Published in final edited form as:
Immunol Rev. 2013 September ; 255(1): 182–196. doi:10.1111/imr.12085.

Role of Regulatory T Cells during Virus Infection

Tamara Veiga-Parga[1], **Sharvan Sehrawat**[2], and **Barry T. Rouse**[1]

[1]Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA

[2]Institute of Life Sciences, Nalco Square, Bhubaneswar, Orissa, India

Summary

The host response to viruses includes multiple cell types that have regulatory function. Most information focuses on $CD4^+$ regulatory T cells that express the transcription factor $Foxp3^+$ (Tregs), which are the topic of this review. We explain how viruses through specific and non-specific means can trigger the response of thymus-derived natural Tregs as well as induce Tregs. The latter derive under appropriate stimulation conditions either from uncommitted precursors or from differentiated cells that convert to become Tregs. We describe instances where Tregs appear to limit the efficacy of antiviral protective immunity and other perhaps more common immune-mediated inflammatory conditions, where the Tregs function to limit the extent of tissue damage that occurs during a virus infection. We discuss the controversial roles that Tregs may play in the pathogenesis of human immunodeficiency and hepatitis C virus infections. The issue of plasticity is discussed, since this may result in Tregs losing their protective function when present in inflammatory environments. Finally, we mention approaches used to manipulate Treg numbers and function and assess their current value and likely future success to manage the outcome of virus infection, especially those that are responsible for chronic tissue damage.

Keywords

regulatory T cells; virus; infection; immunopathology; plasticity; therapy

Background

In the mid-1990s, an old rejected concept reemerged that captured the imagination of most cellular immunologists and converted some notable skeptics to become believers and even enthusiasts (1). These were the regulatory T cells (Treg) rediscovered by Simon Sakaguchi and colleagues (2). Their predecessors, suppressor cells, championed by Richard Gershon 30 years ago, were laid to rest soon after Richard himself met an early death. His suppressor cells could not withstand the scientific scrutiny of molecular biologists and the concept emerged prior to the development of sophisticated genetic systems in mice that could have proven their worth. This time around the cells, now referred to as regulatory cells, have found acceptance for several reasons. These include their possession of a distinguishing phenotype, the fact that regulatory T cells express a canonical transcription factor [forkhead box protein 3 (Foxp3)], as well as the discovery that some natural diseases in mice and humans were the consequence of lacking regulatory T cells (3, 4). After the Sakaguchi paper, an explosion of others soon followed linking regulatory cells eventually to many

Correspondence to: Barry T. Rouse, Department of Biomedical and Diagnostic Sciences, College of Veterinary Medicine, 1414 Cumberland Avenue, Rm. F403, University of Tennessee, Knoxville, TN 37996, USA, Tel.: +1 865 974 4026, Fax: +1 865 974 7817, btr@utk.edu.

T.V-P., S.S, and B.T.R. declare no conflict of interest.

events, but in particular to autoimmunity (5), some cancers (6), allergic disease (7), and the outcome of infectious disease (8). Tregs and other types of regulatory cells are here to stay, and it seems likely that manipulating their numbers and function will one day be a useful medical procedure.

The seminal findings of groups led by Sakaguchi, Powrie, and Shevach (9-11) firmly established a role for Tregs in autoimmunity. However, it was a fascinating report by Belkaid in the David Sachs group (12) at NIH that showed that the pathogenesis of an infectious disease was critically influenced by the nature of the Treg response. Reports that Tregs also control responses to virus infections soon followed (13-15), and it is conceivable that regulatory cells of one kind or another impact on one or more steps in the pathogenesis of virus infections. In this review, we discuss the influence of regulatory cells on the outcome of virus infections focusing on the most studied type $CD4^+Foxp3^+$ regulatory T cells (Tregs). There are basically two types of $Foxp3^+$ Tregs that influence the pathogenesis of virus infections. One group is natural Tregs (nTregs) that are generated in the thymus and which are in large part self-reactive and help prevent autoimmunity. If any of this population reacts specifically with viral antigens, their part of the repertoire is expected to be small and may also be cross reactive with autoantigens. The second population are called adaptive, induced, or converted Tregs (iTregs). This population is derived from naive, or occasionally committed, $CD4^+$ T cells exposed in an appropriate environment of cytokines, cognate antigen, and costimulation to become Tregs (16). This viral antigen-specific population could be larger than that in the nTreg population and act mainly but not exclusively in an antigen-specific way to modulate antiviral responses. The iTregs differ in stability from nTregs and can be distinguished from them phenotypically. The most reliable marker distinguishing the two populations may be neuropilin 1, expressed only by nTregs (17, 18). As mentioned subsequently, other features may also differ between the two populations, the most relevant of which may be their accessibility for therapeutic manipulation. Many additional types of cells may act as regulators, although not all have been linked to the outcome of virus infections. These include invariant natural killer T (iNKT) cells that respond to lipid antigens, double negative $CD3^+$ T cells, γδ-T cells, B-cell regulators, myeloid suppressor cells, highly polarized T-helper 1 (Th1) and $CD8^+$ T cells perhaps involved in Hepatis C virus response regulation (19). See Table 1 for some virus infections where regulatory cells of various types influence the response pattern. We make minimal mention of these other types, but they were recently discussed in other reviews (20).

The impact of Tregs on antiviral immunity

Demonstrating a role for Tregs in disease pathogenesis was made possible when procedures were developed to selectively remove them prior to infection or during the disease process, or by showing with adoptive transfer approaches that the immune function of effector cells could be modulated when Tregs were co-administered (13). In addition, some have measured the consequences of expanding Tregs on the outcome of infection (21). The initial reports relating Treg responses to virus infections both showed that the protective function of $CD8^+$ T cells was compromised by the presence of Tregs (14, 15). For instance, Suvas *et al.* (14) demonstrated that acute infection of mice with herpes simplex virus (HSV) was controlled more effectively in animals that were depleted of Tregs prior to infection. The depleted animals cleared virus more rapidly after footpad infection, and following genital infection, mice were more susceptible to HSV-2 induced fatal encephalitis (22). Adoptive transfers of effector cells into RAG recipients protected animals against encephalitis, but this effect was abrogated when Tregs were co-transferred (14). Similarly in a retrovirus-induced cancer system, the protective effects of adoptive transfers of $CD8^+$ T cells were diminished if Tregs were present (15). Explanations for the immune blunting activity of Tregs were multiple. These included a reduction in the magnitude of the protective T-cell response, an

inhibitory effect on antiviral cytokine production by effector cells, as well as an inhibitory effect on cell trafficking of protective T cells to infected sites (13-15, 22). For example, Lund *et al.* (22) showed with HSV vaginal infection that recruitment of protective $CD8^+$ T cells to the infected genital mucosal was delayed in the presence of Tregs, since they interfered with the establishment of a chemokine gradient. The consequence was facilitated infection of the central nervous system (CNS) and mouse mortality. Effects of Tregs on cell trafficking were also noted by other groups (23). In an animal model of respiratory syncytial virus (RSV) infection, the $CD8^+$ T-cell response was enhanced in the absence of Tregs, but there was a critical delay in trafficking of these protective $CD8^+$ T cells to the lungs and illness was increased (23). Curiously, the effects of Tregs on the magnitude of $CD8^+$ T-cell responses to RSV was not uniform in that inhibitory effects of Tregs were greater on the dominant as compared to the sub-dominant $CD8^+$ T-cell responses to RSV. Reasons for this observation are yet to be found.

Few studies have focused on the role of Tregs in acute infections, where lesions are principally the consequence of direct effects of infection. Moreover, in some instances, the effect of Treg removal has had little or no effect, such as appears to be true with perhaps the most studied of all acute mouse infections – lymphocytic choriomeningitis virus (LCMV) and influenza (24, 25). It seems likely that Tregs play only a minor role during many acute infections. For example, if the infection results in a highly inflamed infection site, many of the cytokines that would be present are inhibitory to the activation of existing Tregs and particularly to the formation of expanded populations of induced Tregs derived from naive T-cell precursors. However, in circumstances where the infecting virus can interact directly with Tregs, this could cause their expansion and activation and in so doing exert a regulatory response in the acute infection. This may explain how the Treg response influences acute infection with HSV in mice. In this instance, the gD envelope glycoprotein of HSV can bind to the HVEM (herpes virus entry mediator) receptor expressed by Tregs (26). A second example could be hepatitis A virus, which can interact with the TIM-1 receptor, which may also be expressed by Tregs (27). This interaction inhibits Treg function, and virus is cleared more efficiently perhaps explaining why chronicity is uncommon in hepatitis A infection. However with hepatitis infection in humans, it is not clear if Tregs affect the magnitude of the primary T-effector cell response.

It seems likely that Tregs could play a more consequential role in responses to chronic and persistent infections, where ample time is available for Treg induction and activation to occur. Indeed, many have attempted to link the variable outcomes typical of chronic infections to the pattern of Treg responsiveness (8, 28, 29). Moreover, as we shall discuss in a later section, there is far more convincing evidence that Tregs play a pivotal role to modulate immunoinflammatory responses to viruses, and many chronic infections cause tissue damage by interacting with one or more host response systems.

Two human viruses, human immunodeficiency virus (HIV) and hepatitis C virus (HCV), have received the most attention, since they are major causes of illness and death and both lack effective prophylactic or, what is even more needed, therapeutic vaccines. There are hints that vaccines could be developed once we sort out how the many components of host responses interplay with the viruses and if the Treg response forms part of the scenario. In fact, Tregs of multiple types have been implicated as participants in the pathogenesis of disease, but the topic remains confusing, controversial, and plagued by skeptics. Both infections are difficult to investigate and mostly rely on *ex vivo* studies on blood, yet the critical events that involve Tregs in HIV infections may be occurring mainly in lymphoid and mucosal tissues and in the liver with HCV. At least HIV does have useful animal models that permit experimentation, but reliable models are lacking for HCV, especially

now that chimpanzees cannot be used. We provide a brief overview of our take on the role of Tregs in both infections. Brief reviews were recently published for both viruses (28, 29).

HIV

Regulatory T cells have been assigned several roles during HIV infection, and all could be occurring at different times during the infection process and the response to therapy. Roles suggested include inhibitory effects on initial infection of $CD4^+$ T cells (30), targets for HIV replication (31), detrimental effects on the protective components of host defense (32), and a beneficial function by minimizing the extent of T-cell activation (33), a critical step that results in acquired immunodeficiency syndrome (AIDS). Two unresolved issues that involve Tregs are of particular interest, since their resolution might impact on future therapeutic measures. Firstly, we need to know if Treg activity is in part responsible for those patients that respond favorably to drug treatment and maintain control even when treatment is discontinued. Secondly, we should establish if Treg activity helps explain how elite controller patients fail to develop clinical disease yet they have never received anti-retroviral treatment.

In vitro studies have shown that HIV can infect and replicate in Tregs (31), but it is doubtful if such cells are the usual targets for HIV *in vivo*. In fact, strong evidence favors the possibility that Tregs might serve to inhibit replication in target $CD4^+$ T cells early during infection (34), and this might assist in preventing the initial spread of virus from the mucosal infection site to lymph nodes. This time-span is when the virus is most vulnerable and where a ‘smart’ vaccine might prevent the establishment of permanent infection. If indeed Tregs are involved in preventing infection of the lymphoid tissue, then expanding their activity and number might represent a useful maneuver.

Many groups have advocated that the Treg response could act, particularly in the early stages of infection, in a detrimental way by impeding the function of the protective aspects of immunity, such as $CD8^+$ T-cell activity (32, 33, 35). Usually, there is a relative and sometimes overall increase in Tregs following infection (36), although this is not observed by all investigators (28, 37). The increased Treg representation can be the consequence of enhanced generation (38), reduced HIV-mediated destruction (39), or conversion of uncommitted cells to become Tregs following contact with dendritic cells (DCs) (40). In addition, most but not all studies indicate that successful treatment results in normalized Treg frequencies, and a similar pattern as is found in controls (41). Unsuccessfully treated patients, in contrast, often retain high Treg frequencies (42). Furthermore, long-term non-progressors and elite controllers may have low levels of Tregs, and these cell numbers may be lower than those found in healthy individuals (43). All of the above observations are consistent with Tregs playing a detrimental role, but more studies are needed to clinch the concept using tissues such as lymph nodes and mucosal sites where relevant events are occurring. Conceivably, the issue could also be resolved by manipulating Treg numbers and function in appropriate monkey models using simian immunodeficiency virus (SIV), but so far this approach has not been satisfactorily accomplished.

There is more enthusiasm for the idea that Tregs may function in a positive way in later phases of the disease to limit exaggerated immune activation, which is a prelude to the onset of AIDS (44). Evidence comes largely from observing changes in Treg frequency following successful treatment of chronically infected patients. Additional support comes from *in vitro* studies (28). Overall, it seems that Tregs may be able to control low levels of T-cell activation, but the effect may not be adequate to control high levels of immune activation as is often present, especially when levels of viral replication are high (28). The case for the beneficial effects of Tregs still needs to be proven, since human studies are at best only

correlative and lack causality. One anticipates that appropriate studies in primates should be able to resolve the issue, since both T-cell blocking and depletion studies can be performed.

HCV

The role of Tregs during the pathogenesis of HCV is even less clear-cut and more difficult to investigate than HIV. Accordingly, to understand HCV pathogenesis, it would be best to perform *ex vivo* analysis with liver tissue where the relevant immunological events are occurring. In untreated HCV persons, the majority eventually succumbs to chronic hepatitis and perhaps liver failure. However, around 20% resolve their infection, likely because they develop a vigorous $CD8^+$ and $CD4^+$ T-cell response to multiple structural and nonstructural viral antigens (45). The challenge is to explain the variable outcome and to design immunological procedures that could push more persons into the resolution category. Alas, any maneuver would need to be started in the face of disease, since most patients are unaware that they are infected until they develop hepatitis. Some have advocated that the variable Treg response in the initial phases of infection may help establish the pattern of subsequent events (46). Support for this idea came from an unfortunate event where many persons were accidentally infected with HCV (47). Studies of blood samples from these patients showed that clones of interleukin-10 (IL-10)-producing Tr1 cells could readily be developed from patients with chronic infection but not from those who controlled their infection (48). Unfortunately, these patients were not followed longitudinally. Others have advocated a role for $CD8^+$ Tregs as instrumental in controlling the outcome of HCV (49).

An idea gaining favor is that liver damage in HCV infection occurs because of a heightened $Foxp3^+$ Treg response that diminishes protective immunity (reviewed in 29). The reasons why some individuals produce high numbers of Tregs is not fully defined. A favored hypothesis suggests it results from changes in the function and trafficking pattern of DCs, which develop increased ability to induce Treg responses (50). Further studies are needed focusing on liver samples studied at multiple time points after known infection, which might only be achievable with drug addict volunteers. Conceivably, suppressing Tregs along with inhibition of viral replication might be a more effective approach to control chronic hepatitis. However, if as others advocate (49) Tregs are playing a useful role in the chronic phase to limit exaggerated T-cell responses that contribute to liver damage and viral persistence, there would be a case for stimulating Tregs along with viral control. Unfortunately, it may not be possible to perform experiments that could clarify if Tregs in chronic HCV are good or evil.

Role of Tregs in controlling immunopathology

From an evolutionary prospective, developing a system that acts to limit the effectiveness of immunity against a pathogen makes little sense. However, with many pathogens, tissue damage is not the direct consequence of viral replication but results from an exuberant inflammatory response to the infection. Indeed, some viruses lack cytopathogenicity, and any tissue damage that occurs is the consequence of a T-cell-mediated response to the infection. The poster child for this situation is LCMV in mice, where lesions do not occur in the absence of an immune response (51). With most virus infections, the host control process may be responsible for some tissue damage, and this feature becomes prominent with chronic infections. Controlling the extent of such tissue damage appears to be a major responsibility of $Foxp3^+$ Tregs. The first clue that suppressive $CD4^+$ T cells were valuable to the host came from the Hasenkrug laboratory (52) studying a chronic retrovirus infection in mice. Subsequently, a series of studies on ocular immunoinflammatory lesions [stromal keratitis (SK)] caused by HSV firmly established a protective role for Tregs (13, 53). Mice lacking Tregs developed more severe SK lesions, and these could be induced at lower infection levels in Treg-suppressed mice than in normal animals. Using adoptive transfer

experiments in $Rag^{-/-}$ recipients, the pathogenic activity of pro-inflammatory Th1 T cells could be suppressed if Tregs were co-administered (13). Additional approaches also supported a protective role for Tregs, including adoptively transferring populations of activated Tregs to normal mice as well as using procedures that expand the Treg population in normal animals in the early phases of infection (21, 54, 55). Other approaches that proved anti-inflammatory were to expose animals to procedures that changed the balance between Treg and T effectors serving to favor the Treg response (55). Some of these maneuvers acted by causing the conversion of naive T cells to become $Foxp3^{+}$ Tregs, an issue that is discussed in a subsequent section. Finally arguing for a protective role for Treg in the SK system, when Tregs were removed from animals with ongoing lesions, these lesions became significantly more severe (53). At least in the ocular immunoinflammatory disease caused by HSV, the case for Tregs as protectors from immune tissue damage is compelling. Whether Tregs play the same role in the natural lesions in humans, which is an important cause of blindness, remains to be substantiated.

Further support for the concept that Tregs limit the extent of virus-induced immunopathology was shown with several other virus infections (Table 2). In the previous section, we mentioned how Tregs may act in a useful way during HIV and HCV, and the same may be true for hepatitis B (56). There is also a strong suspicion that the severe respiratory consequences of RSV infection in children represents an imbalance between an otherwise protective T-cell reaction to virus not being constrained by an adequate Treg response (57). Proving this contention in humans may not be feasible, but animal studies provide strong support for the possibility. For example, the Varga group (58) could show that when mice were depleted of Tregs, inflammatory T cells increased in number, and disease severity was enhanced. Increased tissue damage in experimental RSV infection was also observed by another group using the DEREG depletion strategy to remove Treg (59). When Treg numbers were boosted using IL-2 immune complexes, inflammatory reactions were diminished (60). In the case of RSV, there is some evidence that the infection may damage the function of Tregs that act usefully to control the severity of other syndromes such as allergic asthma (61). This may explain the strong association known to occur between RSV infections requiring hospitalization and the development of asthma in subsequent years.

In instances where viruses cause lesions in the central nervous system (CNS), oftentimes the tissue damage involves immunopathology, and not surprisingly, there is evidence that lesion severity is constrained by a Treg response. Direct evidence for the notion has come from experimental mouse systems, but in humans the severity of encephalitis caused by west Nile virus (WNV) and the subacute sclerosing panencephalitis (SSPE) syndrome that may follow measles infection could both be influenced by the Treg response (62, 63). The direct evidence that Tregs act as modulators of CNS lesion severity comes from the Miller group (64) working with Theiler's virus in susceptible and resistant mouse strains. It seems that susceptible strains develop the autoimmune type CNS lesions because they fail to generate adequate Treg responses. Moreover, when Tregs were depleted in resistant strains, they became susceptible to CNS lesions caused by more intense inflammatory T-cell reactivity. A protective role for a Treg response was also shown in a mouse model for west Nile fever (62), supporting the concept that the same may be occurring in the natural human disease, but this is yet to be formally demonstrated.

Several experimental systems have demonstrated that Tregs play a valuable role during viral pathogenesis acting to minimize tissue damage in the later phases of infection and perhaps serving a critical role to dampen lesion severity with persistent infections. One anticipates a similar beneficial effect in human persistent viral infections, especially with hepatitis

viruses, but so far the case for this opinion has not been substantiated and is difficult to study.

How do viruses trigger and expand nTreg responses?

How viruses trigger and expand nTreg responses is still very much an unsettled issue and almost certainly has multiple answers with these varying with different viruses. Basically, the Treg response could represent the expansion of the existing nTreg population that happens to be cross-reactive between self and viral antigens. Alternatively, the nTreg response could represent non-specific stimulation by innate ligand activity of the virus, damaged tissue components released by the infection, or reaction products produced by the host. Another idea is that Tregs involved in the antiviral response represent mainly induced Tregs derived from viral antigen-specific naive $CD4^+$ T cells (discussed in the next section). A combination of these possibilities could also be occurring with the major origin of the functional Tregs changing during the course of the infection process and its resolution. We briefly discuss some of these issues but advocate that the topic merits much more investigation.

It is evident that Tregs can be especially abundant at initial sites of virus infections, such as the mucosa and skin. Indeed, estimates are that 80% of the total nTreg population is present in the skin (65). However, most Tregs are self-reactive, and the population that is cross reactive with any particular virus remains unknown and is likely to be small. However, when viruses appear to directly trigger rapid expansion of the nTreg population, this is likely to result from innate immune activity of the virus rather than TCR stimulation of antigen-specific nTregs. The logical alternatives to TCR stimulation include PAMP activity of viruses that engages receptors either on the nTreg themselves or on intermediary cells of the immune system, which will then activate Tregs. In line with this notion, several viruses do have one or more known PAMPs, and nTregs express several innate receptors, such as multiple TLRs (66). There is also evidence that exposure of nTregs to some TLR ligands can cause their proliferation and activation, although this topic is controversial and was discussed by Conroy *et al.* (66). In addition, certain products released from damaged tissues have TLR-stimulating activity. These include some heat shock proteins, β-defensins, self-DNA, and some compounds derived from the matrix protein hyaluronidase (25, 67, 68). Some other molecules derived from the host during an inflammatory reaction might also expand and activate nTregs or be involved in Treg conversion (69). Examples include the galectins 1 and 9, some cytokines, and other molecules as we discuss subsequently. The innate receptors TLR2 and TLR4 have been the focus for most studies that show effects on n Tregs. TLR2 is generally found to expand nTregs, and this could explain the acute Treg expansion in mice infected with HSV (70). TLR4 engagement can also expand nTregs (66, 67), and viruses such as RSV have TLR4 ligand activity (66, 71). Not all TLRs provide positive signals to Tregs. For example, TLR8 and TLR9 can be suppressive, with TLR9 also having inhibitory effects on the conversion of conventional T cells into induced Tregs (72). The many discrepancies on the Treg TLR/Treg scenario require resolution. The explanation may be quantitative or be the combined consequence of multiple TLRs and perhaps other innate receptor involvement.

Other non-TCR-mediated mechanisms may also explain how viruses trigger regulation by nTregs. In some instances, this could include possession of a protein that binds to a non-TCR receptor on nTregs and could cause their activation. An example could be the HIV protein gp120, which was shown to activate human nTregs via its ability to bind to the CD4 molecule (73). Another example could be the gD protein of HSV, which binds to the cell entry receptor HVEM, which is expressed on Tregs. Some have shown ligand engagement of HVEM on Tregs can cause their expansion (74), and preliminary results indicate that

HSV interaction with nTregs has a similar outcome (Sharma S. and Rouse B.T., unpublished observations). A third example could derive from the observation that resting nTregs express the molecule TNF receptor 25 and can be expanded when this receptor is engaged by its ligand TL1A (75). Since this ligand is increased in availability during inflammatory reactions, this could be a mechanism to expand Tregs, as was recently shown in a model system involving HSV (76).

Cytokines generated in the microenvironment of infection could also boost nTreg activity. These include IL-2 (which is essential for nTreg survival and expansion), TNF-α, and TGF-β. TGF-β is of particular interest, since as can readily be shown *in vitro*, this molecule is needed for T cells to differentiate to become Tregs (77). Some infections result in TGF-β production by infected or reacting cells (25, 78), but this has not been shown with virus infections. However, since apoptotic body engulfment by DCs or macrophages can result in TGF-β production (79), this process might result in those virus infections that cause apoptosis of infected cells.

During viral infections, many additional host-derived molecules might act to expand and activate Tregs. Of particular interest is the family of galectin molecules that are upregulated or secreted during inflammatory responses. Galectins either form lattices on the surface of cells by binding to cell surface glycoproteins and glycolipids or to specific receptors. The outcome is variable but includes apoptosis, proliferation, and changes of function (80). Galectin-1 and galectin-9 are of particular interest, since both molecules may be produced during inflammatory reactions either by infected cells or by cells that respond to them, such as NK cells. Galectin-9 was shown to modulate the function of T cells that are involved in inflammatory reactions such as those caused by HSV infection of the eye (81). Galectin-9 mainly acts by binding to its receptor TIM-3, which is upregulated on activated cells but is also expressed by a high proportion of nTregs (82). Whereas galectin-9 binding to activated effector cells causes them to undergo apoptosis, its binding to TIM-3 on Tregs may lead to the expansion and activation of these cells, as we have shown with HSV-induced ocular lesions (81). Galectin-1 functions in a similar way to galectin-9 (83), although different recognition systems are involved, and it is not clear if galectin-1 similarly acts to expand and activate nTregs. However, galectin-1 has been reported to expand IL-10-producing Tregs (80).

We can conclude that microbial infections could trigger the expansion and activation of nTregs in several ways, none of which may involve engagement of the nTreg-specific TCR, although this latter event might facilitate responses. These various stimulatory processes are summarized in Fig. 1.

Inducible and converted Tregs in virus infections

It seems likely that when any virus infection causes the induction of antigen-specific functional Tregs, their main origin is either uncommitted T cells TCR-stimulated in an appropriate environment, or they represent the conversion of differentiated T cells that were previously non-regulatory. This latter origin is perhaps less common than *de novo* induction, and when it does occur, it may happen more often in the later phases of chronic infections, being in part responsible for their resolution. T-cell functional conversion involves the phenomenon of plasticity that is discussed subsequently. In fact, recent studies on plasticity seem to favor the concept that conversion of Tregs to other functions is accomplished more readily than conversion to become Tregs (84). Should this be true with virus infections, then iTregs originating from differentiated T effectors may be an uncommon event. However, the gut environment might represent an exception since this site is rich in the many components, which includes dietary substances such as curcumin that can drive conversion (85). The

issue of Treg conversion in the context of virus infections has been poorly explored but is particularly relevant with regard to therapy. Thus, procedures that can foster conversion to Tregs in situations of viral immunopathology could be most beneficial.

The production of induced Tregs requires antigen exposure presented usually by DCs in the presence of one or more of the host-derived molecules discussed in the previous section. Some subsets of DCs appear more adept at inducing Tregs than others. Properties that favor Treg induction include TGF-β and IL-10 production as well as the ability to convert vitamin A into retinoic acid (86). These types of DCs are prominent in the gut mucosa, and it is likely that the majority of systemic iTregs [which can be distinguished from nTregs by their expression of neuropilin-1 (17, 18)] are specific for gut commensal organisms (87). There is also some reason to expect that certain gut pathogens, such as caliciviruses, could infect DCs, pushing their function towards become Treg inducing. Evidence that DCs infected with a virus can become more Treg inducing was demonstrated in a feline virus infection (88). Although virus infection might facilitate the Treg-inducing capacity of DCs, already it is known that the gut environment can be highly tolerogenic, with Tregs being one mechanistic component of the tolerance (89, 90).

With regard to virus infections, information about iTregs has focused on IL-10-induced $Foxp3^{-}CD4^{+}$ T cells that are referred to Tr1 cells (91). These cells, which act by producing IL-10, were one of the first types of regulators linked to the outcome of a virus infection. There is evidence they play a key role in the disease pattern HCV infection (48), although enthusiasm for this notion seems to have evaporated with recent studies on HCV focusing on $Foxp3^{+}$ T cells, as we discussed previously. The interest in Tr1 cells is maintained by the fact that such cells with regulatory activity can be generated *in vitro* against many antigens, and these cells can be used in experimental therapeutic situations (92). However, iTregs can also be generated *in vitro*, and these may function too in part by producing IL-10. The evidence that antigen-specific iTregs exist has notable therapeutic implication, since apart from expanding them *in vitro* for therapy, we should be able to learn how to cause their expansion and activation *in vivo.* Thus, studies from laboratories working on autoimmunity have documented convincingly that antigen-specific Tregs have greater efficacy than activated Tregs of random specificity (93).

Implications of Treg plasticity

Although there are many reasons to believe that Tregs play a beneficial role to dampen tissue damage caused by immunoinflammatory reactions to viruses, many investigators remain unconvinced, especially in the case of HIV and HCV. One reason for the skepticism could relate to the mounting evidence that Tregs are highly prone to functional plasticity (84). Accordingly, under appropriate conditions, the function and phenotype of a $Foxp3^{+}$ Tregs can change. Tregs can begin to express other canonical transcription factors such as Tbet expressed by Th1 cells or ROR-γt characteristic of Th17 T cells and become bifunctional. Alternatively, the Tregs can lose their expression of Foxp3 but express other transcription factors, such as ROR-γt, and function as proinflammatory T cells (reviewed in 94). Studies *in vitro* have identified the types of environments which can drive plasticity, and these changes can be explained on the basis of differences in epigenetic status (95). For example, when Tregs of known specificity are triggered by antigen in the presence of IL-6, they convert to become Th17 cells and lose the expression of Foxp3 (84, 96). Similarly, exposure *in vitro* to IL-12 or IL-6 and IL-21 causes them to become Th1 or T-follicular helper cells, respectively (84). However, the reprogramming to become Th17 T cells is the most usual event, and this is thought to occur commonly in the gut mucosa, accounting for the origin of many of the Th17 populations at that site (97). *In vitro* polarization studies have also revealed that iTregs, the population most likely to participate in regulating responses

during virus infections, show more plasticity than nTregs, with this related to the epigenetic stability of the Foxp3 locus (98, 99). Accordingly, the state of methylation at the so-called Treg cell-specific demethylation region (TSDR) is relevant (98). When the TSDR is demethylated, as occurs with nTregs, the expression of Foxp3 is stable, but with iTregs, the TSDR is partially methylated, which results in less stability of Foxp3 expression and more plasticity (98). Conceivably, the greater stability of nTregs is associated with their main function of constraining autoimmunity.

Some relevant issues with regard to plasticity are whether or not it occurs *in vivo*, the conditions *in vivo* needed for plasticity to occur, and whether such events negate the types of regulatory functions attributed to Tregs and could act to blunt any therapeutic maneuver used to boost the protective effects of Tregs. The answer to the first question is clearly in the affirmative and has been demonstrated in mice by some elegant studies using green fluorescence protein (GFP)-tagged Foxp3$^+$ Tregs (84). The environment that could cause such plasticity, namely one rich in proinflammatory cytokines and Treg-attracting chemokines, is common in many virus-induced inflammatory lesions. Moreover, molecules, such as TGF-β and retinoic acid, that can act to preserve Treg function (89), are unlikely to be present in the lesions in most instances. Thus, it is entirely possible that Tregs can enter inflammatory locations, but after an initial anti-inflammatory effort they could change sides and contribute to causing tissue damage. This change in alliance would be more likely to occur in highly inflamed environments. Such a scenario might help explain the observation that Tregs might be effective at controlling lower levels of inflammatory T-cell activation during HIV infection but seem to be ineffective to turn off the hyperactivation that is a prelude to AIDS (100).

Treg plasticity is currently a hot topic, with many issues yet to be solved. Of particular interest is the observation that plasticity appears to be heterogeneous, with different horses for different courses. Tregs may express Foxp3 along with some expression of the transcription factor characteristic of the effector cells involved in an inflammatory process (94, 97). For instance, in Th1-orchestrated inflammatory sites, Tregs may additionally express Tbet, which could relate to CXCR3 expression needed for their migration to the site as was observed in a *Toxoplasma* system (101). Some Tregs may also co-express IFN-regulatory factor 4 (IRF4), a transcription factor associated with Th2 cells involved in immunopathology (102). Finally, there is an association of transcription factor co-expression by Tregs when they are involved in regulation of Th17-orchestrated inflammatory lesions (103). We must conclude the Treg plasticity is a complex process and its management must be fully understood, since it impacts on the success of any attempts to boost Treg responses in clinical situations, as discussed in a later section.

How do Tregs act?

Much of our mechanistic understanding about the regulatory functions of Tregs come from *in vitro* investigations, and it is far from clear as to how Tregs act *in vivo*, particularly in inflammatory environments. The *in vitro* studies have revealed basically two types of mechanisms: those that necessitate direct contact between Tregs and cells they regulate and those that are independent of cell contact and act by producing inhibitory mediators that in turn are responsible for the inhibitory effects. In situations where direct contact is the mechanism, the effect is often antigen specific with little or no bystander suppression, as would be expected if soluble inhibitory mediators were involved. The requirement for antigen specificity implies that a third cell type that presents antigen must also be involved in the mechanism. In fact, Tregs that are activated can directly mediate regulation in the absence of antigen-presenting cells (APCs) (104). Direct contact effects can either be directed at effector cell function and survival or on DCs, which become impaired APCs for a

number of reasons. These include downregulation of essential costimulator molecules such as CD80 and CD86 (105), perhaps mediated by Treg cytotoxic T-lymphocyte antigen-4 (CTLA-4) expression (106), and the production of suppressive molecules such as indoleamine 2,3-dioxygenase (107) as well as the catalytic inactivation of ATP released from damaged cells (108). The ATP is hydrolyzed by an ectoenzyme expressed by Tregs, which results in the failure of DCs to become inducers of effector cells (104). Exactly why cell contact is needed for Treg activity, which is more evident for nTregs than iTregs, is not clear. Ideas include the need for cell bridging to permit the passage of soluble inhibitors into or to engage receptors on responding cells. This may explain the role of granzyme B, which results in effector cell cytolysis and galectin 1 which causes effector cell apoptosis (109, 110). Direct contact may also permit membrane-tethered TGF-β to cause inhibitory effects by Tregs (111). These issues, which involve many controversies, were lucidly discussed in some recent reviews (104, 112, 113).

The second major mechanism by which Tregs exert their function is to release mediators such as the cytokines IL-10, TGF-β, and IL-35 as well as products such as galectin 1 and some other molecules. These mediators can act at a distance (but usually very close), binding to receptors on cells that are inhibited. This mechanism is more characteristic of iTregs than nTregs, and bystander inhibition is a possibility. Whether Tregs can act by producing TGF-β is quite controversial (104), and TGF-β may not be a major player. Similarly, evidence that IL-35 plays a significant regulatory effect *in vivo* has not been forthcoming (104, 113). The main focus has been on IL-10, a cytokine that can exert a range of inhibitor activities on several cell types that express IL-10 receptors. These effects include inhibition of the proliferation and cytokine producing capacity of effector T cells, along with inhibitory effects on APC function, as well as and inhibition of the proinflammatory activity of macrophages and neutrophils (91).

In vitro studies have certainly revealed how Tregs could exert regulatory functions, but many of the potential mechanisms described might not be occurring *in vivo*. Moreover, there appears to be redundancy as regards regulatory mechanisms because blunting of a single function invariably provokes only partial consequences *in vivo* (104, 113). Other important concepts impact on the potential *in vivo* activity of Treg. These include the location where regulatory effects occur, lymphoid tissues or inflammatory sites, whether or not antigen specificity is needed and the question of Treg functional stability, particularly in inflammatory environments. We have already discussed this latter aspect and have concluded that Tregs have a tendency to lose their inhibitory effects in inflammatory environments and even become proinflammatory participants.

The issue of specificity has received minimal evaluation with viral infections but has been analyzed in some detail in other events that involve Tregs (reviewed in 114). Such studies indicate that antigen-specific Tregs do function more effectively, but once they become activated, they can act nonspecifically (115). Our own studies on the modulatory effects of adoptive transfers of Tregs in the HSV-induced SK system support nonspecific activity (54). We showed that Tregs specific to antigens unrelated to HSV were clinically effective as long as they expressed the activation phenotype (54).

A topic of major relevance to the function of Tregs *in vivo* is where they act and whether heterogenicity exits with regard to the sites and situations where they mediate regulatory effects. Evidence supports regulatory effects occurring in lymphoid tissues, and these may be mainly responsible for the reduced magnitude and changed balance of primary and memory responses to virus infections. However, Tregs may be exerting their main tissue protective modulatory effects by acting within inflammatory sites and not all Treg can access, or act, at such sites. To gain access, Tregs need to express appropriate chemokines

receptors (112). For instance, to get into Th1-orchestrated sites requires the expression of CXCR3 (116). Once in such sites, the transcription factor expression also appears relevant. Thus, Tregs that co-express Tbet along with Foxp3 appear to be more functional that those without Tbet. Other rules apply to get into and function in inflammatory sites that involve different types of orchestrators (103, 117).

We must conclude there are no simple answers to the question of how Tregs act *in vivo*. Almost certainly multiple mechanisms are involved, and these are likely to differ in relevance during the course of a virus infection particularly those that are chronic.

Could Treg manipulation be useful for therapy?

Optimistically, the answer to this question is yes, but so far success has been confined to experimental models of viral disease. Two strategies can be considered. Firstly, Tregs could be expanded *in vitro*, preferably the viral antigen-specific population, and these cells could be administered to animals with immunoinflammatory lesions. This approach is most unlikely to become practical, but its efficacy has been demonstrated in some model systems (54, 118). Our group showed that *in vitro* expanded Tregs could reduce HSV-induced SK lesions, although success was greater the earlier therapy was commenced (54). In the SK model, the Tregs were not specific to viral antigens, but they likely functioned because they expressed an activation phenotype. Conceivably, viral antigen-specific Tregs could be even more effective and might function also at later stages of the disease, but this has yet to be formally demonstrated. One challenge with adoptive transfer approaches is to maximize Treg access to inflammatory sites where they need to act. This challenge might be met by using the type of approach recently described by the Iwasaki group (119). They showed that chemokines administered to local sites could attract T cells from the circulation with them remaining at the site and functioning for prolonged periods. Such a strategy has yet to be reported for Treg site mobilization and is being explored by our group. Ideally, Tregs used for such an approach need to have a fixed epigenetic program, otherwise they may acquire an effector function at the inflammatory site.

A second major strategy for Treg therapy would be to expand the host's own Treg population, preferably in an antigen-specific manner. This approach promises to be more practical. The approach was pioneered by the Von Boehmer group (120), who showed that low concentrations of antigens administered using osmotic pumps could induce Tregs. A modification was to target antigens to a specialized group of DCs that expressed DEC205 (121). The Von Boehmer approach proved valuable in a model for autoimmune diabetes and might merit a trial in humans.

Other approaches have also shown promise, but these only expand Tregs polyclonally which could limit their usefulness, especially for long term efficacy. One example was reported by Sprent and colleagues (122), who showed that the administration of IL-2 complexed with monoclonal antibody (mAb) to IL-2 was effective at expanding the Treg population. The effect had some efficacy in an autoimmune model of multiple sclerosis, but the strategy has not been successful in our SK system. Success has been achieved, however, with a second approach that expands Treg nonspecifically. This is the approach reported by the Podack group (75), which showed that nTregs, which unlike other naive T cells express the TNFR25, can be dramatically expanded when given an agonistic mAb to TNFR25. We showed that mAb administration early after HSV infection resulted in reduced SK lesions. In contrast, when treatment was given in the face of disease, when effector T cells also express TNFR25, lesions became even more severe (76). This latter unwanted consequence could be managed, however, by additionally administering galectins-9 (76). This

combination therapy was shown to function because the galectin-9 acted selectively to cause apoptosis of the proinflammatory T cells.

There are other approaches that cause the induction, or perhaps conversion, of Tregs, which can minimize viral induced inflammatory disease. One such molecule is galectins-9, since this molecule along with galectins-1 will expand at least modestly the Treg population (83, 123). Additional approaches include the use of TGF-β (124), compounds such as FTY720 (21), and agonists of the aryl hydrocarbon receptor (55), all of which succeed in expanding Tregs and achieving some measure of therapeutic success. Additional molecules have also been reported to act with a similar outcome, and these are discussed in other reviews (115, 124).

Using therapeutic approaches to expand Tregs, preferably in an antigen-specific way, holds promise to control viral inflammatory disease, but we are still far from clinical application. There will be many problems to solve, the most important of which may be the durability and stability of any therapeutic approach, especially when the inflammatory milieu contains components that may seduce Tregs to switch their function.

Acknowledgments

This work was supported by National Institute of Allergy and Infectious Diseases Grant AI 063365 and National Institutes of Health Grant EY 005093.

REFERENCES

1. Germain RN. Special regulatory T-cell review: A rose by any other name: from suppressor T cells to Tregs, approbation to unbridled enthusiasm. Immunology. 2008; 123:20–27. [PubMed: 18154615]
2. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995; 155:1151–1164. [PubMed: 7636184]
3. Schubert LA, Jeffery E, Zhang Y, Ramsdell F, Ziegler SF. Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. J Biol Chem. 2001; 276:37672–37679. [PubMed: 11483607]
4. Wildin RS, Smyk-Pearson S, Filipovich AH. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. J Med Genet. 2002; 39:537–545. [PubMed: 12161590]
5. Sakaguchi S, et al. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev. 2006; 212:8–27. [PubMed: 16903903]
6. Zou W. Regulatory T cells, tumour immunity and immunotherapy. Nat Rev Immunol. 2006; 6:295–307. [PubMed: 16557261]
7. Robinson DS, Larché M, Durham SR. Tregs and allergic disease. J Clin Invest. 2004; 114:1389–1397. [PubMed: 15545986]
8. Belkaid Y. Rouse BT. Natural regulatory T cells in. Nat Immunol 2005. 6:353–360.
9. Sakaguchi S. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annu Rev Immunol. 2004; 22:531–562. [PubMed: 15032588]
10. Groux H, Powrie F. Regulatory T cells and inflammatory bowel disease. Immunol Today. 1999; 20:442–445. [PubMed: 10500290]
11. Suri-Payer E, Amar AZ, Thornton AM, Shevach EM. CD4+CD25+ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. J Immunol. 1998; 160:1212–1218. [PubMed: 9570536]

12. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature. 2002; 420:502–507. [PubMed: 12466842]
13. Suvas S, Azkur AK, Kim BS, Kumaraguru U, Rouse BT. CD4+CD25+ regulatory T cells control the severity of viral immunoinflammatory lesions. J Immunol. 2004; 172:4123–4132. [PubMed: 15034024]
14. Suvas S, Kumaraguru U, Pack CD, Lee S, Rouse BT. CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. J Exp Med. 2003; 198:889–901. [PubMed: 12975455]
15. Dittmer U, et al. Functional impairment of CD8(+) T cells by regulatory T cells during persistent retroviral infection. Immunity. 2004; 20:293–303. [PubMed: 15030773]
16. Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? Immunity. 2009; 30:626–635. [PubMed: 19464985]
17. Weiss JM, et al. Neuropilin 1 is expressed on thymus-derived natural regulatory T cells, but not mucosa-generated induced Foxp3+ T reg cells. J Exp Med. 2012; 209:1723–1742. S1721. [PubMed: 22966001]
18. Yadav M, et al. Neuropilin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo. J Exp Med. 2012; 209:1713–1722. S1711–1719. [PubMed: 22966003]
19. Shevach EM. From vanilla to 28 flavors: multiple varieties of T regulatory cells. Immunity. 2006; 25:195–201. [PubMed: 16920638]
20. Belkaid Y, Tarbell K. Regulatory T cells in the control of host-microorganism interactions. Annu Rev Immunol. 2009; 27:551–589. [PubMed: 19302048]
21. Sehrawat S, Rouse BT. Anti-inflammatory effects of FTY720 against viral-induced immunopathology: role of drug-induced conversion of T cells to become Foxp3+ regulators. J Immunol. 2008; 180:7636–7647. [PubMed: 18490766]
22. Lund JM, Hsing L, Pham TT, Rudensky AY. Coordination of early protective immunity to viral infection by regulatory T cells. Science. 2008; 320:1220–1224. [PubMed: 18436744]
23. Ruckwardt TJ, Bonaparte KL, Nason MC, Graham BS. Regulatory T cells promote early influx of CD8+ T cells in the lungs of respiratory syncytial virus-infected mice and diminish immunodominance disparities. J Virol. 2009; 83:3019–3028. [PubMed: 19153229]
24. Betts RJ, Ho AW, Kemeny DM. Partial depletion of natural $CD4^{+}CD25^{+}$ regulatory T cells with anti-CD25 antibody does not alter the course of acute influenza A virus infection. PLoS One. 2011; 6:e27849. [PubMed: 22125630]
25. Rouse BT, Sarangi PP, Suvas S. Regulatory T cells in virus infections. Immunol Rev. 2006; 212:272–286. [PubMed: 16903920]
26. Whitbeck JC, et al. Glycoprotein D of herpes simplex virus (HSV) binds directly to HVEM, a member of the tumor necrosis factor receptor superfamily and a mediator of HSV entry. J Virol. 1997; 71:6083–6093. [PubMed: 9223502]
27. Manangeeswaran M, et al. Binding of hepatitis A virus to its cellular receptor 1 inhibits T-regulatory cell functions in humans. Gastroenterology. 2012; 142:1516–1525.e1513. [PubMed: 22430395]
28. Chevalier MF, Weiss L. The split personality of regulatory T cells in HIV infection. Blood. 2013; 121:29–37. [PubMed: 23043072]
29. Losikoff PT, Self AA, Gregory SH. Dendritic cells, regulatory T cells and the pathogenesis of chronic hepatitis C. Virulence. 2012; 3:610–620. [PubMed: 23076334]
30. Weiss L, Donkova-Petrini V, Caccavelli L, Balbo M, Carbonneil C, Levy Y. Human immunodeficiency virus-driven expansion of CD4+CD25+ regulatory T cells, which suppress HIV-specific CD4 T-cell responses in HIV-infected patients. Blood. 2004; 104:3249–3256. [PubMed: 15271794]
31. Moreno-Fernandez ME, Zapata W, Blackard JT, Franchini G, Chougnet CA. Human regulatory T cells are targets for human immunodeficiency Virus (HIV) infection, and their susceptibility differs depending on the HIV type 1 strain. J Virol. 2009; 83:12925–12933. [PubMed: 19828616]

32. Kinter A, McNally J, Riggin L, Jackson R, Roby G, Fauci AS. Suppression of HIV-specific T cell activity by lymph node CD25+ regulatory T cells from HIV-infected individuals. Proc Natl Acad Sci USA. 2007; 104:3390–3395. [PubMed: 17360656]
33. Eggena MP, et al. Depletion of regulatory T cells in HIV infection is associated with immune activation. J Immunol. 2005; 174:4407–4414. [PubMed: 15778406]
34. Moreno-Fernandez ME, Rueda CM, Rusie LK, Chougnet CA. Regulatory T cells control HIV replication in activated T cells through a cAMP-dependent mechanism. Blood. 2011; 117:5372–5380. [PubMed: 21436067]
35. Aandahl EM, Michaëlsson J, Moretto WJ, Hecht FM, Nixon DF. Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. J Virol. 2004; 78:2454–2459. [PubMed: 14963140]
36. Angin M, et al. Preserved function of regulatory T cells in chronic HIV-1 infection despite decreased numbers in blood and tissue. J Infect Dis. 2012; 205:1495–1500. [PubMed: 22427677]
37. Simonetta F, et al. Early and long-lasting alteration of effector CD45RA(-)Foxp3(high) regulatory T-cell homeostasis during HIV infection. J Infect Dis. 2012; 205:1510–1519. [PubMed: 22457280]
38. Bandera A, et al. CD4+ T cell depletion, immune activation and increased production of regulatory T cells in the thymus of HIV-infected individuals. PLoS One. 2010; 5:e10788. [PubMed: 20520721]
39. Fritzsching B, et al. In contrast to effector T cells, CD4+CD25+FoxP3+ regulatory T cells are highly susceptible to CD95 ligand- but not to TCR-mediated cell death. J Immunol. 2005; 175:32–36. [PubMed: 15972628]
40. Hsieh SM, Chen MY, Pan SC, Hung CC, Chang SC. Aberrant induction of regulatory activity of CD4+CD25+ T cells by dendritic cells in HIV-infected persons with amebic liver abscess. J Acquir Immune Defic Syndr. 2007; 44:6–13. [PubMed: 17003690]
41. Montes M, et al. Normalization of FoxP3(+) regulatory T cells in response to effective antiretroviral therapy. J Infect Dis. 2011; 203:496–499. [PubMed: 21177309]
42. Piconi S, et al. Immune activation, apoptosis, and Treg activity are associated with persistently reduced CD4+ T-cell counts during antiretroviral therapy. Aids. 2010; 24:1991–2000. [PubMed: 20651586]
43. Shaw JM, et al. Increased frequency of regulatory T cells accompanies increased immune activation in rectal mucosae of HIV-positive noncontrollers. J Virol. 2011; 85:11422–11434. [PubMed: 21880771]
44. Hazenberg MD, et al. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. Aids. 2003; 17:1881–1888. [PubMed: 12960820]
45. Lechner F, et al. Analysis of successful immune responses in persons infected with hepatitis C virus. J Exp Med. 2000; 191:1499–1512. [PubMed: 10790425]
46. Cabrera R, et al. An immunomodulatory role for CD4(+)CD25(+) regulatory T lymphocytes in hepatitis C virus infection. Hepatology. 2004; 40:1062–1071. [PubMed: 15486925]
47. Power JP, et al. Hepatitis C viraemia in recipients of Irish intravenous anti-D immunoglobulin. Lancet. 1994; 344:1166–1167. [PubMed: 7934528]
48. Brady MT, MacDonald AJ, Rowan AG, Mills KH. Hepatitis C virus non-structural protein 4 suppresses Th1 responses by stimulating IL-10 production from monocytes. Eur J Immunol. 2003; 33:3448–3457. [PubMed: 14635055]
49. Accapezzato D, et al. Hepatic expansion of a virus-specific regulatory CD8(+) T cell population in chronic hepatitis C virus infection. J Clin Invest. 2004; 113:963–972. [PubMed: 15057302]
50. Oo YH, et al. Distinct roles for CCR4 and CXCR3 in the recruitment and positioning of regulatory T cells in the inflamed human liver. J Immunol. 2010; 184:2886–2898. [PubMed: 20164417]
51. Buchmeier MJ, Welsh RM, Dutko FJ, Oldstone MB. The virology and immunobiology of lymphocytic choriomeningitis virus infection. Adv Immunol. 1980; 30:275–331. [PubMed: 6160740]
52. Iwashiro M, Peterson K, Messer RJ, Stromnes IM, Hasenkrug KJ. CD4(+) T cells and gamma interferon in the long-term control of persistent friend retrovirus infection. J Virol. 2001; 75:52–60. [PubMed: 11119573]

53. Veiga-Parga T, et al. On the role of regulatory T cells during viral-induced inflammatory lesions. J Immunol. 2012; 189:5924–5933. [PubMed: 23129753]
54. Sehrawat S, Suvas S, Sarangi PP, Suryawanshi A, Rouse BT. In vitro-generated antigen-specific CD4+ CD25+ Foxp3+ regulatory T cells control the severity of herpes simplex virus-induced ocular immunoinflammatory lesions. J Virol. 2008; 82:6838–6851. [PubMed: 18480441]
55. Veiga-Parga T, Suryawanshi A, Rouse BT. Controlling viral immuno-inflammatory lesions by modulating aryl hydrocarbon receptor signaling. PLoS Pathog. 2011; 7:e1002427. [PubMed: 22174686]
56. Franzese O, et al. Modulation of the CD8+-T-cell response by CD4+ CD25+ regulatory T cells in patients with hepatitis B virus infection. J Virol. 2005; 79:3322–3328. [PubMed: 15731226]
57. Li B, Wu FL, Feng XB, Sun DK, Cui QQ, Zhao ZX. Changes and the clinical significance of $CD4^+$ $CD25^+$ regulatory T cells and Th17 cells in peripheral blood of infants with respiratory syncytial virus bronchiolitis. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2012; 28:426–428. [PubMed: 22482417]
58. Fulton RB, Meyerholz DK, Varga SM. Foxp3+ CD4 regulatory T cells limit pulmonary immunopathology by modulating the CD8 T cell response during respiratory syncytial virus infection. J Immunol. 2010; 185:2382–2392. [PubMed: 20639494]
59. Loebbermann J, et al. Regulatory T cells expressing granzyme B play a critical role in controlling lung inflammation during acute viral infection. Mucosal Immunol. 2012; 5:161–172. [PubMed: 22236998]
60. Loebbermann J, Durant L, Thornton H, Johansson C, Openshaw PJ. Defective immunoregulation in RSV vaccine-augmented viral lung disease restored by selective chemoattraction of regulatory T cells. Proc Natl Acad Sci U S A. 2013; 110:2987–2992. [PubMed: 23382205]
61. Krishnamoorthy N, et al. Early infection with respiratory syncytial virus impairs regulatory T cell function and increases susceptibility to allergic asthma. Nat Med. 2012; 18:1525–1530. [PubMed: 22961107]
62. Lanteri MC, et al. Tregs control the development of symptomatic West Nile virus infection in humans and mice. J Clin Invest. 2009; 119:3266–3277. [PubMed: 19855131]
63. Sellin CI, et al. Interplay between virus-specific effector response and Foxp3 regulatory T cells in measles virus immunopathogenesis. PLoS One. 2009; 4:e4948. [PubMed: 19319188]
64. Richards MH, Getts MT, Podojil JR, Jin YH, Kim BS, Miller SD. Virus expanded regulatory T cells control disease severity in the Theiler's virus mouse model of MS. J Autoimmun. 2011; 36:142–154. [PubMed: 21273044]
65. Belkaid, Y.; Sehrawat, S.; Rouse, BT. Regulation of antimicrobial immunity. In: Kaufmann, S.; Rouse, BT.; Sacks, D., editors. The immune response to infection. ASM Press; Washington, DC: 2011. p. 109-120.
66. Conroy H, Marshall NA, Mills KH. TLR ligand suppression or enhancement of Treg cells? A double-edged sword in immunity to tumours. Oncogene. 2008; 27:168–180. [PubMed: 18176598]
67. Asea A, et al. Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. J Biol Chem. 2002; 277:15028–15034. [PubMed: 11836257]
68. Biragyn A, et al. Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. Science. 2002; 298:1025–1029. [PubMed: 12411706]
69. Wang Z, et al. Role of IFN-gamma in induction of Foxp3 and conversion of CD4+ CD25- T cells to CD4+ Tregs. J Clin Invest. 2006; 116:2434–2441. [PubMed: 16906223]
70. Sarangi PP, Kim B, Kurt-Jones E, Rouse BT. Innate recognition network driving herpes simplex virus-induced corneal immunopathology: role of the toll pathway in early inflammatory events in stromal keratitis. J Virol. 2007; 81:11128–11138. [PubMed: 17686871]
71. Kurt-Jones EA, et al. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. Nat Immunol. 2000; 1:398–401. [PubMed: 11062499]
72. Hall JA, et al. Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. Immunity. 2008; 29:637–649. [PubMed: 18835196]
73. Becker C, et al. Protection from graft-versus-host disease by HIV-1 envelope protein gp120-mediated activation of human CD4+CD25+ regulatory T cells. Blood. 2009; 114:1263–1269. [PubMed: 19439734]

74. Tao R, Wang L, Murphy KM, Fraser CC, Hancock WW. Regulatory T cell expression of herpesvirus entry mediator suppresses the function of B and T lymphocyte attenuator-positive effector T cells. J Immunol. 2008; 180:6649–6655. [PubMed: 18453584]
75. Schreiber TH, et al. Therapeutic Treg expansion in mice by TNFRSF25 prevents allergic lung inflammation. J Clin Invest. 2010; 120:3629–3640. [PubMed: 20890040]
76. J Reddy PB, et al. TNFRSF25 agonistic antibody and galectin-9 combination therapy controls herpes simplex virus-induced immunoinflammatory lesions. J Virol. 2012; 86:10606–10620. [PubMed: 22811539]
77. Chen W, et al. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med. 2003; 198:1875–1886. [PubMed: 14676299]
78. Belkaid Y. Regulatory T cells and infection: a dangerous necessity. Nat Rev Immunol. 2007; 7:875–888. [PubMed: 17948021]
79. Huynh ML, Fadok VA, Henson PM. Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-beta1 secretion and the resolution of inflammation. J Clin Invest. 2002; 109:41–50. [PubMed: 11781349]
80. Rabinovich GA, Toscano MA. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. Nat Rev Immunol. 2009; 9:338–352. [PubMed: 19365409]
81. Sehrawat S, Suryawanshi A, Hirashima M, Rouse BT. Role of Tim-3/galectin-9 inhibitory interaction in viral-induced immunopathology: shifting the balance toward regulators. J Immunol. 2009; 182:3191–3201. [PubMed: 19234217]
82. Zhu C, et al. The Tim-3 ligand galectin-9 negatively regulates T helper type 1 immunity. Nat Immunol. 2005; 6:1245–1252. [PubMed: 16286920]
83. Rajasagi NK, et al. Galectin-1 reduces the severity of herpes simplex virus-induced ocular immunopathological lesions. J Immunol. 2012; 188:4631–4643. [PubMed: 22467659]
84. Zhou L, Chong MM, Littman DR. Plasticity of CD4+ T cell lineage differentiation. Immunity. 2009; 30:646–655. [PubMed: 19464987]
85. Cong Y, Wang L, Konrad A, Schoeb T, Elson CO. Curcumin induces the tolerogenic dendritic cell that promotes differentiation of intestine-protective regulatory T cells. Eur J Immunol. 2009; 39:3134–3146. [PubMed: 19839007]
86. Sun CM, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J Exp Med. 2007; 204:1775–1785. [PubMed: 17620362]
87. Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science. 2010; 330:1768–1773. [PubMed: 21205662]
88. Joshi A, Garg H, Tompkins MB, Tompkins WA. Preferential feline immunodeficiency virus (FIV) infection of CD4+ CD25+ T-regulatory cells correlates both with surface expression of CXCR4 and activation of FIV long terminal repeat binding cellular transcriptional factors. J Virol. 2005; 79:4965–4976. [PubMed: 15795282]
89. Coombes JL, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med. 2007; 204:1757–1764. [PubMed: 17620361]
90. Hadis U, et al. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. Immunity. 2011; 34:237–246. [PubMed: 21333554]
91. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol. 2001; 19:683–765. [PubMed: 11244051]
92. Battaglia M, Gregori S, Bacchetta R, Roncarolo MG. Tr1 cells: from discovery to their clinical application. Semin Immunol. 2006; 18:120–127. [PubMed: 16464609]
93. Brusko TM, Putnam AL, Bluestone JA. Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. Immunol Rev. 2008; 223:371–390. [PubMed: 18613848]
94. Wohlfert E, Belkaid Y. Plasticity of T reg at infected sites. Mucosal Immunol. 2010; 3:213–215. [PubMed: 20237465]
95. Polansky JK, et al. DNA methylation controls Foxp3 gene expression. Eur J Immunol. 2008; 38:1654–1663. [PubMed: 18493985]

96. Weaver CT, Hatton RD. Interplay between the TH17 and TReg cell lineages: a (co-)evolutionary perspective. Nat Rev Immunol. 2009; 9:883–889. [PubMed: 19935807]

97. Murai M, Krause P, Cheroutre H, Kronenberg M. Regulatory T-cell stability and plasticity in mucosal and systemic immune systems. Mucosal Immunol. 2010; 3:443–449. [PubMed: 20505662]

98. Miyao T, et al. Plasticity of Foxp3(+) T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. Immunity. 2012; 36:262–275. [PubMed: 22326580]

99. Ohkura N, et al. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. Immunity. 2012; 37:785–799. [PubMed: 23123060]

100. Xing S, et al. Increased turnover of FoxP3high regulatory T cells is associated with hyperactivation and disease progression of chronic HIV-1 infection. J Acquir Immune Defic Syndr. 2010; 54:455–462. [PubMed: 20585263]

101. Oldenhove G, et al. Decrease of Foxp3+ Treg cell number and acquisition of effector cell phenotype during lethal infection. Immunity. 2009; 31:772–786. [PubMed: 19896394]

102. Zheng Y, et al. Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T(H)2 responses. Nature. 2009; 458:351–356. [PubMed: 19182775]

103. Chaudhry A, et al. CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner. Science. 2009; 326:986–991. [PubMed: 19797626]

104. Shevach EM. Mechanisms of foxp3+ T regulatory cell-mediated suppression. Immunity. 2009; 30:636–645. [PubMed: 19464986]

105. Misra N, Bayry J, Lacroix-Desmazes S, Kazatchkine MD, Kaveri SV. Cutting edge: human CD4+CD25+ T cells restrain the maturation and antigen-presenting function of dendritic cells. J Immunol. 2004; 172:4676–4680. [PubMed: 15067041]

106. Onishi Y, Fehervari Z, Yamaguchi T, Sakaguchi S. Foxp3+ natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. Proc Natl Acad Sci U S A. 2008; 105:10113–10118. [PubMed: 18635688]

107. Mellor AL, Munn DH. IDO expression by dendritic cells: tolerance and tryptophan catabolism. Nat Rev Immunol. 2004; 4:762–774. [PubMed: 15459668]

108. Borsellino G, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood. 2007; 110:1225–1232. [PubMed: 17449799]

109. Gondek DC, Lu LF, Quezada SA, Sakaguchi S, Noelle RJ. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol. 2005; 174:1783–1786. [PubMed: 15699103]

110. Garín MI, Chu CC, Golshayan D, Cernuda-Morollón E, Wait R, Lechler RI. Galectin-1: a key effector of regulation mediated by CD4+CD25+ T cells. Blood. 2007; 109:2058–2065. [PubMed: 17110462]

111. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med. 2001; 194:629–644. [PubMed: 11535631]

112. Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol. 2012; 30:531–564. [PubMed: 22224781]

113. Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nat Rev Immunol. 2008; 8:523–532. [PubMed: 18566595]

114. Simons DM, et al. How specificity for self-peptides shapes the development and function of regulatory T cells. J Leukoc Biol. 2010; 88:1099–1107. [PubMed: 20495071]

115. Sehrawat S, Rouse BT. Tregs and infections: on the potential value of modifying their function. J Leukoc Biol. 2011; 90:1079–1087. [PubMed: 21914856]

116. Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ. The transcription factor T-bet controls regulatory T cell homeostasis and function during type 1 inflammation. Nat Immunol. 2009; 10:595–602. [PubMed: 19412181]

117. Linterman MA, et al. Foxp3+ follicular regulatory T cells control the germinal center response. Nat Med. 2011; 17:975–982. [PubMed: 21785433]

118. Tang Q, et al. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. J Exp Med. 2004; 199:1455–1465. [PubMed: 15184499]
119. Shin H, Iwasaki A. A vaccine strategy that protects against genital herpes by establishing local memory T cells. Nature. 2012; 491:463–467. [PubMed: 23075848]
120. Apostolou I, von Boehmer H. In vivo instruction of suppressor commitment in naive T cells. J Exp Med. 2004; 199:1401–1408. [PubMed: 15148338]
121. Kretschmer K, Apostolou I, Jaeckel E, Khazaie K, von Boehmer H. Making regulatory T cells with defined antigen specificity: role in autoimmunity and cancer. Immunol Rev. 2006; 212:163–169. [PubMed: 16903913]
122. Boyman O, Surh CD, Sprent J. Potential use of IL-2/anti-IL-2 antibody immune complexes for the treatment of cancer and autoimmune disease. Expert Opin Biol Ther. 2006; 6:1323–1331. [PubMed: 17223740]
123. Sehrawat S, Reddy PB, Rajasagi N, Suryawanshi A, Hirashima M, Rouse BT. Galectin-9/TIM-3 interaction regulates virus-specific primary and memory CD8 T cell response. PLoS Pathog. 2010; 6:e1000882. [PubMed: 20463811]
124. Bluestone JA. Regulatory T-cell therapy: is it ready for the clinic? Nat Rev Immunol. 2005; 5:343–349. [PubMed: 15775994]
125. van der Vliet HJ, et al. Selective decrease in circulating V alpha 24+V beta 11+ NKT cells during HIV type 1 infection. J Immunol. 2002; 168:1490–1495. [PubMed: 11801694]
126. Garba ML, Pilcher CD, Bingham AL, Eron J, Frelinger JA. HIV antigens can induce TGF-beta(1)-producing immunoregulatory CD8+ T cells. J Immunol. 2002; 168:2247–2254. [PubMed: 11859112]
127. Andrade RM, et al. High IL-10 production by aged AIDS patients is related to high frequency of Tr-1 phenotype and low in vitro viral replication. Clin Immunol. 2012; 145:31–43. [PubMed: 22922271]
128. George J, et al. Early short-term antiretroviral therapy is associated with a reduced prevalence of CD8(+)FoxP3(+) T cells in simian immunodeficiency virus-infected controller rhesus macaques. AIDS Res Hum Retroviruses. 2011; 27:763–775. [PubMed: 21142402]
129. Brincks EL, et al. Antigen-Specific Memory Regulatory CD4+Foxp3+ T Cells Control Memory Responses to Influenza Virus Infection. J Immunol. 2013; 190:3438–3446. [PubMed: 23467933]
130. Robertson SJ, Messer RJ, Carmody AB, Hasenkrug KJ. In vitro suppression of CD8+ T cell function by Friend virus-induced regulatory T cells. J Immunol. 2006; 176:3342–3349. [PubMed: 16517701]
131. Lu W, Chen S, Lai C, Guo W, Fu L, Andrieu JM. Induction of CD8+ regulatory T cells protects macaques against SIV challenge. Cell Rep. 2012; 2:1736–1746. [PubMed: 23260669]
132. Vukmanovic-Stejic M, et al. Varicella zoster-specific CD4+Foxp3+ T cells accumulate after cutaneous antigen challenge in humans. J Immunol. 2013; 190:977–986. [PubMed: 23284056]
133. Barros N, Woll F, Watanabe L, Montes M. Are increased Foxp3+ regulatory T cells responsible for immunosuppression during HTLV-1 infection? Case reports and review of the literature. BMJ Case Rep. 2012; 2012
134. Silva-Campa E, Mata-Haro V, Mateu E, Hernández J. Porcine reproductive and respiratory syndrome virus induces CD4+CD8+CD25+Foxp3+ regulatory T cells (Tregs). Virology. 2012; 430:73–80. [PubMed: 22609353]
135. Yang G, et al. Association of CD4+CD25+Foxp3+ regulatory T cells with chronic activity and viral clearance in patients with hepatitis B. Int Immunol. 2007; 19:133–140. [PubMed: 17182968]
136. Beilharz MW, et al. Timed ablation of regulatory CD4+ T cells can prevent murine AIDS progression. J Immunol. 2004; 172:4917–4925. [PubMed: 15067071]
137. Marshall NA, Vickers MA, Barker RN. Regulatory T cells secreting IL-10 dominate the immune response to EBV latent membrane protein 1. J Immunol. 2003; 170:6183–6189. [PubMed: 12794149]
138. Wingate PJ, McAulay KA, Anthony IC, Crawford DH. Regulatory T cell activity in primary and persistent Epstein-Barr virus infection. J Med Virol. 2009; 81:870–877. [PubMed: 19319950]

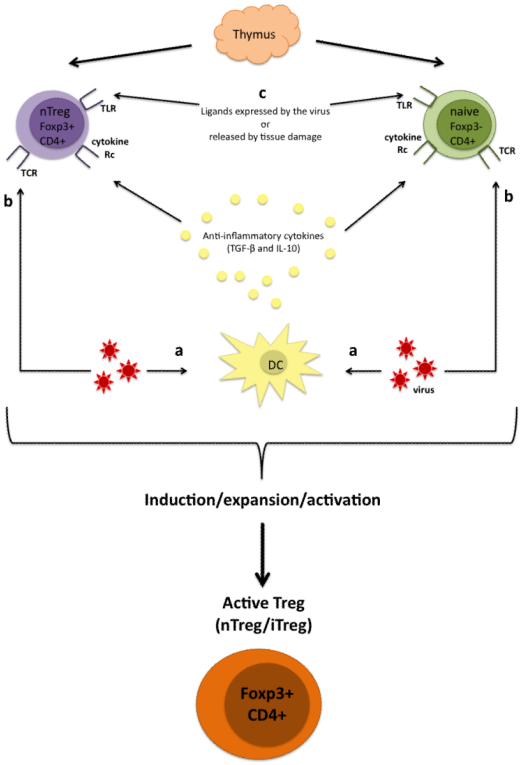

Fig. 1. Possible pathways that viruses may use to induce/activate/expand Tregs as a mechanism to survive within the host

(A) Viruses can manipulate APCs by three different mechanisms: inducing anti-inflammatory cytokine production, modulating antigen presentation, or by interfering with co-stimulatory molecule expression. (B) Some viruses may have antigens that cross-react with self-antigens and will be recognized by the TCR on nTregs, or have antigens that are recognized by the TCR on naive Th0 cells that will become iTregs. iTregs can also recognize non-self antigens through their TCRs, which will induce them to proliferate Moreover, self-antigens released as a result of tissue damage could stimulate nTregs through their TCR. (C) Stimulation of TLRs expressed on Tregs by PAMPs from the virus, or DAMPs such as heat shock proteins, β-defensins, nucleic acid can directly induce regulatory T-cell activation. Stimulation independent of TCR can also happen by host-derived products such as cytokines released after infection (TGF-β, IL-2, IFN-γ, TNF-α), galectins (galectin-1 and galectin-9), cellular metabolites (like retinoic acid), and other molecules.

Table 1

Types of regulatory cells involved in influencing the outcome of some virus infections

VIRUS	Type of regulatory cells involved	Reference
HSV	$CD4^+CD25^+Foxp3^+$	13
HIV	$CD4^+CD25^+Foxp3^+$	30, 32
	NKT cells	125
	$CD8^+CD25^-$	126
	Tr1 cells	127
	$CD8^+Foxp3^+$	128
HCV	$CD4^+CD25^+Foxp3^+$	46
	Tr1	48
	$CD8^+$ regulatory cells	49
Influenza virus	$CD4^+CD25^+Foxp3^+$	129
RSV	$GrB^+CD4^+CD25^+Foxp3^+$	59
	$CD4^+CD25^+Foxp3^+$	60
Friend virus	$CD4^+CD25^+Foxp3^+$	130
SIV	$CD4^+CD8^+$ Tregs	131
Varicella zoster virus	$CD4^+Foxp3^+$	132
Human T-lymphotropic virus	$CD4^+CD25^+Foxp3^+$	133
Measles vims	$CD4^+CD25^+Foxp3^+$	63
Feline immunodeficiency virus	$CD4^+CD25^+Foxp3^+$	88
Porcine Reproductive and Respiratory Syncytial virus	$CD4^+CD25^+Foxp3^+$ $CD8^+CD25^+Foxp3^+$	134
HBV	$CD4^+CD25^+Foxp3^+$	135
Murine AIDS	$CD4^+CD25^+$	136
Cytomegalovirus	$CD4^+CD25^+$	35
Epstein–Barr virus	Trl	137
	$CD4^+CD25^+Foxp3^+$	138

Table 2
Some examples of Treg effects on immunopathology

VIRUS	Effect of Tregs on immunopathology	Reference
HSV-1	control of eye immunopathology by limiting effector function and migration	13, 53
HIV	control of T cell hyperactivation and inhibits HIV replication T cells	28, 30, 33
HCV	suppress CTLs reducing liver damage	49
HBV	reduce liver damage by suppressing HBV-specific adaptive immune responses and regulating influx of macrophages and DCs	56
RSV	limit pulmonary immunopathology by modulating the CD8 T cell response	59, 60
WNV	protect from CNS immunopathology by controlling CD8 T cell responses	62