nature immunology

REVIEW

Natural regulatory T cells in infectious disease

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This review discusses the control exerted by natural CD4$^+$ CD25$^+$ regulatory T cells (natural T_{reg} cells) during infectious processes. Natural T_{reg} cells may limit the magnitude of effector responses, which may result in failure to adequately control infection. However, natural T_{reg} cells also help limit collateral tissue damage caused by vigorous antimicrobial immune responses. We describe here various situations in which the balance between natural T_{reg} cells and effector immune functions influences the outcome of infection and discuss how manipulating this equilibrium might be exploited therapeutically.

Infectious challenges to the host are met by a wealth of humoral and cellular responses. Some agents are difficult to control and the host response to them often results in tissue damage. This tissue damage might be more intense were it not for many regulatory mechanisms that contain the 'zeal' of both innate and adaptive effector responses. The once-disfavored idea that suppressor cells with antigen specificity form part of the regulatory mechanisms has now been revitalized[1]. Indeed, it is has been conceded that several types of regulatory cells exist, some of which are induced in response to infectious challenge and some that are considered natural regulators[2,3]. Inducible regulatory T cells (T_{reg} cells) such as T_R1 or T helper type 3 (T_H3) cells can develop from conventional CD4$^+$ T cells that are exposed to specific stimulatory conditions such as the blockade of costimulatory signals, deactivating cytokines or drugs. These cell types have been discussed in several reviews[2,4,5]. Natural T_{reg} cells, however, arise during the normal process of maturation in the thymus and survive in the periphery as T_{reg} cells. This segregation between natural T_{reg} cells and induced T_{reg} cells could prove to be arbitrary, with the relationship between the populations requiring clarification. Nevertheless, natural T_{reg} cells obey defined rules and express a specific set of markers[3,6]. For example, only natural T_{reg} cells constitutively express CD25, the T cell inhibitory receptor CTLA-4 and the glucocorticoid-inducible tumor necrosis factor receptor (GITR). The unique transcription factor Foxp3 is required for the generation of natural T_{reg} cells, and this represents their most specific marker identified so far (reviewed by Fontenot and Rudensky[6] in this issue). Natural T_{reg} cells can respond to a large variety of self antigens, although growing evidence suggests that these cells may also respond to antigens expressed by microbes. Although inducible T_{reg} cells may control various infectious processes[4], our review focuses only on infections for which an association with natural T_{reg} cells has been suggested (**Table 1**). Understanding the unique properties of natural T_{reg} cells and their mode of action may result in new therapeutic avenues useful for the control of infectious diseases.

In most cases in which natural T_{reg} cells participate in responses to infection, these are chronic infections. As discussed below, the influence of natural T_{reg} cells may favorably affect the outcome or can be harmful to the host. However, the outcome is also affected by other factors. These include the stage of infection, dose of the pathogen and genotype and immunological status of the host as well as the presence of concomitant disease or other infections. We also discuss whether enhanced pathogen survival is one consequence of natural T_{reg} cell function.

Influence of regulatory: effector cell balance

Some of the earliest studies of natural T_{reg} cells emphasized that such cells help control the extent of immune-mediated pathology. In fact, a chief function of natural T_{reg} cells may be to respond to signals associated with tissue destruction and then to minimize collateral tissue damage they cause[7]. A well documented example of this situation is the involvement of natural T_{reg} cells in gastrointestinal homeostasis. Commensal gut bacteria can, in cases of immune dysregulation, trigger harmful inflammatory diseases. Extensive work in mouse models of colitis has demonstrated that natural T_{reg} cells act as chief regulators of such lesions. Adoptive transfer of naive T cell populations lacking natural T_{reg} cells into T cell–deficient mice causes massive gut inflammation. Transfer of CD4$^+$CD25$^+$CD45RB^{lo} T cells together with those naive T cells suppresses disease development, an effect mediated by interleukin-10 (IL-10), transforming growth factor-β (TGF-β) and CTLA-4. Similarly, in mice deficient in the recombination-activating gene(s), *Helicobacter hepaticus* causes

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Published online 22 March 2005; doi:10.1038/ni1181

mild intestinal inflammation, but this is enhanced considerably by the adoptive transfer of $CD4^+CD25^-CD45RB^{hi}$ T cells or T cells from IL-10-deficient mice[8,9]. However, transfer of $CD4^+CD45RB^{lo}CD25^-$ or $CD25^+$ cells together with those cells prevents inflammation in an IL-10- and TGF-β-dependent way[10]. This model was the first, to our knowledge, to demonstrate that the targets of natural T_{reg} cells could include components of the innate immune system as well as pathogenic T cells[9]. In T cell–deficient mice infected with *Helicobacter pylori*, transfer of $CD4^+CD25^-$ T cells provides better control of the infection, but enhanced gastric inflammation also occurs[11]. Natural T_{reg} cells may also control *H. pylori* infection in humans[12]. When isolated from a chronically infected person, T_{reg} cells are able to suppress *H. pylori*–specific T cell responses but not responses to unrelated antigens[12].

The preservation of host homeostasis by natural T_{reg} cells is not restricted to inflammation caused by gastrointestinal bacteria. There is similar involvement in other infection models in which the infected sites require more control. These include the lung, skin and liver as well as the eye. For example, *Pneumocystis carinii* infects mice deficient in recombination-activating gene 2 without inducing detectable pathology. The transfer of $CD4^+CD25^-$ T cells results in better control of infection but also triggers florid pneumonitis that becomes lethal. This outcome can be prevented by transfer of natural T_{reg} cells[13]. During infection of mice with *Candida albicans*, a reduction in natural T_{reg} cell numbers induces better control of the infection, but unfortunately enhanced inflammatory gastrointestinal pathology also occurs[14]. In a nonhealing model of *Leishmania major* infection, cutaneous infection results in progressive lesions caused by a robust T_H2 response[15]. In this model, the amplitude of the response and subsequent pathology is held in check by natural T_{reg} cells[16,17]. This model exemplifies the dual function of natural T_{reg} cells during a given infection. The early disease exacerbation in the absence of natural T_{reg} cells is followed by better control of infection in the later stages of the syndrome than in control mice[18].

The outcome of schistosomal infection in mice depends on T_H2 polarization. The inhibitory effects of natural T_{reg} cells on the T_H1 response have been shown to promote T_H2 polarization and to protect the host from lethal inflammatory pathology[19]. One of the main targets of natural T_{reg} cells in this model is the production of IL-12 by activated dendritic cells, an inhibitory effect that is mediated by IL-10. In the late stages of the schistosomal infection, most of the pathology is a granulomatous fibrosis. IL-10-producing natural T_{reg} cells purified from parasite egg–induced granulomas are an important factor for host survival[20]. Natural T_{reg} cells also seem to be as important in the disease caused by hepatitis C virus (HCV). A chief complication of this chronic infection is massive liver damage that often requires organ transplant. Liver biopsies obtained at the time of the transplant show an inverse correlation between the number of natural T_{reg} cells in the periphery and the histological inflammatory score[21].

Control of inflammatory reaction by natural T_{reg} cells might be especially important in delicate tissues such as the eye. This organ's function requires that the path of light to the retina not be impeded by defracting inflammatory cells. In a model in which a blinding keratitis was caused by herpes virus infection induced by a $CD4^+$ T cell–orchestrated reaction, lesions were much more severe in mice whose natural T_{reg} cells had been depleted[22]. Indeed in the absence of natural T_{reg} cells, nonpathological doses of virus can readily induce keratitis[22].

Table 1 Microbial infections for which a regulatory function for natural T_{reg} cells has been suggested

Microbe	Species	Antigen specificity	Reference
Helicobacter hepaticus	Mouse	ND	8,10
	Human		
Helicobacter pylori	Mouse	ND	12,49
	Human		
Listeria monocytogenes	Mouse	ND	38
Pneumocistis carinii	Mouse	ND	13
Leishmania major	Mouse	Yes	16–18,23,37
Schistosoma masoni	Mouse	Yes	19,20
Candida albicans	Mouse	ND	14,80
Herpes simplex virus	Mouse	ND	22,28
Friend virus	Mouse	ND	30,75
Human immunodeficiency virus	Human	Yes	32–34,59,60
Hepatitis C virus	Human	Yes	21,35,36
Cytomegalovirus	Human	ND	32
Murine AIDS	Mouse	ND	31
Feline immunodeficiency virus	Cat	ND	56,81

ND, not done.

One other consequence of the modulation of excessive immune responses by natural T_{reg} cells is enhanced pathogen survival and, in some cases, long-term persistence. Thus, pathogen persistence may represent a 'compromise' reached by the host with pathogens when new homeostatic conditions become established (**Table 2**). The mouse model of *L. major* infection in which natural T_{reg} cells are a necessary component of the pathogen's survival provides a good example of this[23]. Self-healing C57BL/6 mice infected with a low dose of parasites develop small self-healing lesions, and immunity to re-infection requires persistent infection[24]. Natural T_{reg} cells accumulate at sites of infection and limit the efficacy of T_H1 immune responses (by both IL-10-dependent and IL-10-independent pathways). As a consequence, the natural T_{reg} cells promote pathogen persistence and potential transmission to other hosts. Removal of natural T_{reg} cells leads to 'sterile cure', a state that is not compatible with the preservation of long-term immunity[23].

Another example of this 'entente' between host and pathogen has been provided by ocular infection of mice with herpes simplex virus (HSV). A low dose of virus infection protects mice from $CD4^+$ T cell–mediated pathology by promoting natural T_{reg} cells, a situation that is compatible with the establishment of immunity to re-infection[22].

Table 2 Functions of T_{reg} cells during infection

	Reduced number or function of natural T_{reg} cells	Equilibrium between natural T_{reg} cells and effector cells	Excess number or function of natural T_{reg} cells
Advantages for:			
Host	Pathogen clearance	Maintenance of protective immunity	
		Control of excessive immune responses (immunopathology)	
Microbe		Persistence and/or transmission	Transmission
Disadvantages for:			
Host	Tissue damage	Maintenance of reservoir	Prevention of effector immune responses
			Disease reactivation
Microbe	Microbe clearance		Host destruction

Similarly, reducing natural T_{reg} cells in mice promotes better control of primary *C. albicans* infection. However, enhanced pathology as well as loss of immunity to re-infection occurs unless natural T_{reg} cells are reconstituted[14]. Natural T_{reg} cells may also maintain immunity to other chronic infections in which 'poor-quality' effectors are generated and pathogen persistence is required. All of these models show that a natural T_{reg} cell–dependent balance can be established between host and pathogen that benefits both.

Consequences of natural T_{reg} cell dysregulation

As discussed above, the overall equilibrium between effector and regulatory mechanisms may determine the outcome of an infection and this may in some cases be mutually beneficial to the host and the pathogen (**Table 2**). However, in some situations, the control exerted by natural T_{reg} cells seems to contribute to an unbalanced state, with the host experiencing damage. An example of this seems to be malaria, a disease that causes the death of up to 2 million people annually. Natural immunity to malaria in endemic regions occurs, but it requires several years to develop and the many factors involved are still poorly understood[25]. One factor involved may be the participation of natural T_{reg} cells. In mouse models of malaria, depletion of natural T_{reg} cells protects mice from death caused by the lethal strain of *Plasmodium yoelii*[26]. Removal of natural T_{reg} cells during mouse malaria allows the restoration of a vigorous effector immune response against the parasite and control of infection[26]. Similarly, removal of natural T_{reg} cells after *Plasmodium berghei* infection in mice reduces parasitemia[27]. Whether natural T_{reg} cells function in human malaria remains to be addressed.

Other examples in which natural T_{reg} cells may be detrimental to the host-pathogen immune balance are accumulating. Comparisons of the HSV immune response in natural T_{reg} cell–depleted and intact mice have shown that responses are considerably enhanced in the absence of natural T_{reg} cells[28]. This is evident for many parameters of immunity, including $CD4^+$ and $CD8^+$ T cell responses[28,21] as well as mucosal antibody concentration (F. Toka and B.T.R., unpublished observations). Furthermore, immunized mice lacking natural T_{reg} cells also show greater resistance to viral challenge[28]. The suppressive effect mediated by natural T_{reg} cells has also been found in several retrovirus systems. For example, in mice chronically infected with Friend leukemia virus, the increased number of natural T_{reg} cells compromises the efficacy of protective $CD8^+$ T cell responses[29,30]. When the natural T_{reg} cell 'pressure' is removed, the effector function of $CD8^+$ T cells is restored and persistent viral loads are reduced considerably[30]. In the mouse model of AIDS, disease progression is associated with an increase of putative natural T_{reg} cells in the periphery[31].

Accumulating evidence also indicates that immunity to human immunodeficiency virus (HIV) infection may be controlled by natural T_{reg} cells[32–34]. AIDS is associated with loss of $CD4^+$ T cells and progressive immune dysfunction. Removal of T_{reg} cells from peripheral blood mononuclear cell populations results in increased anti-HIV $CD4^+$ T cell responses[32]. In addition, the *in vitro* HIV-specific CD4 and CD8 T cell responses in most HIV-infected people is substantially abrogated by T_{reg} cells[33]. Such suppression is cell contact dependent and cytokine independent, supporting the idea of involvement of natural T_{reg} cells.

HCV is another disease in which involvement of natural T_{reg} cells is considered to impede immune defense. People chronically infected with HCV have more circulating natural T_{reg} cells in peripheral blood than do uninfected people, and depletion of T_{reg} cells enhances antigen-specific $CD8^+$ T responses *in vitro*[35]. T_{reg} cell suppression is dependent on TGF-β and cell contact[21]. Notably, patients chronically infected with HCV who develop autoimmunity show considerable reduction in their peripheral natural T_{reg} cell numbers[36]. A link between chronic infections, autoimmune disorders and dysregulation of natural T_{reg} cells function requires further analysis.

Natural T_{reg} cells can also control the intensity of secondary responses to infections such as listeria, HSV or leishmania[28,37,38] and may also influence the magnitude of memory[37–39]. In studies using a DNA vaccine against listeria, natural T_{reg} cells strongly restricted the number of antigen specific $CD8^+$ T cell responses[38]. A similar although less notable difference between natural T_{reg} cell–depleted versus intact mice has been reported for the recall response to HSV as well as to some HSV vaccine preparations[39]. Thus, natural T_{reg} cells may influence the extent of secondary immune responses to pathogens and probably affect the host's ability to resist to challenge. Natural T_{reg} cells may also affect the magnitude and homeostatic turnover of long-term memory cells, but this needs further investigation. A better understanding of those issues could affect vaccine design.

Although pathogen persistence is compatible with and in some cases required for the maintenance of immunity to reinfection, persistence can also result in disease reactivation. Reactivation or acti-

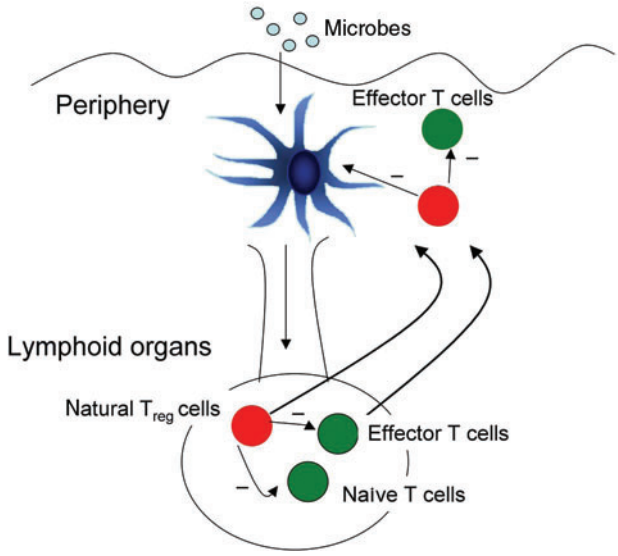

Figure 1 Function and localization of natural T_{reg} cells during infections. Antigen-presenting cells exposed to pathogens in the periphery will initiate effector and natural T_{reg} cell responses. The priming and expansion of effector populations is controlled in the lymph node by natural T_{reg} cells. After chronic infection, both populations migrate to the infected sites and, according to the environment, differentially effect their functions. Some conditions will enhance natural T_{reg} cell functions or survival, leading to local control of effector cells and antigen-presenting cells. Other conditions will abrogate their regulatory functions. Resident natural T_{reg} cells may contribute to the local regulation of tissues in steady-state conditions.

vation of latent or chronic bacterial (for example, *Mycobacterium tuberculosis*), protozoal (for example, *Leishmania* sp. or toxoplasma) and viral (for example, herpes viruses) infection causes an immense burden of morbidity and mortality. Reactivation can occur as a result of immunosuppression or environmental insults or with advancing age[40,41], but a definite cause for the reactivation or primary activation of dormant infections is often not apparent. In some situations, increased numbers of natural T_{reg} cells can lead to disease reactivation. In the mouse model of chronic leishmania infection, for example, the transfer of purified natural T_{reg} cells derived from infected mice into other chronically infected animals is sufficient to trigger disease reactivation and inhibit effector memory response[37]. Furthermore, secondary challenge of mice chronically infected with leishmania at a site distant from the initial infection site induces transient disease reactivation at the primary inoculation site despite induction of a powerful immune response at the challenge site. Reactivation is associated with a local increase in the number of natural T_{reg} cells[37]. These results demonstrate that the equilibrium between natural T_{reg} cells and effector lymphocytes can be disturbed by superinfection, thereby altering immune efficacy and disease reactivation.

Studies of T cell receptor–transgenic mice have shown that antigen-specific natural T_{reg} cells divide when appropriately presented with antigen. Natural T_{reg} cells expressing a transgenic T cell receptor specific for hemagglutinin or ovalbumin divide extensively when exposed to their respective antigens *in vivo*[42,43]. In addition, natural T_{reg} cells strongly proliferate *in vivo* in steady-state conditions[44]. Except in gnotobiotic facilities, all hosts are massively colonized by commensal microbes as well as with some persistent pathogens. Such stimuli are thought to induce natural T_{reg} cells, although this needs verification. Natural T_{reg} cells specific for leishmania can be recovered from chronically infected mice. These cells maintain a strong proliferative capacity in response to the antigen in regional lymph nodes and at sites of infection and continue to accumulate proportionally with age. This contrasts with the activity of effector T cells in these chronically infected mice that still produce interferon-γ but proliferate poorly in response to the antigen (I. Suffia and Y.B., unpublished observations).

In addition to their direct effect on effector T cells, natural T_{reg} cells may exert a 'bystander' influence on the local environment through cytokine release or through a direct effect on antigen-presenting cells. They may also orchestrate inhibitory effects by inducing dendritic cells to produce indoleamine 2,3 dioxygenase, which prevents effector T cells from responding to antigen[45]. Thus, the continual accumulation of natural T_{reg} cells at sites of infection can upset the homeostasis of the infected organ and cause local immunosuppression. Such an effect might be particularly substantial in the context of chronic systemic infection, such as with HIV or HCV. We have also found natural T_{reg} cells among the inflammatory cells in HSV-infected sensory ganglia. Here we believe the natural T_{reg} cells may serve to prevent effector T cells from destroying the infected but irreplaceable neurons (S. Suvas and B.T.R., unpublished data).

A decrease in immune function is well described in the elderly[46]. In particular, their decreasing T cell function seems to be associated with increased risk and severity of infection, impaired response to vaccination and poorer control of cancer. Reactivation or activation of persistent infection occurs with increased frequency in the elderly. An increased accumulation of cells with regulatory functions has been shown in older mice compared with younger ones[47,48]. Whether changes in natural T_{reg} cell function account for the diminished immunity of older humans remains to be addressed.

Antigen specificity of natural T_{reg} cells

Natural T_{reg} cells have a polyclonal T cell receptor repertoire in normal mice and are thought to recognize a wide array of self antigens. Whether they also recognize foreign antigens and the extent of their repertoire for such antigens is unknown. We believe the case is strong for natural T_{reg} cell recognition of antigens derived from pathogens and that such recognition is an essential step in their regulatory function. Evidence for this comes from studies of schistosoma and leishmania infection. Natural T_{reg} cells from chronically infected mice can produce IL-10 in response to parasite antigens but not to other stimuli or pathogens[19,20,23]. The activated phenotype of natural T_{reg} cells populations in various models of infection also suggest their antigen specificity. Some of the most convincing data are from human studies. In HIV-infected people, cells with the characteristics of natural T_{reg} cells mediate suppression in an antigen-specific way[33]. Similarly, in patients infected with *H. pylori*, T_{reg} cell–mediated suppression can be shown only with *H. pylori* antigens[49]. In addition, T_{reg} cells purified from peripheral blood mononuclear cell samples of HIV-infected patients on highly active antiretroviral therapy produce large amounts of IL-10 in response to p24 antigen[34]. Similarly, IL-10 production by T_{reg} cells also occurs in HCV-infected patients in response to viral antigens[21,50]. Although expression of Foxp3 by microbe-specific T_{reg} cells remains to be formally demonstrated in the human studies, these results are consistent with the idea that natural T_{reg} cells can recognize foreign antigen.

A possibility not mutually exclusive with the discussion above would be that other antigenic specificities could also influence natural T_{reg} cell function during infection. Infections are often associated with tissue damage and therefore self antigens can potentially be presented in a nontolerogenic way. Thus, some natural T_{reg} cells may respond

to microbial antigens that are cross-reactive with self. Many models have advocated a mechanism of molecular mimicry by showing that foreign antigens can activate T cells that are cross-reactive with self antigen[51,52]. However, molecular mimicry remains a contentious issue[53]. Pathogens can also present antigens cross-reactive with gut flora antigens. In fact, because natural T_{reg} cells are prominent in the control of gut homeostasis, the gut flora may shape the repertoire of natural T_{reg} cells. In several models of infection, removal or modification of gut flora can influence the susceptibility of the host to infection[54,55]. As host-pathogen interactions change over time, it is very likely that the nature of the antigens 'seen' by natural T_{reg} cells and their antigen specificities at sites of infection varies according to the site or the stage of the infection. However, whether natural T_{reg} cells recognize specific pathogen antigens needs further investigation.

Microbes may favor natural T_{reg} cell induction

Most pathogens delay or prevent host destruction. For this, they have evolved multiple strategies to manipulate both the innate and the adaptive immune systems. Pathogens may also have evolved strategies to establish conditions favoring natural T_{reg} cell priming (manipulation of antigen-presenting cells), recruitment (triggering of chemokines) and survival (creating a favorable cytokine environment; **Fig. 1**). Indeed, natural T_{reg} cells are 'activated' by infections. This activation is demonstrated by enhanced natural T_{reg} cell cytokine production (IL-10 or TGF-β) in response to polyclonal stimuli or, in some cases, exposure to antigens in infected hosts[19–21,23]. Activation is also evident by increased expression of activation markers at the surface of natural T_{reg} cells and their enhanced suppressive function both *in vitro* and *in vivo*[28,56]. In addition to responding to exposure to antigens, natural T_{reg} cells can also respond to microbial products. For example, natural T_{reg} cells selectively express Toll-like receptors 4, 5, 7 and 8. Moreover, exposure of natural T_{reg} cells to lipopolysaccharide induces upregulation of activation markers on their cell surfaces, and this enhances natural T_{reg} cell survival and proliferation[57]. In addition, lipopolysaccharide treatment increases natural T_{reg} cell–mediated suppression *in vivo* and *in vitro*. Many pathogen-associated molecular patterns or pattern-recognition receptors and inflammatory tissue factors could also favor natural T_{reg} cell function and survival. The amount of activation of the antigen-presenting cells is critical to the development and induction of natural T_{reg} cells. For example, mature dendritic cells are more efficient at inducing the proliferation of transgenic natural T_{reg} cells than are immature cells[58].

It seems that natural T_{reg} cells may themselves be infected by pathogens such as HIV[59]. Similarly, conventional $CD4^+$ T cells transduced with Foxp3 to generate functional natural T_{reg} cells are also easily infected by HIV[59]. It is not apparent if such infected natural T_{reg} cells remain effective as regulators. In the feline lentivirus system, too, $CD4^+$ T cells may be targets of infection, but only natural T_{reg} cells sustain virion production when cultured with IL-2 (ref. 56).

One mechanism by which pathogens might manipulate natural T_{reg} cell function would be to create an environment that favors pathogen retention and survival. A decrease in the number of functional natural T_{reg} cells has been found in the peripheral blood of symptomatic patients infected with HIV or HCV[33,36,59]. It is very likely that such decreases reflect the redistribution of natural T_{reg} cells rather than an overall decrease. In various experimental models such as infection with leishmania, HSV and schistosoma, regulatory cells preferentially accumulate at sites of disease[20,22,23]. Natural T_{reg} cells with powerful suppressive function also accumulate at sites of human cutaneous leishmaniasis (J.S. Silva, personal communication). The segregation of human natural T_{reg} cells at sites of infection is also supported by the finding that the number of these cells is greatly enhanced in the lymphoid organs of HIV-infected people[60]. Thus, natural T_{reg} cells may preferentially accumulate at sites of infection and, as a consequence, the peripheral blood may not always be the most appropriate compartment in which to investigate the function of natural T_{reg} cells in human chronic infections.

The conditions created by the infectious process obviously favor the recruitment and/or local survival of natural T_{reg} cells. The anti-inflammatory cytokine TGF-β, often produced in high concentration during chronic infections, is also an important factor for the local survival and function of natural T_{reg} cells[61]. Several pathogens can directly trigger TGF-β production by the cells they infect. In addition, TGF-β is highly expressed in the vicinity of tissues such as the gut, the eye, the skin or the lung. Cells and molecules 'downstream' of the inflammatory response are also associated with anti-inflammatory processes such as TGF-β production. Conventional $CD4^+$ T cells exposed to high concentrations of TGF-β can become suppressive, $Foxp3^+$ cells[62,63]. The involvement of this pathway in the local induction of $Foxp3^+$ T_{reg} cells during infection remains to be addressed. In addition, large amounts of TGF-β could also promote the local survival or retention of natural T_{reg} cells.

Whether pathogens can trigger the production of chemokines favoring natural T_{reg} cells recruitment remains to be determined. Differences in chemokine responsiveness or receptor expression between natural T_{reg} cells and effector T cells have been demonstrated in various models[64–66]. However, most available data have been obtained using natural T_{reg} cells purified from lymphoid organs

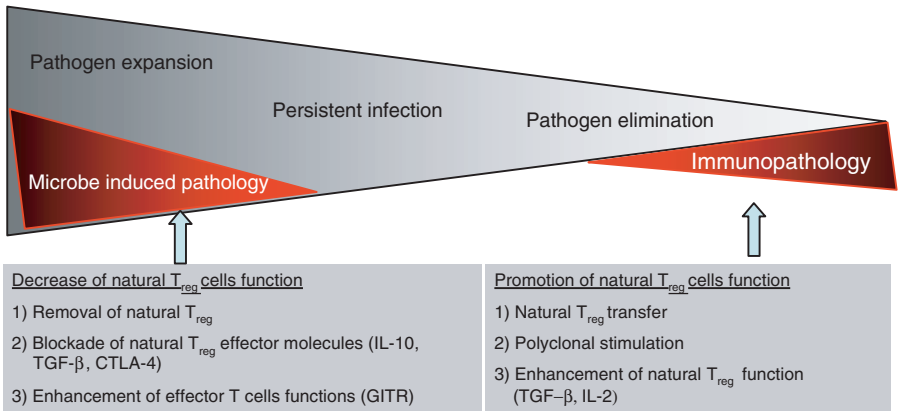

Figure 2 Manipulation of natural T_{reg} cells as a therapeutic approach during infection. The interactions between a host and a pathogen range from uncontrolled pathogen growth to sterile elimination. Blockade or enhancement of natural T_{reg} cell function may represent a therapeutic approach at each 'extreme' of the host-pathogen interaction. Excess control of effector immune responses by natural T_{reg} cells can lead to uncontrolled growth of the pathogen and eventual death of the host. In such cases, prevention of natural T_{reg} cell function may restore the capacity of the host to efficiently control infection. At the other 'extreme' of the host-pathogen interaction, effector immune responses can efficiently eliminate pathogens. This situation can lead to 'unleashed' effector immune responses and immunopathology. In the most extreme scenario, the host can die from uncontrolled immune responses. For controlling immnopathology, enhancement of natural T_{reg} cells function may represent a therapeutic approach.

in mice or peripheral blood in humans. There are almost no data on the signals and molecules involved in the traffic and retention of natural T_{reg} cells at sites of infection where regulation would be required. However, in the eye, the antigen VLA-4 seems to be involved in homing of natural T_{reg} cells[22], and in the skin, the $\alpha_E\beta_7$ integrin is required for the retention of natural T_{reg} cells during leishmania infection (I. Suffia and Y.B., unpublished data).

Counter-regulation or enhancement of natural T_{reg} cell function

The capacity of a host to mount an effective immune response can be limited by the preexistence of counter-regulatory elements. Thus, controlling regulatory mechanisms may represent a powerful strategy for controlling chronic infection or enhancing the efficiency of vaccines (**Fig. 2**). Many mechanisms that boost immune responses and favor the control of pathogens also abrogate natural T_{reg} cell function[67–69] (**Fig. 1**). When activated dendritic cells are used as source of antigen-presenting cells, the *in vitro* suppression mediated by natural T_{reg} cells is abolished, an effect partially mediated by IL-6 (ref. 67). In this setting, the suppressive function of natural T_{reg} cells is abrogated by a proinflammatory environment. Other reports have challenged this hypothesis. In fact, the main target of this control seems to be activation of the effector T cells that become unresponsive to natural T_{reg} cell suppression. Far from being 'switched off' by activation, the proliferative and suppressive functions of natural T_{reg} cell are boosted by encounters with activating signals. For example, in ovalbumin-specific T cell receptor–transgenic mice, mature dendritic cells with high expression of costimulatory molecules can more efficiently induce antigen-specific proliferation of natural T_{reg} cells than can immature dendritic cells[58]. Natural T_{reg} cells that have undergone massive proliferation in these conditions remain potent suppressor cells *in vitro*[58]. Microbial products can also directly enhance natural T_{reg} cell functions[57]. Thus, in general, activation enhances rather than abrogates natural T_{reg} cell function. However, we cannot discount the possibility that the suppressive function of natural T_{reg} cells could be differentially regulated in lymphoid organs versus tissues. Natural T_{reg} cells might be expected to preserve the integrity of the host, reaching sites of inflammation in the periphery but abrogating their function when antigen or inflammation is absent.

Strategies aimed at manipulating natural T_{reg} cell function or numbers have therapeutic potential (**Fig. 2**). In many infections in both mice and humans, removal of natural T_{reg} cells (as assessed by expression of CD25) has resulted in enhanced effector immune responses[21,28,33,37]. Targeting the molecules involved in regulatory cell activity *in vivo*, such as CTLA-4, TGF-β or IL-10, alone or in combination, has often proven effective in controlling many chronic infections[70–72]. GITR shows constitutively high expression by natural T_{reg} cells as well as by activated effector T cells[73]. Suppression mediated by natural T_{reg} cells is blocked by GITR engagement with GITR ligand (or agonist antibody) *in vitro* because of strong activation of effector cells that become unresponsive to T_{reg} cell–mediated suppression[74]. The target of this effect *in vivo* remains to be determined and, based on the pattern of GITR expression, is likely to change during the course of the infection. Nevertheless, targeting GITR *in vivo* has produced some notable results. During Friend virus infection in mice, treatment with agonist antibody to GITR reverses the effect of natural T_{reg} cells, leading to enhanced T_H1 and $CD8^+$ T cell responses, reduction of viral load and pathology and restoration of $CD8^+$ T cell–mediated antitumor responses[75]. Similarly, treatment of mice with agonist antibody to GITR diminishes herpetic keratitis, although it is not apparent if this effect involves the participation of natural T_{reg} cells (S. Suvas and B.T.R., unpublished data).

Induction or activation of natural T_{reg} cells represents a therapeutic objective when tissue damage is excessive (**Fig. 2**). In a model of mouse colitis, the transfer of natural T_{reg} cells was found to be sufficient to control established inflammatory disease[76]. The polyclonal activation of natural T_{reg} cells *in vivo* could also favor the control of other immune-mediated lesions. For example, nonmitogenic antibody to CD3 may activate natural T_{reg} cells selectively[77]. Such an approach has been shown to control autoimmune diabetes in nonobese diabetic mouse models[77]. Increasing natural T_{reg} cell function or numbers could potentially be achieved by providing cytokines that favor natural T_{reg} cell activity or survival, such as IL-2 or TGF-β. After incubation with TGF-β and IL-2, natural T_{reg} cells become more powerful than untreated cells in protecting the host from acute graft-versus-host disease[78]. In addition, TGF-β may convert conventional T cells into $Foxp3^+$ natural T_{reg} cells that act to modulate lesions in an experimental asthma model[62]. When conventional human $CD4^+$ T cells were transfected with Foxp3, those cells also acquired the phenotype and function of natural T_{reg} cells[59]. A similar approach in mice may prevent colitis[79] and this could represent a powerful way to generate many antigen-specific natural T_{reg} cells that could target sites of infection.

In conclusion, natural T_{reg} cells participate in the immune response to many and perhaps all infectious agents. Usually they serve to restrain exuberant immune reactivity, which in many chronic infections benefits the host by limiting tissue damage. However, the natural T_{reg} cell response may handicap the efficacy of protective immunity, including that induced by vaccines. The challenge for immunologists is to harness understanding of the 'ins and outs' of natural T_{reg} cells and their 'cousins', learning how to tailor their function to achieve the proper balance between protection and pathology.

ACKNOWLEDGMENTS

We thank C.L. Karp and S. Suvas for critical reading of the manuscript.

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Published online at http://www.nature.com/natureimmunology/

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