Regulatory T cells and infection: a dangerous necessity

Yasmine Belkaid

Abstract | Surviving a given infection requires the generation of a controlled immune response. Failure to establish or restore homeostatic conditions during or following the onset of an infection can lead to tissue damage. Investigation of the immunoregulatory network that arises in response to the infectious process or that is induced by the pathogen itself should provide insight into therapeutic approaches for the control of infection and any subsequent immunopathology. In this Review, I discuss current hypotheses and points of polemic associated with the origin, mode of action and antigen specificity of the various populations of regulatory T cells that arise during infection.

Surviving an infection requires the generation of a controlled immune response in the host that recognizes and eliminates the invading pathogen while limiting the collateral damage to self tissues that can result from an exuberant immune response. At the same time, most microorganisms have to avoid elimination by the host immune response to guarantee their successful transmission. So during an infection, immune regulation can arise as a result of the host response to the infectious process in a bid to maintain or restore a homeostatic environment and/or it can be actively induced by the pathogen to promote pathogen survival.

Many pathogens have evolved mechanisms to manipulate the regulatory network of the host to their advantage, thereby generating conditions that ensure their survival for an extended period of time. These strategies include evasion of humoral and cellular immunity by antigenic variation, interference with antigen processing or presentation, and subversion of phagocytosis or killing by cells of the innate immune system (reviewed in REF. 1). Another common strategy used by microorganisms to extend their survival involves the induction of regulatory responses that are normally associated with the termination of effector immune responses of the host. This can be achieved directly through the induction of host immune regulatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor-β (TGFβ), which are produced by innate immune cells in response to pathogen-derived molecules, or indirectly through the generation of regulatory cells. Although it has long been recognized that T cells with suppressive or anergic activity, or IL-10-producing T cells are generated *in vivo* during infection[2], it has only recently emerged that specialized subsets of regulatory T cells also contribute to this regulatory network.

Several types of regulatory T cell have been described on the basis of their origin, generation and mechanism of action, with two main subsets identified: naturally occurring $CD4^+CD25^+$ regulatory T cells (referred to here as natural T_{Reg} cells), which mainly develop in the thymus and regulate self-reactive T cells in the periphery, and inducible regulatory T cells, which develop in the periphery from conventional $CD4^+$ T cells after exposure to signals such as regulatory cytokines, immunosuppressive drugs or antigen-presenting cells (APCs) conditioned by microbial products[3] (FIG. 1). Both types of regulatory T cell, by virtue of their capacity to control the intensity of effector responses, have been shown to have a major role in infection. However, the recent discovery that expression of forkhead box P3 (FOXP3), a transcription factor known to be crucial for the development and function of natural T_{Reg} cells, can be induced *de novo* by conventional $CD4^+$ T cells renders the distinction between natural T_{Reg} cells and inducible regulatory T cells less obvious. In addition, we still need to understand how interchangeable or reversible some of these populations are. For the purpose of this Review, I define natural T_{Reg} cells as the population of regulatory T cells that is present in the host before pathogen exposure, and inducible regulatory T cells as those cells that acquire regulatory function in the context of a given infection. Inducible regulatory T-cell populations include T regulatory 1 (T_R1) cells, which secrete IL-10, T helper 3 (T_H3) cells, which secrete TGFβ, and converted $FOXP3^+$ regulatory T cells.

Mucosal Immunology Unit, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 4 Center Drive, Room 4/126, Bethesda, Maryland 20892, USA.
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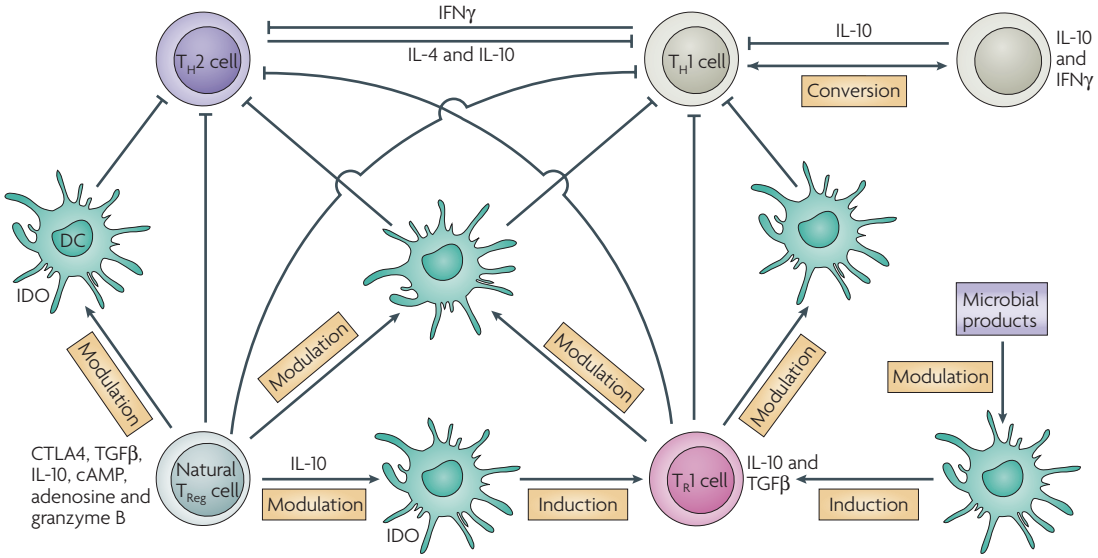

Figure 1 | **Regulatory T cells during infection.** Various populations of regulatory T cells have been shown to have a role during infection. T helper 1 (T_H1)-cell and T_H2-cell populations can regulate each other via their production of cytokines. Naturally occurring forkhead box P3 (FOXP3)$^+$CD4$^+$CD25$^+$ regulatory T cells (natural T_{Reg} cells) can limit T_H1- and T_H2-cell responses either indirectly by modulating antigen-presenting cell (APC) function or directly by cell–cell contact. Indirect regulatory mechanisms involve the release of transforming growth factor-β (TGFβ), interleukin-10 (IL-10) or adenosine, or the induction of the tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) by APCs recognizing cytotoxic T-lymphocyte antigen 4 (CTLA4) on natural T_{Reg} cells. Direct regulation of effector T cells includes the release of cyclic AMP (cAMP) by T_{Reg} cells. IL-10-producing T regulatory 1 (T_R1) cells can be induced by dendritic cells (DCs) under certain conditions (such as following manipulation of DCs by microbial products). Natural T_{Reg} cells can also favour the development of T_R1 cells via their modulation of APC functions. T_R1 cells can limit immune responses during infections through their ability to release IL-10 and/or TGFβ. During sustained T_H1-cell responses, T_H1 cells can secrete IL-10 that in a regulatory-feedback loop will limit T_H1-cell responses. These cells can revert to a T_H1-cell phenotype. IFNγ, interferon-γ.

In this Review, I discuss how regulatory T cells can control infection as part of the microbial life cycle or as a by-product of the diseases they produce. I discuss recent findings involving regulatory T cells in the control of primary and secondary responses against pathogens, and how this control can be beneficial or detrimental to the host.

Natural T_{Reg} cells

Role of natural T_{Reg} cells during infection in mouse models. Natural T_{Reg} cells were initially described as a unique population of CD4$^+$ T cells that prevent the expansion of self-reactive lymphocytes and subsequent autoimmune disease (reviewed in REFS 4,5). Natural T_{Reg} cells are classically defined by their constitutive expression of CD25 (also known as the IL-2 receptor α-chain). These cells also express cytotoxic T-lymphocyte antigen 4 (CTLA4) and the tumour-necrosis factor (TNF)-receptor family members GITR (glucocorticoid-induced TNF-receptor-related protein), OX40 (REF. 5), CD39 and CD73 (REF. 6) and high levels of folate receptor 4 (FR4)[7]. However, none of these markers are specific for natural T_{Reg} cells, as they can also be expressed by activated T cells. Expression of the transcription factor FOXP3 is the most definitive signature of natural T_{Reg} cells in mice[5], but its expression can also be transiently upregulated by activated human T cells.

Despite extensive studies in various models, the mechanism by which natural T_{Reg} cells limit effector responses *in vivo* remains poorly understood. Recently performed *in vivo* imaging indicates that natural T_{Reg} cells form long-lasting interactions with dendritic cells (DCs) soon after they enter the lymph nodes, and this impairs the ability of DCs to subsequently activate effector T cells, indicating that, *in vivo*, natural T_{Reg} cells may inhibit T-cell responses indirectly by modulating the function of APCs[8]. In addition, the production of anti-inflammatory cytokines, such as TGFβ and IL-10, has been shown to also contribute to natural T_{Reg}-cell suppressive activity *in vivo*[9]. CTLA4-expressing natural T_{Reg} cells induce the expression by APCs of the enzyme indoleamine 2,3-dioxygenase (IDO), which degrades tryptophan, and lack of this essential amino acid has been shown to inhibit T-cell activation and promote T-cell apoptosis[10]. More recently, adenosine and cyclic AMP have also been shown to contribute to T_{Reg}-cell suppressive activity[6,11]. However, in most cases, the mechanisms of suppression by T_{Reg} cells are still largely unclear. During infection, such mechanisms are likely to be redundant and vary according to the site of infection or the degree of inflammation (FIG. 2).

Some of the earliest studies of natural T_{Reg} cells emphasized that such cells control the extent of immune-mediated pathology. Activated natural T_{Reg} cells efficiently control self-reactive T cells and innate

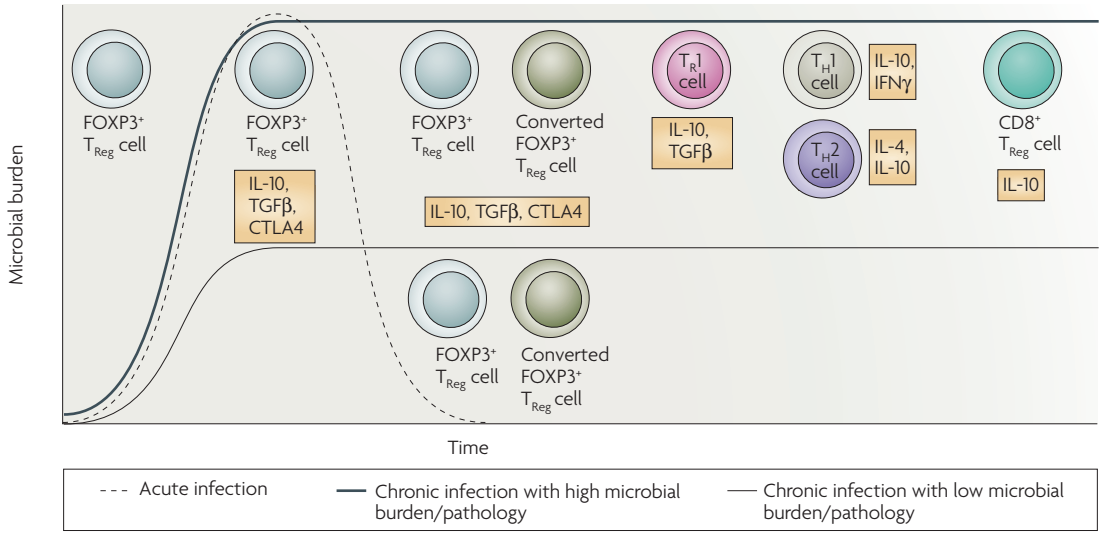

Figure 2 | **The nature of regulatory T cells involved and the mechanism of suppression depend on the strength and stage of the pathological process.** During an acute infection, polyclonal natural regulatory T (T_{Reg}) cells may contribute to the control of the inflammatory process. During chronic infections with sustained T helper 1 (T_H1)- or T_H2-cell responses, several regulatory processes may contribute to immune regulation. Natural T_{Reg} cells could produce cytokines, such as interleukin-10 (IL-10) or transforming growth factor-β (TGFβ), to prevent tissue damage; those that accumulate at the site of infection may be enriched in pathogen-specific T_{Reg} cells. T regulatory 1 (T_R1) cells can be induced because of the effect (deactivation and induction of cytokine production) of pathogens on antigen-presenting cells (APCs) or chronic exposure to microbial antigen. Forkhead box P3 $(FOXP3)^-$ T cells could be converted into $FOXP3^+$ regulatory T cells at sites enriched in TGFβ, such as the skin or the gut. Other populations of T_{Reg} cells can also contribute to the control of immunopathology, such as regulatory $CD8^+$ T cells. During infections that induce a sustained T_H1-cell response, T_H1 cells themselves may also contribute to the limitation of immune responses and tissue damage through the release of IL-10. CTLA4, cytotoxic T lymphocyte antigen 4; IFNγ, interferon-γ.

responses in mouse models of colitis, thereby minimizing collateral tissue damage[12]. A similar scenario probably occurs during chronic infection, whereby natural T_{Reg} cells would be required to monitor the constant immune response by the host and to prevent detrimental tissue damage. Natural T_{Reg}-cell-mediated control of immunopathology may be particularly important for protecting immune-privileged environments or tissues with highly specialized functions, such as the liver or eyes. In a model in which mice were infected in the eye with herpes simplex virus (HSV), natural T_{Reg} cells were shown to protect against the development of virus-induced inflammatory lesions[13]. Chronic infection with *Schistosoma mansoni* in mice also illustrates the protective role of natural T_{Reg} cells against immunopathology, as their removal increases damage to the liver[14].

Even when natural T_{Reg} cells successfully preserve homeostasis in the host by controlling excessive immune responses, one consequence of such control is enhanced pathogen survival and, in some cases, long-term pathogen persistence. For example, in a resistant mouse model of *Leishmania major* infection, mice remain chronically infected at the site of primary infection. Natural T_{Reg} cells that accumulate at this site regulate the function of local effector cells, through IL-10-dependent and IL-10-independent mechanisms, and this prevents efficient elimination of the parasite[15]. In this model of infection, parasite persistence, as a result of immune suppression by natural T_{Reg} cells, is necessary for the maintenance of protective immunity against the parasite[13,15]. So, in some cases, natural T_{Reg} cells can control the fine balance that is sometimes established between the pathogen and its host, and thereby mediate an equilibrium that can become mutually beneficial. In other cases, regulatory control is too excessive, allowing the pathogen to replicate without restraint and overwhelm the host, thereby compromising the survival of the host. For example, the depletion of natural T_{Reg} cells can protect mice infected with *Plasmodium yoelii* — a parasite strain responsible for lethal rodent malaria — from death by restoring a vigorous effector immune response that eradicates the parasites[16]. Filarial disease caused by infection with a filarial nematode is associated with a profound suppression of the host immune system. In a model of this disease, the infection and subsequent immunosuppression are associated with an accumulation of natural T_{Reg} cells in the thoracic cavity, and removal of these cells results in clearance of the parasite and protects the animals from the disease[17]. Similarly, in humans infected with *Plasmodium falciparum*, a causative agent of human malaria, the removal of natural T_{Reg} cells enhances *in vitro* proliferation of peripheral-blood mononuclear cells and their production of interferon-γ (IFNγ) in response to malaria antigens[18].

Role of natural T_{Reg} cells during infections in humans. In humans, reliable identification of natural T_{Reg} cells is complicated by the fact that FOXP3 expression does not always correlate with regulatory properties and it can be transiently expressed by activated T cells. Likewise,

Colitis
An inflammatory disease of the colon. In humans, colitis is most commonly classified as ulcerative colitis or Crohn's disease, two inflammatory bowel diseases that have unknown aetiology. Various hereditary and induced mouse models of human colitis have been developed.

Filarial diseases
Diseases such as human river blindness and elephantiasis that are caused by filarial nematodes.

CD25 or other natural T_{Reg}-cell markers cannot be used to reliably discriminate between natural T_{Reg} cells and highly activated T cells. Furthermore, in the lymphoid tissues of subjects infected with HIV, for example, most $CD4^+FOXP3^+$ T cells are $CD25^{-/low}$ (REF. 19). Most studies that evaluate human natural T_{Reg}-cell function or numbers are done using peripheral blood as this is the most accessible compartment. However, these measurements may not be representative of all tissues, as in some chronic infections in humans, natural T_{Reg} cells accumulate in infected tissues and are rarely detectable in the blood. Despite these caveats, some reports provide convincing evidence for a role of natural T_{Reg} cells in many human viral infections (reviewed in REF. 20) (TABLE 1).

Decreased numbers of natural T_{Reg} cells have been reported in patients who are chronically infected with HIV[21]. This observation suggests that T_{Reg} cells, as conventional T cells, are progressively lost during HIV infection. Furthermore, cells from infected individuals that show strong HIV-specific T_{Reg}-cell function *in vitro* had significantly lower levels of plasma viraemia and higher $CD4^+$ to $CD8^+$ T-cell ratios than individuals with undetectable T_{Reg}-cell activity[21]. However, the observation that the expression of natural T_{Reg}-cell markers, such as FOXP3, CD25 and CTLA4, is increased in lymphoid tissues from patients infected with HIV and macaques infected with SIV (simian immunodeficiency virus) suggests that the accumulation of T_{Reg} cells in infected tissues could account for the decreased frequency of natural T_{Reg} cells in the blood[22,23]. Interestingly, the frequency of $FOXP3^+$ T_{Reg} cells was much higher in the duodenal mucosa of patients infected with HIV compared with healthy controls[24]. Such accumulation, however, may be associated with the high level of infection in these tissues. In several reports, the removal of $CD4^+CD25^+$ T cells from cultures of peripheral or lymphoid leukocytes from patients infected with HIV or macaques infected with SIV results in an increase in virus-specific immune responses *in vitro*[21,25]. These findings suggest that natural T_{Reg} cells, by suppressing virus-specific immunity, may contribute to uncontrolled viral replication and therefore have a detrimental role in HIV infection.

Infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) is the most common cause of liver disease worldwide, failure to control infection with either virus results in an immune-mediated acute and chronic necroinflammatory liver disease. In patients with chronic HBV infection, the number of $FOXP3^+$ T_{Reg} cells is highly enriched both in the periphery and in the liver[26]. Furthermore, antigen-specific suppression of effector responses *in vitro* suggests that the expansion of antigen-specific T_{Reg} cells during this type of infection may contribute to the associated liver pathology[26]. HCV-associated liver disease also seems to involve natural T_{Reg} cells, which could impede immune defence against the virus. Individuals who are chronically infected with HCV have a higher number of T_{Reg} cells in the blood compared with uninfected individuals, and depletion of these cells enhances antigen-specific $CD8^+$ T-cell responses *in vitro*[27]. Interestingly, T_{Reg}-cell suppression is TGFβ and cell-contact dependent[28]. The inverse correlation between the HCV-specific TGFβ response by $CD4^+CD25^+$ T cells and liver damage strongly supports the idea that natural T_{Reg} cells also have a role in controlling chronic inflammatory responses and liver damage in HCV carriers[29]. Interestingly, patients who are chronically infected with HCV and who subsequently develop autoimmunity have fewer peripheral natural T_{Reg} cells[30]. However, the link between chronic infection, autoimmune disorders and dysregulation of T_{Reg}-cell function requires further analysis.

Antigen specificity of natural T_{Reg} cells. Whereas the antigen specificity of inducible regulatory T cells (T_R1 and T_H3 cells) is associated with microbial antigens, the nature of the antigens recognized by natural T_{Reg} cells is less obvious. Natural T_{Reg} cells are believed to recognize a wide array of self antigens as a consequence of their development and selection in the thymus[31]. During the onset of acute disease, natural T_{Reg} cells could recognize self antigens that are released by tissue damage; however, evidence from chronic infection suggests that natural T_{Reg} cells recognize microbial antigens[14,15,21,28,32–35]. In a mouse model of leishmaniasis, natural T_{Reg} cells that accumulate at the site of infection can recognize parasite-derived antigens[36]. In addition, far from being anergic, as *in vitro* experiments had suggested, natural T_{Reg} cells proliferate vigorously when they encounter their cognate microbial antigens[36]. Notably, these cells are restricted to the site of infection and depend on antigen for their maintenance[36]. Such compartmentalization provides a potential explanation to the concept of concomitant immunity, in which the host is immune to re-infection at a secondary site while maintaining a local chronic infection[37].

Inducible populations of regulatory T cells

IL-10-producing T cells. The role of IL-10 as an immunoregulatory cytokine in infection has been mainly documented in the context of chronic infections[38]. IL-10 has been shown to inhibit the immune responses (by both T_H1 cells and T_H2 cells) to many pathogens in experimental models[39–41] and in human infectious diseases, such as tuberculosis, malaria, hepatitis C, filariasis, leishmaniasis and schistosomiasis[35,42–46]. The most remarkable example of this control is illustrated by its crucial role during acute infection of mice with *Toxoplasma gondii*. In this model, IL-10 produced by T cells is the key regulator of effector-cell responses, as IL-10-deficient mice can control parasite number but they succumb to lethal immunopathology driven by unrestrained effector-cell responses[47]. During T_H2-cell-dominated infection with helminths most IL-10 is produced by T_H2 cells[38]. Besides T cells, IL-10 can be produced by numerous cell types, including macrophages, DCs, B cells and natural killer (NK) cells (reviewed in REF. 38). In mice, it has been recently shown that macrophages activated in the presence of immunoglobulin-containing immune complexes secrete high levels of IL-10, and this enhances mouse susceptibility to infection with *L. major*[49]. This observation is highly relevant to human visceral leishmaniasis, which is characterized by a polyclonal expansion of immunoglobulin-secreting B cells.

Table 1 | **Microbial infections known to involve natural regulatory T-cell induction**

Microorganism	Host	Effect of T_{Reg} cells on immunopathology and pathogen load	Refs
Parasitic infections			
Schistosoma mansoni	Mouse	Control of liver pathology through IL-10; favours host survival	120,121
Schistosoma japonicum	Mouse	Suppression of antigen-specific T-cell proliferation *in vitro*	122
Leishmania major	Mouse	In resistant strains: control of T_H1-cell responses by IL-10-dependent and -independent mechanisms; favours parasite persistence	120
		In susceptible strains: control of T_H2-cell responses; T_{Reg}-cell depletion transiently exacerbates disease	
Leishmania amazonensis	Mouse	T_{Reg}-cell depletion leads to enhanced parasite numbers and enhanced pathology	120
Leishmania braziliensis	Human	Accumulation of T_{Reg} cells at cutaneous sites of infection	123
Plasmodium yoelii	Mouse	Control of effector immune responses; uncontrolled parasite expansion leads to host death	120
Plasmodium berghei	Mouse	Parasite expansion by limiting effector responses	98
Plasmodium falciparum	Human	Early burst of TGFβ production with T_{Reg}-cell expansion in the blood; correlation between high parasite burden and increase in T_{Reg} cells; T_{Reg}-cell depletion enhances immune responses *in vitro*	120
Brugia pahangi	Mouse	T_H2-cell responses reduced	120
Litomosoides sigmodontis	Mouse	Control of effector responses through an IL-10-independent mechanism; promotes parasite persistence	120
Intestinal nematodes	Mouse	T_{Reg} cells activated or induced by infection provide protection in an asthma model	120,124
Viral infections			
Friend virus	Mouse	Viral persistence by limiting $CD8^+$ effector T cells; *in vitro* suppression through cell contact	98,125,126
Murine AIDS	Mouse	Effector responses limited; favours viral replication	98
HSV	Mouse	$CD8^+$ T-cell proliferation and effector functions limited; favours viral replication; control of eye immunopathology	98
HIV	Human	Increase in T_{Reg} cells in lymphoid organs and mucosal tissues; T_{Reg}-cell depletion from the blood increases virus-specific immune responses *in vitro*	24,91,98
HCV	Human*	Suppression of IFNγ production, expansion and AICD of HCV-specific T cells after recovery and during persistent infection; negative correlation between percentage of T_{Reg} cells and inflammation; HCV peptide can stimulate T_{Reg} cells from HCV patients *in vitro*	29, 98, 127, 128
HBV	Human	T_{Reg} cells accumulate in the liver during chronic severe HBV infection; correlation between frequency of T_{Reg} cells and viral load; T_{Reg}-cell depletion increases antigen-specific IFNγ production; T_{Reg} cells suppress the proliferation of autologous PBMCs mediated by HBV antigens	26,129, 130
HTLV-1	Human	Dysfunction of T_{Reg} cells; inverse correlation between FOXP3 expression and viral load	131–133
CMV	Human	Antiviral T-cell responses controlled	134
Vaccinia virus	Mouse	$CD8^+$ T-cell responses controlled; responses to immunodominant epitopes suppressed	109
Influenza virus	Mouse	$CD8^+$ T-cell responses controlled; responses to immunodominant epitopes suppressed	109
SIV	Macaque	Viral replication and immune activation in lymphatic tissue correlates with increased T_{Reg}-cell numbers; frequency of T_{Reg} cells inversely correlates with magnitude of CTL responses	23,25, 135
FIV	Cat	In chronic infection, increased frequency of activated T_{Reg} cells in the blood and lymph nodes	98
Fungal infections			
Candida albicans	Mouse	Antifungal T_H1-cell responses limited; immunopathology controlled	98
Paracoccidioides brasiliensis	Human	Increased frequency of $CTLA4^+GITR^+TGFβ^+FOXP3^+$ T_{Reg} cells in the blood and fungi-induced granuloma; increased suppressive activity *in vitro*	136
Aspergillus fumigatus	Mouse	Recruitment of T_{Reg} cells to the site of infection; neutrophil control (IL-10, CTLA4 and IDO)	71
Bacterial infections			
Helicobacter hepaticus	Mouse	Innate immune responses controlled	98
Helicobacter pylori	Mouse	Antibody and T-cell responses limited	98,137
Listeria monocytogenes	Mouse	Primary and secondary $CD8^+$ T-cell responses limited	98
Pneumocystis carinii	Mouse	Pro-inflammatory cytokines and lung pathology limited	98,138
Mycobacterium tuberculosis	Mouse	Effector responses limited	139,140
	Human	T_{Reg}-cell numbers increase in the blood and sites of infection in patients with active TB; frequency of $FOXP3^+$ cells inversely correlates with local mycobacteria-specific immunity; mycobacteria-specific IFNγ and IL-10 production suppressed	
Chlamydia trachomatis	Human	$FOXP3^+$ cells accumulate in infected conjunctiva; potential role in control of immunopathology	141

*Or chimpanzee. AICD, activation-induced cell death; CMV, cytomegalovirus; CTL, cytotoxic T lymphocyte; CTLA4, CTL antigen 4; FIV, feline immunodeficiency virus; FOXP3, forkhead box P3; GITR, glucocorticoid-induced tumour-necrosis-factor-receptor-related protein; HBV, hepatitis B virus; HCV, hepatitis C virus; HSV, herpes simplex virus; HTLV-1, human T-cell lymphotrophic virus 1; IDO, indoleamine 2,3-dioxygenase; IFNγ, interferon-γ; IL-10, interleukin-10; PBMCs, peripheral-blood mononuclear cells; SIV, simian immunodeficiency virus; TB, tuberculosis; TGFβ, transforming growth factor-β; T_H, T helper; T_{Reg}, T regulatory.

IL-10 can also be produced by natural T_{Reg} cells and, in some cases, is associated with their function; however, in most cases, the inducible T_R1-cell population is the relevant source of this cytokine during infection. During various infections, T_R1 cells develop from conventional T cells after encounter with certain signals, such as exposure to deactivated or immature APCs, repeated exposure to antigen or IL-10 itself (reviewed in REFS 3,50). Of note, these conditions prevail during chronic infections in which APC functions are often targeted by the pathogen, and cells of the immune system are chronically exposed to microbial antigens. Consistent with a role for these cells in human disease, T_R1-cell clones can be isolated from patients who are chronically infected with HCV[35]. Interestingly, these regulatory clones had similar viral antigen specificity to protective T_H1-cell clones isolated from the same patients[35].

Pathogens themselves can induce the production of IL-10 by the cells they infect or are in contact with. For example, filamentous haemagglutinin from *Bordetella pertussis* was shown to induce IL-10 production by DCs, which favoured the differentiation of naive T cells into T_R1 cells[51]. Similarly, T_R1 cells can be generated from naive T cells by co-culturing with DCs stimulated with phosphatidylserine from *S. mansoni*[52]. The importance of this mechanism is further illustrated by the fact that pathogens such as human cytomegalovirus and Epstein–Barr virus encode homologues of IL-10 (REF. 53), which may favour viral persistence, and potentially contribute to immune suppression in Hodgkin's lymphoma[54], through the induction of T_R1 cells.

Although T_R1 cells define a population of T cells that can produce IL-10 and/or TGFβ, some IL-10-producing T cells can also produce IFNγ. The autocrine regulation of T_H1 and T_H2 cells by IL-10 was initially described in human clones[55]. In the context of an infectious disease, cells that produce both IFNγ and IL-10 were first described in the bronchoalveolar lavage of patients with tuberculosis[56] and in individuals chronically infected with *Borrelia burgdorferi*[57]. Indeed, in many chronic infections, in humans and experimental animals, the presence of $CD4^+$ T cells that produce high levels of both IL-10 and IFNγ has been documented (reviewed in REF. 58).

Recently, it was shown that IFNγ- and IL-10-producing $CD4^+$ T cells emerge during experimental infection with *T. gondii* and in a model of non-healing leishmaniasis, and that these cells share many features with T_H1 cells and are the main source of protective IL-10 (REFS 59,60). These T cells were identified as activated T-bet$^+$ T_H1 cells that were distinct from T_H2 cells, natural T_{Reg} cells or other subsets of inducible regulatory T cells. Unlike IFNγ production, IL-10 production was transient, observed in only a fraction of these cells and was produced more rapidly by recently activated T cells than by resting T cells[59]. The instability of IL-10 synthesis, which was observed only when the T_H1 cells were fully activated, is probably necessary to prevent sustained suppression of effector functions. So, it appears that, in some cases, cells with regulatory properties could arise from fully differentiated T_H1 cells to provide a negative-feedback loop. It is likely that numerous previous studies of T_R1 cells in fact also implicated these IFNγ- and IL-10-producing cells or similar populations. The emergence of these IFNγ- and IL-10-producing T cells may represent a dominant regulatory response to infections that induce highly polarized T_H1-cell responses.

Potential role for converted FOXP3$^+$ regulatory T cells during infection. *In vitro* studies have shown that conversion of naive peripheral $CD4^+CD25^-$ T cells into FOXP3$^+$ regulatory T cells could be achieved through ligation of the T-cell receptor (TCR) in the presence of TGFβ (REFS 61–64). Such conversion can be mimicked *in vivo* by delivering antigen under subimmunogenic conditions (such as delivery of antigen using osmotic pumps)[65] or by targeting antigen to DCs via the regulatory receptor CD205 (also known as LY75 or DEC205)[66]. The targeting or the manipulation of DCs by pathogens, as well as chronic exposure to low doses of antigen, is characteristic of many chronic infections. During infection, the downstream effects of inflammatory responses are also often associated with anti-inflammatory processes including TGFβ production. Furthermore, some pathogens target sites in which TGFβ is highly produced, such as the gastrointestinal tract, the skin and the eye[67], as TGFβ may assist in the conversion of FOXP3$^+$ T_{Reg} cells, and this could ensure pathogen persistence in the host. TGFβ can be also produced by infected cells or by cells with which the microorganisms are in contact, or as a result of an inflammatory process.

In a mouse model of *Toxoplasma* infection, intraepithelial lymphocytes secrete TGFβ that prevents the development of lethal ileitis[68]. Compelling data in a mouse model of malaria also suggest that TGFβ and regulatory T cells are central regulators of immunopathology and parasite expansion[18]. Similarly, after experimental malaria infection of human volunteers, enhanced TGFβ production and FOXP3$^+$ T_{Reg}-cell responses were detected in the peripheral blood and this correlated with a faster parasitic growth rate[18]. Cells with natural T_{Reg}-cell characteristics are rapidly induced following blood-stage infection and are associated with a decrease in pro-inflammatory cytokine production and antigen-specific immune responses, as well as the burst of TGFβ production. Monocytes are a probable source of the early TGFβ production in malaria blood-stage infection[18]. One intriguing observation is that only a fraction of infected individuals displayed this early TGFβ burst. Whether there is a genetic predisposition in the capacity of individuals to produce cytokines that are known to promote regulatory T-cell induction and how this could correlate with their susceptibility to infectious diseases remains to be addressed.

Although acute infection with *Listeria monocytogenes* failed to induce *de novo* expression of FOXP3 by conventional $CD4^+$ T cells[69], we speculate that chronic infections may require an additional layer of regulation, which would be provided by converted FOXP3$^+$ regulatory T cells. The gut in particular may represent a unique environment that favours regulatory T-cell conversion. This site requires additional levels of control because it has to maintain the delicate balance between tolerance to commensal bacteria and food products and the capacity

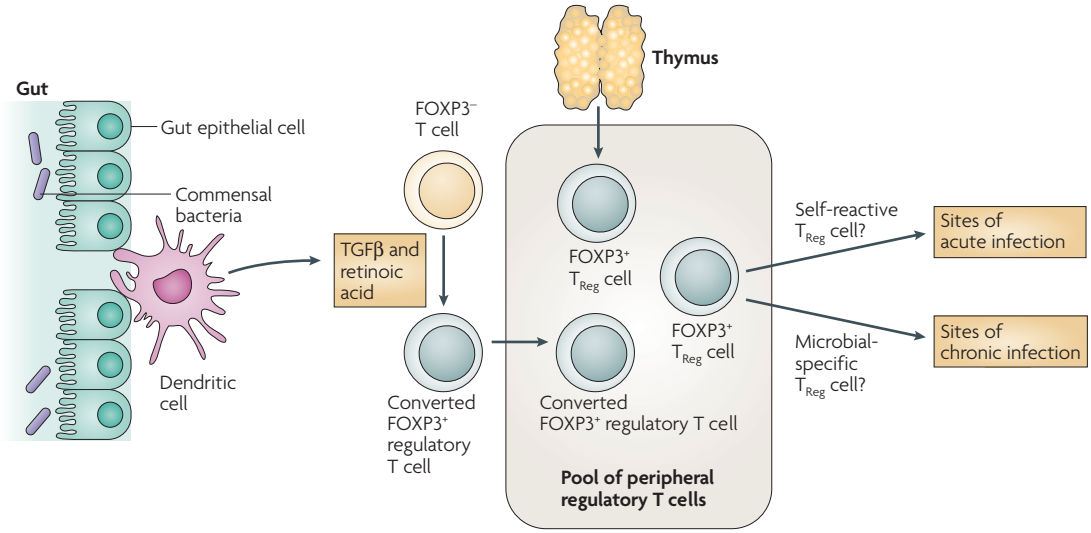

Figure 3 | **Origin and specificity of natural regulatory T cells during infections.** The origin and antigen specificity of natural regulatory T (T_{Reg}) cells may vary according to the site and the nature of the infection. In acute infection, tissue damage may be associated with enhanced presentation of self antigens. In this case, self-reactive natural T_{Reg} cells may be activated and could, in a bystander manner, limit effector responses against the pathogen. In some chronic infections, there is evidence that natural T_{Reg} cells derived from the thymus may accumulate at sites of infection and can recognize microbial antigens. In an environment that is rich in transforming growth factor-β (TGFβ) and the vitamin A metabolite retinoic acid, such as the gut, peripheral conversion of forkhead box P3 (FOXP3)$^-$ T cells into FOXP3$^+$ T_{Reg} cells may occur in response to food or gut-flora antigen or during oral infection. These converted FOXP3$^+$ T cells could potentially limit immune responses and some of them may be able to recirculate and thereby could contribute to the control of peripheral homeostasis.

to mount an effective immune response against ingested pathogens. This hypothesis is supported by the observation that the main site in which peripheral conversion can be observed is the gut-associated lymphoid tissues[70]. Such conversion was associated with the observation that DCs from the lamina propria of the small intestine have the unique ability to generate regulatory T cells *in vitro* through a mechanism that depends on TGFβ and the vitamin A metabolite retinoic acid[70] (FIG. 3). A compelling hypothesis would be that these gut-converted regulatory T cells could become part of the peripheral regulatory T-cell pool. So over time, the gut flora, oral pathogens or food may have an important role in shaping the repertoire of peripheral FOXP3$^+$ regulatory T cells. The relative contribution of these converted regulatory T cells to peripheral tolerance and the outcome of infections remains to be addressed (FIG. 3).

Peripheral regulatory T-cell conversion could be particularly relevant in long-term chronic infections, such as infection with *Mycobacterium tuberculosis* or *T. gondii*, as the process of thymic involution during ageing is expected to limit the output of natural T_{Reg} cells. However, in the absence of definitive markers to distinguish natural T_{Reg} cells from converted FOXP3$^+$ regulatory T cells, these issues will remain difficult to address.

Crosstalk between regulatory T-cell populations

The distinction between natural T_{Reg} cells and inducible regulatory T cells *in vivo* is not always clear, particularly in highly inflammatory settings. Moreover, different regulatory T-cell populations may have the capacity to influence the emergence or function of one another. This notion was recently suggested in a mouse model of *Aspergillus fumigatus* conidia infection[71]. In this model, control of allergic immunopathology induced by the fungus requires the sequential activity of various populations of regulatory T cells. Early in infection, inflammation is controlled by the expansion and local recruitment of natural T_{Reg} cells that are capable of limiting innate immune responses through the combined action of IL-10 and CTLA4 to induce the production of IDO by APCs. This control of innate immune responses, in particular of DCs, leads to the subsequent activation and expansion of T_R1 cells that produce both IL-10 and TGFβ. In turn, T_R1 cells can inhibit T_H2 cells, which are responsible for the allergic response to the fungus[71]. This sequential role for various populations of regulatory T cells may not be an exception but the rule, as most infections proceed through various stages and therefore require various layers of regulation (FIG. 2).

Bystander effect of regulatory T cells

Following activation, regulatory T cells can suppress unrelated immune responses in a non-antigen-specific manner either through cell contact or through the regulatory cytokines they produce — a mechanism known as bystander suppression (reviewed in REF. 9). Recent evidence supports the idea that infection-induced regulatory T cells can have a major role in the outcome of secondary infections, as well as in autoimmune or allergic responses (FIG. 4).

Thymic involution
The age-dependent decrease of thymic epithelial volume, which results in decreased production of T cells.

Bystander suppression
Inhibition of effector T-cell function by regulatory T cells of different antigen specificity.

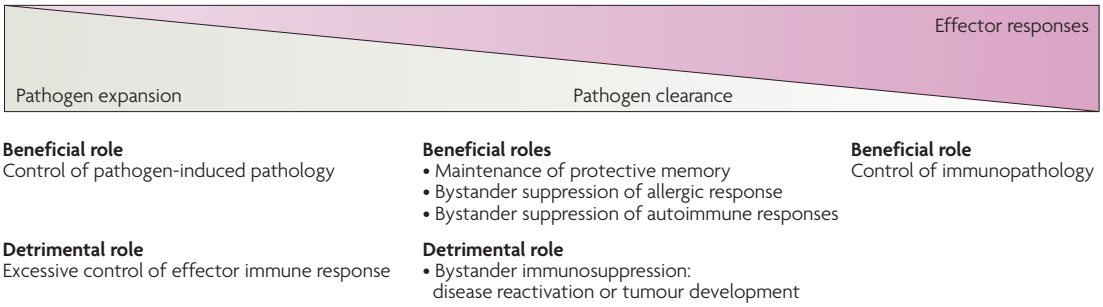

Figure 4 | **Positive and negative roles of regulatory T cells during infection.** The interaction between a host and a pathogen ranges from uncontrolled pathogen growth to sterile elimination. Regulatory T cells have been shown to have a role at both extremes. One major beneficial role of regulatory T cells is the limitation of pathogen-induced pathology and immunopathology. In some cases, this control is excessive and leads to limited immune responses and enhanced pathogen expansion. During chronic infection, regulatory T cells have been shown to mediate a compromise by limiting tissue damage while favouring long-term maintenance of immunity. Regulatory T cells activated by infections can also contribute to the limitation of allergic or autoimmune responses. By contrast, chronic activation of regulatory T cells during persistent infections or during ageing could lead to disease reactivation or tumour development.

Some parasitic infections such as *S. mansoni* infection in humans can generate a highly polarized T_H2-cell response, which in turn can negatively modulate T_H1-cell responses to unrelated antigens, thereby diminishing the strength of immune responses against secondary infections. Protection against *P. falciparum* malaria is associated with the production of IgG1 and IgG3, which is dependent on the provision of help to B cells by T_H1 cells; so, a highly T_H2-cell-polarized environment may account for the increased susceptibility to malaria in individuals co-infected with *S. mansoni* compared with control individuals[72]. Similarly, prior infection with *S. mansoni* or exposure to non-viable *S. mansoni* reduce both the incidence and severity of experimental autoimmune encephalomyelitis (EAE)[73], as well as the development of insulitis in non-obese diabetic (NOD) mice[74] and the induction of colitis by trinitrobenzene sulphonic acid[75], which are all regarded as T_H1-cell-associated diseases. Although some of these observations could be the consequence of cross-regulation between T_H2-cell and T_H1-cell responses, experimental and clinical evidence support the idea that activated regulatory T cells induced by prior infection also contribute to this control[76]. In a cohort of patients with multiple sclerosis, helminth infections were associated with significantly fewer disease exacerbations (relapses) compared with uninfected patients with multiple sclerosis[77]. In these patients, infection also correlated with the emergence of myelin-specific regulatory T cells that produced IL-10 and TGFβ (REF. 77). However, these results have to be confirmed in longitudinal studies to determine whether the occurrence of helminth infection directly correlates with the amelioration of symptoms of multiple sclerosis.

A few years ago, the concept of the 'hygiene hypothesis' emerged, stating that increasing rates of allergy and asthma in Western countries are a consequence of reduced infectious stresses during early childhood[78]. The mechanistic explanations appear to be associated with a 'counter-regulatory' model that involves the induction of various regulatory T-cell populations during infection. Experimental work has lent strong support to this hypothesis[79]. For example, during gastrointestinal infection, the helminth-driven natural T_{Reg}-cell suppression of the function of effector cells is responsible for protection against subsequent airway inflammation[80]. It is likely that part of this mechanism has evolved as a result of our symbiotic relationship with gut flora. Interestingly, probiotic microorganisms have been shown to have beneficial effects in the treatment of inflammatory bowel diseases through their induction of regulatory T-cell populations[81]. Therefore, the presence of symbiotic and pathogenic microorganisms in the gut or other peripheral tissues could lead to the maintenance of a pool of activated regulatory T cells (both natural and inducible) that would maintain host immune homeostasis and enhance the threshold required for immune activation and induction of an immune response[79]. The benefit of such deactivation would be to decrease the instances of aberrant immune responses, such as those contributing to allergic and autoimmune disorders. Pathogenic microorganisms may also have evolved to express antigens that crossreact with gut flora antigens. In infections, the removal or modification of the gut flora is associated with a modification of the phenotype of the host immune responses[82,83]. So some microorganisms may hijack regulatory T cells that are induced or activated in the gut to limit pathogenic responses against gut flora to ensure their own survival.

In the gastric mucosa of patients with gastric adenocarcinoma induced by *Helicobacter pylori*, higher numbers of regulatory T cells could be detected compared with tumour-free subjects[84]. Interestingly, regulatory T cells purified from the gastric tumours could suppress effector-cell responses specific for *H. pylori in vitro*. So, the presence of functional antigen-specific regulatory T cells could contribute to bacterial persistence and potentially to gastric-tumour progression by suppressing both antibacterial and antitumoral immune responses through bystander suppression.

Experimental autoimmune encephalomyelitis
(EAE). An experimental model of the human disease multiple sclerosis. EAE is an autoimmune disease mediated by $CD4^+$ T helper 1 (T_H1) cells and interleukin-17-producing T_H17 cells reactive to components of the myelin sheath that infiltrate the nervous parenchyma, release pro-inflammatory cytokines and chemokines, promote leukocyte infiltration and contribute to demyelination.

Non-obese diabetic mice
(NOD mice). A strain of mice that normally develops idiopathic autoimmune diabetes that very closely resembles type 1 diabetes in humans. The target antigen(s) that is recognized by the pathogenic $CD4^+$ T cells that initiate disease is expressed by pancreatic islet cells, but its identity has remained elusive.

Probiotic
Viable bacteria used therapeutically or prophylactically for colonization of the intestine for the purpose of modifying the intestinal microflora in ways presumed to be beneficial to the host.

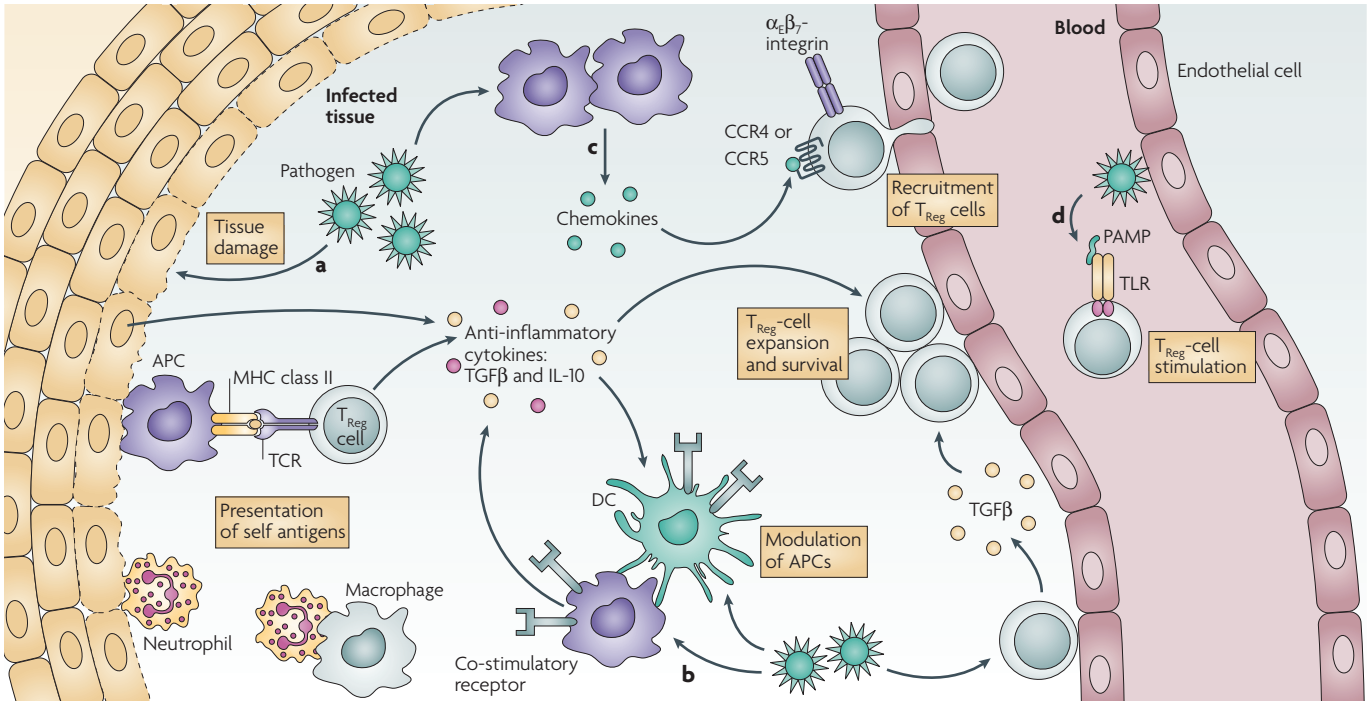

Figure 5 | **Potential strategies used by pathogens to promote regulatory T-cell induction and functions.** Microorganisms can promote the induction of regulatory T cells to secure their own survival in their host. Tissue damage induced by the pathological process could contribute to increase regulatory T-cell activity at sites of infection by favouring self-antigen presentation or by inducing cytokines that promote regulatory T-cell survival or induction (**a**). Some pathogens may have evolved such that their antigens now crossreact with self antigens and thereby can stimulate natural regulatory T (T_{Reg}) cells (not shown). In addition, microorganisms can manipulate antigen-presenting cells (APCs) by interfering with co-stimulatory molecule expression, by modulating antigen presentation or by favouring the induction of regulatory cytokine production (**b**). Other strategies may involve the induction of chemokines that promote regulatory T-cell recruitment (**c**), and the release of PAMPs (pathogen-associated molecular patterns) that directly induce regulatory T-cell activation (**d**). CCR, CC-chemokine receptor; DC, dendritic cell; IL-10, interleukin-10; TCR, T-cell receptor; TGFβ, transforming growth factor-β; TLR, Toll-like receptor.

During chronic infection with *L. major*, natural T_{Reg} cells that accumulate at the site of infection favour the growth of B16 melanoma by limiting local antitumoral responses (G. Pothiawala and Y.B., unpublished observations). So, the continued presence of regulatory T cells at sites of infection can upset the homeostasis of the infected organ and can cause local immunosuppression potentially leading to disease reactivation or tumour development.

We are just beginning to grasp the importance of counter-regulation induced by the infectious process. As discussed later, these concepts have provided the basis for new therapeutic approaches in which microbial molecules could be used to induce regulatory T cells to control allergic and autoimmune diseases.

Impact of pathogens on regulatory T cells

Pathogens favour regulatory T-cell function. Because natural T_{Reg} cells generate favourable conditions for the persistence of microorganisms, it is conceivable that the induction, maintenance and function of natural T_{Reg} cells could also be manipulated by microorganisms (FIG. 5). In addition to the TCR recognition of specific antigens, whether they be host or pathogen derived, natural T_{Reg} cells can also respond to microbial products independent of TCR signals. Natural T_{Reg} cells have been shown to be controlled directly or indirectly by Toll-like receptor (TLR) signalling (reviewed in REF. 85). Consistent with a direct role for TLRs, human natural T_{Reg} cells express TLR5 at levels that are comparable to APCs, and co-stimulation with the TLR5 ligand flagellin increases their suppressive capacity and enhances their expression of FOXP3 (REF. 86). This feature could offer certain pathogens an opportunity to enhance immunosuppression. Although some ligand interactions with TLRs have been proposed to increase natural T_{Reg}-cell suppressive capacity, others have been shown to limit their function[87]. For example, TLR2 signalling temporally abrogates the suppressive phenotype of natural T_{Reg} cells and decreases their FOXP3 expression[85,88]. A decrease in total numbers of natural T_{Reg} cells has been shown in TLR2-deficient mice, which could be explained by the fact that TLR2 agonists induce natural T_{Reg}-cell proliferation[88,89].

An indirect role for TLRs is provided by the observation that mature DCs are more efficient at inducing the proliferation of transgenic natural T_{Reg} cells than immature DCs[90]. Therefore, microbe-associated DC maturation, stimulation of TLRs or other pattern-recognition receptors, induction of cytokine production, and release

B16 melanoma
A widely used experimental mouse melanoma. B16 melanoma is poorly immunogenic and therefore is difficult for the immune system to eliminate. Largely because of this, it makes a good model for testing cancer immunotherapies.

of factors and antigens from pathogen-mediated tissue damage could all favour natural T_{Reg}-cell activation and thereby support survival of the pathogen.

Pathogens can favour regulatory T-cell survival. Recent reports have suggested that HIV may provide a survival and/or proliferative signal to natural T_{Reg} cells[91]. In an *in vitro* model of HIV infection, exposure of T_{Reg} cells to inactivated HIV increased the numbers of T_{Reg} cells in an HIV gp120-dependent manner[91]. The increase in T_{Reg}-cell number was not a result of enhanced resistance to apoptosis, suggesting that these cells may exhibit a survival advantage over effector T cells. This advantage could protect T_{Reg} cells from destruction in lymphoid sites of high viral replication, such as in gut-associated lymphoid tissue. Another means by which HIV may protect itself while favouring regulatory responses would be to avoid the infection of natural T_{Reg} cells. Some data suggest that the expression of FOXP3 by natural T_{Reg} cells may itself interfere with the suppression of HIV-1 promoter transcription and thereby limit viral replication; this could contribute to the general immunosuppression observed in HIV patients[92]. This finding is supported by the observation that purified $CD4^+CD127^-$ T cells, a subset that contains both regulatory T cells and recently activated effector T cells, exhibited relatively lower levels of viral DNA *in vivo* than did $CD4^+CD127^+$ T cells[93].

Pathogens favour regulatory T-cell recruitment and retention. One mechanism by which microorganisms might manipulate regulatory T-cell function is by creating an environment that favours the retention of regulatory cells. Integrin $\alpha_E\beta_7$ (also known as CD103 or ITGAE), the expression of which is positively modulated by TGFβ, has been shown to favour natural T_{Reg}-cell retention at sites infected by the parasite *L. major*[94]. In the same model of infection, CC-chemokine receptor 5 (CCR5) expression by natural T_{Reg} cells was shown to be required for their migration to the infected sites[95]. Of interest, exposure of T cells to parasite-infected DCs also enhances T-cell expression of $\alpha_E\beta_7$-integrin. Furthermore, infection of APCs by *L. major* favours the production of ligands for CCR5 by the APCs[95], suggesting that the pathogen itself manipulates its environment to favour natural T_{Reg}-cell recruitment and retention. Notably, natural T_{Reg} cells that respond to parasite antigen are restricted to the site of infection[36], whereas antigen-specific IFN-producing effector T cells are found at distal sites. Furthermore, within the infected site, the percentage of natural T_{Reg} cells undergoing apoptosis was twice as high as it was for non-T_{Reg} cells. These results suggest that one mechanism by which strongly proliferating natural T_{Reg} cells that accumulate in infected sites are controlled *in vivo* is through rapid death. Such a mechanism could allow for the compartmentalization of T_{Reg}-cell function and prevent a general immunosuppression that would be associated with the dissemination of activated T_{Reg} cells.

Human T_R1 cells express high levels of CCR8 following activation, and these cells respond preferentially to CC-chemokine ligand 1 (CCL1), which is the ligand for CCR8 (REF. 96). This chemokine seems to be relevant *in vivo* as in a model of helminth infection its expression is strongly associated with T_R1 cells that limit T_H2-cell-mediated granuloma formation[97].

Targeting regulatory T cells

In some circumstances, the regulation exerted by regulatory T cells is excessive and prevents the establishment of protective immune responses, whereas in other circumstances, this control is not sufficient to prevent immunopathology. At both extremes, manipulation of regulatory T cells could offer therapeutic potential.

To control infection. The capacity of a host to mount an effective immune response to infection or vaccination is limited by the pre-existence of counter-regulatory elements. Targeting the molecules involved in regulatory T-cell activity *in vivo*, such as CTLA4, TGFβ or IL-10, alone or in combination, has proved effective in controlling many chronic infections (reviewed in REF. 98). Many mechanisms that boost immune responses and favour the control of pathogens also abrogate T_{Reg}-cell functions[98]; this seems to be achieved mainly by rendering effector T cells unresponsive to T_{Reg}-cell suppression. Targeting the T-cell-expressed receptor GITR *in vivo* has a beneficial outcome in infection models[17,99]. Although the target of such treatment (T_{Reg} cells or effector T cells) has not been identified in these models, its main mechanism might be associated with enhanced effector T-cell responses[100]. Blockade of other molecules that are highly expressed by T_{Reg} cells, such as FR4, to enhance immune responses against pathogens remains to be addressed[7]. Recent findings suggesting a role for adenosine[6] and cyclic AMP[11] in T_{Reg}-cell suppressive function offer new potential means to limit their function.

Similar to naive and effector T cells, the proliferation and the suppressive functions of natural T_{Reg} cells are boosted by encounters with activating signals, such as activated APCs and some microbial products (such as flagellin)[86,90]. Strategies to manipulate natural T_{Reg}-cell function or number clearly have good therapeutic potential. In many infections in both mice and humans, depletion of natural T_{Reg} cells (using CD25-specific antibodies) has resulted in enhanced effector immune responses[21,28,37,101]. However, recently it has been shown that complete ablation of FOXP3 expression in adult mice leads to the development of autoimmunity[102]. So, systemic strategies that target natural T_{Reg} cells may not be applicable in humans as they may run the risk of triggering autoimmune disorders or uncontrolled pathological immune responses. The identification of molecules that favour tissue-specific migration of T_{Reg} cells such as CCR4 (REF. 103) could allow targeted manipulation of their functions and should minimize such risk.

To establish memory. At present, no vaccines are available against many life-threatening diseases such as malaria, tuberculosis and AIDS. The failure of traditional vaccine approaches and the growing understanding that most pathogens thrive in the presence of regulatory responses support the idea that efficient protective

immune responses have to be initiated under conditions that prevent the initiation of regulatory responses.

It is now clear that regulatory T cells can control the intensity of secondary responses to infections. In a model of HCV infection of chimpanzees, natural T_{Reg} cells have been shown to control HCV-specific effector T cells not only during chronic infection but also after recovery[104]. Likewise, these cells can hamper the efficacy of vaccines against infectious agents. In studies using a vaccine against *L. monocytogenes*, natural T_{Reg} cells restricted the magnitude of pathogen-specific $CD8^+$ T-cell responses upon secondary challenge with the bacterium or the vaccine[105]. Similarly, control of the number of natural T_{Reg} cells before DNA vaccination against herpes simplex virus 1 (HSV1) or HBV had an adjuvant effect on the quality and the intensity of the effector responses in both acute and memory stages[106,107].

In a model of vaccination against mouse malaria, the depletion of natural T_{Reg} cells during vaccination resulted in more durable immunity and better control of parasite burden after challenge compared with vaccination alone[108]. Interestingly, such depletion also led to enhanced T-cell responses to subdominant parasite epitopes[108]. Natural T_{Reg}-cell depletion also significantly increases $CD8^+$ T-cell responses following exposure to influenza A virus and vaccinia virus[109]. In this study, natural T_{Reg} cells selectively suppress responses to the most immunodominant $CD8^+$ T-cell epitopes, therefore influencing immunodominance hierarchies[109]. This point may be particularly important for vaccines against parasitic infections, in which responses to only a few, if any, dominant antigens can be detected.

The importance of preventing the induction of regulatory responses during vaccination has been highlighted by recent findings. Conventional antigen-specific T cells converted into regulatory T cells in the periphery under subimmunogenic conditions can be subsequently expanded by the delivery of antigen under immunogenic conditions[66]. So, if not done in optimal conditions, vaccination itself can generate regulatory T cells. In a mouse model of vaccination against *T. gondii*, the production of IL-10 by $CD4^+$ T cells that were reactivated following secondary challenge is controlled by IFNγ. This production of IL-10 upon secondary exposure to the parasite interferes with the efficiency of vaccination and leads to the death of the animal[110]. Previous reports clearly show that vaccination with the *Leishmania* antigen LACK, when used with an adjuvant, protected mice against re-challenge[111]. Surprisingly, vaccination of mice with the LACK antigen in the absence of adjuvant can favour the emergence of IL-10-producing regulatory T cells[112]. The presence of these cells predicts vaccination failure. Removal of $CD25^+$ cells abrogated IL-10 production and restored protection by the vaccine[112]. These results highlight the need to address the potential of each microbial antigen to trigger regulatory T cells following vaccination and also highlight the importance of defining adjuvants that prevent regulatory T-cell priming or activation.

Another approach to promote protective immune responses in the face of counter-regulation would be to select a site of vaccination in which regulatory T cells are not overrepresented. For example, the skin (dermis) contains the highest percentage of natural T_{Reg} cells in the body (Y.B., unpublished observations). Therefore, in infection with *L. major*, the site of primary exposure to the pathogen — dermal versus subcutaneous — conditions the efficiency of control of a secondary infection at a distal site[113].

Although preventing regulatory T-cell induction or function as a vaccination strategy may favour the establishment of protective immunity, we need to take into account the possibility that in some situations secondary responses also contribute to immunopathology. For example, in a model of vaccination against *B. burgdorferi*, destructive osteoarthropathy ensues after bacterial challenge[114], a model that has been proposed to address the mechanism underlying lyme arthritis in humans. In this particular case, the presence of natural T_{Reg} cells prevented the development of arthritis. Therefore, secondary exposure to the antigen in the presence of regulatory elements may prevent exuberant responses in the context of vaccination.

Finally, the efficiency of protective responses either induced by vaccination or in response to infection can be conditioned by the pre-existence of regulatory responses in the host. Murine $CD4^+$ T cells specific for the LACK antigen are present in naive mice and may have arisen owing to crossreactivity between LACK and antigens present in the gut flora[115]. Chronic exposure to low doses of microbial antigen could also favour the emergence of a regulatory population that could limit subsequent immune responses.

To minimize immunopathology. Most pathologies are the consequence of uncontrolled immune responses. The induction or activation of regulatory elements is therefore a key approach to treat or prevent tissue damage. In a mouse model of colitis, the transfer of natural T_{Reg} cells was sufficient to control established inflammatory disease[116]. Increasing natural T_{Reg}-cell function or number could potentially be achieved by providing a cytokine milieu that favours natural T_{Reg}-cell activity or survival, such as IL-2 or TGFβ. Enhancing the number or function of $FOXP3^+$ cells can be also achieved *in vivo* by retroviral transfer of *FOXP3* (REF. 117). In a mouse model of *S. mansoni* infection, such an approach at the onset of granuloma formation enhances FOXP3 expression in the granuloma and strongly suppresses granuloma development[117]. The demonstration that TCR ligation in the presence of TGFβ can lead to the generation of functional $FOXP3^+$ regulatory T cells *in vitro* and *in vivo* offers great therapeutic potential. However, we still need to evaluate the relative stability of these converted cells, as reversion to an effector phenotype against the target antigen could have severe consequences *in vivo*. Furthermore, enhancing regulatory T-cell numbers or functions *in vivo* can potentially lead to the reactivation of dormant infections[37] or the suppression of antitumoral responses.

One promising therapeutic approach has emerged from the observation that microbial products can

favour the induction of T_R1-cell populations *in vivo*. IL-10-producing T_R1 cells can be induced *in vitro* by DCs stimulated with phosphatidylserine isolated from *S. mansoni*[118]. Exposure of mice to *S. mansoni* antigen prevents the development of type 1 diabetes in NOD mice[74], as well as experimental colitis[75]. The use of single microbial molecules as therapeutic agents has been recently shown, as filamentous haemagglutinin of *B. pertussis* can efficiently treat experimental colitis[119].

Concluding remarks

Although regulatory T-cell populations have taken centre stage over the past few years, it is important to remember that virtually all populations of cells can acquire regulatory properties. The challenge of the next few years will be to decipher their relative dependence and contribution to the regulatory responses against infections. We have only just begun to understand their specific role in various infections. Because most microorganisms have co-evolved with their hosts, they have developed mechanisms to manipulate the most central elements of the regulatory network of their host. Therefore, they may represent a powerful tool to decipher the mechanisms that favour regulatory T-cell functions. Understanding the mechanisms by which regulatory T cells are mobilized and activated and the nature of the antigens they recognize will be the next step in the design of rational approaches to achieve the appropriate balance between protection and pathology during infections.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
CD4 | CD25 | CD205 | CTLA4 | FOXP3 | IDO | IFNγ | IL-10 | TGFβ

FURTHER INFORMATION

Yasmine Belkaid's homepage: http://www3.niaid.nih.gov/labs/aboutlabs/lpd/mucosalImmunology

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