 1997; 185: 2043-2051.
  58. Mars LT, Bauer J, Gross DA, Bucciarelli F, Firat H, Hudrisier D, et al. CD8 T cell responses to myelin oligodendrocyte glycoprotein-derived peptides in humanized HLA-A\*0201-transgenic mice. *J Immunol* 2007; 179: 5090-5098.
  59. de Jersey J, Snelgrove SL, Palmer SE, Teteris SA, Mullbacher A, Miller JF, Slattery RM. Beta cells cannot directly prime diabetogenic CD8 T cells in nonobese diabetic mice. *Proc Natl Acad Sci USA* 2007; 104: 1295-1300.
  60. Gagnerault MC, Luan JJ, Lotton C, Lepault F. Pancreatic lymph nodes are required for priming of beta cell reactive T cells in NOD mice. *J Exp Med* 2002; 196: 369-377.
  61. Turley S, Poirot L, Hattori M, Benoist C, Mathis D. Physiological beta cell death triggers priming of self-reactive T cells by dendritic cells in a type-1 diabetes model. *J Exp Med* 2003; 198: 1527-1537.
  62. Yamanouchi J, Verdaguer J, Han B, Amrani A, Serra P, Santamaria P. Cross-priming of diabetogenic T cells dissociated from CTL-

- induced shedding of beta cell autoantigens. *J Immunol* 2003; 171: 6900-6909.
63. Trudeau JD, Dutz JP, Arany E, Hill DJ, Fieldus WE, Finegood DT. Neonatal beta-cell apoptosis: A trigger for autoimmune diabetes? *Diabetes* 2000; 49: 1-7.
  64. Zhang Y, O'Brien B, Trudeau J, Tan R, Santamaria P, Dutz JP. In situ beta cell death promotes priming of diabetogenic CD8 T lymphocytes. *J Immunol* 2002; 168: 1466-1472.
  65. Kurts C, Sutherland RM, Davey G, Li M, Lew AM, Blanas E, et al. CD8 T cell ignorance or tolerance to islet antigens depends on antigen dose. *Proc Natl Acad Sci USA* 1999; 96: 12703-12707.
  66. Harbers SO, Crocker A, Catalano G, D'Agati V, Jung S, Desai DD, Clynes R. Antibody-enhanced cross-presentation of self antigen breaks T cell tolerance. *J Clin Invest* 2007; 117: 1361-1369.
  67. Rock KL, Shen L. Cross-presentation: Underlying mechanisms and role in immune surveillance. *Immunol Rev* 2005; 207: 166-183.
  68. Frigerio S, Junt T, Lu B, Gerard C, Zumsteg U, Hollander GA, Piali L. Beta cells are responsible for CXCR3-mediated T-cell infiltration in insulinitis. *Nat Med* 2002; 8: 1414-1420.
  69. Lang KS, Recher M, Junt T, Navarini AA, Harris NL, Freigang S, et al. Toll-like receptor engagement converts T-cell autoreactivity into overt autoimmune disease. *Nat Med* 2005; 11: 138-145.
  70. Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN. TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. *Nat Med* 2007; 13: 543-551.
  71. Graser RT, DiLorenzo TP, Wang F, Christianson GJ, Chapman HD, Roopenian DC, et al. Identification of a CD8 T cell that can independently mediate autoimmune diabetes development in the complete absence of CD4 T cell helper functions. *J Immunol* 2000; 164: 3913-3918.
  72. Heath WR, Carbone FR. Cross-presentation, dendritic cells, tolerance and immunity. *Annu Rev Immunol* 2001; 19: 47-64.
  73. Probst HC, McCoy K, Okazaki T, Honjo T, van den Broek M. Resting dendritic cells induce peripheral CD8<sup>+</sup> T cell tolerance through PD-1 and CTLA-4. *Nat Immunol* 2005; 6: 280-286.
  74. Perez VL, Van Parijs L, Biuckians A, Zheng XX, Strom TB, Abbas AK. Induction of peripheral T cell tolerance in vivo requires CTLA-4 engagement. *Immunity* 1997; 6: 411-417.
  75. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 1995; 3: 541-547.
  76. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, et al. Lymphoproliferative disorders with early lethality in mice deficient in Ctl4. *Science* 1995; 270: 985-988.
  77. Perrin PJ, Maldonado JH, Davis TA, June CH, Racke MK. CTLA-4 blockade enhances clinical disease and cytokine production during experimental allergic encephalomyelitis. *J Immunol* 1996; 157: 1333-1336.
  78. Keir ME, Freeman GJ, Sharpe AH. PD-1 regulates self-reactive CD8<sup>+</sup> T cell responses to antigen in lymph nodes and tissues. *J Immunol* 2007; 179: 5064-5070.
  79. Wang J, Yoshida T, Nakaki F, Hiai H, Okazaki T, Honjo T. Establishment of NOD-Pdcd1<sup>-/-</sup> mice as an efficient animal model of type I diabetes. *Proc Natl Acad Sci USA* 2005; 102: 11823-11828.
  80. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 1999; 11: 141-151.
  81. Salama AD, Chitnis T, Imitola J, Ansari MJ, Akiba H, Tushima F, et al. Critical role of the programmed death-1 (PD-1) pathway in regulation of experimental autoimmune encephalomyelitis. *J Exp Med* 2003; 198: 71-78.
  82. Latchman YE, Liang SC, Wu Y, Chernova T, Sobel RA, Klemm M, et al. PD-L1-deficient mice show that PD-L1 on T cells, antigen-presenting cells, and host tissues negatively regulates T cells. *Proc Natl Acad Sci USA* 2004; 101: 10691-10696.
  83. Keir ME, Liang SC, Guleria I, Latchman YE, Qipo A, Albacker LA, et al. Tissue expression of PD-L1 mediates peripheral T cell tolerance. *J Exp Med* 2006; 203: 883-895.
  84. Ansari MJ, Salama AD, Chitnis T, Smith RN, Yagita H, Akiba H, et al. The programmed death-1 (PD-1) pathway regulates autoimmune diabetes in nonobese diabetic (NOD) mice. *J Exp Med* 2003; 198: 63-69.
  85. Guleria I, Gubbels Bupp M, Dada S, Fife B, Tang Q, Ansari MJ, et al. Mechanisms of PDL1-mediated regulation of autoimmune diabetes. *Clin Immunol* 2007; 125: 16-25.
  86. Fife BT, Guleria I, Gubbels Bupp M, Eagar TN, Tang Q, Bour-Jordan H, et al. Insulin-induced remission in new-onset NOD mice is maintained by the PD-1-PD-L1 pathway. *J Exp Med* 2006; 203: 2737-2747.
  87. Gao W, Demirci G, Strom TB, Li XC. Stimulating PD-1-negative signals concurrent with blocking CD154 co-stimulation induces long-term islet allograft survival. *Transplantation* 2003; 76: 994-999.
  88. Rodgers JR, Cook RG. MHC class Ib molecules bridge innate and acquired immunity. *Nat Rev Immunol* 2005; 5: 459-471.
  89. Hu D, Ikizawa K, Lu L, Sanchirico ME, Shinohara ML, Cantor H. Analysis of regulatory CD8 T cells in Qa-1-deficient mice. *Nat Immunol* 2004; 5: 516-523.
  90. Gaur A, Ruberti G, Haspel R, Mayer JP, Fathman CG. Requirement for CD8<sup>+</sup> cells in T cell receptor peptide-induced clonal unresponsiveness. *Science* 1993; 259: 91-94.
  91. Jiang H, Kashleva H, Xu LX, Forman J, Flaherty L, Pernis B, et al. T cell vaccination induces T cell receptor V<sub>β</sub>-specific Qa-1-restricted regulatory CD8<sup>+</sup> T cells. *Proc Natl Acad Sci USA* 1998; 95: 4533-4537.
  92. Madakamutil LT, Maricic I, Sercarz E, Kumar V. Regulatory T cells control autoimmunity in vivo by inducing apoptotic depletion of activated pathogenic lymphocytes. *J Immunol* 2003; 170: 2985-2992.
  93. Tang X, Maricic I, Purohit N, Bakamjian B, Reed-Loisel LM, Beeston T, et al. Regulation of immunity by a novel population of Qa-1-restricted CD8 $\alpha\alpha$ :TCR $\beta$ <sup>+</sup> T cells. *J Immunol* 2006; 177: 7645-7655.
  94. Panoutsakopoulou V, Huster KM, McCarty N, Feinberg E, Wang R, Wucherpfennig KW, Cantor H. Suppression of autoimmune disease after vaccination with autoreactive T cells that express Qa-1 peptide complexes. *J Clin Invest* 2004; 113: 1218-1224.
  95. Jiang H, Wu Y, Liang B, Zheng Z, Tang G, Kanellopoulos J, et al. 2005. An affinity/avidity model of peripheral T cell regulation. *J Clin Invest* 115: 302-312.
  96. Shmidt TE, Zhuchenko TD, Iakhno NN. Glatiramer acetate (Copaxone) influence on different stages of multiple sclerosis pathogenesis. *Zh Nevrol Psikhiatr Im S S Korsakova* 2003; (Spec No 2): 79-82.

97. Biegler BW, Yan SX, Ortega SB, Tennakoon DK, Racke MK, Karandikar NJ. Glatiramer acetate (GA) therapy induces a focused, oligoclonal CD8<sup>+</sup> T-cell repertoire in multiple sclerosis. *J Neuroimmunol* 2006; 180: 159-171.
98. Tennakoon DK, Mehta RS, Ortega SB, Bhoj V, Racke MK, Karandikar NJ. Therapeutic induction of regulatory, cytotoxic CD8<sup>+</sup> T cells in multiple sclerosis. *J Immunol* 2006; 176: 7119-7129.
99. Suzuki H, Kundig TM, Furlonger C, Wakeham A, Timms E, Matsuyama T, et al. Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. *Science* 1995; 268: 1472-1476.
100. Rifa'i M, Kawamoto Y, Nakashima I, Suzuki H. Essential roles of CD8<sup>+</sup>CD122 regulatory T cells in the maintenance of T cell homeostasis. *J Exp Med* 2004; 200: 1123-1134.
101. Endharti AT, Rifa'i M, Shi Z, Fukuoka Y, Nakahara Y, Kawamoto Y, et al. Cutting edge: CD8<sup>+</sup>CD122<sup>+</sup> regulatory T cells produce IL-10 to suppress IFN-gamma production and proliferation of CD8<sup>+</sup> T cells. *J Immunol* 2005; 175: 7093-7097.
102. Saitoh O, Abiru N, Nakahara M, Nagayama Y. CD8<sup>+</sup>CD122<sup>+</sup> T cells, a newly identified regulatory T subset, negatively regulate Graves' hyperthyroidism in a murine model. *Endocrinology* 2007; 148: 6040-6046.
103. Lee YH, Ishida Y, Rifa'i M, Shi Z, Isobe K, Suzuki H. Essential role of CD8<sup>+</sup>CD122<sup>+</sup> regulatory T cells in the recovery from experimental autoimmune Encephalomyelitis. *J Immunol* 2008; 180: 825-832.
104. Ciubotariu R, Colovai AI, Pennesi G, Liu Z, Smith D, Berlocchio P, et al. Specific suppression of human CD4<sup>+</sup> Th cell responses to pig MHC antigens by CD8<sup>+</sup>CD28<sup>-</sup> regulatory T cells. *J Immunol* 1998; 161: 5193-5202.
105. Colovai AI, Liu Z, Ciubotariu R, Lederman S, Cortesini R, Suciuc Foca N. Induction of xenoreactive CD4<sup>+</sup> T-cell anergy by suppressor CD8<sup>+</sup>CD28<sup>-</sup> T cells. *Transplantation* 2000; 69: 1304-1310.
106. Najafian N, Chitnis T, Salama AD, Zhu B, Benou C, Yuan X, et al. Regulatory functions of CD8<sup>+</sup>CD28<sup>-</sup> T cells in an autoimmune disease model. *J Clin Invest* 2003; 112: 1037-1048.
107. Ben-David H, Sharabi A, Dayan M, Sela M, Mozes E. The role of CD8<sup>+</sup>CD28 regulatory cells in suppressing myasthenia gravis-associated responses by a dual altered peptide ligand. *Proc Natl Acad Sci USA* 2007; 104: 17459-17464.
108. Paas-Rozner M, Sela M, Mozes E. The nature of the active suppression of responses associated with experimental autoimmune myasthenia gravis by a dual altered peptide ligand administered by different routes. *Proc Natl Acad Sci USA* 2001; 98: 12642-12647.
109. Chang CC, Ciubotariu R, Manavalan JS, Yuan J, Colovai AI, Piazza F, et al. Tolerization of dendritic cells by T(S) cells: the crucial role of inhibitory receptors ILT3 and ILT4. *Nat Immunol* 2002; 3: 237-243.
110. Manavalan JS, Kim-Schulze S, Scotto L, Naiyer AJ, Vlad G, Colombo PC, et al. Alloantigen specific CD8<sup>+</sup>CD28<sup>-</sup> FOXP3<sup>+</sup> T suppressor cells induce ILT3<sup>+</sup> ILT4<sup>+</sup> tolerogenic endothelial cells, inhibiting alloreactivity. *Int Immunol* 2004; 16: 1055-1068.
111. Singh RP, La Cava A, Wong M, Ebling F, Hahn BH. CD8<sup>+</sup> T cell-mediated suppression of autoimmunity in a murine lupus model of peptide-induced immune tolerance depends on Foxp3 expression. *J Immunol* 2007; 178: 7649-7657.
112. Kang HK, Michaels MA, Berner BR, Datta SK. Very low-dose tolerance with nucleosomal peptides controls lupus and induces potent regulatory T cell subsets. *J Immunol* 2005; 174: 3247-3255.
113. Seo SK, Choi JH, Kim YH, Kang WJ, Park HY, Suh JH, et al. 4-1BB-mediated immunotherapy of rheumatoid arthritis. *Nat Med* 2004; 0: 1088-1094.