The role of virus-induced regulatory T cells in immunopathology

Shelly J. Robertson · Kim J. Hasenkrug

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.

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.
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 IFN-γ-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

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 | IL-10             | Cell-contact-dependent | No APC required | [8, 50, 139] |
| MAIDS virus    | Tr1                         | CD4+CD25+ | IL-10             | Disease progression | (Unpublished) | [51] |
| complex        |                             |         |                   |                     |                  |           |
| FIV            | CD4+CD25+                  | CD25, CD69, CTLA-4, B7 | IL-2 production  | IL-2 production     | Cell-contact-dependent | [71] |
| SIV            | CD4+CD25+                  | CD25, CTLA-4, IDO | TGFβ, IL-10       | T cell proliferation | CD4+ and CD8+ T cell proliferation | [81, 83] |
| EBV            | CD4+CD25+                  | GITR    | No IL-10/TGFβ    | CD4+ T cell proliferation | CD4+ T cell production of IL-2 | Cell-contact-dependent | [55] |
| HSV            | Tr1                         | CD4     | IL-10            | T cell proliferation | IL-10-dependent | [56] |
|                |                             |         |                   |                     |                  |           |
| HBV            | Tr1                         | CD4     | IL-10            | T cell proliferation | IL-10-dependent | [56] |
| HIV            | Tr1                         | CD4     | IL-10            | IFNγ production    | IL-10-dependent | [99] |
|                | CD8+                       | IL-10   | PBMC proliferation| IL-dependent      |                  |           |
|                |                             |         |                   |                     |                  |           |
| HTLV-1         | CD4+CD25+                  | GITR, CTLA-4 | IL-10/TGFβ      | PBMC proliferation  | IL-10-dependent | [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
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 defenses, 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 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 cytolise 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-infected 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.

References

1. Nishizuka Y, Sakakura T (1969) Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. Science 166:753–755
2. Gershon RK, Kondo K (1970) Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunology 18:723–737
3. Gershon RK, Cohen P, Hencin R, Liebhäber SA (1972) Suppressor T cells. J Immunol 108:586–590
4. Kobori JA, Strauss E, Minard K, Hood L (1986) Molecular analysis of the hotspot of recombination in the murine major histocompatibility complex. Science 234:173–179
5. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M (1995) Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 155:1151–1164
6. Boussiotis VA, Tsai EY, Yunis EJ, Thim S, Delgado JC, Dasher CC, Berezovskaya A, Rousset D, Reynes JM, Goldfeld AE (2000) IL-10-producing T cells suppress immune responses in anergic tuberculosis patients. J Clin Invest 105:1317–1325
7. Doetze A, Satoguina J, Burchard G, Rau T, Loliger C, Fleischer D, Hasenkrag KJ (2001) Immunosuppression by CD4+ regulatory T cells induced by chronic retroviral infection. Proc Natl Acad Sci U S A 98:9226–9230
8. MacDonald AJ, Duffy M, Brady MT, McKiernan S, Hall W, Herzenberg AA, Herzenberg LA, Jedd DA, Jedd AH, Mackay IR, McMichael AJ, Neuberger MS, Nossal GJ, Parrott DE, Pollock BL, Sercarz EE, Shearer GM, Sprent J, Tonegawa S, Tyrrell DA, Waksman H, Woolcock AJ, Zinkernagel RM (1998) A definition of T regulatory (Treg) cells. J Clin Immunol 18:720–725
9. McGuirk P, McCann C, Mills KH (2002) Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by Bordetella pertussis. J Exp Med 195:221–231
10. Kullberg MC, Jankovic D, Gorelick PL, Caspar P, Letterio JJ, Cheever AW, Sher A (2002) Bacteria-triggered CD4+ T regulatory cells suppress Helicobacter hepaticus-induced colitis. J Exp Med 196:505–515
11. Guyot-Revol V, Innes JA, Hackforth S, Hinks T, Lalvani A (2006) Regulatory T cells are expanded in blood and disease sites in patients with tuberculosis. Am J Respir Crit Care Med 173:803–810
12. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL (2002) CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature 420:507
13. Mills KH (2004) Regulatory T cells: friend or foe in immunity to infection? Nat Rev Immunol 4:841–855
14. Fehervari Z, Sakaguchi S (2004) CD4+ Tregs and immune control. J Clin Invest 114:1209–1217
15. Bellard Y, Rouse BT (2005) Natural regulatory T cells in infectious disease. Nat Immunol 6:353–360
16. Maloy KJ, Powrie F (2001) Regulatory T cells in the control of immune pathology. Nat Immunol 2:816–822
17. Rouse BT, Suvas S (2004) Regulatory cells and infectious agents: detentes cordiale and contraire. J Immunol 173:2211–2215
18. Maloy KJ, Powrie F (2001) Regulatory T cells in the control of immune pathology. Nat Immunol 2:816–822
19. Rouse BT, Suvas S (2004) Regulatory cells and infectious agents: detentes cordiale and contraire. J Immunol 173:2211–2215
20. De Boer RJ, Oprea M, Antia R, Murali-Krishna K, Ahmed R, Perelson AS (2001) Recruitment times, proliferation, and apoptosis rates during the CD8(+) T-cell response to lymphocytic choriomeningitis virus. J Virol 75:10663–10669
21. Hassett DE, Silika MK, Zhang J, Whitton JL (2000) Direct ex vivo kinetic and phenotypic analyses of CD8(+) T-cell responses induced by DNA immunization. J Virol 74:8286–8291
22. Liu F, Feuer R, Hassett DE, Whitton JL (2006) Peptide vaccination of mice immune to LCMV or vaccinia virus causes serious CD8 T cell-mediated, TFN-dependent immunopathology. J Clin Invest 116:465–475
23. Xu L, Yoon H, Zhao MQ, Liu J, Ramana CV, Enelow RI (2004) Cutting edge: pulmonary immunopathology mediated by antigen-specific expression of TNF-alpha by antiviral CD8+ T cells. J Immunol 173:721–725
24. Wells MA, Albrecht P, Emms FA (1981) Recovery from a viral respiratory infection. I. Influenza pneumonia in normal and T-deficient mice. J Immunol 126:1036–1041
25. Yeldandi AV, Colby TV (1994) Pathologic features of lung biopsy specimens from influenza pneumonia cases. Hum Pathol 25:47–53
26. Rainer TH, Chan PK, Ip M, Lee N, Hui DS, Smit D, Wu A, Ahuja AT, Tam JS, Sung JJ, Cameron P (2004) The spectrum of severe acute respiratory syndrome-associated coronavirus infection. Ann Intern Med 140:614–619
27. Deeks SG, Kitchen CM, Liu L, Guo H, Gascon R, Narvaez AB, Hunt P, Martin JN, Kahn JO, Levy J, McGrath MS, Hecht FM (2004) Immune activation set point during early HIV infection predicts subsequent CD4+- T-cell changes independent of viral load. Blood 104:942–947
28. Hazenberg MD, Otto SA, van Benthem BH, Roos MT, Coutinho RA, Lange JM, Hamann D, Prins M, Miedema F (2003) Persistent immune activation in HIV-1 infection is associated with progression to AIDS. AIDS 17:1881–1888
29. Sousa AE, Carneiro J, Meier-Schellersheim M, Grossman Z, Victorino RM (2002) CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. J Immunol 169:3400–3406
30. van Asten L, Danisman F, Otto SA, Borghans JA, Hazenberg MD, Coutinho RA, Prins M, Miedema F (2004) Pre-seroconversion immune status predicts the rate of CD4 T cell decline following HIV infection. AIDS 18:1885–1893
31. Garba ML, Pilcher CD, Bingham AL, Eron J, Frelinger JA (2002) HIV antigens can induce TGF-beta(1)-producing immunoregulatory CD8+ T cells. J Immunol 168:2247–2254
32. Sakaguchi S (2005) Naturally arising Foxp3-expressing CD4+ regulatory T cells in immunological tolerance to self and non-self. Nat Immunol 6:345–352
33. O’Garra A, Vieira PL, Vieira P, Goldfeld AE (2004) IL-10-producing and naturally occurring CD4+ Tregs: limiting collateral damage. J Clin Investig 114:1372–1378
34. Curotto de Lafaille MA, Lino AC, Kutchukhidze N, Lafaille JJ (2004) CD25− T-cells generate CD25+Foxp3+ regulatory T cells by peripheral expansion. J Immunol 173:7259–7269
35. Apostolou I, Sarukhan A, Klein L, von Boehmer H (2002) Origin of regulatory T cells with known specificity for antigen. Nat Immunol 3:76–79
36. Fantini MC, Becker C, Monteleone G, Pallone F, Galle PR, Neurath MF (2004) Cutting edge: TGF-beta induces a regulatory...
phenotype in CD4+CD25− T cells through Foxp3 induction and down-regulation of Smad7. J Immunol 172:5149–5153
37. Karim M, Kingsley CI, Bushell AR, Sawizki BS, Wood KJ (2004) Allotransplant-induced CD25+CD4+ regulatory T cells can develop in vivo from CD25−CD4+ precursors in a thymus-independent process. J Immunol 172:923–928
38. Kretschmer K, Apostolou I, Hawiger D, Khazaie K, Nussenzweig MC, von Boehmer H (2005) Inducing and expanding regulatory T cell populations by foreign antigen. Nat Immunol 6:1219–1227
39. Thorstenson KM, Khoruts A (2001) Generation of anergic and potentially immunoregulatory CD25+CD4+ T cells in vivo after induction of peripheral tolerance with intravenous or oral antigen. J Immunol 167:188–195
40. Rao PE, Petrone AL, Ponate PD (2005) Differentiation and expansion of T cells with regulatory function from human peripheral lymphocytes by stimulation in the presence of TGF-β. J Immunol 174:1446–1455
41. Zheng SG, Gray JD, Ohtsuka K, Yamagishi S, Horwitz DA (2002) Generation ex vivo of TGF-β-beta-producing regulatory T cells from CD4+CD25− precursors. J Immunol 169:4183–4189
42. Chen ZM, O’Shaughnessy MJ, Gramaglia I, Panoskalsit-Mortar A, Murphy WJ, Narula S, Roncarolo MG, Blazar BR (2003) IL-10 and TGF-beta induce alloreactive CD4+CD25− T cells to acquire regulatory cell function. Blood 101:5076–5083
43. Lehmann J, Hueln J, de la Rosa M, Massyna F, Kretschmer U, Krenn V, Brunner M, Schoeffeld A, Hamann A (2002) Expression of the integrin alpha Ebeta 7 identifies unique subsets of CD25+ as well as CD25− regulatory T cells. Proc Natl Acad Sci U S A 99:13031–13036
44. Uraushihara K, Kanai T, Ko K, Totsuka T, Makita S, Iiyama R, Okamoto R, Koyanagi A, Akiba H, Okumura K, Yagita H, Liblau R, Salomon BL (2003) Continuous activation of CD4+CD25− T cells leads to the abrogation of their suppressive activity in vitro. Eur J Immunol 33:1090–1099
45. Horii S, Nomura T, Sakaguchi S (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 299:1057–1061
46. Khattri R, Cox T, Yasayko SA, Ransdell F (2003) An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol 4:337–342
47. Robertson JS, Messer RJ, Carmody AB, Hasenkruk KJ (2006) In vitro suppression of CD8+ T cell function by friend virus-induced regulatory T cells. J Immunol 176:3342–3349
48. Beilharz MW, Sammels LM, Paum A, Shaw K, van Eeden P, Watson MW, Ashdown ML (2004) Timed ablation of regulatory CD4+ T cells can prevent murine AIDS progression. J Immunol 172:4917–4925
49. Fisson S, Darrasse-Jeze G, Litvinova E, Septier F, Klatzmann D, Liblau R, Salomon BL (2003) Continuous activation of autoreactive CD4+CD25+ regulatory T cells in the steady state. J Exp Med 198:737–746
50. Nolte’t Hoen EN, Wagenaar-Hilbers JP, Boot EP, Lin CH, Arkesteijn GJ, van Eden W, Taams LS, Wubben MH (2004) Identification of a CD4+CD25+ T cell subset committed in vivo to suppress antigen-specific T cell responses without additional stimulation. Eur J Immunol 34:3016–3027
51. Krathwohl MD, Schacker TW, Anderson JL (2006) Abnormal presence of semimature dendritic cells that induce regulatory T cells in HIV-infected subjects. J Infect Dis 193:494–504
52. Voo KS, Peng G, Guo Z, Fu T, Li Y, Frappier L, Wang RF (2005) Functional characterization of EBV-encoded nuclear antigen 1-specific CD4+ helper and regulatory T cells elicited by in vitro peptide stimulation. Cancer Res 65:1577–1586
53. Marshall NA, Vickers MA, Barker RN (2003) Regulatory T cells secreting IL-10 dominate the immune response to EBV latent membrane protein 1. J Immunol 170:6183–6189
54. Caton AJ, Cozzo C, Larkin J 3rd, Lerman MA, Boe streakan A, Jordan MS (2004) CD4+CD25+ regulatory T cell selection. Ann N Y Acad Sci 1029:101–114
55. Fujishima M, Hirokawa M, Fujishima N, Sawada K (2005) TRCAlphabeta repertoire diversity of human naturally occurring CD4+CD25+ regulatory T cells. Immunol Lett 99:193–197
56. Tacholoziky R, Kraj P, Ignatowicz L (2002) Peptide specificity of thymic selection of CD4+CD25+ T cells. J Immunol 168:613–620
57. Taams LS, Vukmanovic-Stjetcj M, Smith J, Dunne PJ, Fletcher JM, Plunkett FJ, Ebeling SB, Lombardi G, Rustin MH, Bijlsma JW, Lafeber FP, Salmon M, Akbar AN (2002) Antigen-specific T cell suppression by human CD4+CD25+ regulatory T cells. Eur J Immunol 32:1621–1630
58. Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, Shimizu J, Sakaguchi S (1998) Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. Int Immunol 10:1969–1980
59. Walker LS, Chodos A, Eggema M, Dooms H, Abbas AK (2003) Antigen-dependent proliferation of CD4+ CD25+ regulatory T cells in vivo. J Exp Med 198:249–258
60. Hayashi Y, Tsukumo S, Shioti H, Kishihara K, Yasutomo K (2004) Antigen-specific T cell repertoire modification of CD4+ CD25+ regulatory T cells. J Immunol 172:5240–5248
61. Yamazaki S, Iyoa T, Tarbell K, Olson K, Velinon K, Inaba K, Steinman RM (2003) Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. J Exp Med 198:235–247
62. Taams LS, Akbar AN (2005) Peripheral generation and function of CD4+CD25+ regulatory T cells. Curr Top Microbiol Immunol 293:115–131
63. Cohn M (2004) Whither T-suppressors: if they didn’t exist would we have to invent them? Cell Immunol 227:81–92
64. Peng Y, Laouar Y, Li MO, Green EA, Flavell RA (2004) TGF-beta regulates in vivo expansion of Foxp3-expressing CD4+CD25+ regulatory T cells responsible for protection against diabetes. Proc Natl Acad Sci U S A 101:4572–4577
65. Levings MK, Bacchetta R, Schulz U, Roncarolo MG (2002) The role of IL-10 and TGFβ in the differentiation and effector function of T regulatory cells. Int Arch Allergy Immunol 129:263–276
66. Moore KW, Viepra P, Fiorentino DF, Trounstine ML, Khan TA, Mosmann TR (1990) Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein–Barr virus gene BCRFI. Science 248:1230–1234
67. Joshi A, Garg H, Tompkins MB, Tompkins WA (2005) Preferential in vivo immunodeficiency virus (FIV) infection of CD4+CD25+ T-regulatory cells correlates both with surface expression of CXCR4 and activation of FIV long terminal
null
110. Oswald-Richter K, Grill SM, Shariat N, Leelawong M, Sundrud M, Mahalingam M, Peakman M, Davies ET, Pozniak A, McManus Kinter AL, Hennessey M, Bell A, Kern S, Lin Y, Daucher M, Appay V, Nixon DF, Donahoe SM, Gillespie GM, Dong T, King Trimble LA, Lieberman J (1998) Circulating CD8 T

111. Clerici M, Stocks NI, Zajac RA, Boswell RN, Lucey DR, Aandahl EM, Michaelsson J, Moretto WJ, Hecht FM, Nixon DF

112. Unitt E, Rushbrook SM, Marshall A, Davies S, Gibbs P, Morris LS, Coleman N, Alexander GJ (2005) Compromised lymphocytes infiltrate hepatocellular carcinoma: the role of T-regulatory cells. Hepatology 41:722–730

113. Weiss L, Donkova-Petriini V, Caccavelli L, Balbo M, Carbonelli C, Levy Y (2004) Human immunodeficiency virus-driven expansion of CD4+CD25+ regulatory T cells which suppress HIV-specific CD4 T-cell responses in HIV-infected patients. Blood 104:3249–3256

114. Aandahl EM, Michaelsson J, Moretto WJ, Hecht FM, Nixon DF (2004) Human CD4+CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytokomelavirus antigens. J Virol 78:2454–2459

115. Kinter AL, Hennessey M, Bell A, Kern S, Lin Y, Daucher M, Planta M, McLaughlin M, Jackson R, Ziegler SF, Faulki AS (2004) CD25+CD4+ regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4+ and CD8+ HIV-specific T cell immune responses in vitro and are associated with favorable clinical markers of disease status. J Exp Med 200:331–343

116. Mahalingam M, Peakman M, Davies ET, Pozniak A, McManus TJ, Vergani D (1993) T cell activation and disease severity in HIV infection. Clin Exp Immunol 93:337–343

117. Oswald-Richter K, Grill SM, Shariat N, Leelawong M, Sundrud MS, Haas DW, Unutmaz D (2004) HIV infection of naturally occurring and genetically reprogrammed human regulatory T-cells. PLoS Biol 2:955–966

118. Clerici M, Stocks NI, Zajac RA, Boswell RN, Lucey DR, Via CS, Shearer GM (1989) Detection of three distinct cytokine expression patterns during acute human immunodeficiency virus type 1 infection. J Infect Dis 185:1355–1358

119. Groux H, Torpier G, Monte D, Mouton Y, Capron A, Amesen JC (1992) Activation-induced death by apoptosis in CD4+ T cells from human immunodeficiency virus-infected asymptomatic individuals. J Exp Med 175:331–340

120. Grossman Z, Meier-Schellenbier M, Sousa AE, Victorinio RM, Paul WE (2002) CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? Nat Med 8:319–323

121. Lenardo MJ, Angelman SB, Bounkeu V, Dimas J, Duvall MG, Graubard MB, Hornung F, Selkirk MC, Speirs CK, Trageser C, Orenstein JO, Bolton DL (2002) Cytopathic killing of peripheral blood CD4+ T lymphocytes by human immunodeficiency virus type 1 appears necrotic rather than apoptotic and does not require env. J Virol 76:5082–5093

122. Andersson J, Boasso A, Nilsson J, Zhang R, Shire NJ, Lindback S, Shearer GM, Chougnet CA (2005) Cutting edge: the prevalence of regulatory T cells in lymphoid tissue is correlated with viral load in HIV-infected patients. J Immunol 174:3143–3147

123. Guadalupe M, Reay E, Sankaran S, Prindiville T, Flamm J, McNiel A, Dandeker S (2003) Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol 77:11708–11717

124. Haase AT (1999) Population biology of HIV-1 infection: viral and CD4+ T cell demographics and dynamics in lymphatic tissues. Annu Rev Immunol 17:625–656

125. Sankaran S, Guadalupe M, Reay E, George MD, Flamm J, Prindiville T, Dandeker S (2005) Gut mucosal T cell responses and gene expression correlate with protection against disease in long-term HIV-1-infected nonprogressors. Proc Natl Acad Sci USA 102:9860–9865

126. Moore KW, de Waal-Malefyt R, Coffman RL, Orenstein JO, Bolton DL (2002) Cytopathic killing of peripheral blood CD4+ T lymphocytes by human immunodeficiency virus type 1 appears necrotic rather than apoptotic and does not require env. J Virol 76:5082–5093

127. Barrat FJ, Cua DJ, Boonstra A, Richards DF, Crain C, Savelkoul HF, de Waal-Malefyt R, Coffman RL, Hawrylowicz CM, O’Garra A (2001) Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19:683–765

128. Ostrowski MA, Gu JX, Kovacs C, Freedman J, Luscher TF, Korangy F (2005) Increased populations of regulatory T cells are associated with reduction in liver inflammation and fibrosis in patients with autoimmune hepatitis. J Autoimmun 25:63–71

129. Migueles SA, Laborico AC, Sabbaghian MS, Rabin HS, Hallahan CW, Van Baarle D, Kostense S, Miedema F, McLaughlin M, Ehler L, Metcalf JI, Liu S, Connors M (2002) HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. Nat Immunol 3:1061–1068

130. Monte D, Groux H, Raharinivo B, Plouvier B, Dewulf J, Cacoub P (1992) Productive human immunodeficiency virus-1 infection of megakaryocytes is enhanced by tumor necrosis factor-alpha. Blood 79:2670–2679

131. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M (1995) Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 373:123–126

132. Groux H, Torpier G, Monte D, Mouton Y, Capron A, Ogg GS, Spiegel HM, Conlon C, Spina CA, Havlir DV, Levy Y (2004) Human immunodeficiency virus-driven expression of perforin and FAS ligand by CD8+ T lymphocytes in lymphoid tissue of infected individuals with differential disease progression: reciprocal interferon-gamma and interleukin-10 responses. J Infect Dis 184:1268–1278
129. Nakamura K, Kitani A, Fuss I, Pedersen A, Harada N, Nawata H, Strober W (2004) TGF-beta1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice. J Immunol 172:834–842
130. Chen ML, Pittet MJ, Gorelik L, Flavell RA, Weisleder R, von Boehmer H, Khazaie K (2005) Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals in vivo. Proc Natl Acad Sci U S A 102:419–424
131. Green EA, Gorelik L, McGregor CM, Tran EH, Flavell RA (2003) CD4+CD25+ T regulatory cells control anti-islet CD8+ T cells through TGF-beta–TGF-beta receptor interactions in type 1 diabetes. Proc Natl Acad Sci U S A 100:10878–10883
132. von Boehmer H (2005) Mechanisms of suppression by suppressor T cells. Nat Immunol 6:338–344
133. Fontenot JD, Rudensky AY (2005) A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. Nat Immunol 6:331–337
134. Fontenot JD, Gavin MA, Rudensky AY (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4:330–336
135. Hori S, Sakaguchi S (2004) Foxp3: a critical regulator of the development and function of regulatory T cells. Microbes Infect 6:745–751
136. Fallarino F, Grohmann U, Hwang KW, Orabona C, Vacca C, Bianchi R, Belladonna ML, Fioretti MC, Alegre ML, Puccetti P (2003) Modulation of tryptophan catabolism by regulatory T cells. Nat Immunol 4:1206–1212
137. Mellor AL, Baban B, Chandler P, Marshall B, Jhaever K, Hansen A, Koni PA, Iwashima M, Munn DH (2003) Cutting edge: induced indoleamine 2,3 dioxygenase expression in dendritic cell subsets suppresses T cell clonal expansion. J Immunol 171:1652–1655
138. Munn DH, Sharma MD, Lee JR, Jhaever KG, Johnson TS, Keskin DB, Marshall B, Chandler P, Antonia SJ, Burgess R, Slingluff CL Jr, Mellor AL (2002) Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. Science 297:1867–1870
139. Dittmer U, He H, Messer RJ, Schimmer S, Olbrich AR, Oehlen C, Greenberg PD, Stromnes IM, Iwashiro M, Sakaguchi S, Evans LH, Peterson KE, Yang G, Hasenkugl KJ (2004) Functional impairment of CD8(+) T cells by regulatory T cells during persistent retroviral infection. Immunity 20:293–303
140. Thornton AM, Shevach EM (2000) Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. J Immunol 164:183–190
141. Zelinskkyy G, Robertson SJ, Schimmer S, Messer RJ, Hasenkugl KJ, Dittmer U (2005) CD8+ T-Cell dysfunction due to cytolytic granule deficiency in persistent Friend retrovirus infection. J Virol 79:10619–10626
142. Bushell A, Jones E, Gallimore A, Wood K (2005) The generation of CD25+ CD4+ regulatory T cells that prevent allograft rejection does not compromise immunity to a viral pathogen. J Immunol 174:3290–3297
143. Suvas S, Kumaraguru U, Pack CD, Lee S, Rouse BT (2003) CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. J Exp Med 198:889–901
144. Toka FN, Suvas S, Rouse BT (2004) CD4+CD25+ T cells regulate vaccine-generated primary and memory CD8+ T-cell responses against herpes simplex virus type 1. J Virol 78:13082–13089
145. Franzese O, Kennedy PT, Gehring AJ, Gotto J, Williams R, Maini MK, Bertoletti A (2005) Modulation of the CD8+T-cell response by CD4+ CD25+ regulatory T cells in patients with hepatitis B virus infection. J Virol 79:3322–3328
146. Stoop JN, van der Molen RG, Baan CC, van der Laan LJ, Kuipers EJ, Kusters JG, Janssen HL (2005) Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. Hepatology 41:771–778
147. Eggema MP, Barughare B, Jones N, Okello M, Mutalya S, Kityo C, Mugenyi P, Cao H (2005) Depletion of regulatory T cells in HIV infection is associated with immune activation. J Immunol 174:4407–4414
148. Kohno T, Yamada Y, Akamatsu N, Kamihira S, Imaizumi Y, Tomonaga M, Matsuyama T (2005) Possible origin of adult T-cell leukemia/lymphoma cells from human T lymphotropic virus type-1-infected regulatory T cells. Cancer Sci 96:527–533