2
Priming Regulatory T Cells and Antigen-Specific Suppression of Autoimmune Disease

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1 Summary

It is now widely recognized that regulatory T cells (Treg) play an important role in protecting the body from autoimmune diseases. T cells with suppressor activity have been identified in both CD4+ and CD8+ T cell populations in humans and animal models of inflammatory disease. Here we provide a brief review of the field of T cell suppression with special emphasis on CD8+ T cell-mediated regulation of immune responses to self. We focus on the role of CD8+ Treg in the control of myelin basic protein-reactive T cells in experimental autoimmune encephalomyelitis, a model for human multiple sclerosis. We address how Treg can be specifically induced to downregulate an immune response.

2 Introduction

Central tolerance in the thymus is the immune system's primary mechanism of purging the body of self-reactive T cells. However, thymic tolerance is not absolute, and potentially pathogenic T cells with specificity to self-antigens are present in the

periphery (Bouneaud et al., 2000). Once activated such T cells are capable of mediating inflammatory reactions against one's own tissues, and these responses may manifest as autoimmune disease (e.g., multiple sclerosis, type 1 diabetes, Crohn's disease). The immune system has thus developed peripheral mechanisms to control aberrant autoimmune reactions. In addition to T cell anergy (Schwartz, 2003) and activation-induced cell death (AICD) (Kabelitz et al., 1993), active suppression by regulatory T cells has now been recognized as a major peripheral mechanism to protect against detrimental autoimmune reactions (Kumar, 2004; Sakaguchi, 2005; Shevach, 2000). In experimental systems where Treg has been depleted or disabled, autoimmune disease has been frequently observed (Piccirillo and Shevach, 2004). In turn, experimental strategies targeting Treg are likely to prove effective in the treatment of autoimmunity (Hori et al., 2003).

Here we discuss the importance of Treg, describing how they can be targeted to help protect against or treat autoimmune disease. We focus on the studies performed in our laboratory that have deciphered the role of CD4 and CD8 Treg in mediating antigen-specific protection from experimental autoimmune encephalomyelitis (EAE), a surrogate mouse model for multiple sclerosis in humans.

3 Identification of T Cells With Suppressor Function

The existence of a T cell population with a regulatory role in autoimmune disease was proposed after the experiments performed by Nishizuka and Sakakura in 1969. They reported that thymectomy in a neonatal (day 3) rodent resulted in the development of autoimmune disease. Further experiments demonstrated that reconstitution of the day 3 thymectomized rodent's T cell population with thymocytes from an adult mouse protected against autoimmune disease. T cells with suppressor function were first described by Gershon and Kondo during the 1970s. It was demonstrated that adoptive transfer of T cells derived from an animal tolerant to a given antigen suppressed antibody responses to that antigen in the recipient (Gershon and Kondo, 1970). In similar systems the downregulation of type I hypersensitivity or cell-mediated delayed hypersensitivity reactions, after the transfer of T cells derived from tolerized animals, was reported (Askenase et al., 1975; Takatsu and Ishizaka, 1975). The suppressor T cell activity was found to reside in the Lyt2+ (CD8) T cell population (Cantor et al., 1976). However, further characterization of this T suppressor cell population was hindered by the inability to characterize these cells molecularly in a well defined antigenic system. Furthermore, realization of T cells with different type 1 and type 2 cytokine secretion during the mid-1980s led many immunologists to believe that T cell-mediated suppression could be explained by conventional T cell subsets secreting counterinhibitory cytokines, not by a population of T cells with dedicated suppressor function (Green and Webb, 1993; Salgame et al., 1991).

Despite skepticism, several laboratories continued to study T cell-mediated suppression (Kumar and Sercarz, 1993; Sakaguchi et al., 1985, 1995). In 1995, Sakaguchi and colleagues published the seminal paper identifying a subpopulation

of T-helper cells constitutively expressing the CD25 cell surface marker with the ability to protect against autoimmune disease. Athymic BALB/c mice inoculated with T cell suspensions depleted of CD4+CD25+ T cells developed spontaneous autoimmune disease. Disease could be prevented if mice were reconstituted with CD4+CD25+ T cells shortly after inoculation with the CD4+CD25− T cells (Sakaguchi et al., 1995). Discovery of a cell surface phenotype to identify a population of T cells with suppressor function rekindled the interest of many immunologists into T cells with dedicated suppressor function. CD4+CD25+ Tregs have been characterized as a naturally occurring anergic population that is FoxP3+, CTLA-4+ in both humans and animals (Piccirillo and Shevach, 2004). To date the mechanism of suppression by CD4+CD25+ T cells has yet to be fully defined. However, it is generally agreed that the mechanism is contact-dependent, and it involves different molecules, including interleukin-10 (IL-10) or transforming growth factor-β (TGFβ) in some models. It is likely that within the CD4+CD25+ T cell population there are multiple subsets of T cells utilizing different mechanisms of suppression, as most studies are based on the isolation of polyclonal populations.

Other experimentally induced CD4+ Treg subsets have been described. These include Th3 cells, which can be induced by oral tolerance protocols (Weiner, 2001). Such cells mediate their suppressor function through secretion of TGFβ. Tr1 cells mediate suppression by secretion of IL-10 and can be induced by various immunization protocols that may be dependent on naive T cell interactions with antigen-presenting cells (APCs) in a non-activated/immature state (Jonuleit et al., 2000).

CD8 Treg populations have been described in many systems. They include CD8+CD28− Treg (Filaci and Suciu-Foca, 2002), CD8+CD75+ Treg (Zimring and Kapp, 2004), CD8+CD25+ Treg (Cosmi et al., 2003), and anti-T cell receptor (TCR) CD8αα+ Treg (Kumar, 2004; Tang et al., unpublished data). CD8+CD28− Treg can be generated in vitro from naive T cells in an allogeneic mixed leukocyte reaction (MLR) or by the direct use of IL-10 (Filaci and Suciu-Foca, 2002). CD8+CD28− Tregs have been shown to mediate allosuppression by inhibiting APC maturation. Analogous to CD4+CD25+ Treg cells, CD8+ T cells co-expressing the CD25 molecule with suppressive ability have been identified. Akin to their CD4+ counterparts, CD8+CD25+ Tregs appear to be a naturally occurring population, FoxP3+ and CTLA4+; and their regulatory function is cell–cell contact-dependent (Cosmi et al., 2003). Peripheral CD8+CD25+ T cells with in vitro suppressor ability have also been shown to be a naturally occurring population in major histocompatibility complex II (MHC II)-deficient mice (Bienvenu et al., 2005). Although similar to CD4+CD25+ T cells with respect to CTLA-4 and Foxp3 expression, murine CD8+CD25+ T cells strongly proliferate and produce interferon-γ (IFNγ) upon in vitro stimulation in the absence of exogenous IL-2. It has yet to be determined if CD8+CD25+ Treg cells function in vivo to protect against autoimmune disease or whether their mechanism of action is IFNγ-dependent. However, naturally occurring rat CD8+CD45RC^{low} have been shown in vivo to protect against CD4+ T cell-mediated graft-versus-host-disease by a mechanism that appears to be cell–cell contact-dependent (Xystrakis et al., 2004).

3.1 Studying Treg Populations at the Clonal Level Using the EAE Model

Experimental autoimmune encephalomyelitis can be induced in susceptible animals by adoptive transfer of T cell clones or lines reactive to myelin antigens or by immunization with myelin proteins, such as myelin basic protein (MBP) emulsified in complete Freund's adjuvant (CFA). PL/J and B10.PL mice spontaneously recover from and become refractory to subsequent induction of EAE (Kumar, 2004). Evidence suggests that CD8 Tregs arise naturally to protect from EAE (Madakamutil et al., 2003; Tang et al., in press). In 1992, it was demonstrated that after CD8+ T cell depletion prior to induction of EAE the mice were no longer protected from reinduction of EAE (Jiang et al., 1992). Additionally, a more chronic form of EAE was seen in CD8-/-PL/J H-2^u mice (Koh et al., 1992). Our laboratory has elucidated a feedback inhibition regulatory mechanism that incorporates both anti-TCR CD4+ and CD8+ Tregs that control MBP-reactive CD4+ T cells and thereby protect animals from EAE (Kumar, 2004). The induction of anti-TCR Treg has also been demonstrated in Lewis rats and B10.PL or PL/J mice during the recovery from EAE (Vandenbark et al., 1996; Jiang and Chess, 2000). The EAE model in the B10.PL or PL/J mouse provides the ideal system to study the induction and function of Treg. First, immunization with MBP emulsified in CFA induces a clonotypic CD4+ T cell response to MBP directed toward a single determinant (Kibler et al., 1977; Zamvil et al., 1986); second, most of the pathogenic CD4+ T cells responding to this determinant express the Vβ8.2 TCR chain (Acha-Orbea et al., 1988; Kumar et al., 1989; Urban et al., 1988). These characteristics provide a specific target for regulation. Third, disease is generally monophasic; most of the animals recover spontaneously and are refractory to disease reinduction. The observation that animals became resistant to disease indicates the induction of an active regulatory mechanism to downregulate the pathogenic CD4+ T cell population. Further investigation demonstrated that immunization with peptides derived from the Vβ8.2+ TCR chain in both mice and rats could protect the animal from EAE, indicating that a population of Tregs specific for TCR determinants on the pathogenic T cell population could regulate disease (Howell et al., 1989; Kumar and Sercarz, 1993; Kumar et al., 1995; Vandenbark et al., 1989). Importantly, these TCR-derived peptides are also immunogenic in the human population and have been used to halt progression of multiple sclerosis (Saruhan-Direskeneli et al., 1993; Vandenbark et al., 1996).

3.2 Role of Regulatory T Cells in Human Autoimmune Disease

Studies confirming a role for Treg in human autoimmune disease are limited and fraught with practical difficulties. Major hindrances include identification of disease-associated autoantigens, heterogeneity of the human T cell repertoire, sample collection, and a lack of in vivo experimental techniques. Despite such limitations, studies have detected defects in functional Treg populations in

TABLE 2.1. Treg in Autoimmune Diseases.

Regulatory cell	Arthritis		Multiple sclerosis		Diabetes		Inflammatory bowel disease	
	Human	Rodent	Human	Rodent	Human	Rodent	Human	Rodent
CD4+, CD25+, Treg	+[1]	+[2]	+[3]	+[4]	±[5/6]	+[7]	+[8]	+[9]
Tr1	±	+[10]	±[11]	+[12]	±	+[13]	±	+[14]
Th3	ND	ND	+[5]	+[16]	ND	ND	ND	+
Anti-TCR, Treg/ CD8, Treg	ND	+[17]	+[18]	+[19]	+[20]	+[21]	+[22]	ND

Table supplies the evidence for a protective role of different subsets of Treg in humans and the experimental rodent model of various autoimmune diseases. Rodent model for arthritis, collagen-induced arthritis; for multiple sclerosis, EAE; for diabetes, nonobese diabetic (NOD) mouse; for inflammatory bowel disease, experimental colitis.

+, evidence for; ±, evidence for and against; ND, not determined.

[1]Ehrenstein et al., 2004; [2]Morgan et al., 2005; [3]Vigiletta et al., 2004; [4]Kohm et al., 2002; [5]Putnam et al., 2005; [6]Lindley et al., 2005; [7]Chen et al., 2005; [8]Kelsen et al., 2005; [9]Martin et al., 2004; [10]Quattrocchi et al., 2001; [11]Aharoni et al., 2005; [12]Cua et al., 2001; [13]You et al., 2004; [14]Groux et al., 1997; [15]Fukaura et al., 1996; [16]Weiner, 2001; [17]Honda et al., 2004; [18]Zhang et al., 1995; [19]Madakumatil et al., 2003; [20]Bisikiriska et al., 2005; [21]Panoutsakopoulou et al., 2004; [22]Brimnes et al., 2005.

autoimmune disease and allergy, and immunotherapeutic studies suggest that targeting Treg populations may be critical for efficacy (Bisikirska et al., 2005; Hong et al., 2005; Kumar et al., 2001; Lan et al., 2005).

Both CD4+ and CD8+ T cells with regulatory function have been described in many autoimmune settings and represent a target for disease intervention. Table 2.1 summarizes studies in the autoimmune disease setting in which regulatory T cells have been shown to be associated.

4 Immunotherapeutic Strategies to Treat Autoimmune Disease

The prevalence of autoimmune disease in the human population is steadily rising, with approximately 5% of the U.S. population currently afflicted (Jacobson et al., 1997). The mainstay immunosuppressive treatments include corticosteroid and nonsteroidal antiinflammatory or cytotoxic drugs. These treatments are palliative aimed at nonspecifically reducing inflammation or killing dividing cells. The usage of such drugs is hindered by their limited selectivity and significant toxicity. However, novel therapeutic strategies based on immunomodulation of inflammatory cytokine networks are now available, and therapies targeting Treg are currently being developed (see below).

Inflammatory cytokine networks can be modulated to curtail autoimmune manifestations. For example, an anti-tumor necrosis factor-α (TNFα) monoclonal antibody (mAb) has been approved by the Food and Drug Administration (FDA) and has shown efficacy in the treatment of rheumatoid arthritis (RA) and Crohn's disease (Feldman and Steinman, 2005). IFNβ has demonstrated efficacy in the treatment of relapsing remitting multiple sclerosis (MS) (Revel, 2003). However, both of these treatment regimens still suffer from a lack of specificity, which hinders both their efficacy and safety. For example, IFNβ treatment is effective in only 30% of relapsing remitting MS patients, and its mechanism of action is still unclear (Revel, 2003). Anti-TNFα treatment has been associated with exacerbation of latent tuberculosis in some patients (Keane et al., 2001).

4.1 Induction of Treg (Nonspecific)

As discussed above, Treg populations can downregulate pathogenic immune responses and represent a valid target for immune intervention. However, the techniques employed to induce these cells have yet to be optimized. In experimental TCR-transgenic models disease-regulating CD4+CD25+ Treg can be generated and/or activated by antigen vaccination, (Thorstenson and Khoruts, 2001; Zhang et al., 2001). The generation of regulatory cells has also been demonstrated by the use of general immunosuppressive drugs. Barrat and colleagues demonstrated that a cocktail of vitamin D_3 and dexamethasone could induce IL-10-producing CD4+ T cells with in vivo suppressor ability (Barrat et al., 2002). Recently, Bisirikia and colleagues demonstrated that OKT3 (a modified anti-CD3 mAb) activated a population of CD8+CD25+ Treg cells that could suppress the in vitro responses of CD4+ T cells derived from type 1 diabetes patients (Bisirikia et al., 2005). Furthermore, treatment with copolymer-I, a polymer consisting of four randomly joined amino acids that has proven efficacy in the treatment of MS, increased the number of Foxp3+ CD4+CD25+ T cells in the peripheral blood of MS patients. In vitro analysis showed copolymer-I generated CD4+CD25+ T cells expressing high levels of FoxP3 and exhibiting increased regulatory ability (Hong et al., 2005). Karandikar and colleagues have also published data suggesting that copolymer-I treatment targets CD8+ Treg (Karandiker et al., 2002). Initially CD8+ T cells from MS patients were found to respond poorly to copolymer-I. However, after copolymer-I treatment the patients' CD8+ T cell responses were restored. Interestingly, significantly enhanced IFNγ expression by the CD8 T cells was recorded, and it was proposed that copolymer-I treatment restored the IFNγ-dependent suppressor ability of CD8 T cells. The above studies indicate that the augmentation of Treg ability correlates with the efficacy of immunotherapy.

4.2 Induction of Treg (Antigen-Specific)

The ideal immunosuppressive therapeutic strategy would be one that specifically targets the disease driving immune cells (i.e., the cells reacting directly to the body's self-antigens). By blocking or modulating the function of these cells one

would be able to manipulate the underlying cause of disease. Such treatment strategies are dependent on identification of the autoantigen or antigens involved in disease pathogenesis and the cells reacting to these antigens. Strategies based on inducing tolerance to defined antigens have shown much promise in experimental systems where the inflammatory response is directed toward a single autoantigen on a defined genetic background (Bielekova and Martin, 2001). In such systems the animal could be vaccinated in a tolerogenic manner with a peptide (e.g., p87-99 epitope of MBP) or whole antigen (e.g., MBP) to modulate the disease course (Brocke et al., 1996). However, on translation onto heterogeneous human backgrounds, the efficacy of such treatments has been unpredictable (Bielokova et al., 2000; Kappos et al., 2000). One hindrance to therapies based on targeting tissue antigens is the phenomenon of epitope spreading (Sercarz et al., 1993). Although the initial immunological response may be toward a single determinant, during the period between the initial insult and the manifestation of clinical disease the focus of autoimmune reaction may have spread to other tissue antigens. Thus peptide therapy targeting only one or a few autoantigens may be insufficient at the time of clinical disease.

Another therapeutic strategy with antigen specificity targets receptors expressed on pathogenic disease-mediating T cell populations. When treating autoimmune disease, the aim of T cell vaccination (TCV) is to induce a population of T cells that react specifically against and inhibit the disease causing the pathogenic T cell population. Evidence suggests that TCV induces a population of Tregs that recognize clonotypic antigens on pathogenic T cells (Sun et al., 1988; Zhang et al., 1995). Experimental evidence suggests that these antigens are expressed within the variable region of the TCR on the pathogenic T cell (Jiang et al., 1998). Studies have demonstrated that vaccination with TCR-derived peptides can protect mice from EAE (Kumar and Sercarz, 1993; Kumar et al., 1995; Vandenbark et al., 1989). This indicates that within the bodies' immune system there is a naturally occurring subset of Treg that recognize conserved TCR regions with the ability once primed to downregulate cells expressing a particular TCR. Tregs have been shown to reside in both CD4+ and CD8+ T cell populations (Kumar and Sercarz, 1993; Tang et al., unpublished data). The induction of Treg can explain how the B10.PL mouse spontaneously recovers from EAE and becomes resistant to further disease induction. In this model we have shown that MBP immunization plus adjuvant leads to a TCRVβ8.2+ CD4+ T cell-mediated inflammatory disease, and recovery is associated with the activation of CD4+ and CD8+ T cells reactive against epitopes within the TCRVβ8.2+ (Kumar, 2004).

Over the last decade our laboratory has put together the pieces and deciphered the regulatory network engaged in suppressing the EAE-mediating TCRVβ8.2+ CD4+ T cell responses in the B10.PL mouse. We have now characterized two Treg (CD4+ and CD8+) populations that work together specifically to downregulate the pathogenic T cells. Injection of a peptide from the Vβ8.2+ TCR framework 3 region was demonstrated to protect mice from EAE,

and characterization of the reacting T cells revealed that this MHC II-restricted peptide induces a Vβ14+ TCR CD4+ Treg population (Kumar and Sercarz, 1993). Additionally, CD8 depletion revealed that CD8+ T cells are also essential for disease protection and downregulation of the Vβ8.2+ TCR T cell population (Kumar et al., 1997). Recent studies have characterized a novel population of CD8+ Tregs. The CD8+ Treg population recognizes a different determinant in the CDR2 region of the Vβ8.2+ TCR in the context of the nonclassical MHC I molecule Qa-1(Tang et al., in press). The data suggest that CD4+ Tregs help in the recruitment and activation of the CD8 Tregs. It is the CD8 Tregs that ultimately kill the pathogenic CD4+ T cell population by apoptosis induction (Madakamutil et al., 2003). This mechanism of clonotypic feedback regulation is depicted in Figure 2.1.

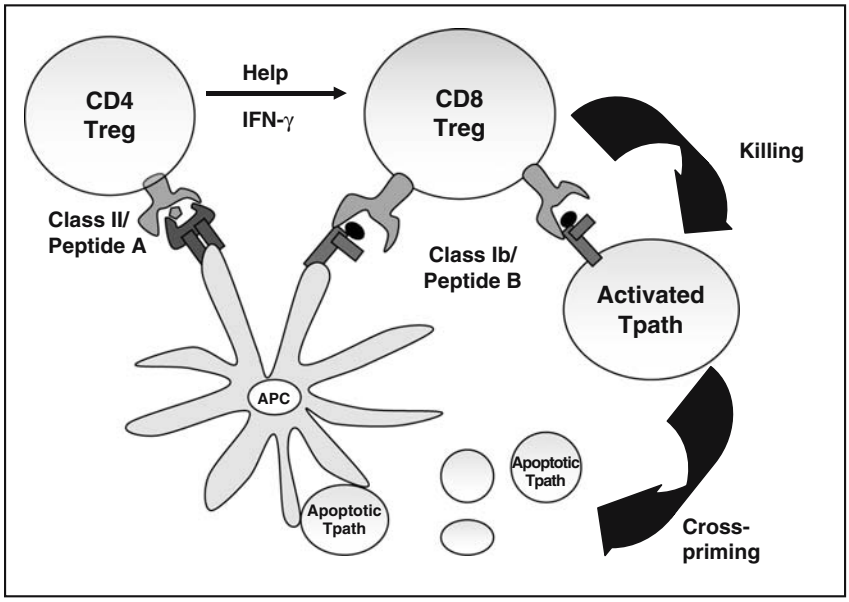

FIGURE 2.1. Negative-feedback mechanism targeting T cell receptors (TCRs). The working model of negative feedback TCR-based regulation depicts Treg-mediated killing of activated Tpath cells in a TCR-specific manner. CD4+ Treg recognizes a TCR-derived peptide in the context of a major histocompatibility complex II (MHC II) molecule and provides help for the CD8+ Treg. CD8+ Treg, recognizing another distinct peptide from the same TCR in the context of the nonclassic MHC class Ib molecule Qa-1, kills the activated pathogenic T cell population by inducing apoptosis. To complete the negative feedback loop, the apoptotic T cells are captured by antigen-presenting cells (APCs), and their TCR-derived peptides are processed and presented to cross-prime Treg.

5 Antigen-Presenting Cells and Priming of Treg

Antigen-specific immunotherapy is dependent on Tregs recognizing a specific peptide determinant in the context of an MHC molecule displayed on the surface of an APC. Therefore, the APC is central to the immunotherapeutic mechanism. The most effective APC in the priming of T cells is the dendritic cell (DC). The ability of a DC to prime an immune response is dependent on its maturation state. Immature DCs are highly adept at capturing antigens but have low cell surface levels of co-stimulatory molecules hindering their ability to prime T cells strongly. On the other hand, under certain conditions such as inflammation, DCs receive signals to become activated. For example, DCs can be innately stimulated through their Toll-like receptors by products derived from microbes such as lipopolysaccharide (LPS) or unmethylated CG dinucleotides (CpGs) (Pasare and Medzhitov, 2004). Activation of the DC results in upregulation of co-stimulatory molecules and secretion of soluble mediators, resulting in an enhanced ability to stimulate T cells. The activation state thus determines the type of immune response a DC can mediate. In the "nonactivated," immature state DCs may present antigens to naive T cells in such a context that induces peripheral tolerance (Hawiger et al., 2001), although mature DCs can mediate strong "productive" immune responses to fight infection.

Various types of Treg may be primed by activated and nonactivated DCs. Immature DCs have been demonstrated to be essential in the development of Tr1-like cells. Jonuleit and colleagues demonstrated the in vitro generation of IL-10-producing T cells after stimulating naive CD4+ T cells with immature DCs (Jonuleit et al., 2000). The in vivo generation of CD8 Treg has been demonstrated after immunization with immature DCs pulsed with influenza matrix protein (IMP). Here it was shown that CD8 T cells isolated 7 days after immunization could suppress T effector cell responses to IMP in vitro (Dhodapkar and Steinman, 2002). Faunce et al. demonstrated that DCs rendered tolerogenic by exposure to TGFβ and MBP could induce CD8+ Tregs that could protect against EAE (Faunce et al., 2004). In turn, CD8+ Treg cells have been shown to mediate the APC function. Chang and colleagues demonstrated that CD8+CD28− T suppressor cells acted on immature DCs, inducing upregulation of the inhibitory receptors ILT3 and ILT4, rendering the APCs capable of anergizing T-helper cells (Chang et al., 2002). Furthermore, the group showed that human CD8+CD28− T suppressor cells from heart allograft recipients acted on both professional APCs and endothelial cells carrying donor MHC I antigens, converting them to a tolerogenic state (Chang et al., 2002; Manavalan et al., 2004). The above studies are examples of how CD8 Tregs can both mediate and be mediated by APCs. The CD8 Treg function is absolutely dependent on interactions with APCs.

The mechanisms involved in the priming of anti-TCR CD8+ Tregs are currently under investigation. Anti-TCR CD8 Treg cells recognize their cognate antigens in the context of the nonclassical MHC I molecule Qa-1. Evidence that Qa-1 molecules may be involved in this regulation came from studies that demonstrated that the activity of CD8+ T cell hybridomas generated from T cell-vaccinated mice

appeared to be Qa-1b-dependent (Jiang and Chess, 2000; Jiang et al., 1995, 1998). Our laboratory has now generated functional CD8+ Treg clones reactive to a specific TCR peptide. We have shown that upon adoptive transfer these Qa-1-restricted CD8+ Treg clones protect against Vβ8.2+ TCR T cell-mediated autoimmune disease. Furthermore, Tregs react to the p42-50 peptide derived from the Vβ8.2+ TCR (Tang et al., in press). Functional Qa-1 molecules are upregulated on the surface of activated DCs and T and B lymphocytes (Sarantopoulis et al., 2004; Tang et al., in press) and, as depicted in Figure 2.1, are targets for regulation in our model. Currently, we are investigating how the Qa-1 molecules acquire their cognate antigen p42-50 derived from the Vβ8.2+ TCR in the EAE model. We know that in the B10.PL mouse 20% to 30% of the peripheral TCR repertoire is Vβ8+; and during an immune response a significant proportion of these cells undergo apoptotic death (in press)). The uptake of apoptotic cells and the cross-presentation of antigen determinants derived from these cells has been widely described (Albert et al., 1998; Mougneau et al., 2002).

Our data suggest that DCs can capture apoptotic CD4 T cells and process and present Vβ8.2+ TCR-derived peptides to prime CD4+ and CD8+ Tregs. Such a mechanism explains how EAE can be naturally downregulated in the B10-PL mouse without the need for exogenous antigens to stimulate Treg. This mechanism may also be applicable in the downregulation of an antiviral immune response (Kumar, 2004). For example, cells expressing a unique TCR-Vβ chain can account for up to 50% of the peripheral T cell population during an antiviral immune response (Murali-Krishna et al., 1998). We would predict specific TCR-derived epitopes, captured from apoptotic T cells generated during an antiviral response, to be presented by APCs to prime Treg. The primed Treg cells would orchestrate the TCR-specific downregulation of the antiviral response.

One caveat to this proposed mechanism was that APCs may present TCR-derived peptides from apoptotic T cells during normal peripheral turnover. If Tregs were primed under such circumstances, the body's ability to launch productive immune responses to evading pathogens would be hindered. However, our preliminary data indicate that the effector function of the Tregs is under strict control of the APCs. Using immature/nonactivated DCs pulsed with apoptotic Vβ8.2TCR+ T cells, we have been able to demonstrate only weak CD4+ Treg priming in in vitro co-cultures. However, if after pulsing with apoptotic Vβ8.2TCR+ T cells the DCs were activated, strong CD4+ and CD8+ Treg priming was detectable. These observations suggest that Treg may be functionally primed only under conditions when they encounter activated DCs. Such DCs would be constantly sampling apoptotic T cells but functionally priming Tregs only when they receive activation signals. Thus CD8+ Treg would function to downregulate TCR-specific responses only during inflammation and not under steady-state conditions (Fig. 2.2). Furthermore, we have found that $CD11c^+$ DCs isolated from mice during EAE could stimulate CD4Treg in vitro, whereas DCs isolated from naive mice could not (unpublished data). We have additional data indicating that only the activated Vβ8.2TCR+ T cells are regulated. Nonactivated T cells have low-level Qa-1 cell surface expression and are not targeted by the

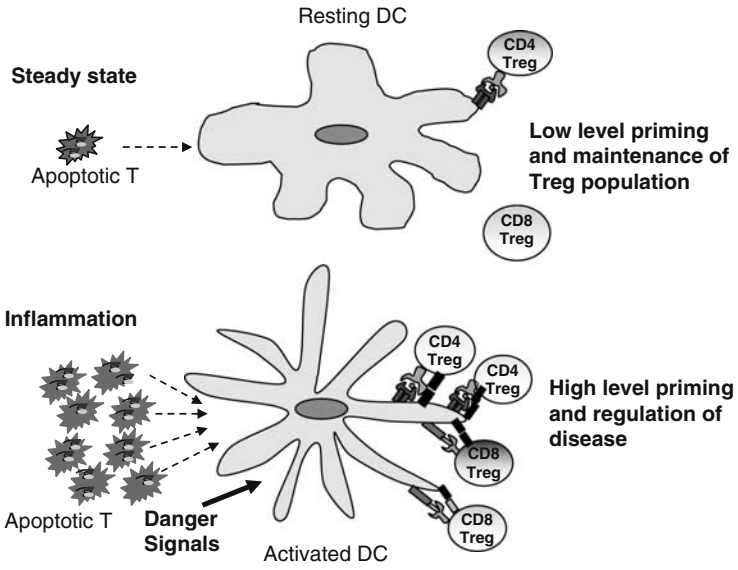

FIGURE 2.2. Dendritic cells (DCs) present TCR-derived peptides from apoptotic T cells to Treg. For priming of TCR-reactive Treg, we predict that apoptotic TCRVβ8.2+ T cells are captured by the APCs, and the TCR-derived peptides are processed and presented. In the steady state, this may occur during natural apoptotic turnover of T cells. During inflammatory disease, when there is a high level of apoptosis, capture may be increased. The apoptotic T cell's TCR-derived peptides may then be cross-presented via the MHC II pathway to prime CD4+ Treg and via the nonclassic MHC class Ib pathway to prime CD8+ Treg. In an inflammatory milieu, activated DCs would provide stronger Treg priming and thus, predictably, boost efficient immune regulation.

Qa-1-restricted Treg. Therefore, only the MBP-reactive T cells that have been activated are targeted, and most of the TCRVβ8.2+ T cells are not subjected to Treg-mediated suppression.

6 Conclusion

Here we have discussed the involvement of Tregs in both human and experimental animal autoimmune disease settings. There is now overwhelming evidence that Tregs play an important role in suppressing aberrant immune responses to self. Several mechanisms of suppression have been proposed, and identifying the appropriate regulatory T cell to treat specific disease processes may be essential for effective treatment protocols. We have focused on a TCR-based regulatory mechanism that specifically operates on activated T cells. We believe that therapies targeting this mechanism will downregulate and confer protection against aberrant immune reactions involving T cell responses.

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