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Regulatory T Cells and Viral Infections

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1. ABSTRACT

Viral infections often lead to generalized immunosuppression characterized by the downregulation of virus-specific $CD4^+$ and $CD8^+$ T cell responses and/or non-specific inflammation. One of the mechanisms for the virus-induced immunosuppression is the induction of $CD4^+CD25^+$ regulatory T (Treg) cells that act to suppress effector T cell functions during infection. Depending on the situation, the $CD4^+CD25^+$ Treg cell-mediated suppression can be either beneficial or detrimental to the host. On one hand, they play a critical role in maintaining host homeostasis by controlling exaggerated and destructive inflammations paralleling strong antiviral immune responses and thus contribute to host protection. On the other hand, suppression of virus-specific T cell responses by the Treg cells depresses host antiviral immune responses and thus facilitates viral persistence and disease progression. Despite numerous reports on induction of $CD4^+CD25^+$ Treg cells in viral infections, it is still not fully clear how they are induced and how they act to suppress effector T cell functions in viral infections. This review discusses recent progress in our understanding of the role of virus-induced $CD4^+CD25^+$ Treg cells and the mechanisms by which they are induced and exert their function during infection.

2. INTRODUCTION

Regulatory T cells, particularly $CD4^+CD25^+$ Treg cells, have been shown to play an important role in regulating immune responses and maintaining homeostasis under various disease conditions including autoimmune disease, inflammation, cancer, and microbial infections. Indeed, a growing body of studies has indicated that $CD4^+CD25^+$ Treg cells actively regulate host antiviral responses by suppressing effector T cell activation and functions during viral infections. They are initially recognized by their roles in mediating immunologic self-tolerance to prevent the activation of self-reactive T cells and thus prevent harmful autoimmune responses to self and foreign antigens (1, 2). The finding by Belkaid *et al* in 2002 on the ability of $CD4^+CD25^+$ Treg cells to control *Leishmania major* persistence and immunity uncovered a new role of these Treg cells in regulation of T cell responses to foreign pathogens (3), and thus extended their role in maintaining host homeostasis beyond autoimmunity. Accordingly, their target cell repertoire for suppression also expanded to include pathogen-specific $CD4^+$ and $CD8^+$ T cells that are not self-reactive in nature. Because of their involvement in a wide variety of disease types and their unique role in helping both pathogens and the host, $CD4^+CD25^+$ Treg cells have been under intensive

investigation in recent years, and are the best characterized member in the regulatory T cell family which includes several subpopulations of T cells with similar suppressive activities but distinct phenotypes, origins, and mechanisms of action, such as T regulatory type 1 (Tr1) (4), Th3 (5), and $CD8^+$ (6, 7) Treg cells. Although all these groups of regulatory T cells have been shown to be involved in suppression of T cell immune responses during viral infections, this review will mainly focus on the $CD4^+CD25^+$ Treg cells, since they are likely the major player in the Treg cell family and most of the Treg cell-associated suppressive effects observed in viral infections are mediated by this group of Treg cells.

Effective control of viral infection relies on the function of effector T cells to mount strong virus-specific immune responses characterized by the activation of cytotoxic T cells (CTL). However, this function is often suppressed or impaired in viral infections, especially in chronic or persistent viral infections such as those caused by human immunodeficiency virus (HIV), hepatitis C virus (HCV), and Epstein–Barr virus (EBV). This suppression enables viruses to evade host immune surveillance, prevents them from being eradicated by host immune cells, and facilitates the establishment of their persistence or latency. A number of mechanisms have been proposed for the virus-induced immunosuppression, and different mechanisms have been defined in different viral infections. Some examples include direct infection of immune cells leading to the impairment in their capability to mount vigorous antiviral immune responses, induction of immunosuppressive soluble factors elaborated by host immune cells or infected cells of other origins, induction of immunoregulatory cells, suppressive effect of viral proteins, or dysregulation of immune responses such as cytokine shift from Th1 to Th2 responses (8-10). During the recent years, $CD4^+CD25^+$ Treg cells that are expanded and activated upon virus infection have been found to possess strong suppressive effect on virus-specific effector $CD4^+$ helper (Th) and $CD8^+$ cytotoxic T cells (11-15). With their potent immunosuppressive properties, $CD4^+CD25^+$ Treg cells represent another mechanism by which viruses induce immunosuppression to aid their efforts to overcome or evade host antiviral responses. By suppressing host antiviral responses, $CD4^+CD25^+$ Treg cells contribute to the development of chronic or persistent infections and thus are beneficial to the virus.

However, the combat between viruses and the host is a dynamic process. While viruses endeavor every effort to overcome or evade host antiviral defensive responses, the host immune system strives to mount vigorous antiviral responses to eliminate invading viruses. The activation of immune cells to generate responses to viral antigens involves a series of interactions and events that culminates in the formation of both non-specific inflammatory and specific humoral and/or cell-mediated immune responses. These responses need to be strong so that viruses can be eliminated and the infection can be confined quickly. However, strong responses, both non-specific inflammatory and specific immune responses, can be harmful to the host if the activated immune cells overreact. Obviously, these antiviral responses require a high degree of regulation to be effective. $CD4^+CD25^+$ Treg cells are likely to play a key role in this process to control excessive inflammatory and immune responses. This has been demonstrated by an increasing number of studies showing that $CD4^+CD25^+$ Treg cells function to suppress immune-mediated pathogenesis resulting from viral infections (14, 16-19). Apparently, they function to benefit the host.

Thus, $CD4^+CD25^+$ Treg cells induced during viral infections can be a double-sided sword that, depending on the situation, can be either beneficial or detrimental to the host. Their suppressive function is essential for the maintenance of host homeostasis during the course of antiviral immune responses and, therefore, constitutes an indispensable component of host defense to viral infections. Despite the large number of studies showing the activation and involvement of $CD4^+CD25^+$ Treg cells in viral infections, the mechanisms for their induction and action are still not fully understood. This review intends to provide a brief summary of recent studies on how these cells are induced and how they act to suppress effector T cell functions in viral infections. A better understand of the dynamics and mechanisms of $CD4^+CD25^+$ Treg cell induction and action during infection will help us develop appropriate strategies to harness these cells towards desired clinical outcomes that benefit the host.

3. NATURAL VS INDUCIBLE $CD4^+CD25^+$ TREG CELLS

Based on their origins, $CD4^+CD25^+$ Treg cells can be further divided into two subpopulations, the natural Treg cells that emerge from the thymus (1, 20-22) and the inducible Treg cells that are induced and converted in the periphery from $CD4^+CD25^-$ naive T cells (22-24). The $CD4^+CD25^+$ Treg cells isolated from clinical samples of viral infections may contain either or both of these two subsets of Treg cells. Phenotypically, they are indistinguishable in peripheral blood or tissues.

3.1. Natural $CD4^+CD25^+$ Treg cells

This type of Treg cells are generated and matured in the thymus and then exported to the periphery. They are known as natural Treg cells. They represent 5 - 10% of peripheral $CD4^+$ T cells in mice and less than 5% in humans and constitutively express CD25, the α subunit of the IL-2 receptor, on their surface (1, 25, 26). The thymus origin of these cells was demonstrated by Asano *et al* who showed that neonatal mice thymectomized on day 3 after birth lacked $CD4^+CD25^+$ Treg cells and developed autoimmune gastritis and high titers of circulating antiparietal cell autoantibodies (25). Further studies have demonstrated that natural $CD4^+CD25^+$ Treg cells are potent suppressor T cells that mediate strong immunosuppression in a contact-dependent but cytokine-independent manner (26-30). *In vitro* studies have shown that they are anergic to polyclonal, allogenic, or antigen-specific stimulation (27, 31, 32). However, they are able to proliferate *in vivo* in response to T cell receptor (TCR)-mediated stimulation

(33). Their suppressive function requires their activation via TCR stimulation, and this activation is independent of co-stimulation mediated by CD28/CTLA-4 interactions with CD80/CD86 (26-29, 34-36). In addition to CD25, they also express, either constitutively or specifically, several receptors and molecules that may participate in their development and function. These include cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) (37), glucocorticoid-induced tumor necrosis factor receptor (GITR) (38), and Forkhead box P3 transcription factor (FOXP3) (39).

3.2. Inducible $CD4^+CD25^+$ Treg cells

This type of Treg cells are generated in the peripheral and are converted from $CD4^+CD25^-$ T cells under certain conditions. Generation of $CD4^+CD25^+$ Treg cells from $CD4^+CD25^-$ precursor has been demonstrated both *in vitro* (40, 41) and *in vivo* (42). The conversion can be induced by cytokines such as IL-10 (43) and TGFβ (24, 41, 44), dendritic cells (DC) (23, 45), alloantigens (42), superantigens (46), or costimulatory signals (40). Unlike thymus-derived natural Treg cells that show exclusively cytokine-independent suppression, peripheral-converted $CD4^+CD25^+$ Treg cells may act through a cytokine-dependent mechanism (41). Notably, not all conditions that induce CD25 expression to convert the phenotype from $CD4^+CD25^-$ to $CD4^+CD25^+$ will result in the generation of regulatory T cells with suppressive function. This is illustrated by Thornton *et al* who showed that $CD4^+CD25^+$ T cells generated by Con A stimulation of $CD4^+CD25^-$ T cells purified from normal mice failed to suppress the proliferative responses of the $CD4^+CD25^-$ cells (27). This suggests that the conversion of naïve $CD4^+CD25^-$ T cells to $CD4^+CD25^+$ Treg cells is likely a complex process which may require appropriate signals and cytokine milieu, and that mere expression of CD25 is insufficient to render the cells capable to mediate suppression.

4. INDUCTION OF $CD4^+CD25^+$ TREG CELLS IN VIRAL INFECTIONS

The existence of Treg cells and their involvement in immunosuppression during viral infection was observed decades ago in several animal models. In 1975, Toy *et al* observed that cellular immunity was suppressed *in vitro* by Friend virus-infected mouse spleen cells (47). Similarly, Carpenter *et al* observed that suppressor cells from the spleens of reticuloendotheliosis virus (REV)-infected chicken severely inhibited the ability of spleen cells from uninfected chickens to respond to PHA, and the inhibition required cell-cell contact between suppressor and target cells (48). In fact, such virus-induced suppressor cells were reported in a large number of viral infections in various animal models such as in mice infected by Moloney sarcoma virus (49, 50), Friend leukemia virus (47) and AK virus (51), in cats infected by feline leukemia virus (52) and feline immunodeficiency virus (FIV) (53), and in chickens infected by REV (54) and Marek's disease virus (55). In humans, suppressor cells were found in patients infected with measles virus (56), herpes simplex virus (HSV) (57), and reovirus (58). Despite the clear evidence for the presence of such suppressor cells in various types of viral infections, the nature of these suppressor cells were not clear at that time.

It was not until recent years that the role of some T cells, particularly $CD4^+$ T cells, in mediating immunosuppressive effects during virus infection was demonstrated. In 2001, Iwashiro *et al* showed that $CD4^+$ T cells from mice chronically infected with Friend virus were able to suppress antitumor immune responses *in vivo* after being adoptively transferred into uninfected mice and also inhibit CTL generation in mixed lymphocyte cultures *in vitro* (11). This was the first evidence showing the induction of $CD4^+$ Treg cells in viral infection. Since then, the induction of $CD4^+$ Treg cells, particularly $CD4^+CD25^+$ Treg cells, has been demonstrated in many human and animal viral infections including HIV (13, 46), hepatitis B virus (HBV) (59, 60), HCV (15, 61, 62), HSV (12, 16), EBV (63) cytomegalovirus (CMV) (46), human T cell lymphotropic virus type 1 (HTLV-1) (64), FIV (53), murine acquired immunodeficiency syndrome (MAIDS) virus (65), Coxsackievirus B3 (CVB3) (66), dengue virus (19), and simian immunodeficiency virus (SIV) (67). The induction is often manifested by the increased number of $CD4^+CD25^+$ Treg cells in the peripheral blood or local tissues of virus-infected patients, and this increase is always associated with certain changes in host antiviral immune responses or disease status. For example, in HIV infection, the increased frequency of $CD4^+CD25^+$ Treg cells in patient peripheral blood has been related to low peripheral blood $CD4^+$ T cell counts and polarization toward a Th2 immune response (68). In chronic severe HBV infection, the frequencies of $CD4^+CD25^+$ Treg cells in both peripheral blood and liver-infiltrating lymphocytes were significantly increased, and this increase correlated with serum viral load (69). Since it is impossible to differentiate natural from inducible $CD4^+CD25^+$ Treg cells in the peripheral, it is not known whether the increase in $CD4^+CD25^+$ Treg cell frequency after virus infection results from the expansion of natural Treg cells or the conversion of naïve $CD4^+CD25^-$ T cells, or both. Another indication of $CD4^+CD25^+$ Treg cell induction in viral infections is their activation. Normal $CD4^+CD25^+$ Treg cells require activation through the engagement of their TCR to become suppressive (27). However, $CD4^+CD25^+$ Treg cells isolated from virus-infected animals or patients are fully functional and do not need additional TCR-mediated activation. Evidently, these cells have been activated *in vivo* after virus infection, most likely through the engagement of their TCR with viral antigens or other undefined mechanisms.

5. ROLE OF VIRUS-INDUCED $CD4^+CD25^+$ Treg CELLS

As a component of the immune system, $CD4^+CD25^+$ Treg cells are expected to participate in host antiviral immune responses with the goal to protect the host from undue damage by viruses and restore homeostasis. However, their role in viral infections appears complicated and it is still difficult to predict the exact role that they will play in any given virus infection. Whether they act to benefit the host or virus may depend on various factors

such as the nature and the stage of the infection and immune activation.

5.1. Role of $CD4^+CD25^+$ Treg cells in viral pathogenesis

Numerous studies have shown that $CD4^+CD25^+$ Treg cells suppress host antiviral immune responses. Although it is not known whether the suppression represents an active strategy of viruses to weaken host defense against them or just a "side-effect" of host antiviral immune responses, it provides viruses with the opportunity to evade host immune attack and facilitate their spread and pathogenic effects. The targets of suppression by $CD4^+CD25^+$ Treg cells include both $CD4^+$ and $CD8^+$ effector T cells as well as other cells that may be involved in antiviral immune responses such as dendritic cells (70), monocytes/macrophages (71), and NK cells (72, 73). Since $CD8^+$ cytotoxic T cells play a major role in antiviral immune responses, it is not surprising that they are the prime target of $CD4^+CD25^+$ Treg cell suppression. Indeed, a large body of studies have shown that virus-specific $CD8^+$ T cell responses including their proliferation, IFNγ production, perforin expression, and cytotoxic activities are suppressed by $CD4^+CD25^+$ Treg cells in many human and animal viral infections (14, 15, 46, 60, 69, 74-77). As a result, host antiviral immune responses are impaired and infections progress. Interestingly, not all functions of $CD8^+$ T cells are suppressed in every virus infection. For example, suppression of $CD8^+$ T cell effector functions without affecting their proliferation and activation was reported in mouse chronically infected with Friend retrovirus (74, 77), although suppression of $CD8^+$ T cell proliferation was observed in many other viral infections. The question is what influences the $CD4^+CD25^+$ Treg cells to selectively suppress one function of $CD8^+$ T cells but not another. In addition to $CD8^+$ T cells, the proliferation and effector functions of $CD4^+$ T cells are also suppressed by virus-induced $CD4^+CD25^+$ Treg cells in many viral infections (13-15, 18, 19).

For many chronic or persistent infections, how viruses escape host immune surveillance and what leads to the development of persistent infection have been the hot topics of investigation. One possible mechanism appears to be the induction of $CD4^+CD25^+$ Treg cells at the early stage of acute infection that dampens the T cell antiviral responses resulting in their inability to completely clear the virus. For example, in HCV infection, virus-specific T cells have been shown to arise early after infection and strong T cell responses are induced during acute phase of infection (78, 79). This rapid response of immune system is necessary for quick clearance of the virus and control of the infection. However, complete clearance of the virus and full recovery only occur in a small percentage of HCV-infected patients and most patients develop chronic disease with persistent infection. The reason for the failure to clear the virus in most of the patients during the initial antiviral T cell immune responses still remains unknown. However, weak or unsustained acute CTL responses have been linked to the development of chronic disease (79, 80). One possible explanation for the weak $CD8^+$ T cell responses during acute viral infection is the early induction of $CD4^+CD25^+$ Treg cells upon infection. Recently, Perrella *et al* observed that the frequency and suppressive activity of $CD4^+CD25^+$ Treg cells were increased in the majority of the patients with acute HCV infection (81). Similarly, in SIV-infected rhesus macaque and African green monkey models, Estes *et al* (82) and Kornfeld *et al* (67) also observed a rapid induction of $CD4^+CD25^+$ Treg cells during acute SIV infections. More significantly, the early induction of $CD4^+CD25^+$ Treg cells during acute infection was associated with the failure of viral clearance, as patients with elevated frequency and function of $CD4^+CD25^+$ Treg cells progressed to chronic infection, whereas those who showed no significant increase in Treg frequency or function during acute phase of infection cleared the virus and fully recovered from the disease (81). It is highly likely that these Treg cells induced at the early stages of infection suppressed $CD8^+$ T cell functions during acute infection, resulting in the failure of viral clearance. In support of this hypothesis, Suvas *et al* demonstrated in a mouse model of acute HSV infection that depletion of $CD4^+CD25^+$ Treg cells before infection noticeably accelerated the viral clearance (12). These observations provided support for the proposition of early induction of $CD4^+CD25^+$ Treg cells as one of the mechanisms for developing chronic or persistent viral infections and raised the possibility to use this induction as a clinical predictor for the prognosis of acute viral infection.

Early induction of $CD4^+CD25^+$ Treg cells is likely programmed to control the magnitude of $CD8^+$ T cell activity to ensure that no host damage will result from the strong antiviral immune responses which are often accompanied by local or systemic inflammation. However, the induction may have been exaggerated or occurred too early, as in the case demonstrated by Estes *et al* in acute SIV infections (82), where it dampens the $CD8^+$ T cell response before the viruses have been completed cleared. What triggers the premature induction of these Treg cells remains to be determined. Rapid replication and spread of viruses, together with acute inflammation or massive activation of antiviral immune responses during acute infections, may create a microenvironment that facilitates premature expansion and activation of $CD4^+CD25^+$ Treg cells. Thus, it appears that the magnitude and timing of the early induction of $CD4^+CD25^+$ Treg cell activity may play a decisive role in the fate of the acute infection.

Amazingly, viruses can not only induce $CD4^+CD25^+$ Treg cells to suppress host antiviral immune responses to aid their infection, but also inhibit the function of these cells leading to the dysregulation of host immune response that favors their pathogenic effects and development of latency. This was demonstrated in a mouse model with γ-herpesvirus 68 infection, in which direct viral infection of $CD4^+CD25^+$ Treg cells decreased the frequency and function of these cells and resulted in an uncontrolled expansion of leukocytes and the establishment of viral latency (83). Such inhibition of $CD4^+CD25^+$ Treg cell function was also observed in a human retroviral infection in which $CD4^+CD25^+$ Treg cells defective in their suppressive function were found in patients infected with HTLV-1 virus (17). An HTLV-1 viral protein, Tax, was identified as responsible for the direct inhibition of Treg

cell function. These patients showed high levels of immune activation and developed a neurological inflammatory disease termed HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP), which may result from a break in immunological self tolerance. In these studies, both γ-herpesvirus 68 and HTLV-1 viruses directly infected $CD4^+CD25^+$ Treg cells. Conceivably, the expression of viral proteins, such as HTLV-1 Tax protein, or replication of these viruses within the Treg cells inhibited the expression of certain cellular proteins essential for the suppressive activities of $CD4^+CD25^+$ Treg cells, such as FOXP3, GITR and HTLA-4 (17), and thus impaired the function of these cells. Interestingly, another virus, FIV, that also directly infects $CD4^+CD25^+$ Treg cells, did not show suppressive effects on the cells (53). Instead, infection by FIV phenotypically and functionally activated the Treg cells.

5.2. Role of $CD4^+CD25^+$ Treg cells in host defense

5.2.1. Control of inflammatory responses

Virtually all viral infections induce host inflammatory responses, which may represent an essential component of host defense mechanisms against viral infections. However, excessive inflammation can cause tissue damage and thus be harmful to the host if left uncontrolled. $CD4^+CD25^+$ Treg cells have been suggested to play a critical role in controlling exaggerated inflammatory responses accompanied with many acute and chronic viral infections. A recent study on acute dengue virus infection showed that $CD4^+CD25^+$ Treg cells, which were induced during the acute dengue virus infection, are able to suppress the production of inflammatory cytokines including IFNγ, TNFα, and IL-6 from T cells and monocytes *in vitro* in response to dengue virus antigens (19). The disproportionate production of these inflammatory cytokines has been thought to contribute to the severity of dengue disease since they cause plasma leakage in the patients (84). More importantly, a significant increase in $CD4^+CD25^+$ Treg cells was found in patients with mild disease symptoms but not in those with severe disease. This is a strong indication that the activity of $CD4^+CD25^+$ Treg cells contributes to the control of inflammatory phase of the disease and is beneficial for disease outcome. The role of $CD4^+CD25^+$ Treg cells described here in acute dengue virus infection seems not in agreement with that described above in acute HCV infection. This may be due to the difference between the two viruses and the infections they cause. Unlike HCV, Dengue virus rarely causes chronic infection and the virus-induced inflammation is much more severe during the acute infection. This is perhaps a good example for the conception that the role of $CD4^+CD25^+$ Treg cells varies with the nature of the virus and infection.

If $CD4^+CD25^+$ Treg cells are critical in controlling virus-induced inflammation, one would expect that the absence of these cells would negatively impact the disease outcome. Indeed, in a mouse model of human HSV stromal keratitis, which usually results from a T cell-mediated immunoinflammatory response to HSV infection in the corneal stroma, the pathological lesion is significantly more severe if mice were depleted of $CD4^+CD25^+$ Treg cells before infection (16). Similarly, in an influenza virus hemagglutinin (HA)-induced mouse uveoretinitis model, depletion of $CD4^+CD25^+$ Treg cells exacerbated intraocular inflammation, whereas injection of mice with HA-specific $CD4^+CD25^+$ T cells controlled the disease (85).

One negative aspect for the function of $CD4^+CD25^+$ Treg cells in controlling virus-induced inflammatory responses is that the suppression is not selective or specific. Thus, while $CD4^+CD25^+$ Treg cells exert their suppression on inflammatory responses, simultaneous suppression may occur on virus-specific T cell responses necessary for virus clearance. This is best illustrated in chronic HCV infection. While a significant inverse correlation was found between the frequency and HCV-specific TGFβ response of $CD4^+CD25^+$ Treg cells and liver inflammation (15, 18), the same group of chronic HCV patients also showed a positive correlation between these Treg cells and viral load (15). Thus, $CD4^+CD25^+$ Treg cells acted to protect the host through controlling viral infection-associated liver inflammation. However, this protection is at the expense of reduced antiviral T cell responses and increased risk for virus spread.

5.2.2. Control of hyperactivation of immune responses

Chronic or persistent immune activation is a characteristic of certain human and animal retroviral infections such as HIV (86), and has been suggested to be responsible for the disease deterioration. In HIV and SIV infections, elevated immune activation induced by virus infection has been thought to result in $CD4^+$ T cell depletion (87-89), a hallmark of AIDS progression. An increasing line of evidence indicates that the hyperactivation of T cells may be controlled by $CD4^+CD25^+$ Treg cells, which are induced in most retroviral infections (13, 46, 53, 64, 65, 67, 82). The induced $CD4^+CD25^+$ Treg cells are able to suppress virus-specific $CD4^+$ and $CD8^+$ T cell responses *in vitro* in a cell contact-dependent and cytokine-independent manner. Decrease in the frequency of these Treg cells as HIV infection progresses has been found to be associated with immune activation of $CD4^+$ and $CD8^+$ T cells in HIV patients (90). Importantly, patients with strong HIV-specific $CD4^+CD25^+$ Treg cell functions *in vitro* showed favorable clinical markers of disease status such as significantly lower levels of plasma viremia and higher $CD4^+$ to $CD8^+$ T cell ratios than those without Treg cell activity (14). This suggests that $CD4^+CD25^+$ Treg cells may play an important role in protecting HIV patients from progression to AIDS. In support of this, a recent study found a direct correlation between the preservation of $CD4^+$ T cell counts and high percentage of $CD4^+CD25^+$ Treg cells in SIV-infected sooty mangabeys (91) which, in marked contrast to HIV-infected humans, usually show normal $CD4^+$ T cell counts and lack of generalized immune activation, and rarely progress to AIDS despite chronic high levels of virus replication (92).

Another piece of evidence supporting the beneficial role of $CD4^+CD25^+$ Treg cell suppression of HIV-specific T cell responses is the study of HIV-1

infection in young children. It has been observed that vertical transmission of HIV-1 *in utero* only occurs in a very low percentage (only 3% - 15%) of HIV-1-exposed infants (93). A recent study has found that the virus-specific T cell responses are weak in HIV-1-exposed children. The weak T cell responses are likely due to the suppression by $CD4^+CD25^+$ Treg cells, since the responses become strong after the Treg cells are removed (94). This suggests that $CD4^+CD25^+$ Treg cells may contribute to suppress the activation of virus-specific T cells and thus protect against infection in young children.

The studies described above demonstrate the benefit of $CD4^+CD25^+$ Treg cell induction in viral infections that induce immune hyperactivation. Conceivably, their absence or a deficiency in their suppressive function may lead to progression of the infection and deterioration of the disease. Indeed, in patients with HTLV-1-associated HAM/TSP, the frequency of $CD4^+CD25^+$ Treg cells actually increased when compared to the HTLV-1–infected asymptomatic carriers and uninfected healthy donors (17). However, these cells are defective in their suppressive functions and unable to control the hyperactivation of virus-specific T cell immune responses in HTLV-1 infection (17). This leads to the progression of HTLV-1 infection and the manifestation of HTLV-1-associated HAM/TSP. As discussed above, the loss of suppressive function may result from their direct infection by HTLV-1 virus. This could also represent one of the potential mechanisms for the failure of these Treg cells to successfully control chronic activation of T cells in AIDS patients.

5.2.3. Control of virus-associated autoimmune disorders

Viral infection has long been associated with the development of autoimmune diseases (95). For example, patients with chronic HCV infection often develop mixed cryoglobulinemia (MC), an autoimmune disorder characterized by polyclonal B cell activation and autoantibody production (96). Other autoimmune disorders have also been associated with chronic HCV infection, including membranoproliferative glomerulonephritis, autoimmune thyroiditis and lymphoproliferative disorders, autoimmune thrombocytopenia, pruritus, and type II diabetes mellitus (97-99). In addition, many viruses are associated with the development of type I diabetes (100), multiple sclerosis (101, 102), myocarditis (66, 103, 104), and systemic lupus erythematosus (105) in human and animals. One mechanism for the onset of the autoimmune diseases after virus infection may be the reduction or impairment of the $CD4^+CD25^+$ Treg cell suppressive function following virus infection, which leads to the breakdown of self tolerance and the enhancement of autoimmune T cell responses. This is implicated by the finding that the frequency of $CD4^+CD25^+$ Treg cells was significantly reduced in chronic HCV-infected patients who developed MC symptoms as compared to those with asymptomatic MC, no MC, or healthy controls (106). In this particular case, the $CD4^+CD25^+$ Treg cell deficiency likely enhanced the $CD4^+$ T cell help to autoantibody-secreting B cells leading to the development of MC symptoms. Consistently, in an animal model, depletion of $CD4^+CD25^+$ Treg cells in reovirus-infected mice resulted in the development of severe insulitis leading to an overt early diabetes (107). Thus, failure to maintain Treg cell function may be a critical determinant for disease expression in virus-induced autoimmune diseases.

6. MECHANISMS OF $CD4^+CD25^+$ TREG CELL INDUCTION IN VIRAL INFECTION

To date, many questions regarding the mechanisms for $CD4^+CD25^+$ Treg cell induction during viral infections still remain to be answered. Direct evidence on the induction of these cells by viruses or viral proteins is very limited. How these cells are generated and activated following virus infection is largely unknown, especially for the natural Treg cells since they are generated in thymus and are assumed to respond only to self-antigens (108). The induction of $CD4^+CD25^+$ Treg cells is not likely a selective event, since other $CD4^+$ and/or $CD8^+$ T cells are also induced at the onset of infection. In addition, both $CD4^+CD25^+$ Treg cells and $CD4^+$ helper T cells have been induced against the same epitopes on the viral protein in HCV-infected patients (61), suggesting that the same signal that induces regular $CD4^+$ T cells can also induce Treg cells simultaneously. One possibility is that, instead of selective induction of the Treg cell population, all $CD4^+$ T cells are induced upon infection, and then the activated $CD4^+CD25^+$ Treg cells suppress effector $CD4^+$ T cell functions. If this is the case, a kinetic change in activation of the $CD4^+$ T cell populations should be detectable. Data from many studies have suggested that $CD4^+CD25^+$ Treg cells can be induced through a number of mechanisms during virus infection.

6.1. Viral antigens

To date, information on direct induction of $CD4^+CD25^+$ Treg cells *in vivo* by viral antigens is still limited, although *in vitro* responses of these cells to viral proteins and peptides have been reported in several studies (13, 15, 109). Whether viral antigens directly activate and expand $CD4^+CD25^+$ Treg cells *in vivo* still remains to be demonstrated. However, this possibility is strongly suggested by observations from a large number of studies. First, virus-specific $CD4^+CD25^+$ Treg cells can be rapidly induced *in vitro* by culturing PBMC from virus-infected patients with viral peptides, as shown in HCV infection (110), suggesting that these cells have previously been primed by cognate HCV viral antigens *in vivo*. Furthermore, it has been found that only a small number of peptides from a peptide pool can induce Treg cells and the actual peptides that can stimulate Treg cells vary between patients, indicating the existence of dominant epitopes for $CD4^+CD25^+$ Treg cells. Second, $CD4^+CD25^+$ Treg cells specific to viral antigens can be found in virus-infected patients. Studies from Weiss *et al* showed that HIV viral protein p24 readily induced IL-10 production and TGFβ mRNA expression by $CD4^+CD25^+$ Treg cells from HIV-infected patients (13). This induction was p24-specific since CMV and PPD showed no or low stimulation of IL-10 and TGFβ expression in these cells. Similar specific responses to viral antigens has also been observed for $CD4^+CD25^+$ Treg cells from patients with HCV infection (15). These studies demonstrated the existence of virus-

specific $CD4^+CD25^+$ Treg cells *in vivo* and suggested that expansion and activation of $CD4^+CD25^+$ Treg cells *in vivo* may be triggered by viral antigens. Third, as discussed above, $CD4^+CD25^+$ Treg cells isolated from virus-infected patients are fully functional and do not need additional activation for their suppressive function. Apparently, they have been activated *in vivo* following virus infection. Since the acquisition of their suppressive function requires their activation via TCR (27), it is highly likely that this TCR-dependent activation *in vivo* is achieved through the engagement of their TCR with viral antigens. Finally, a more direct implication comes from a study by Huber *et al*, who showed that a single nonconserved amino acid difference in the nuclear capsid proteins of Coxsackievirus B3 H3 and H310A1 strains completely changed their capabilities to induce $CD4^+CD25^+$ Treg cells (66). Thus, a direct interaction between viral proteins or peptides and Treg cells is likely involved in Treg cell induction.

Several possibilities exist for direct induction of $CD4^+CD25^+$ Treg cells by viral antigens *in vivo*. As discussed above, viral infections often trigger autoimmune responses, and one of the ways this could occur is through the mechanism of molecular mimicry. It has been shown that viral peptides with sufficient structural similarity to self-peptides can activate T cell clones that are specific only for autoantigens such as myelin basic protein (111, 112). Such molecular mimicry between viral and host proteins has been observed in a number of viruses such as measles virus (113), HBV (111), HSV (112, 113), EBV (63, 112), and Adenovirus type 12 (112). Autoantibodies have been found frequently in the sera of patients infected with hepatitis, chickenpox, herpes, mumps, or measles viruses (114). In fact, it was concluded a long time ago that molecular mimicry is a common phenomenon following the finding that many human and animal viruses share antigenic determinants with host cell proteins (114, 115). Although the mechanism of molecular mimicry has not been confirmed for $CD4^+CD25^+$ Treg cell induction in viral infections, it still remains a plausible hypothesis for viral antigen-mediated direct induction of $CD4^+CD25^+$ Treg cell activation, especially for the induction of thymus-derived natural Treg cells since they are considered to only respond to autoantigens. In addition, many human and animal retroviruses contain highly conserved immunosuppressive peptides in their envelope proteins (116-119). These peptides are able to suppress immune responses of lymphocytes, monocytes, NK cells and macrophages. It remains to be determined whether these immunosuppressive viral peptides directly induce $CD4^+CD25^+$ Treg cell generation and/or activation. However, this proposition is encouraged by the study of Walker *et al*, who demonstrated that antigen-specific $CD4^+CD25^+$ Treg cells can be generated *in vitro* from either naïve or memory $CD4^+$ T cells using influenza virus hemagglutinin epitopes (120). Moreover, viral antigen may induce generation of $CD4^+CD25^+$ Treg cells from regular $CD4^+$ T cells in chronic viral infections by long-term, low-dose stimulation of these cells. This resembles the so-called "low-zone tolerance" that describes the antigen-specific tolerance resulting from long-term stimulation with sub-immunogenic doses of antigens (121). Finally, the induction may also be achieved by superantigens expressed by some viruses such as mouse mammary tumor virus (122).

6.2. Virus-induced cytokines

The cytokines that have been studied extensively in the context of Treg cell generation and activation are TGFβ and IL-10, due to their pronounced immunosuppressive nature. Accumulating data indicate that TGFβ plays an important role in induction of $CD4^+CD25^+$ Treg cells. However, this induction seems limited only in peripheral through either expansion of a small number of thymus-derived natural $CD4^+CD25^+$ Treg cells maintained in peripheral (123, 124) or conversion of peripheral naïve $CD4^+CD25^-$ T cells into FOXP3-expressing $CD4^+CD25^+$ Treg cells (24, 125). The latter may be due, at least partly, to the capability of TGFβ to upregulate the expression of CD25, FOXP3, and CTLA-4 on activated naïve $CD4^+$ T cells (24, 41, 44, 125-127). It seems clear that TGFβ is not required for thymic generation of $CD4^+CD25^+$ Treg cells, since several studies have shown that $CD4^+CD25^+$ Treg cells develop normally in thymus in both $TGF\beta1^{-/-}$ mice (128, 129) and transgenic mice defective in TGFβ1 signaling specifically in T cells and thymocytes (127, 130, 131). Thus, the role of TGFβ is most likely to maintain the peripheral $CD4^+CD25^+$ Treg cell pool and expand the cells when necessary.

The effect of virus-induced cytokines on the induction of $CD4^+CD25^+$ Treg cells during viral infections still remains to be fully illustrated. Elevated levels of TGFβ expression and production have been detected in many viral infections including HIV (132, 133), SIV (82), HCV (134, 135), EBV (136), CMV (137), dengue virus (138), influenza virus (139), HTLV-1 (140), and vaccinia virus (141) infections. The elevated TGFβ levels may represent either a strategy utilized by virus to induce immunosuppression for their invasion, or host protective anti-inflammatory response against virus-induced inflammation, or both. The induction of TGFβ may occur very rapidly after infection, as shown in a SIV-infected Africa green monkey model, in which the upregulated TGFβ expression was detected as early as 24 hours after infection (67). Importantly, the strong induction of TGFβ1 was correlated with both upregulation of FOXP3 expression and increased levels of $CD25^+$ T cells in the infected animals, indicating an active role of virus-induced TGFβ in expansion and activation of $CD4^+CD25^+$ Treg cells in viral infection.

The capability of IL-10 to induce $CD4^+CD25^+$ Treg cells *in vivo* was demonstrated in a mouse model of type I diabetes, in which overexpression of IL-10 using an IL-10-expressing adeno-associated virus vector induced $CD4^+CD25^+$ Treg cells *in vivo* and ameliorated the disease (142). The induction of the Treg cells by IL-10 seems to be an indirect event mediated through dendritic cells. IL-10 has been shown to inhibit the upregulation of the costimulatory molecules including CD40, CD80, CD86 on DC (143, 144). Consequently, it arrests the development of fully matured DC but leads to the generation of the so-

called "regulatory DC" with reduced expression of the costimulatory molecules similar to immature DC. These regulatory DC have been shown to induce $CD4^+CD25^+$ Treg cells (23, 144). Similar to TGFβ, IL-10 production is also induced in many human and animal viral infections (145-150). Notably, some viruses, such as EBV (151) and parapoxvirus orf virus (152) and equine herpesvirus 2 (153), encode a viral homolog of cellular IL-10. Like cellular IL-10, viral IL-10 is also able to inhibit DC maturation and function and promote generation of immature DC that promote T cell anergy (154, 155).

6.3. Virus-induced DC

DC are potent antigen-presenting cells (APC) essential for activation of both virus-specific $CD4^+$ and $CD8^+$ T cells. Like TGFβ, DC have no effect on thymic generation of $CD4^+CD25^+$ Treg cells. Increasing evidence from recent studies have shown that DC, under appropriate conditions, are able to either directly induce antigen-specific expansion of functional $CD4^+CD25^+$ Treg cells both *in vitro* and *in vivo* by stimulating extensive proliferation of these Treg cells (156, 157)**,** or induce naïve $CD4^+$ T cells to differentiate into $CD4^+CD25^+$ Treg cells with strong suppressive function (45). Thus it is perceivable to expect the involvement of DC, whether active or passive, in expansion and activation of $CD4^+CD25^+$ Treg cells in viral infections. In fact, many viral infections have shown dysregulation of DC during infection. This includes reduced expression of MHC class II and co-stimulatory molecules such as CD40, CD80, and CD86, and impaired function in effector T cell stimulation (158-161). The dysregulation of DC may result from direct infection by viruses (162) or from the suppression by virus-induced immunosuppressive cytokines such as IL-10, as described above. These DC are able to induce $CD4^+CD25^+$ Treg cells (23, 144). Interestingly, the study by Moseman *et al* showed that activated allogeneic plasmacytoid dendritic cells (PDC) can efficiently induce naïve $CD4^+CD25^-$ T cells to differentiate into suppressive $CD4^+CD25^+$ Treg cells (45). This raised the possibility for the existence of a specialized DC subset dedicated to the $CD4^+CD25^+$ Treg cell induction. Similar function of virus- or CpG-stimulated PDC to induce other types of Treg cells such as Tr1 and $CD4^+$ cytotoxic Treg cells has also been reported (163, 164). PDC have been shown to play a central role in innate immunity against viral infections (165) and many studies have shown that PDC are activated by viral infections (164, 166-168). A striking feature of the activated PDC is their production of high levels of IFNα upon viral infections (169), which is an essential component of innate immunity against viral infections (170). Interestingly, IFNα has been shown to synergize with IL-10 in priming $CD4^+$ T cells to differentiate into Tr1 regulatory T cells (164, 171).

7. MECHANISMS OF SUPPRESSION BY VIRUS-INDUCED $CD4^+CD25^+$ TREG CELLS

The molecular mechanisms by which Treg cells suppress effector T cells are still not fully understood. However, enormous progress has been made towards our understanding on how virus-induced Treg cells exert their suppressive functions on effector T cells. It has been generally accepted that $CD4^+CD25^+$ Treg cells exert their suppressive function through direct cell-to-cell contact with effector T cells or APC. However, controversial results have been obtained regarding cytokine involvement in mediating $CD4^+CD25^+$ Treg cell suppression. While most *in vitro* studies have shown that suppression by $CD4^+CD25^+$ Treg cells is cytokine-independent, a large body of *in vivo* studies has demonstrated the requirement of TGFβ and IL-10 for suppression by $CD4^+CD25^+$ Treg cells (3, 130, 172-176). Nevertheless, accumulating evidence has suggested that suppression by $CD4^+CD25^+$ Treg cells may be mediated by a variety of mechanisms and that the molecules and pathways involved in mediating the suppression may vary with conditions or stages of disease and/or immune responses.

7.1. FOXP3

FOXP3 is specifically expressed in $CD4^+CD25^+$ Treg cells and has been suggested to be a master regulator for the development and function of $CD4^+CD25^+$ Treg cells (39, 177-179). This is based the observations that induction of FOXP3 expression is usually accompanied by an acquisition of suppressive function of $CD4^+CD25^+$ Treg cells, and loss of FOXP3 expression abrogates the ability of the cells to suppress effector T cell responses. In viral infections, this is best illustrated in patients infected with HTLV-1 virus. Infection of $CD4^+CD25^+$ Treg cells by HTLV-1 virus leads to the reduction of FOXP3 mRNA expression and protein production. This abrogates the ability of HTLV-1-infected Treg cells to suppress the proliferation of $CD4^+CD25^-$ T cells, resulting in the development of HAM/TSP in HTLV-1-infected patients (17, 180). Since FOXP3 is essential for the development of peripheral $CD4^+CD25^+$ Treg cells (39, 177-179, 181, 182), it is not clear whether the loss of $CD4^+CD25^+$ Treg cell activity is due to a decrease in the frequency of the $CD4^+CD25^+$ T cells with regulatory capability in the total $CD4^+CD25^+$ T cell population, or due to the loss of function of $CD4^+CD25^+$ Treg cells. However, increased production of IL-2 was detected in these cells, suggesting that reduction in FOXP3 expression may lead to a defect in their regulatory function rather than a decrease in their frequency, since normal functional $CD4^+CD25^+$ Treg cells do not produce IL-2 (27, 28, 31). Interestingly, Sereti *et al* found that high levels of FOXP3 expression can be induced in a long-lived population of cytokine-expanded naïve (CEN) $CD4^+CD25^+$ T cells in HIV-infected patients receiving IL-2 immunotherapy (183). These IL-2-expanded $FOXP3^+CD4^+CD25^+$ T cells are phenotypically and functionally distinct from naturally occurring $CD4^+CD25^+$ Treg cells and do not display strong suppressive activity. This raised the question about the previous statement that only $CD4^+CD25^+$ Treg cells express FOXP3 and suggested that expression of FOXP3 may not necessarily confer immunosuppressive activity on the cells.

7.2. CTLA-4

Because $CD4^+CD25^+$ Treg cell suppression is dependent on direct cell contact, the surface molecules on Treg cells, target cells, and the cells with potential involvement in $CD4^+CD25^+$ Treg cell function have gained much attention in recent years. Among them, CTLA-4 has

drawn widespread interest because of its high constitutive expression on $CD4^+CD25^+$ and other Treg cells (37). A number of studies have implicated that CTLA-4 expression plays a key role in mediating the suppressive function of $CD4^+CD25^+$ Treg cells, as *in vivo* blockade of CTLA-4 abolished the suppressive activities of these Treg cells to control inflammatory (174, 175) or autoimmune (37) diseases. A similar role of CTLA-4 has also been observed in viral infections. In mice infected with Friend virus, virus infection induced $CD4^+CD25^+$ Treg cells and these cells suppressed allogeneic CTL generation *in vitro* (11). Blocking CTLA-4 activity with a monoclonal anti-CTLA-4 antibody reversed this Treg cell-mediated suppression and restored the CTL generation. In another study with SIV-infected macaques, increased effector function of both SIV-specific $CD4^+$ and $CD8^+$ T cells and decreased viral load in lymph nodes were detected after treating the animals with the anti-CTLA-4 antibody that blunted $CD4^+CD25^+$ Treg cell suppression (184). These studies provide evidence for the essential role of CTLA-4 in mediating the suppressive effect of $CD4^+CD25^+$ Treg cells on host antiviral immune response. In many viral infections, such as in HIV (185, 186) (187), SIV (82), HCV (188, 189), influenza (190), and EBV (191) infections, upregulated expression of CTLA-4 has been observed in parallel to the induction of $CD4^+CD25^+$ Treg cells. There is also a recent report on the direct effect of HIV-1 viral protein Vpr on the induction of CTLA-4 in HIV-infected T cells (192).

How CTLA-4 mediates the suppressive effect of $CD25^+CD4^+$ Treg cells is still under investigation. One commonly accepted mechanism is that it functions through its interaction with costimulatory molecules CD80 and CD86 on APC and/or effect T cells (193). Ligation of CTLA-4 to CD80/CD86 on APC either induces the production of the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) (194, 195) and the generation of inhibitory tryptophan metabolites (196-198), or competes with CD28 on effector T cells to bind to CD80/CD86 on APC and thus blocks the costimulatory signaling pathway for effector T cell activation. This seems true for CTLA-4 in viral infections, as elevated IDO expression was detected in viral infections in both animal model (188, 189) and patients infected with HCV (188). More convincingly, decreased IDO expression was detected in SIV-infected macaques following CTLA-4 blockade, which apparently diminished the suppressive effect of $CD4^+CD25^+$ Treg cells, as increased SIV-specific effector T cell function and decreased viral load were detected in these animals (184).

7.3. TGFβ

Because of its marked immunosuppressive property and elevated production following $CD4^+CD25^+$ Treg cell activation in many situations, TGFβ has been examined extensively for its role in mediating $CD4^+CD25^+$ Treg cell suppression. The results obtained are rather controversial or confusing. While a number of *in vitro* studies have shown that suppression by $CD4^+CD25^+$ Treg cells does not involve the activity of TGFβ (27, 28, 31, 199, 200), several *in vivo* studies have demonstrated the importance of TGFβ in $CD4^+CD25^+$ Treg cell suppression (130, 172, 174-176). However, the latter is debatable as well, as some studies have also shown the independence of $CD4^+CD25^+$ Treg cell function on TGFβ *in vivo* (66, 128, 200). In an effort to explain these above discrepancies, Nakamura *et al* made an interesting finding that mouse $CD4^+CD25^+$ Treg cells express the latent form of TGFβ1 on their surface, and suggested that the cell contact-dependent suppression by $CD4^+CD25^+$ Treg cells was mediated by this cell membrane-bound TGFβ1, which is activated upon cell contact between the Treg and target T cells (34). However, this mechanism was not confirmed by others in either human (35) and mouse systems (200), as $CD4^+CD25^+$ Treg cells strongly suppressed $CD4^+$ T cells from mice deficient in Smad3, a signaling protein essential for TGFβ suppression of T cell proliferation and activation, and the $CD4^+CD25^+$ T cells from $Smad3^{-/-}$ mice were as efficient in mediating suppression as those from wild-type mice, indicating that no TGFβ signaling is involved in the suppression (200).

To date, data about the role of TGFβ in virus-induced $CD4^+CD25^+$ T cell suppressive function are derived exclusively from *in vitro* studies, and inconsistent results similar to those mentioned above have also been observed for $CD4^+CD25^+$ T cells from virus-infected subjects. Most *in vitro* studies using these Treg cells indicate that TGFβ is not essential for $CD4^+CD25^+$ Treg cell-mediated suppression because anti-TGFβ antibody cannot block the suppressive effect of these Treg cells on virus-specific $CD4^+$ or $CD8^+$ T cell responses (13, 14, 75, 76, 201). However, unlike the *in vitro* results described above in other systems, there is also a report that *in vitro* suppression by $CD4^+CD25^+$ Treg cells is TGFβ-dependent. In this study, addition of anti-TGFβ antibody to the cell culture abrogated $CD4^+CD25^+$ Treg cell-mediated suppression and increased HCV-specific IFNγ production in HCV-stimulated PBMC (15). Thus, further studies, particularly *in vivo* studies, are needed to clarify the role of TGFβ in mediating $CD4^+CD25^+$ Treg cell function in viral infections.

Although there is still a lack of *in vivo* demonstration for the role of TGFβ in mediating $CD4^+CD25^+$ Treg cell function in viral infections, it can be anticipated that TGFβ may contribute to the suppression by virus-induced $CD4^+CD25^+$ Treg cells *in vivo*. This is based on the fact that almost all viral infections lead to immunosuppression and virus-induced TGFβ has been suggested as the key mediator of immunosuppression in viral infections (137, 202, 203). Many studies have shown that $CD4^+CD25^+$ Treg cells from virus-infected patients produce high levels of TGFβ (13, 15, 67, 82, 134). In addition, a wide variety of cell types are capable to produce TGFβ upon virus infection, including other types of regulatory T cells such as Th3 (204) and $CD8^+$ Treg cells (205). Thus, the suppressive effect observed *in vivo* represents a total effect of TGFβ from all sources. It is difficult to conceive that the TGFβ produced by virus-induced $CD4^+CD25^+$ Treg cells does not join the total TGFβ pool in mediating immunosuppression during viral infections.

7.4. IL-10

Another immunosuppressive cytokine potentially involved in mediating $CD4^+CD25^+$ Treg cell function is IL-10. Similar to TGFβ, studies on the role of IL-10 in mediating $CD4^+CD25^+$ Treg cell function in viral infections have shown controversial results. Most *in vitro* studies using $CD4^+CD25^+$ Treg cells from virus-infected patients showed that blocking IL-10 activity *in vitro* by using anti-IL-10 or anti-IL-10 receptor antibodies could not abrogate the suppression by $CD4^+CD25^+$ Treg cells, and thus concluded that the suppression was not mediated by IL-10 (13, 14, 201). However, a study by Huber *et al* showed that coxsackievirus B3 variant H310A1-induced $CD4^+CD25^+$ Treg cells were dependent on IL-10 for their suppressive function *in vivo*, and anti–IL-10 treatment abrogated the protection by $CD4^+CD25^+$ Treg cells against viral myocarditis (66). This seems consistent with the results from other *in vivo* studies that showed the dependence of $CD4^+CD25^+$ Treg cells on IL-10 for their suppressive functions in controlling expansion of peripheral $CD4^+$ T cells (206), over-production of cytokines (207), Th1-mediated inflammation (208), and autoimmune pneumonitis (209).

7.5. IL-2

Inhibition of IL-2 production was the first mechanism suggested for $CD4^+CD25^+$ Treg cell-mediated suppression when Thornton *et al* observed that $CD4^+CD25^+$ Treg cells inhibited the proliferation and IL-2 production of $CD4^+CD25^-$ effector T cells, and that addition of exogenous IL-2 not only markedly enhanced the effector $CD4^+$ T cell responses but almost completely abrogated the suppressive effects of $CD4^+CD25^+$ Treg cells (27). However, this mechanism was questioned since IL-2 is not required for the development and function of T cells *in vivo* (210, 211), except for Treg cells themselves (212). In viral infections, little is known about the role of IL-2 in mediating the suppressive function of virus-induced $CD4^+CD25^+$ Treg cells. The only information is a study on HIV infection showing that suppression of HIV p24-stimulated $CD4^+$ T cell proliferation by $CD4^+CD25^+$ Treg cells is associated with the inhibition of IL-2 production (14). Whether IL-2 plays a role in virus-induced $CD4^+CD25^+$ Treg cell function still remains to be determined.

7.6. GITR

GTIR has been shown to be constitutively and predominantly expressed at high levels on $CD4^+CD25^+$ Treg cells (38, 213). Initially, data from both *in vitro* and *in vivo* studies suggested an essential role of GITR in mediating $CD4^+CD25^+$ Treg cell function. This was based on the observations that $CD4^+CD25^+$ Treg cell-mediated suppression of anti-CD3-stimulated or peptide-specific $CD4^+$ T cell proliferation was abrogated after blocking GITR signaling with anti-GITR antibodies *in vitro* (38, 213), and that injection of normal mice with the antibodies led to the development of autoimmune gastritis and autoantibodies against parietal cells (38), a typical consequence from the loss of $CD4^+CD25^+$ Treg cell function in maintaining self-tolerance. However, it was later found that addition of anti-GITR antibody could not reverse the $CD4^+CD25^+$ Treg cell-mediated suppression of $CD4^+$ T cells from $GITR^{-/-}$ mice (214), although these Treg cells from normal mice expressed GITR. Furthermore, $CD4^+CD25^+$ Treg cells from $GITR^{-/-}$ mice showed comparably potent suppressive activity to those from normal mice, and the suppression by $CD4^+CD25^+GITR^{-/-}$ Treg cells could be reversed by the addition of anti-GITR antibody. Collectively, these results clearly indicate that suppression by $CD4^+CD25^+$ Treg cells is mediated through the GITR on the target cells and not on the $CD4^+CD25^+$ Treg cells. Thus, engagement of the GITR on $CD4^+CD25^+$ Treg cells plays no role in abrogation of the $CD4^+CD25^+$ T cell-mediated suppression. Instead, engagement of GITR on target T cells, which turned out to render the target T cell resistant to the suppression by $CD4^+CD25^+$ Treg cells, is required to overcome $CD4^+CD25^+$ Treg cell-mediated suppression. Nonetheless, engagement of GITR with its ligand or antibody will likely change the status of effector T cell responses to pathologic stimuli, and thus may possess therapeutic potential. In support of this, studies on a mouse Friend virus model of retrovirus infection showed that administration of anti-GITR antibody to the infected mice increased Th1 cytokine production by both $CD4^+$ and $CD8^+$ T cells, ameliorated disease manifestation, and reduced virus load (74, 215). Consistently, La *et al* demonstrated that a single injection of anti-GITR antibody to HSV-infected mice immediately after viral infection significantly increased both the number of activated, antigen-specific $CD4^+$ Th1 and $CD8^+$ T cells and their IFNγ production (216). Thus, signaling through GITR enhances the antiviral immune responses. This concept has been tested in animal models for its potential clinical application in antiviral therapy. One approach is to use GITR ligand (GITRL) as an adjuvant to enhance viral vaccine efficacy. In a recent study on the development of DNA vaccine for HIV infection, Stone *et al* generated plasmids encoding a multimeric soluble GITRL fusion protein to use as an adjuvant (217). Immunization of mice with HIV DNA vaccine plus GITRL adjuvant plasmids significantly augmented virus-specific $CD4^+$ and $CD8^+$ T cell responses including their proliferation, IFNγ production, cytolytic activity, and antibody production. Since ligation of GITR provides costimulatory signals to both $CD4^+CD25^+$ Treg cells and $CD4^+CD25^-$ effector T cells (218), the enhanced effector T cell responses may represent combined effects of direct activation upon ligation of their GITR with GITRL and indirect activation through the reduction of Treg cell suppressive effect following the ligation of GITR on Treg cells.

In summary, more studies are still needed to clarify the mechanisms by which $CD4^+CD25^+$ Treg cells execute their suppressive functions. Whether there are more molecules potentially involved in the function of these Treg cells remains to be determined. Giving the fact that the suppression of $CD4^+CD25^+$ Treg cells can be abrogated by blocking one molecule but not another and that all molecules described above are potentially capable of mediating $CD4^+CD25^+$ Treg cell function, it is likely that the suppression of $CD4^+CD25^+$ Treg cells is mediated by different molecules under different conditions such as the nature of virus and infection, or the stage, magnitude, and

nature of inflammation and immune activation. It is important to find out whether the suppression observed is mediated by a single molecule or results from the collective effects of multiple molecules. In fact, the role of these molecules in mediating Treg cell function may change during the course of infection as disease condition changes. For example, as shown in the study on *Leishmania major* infection, IL-10 is not involved in suppression by $CD4^+CD25^+$ Treg cells at the early stage of infection, but is required at a later stage when chronic infections are well-established (3).

8. CONCLUSIONS AND PERSPECTIVE

In a productive and successful immune response to a viral infection, the processes of mounting a vigorous antiviral immune response and restricting excessive inflammatory responses to minimize collateral damage to host tissues are appropriately regulated and homeostasis is maintained. Because of their unique suppressive function, $CD4^+CD25^+$ Treg cells appear to play a key role in controlling undue inflammatory and hyperimmune responses in viral infections. However, this benefit is achieved at the cost of reduced antiviral immune responses and increased risk of developing chronic or persistent viral infection. The active role of $CD4^+CD25^+$ Treg cells in regulating antiviral immune responses makes them promising targets for clinical antiviral therapy. The biggest challenge is how to direct the function of these Treg cells precisely towards a desired therapeutic outcome to benefit the host, and, at the same time, keep their detrimental effect to the minimum. This requires a clear understanding of the biological and functional properties of $CD4^+CD25^+$ Treg cells in viral infections. To date, information on many aspects of $CD4^+CD25^+$ Treg cells in viral infections is still very limited. Many questions still remain to be answered and controversial results to be clarified. Further studies are needed to depict precise mechanisms on how $CD4^+CD25^+$ Treg cells are induced and regulated during viral infections and how they execute their function. Special attention should be paid to the discrepancy between *in vitro* and *in vivo* observations. To date, there is still no explanation for this discrepancy. Since *in vitro* assay conditions usually do not reflect the actual physiological environment *in vivo*, data from *in vitro* studies should be interpreted with caution. Accordingly, the functional property of $CD4^+CD25^+$ Treg cells *in vivo* will very likely differ from that predicted from *in vitro* studies. This is particularly important in the design of therapeutic strategies targeting $CD4^+CD25^+$ Treg cells for clinical application since strategies based on in vitro observations may not generate the anticipated effect in vivo. Finally, it should also be kept in mind that other types of regulatory T cells, such as Tr1, Th3, CD8+, and CD45RBlow Treg cells, may also regulate immune responses in viral infections. The relationship between these Treg cells and CD4+CD25+ Treg cells and how they affect each other in viral infections are unclear. Detailed *in vivo* analysis should provide information for our understanding of the biological effect of these Treg cells in viral infections and help with the development of strategies for the prevention and treatment of viral diseases.

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