

Review

Specific Control of Immunity by Regulatory CD8 T Cells

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T lymphocytes with dedicated suppressor function (Treg) play a crucial role in the homeostatic control of immunity in the periphery. Several Treg phenotypes have now been identified in the CD4 and CD8 T cell populations, suggesting their down-regulatory function in both human and animal models of autoimmunity, transplantation and tumor immunity. Here we will focus on the CD8 Treg population and their ability to specifically inhibit a pathogenic autoimmune response. This review will detail the current advances in the knowledge of CD8 Treg in the context of antigen specificity, phenotype, MHC restriction, mechanism of action, and priming. *Cellular & Molecular Immunology*. 2005;2(1):11-19.

Key Words: regulatory T cell, Qa-1, TCR-peptides, apoptosis, immune deviation, cross-priming, EAE

Introduction

Negative selection in the thymus (central tolerance) does not eliminate all potentially pathogenic auto-reactive lymphocytes, and such cells can be found frequently in the periphery (1-3). Therefore, in addition to central tolerance, mechanisms controlling potentially harmful autoreactivity in the periphery (peripheral tolerance) must also exist. Multiple mechanisms contribute to peripheral tolerance, including clonal deletion, clonal anergy, and active suppression by suppressor T cells (regulatory T cells or Treg) (4-10). Since the identification of CD25 as a marker for a naturally occurring CD4⁺ suppressor T cell, there has been a renewal of interest in the area of active T cell-mediated suppression (11, 12). The suppression of immunity by the naturally occurring CD4⁺CD25⁺ regulatory T cells (CD4 Treg) has been suggested in many different animal models and in human studies (13). In addition to CD4 Treg, regulatory CD8 T cells (CD8 Treg) have also been demonstrated to play an important role in neonatal tolerance (14), allo-graft (15) or xeno-graft tolerance (16), and autoimmune diseases (17).

There are two broad categories of immune regulation: antigen specific and non-specific. Several subsets of Treg have been suggested to suppress immunity in a nonspecific manner, including CD4⁺CD25⁺ Treg (13) and some CD8

Treg (CD8⁺CD25⁺, CD8⁺CD122⁺, CD45RC^{low} and IL-2/GM-CSF-induced CD8 Treg) (18-23). In contrast, antigen specific Treg are primed in the process of the immune response to foreign or self-antigens. The primed Treg then specifically down-regulate that immune response. The mechanism is referred to as feedback inhibition, a mechanism that has long been described in macromolecular synthesis. These Treg include CD8⁺CD28⁻ Treg (24), CD8⁺CD75s⁺ Treg (25), plasmacytoid dendritic cell (DC2)-induced CD8 Treg (26), CD8⁺CD45RC^{high} Tc1 Treg (27) and TCR peptide specific CD8 α Treg (17).

While much has been learned about the characteristics and roles of Treg, several important questions have not been answered. First, antigen fine specificity for most of the Treg is not known due to their polyclonal nature. Second, the study of MHC restriction has been mostly ignored in this area because of the unknown antigen specificity. Third, the relationship between CD4 and CD8 Treg is unclear. Resolution of these questions requires further investigation of the Treg population in systems where the antigen specificity of each T cell involved can be determined. This enables functional cells to be cloned and expanded to study their role *in vitro* and, by adoptive transfer, *in vivo*. An overview of the investigations we have performed concerning the role of CD8 T suppressors in various models will shed light on future directions for this area.

Regulatory/suppressor CD8 T cells: Early investigations

In the early 1970's Gershon and colleagues demonstrated that T cells from animals tolerant to antigen A could be adoptively transferred to specifically suppress antibody responses to antigen A in recipient animals (28). Later studies reported that T suppressor cells could down-regulate type I

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hypersensitivity and cell-mediated delayed type hypersensitivity (DTH) reactions (29, 30). In addition to inducing T suppressor cells by means of tolerance-inducing protocols, *in vitro* generation of specific suppressor T cells was also reported (31). At that time Lyt allo-antisera became available which were able to differentiate between helper T cells (Lyt1⁺) and cytotoxic T cells (Lyt2⁺). It was demonstrated that the suppressor activity resided within the Lyt2⁺ (CD8⁺) subsets (32). Further allo-antisera phenotyping suggested that the Lyt2⁺ T cell population expressed a serologically defined I-J determinant from the mouse MHC class II region (33). However, later studies failed to confirm the existence of the I-J subregion within the MHC class II region or to identify an mRNA transcript for the I-J determinant (34).

When analyzed *in vitro* Lyt2⁺ (CD8⁺) T suppressor cell function appeared to be dependent on presence of activated CD4⁺ T cells (35, 36). In an experiment to demonstrate negative feedback mechanism, the responding CD4⁺ T cells were found to activate CD8⁺ T cells to mediate their own down-regulation. Cantor and colleagues serologically identified the cell surface expression of Qa-1 (later confirmed to be a non-classical MHC class Ib molecule) on the CD4⁺ inducer cells. Qa-1 was found to be necessary for the induction of negative feedback (36). The role of Qa-1 in CD8⁺ Treg-mediated suppression will be discussed in depth later.

While many of the conclusions drawn from the 1970's studies are still relevant, they lacked molecular confirmation. Many major molecular immunological questions such as the nature of MHC restriction that would have obvious implications on the interpretation of the I-J and Qa-1 roles in T cell-mediated suppression, had yet to be answered. It was due to a lack of molecular characterization of serologically defined markers, along with the realization in the mid-80s that cytokines secreted from conventional T cells could be inhibitory to other T cell responses, that led to much skepticism within the immunological community concerning the existence of a unique cell population with dedicated suppressor function.

Early T cell vaccination (TCV) investigations showed that immunization with syngeneic allo-reactive T lymphoblasts induced unresponsiveness to transplantation antigens (37). Later experiments expanded this earlier work towards antigen specificity, showing that allospecific T lymphoblasts can specifically immunize against graft versus host disease (GVHR) by induction of an anti-idiotypic response (38). For example, immunization of F1 rats with alloreactive T cell populations of parental strain origin induced a host-mediated T cell response that was specific for the anti-MHC receptors on the alloreactive parental T cells. The cells responsible for resistance to GVHR were later shown to be cytotoxic (38). In another system, Irun Cohen's laboratory initiated a series of experiments in the 1980s using experimental autoimmune encephalomyelitis (EAE) disease in rodents, a model of human multiple sclerosis (MS), which led to the coining of the current concept of TCV (39-41). EAE can be induced either by active immunization with a

self-antigen, e.g. myelin basic protein (MBP), or by passive transfer of Th1 CD4 T cells reactive with MBP. Animals contract paralytic disease, recover spontaneously, and become resistant to re-induction of EAE. Animals vaccinated with attenuated pathogenic CD4 T cell lines reactive to MBP are resistant to induction of the disease (39-41). To demonstrate the mechanisms of TCV, Sun et al. generated a T cell line from spleens of Lewis rats that had recovered from MBP-reactive T cell line S1-induced EAE. The spleen-derived T cell lines expressed a CD8 phenotype and specifically responded to determinants on the inducing S1 line but not the autoantigen MBP (42). The anti-S1 cells selectively lysed the encephalitogenic S1 T cell lines *in vitro* and neutralized their encephalitogenic capacity *in vivo* (42). CD8 T cell lines with similar cytotoxic function were also isolated from human subjects who had received TCV (43, 44).

An important characteristic of EAE in B10.PL and PL/J mice is that recovered mice are resistant to subsequent re-induction of EAE. The role of CD8 Treg in the resistance to re-induction of EAE was first demonstrated in two separate studies. First, mice depleted of CD8⁺ T cells prior to the EAE induction are no longer resistant to re-induction of EAE (45). Second, CD8^{-/-} PL/J H-2^u mice have a more chronic form of EAE reflected by a higher frequency of relapses (46).

Indeed, CD8 T cells were found not only to regulate autoimmune T cells, but also to regulate general immune responses. Early experiments indicated that CD8 Treg control the normal T cell repertoire in the periphery. Nanda et al. showed that Vβ^a mice that lacked 10 TCR Vβ gene segments responded to a peptide of hen egg-white lysozyme (HEL), whereas in wild-type Vβ^b mice a response to the same peptide could not be detected. Peptide-specific T cell responsiveness was revealed in wild-type (Vβ^b) mice when they were treated *in vivo* with anti-CD8 antibody (47).

It is clear that CD8 Treg play an important role in protecting the body from pathogenic immune responses in a variety of settings. In the following sections we will review our current knowledge regarding CD8 Treg in the context of antigen specificity, phenotype, MHC restriction, mechanism of action and priming.

Antigen specificity of CD8 Treg

As mentioned, two broad categories of CD8 Treg have been described: antigen nonspecific and antigen specific. Antigen nonspecific CD8 Treg are either naturally occurring (CD8⁺ CD25⁺ thymocytes, CD8⁺CD122⁺ and CD8⁺CD45RC^{low}) or induced *in vitro* in the presence of cytokines (IL-2/GM-CSF-induced CD8 Treg). On the other hand, antigen specific CD8 Treg are induced either *in vitro* (CD8⁺CD28⁻ Treg and DC2-induced CD8 Treg) or *in vivo* (CD8⁺CD75s⁺ Treg, CD8⁺CD45RC^{high} Tc1 Treg, and TCR peptide specific CD8αα Treg) after priming with a specific antigen (Table 1). Antigens recognized by CD8 Treg can be the priming antigens, molecules on the effector cells, or others. Though

Table 1. Summary of CD8 Treg and their characteristics

Categories	Generation	Antigen specificity	Phenotype	MHC restriction	Mechanisms of action
CD8 $\alpha\alpha$	Spontaneously primed during disease	TCR, CDR1/2	CD8 $\alpha\alpha$	Qa-1a	Cytotoxicity
CD8 ⁺ CD28 ⁻	Allogeneic MLR, Xenogeneic MLR, and <i>in vitro</i> nominal antigen	?	CD28 ⁻	MHC I	Inhibit APC maturation
	IL-2 + IL-10	?	CD28 ⁻	?	Cytokine: IFN- γ , IL-6, IL-10
CD8 ⁺ CD75s ⁺	Allogeneic MLR, oral tolerance	?	CD75s ⁺	?	Non-lytic
CD8 ⁺ CD45RC ^{high}	Neonatal tolerance	?	CD45RC ^{high} , Tc1	?	?
DC2-induced	Priming with CD40L activated DC2	?	?	?	Cytokine: IL-10
CD8 ⁺ CD122 ⁺	Naturally occurring	?	CD122 ⁺	?	?
CD8 ⁺ CD25 ⁺ thymocytes	Naturally occurring	?	CD25 ⁺	?	non-lytic, membrane CTLA-4/TGF- β
CD8 ⁺ CD45RC ^{low}	Naturally occurring	?	CD45RC ^{low}	?	non-lytic

the induction of CD8⁺CD75s⁺ Treg, DC2-induced CD8 Treg and CD8⁺CD45RC^{high} Tc1 Treg depends on antigen priming, the antigens recognized by these CD8 Treg remain unknown (25-27). In the early allo-suppression experiments, it was proposed that CD8⁺CD28⁻ Treg recognize the MHC class I molecules on the allogeneic stimulator (15, 16). However, data from *in vitro* induced CD8⁺CD28⁻ Treg after priming with specific antigens suggested that the CD8⁺CD28⁻ Treg did not respond to the priming antigens (48). Therefore, the actual antigens recognized by this subset of CD8 Treg remains to be discovered.

Knowing the antigens recognized by CD8 Treg is critical to understanding their targets and designing efficient therapies based on their induction or transfer. In contrast to many of the CD8 Treg described above, the antigen specificity of CD8 Treg induced by TCV or primed during the course of EAE has been extensively investigated both in human and in animal models. CD8 T cells were identified to be the cells responsible for the resistance to further induction of EAE after spontaneous recovery or TCV (42, 45, 46, 49). It was initially proposed that TCV induced both an anti-idiotypic and an anti-ergotypic network response (40, 50). Later studies demonstrated that immunization with TCR peptides derived from the TCR β chain of pathogenic T cells could render rats resistant to the induction of EAE (51, 52). Therefore the TCR utilized by pathogenic CD4 T cells was proposed to be the target of Treg in EAE. However, little evidence has been presented to show that the TCR is actually targeted by CD4 and CD8 Treg. Previously, TCR peptides had been reported to induce the expansion of CD8 Treg *in vitro* both in humans and in mice (53-56). However, the functional relevance of these TCR peptide-induced CD8 Treg has not always been demonstrated. We have now shown conclusively that the TCR is targeted by both CD4 and CD8 Treg. To fully illustrate this issue, we have been able to clone both CD4 and CD8 Treg that respond to TCR peptides from

the framework III region (framework III peptide) and from the CDR1/2 region (CDR1/2 peptide) of the TCR V β 8.2 chain, respectively. Both CD4 and CD8 Treg clones are able to transfer protection against EAE induction *in vivo* and are physiologically primed during the course of EAE (17, 57). In addition both TCR peptides can immunize against EAE. Important to demonstrating the specificity of the regulation, TCR peptide-reactive CD8 Treg clones can kill only V β 8.2⁺ CD4 T cell clones (a dominant pathogenic clone in EAE in B10.PL mice) but not irrelevant V β 8.2⁻ clones (Tang et al, manuscript in preparation). Immunization with the framework III region peptide is associated with the recruitment of CD8 Treg that specifically kill TCR V β 8.2⁺ MBPAc1-9 activated CD4 T cells *in vivo*. (58). For the first time we have been able to demonstrate the functionality of the TCR-reactive CD8 Treg at the clonal level.

Phenotypes of CD8 Treg

One reason for the difficulty in characterizing Treg is the lack of specific markers available to identify these small subsets of T cells. The finding that peripheral CD4⁺CD25⁺ T lymphocytes in naïve animals represent a unique lineage of immunoregulatory T cells has helped expedite the understanding of a regulatory population *in vitro* and *in vivo* (11, 12). In the past years, immunologists have been actively searching for specific phenotypes to identify CD8 Treg. Indeed, several cell surface molecules have been proposed, including CD8⁺CD28⁻ (59), CD8⁺CD25⁺ thymocytes (18, 19), CD8⁺CD122⁺ (20), CD8⁺CD45RC^{low} (21, 22), CD8⁺CD75s⁺ (25, 60) and CD8 $\alpha\alpha$ ⁺ regulatory T cells (Tang et al, manuscript in preparation). Table 1 summarizes the varied phenotypes of currently reported CD8 Treg.

As mentioned above we have been able to demonstrate, at the clonal level, that TCRs are targeted by both CD4 and

CD8 Treg. Interestingly, the CD8 Treg appear to lack expression of CD8 β chain and express a CD8 $\alpha\alpha$ homodimer. Data from previous studies suggested that CD8 $\alpha\alpha$ T cells predominantly reside in the intraepithelial lymphocyte (IEL) population and originate in the thymus because double positive cells from thymus can acquire the CD8 $\alpha\alpha$ homodimer upon arrival at intraepithelial sites (61, 62). Furthermore, recent data indicated that CD8 $\alpha\alpha$ T lymphocytes show self-reactivity and CD8 $\alpha\alpha$ T cells, within the IEL population, have been shown to transfer protection against colitis (63, 64). While peripheral lymphoid organs have barely detectable CD8 $\alpha\alpha$ T cells, spleens of classical MHC I knockout mice have an elevated number of these T cells (65), suggesting that CD8 $\alpha\alpha$ T cells do have the ability to circulate through these sites to regulate systemic immune response (7).

MHC restriction elements for CD8 Treg

Investigation of MHC restriction has been hindered in the past due to unknown antigen specificities of the CD8 Treg. Through the late 1970s to early 1980s, an I-J determinant was serologically and biologically defined to be a restriction element for suppressor T cells and suppressor factors (66). However, genetic analysis of MHC I region did not support the I-J hypothesis (34). Though several explanations for the discrepancy of genetic data and biological/serological data were proposed, the puzzle has still not been resolved (67). As shown in Table 1, most of the currently proposed CD8 Treg have unknown MHC restriction elements. Though classical MHC I and II molecules have been suggested to be the restriction element for CD8⁺CD28⁻ Treg (48) and CD8 Treg controlling B cell function (68), respectively. These data are yet to be confirmed in other systems.

It has been demonstrated that TCR peptides can induce both CD4 and CD8 Treg (7). In our system, CD4 Treg specific for the framework III peptide are restricted by MHC II molecules (69, 70). Interestingly, we found that the CD8 $\alpha\alpha$ Treg clones were restricted by the Qa-1a molecules (Tang et al. manuscript in preparation). These data are consistent with recent reports from other laboratories indicating that Qa-1 may serve as a restriction element for CD8 Treg (53, 54, 71, 72, 73). However, serological and biological studies do not provide direct evidence showing Qa-1 restriction of the CD8 Treg. Recently, we have been able to demonstrate direct binding of the CDR1/2 TCR peptide to recombinant Qa-1a molecules. This initially was surprising since the TCR peptide sequence does not satisfy any of the current hypothetical Qa-1 binding motifs that are proposed on the basis of binding of a peptide Qdm (Qa-1 determinant modifier) derived from class Ia MHC leader sequence (74). Even though Qdm represents one of the dominant determinants for Qa-1b, recent experiments have directly shown Qa-1b binding to a diverse repertoire of peptides (75-78).

Another feature of the TCR peptide-reactive CD8 Treg is that the CD8 Treg control only activated but not resting MBP-reactive pathogenic CD4 T cells (58). Earlier work also

indicated that the efficacy of TCV depends on activation of the cells by incubation with MBP or with a T cell mitogen prior to inoculation into recipient rats (50, 79). One may speculate that only activated pathogenic CD4 T cells need to be regulated *in vivo*. Indeed, an interesting finding was that some determinants of Qa-1 are absent on un-activated cells but present on the membranes of mitogen-activated lymphocytes (80). This has relevance to the concept of anti-ergotypic T cells proposed a decade ago to describe those Treg that respond to the state of activation of other T cells (50). Whether the CDR1/2 TCR peptide represents an ergotypic determinant in the context of Qa-1 molecules is currently under investigation.

Mechanisms of regulation by CD8 Treg

CD8 Treg may mediate suppression of an immune response by a number of different mechanisms. Non-specific cytokine or cell contact mechanisms, or specific killing mechanisms of regulation have all been described for CD8 Treg.

CD8 Treg may secrete counter-regulatory Tc1 (IFN- γ), Tc2 (IL-4) or immunosuppressive (IL-10) cytokines to suppress an immune response. Protection from Th1- or Th2-mediated disease has been reported using counter-regulatory Tc1 or Tc2 cells (81). Gilliet and Liu generated human CD8 Treg from naïve CD8 T cells (26). The suppression exerted on naïve CD8 T cells by CD8 Treg was IL-10 dependent. Thus as previously described for Th1, Th2, and IL-10 producing Tr1 cells, CD8 Treg can also mediate non-specific bystander suppression.

CD8⁺CD28⁻ Treg have been reported to modulate APC's stimulatory ability. Data by Chang et al. suggest that human heart transplantation recipient's CD8⁺CD28⁻ Treg interfere with donor's immature DC's CD40 signaling pathways (82). Such interference hinders NF- κ B activation and the maturation of the DC, resulting in the generation of a tolerogenic APC. Alloreactive CD4 T cells interacting with these tolerogenic DCs were anergized in an HLA-restricted manner.

Balashov et al. generated CD8 Treg by activating CD8 T cells in an autologous mixed lymphocyte reaction (AMLR) in the presence of IL-2 and GM-CSF (23). The CD8 Treg were non-cytotoxic, and suppressor function was completely abrogated by the addition of anti-IFN- γ but not anti-IL-4, anti-IL-10, or anti-TGF- β monoclonal antibodies into the cultures. Evidence that such CD8 Treg may not be just an *in vitro* phenomenon is supported by the observation that CD8 T cells isolated during active MS and systemic lupus erythematosus (SLE) demonstrated defective IFN- γ production and suppressor function (23, 83).

While the majority of reported CD8 Treg have been generated by *in vitro* manipulations, naturally occurring non-cytolytic CD8 Treg subsets have recently been identified (18, 21). The CD8⁺CD25⁺ and CD8⁺CD45RC^{low} naturally occurring Treg have a comparable phenotype (FoxP3⁺, CTLA-4⁺) and regulatory function (cell-cell contact dependent suppression) to their CD4 Treg counterparts. Xystrakis and colleagues

demonstrated that CD8⁺CD45RC^{low} T cells protected against CD4⁺ T cell-mediated GVHR in rats (21, 22). However, the *in vivo* mechanism of suppression has yet to be deciphered. Human CD8⁺CD25⁺ Treg were found to exert their suppressive activity through both CTLA-4 and TFG- β 1 interactions with their targets, which led to the down-regulation of IL-2R α chain expression on target T cells making them unresponsive to IL-2 (18). Interestingly, clones generated from CD8⁺CD25⁺ human thymocytes showed impaired ability to suppress Th2 cell responses compared to Th1 (19). In this system it appeared that Th2 cells were refractory to suppression by Treg in an IL-4 dependent manner. One may speculate that in Th1-mediated disease regulation of the pathogenic Th1 cell populations could create a favorable and less competitive environment for the non-pathogenic Th2 populations to expand. CD8⁺CD45RC^{low} Treg suppression of IFN- γ responses from autologous CD4 responder T cells has been described (21). It will be important to determine the *in vivo* functions of these cells and whether peripheral CD8⁺CD25⁺ T cells have the same regulatory function as their thymic counterparts.

We have demonstrated the involvement of both CD4 and CD8 Treg in the control of CD4 Th1 pathogenic T cells (7). The dependence of CD4 Treg on CD8 Treg is demonstrated by the fact that transfer of TCR peptide specific CD4 Treg into CD8 knockout mice does not confer protection (70). We recently showed that TCR peptide-reactive T cells induce *in vivo* apoptotic deletion of MBPAc1-9 activated dominant V β 8.2⁺CD4⁺ T cells in wild type but not CD8-depleted B10.PL mice, suggesting that TCR peptide induced-CD8 Treg mediate apoptotic elimination of the target cells (58). This mechanism of regulation appears not to be unique to our model as Jiang et al. demonstrated that TCv induced a CD8 Treg that displayed specific cytotoxicity *in vitro* towards V β 8 transfectants (53, 71). Additionally, others have demonstrated that cytolytic T-T interactions are responsible for the suppression of MBP specific CD4 T cells in TCv vaccinated human and rats (42, 43). We have shown that the CD8 Treg clones show specific cytotoxicity towards V β 8.2⁺, but not V β 8.2⁻ T cell clones (Tang et al. manuscript in preparation).

The induction (and priming) of CD8 Treg

While the direct *in vivo* identification of a natural CD8 Treg has proved difficult, the induction of Treg has been described in many experimental systems following *in vitro* manipulations, including the co-culture of naive T cells with tolerogenic APC and/or exogenous cytokines such as IL-2, TGF- β , GM-CSF and IL-10 (23, 26, 84-86). Gilliet and Liu generated human CD8 Treg by priming naive CD8 T cells with allogeneic CD40 ligand-activated plasmacytoid DCs (DC2) (26). CD8 Treg induction was dependent on antigen presentation and IL-10 production from the DC2. This is in line with the recent report that naive CD8 T cells may differentiate into CD8⁺CD28⁻ Treg cells under the sole influence of IL-10 (87). IL-10 inhibits the maturation of DCs. So it is likely that in these cases the weak stimulation

provided by the DCs induces a regulatory phenotype in the CD8 T cell. However, such systems must be treated with caution as their physiological relevance is often questionable.

CD8 Treg can also be generated *in vivo* using oral or neonatal tolerance-inducing protocols. However, the direct isolation of the Treg has proved difficult. In an oral tolerance model, Ke et al. demonstrated that oral administration of OVA protein activated spleen cells that transferred unresponsiveness to naive syngeneic mice (88). Suppression was mediated by CD4⁺CD8⁺ T cells as demonstrated by CD8 T cell depletion with monoclonal antibody. In a similar setting, neonatal Brown-Norway rats administered with mercury displayed a dominant tolerance specific for the metal. *In vivo* CD8 depletion broke the tolerance, and CD8 T cells could transfer the tolerance to syngeneic naive rats (14, 89, 90).

In addition to tolerogenic antigens and TCv, vaccines incorporating *in vitro* manipulated APCs have recently been reported to induce CD8 Treg *in vivo* (91, 92). In a small human cohort, Dhondapkar and Steinman reported that immunization with immature DCs pulsed with influenza matrix protein (IMP) induced a peptide-specific Treg cell population (91). CD8 T cells isolated 7 days after immunization demonstrated *in vitro* suppression of T effector cell responses to IMP stimulation. Faunce et al. demonstrated that suppression of ongoing EAE was dependent on the development of CD8 Treg after tolerogenic APC immunization (92). Furthermore, adoptive transfer of CD8 T cells from tolerogenic APC treated mice into naive mice could prevent EAE induction in an antigen specific manner.

It has yet to be determined whether the CD8 Treg described above are experimentally manipulated conventional CD8 T cells or expanded naturally occurring CD8 Treg. Najafian and colleagues reported the transfer of purified CD8⁺CD28⁻ T cells from a naive mouse into a CD8^{-/-} recipient was associated with a significant decrease in EAE disease incidence when compared to control groups (93). This observation is suggestive of a population of naturally occurring Treg cells residing within the CD8⁺CD28⁻ T cell population. However, naturally occurring CD8⁺CD28⁻ T cells isolated from healthy humans fail to show suppressive activity (87). This highlights the problems of studying polyclonal populations which may contain Treg, but the identification and function of Treg is masked by the non-Treg majority. Thus until a specific marker is identified studies focusing at the clonal Treg level will prove most valuable.

During the primary response certain pathogenic T cell clones may become dominant (94). Specific down regulation of the late phase of the primary response and protection from secondary inflammatory responses may be mediated by CD4 and CD8 Treg recognizing TCR epitopes associated with pathogenic T cell clones (7). In the B10.PL mouse 20-30% of the peripheral TCR repertoire is V β 8⁺. Uptake of apoptotic cells and cross-presentation of antigenic determinants derived from apoptotic cells has been widely described (95, 96). During the normal peripheral turnover of T cells, APCs may capture dead cells and present low levels of processed TCR peptides to the Treg. However, during an inflammatory event,

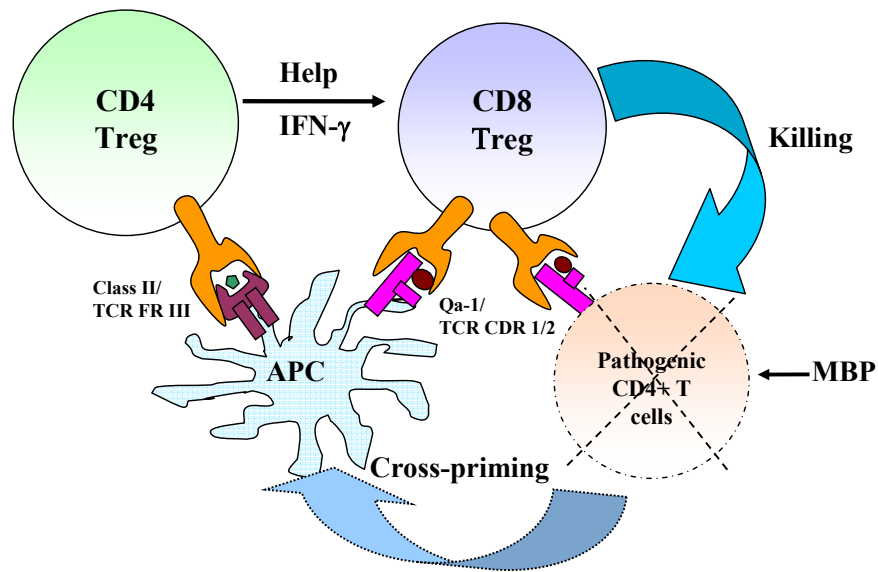


Figure 1. A model of specific immune regulation. During normal peripheral turnover or following the expansion/contraction phase, MBP-reactive CD4⁺ T cells are captured by professional APCs. These APCs process and present distinct TCR peptides in the context of MHC class II and Qa-1 MHC class Ib molecules for the induction of CD4⁺ and CD8⁺ Treg, respectively, a process commonly referred to as cross-priming. CD4⁺ Treg recognize an Fr3 region TCR peptide, and secrete type 1 proinflammatory cytokines, such as IFN- γ , for effective recruitment or activation of CD8⁺ Treg. CD8⁺ Treg recognize CDR1/2 region TCR peptide/Qa-1 complexes on the surface of activated and pathogenic CD4 Th1 cells, resulting in their apoptotic death. Low avidity, slower-reacting Th2 cells that are relatively less susceptible to apoptosis can then eventually expand, resulting in immune deviation of the anti-MBP response at the population level. At this stage, Th2 cell secretion of cytokines such as IL-4 or IL-10 can further enhance the down-regulation of the anti-MBP response.

higher levels of TCR peptide derived from an increased number of apoptotic V β 8.2⁺ T cells would be presented by APCs, along with high levels of co-stimulation signals, to Treg. Under such conditions Treg would receive full activation signals and orchestrate the specific down-regulation of the V β 8.2 pathogenic T cells. This mechanism of down-regulation may not just be applicable to the control of autoimmunity. During an antiviral immune response, T cells expressing unique TCR V β chains and recognizing a specific viral determinant can account for up to 50% of the peripheral T cell repertoire (97). Many of these cells undergo activation-induced cell death. The resulting apoptotic T cells may be captured and their TCRs processed and presented by APCs to anti-TCR CD4 and CD8 Treg. Contraction of the anti-viral response would be associated with the cross-priming of TCR peptide-specific CD4 and CD8 Treg that specifically down-regulate the anti-viral T cell response.

We have proposed in the B10.PL EAE model that capture of apoptotic V β 8.2 T cells by professional APCs and cross-presentation of the TCR peptides to prime CD4 and CD8 Treg is an essential part of the negative feedback mechanism of TCR-based regulation. We have recently found that APCs isolated from the spleen during active disease can stimulate V β 8.2 TCR peptide determinant-reactive CD4 Treg in *in vitro* cultures without the addition of exogenous antigen. Additionally, larger numbers of irradiated splenocytes derived from naive B10.PL mice can activate CD4 Treg clones, again in the absence of exogenously added antigen,

suggesting that the “steady state” APCs are presenting low levels of TCR peptide to Treg and the level of stimulation is increased during inflammatory disease. Data indicate that DCs are the APC that provide the strongest stimuli to TCR-reactive Treg. We have recently demonstrated the efficient uptake of apoptotic V β 8.2 T cells by immature DCs. Furthermore, in co-culture experiments, DCs pre-pulsed with apoptotic V β 8.2 T cells are able to stimulate V β 8.2-reactive CD4 Treg. Preliminary data indicate TCR reactive Qa-1 restricted CD8 Treg are also stimulated in similar co-cultures (Smith et al. unpublished observations). Overall, the results suggest that DCs capture T cells undergoing apoptosis and “cross-present” TCR peptides in both class II and non-classical MHC class I contexts (Figure 1).

Summary:

A model of specific immune regulation

Regulatory CD4 and CD8 T cells specifically reactive with distinct TCR peptides are involved in the resistance to EAE induction mediated by CD4⁺V β 8.2⁺ pathogenic T cells (Figure 1). The CD4 Treg recognize a determinant from framework III region of the TCR V β 8.2 chain and are restricted by MHC class II molecules, whereas the CD8 Treg recognize a determinant from CDR1/2 region of the same TCR V β 8.2 chain and are restricted by Qa-1, non-classical class Ib molecules. In addition CD8 Treg require help from

CD4 Treg and are the final effectors, killing antigen activated pathogenic CD4 T cells bearing the TCR V β 8.2 receptor. The determinants recognized by both CD4 and CD8 Treg are cross-presented by antigen presenting cells (APCs) that capture apoptotic TCR V β 8.2⁺ CD4 T cells *in vivo*. Finally, the combined action of CD4 and CD8 Treg prevents pathogenic CD4 T cells from expanding *in vivo* to mediate clinical EAE. This model defines the mechanisms of TCR-based immune regulation *in vivo*, and has aided in designing better therapeutic strategies for TCV and other related immunotherapies (98-101). This pathway for priming T cells with specific down-regulatory ability may represent a general mechanism for acquiring and maintaining homeostasis following infections, transplantation and autoimmunity (7).

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