Springer Semin Immun (2006) 28:51–62
DOI 10.1007/s00281-006-0019-2

REVIEW

The role of virus-induced regulatory T cells in immunopathology

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Received: 27 April 2006 / Accepted: 17 May 2006 / Published online: 14 July 2006

Abstract In recent years, regulatory T cells have received increased attention for their role in immune responses to microbial infections. The list of microbial pathogens associated with regulatory T cell responses is growing rapidly and includes bacteria, viruses, parasites, and fungi. As the biology of regulatory T cells is revealed, we are discovering that their induction during infection is a normal aspect of immunity, necessary to limit collateral damage from inflammatory responses and aggressive immunological effectors. Thus, these cells play a critical role in maintaining the delicate balance between preventing immunopathology and allowing the immune response to clear infections. While generally successful, there are notable exceptions where regulatory T cell-mediated suppression appears to be responsible for allowing certain viruses to establish and maintain a persistent state. In this review, we will discuss our current understanding of what virus-induced regulatory T cells are, how they are induced, and what mechanisms they use to suppress immunity. The complex role of Tregs in regulating immunity to viral infections, and the consequences their activity has on disease is illustrated by a review of specific viral infections including hepatitis C virus and human immunodeficiency virus.

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Introduction

The concept of immunosuppressive T cells arose more than three decades ago from studies on autoimmunity [1], and B cell [2] and T cell [3] responses to foreign antigens. However, studies on suppressor T cells fell into disfavor in the mid 1980s when the I-J subregion of the MHC that reportedly encoded the restriction elements for suppressor cells was found to contain no genes [4]. Interest was rekindled in 1995 with the finding by Sakaguchi et al. that a subset of T cells constitutively expressing CD25 was immunosuppressive and could protect against autoimmunity [5]. Indications that regulatory T cells (Tregs) were also involved in immune responses to infectious agents began to appear around the turn of the century with reports of immunosuppressive T cell involvement in immunity to *Mycobacterium tuberculosis* [6] and the filarial nematode *Onchocerca volvulus* [7]. The first connection between chronic viral infection and Tregs appeared in a study of mice infected with Friend retrovirus (FV) [8], and this was quickly followed by a study of humans chronically infected with hepatitis C virus (HCV) [9]. At first, the induction of Tregs during a viral infection was considered to be a detrimental response that promoted virus persistence with little or no benefit to the host. However, as will be discussed, it is becoming increasingly clear that pathogen-induced Tregs play a key role in protection from the immunopathology that can occur from hyperactive immune responses to infectious agents [10]. Tregs responses appear common to most if not all infections. Studies have demonstrated that bacterial [6, 11–13], parasitic [7, 14], and viral infections (Table 1) all induce immunosuppressive Tregs, probably as a normal part of the immune response [15–19].

Viral infections are the prototypic inducers of type 1 T helper cell (Th1) responses that generate interferon

gamma (IFNγ) and IL-2 to promote responses by cytolytic T lymphocytes (CTL), the primary effectors of adaptive immunity that kill infected cells. As demonstrated in the lymphocytic choriomeningitis virus model in mice, within the first week of infection, virus-specific CTL expand to very high numbers and develop cytolytic activity [20, 21]. Although CTL killing is specific and relatively directional, the release of cytotoxic granules and cytokines such as tumor necrosis factor [22, 23] can nevertheless result in bystander killing of uninfected cells, leading to extraneous tissue damage. In addition, because CTL numbers can reach many millions of cells during peak responses, even low levels of cross reactivity with uninfected cells can produce pathology. Examples exist in both experimental animal models and in human infections where overactive immune responses lead to lethal damage. It was shown more than two decades ago that T cell-deficient mice infected with influenza virus had less immunopathology and longer survival times than wild-type mice [24]. In humans there is evidence that the high mortality rates of the 1918 influenza pandemic [25] and the recent SARS-associated coronavirus epidemic [26] were due to immunopathological effects. Likewise, immune hyperactivity appears to be one of the most damaging aspects of HIV infections [27–30].

The emerging view is that the immune system has evolved immunoregulatory mechanisms to protect against such overexuberant immune responses, and a major component of this control is immunosuppression by Tregs. Not surprisingly, certain viruses have evolved the means to directly or indirectly subvert the immunosuppressive properties of Tregs to help them evade immunological destruction. The failure to completely eradicate viruses leaves the host susceptible to reactivations of latent viruses and complications such as liver cancer and AIDS in subjects chronically infected with HCV or HIV, respectively. Thus the Tregs response has both positive and negative aspects, and the factors that determine which aspect prevails are complex and still being elucidated.

What are virus-induced Tregs?

For the purposes of this review virus-induced Tregs are simply defined as immunosuppressive T cells that become activated during viral infection. We will focus primarily on CD4+ Tregs, but CD8+ Tregs have also been defined and may contribute to HIV-induced immunosuppression [31]. The relationship between virus-induced Tregs and natural Tregs is only currently becoming clarified. Natural Tregs comprise an immunosuppressive subset of CD4+ T cells that is normally present at a frequency of about 10% of the CD4+ T cell population in mice and approximately 2–5% of the CD4+ T cells in human blood. This subset was initially described as controlling autoimmune reactivity by active suppression in peripheral tissues (peripheral tolerance). More recently it has become evident that these cells suppress reactivity to foreign antigens as well as self antigens [32]. The most common cell surface marker used to identify natural Tregs is CD25, the alpha chain of the IL-2 receptor, which is constitutively expressed at high levels on natural Tregs [5]. However, activated T cells also express CD25 so this marker is not definitive, especially during an infection where many T cells are activated.

Studies on the Leishmania parasite in a mouse model clearly showed that Tregs specific for foreign antigens can develop from the natural Tregs subset [14]. There is also a subset of CD4+ Tregs, known as T regulatory cells type 1 (Tr1), that are developmentally distinct from the CD25+ subset, secrete immunosuppressive IL-10, and appear to be involved in suppressing immune responses to infectious agents (reviewed by O'Garra et al. [33]). In addition, both in vitro [34–36] and in vivo studies [37–39] have shown that CD25-positive Tregs can develop from CD25 negative cells, especially when stimulated in the presence of immunosuppressive cytokines such as transforming growth factor β (TGFβ) or IL-10 [40–42]. In addition to the cytokine microenvironment, the antigen dose and the type of antigen presenting cell (APC) presenting the antigen can strongly influence the conversion of CD25-negative cells into Tregs [38]. Finally, there is a minor subset of CD25-negative CD4+ T cells that do not upregulate CD25, yet suppress reactivity against both self [43–45] and foreign antigens [46]. This subset is increased in aged mice [47]. Thus, the types of Tregs are diverse and the cytokine milieu in which a T cell is activated can be a determining factor in its differentiation to either a conventional effector T cell or a Tregs. Because virus infections have potent effects on cytokine production, it is not surprising that they can influence T cell differentiation and induce a broad range of Tregs subsets (see Table 1).

As pathologists and scientists, we need specific handles with which to identify and/or isolate Tregs. Unfortunately, there is no specific cell surface marker that uniquely identifies these cells. Instead, we rely on combinations of phenotypic markers (Table 1), optimally including at least one functional marker such as an immunosuppressive cytokine (e.g., TGFβ or IL-10), or the transcriptional repressor, Foxp3 (see below) [48, 49]. Levels of activation and expression of adhesion molecules may also be significantly altered in virus-induced Tregs [8, 50–53]. In addition to changes in phenotype, induction of Tregs by viruses can produce localized expansions or accumulation of Tregs, as is seen in the lymph nodes of HIV-infected patients [54]. Thus, Tregs subpopulations can undergo qualitative and/or quantitative changes, following viral infections.

Table 1 Summary of phenotype, suppressed immune responses, and mechanisms of virus-induced regulatory T cells

Virus	Type of regulatory T cell	Markers	Cytokine produced	Responses suppressed	Mechanism	Reference
Friend virus	CD4+CD25+	CD25 CD69 CTLA-4 CD103		Antitumor responses CD8+ T cell effector function No suppression of CD8+ T cell proliferation	Cell-contact-dependent No APC required	[8, 50, 139]
	Tr1		IL-10			(Unpublished)
MAIDS virus complex	CD4+CD25+	CD25 CD69	IL-10	Disease progression		[51]
FIV	CD4+CD25+	CD25 B7 CTLA-4		CD4+ T cell proliferation IL-2 production		[71]
SIV	CD4+CD25+	IDO	TGFβ IL-10	CD8+ T cell responses T cell hyperactivation		[81, 83]
EBV	CD4+CD25+	GITR	No IL-10/ TGFβ	CD4+ and CD8+ T cell proliferation CD4+ T cell production of IL-2	Cell-contact-dependent	[55]
	Tr1	CD4	IL-10	T cell proliferation IFNγ production	IL-10-dependent	[56]
HSV	CD4+CD25+	CD25	IL-10	Th1 and CD8+ T cell responses	Partially IL-10-dependent	[10, 143, 144]
HBV	CD4+CD25+	CD25 CTLA-4		Th1 and CD8+ T cell proliferation IFNγ production		[145, 146]
HCV	CD4+CD25+	CD25 CTLA-4	IL-10 TGFβ	Th1 and CD8+ T cell proliferation IFNγ production	Cell-contact-dependent IL-10/TGFβ-independent TGFβ-dependent	[94–97]
	Tr1	CD4	IL-10			[9]
	CD8+		IL-10	PBMC proliferation	IL-dependent	[99]
HIV	CD4+CD25+	CD25 GITR CTLA-4 CD80 and IDO mRNA	IL-10 TGFβ	CD4+ and CD8+ T cell proliferation IFNγ production	Cell-contact-dependent IL 10/TGFβ-independent	[77, 106–108, 121, 147]
	Tr1		IL-10	T cell proliferation	Partially IL-10-dependent	[77]
	CD8+		TGFβ	CD8+ T cell IFNγ production	TGFβ-dependent	[31]
HTLV-1	CD4+CD25+	GITR CTL-4		PBMC proliferation		[148]

MAIDS murine AIDS, *FIV* feline immunodeficiency virus, *SIV* simian immunodeficiency virus, *EBV* Epstein–Barr virus, *HSV* herpes simplex virus, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *PBMC* peripheral blood mononuclear cell, *HIV* human immunodeficiency virus, *HTLV-1* human T lymphotropic virus type-1, *Tr1* type 1 regulatory T cell, *CTLA-4* cytotoxic T lymphocyte-associated antigen 4, *GITR* glucocorticoid-induced TNFR receptor, *IDO* indole 2,3-dioxygenase, *TGFβ* transforming growth factor β, *IL-10* interleukin 10, *IFNγ* interferon gamma, *Th1* type 1 T helper cell, *APC* antigen presenting cell

Are virus-induced Tregs antigen specific?

In cases where the antigen specificity of virus-induced Tregs has been studied, Tregs specific for viral antigens have been identified. For example, CD4+ Tregs that recognized HCV core antigens were cloned from a cohort of HCV-infected women. Interestingly, the same viral core antigens were recognized by IFNγ-secreting helper T cells from the same patients [9]. A similar finding was made in a study of T cell lines and clones specific for Epstein–Barr virus (EBV) [55]. EBV-encoded nuclear antigen 1 stimulated both helper T cells and Tregs, although there was a suggestion that EBV

peptide specificity might bias the induction of Tregs vs helper. For the EBV latent membrane protein 1, there appears to be a definite bias toward Tregs responses. EBV latent membrane protein 1 is poor at eliciting CTL responses but stimulates IL-10-secreting Tregs [56]. In HIV as well, TGFβ-secreting CD8+ Tregs were generally stimulated by a different set of HIV peptides than IFNγ-secreting effector cells [31]. Thus, it appears that virus-induced Tregs are generally antigen-specific, and there are some suggestions that certain viral antigens may bias responses toward Tregs.

The idea that certain antigens may bias the response toward Tregs implies that the Treg repertoire is different from the effector T cell repertoire. Studies on natural Tregs indicate that their repertoire is just as diverse as that of conventional T cells [57–61]. Tregs also respond to antigenic challenge in a manner similar to conventional T cells, undergoing expansion of antigen-specific subsets followed by contraction [38, 57, 62–64]. One study found that there was at least a 70% overlap in T cell receptor Vβ usage between Tregs and conventional CD4+ T cells [65]. Thus, both the Tregs repertoire and responses appear very similar to conventional T cells, and the preponderance of evidence argues against antigen specificity being a major component in biasing responses toward Tregs [66].

How do viruses induce Tregs?

If certain viral antigens do not preferentially stimulate Tregs in most cases, then how are they induced? Multiple factors can influence the type of immune response that predominates in a given infection. As noted above, the presence of immunosuppressive cytokines such as IL-10 or TGFβ can strongly influence the generation of Tregs [67, 68]. Some viruses can directly stimulate an immunosuppressive microenvironment. For example, EBV encodes a homologue to IL-10 [69] that has the potential to directly influence the induction of Tregs. Another possible direct mechanism of activation is via infection of Tregs. Feline immunodeficiency virus (FIV) preferentially infects CD4+ CD25+ T cells apparently because of high expression of cell-surface coreceptor molecules (CXCR4) and transcription factors important for FIV replication [70]. Infection of these cells activates their immunosuppressive function and may contribute to a loss of T cell effector functions leading to the development of AIDS [71]. While direct mechanisms of Tregs induction are possible, indirect mechanisms are likely more common. Early events in infection such as the production of defensins, cytokines, and chemokines by infected cells or by APCs that have picked up viral particles or cellular debris from infected cells play important roles in shaping the immune response. The type of APC, its level of activation, and its cytokine secretion profile all influence the type of response induced in the responding T cells [72].

One way in which viruses indirectly induce Tregs is by provoking anti-inflammatory cytokine production by APCs. In vitro experiments have shown that human plasmacytoid dendritic cells (pDCs) stimulated with herpes simplex virus (HSV) produce type I IFNs and IL-10 that stimulate CD4+ T cells to differentiate into Tregs [73]. The mechanism of this effect of HSV on pDC is not yet clear but could involve the stimulation of pattern recognition receptors such as toll-like receptors [74]. Infection of dendritic cells (DCs) or even uptake of some noninfectious viruses can affect DC differentiation and antigen presentation leading to induction of peripheral tolerance mechanisms. Normally, the uptake of viral antigens by APCs initiates a cascade of events leading to a maturation and differentiation process typified by migration to draining lymph nodes coupled with upregulation of MHC molecules, costimulatory molecules, cytokines, and chemokines. This process typically leads to the induction of Th1 antiviral responses characterized by the development of antiviral CTL that recognize and kill infected cells. In contrast, presentation of self antigens by nonactivated immature DCs, which express low levels of MHC class II and costimulatory molecules, leads to the induction of Tregs to sustain self-tolerance [75, 76]. Thus, one way for viruses to evade activation of the antiviral immune response is to disrupt the normal activation cascade of DCs and thereby promote the induction of Tregs.

It has been shown that when DCs are infected with HIV-1 in vitro, they maintain an immature phenotype, produce IL-10, and induce Tr1-type Tregs [77]. A very interesting study on HIV-infected patients showed that their lymph nodes had significantly increased levels of “semimature” DCs of both myeloid and plasmacytoid phenotypes [54]. Very few of the semimature DCs were infected, yet they failed to express the costimulatory molecule CD40 or secrete IL-12, factors important in the development of antiviral Th1 responses. In addition, the lymph nodes contained significantly increased percentages of Tregs compared to controls. In vitro assays with the semimature DCs isolated from the lymph nodes showed that they could stimulate the induction of Tregs. Thus, it appears that HIV directly or indirectly interrupts the normal process of DC maturation to drive the immune system toward tolerance rather than immunity.

By poorly understood mechanisms, some viruses, notably HIV and simian immunodeficiency virus (SIV), cause rapid and general hyperactivation of immune responses. Levels of immune hyperactivation during HIV infections correlate with the degree of CD4+ T cell depletion and time of progression to AIDS [27–30]. Tregs may be induced as part of HIV-induced hyperactivation, or alternatively, may be responding as an attempt to control it. Recent evidence from the SIV

model strongly suggests that a very early Tregs response that protects from immune hyperactivation may also protect from AIDS. The predominant correlation between AIDS in SIV-infected macaques and the lack of AIDS in SIV-infected African green monkeys or sooty mangabeys is not virus load, which is roughly equivalent in the different species, but levels of virus-induced hyperactivation [78–80].

In a comparative study between SIV infection of macaques and African green monkeys, the lack of hyperactivation in African green monkeys was associated with significant anti-inflammatory responses within 24 h of infection [81]. These very early anti-inflammatory responses were characterized by the production of TGFβ, a corresponding lack of Th1 cytokines, and evidence that both CD4+ and CD8+ Tregs percentages were increased. An implication of this study is that while very early immunosuppression by Tregs may dampen Th1 responses and facilitate virus persistence, they protect the host from immune hyperactivation, which may be the root cause of pathogenesis and eventual onset of AIDS. Another implication is that the very rapid activation of Tregs may be due to an innate response such as direct stimulation via toll-like receptors [82]. Interestingly, although the Tregs response in macaques is too slow to protect them from SIV-induced immune hyperactivation, it is premature in comparison to Tregs responses to cytomegalovirus infection. The Tregs response was detectable already by day 7 and correlated with dampening CTL responses before the virus was cleared [83]. Thus, the timing of the Tregs response in SIV infection appears to be critical in determining disease outcome.

What is the role of virus-induced Tregs in viral infections and disease?

Regardless of how Tregs are induced by a given virus, the nature of the response is always immunosuppressive. However, multifaceted components of the host–virus interaction determine whether the impact on the disease state will be positive or negative. The importance and complexity of Tregs in viral disease is illustrated by several informative studies on HCV infection. In HCV, virus clearance during acute infection of humans is associated with strong Th1 [84–89] and CTL responses [90–93]. One of the first studies showing a role for Tregs in viral infection was done in a cohort of approximately one thousand women infected with the same virus following transfusion of HCV-contaminated immunoglobulin following childbirth. Roughly half of the women cleared the infection and the other half developed chronic infections. HCV-specific T cells that produced IL-10 (Tr1 cells) were found in a significantly higher proportion of chronically infected patients than in individuals who had cleared the infection [9]. Other studies have confirmed that patients with chronic HCV infection have significantly higher proportions of Tregs in their blood than both normal controls and patients who have recovered from HCV infection [94, 95]. The virus-induced Tregs associated with chronic HCV infection suppress virus-specific CD8+ T cells [94, 96, 97], providing a possible explanation for dysfunctional CD8+ T cell responses in chronic HCV infection [98].

Interestingly, while Treg-mediated suppression of Th1 and CTL responses during acute infection is detrimental in terms of allowing HCV to establish chronic infections, once chronic infection is established it appears that the Tregs are essential in protecting patients from immunopathology. Virus-specific CD8+ T cell-mediated cytolysis of infected liver cells can result in severe immunopathological damage. It has been shown that cirrhosis in chronically infected patients is kept in check by IL-10-producing CD8+ regulatory T cells that suppress the effector function of CTL [99]. In addition, 30–50% of patients with chronic HCV infection develop an autoimmune disorder known as mixed cryoglobulinemia (MC). Patients with symptomatic MC had significantly reduced levels of CD4+CD25+ regulatory T cells in their blood [100, 101]. The reduction of Tregs in these patients was associated with a decreased ability to regulate immunopathological CD8+ T cell responses [102], increased Th1 cytokine levels, higher incidence of cirrhosis, and increased mortality rates [103]. Lest the picture appear too simple, an additional complication in HCV infections is hepatocellular carcinoma (HCC). Patients with HCC had increased populations of Tregs in their blood that suppressed the proliferation and cytokine secretion of activated CD4+CD25− T cells [104]. Perhaps even more interestingly, the tumors themselves contained high levels of Tregs, and the CD8+ tumor infiltrating lymphocytes in the tumor tissues were dysfunctional [105]. Thus, HCV-induced Tregs appear to protect chronically infected patients from immunopathological diseases, but likely contribute to an inability to cytolyse cancer cells and prevent HCC.

Numerous studies have now demonstrated the involvement of Tregs in HIV infections, but as just discussed for HCV, their role in various aspects of disease appears complex. Suppression of both CD4+ T cell [106–108] and CD8+ T cell [107, 108] responses have been described in vitro. HIV induces immune hyperactivation similar to SIV in macaques, and this hyperactivation may play a predominant role in the depletion of CD4+ T cells [29]. The degree of hyperactivation is a powerful prognosticator of AIDS progression and death [27–30, 109]. Interestingly, the loss of peripheral Tregs in HIV patients is also a prognosticator of a poor clinical outcome because it correlates with increased HIV-induced immune hyperactivation [108, 110]. Thus, it appears that Tregs may protect from severe disease by controlling virus-induced immune hyperactiva-

tion. Although Tregs may help protect HIV-infected persons from hyperactivation, they likely also contribute to the previously described dysfunction of both T helper cells [111] and CD8+ T cells [112–115].

It is not known why Tregs levels eventually drop in HIV infections, but human Tregs are highly susceptible to infection by HIV [110], which could lead to their dysfunction or cell death through various mechanisms [116–120]. There is also evidence that the drop in Treg levels in the peripheral blood may be due more to a redistribution of Tregs than to a decrease in total Tregs numbers. In studies of tonsil tissue from HIV-infected patients, Andersson et al. showed the presence of increased levels of Tregs, and there was a positive correlation between the prevalence of Tregs and viral loads [121]. These findings are supported by recent data showing increased levels of Tregs in the lymph nodes of HIV-infected patients [54]. Given that HIV primarily replicates in lymphoid tissues [122, 123], the presence of Tregs that have been shown to suppress both CD4+ and CD8+ T cell functions could have a dramatic and very detrimental impact on the ability of the immune system to clear infected cells. The idea that Treg-mediated suppression of cellular immune responses in lymphoid tissue could increase disease is bolstered by a recent study showing that the maintenance of virus-specific cellular immune responses in gut-associated lymphoid tissue correlates with an asymptomatic state in long-term nonprogressors [124].

How do virus-induced Tregs mediate suppression?

The molecular mechanisms by which Tregs mediate suppression of effector T cell responses are largely unknown. In general, CD4+ Tregs inhibit T cell responses either indirectly through the production of anti-inflammatory cytokines, such as IL-10 or TGFβ, or directly through cell-to-cell contact. IL-10 is a potent immunosuppressive cytokine that exerts its anti-inflammatory effects primarily on APCs, which ultimately leads to the down-regulation of Th1 responses [125]. IL-10-producing Tregs (Tr1) can be generated in vitro by stimulating naïve T cells with chronic antigen [126] or in the presence of immunosuppressive drugs [127]. Among the known virus-induced Tregs, the expression of IL-10 appears to be a common theme (Table 1). In HIV, T cells from infected donors produce IL-10 when cocultured with HIV-infected immature DC and suppress CD4+ T cell proliferation in an IL-10-dependent manner [77]. In addition, the frequency of IL-10-producing CD4+ T cells is significantly increased in HIV patients with progressive disease compared to patients with nonprogressive disease [128]. A role for IL-10 has also been implicated in EBV infections. Peripheral blood mononuclear cells from EBV-seropositive individuals stimulated with EBV-specific latent membrane protein 1 induced high levels of IL-10 secretion and the ability to inhibit T cell proliferation and IFNγ responses in vitro [56]. In these studies, neutralizing anti-IL-10 antibodies completely abrogated the suppressive activity demonstrating the requirement for IL-10. As discussed above, virus-specific CD4+ and CD8+ Tregs cells from HCV-infected patients also produce IL-10 in response to viral antigens and inhibit HCV-specific T cell responses [9, 95, 99]. However, in vitro suppression by these cells was found not to be dependent on IL-10 but rather required cell–cell contact [96, 97]. Although in vitro studies show that IL-10 is not essential for virus-induced, Tregs-mediated suppression, its multiple anti-inflammatory effects are likely important for the development and/or function of virus-induced Tregs in vivo.

TGFβ is another important immunosuppressive cytokine that has been implicated in the function of Tregs [129]. Increased expression of TGFβ in CD4+CD25+ T cells has been reported in HIV-infected individuals [106, 108, 121] and SIV infection of rhesus macaques [83]. HIV infection is associated with the circulation of dysfunctional CD8+ T cells that fail to eliminate chronic viruses. One mechanism that may contribute to this dysfunction is the presence of CD8+ regulatory T cells that secrete TGFβ. HIV-induced CD8+ Tregs were HIV-specific and suppressed CD8+ T cell IFNγ responses in vitro, an effect that was reversible by anti-TGFβ antibodies [31]. The function of TGFβ in Tregs-mediated suppression has been studied most extensively in models of autoimmunity and tumor rejection involving the suppression of CD8+ T cells. These studies demonstrate that TGFβ expressed on the surface of Tregs or APCs interacts with the TGFβ receptor II on CD8+ T cells, resulting in inhibition of activation [129–132]. In addition, TGFβ can induce the expression of the forkhead transcription factor Foxp3 in CD4+CD25− T cells, which confers suppressive activity [36]. Foxp3 is a transcriptional repressor that functions as the Tregs cell lineage specification factor [48, 49, 133–135]. Although the transcriptional programming orchestrated by Foxp3 has not been clearly defined, immunosuppressive activity of T cells is associated with Foxp3 expression. Thus, production of TGFβ during virus infections may both directly suppress effector T cells and help promote Tregs development.

Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), a negative regulator constitutively expressed on CD4+CD25+ Tregs cells, has received significant attention as a potential mediator of Tregs suppressive function. CTLA-4 has been shown to induce the expression of the tryptophan-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) in tolerogenic DCs [136]. Through the depletion of tryptophan, an important growth factor, IDO inhibits clonal expansion of T cells [137, 138]. At present, evidence

supporting a role for IDO in virus-induced Tregs is only circumstantial. In a comparative study with HIV-infected patients undergoing highly active antiretroviral therapy treatment, untreated HIV-infected patients expressed high levels of IDO-specific mRNA in tonsils [121]. Although DCs are the typical source of IDO, recent studies using the rhesus macaque model show a rapid induction of IDO expression in CD4+CD25+FOXp3+ Tregs cells in the lymph nodes during acute SIV infection [83].

Several studies of HIV-infected individuals have now demonstrated intriguing correlations between the presence of Tregs, dysfunctional lymphocytes, virus production, and different disease states [106, 108, 121]. However, direct proof and dissection of the roles of Tregs-mediated suppression in vivo requires an experimental model. To this end, we have used mice infected with FV, the model in which virus-induced Tregs were originally described [8]. Chronic FV infection induces CD4+ Tregs that suppress CD8+ T cell functions [8, 50, 139]. Suppression of CD8+ T cell function can be adoptively transferred into naïve or acutely infected mice with CD4+ T cells purified from chronically infected mice [8, 139]. When CD4+ T cells from chronically infected mice were adoptively transferred into acutely infected mice, they not only produced IL-10 but also promoted IL-10 production by the host's CD4+ T cells [139]. Interestingly, both CD25-positive and negative subsets exhibited suppressive activity in vivo. An in vitro assay designed to further investigate the mechanisms of suppression indicated the presence of two distinct subsets of FV-induced Tregs. The CD4+CD25− subset was the IL-10-producing subset (unpublished data), while the ability to directly suppress IFNγ production by stimulated CD8+ T cells was found only in the CD4+CD25+ subset [50]. Suppression of CD8+ T cell function by FV-induced CD4+CD25+ T cells in vitro occurred in a cell contact-dependent manner with no requirement for APCs. Interestingly, FV-induced Tregs did not inhibit the proliferative responses of stimulated CD8+ T cells or their expression of activation markers. Suppression was limited to effector functions such as the production of cytokines and cytolytic molecules. Furthermore, FV-induced Tregs also suppressed the effector function of virus-specific CD8+ T cells that had been fully activated by exposure to virus in vivo. This ability could be key to their role in preventing immunopathology.

Another interesting finding from the in vitro studies on FV-induced Tregs was that they suppressed CD8+ T cells in vitro regardless of the specificity of the CD8+ T cell [50]. This is consistent with the finding that, although the activation of Tregs is antigen-specific and dependent on T cell receptor signaling, their effector function is nonspecific and can generate "bystander suppression" [140]. The implication is that some degree of general immunosuppression may be associated with virus-induced Tregs activity. In mice chronically infected with FV, virus-induced Tregs suppressed virus-specific CD8+ T cell responses in vivo [8, 139, 141] and in vitro [50]. While the immunosuppression associated with these virus-induced Tregs was strongest to virus-specific responses, it was shown that both in vivo and in vitro responses to nonviral antigens were weakened [8]. This study suggests that, indeed, some general immunosuppression is associated with virus-induced Tregs, but further studies will be needed to determine whether the effect is potent enough to affect immune responses to infectious agents. In that regard, a recent transplantation study showed that the generation of allograft-specific Tregs did not compromise immunity to infection with influenza [142]. A general conclusion from these studies and unpublished data from our lab is that the microenvironmental localization of Tregs plays a more important role in determining which responses get suppressed than does the specificity of the cell being suppressed.

Conclusion

Numerous factors such as the timing, intensity, mechanism of induction, and the microenvironmental location of the Tregs response have considerable impacts on whether the outcome is primarily beneficial or detrimental to the host. Clearly, Tregs responses are highly evolved and critical to the regulation of antiviral immunity. It appears that Tregs respond during infections with all viruses, not just those that become chronic, and they provide a critical governor on immune effector responses that could otherwise cause life-threatening immunopathological damage. They are usually highly successful at their jobs, as evidenced by our ability to recover from most viral infections without serious sequelae. Although some viruses have evolved ways to subvert the Tregs responses, thereby allowing them to establish and maintain persistence, most chronic viral infections are rather benign. Virtually all humans carry chronic viral infections, and they are usually not highly pathogenic unless the person becomes immunocompromised. Of course there are outstanding exceptions such as HIV and HCV that cause a great deal of suffering and death. The studies with the natural hosts of SIV suggest that HIV may be such an extreme example because it has so recently jumped the species barrier, and humans have not had time to evolve and adapt protective Tregs responses as the sooty mangabies and African green monkeys have done. It is clear that Tregs responses are important in HIV infections, but the situation appears to be just as complex as it is in HCV infections, and much remains to be learned. Thus, a great deal of care must be taken with therapeutic intervention because there is a large potential to exacerbate disease rather than cure it. That being said, modulation of

the Tregs response may indeed be a key component in therapies to treat chronic viral infections such as HIV and HCV.

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