Suppressor T cell is an old term, originally found in the 1970s literature (49, 50), but it was short-lived because advances in molecular biology soon afterward proved that the gene locus, thought to be associated with suppression, was nonexistent. Our recent understanding started with the finding that a small proportion of CD4⁺ T cells in mice constitutively expressed the high-affinity interleukin-2 (IL-2) receptor α-chain, CD25, and depletion of these cells (now designated natural regulatory T cells [Treg]) caused autoimmune disease and enhanced responses to foreign antigens (112). This study resulted in a rebound of intense interest in suppressor T cells, and similar cells in humans were identified shortly afterward (78, 98; reviewed in reference 9). It is now well established that natural Treg suppress a diverse range of immune responses in a contact-dependent manner in vitro and in vivo, in response to T-cell receptor (TCR)-mediated stimulation (reviewed in references 43 and 136). Human Treg are less well defined than their murine counterparts and less well studied in general, although the two have features in common. Differences between human and murine Treg, which may complicate the interpretation of human data, have been noted. For instance, in naïve inbred pathogen-free mice, natural Treg can be reliably isolated based on their CD25 expression; however, this population in adult outbred humans is inevitably a mixture of Treg and recently activated T effector cells, with the latter expected particularly during an ongoing infection. Opinions are divided over whether Treg play a pathogenic role in chronic viral infection in humans, especially in infections for which the development of a vaccine has so far failed, such as in chronic viral infection in humans, especially in infections for which the development of a vaccine has so far failed, such as in the case of human immunodeficiency virus (HIV) and hepatitis C virus (HCV). This review considers the key findings in Treg biology and discusses the current position for Treg in viral infections in humans, especially in infections for which the development of a vaccine has so far failed, such as in the case of human immunodeficiency virus (HIV) and hepatitis C virus (HCV). This review considers the key findings in Treg biology and discusses the current position for Treg in viral infections in humans.
Providing IL-2 to IL-2

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tantly, Treg express a more diverse TCR repertoire than non-
Treg. This may explain why Treg can inhibit immune responses
not only to self antigens but also to non-self antigens. Details
of the thymic development of murine Treg were reviewed
elsewhere (84, 101). After exit from the thymus, Treg proliferate
in response to homeostatic signals (38), which are dis-
cussed in later sections. Although extrathymic conversion of
naive T cells to Treg is possible under certain experimental
conditions (6, 24, 66), the classical view is that the peripheral
Treg pool is derived largely from thymic Treg precursors and
that conversion in nature, at least in mice, is rare (42). An
extraordinary example of conversion in nature in mice was
reported recently and is believed to be important for gut and
oral tolerance toward food and environmental antigens (14, 28,
121). The CD103+ dendritic cells (DC) from mesenteric lymph
nodes express transforming growth factor β (TGF-β) and
Aldh1a2, an enzyme that converts vitamin A to retinoic acid
(RA), and the combination of TGF-β and RA effectively con-
vert naïve T cells into Foxp3+ Treg (28). TGF-β- and RA-
mediated conversion are discussed further in a later section.

**FOX3 in human cells.** Similar to results with scurfy and
Foxp3−/− mice, the CD4+CD25+ T cells isolated from IPEX
patients do not express FOXP3 or express nonfunctional
FOX3 and have no suppressive activity (48, 145). In periph-
eral blood mononuclear cells (PBMC) from healthy individu-
als, the CD25high population contains the most potent suppres-
sor cells, and a great majority of these cells express FOXP3
(10, 105). Human Treg have also been isolated from cord
blood (124) and the thymus (118). Because FOXP3 is not
expressed on the cell surface, other flow cytometric gating
strategies were developed to facilitate human Treg isolation.
One example is to gate for the CD45RA−CD4+CD25high pop-
ulation, which can give rise to a homogeneous Treg line upon
vitro expansion (57, 58). It was recently reported that surface
expression of CD127 (the IL-7 receptor) was high on
effector T cells and low on Treg and allows reliable and con-
venient recognition of Treg (CD25+CD127−) from effector
cells (CD25−CD127+) (55, 85, 113).

In mice, Foxp3 was reported to have no intrinsie role in the
response of non-Treg toward foreign antigens, and de novo
upregulation of Foxp3 on recently activated CD25− cells does
not occur in vitro and in vivo during acute infection (42, 43).
However, there is considerable dispute about whether this is
to be true also for humans.

**Activated human T cells express FOX3.** Several groups
reported that in vitro stimulation of PBMC with anti-CD3 with
or without costimulation resulted in de novo FOXP3 expres-
sion by activated T cells (48, 140). However, in contrast to in
vivo-generated Treg (Treg isolated directly from PBMC), in
vitro-induced FOXP3 expression in effector T cells is transient
(48). FOXP3−activated effector cells are highly proliferative
(141), produce IL-2 and gamma interferon (IFN-γ), retain
CD127 expression, and have no suppressor function (3, 141).

Thus, while the expression of FOXP3 in humans is not re-
stricted to the Treg lineage, the role of this molecule in non-
Treg should be differentiated from its role in Treg. Neverthe-
less, the expression of FOXP3 by activated non-Treg
represents a significant complication in human Treg research.

**Thymic or peripheral origin is less obvious.** Several studies
estimated the TCR repertoire diversity of the human Treg
population. Vβ usage (the prevalence of specific Vβ families
within the repertoire) in a particular T-cell population can be
analyzed by flow cytometry. The critical antigen-binding do-
main of a TCR is complementarity-determining region 3
(CDR3), and each clonal TCR has a different CDR3. Thus,
the diversity of a repertoire can be estimated by the diversity of
the CDR3 sequences (52). When analyzed for Vβ usage and
CD3 diversity, the human CD4+CD25+ Treg population was
found to be broad and complex and remarkably similar to the
CD4+CD25+ population, both within the thymus (69) and the
periphery (45, 69, 138). This observation implies that the same
antigen can elicit either an effector response or a Treg re-

tinction of the thymic development of murine Treg were reviewed

The TCR excision circles (TREC) are episomal DNA mol-
ecules generated during TCR gene rearrangement. TREC do
not replicate and are diluted by T-cell proliferation. Kasow and
colleagues reported that thymic CD4+CD25+ cells showed a
remarkably higher level of TREC than that seen in peripheral
CD4+CD25− cells, suggesting that Treg are probably gener-
ated in the thymus and thereafter migrate to the periphery and
proliferate to compensate for the reduced thymic output in
adult life (69). However, a recent study (138) pointed out that
homeostatic expansion of Treg in humans is insufficient to
sustain the obviously stable numbers of Treg throughout life.
Using [3H]glucose labeling of dividing cells in vivo, Vuk-
manovic-Stjecic et al. showed that CD4+CD25high cells in adult
humans proliferated rapidly with a doubling time of ~8 days
(138). However, this cell population has significantly shorter
telomeres than those in the total CD4+ population and is
unable to upregulate telomerase. In the absence of telomerase,
each cell division reduces the length of telomeric DNA by 50 to
100 bp, eventually leading to cell cycle arrest. The telomere
length of CD4+CD25high cells is ~4 kb, indicating that they are
approaching the limit of further division. The authors (138)
argued that since the Treg number is stable throughout life and
since these cells turn over rapidly yet have limited potential for
further proliferation, they must be derived from other existing
T cells in the periphery. The origins of human Treg have
important consequences for their function during viral infec-
tions. Identifying virus-specific Treg and understanding their
origin is a challenging topic.

**Homeostatic proliferation and the critical role of IL-2.**
When a small number of naïve T cells or memory T cells are
transferred to an immune-deficient host, they proliferate rap-
idly and result in functional restoration of the peripheral T-cell
pool. The signals driving homeostatic proliferation are not
known precisely but are unlikely to be foreign antigens. naïve
T cells require TCR/MHC interaction to proliferate with (34,
134) or without (27) self peptides, and memory T cells do not
even require MHC (96). Treg also respond to homeostatic
signals (46), proliferate rapidly upon transfer to a lymphopenic
host, similar to naïve T cells, and are dependent on self peptide
and MHC to proliferate (46, 114).

IL-2 plays a nonredundant role in promoting the homeo-
static proliferation and survival of natural Treg in vivo (41).
Treg do not produce IL-2 but are many times more sensitive to
IL-2 than are effector T cells (111). Mice deficient in IL-2 or
any of the components of IL-2 receptors develop fatal auto-
immune diseases, and providing IL-2 to IL-2−/− mice (4) or
adoptive transfer of wild-type Treg to IL-2Rβ−/− mice pre-
vented disease (11). It was found that IL-2−/− Foxp3+ cells were substantially less effective than wild-type Treg in repopulating immune tissues of immune-deficient recipients (12, 41). The role of IL-2 has been carefully reevaluated in recent years (reviewed in reference 89), and it is currently believed that this potent in vitro T-cell growth factor plays a redundant role in vivo in promoting immunity but a nonredundant role in sustaining tolerance.

In the past decade, IL-2 has been used in multiple clinical trials, mostly in conjunction with highly active antiretroviral therapy (HAART), to treat HIV patients who have persistent low CD4 T-cell counts despite HAART. However, if the primary role of IL-2 is to promote tolerance, the outcome of these trials may to some degree reflect the consequence of manipulating the Treg compartment. IL-2 therapy resulted in a sustained increase in CD4 T-cell counts for most patients (19, 70, 125), and the effect appeared to be long lasting as shown by 10-year follow-up studies (75). In a pooled retrospective analysis of IL-2 given intravenously, plasma HIV RNA levels were significantly lower in IL-2-treated patients than in patients receiving antiretroviral therapy only (32). It is currently unknown if IL-2 treatment would eventually enhance the survival of patients; in particular, IL-2 treatment at high or low doses did not result in enhanced immunological responses (77). The efficacy of IL-2 was addressed in larger phase III studies (126). The effect of IL-2 was more profound on CD4 than CD8 T cells because, although IL-2 enhances the proliferation of both CD4+ T and CD8+ T cells, it prolongs the survival of expanded CD4+ but not CD8+ T cells (116). Among the expanded cells, most are naive CD4+CD45RO− T cells expressing CD25 and FOXP3 (75). This population produces little IL-2 and has a suppressive function, in which sense it is similar to natural Treg, but also not identical to CD25high natural Treg, as they have a higher TREC content and are less susceptible to apoptosis.

**Suppression in vitro and in vivo.** In addition to homeostatic expansion, Treg also proliferate rapidly in vivo in response to TCR ligation by foreign antigens (74, 139). However, Treg exhibit profound anergy in vitro in response to TCR-mediated stimulation, unless supplied with a high dose of IL-2. In response to TCR ligation, Treg suppress the IL-2 production and/or proliferation of other T cells in vivo and in vitro (130, 131). The suppression itself is antigen nonspecific (131) and independent of APC (reviewed in reference 94). The addition of IL-2 to the cultures abolishes Treg anergy but maintains their suppressive function (129). IL-2 may be important for Treg function, because neutralization of IL-2 abolished suppression (128). However, Foxp3+ cells isolated from IL-2−/− mice suppressed as potently as wild-type Treg (41); thus, this issue is currently inconclusive. Treg can also suppress the function of B cells (81, 82), NK cells (51), and CD8+ T cells. Given the diversity of the target cells, it is possible that a universal membrane contact is involved, as it was recently reported that cyclic AMP transfer from Treg into its target cells via membrane gap junctions rendered the target cell unresponsive to a variety of stimuli (18).

**TGF-β and conversion.** TGF-β is an important molecule implicated in many aspects of biological function (see reference 79 for a review). Conversion of peripheral naïve CD4+CD25− T cells into Treg by antigen-specific activation in the presence of TGF-β has been described for both mice and humans (24, 37, 146, 153). TCR engagement is essential, and TGF-β alone cannot mediate conversion. The converted cells expressed Foxp3 de novo, became CD25+, and expressed high levels of TGF-β mRNA, but natural Treg were little affected under the same conditions (24, 37). The expression of Foxp3 inhibits the Smad7 promoter in CD4+CD25+ T cells and rendered them highly susceptible to TGF-β signaling (37). The link between TGF-β and Treg was reviewed recently (109).

The vitamin A metabolite RA spectacularly enhances TGF-β-mediated conversion (14, 28, 95). RA alone cannot mediate conversion, but it somehow lowers an unknown threshold of TGF-β-mediated conversion. The in vitro-converted Treg home to the small intestine in vivo after adoptive transfer, highlighting a relevant role of these cells in gut tolerance (14). Adoptive transfer studies, however, showed that RA-TGF-β-converted Treg are as unstable as TGF-β-converted Treg, as ~50% of injected cells lost Foxp3 expression in vivo within several days of encountering antigen, although in the absence of antigen, RA-TGF-β-converted Treg are more stable than TGF-β-converted Treg (14). Thus, although the function and phenotype of TGF-β-or RA-TGF-β-converted Treg resemble those of natural Treg, their therapeutic potential is currently uncertain.

**IMPLICATIONS OF Treg IN VIRAL INFECTIONS**

In acute, self-limited virus infections, a vigorous CD8 T-cell response leads to viral clearance. However, many viruses induce persistent infections, despite continuous measurable CD8 T-cell responses (103), a situation in which Treg may be involved. Although Treg activity could be beneficial to the host due to a suppression of tissue damage mediated by virus-specific effector T cells, those T cells simultaneously inhibit the host immunity important for viral clearance and thus may contribute to persistent infection.

**Treg in murine chronic viral infections.** (i) FV. The implication of Treg in viral infection was first described for mice persistently infected with Friend virus (FV) and was reviewed in reference 56. CD8+ T cells are critical for the control of acute FV infection but fail to control virus during persistent infection (151). Iwashiro et al. (65) were the first to show that CD4+ Treg were involved in the suppression of CD8 function during persistent FV infection (65). The CD4+ Treg inhibited IFN-γ production and the cytotoxic activity of CD8+ T cells but did not affect their antigen recognition or proliferation (30). The suppressor activity in this model was not contained solely within the CD25+CD4+ fraction, but injection of anti-GITR antibody partly abolished the suppressor effects (30). The characteristic suppression of CD8+ T-cell effector functions but not proliferation could be reproduced in vitro using Foxp3+CD4+CD25+ cells from persistently infected mice and was shown to be contact dependent (104).

(ii) HSV. In the footpad model of herpes simplex virus (HSV) infection, the CD8+ T-cell response to the dominant HSV antigens is suppressed by CD4+CD25+ Treg (123). CD25+ cell depletion enhanced the peptide-specific CD8+ T-cell response (proliferation, IL-2 and IFN-γ production, and cytotoxic T-lymphocyte [CTL] activities in vitro and/or in vivo).
Adoptive transfer experiments demonstrated that HSV infection induced a population of HSV-specific Treg that impaired the viral clearance rate during the acute phase of infection and exercised a long-term effect on the CD8 memory pool, as CD8 memory T cells generated in the absence of Treg mediated viral clearance more effectively (123). In the corneal model of HSV infection, Treg inhibited the HSV-specific CD4+ cell response, but in this case CD25+ cell depletion increased the tissue damage caused by CD4+ effector cells (122). Treg isolated from stromal keratitis lesions in mice expressed high levels of Foxp3 mRNA and exhibited suppressor function in vitro. In the presence of Treg, effector CD4+ T cells showed reduced infiltration into the lesion, perhaps due to a lower expression of VAL-4, a molecule which facilitates T-cell migration into tissue. The beneficial effects of Treg in this model were counterbalanced by CD4+CD25+ antiviral effector cells, because when naïve Treg alone were transferred to SCID mice prior to infection without cotransfer of CD4+CD25+ cells, the mice did not develop corneal lesions but died earlier due to HSV encephalitis (122). These studies highlighted the idea that Treg can be friend or foe during viral infection, depending on the magnitude of the effector T-cell responses that they regulate (reviewed in reference 108).

(iii) Other viruses. The implication that Treg can influence virus-specific CD8+ T-cell responses was further illustrated by influenza A virus and vaccinia virus infections of mice or by animals injected with simian virus 40-transformed cells (54). The immunodominance hierarchies of the major CTL epitopes are well characterized in these models. Regardless of whether CD8+ T effectors were generated by direct priming or cross priming, depletion of CD25+ cells enhanced their function (IFN-γ production and CTL activity). Importantly, the effects were more pronounced for immunodominant epitopes than subdominant epitopes, suggesting that Treg function to narrow the disparities among CD8 T-cell epitopes (54).

Treg in persistent virus infections of humans. (i) HCV. Currently, about 3% of the world population are HCV carriers, a proportion of whom will eventually develop cirrhosis, cancer, or liver failure. Persistent HCV infection is associated with a weak, narrow cell-mediated immune response that is characterized by a low frequency, typically <0.3%, of HCV-specific IFN-γ-producing T cells as measured by ex vivo enzyme-linked immunospot assay (25, 29, 127) and an even lower frequency of IL-2-producing cells (115).

HCV-specific impairment of DC function was hypothesized to result in altered CD4+ T-cell function, because in persistently infected patients, the absolute number of CD4+ T cells was increased but HCV antigen-specific proliferation was not detected (133). Depletion of CD4+ cells in an ex vivo enzyme-linked immunospot assay enhanced IFN-γ secretion by the remaining PBMC (120). Importantly, the effect of CD4+ cell depletion was observed for all major antigenic regions within the HCV polyprotein without significant bias toward any particular region, implying a global effect of HCV-specific T-cell suppression (120). A higher proportion of circulating CD4+CD25+ cells was reported to be present in the blood of HCV carriers than in that of convalescent individuals and of uninfected control individuals (22, 120), and a much higher percentage of CD4+CD25+ cells (110) or FOXP3+ Treg (142) was found within the infected liver than in the blood. The CD4+CD25+ T-cell fraction isolated from PBMC of infected patients suppressed virus-specific CD8+ T-cell proliferation and IFN-γ production, and depletion of these cells resulted in increased IFN-γ production by the remaining cells in response to HCV proteins (17, 22, 110). These studies suggest an important role for Treg in establishing and/or maintaining HCV persistence.

Data from our laboratory supported these findings (80). We found that Foxp3+ IFN-γ Treg were preferentially recalled by HCV peptides in short-term PBMC cultures. In our studies, Foxp3 expression was not de novo; rather, CD25 expression on a proportion of preexisting Foxp3+ cells was upregulated by certain HCV peptides. Importantly, a majority of the peptides tested were unable to upregulate CD25 expression on Foxp3+ IFN-γ Treg, and only a small number showed this capacity, suggesting that dominant Treg epitopes may exist (80). We are currently determining the location and hierarchy of Treg epitopes for HCV, which will be important for vaccine design and development. IL-10 may also play a role in promoting HCV persistence. A significant proportion of HCV tetramer+Foxp3+CD4+ Treg in short-term PBMC cultures produced IL-10 but not TGF-β (N. Nakamoto, H. Ebinuma, Y. Li, W. Kwok, D. Price, B. Levine, and K. -M. Chang, poster abstract, Keystone Symposia on Regulatory T Cells, abstr. 126, Vancouver, Canada, 2007), and HCV-specific IL-10-producing type 1 Treg (Tr1) were also found in persistent HCV infection (2, 87).

We have not been able to detect HCV RNA in ex vivo Treg isolated from HCV-infected patients, and so far we have also failed to infect DC with the cell culture-permissive HCV strain JFH-1 (S. Li and E. Gowans, unpublished observations). Nevertheless, as DC-SIGN was reported to be one of the cellular receptors for HCV (86), it was speculated that even if HCV could not productively infect DC, binding of HCV to DC-SIGN may transduce a tolerance signal and affect the downstream Treg compartment.

In addition to CD4+ Treg, CD8+ Treg in persistent HCV infection were described (15). In the presence of exogenous IL-2, de novo expression of FOXP3 was detected on a proportion of tetramer+CD8+ T cells from short-term PBMC cultures stimulated with HCV peptides. IL-2 was required in this assay because, in the absence of IL-2, only FOXP3+CD8+ effector cells were expanded. Importantly, the CD8+ T effector cells and CD8+ Treg expanded in these cultures had the same antigen specificity, as shown by tetramer staining (15).

In contrast to the results described above, Treg deficiency was reported in patients with persistent HCV infection. These patients often develop mixed cryoglobulinemia (MC), an autoimmune B-cell proliferative disorder (20). In a comparison of HCV/MC patients to healthy donors, circulating Treg frequency was found to be significantly reduced in HCV/MC patients, and these Treg showed reduced immunosuppressive activity. It is known that HCV-infected patients have an IL-2 deficiency (115). Recent data obtained using the sensitive Bioplex assay in persistently infected HCV patients also showed that IL-2 was often undetectable or detected at lower concentrations than IFN-γ in the supernatant of short-term PBMC cultures stimulated with HCV peptides (R. French et al., personal communication). Because IL-2 is important for the survival and homeostatic expansion of Treg, it is possible that the
autoimmune disease in HCV/MC patients is linked to IL-2 deficiency. A recent study of chimpanzees (90) found that Treg from HCV-experienced animals differed from those of naive animals in that they were more proliferative in response to IL-2 and had a lower TREC content, which suggests that HCV infection altered the Treg compartment. The frequency and function of Treg in persistently infected and recovered chimpanzees were similar, and in addition to suppressing HCV-specific IFN-γ secretion, Treg from both groups suppressed apoptosis of HCV peptide-specific CD8+ T cells. This result suggests that Treg may regulate memory responses even after recovery, perhaps by preventing memory cell apoptosis. It is likely that Treg are beneficial or detrimental depending on the circumstances.

(ii) HIV. T-cell dysfunction plays an important role in AIDS disease progression. One explanation for impaired T-cell function arises from recent studies on Treg in HIV-infected patients. Immunosuppressive CD4+ T cells, which express the common Treg marker CD25, have been shown to inhibit HIV-specific T-cell responses (1, 5, 72, 143), affecting both CD4+ (1, 72, 143) and CD8+ (1, 72) T-cell functions. In general, IFN-γ production by T cells and T-cell proliferation were affected by Treg from HIV-infected patients. In addition, the cytolytic and noncytolytic anti-HIV activities of CD8+ T cells were shown to be suppressed by CD25+ Treg (73). The immunosuppressive cytokines IL-10 and TGF-β were produced by the CD25+ CD4+ Treg; however, these molecules might be only surrogate markers for Treg, since their expression in vitro was mostly cell contact dependent (72, 143). HIV antigens caused Treg to proliferate specifically, thus demonstrating the presence of virus-specific cells among the CD4+ CD25+ T-cell subset (143).

Treg could have contrasting effects in HIV infection: on the one hand, they might limit the general, nonspecific immune activation usually found in HIV-infected patients, or they may suppress antiviral immune responses. In fact, strong Treg function in blood was linked to reduced chronic immune activation and low viral load (VL) in patients (31, 72). In contrast, recent results for HIV-positive patients and simian immunodeficiency virus (SIV)-infected macaques (33, 61) showed that the accumulation of Treg in lymphoid tissues was clearly associated with high VL and reduced CTL activity (35).

Thus, Treg in HIV infection might exert different functional properties depending on their localization within the infected host. Alternatively, an active process of viral replication may promote Treg compartmentalization into lymphoid organs, which renders the interpretation of the data concerning Treg in the circulating blood difficult. Another unresolved issue is whether Treg act as a reservoir for efficient HIV replication. It has been reported (99a) that Treg are highly susceptible to HIV infection in vitro; however, in vivo data failed to corroborate this finding, since the level of infected Treg in tissues in the SIV model was relatively low. FOXP3 overexpression in T cells resulted in discrepant findings, leading to either suppression or enhancement (31) of HIV-1 promoter transcription. Clearly, additional work is needed to address the role of different viral factors in the dynamics of Treg infection by HIV.

The question remains whether natural Treg or a population of induced Treg, which are generated from naive CD4+ T cells during infection, play a major role in HIV pathogenesis. In HIV-infected lymph nodes, several groups have found a clear accumulation of Treg (5), which argues for an induction, or at least an expansion, of these cells during infection (35). The mechanism for the induction or expansion during retroviral infection needs to be determined to develop therapeutic means for intervention. Our laboratory has proposed a model (99) in which a direct HIV gp120-CD4 interaction promoted survival and subsequent accumulation of Treg. Alternatively, TGF-β1 could play a role in the expansion/induction of Treg during HIV infection. Indeed, increased levels of TGF-β1 were found in lymphoid tissues from HIV progressors compared to non-progressors and uninfected individuals, although the cellular source of elevated TGF-β was not determined (99). During experiments with acute SIV infection, Estes et al. (35) also showed accumulation of TGF-β-expressing Treg which could play a role in inducing or amplifying Treg expansion/induction. Finally, DC might play an important role in Treg cell induction and/or expansion. During an ongoing infection, the differentiation of naive T cells into effector T cells is controlled by DC, which present processed antigenic peptides to T cells. However, mature DC are necessary for efficient antigen presentation. Recent evidence suggests that immature DC induce Treg rather than Th cells upon antigen presentation to naive T cells (135). This might represent a hitherto unrecognized immune escape mechanism by which viruses induce Treg and subsequently suppress antiviral immune responses. HIV can infect DC and alter their functional properties (117). The accessory HIV protein, vpr, downregulates the expression of costimulatory molecules on DC and interferes with the expression of the DC maturation marker, CD83 (88). In addition, vpr inhibits the production of IL-12 and upregulates the immunosuppressive cytokine IL-10 in DC. Alternatively, our and other laboratories have shown that interactions between DC and HIV gp120 or inactivated virus, which harbors intact surface gp120, lead to impaired DC function through direct or indirect mechanisms (26). These functionally impaired DC fail to transmit optimal signal to T cells but appear to favor the emergence of Tr1 cells in vitro (152). Results from Granelli-Piperno (53) also showed that HIV-infected DC were able to induce Tr1 in vitro that can suppress effector T-cell responses. The suppressive effect was dependent partially on IL-10 production by the Treg. In support of this hypothesis, lymph nodes from untreated HIV-infected subjects contained an abundance of immature DC, the loss of which correlated with the initiation of HAART. Lymph node DC from untreated HIV-infected subjects cultured with natural allogeneic T cells induced these T cells to adopt the phenotype of Treg, an ability of DC that was lost after HAART (76). Indoleamine 2,3-dioxygenase (IDO)-mediated tryptophan catabolism in DC can induce a Treg phenotype in Th cells (36), providing an additional mechanism for DC-mediated Treg expansion/conversion during viral infection. This mechanism is expected to play a critical role in HIV infection, because we have detected high levels of IDO expression in tissues of HIV- and SIV-infected hosts during active viral replication (5, 16a, 99), and in vitro HIV-exposed plasmacytoid DC express high levels of IDO (16). Using the FV animal model, we showed that the virus efficiently infects DC and interferes with their maturation process (9a). As a result, the interaction between infected DC and naive T cells was altered compared to the uninfected DC/T-cell interaction and the infected DC preferentially expanded Foxp3-positive...
Treg, with suppressive activity, rather than Th cells. The timing of maximum DC infection in vivo (9a) correlated with the maximum expansion of Treg and the functional impairment of CTL during acute infection (150). Thus, the infection of DC, and/or the virally induced modulation of DC function, seems to be a common feature of retroviral infections and might be a critical step in the induction or expansion of Treg in HIV infection and subsequent immune evasion by the virus.

(iii) HBV. Hepatitis B virus (HBV) leads to a persistent infection in ~5% of infected adults and ~95% of infected neonates. There are currently ~300 million carriers worldwide. Different groups have published contradictory data regarding the involvement of Treg. A recent study of a large HBV cohort in China (149) found that the frequency of CD25+CD4+Foxp3+ Treg in PBMC and liver biopsy specimens was significantly higher in carriers than in individuals with resolved infection and healthy controls. The increase in Treg was associated with hepatitis B e antigen (HBeAg) status and impaired viral clearance. This is contradictory to an earlier study, in which increased Treg frequency did not correlate with the VL (119). Yang et al. (149) also reported that serum TGF-β levels correlated positively with Foxp3+ Treg frequency, and the authors concluded that TGF-β may play a role in Treg induction during HBV infection (149). Studies by another group (44), however, did not support a possible pathogenic role for Treg in persistent HBV infection, as they found no difference in Treg frequencies between asymptomatic carriers, patients with chronic clinical activity, individuals with resolved infection, or healthy controls (44). Furthermore, Treg frequency was not correlated with HBeAg status or VL or with different regimens of antiviral therapy. In addition, depletion of CD4+CD25+ T cells in vitro enhanced the proliferation of HBV-specific CD8+ T cells and IFN-γ production from these cells in all patients, regardless of their disease stage. Stoop et al. reported that Treg depletion enhanced the proliferation of the remaining PBMC to HBV core antigen, but not tetanus toxoid (119), suggesting that CD4+CD25+ T cells in HBV patients were HBV specific. In contrast, Franzese et al. (44) showed that the CD8+ T-cell responses driven by control peptides were enhanced in a manner equal to that of HBV-driven responses, implying that the CD4+CD25+ T pool in HBV+ patients had no bias in antigen specificity (44).

(iv) HTLV. Human T-cell lymphotropic virus type 1 (HTLV-1) may be an example of a virus which can induce Treg dysfunction. HTLV-1 is a retrovirus which infects both CD4+ (132) and CD8+ (97) T cells and establishes lifelong persistence. A small percentage of HTLV-1 carriers develop adult T-cell leukemia and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/STP). CD4+CD25+ cells are the major reservoir for HTLV-1 replication (148, 149). In STP patients, ~90% of CD4+CD25+ cells contain HTLV-1 proviral DNA and express HTLV-1 tax mRNA at significantly higher levels than CD4+CD25− cells. The percentage of FOXP3+ cells, as well as the level of FOXP3 expression within single cells, was lower in HAM/STP patients than in healthy donors. This FOXP3 downregulation seems to be induced by the HTLV-1 tax protein, since studies showed that when tax was expressed in Treg from healthy donors, the transfected Treg expressed lower levels of Foxp3 mRNA and lost immunosuppressive function. Thus, HTLV-1 infection results in Treg dysfunction as a consequence of direct inhibition of Foxp3 expression by the virus-encoded protein tax (147, 148).

(v) CMV and EBV. In healthy donors, CD25+ cell depletion enhanced IFN-γ and IFN-α production by cytomegalovirus (CMV) tetramer+ CD8+ T cells (1), and the authors concluded that Treg may suppress antiviral immunity in general. Although IL-10-producing Tr1 cells play a central immunosuppressive role in latent Epstein-Barr virus (EBV) infection (92), EBV-specific Foxp3+ Treg may also be involved. EBV-specific CD4+ Treg clones were generated using several T-cell epitopes, and a characterization of these clones revealed that, while most clones showed a Th phenotype, Treg clones expressing high levels of CD25/GITR/Foxp3 also existed. These EBV-specific Treg suppressed other T-cell responses in a contact-dependent manner. Thus, an important point highlighted by this study is that Treg clones and Th clones recognized the same determinants, and the authors called for caution when designing peptide-based vaccines (137). In immunosuppressed organ transplant recipients, EBV-specific CD8+Foxp3+ Treg coexpressing IFN-γ and IL-10 but not TGF-β could be generated ex vivo by stimulating bulk T cells with peptide-loaded Th1-polarized DC. These Treg suppressed proliferation of autologous noncognate CD4+ T cells in a contact-dependent manner. Under the same conditions, CD8+ effector cells, but not CD8 Treg, were generated from the PBMC of healthy donors, suggesting that the DC-CD8+ T-cell interaction was altered in immunosuppressed patients (102).

CONCLUDING REMARKS

Treg dysregulation has been reported to be present in many examples of persistent viral infections. It is unclear if the altered Treg function is a cause or effect of viral persistence in the host. However, since the deficiency in Treg number and/or function may cause virus-associated autoimmune tissue destruction and enhanced Treg frequency and activity may suppress antiviral immunity, Treg dysregulation does not appear to be an innocent coincidence of viral persistence. It is hard to predict whether therapeutic manipulation of Treg could help to resolve chronic viral infection or limit its damage. In patients with persistent HCV infection, virus-specific Treg outnumbers virus-specific T effector cells by far, and we wonder if, during the long course of the disease, Treg are somehow expanded while T effector cells are deleted. A central question remaining for HCV, as well as for other viral diseases, is whether Treg require priming to recognize virus antigen, and if so, how this priming would occur. Our work with HIV and FV supports the hypothesis that certain viruses can subvert DC function so that a naïve T cell is primed to become a Treg rather than an effector T cell, or alternatively, if Treg and T effector cells are distinct lineages, naïve Treg may be preferentially primed over T effector cells. In some situations, Treg are deleted as a consequence of virus replication or by unknown mechanisms, adding one more layer of complexity to this already difficult topic. One important practical consideration is whether dominant Treg epitopes exist. Although it is speculated that Treg and T effector cells may recognize the same epitopes, the location and hierarchy of Treg epitopes in any viral protein are largely unknown. Given that vaccine development has so far failed for HIV and HCV, the removal of
dominant Treg epitopes, if they exist, may increase the chances of developing successful vaccines.

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