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CHAPTER 3

Regulatory T Cells in Infection

Rick M. Maizels and **Katherine A. Smith**

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Abstract

Infectious agents have intimately co-evolved with the host immune system, acquiring a portfolio of highly sophisticated mechanisms to modulate immunity. Among the common strategies developed by viruses, bacteria, protozoa, helminths, and fungi is the manipulation of the regulatory T cell network in order to favor pathogen survival and transmission. Treg activity also benefits the host in many

Institute of Immunology and Infection Research, University of Edinburgh, Edinburgh, United Kingdom

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circumstances by controlling immunopathogenic reactions to infection. Interestingly, some pathogens are able to directly induce the conversion of naive T cells into suppressive Foxp3-expressing Tregs, while others activate pre-existing natural Tregs, in both cases repressing pathogen-specific effector responses. However, Tregs can also act to promote immunity in certain settings, such as in initial stages of infection when effector cells must access the site of infection, and subsequently in ensuring generation of effector memory. Notably, there is little current information on whether infections selectively drive pathogen-specific Tregs, and if so whether these cells are also reactive to self-antigens. Further analysis of specificity, together with a clearer picture of the relative dynamics of Treg subsets over the course of disease, should lead to rational strategies for immune intervention to optimize immunity and eliminate infection.

1. INTRODUCTION

Regulatory T cells are now recognized as an absolute requirement for healthy function of the mammalian immune system to forestall autoimmune pathology by self-reactive lymphocytes and to prevent deleterious reactions against extrinsic commensal and dietary antigens (Kim *et al.*, 2007; Sakaguchi *et al.*, 2008). Tregs also control immune responsiveness to infective pathogens (Belkaid and Tarbell, 2009), and in this context, their influence is not always benign. In this review, we survey our current knowledge of the role of Tregs in a wide range of infection settings and highlight the examples in which these cells are of critical importance in conferring susceptibility, dampening pathogenesis, and maintaining functional immunity.

The significance of Treg involvement in infectious episodes is not limited simply to how the host handles a particular pathogen; there is abundant evidence from infections of all types that adaptation or modulation of immune capacity resulting from infection can profoundly impact on bystander immune responses, in particular allergies, autoimmune diseases, and gastrointestinal disorders (Bach, 2002; Maizels, 2005). While this interaction is popularly termed the ''Hygiene Hypothesis'' (see Section 6 below), it is clearly highly context-dependent, and we also review the emerging evidence that some infectious agents in particular are able to alter the immune status of their host through the regulatory T cell compartment.

2. REGULATORY T CELLS

Regulatory T cells encompass several distinct phenotypes of immune system cells able to block or suppress immune reactivity *in vivo* and *in vitro*. While the most well-characterized Treg subset is a dedicated

lineage selected in the thymus, there is peripheral conversion of potential effector cells into the Treg compartment, and evidence that additional T cell types engage transiently in regulatory activity or combine production of regulatory cytokines with more conventional effector products. The predominant Treg types are $CD4^+$ and express either or both the surface IL-2Rα chain CD25 and the forkhead box transcription factor Foxp3 (Gavin *et al.*, 2007). The $CD4^+CD25^+Foxp3^+$ phenotype also commonly expresses the inhibitory receptor CTLA-4 (Alegre *et al.*, 2001) as well as the GITR receptor which can activate both regulatory and non-regulatory T cells (Shimizu *et al.*, 2002). In addition to Foxp3-expressing Tregs are other functional regulatory cells, which may produce IL-10 (termed Tr1) and IL-35 (Tr35) (Collison *et al.*, 2010). In the case of Tr1, as IL-10 can also be produced by both Th1 ($IFN\gamma^+$ (Jankovic *et al.*, 2007)) and Th2 ($IL\text{-}4^+$) effectors, definition of this subset is relatively fluid. Moreover, T cells producing TGF-β, originally named Th3, can also act in a regulatory capacity. Finally, while Tregs are generally found to be $CD4^+$, $CD8^+$ T cells can express Foxp3 (Nakagawa *et al.*, 2010) and produce the same suppressive cytokines.

$Foxp3^+$ Treg cells exist in two categories, which arise in fundamentally different ways. Thymic, or ''natural,'' regulatory T cells recognize self-antigens in the thymus and are imprinted with regulatory function before being released into the periphery. Thymic Tregs are a $CD4^+CD25^+Foxp3^+$ phenotype, and deficiency of these cells results in fatal autoimmune inflammation; this subset remains committed to this function over time (Rubtsov *et al.*, 2010). In addition, naive peripheral $CD4^+$ T cells can be induced to adopt a regulatory function by initiating expression of Foxp3 (or indeed, TGF-β, IL-10, or IL-35). In particular, induced or ''adaptive'' Tregs can convert from $CD4^+Foxp3^-$ to $CD4^+Foxp3^+$, as discussed further below, thereby expanding the range of Treg specificities to exogenous antigens (Bluestone and Abbas, 2003). However, induced Tregs are not irrevocably programmed and may revert to an effector phenotype, losing expression of CD25 and Foxp3 under certain conditions.

Early experiments with Tregs used CD25 as an accessible surface marker for cell transfer and *in vivo* antibody-mediated depletion with the antibody PC61 (developed by Lowenthal *et al.* (1985)). While landmark studies were achieved with these methods, it was recognized that effector populations can also be compromised by anti-CD25 antibody, and that a substantial minority of $Foxp3^+$ Tregs do not, at any one time, express CD25. In recent years, it has been possible to better target $Foxp3^+$ Tregs, through the construction of transgenic mice expressing the diphtheria toxin receptor (DTR) under the control of the Treg-specific Foxp3 promoter. Constructs described to date include the DEREG (Lahl *et al.*, 2007) and Foxp3LuciDTR (Suffner *et al.*, 2010) mice, both of which have transgenic BACs randomly inserted, and the $Foxp3^{DTR}$ mouse (Kim *et al.*,

2007; Lund *et al*., 2008) containing a construct knocked-in to the Foxp3 locus on the X chromosome. Administration of diphtheria toxin (DTx) selectively depletes Tregs, although transiently and sparing cells which downregulate Foxp3 or (in the case of the BAC transgenics) suppress the randomly integrated locus.

3. INFECTIONS

Infectious agents have developed, over long evolutionary time, effective and often exquisite means of surviving in the host sufficiently long to assure transmission, and very often to establish long-term residence. Among the many strategies infectious organisms employ, exploiting the regulatory T cell compartment is doubtless one of the most effective. However, in the twists and turns of evolving host-pathogen co-adaption, some surprising interactions have developed: thus Tregs do not simply suppress immunity and forestall pathology, but also facilitate appropriate effector mechanisms and maintain long-term memory.

Some thought needs to be given to how infectious diseases of humans are appropriately modeled in laboratory rodents. Acute microbial infections in mice are not reflective of long-term human chronic infections, in which immunoregulation and re-setting of immune homeostasis are the norm. However, mouse models are invaluable for understanding early events, in particular the induction and activation of regulatory networks, and elegant transgenic constructs are available which permit tracking, manipulation, and gene-deletion within individual cell phenotypes.

While the application of these more sophisticated tools to infectious disease systems is still in its infancy, a substantial body of information has accumulated on the expression of Treg subsets, their functions *in vitro*, and the role of either $CD25^+$ or $Foxp3^+$ Tregs in the course of infection *in vivo*. We summarize in the following sections the data from the major infection systems, in humans and experimental models, while presenting more a complete annotation of infections under study in Tables 3.1–3.5 for each of the taxonomic groupings.

3.1. Viruses

Since the early 1900s, virus infections have been associated with immune suppression, variously attributed to functional impairment of lymphocytes, compromised function of antigen presenting cells, and the triggering of a suppressive T cell subset (Rouse and Horohov, 1986). Over recent years, a wide variety of viral infections has been examined for Treg activity (as detailed in Table 3.1), supporting the contention that

TABLE 3.1 Tregs in viral infections

Retroviruses		
FIV	Tregs promote progression and restrain anti-viral responses	Vahlenkamp *et al*. (2004); Mikkelsen *et al*. (2010)
Friend retrovirus	Expansion of Tregs *in vivo* and loss of tumor immunity	Iwashiro *et al*. (2001); Zelinskyy *et al*. (2006)
	Tregs suppress CD8 antiviral immunity	Dittmer *et al*. (2004); Robertson *et al*. (2006); Zelinskyy *et al*. (2009a,b)
	Nonantigen-specific Tregs control pathology in RAG model	Antunes *et al*. (2008)
HIV	Treg numbers correlate with viral load but decline in persistent viremia	Andersson *et al*. (2005); Baker *et al*. (2007); Nilsson *et al*. (2006); Tsunemi *et al*. (2005)
	$CD25^+$ Tregs maintain suppressive capacity in infection	Kinter *et al*. (2007a,b)
	Tregs reduce activation of and inhibit infection of effector T cells	Chase *et al*. (2008); Moreno-Fernandez *et al*. (2011)
	gp120 binding to CD4 may activate Tregs	Becker *et al*. (2009)
	T cells recognising protective HLA allele specificities not suppressed by Tregs	Elahi *et al*. (2011)
LCMV	Blocks diabetes through Tregs	Diana *et al*. (2011)
	Superantigen-mediated expansion of Tregs	Punkosdy *et al*. (2011)
MAIDS	Tregs promote infection, ablation blocks	Beilharz *et al*. (2004)
MMTV	Tregs reduce viral load at outset, increase later. Superantigen-specific Tregs	Cabrera *et al*. (2008)
SIV	Foxp3 in both $CD25^-$ and $CD25^+$ T cells correlates with high viremia	Boasso *et al*. (2007)

(*continued*)

TABLE 3.1 (*continued*)

RNA viruses		
Hepatitis C	Elevated functional Foxp3$^+$ Tregs	Ebinuma *et al*. (2008)
	Disease resolution associated with reduced Treg activity	Smyk-Pearson *et al*. (2008)
Influenza A	CD25$^+$ depletion raises CD8$^+$ response	Haeryfar *et al*. (2005)
MHV coronavirus	Treg depletion can be fatal	Anghelina *et al*. (2009)
	Tregs reduce demyelination	Trandem *et al*. (2010)
	Viral epitope-specific Tregs	Zhao *et al*. (2011)
Rhinovirus in humans	Induce IL-35$^+$Foxp3$^-$ Tregs via DCs	Seyerl *et al*. (2010)
RSV	Tregs dampen response and limit pathology but depletion does not change viral load	Fulton *et al*. (2010); Lee *et al*. (2010)
DNA viruses		
CMV	CD25$^+$ Tregs suppress CMV response	Aandahl *et al*. (2004)
EBV	CD8$^+$ Tregs in active infection	Popescu *et al*. (2007)
Hepatitis B	CD4$^+$FoxP3$^+$ Treg numbers correlate with viral load and serum TGF-β	Barboza *et al*. (2007); Yang *et al*. (2007a)
HSV-1	Tregs expand in infection, restraining responsiveness and pathology	Suvas *et al*. (2003, 2004)
	Ocular pathology controlled by Tregs (and IL-10), including *in vitro*-generated viral-specific Tregs	Sarangi *et al*. (2008); Sehrawat *et al*. (2008)
HSV-2	Treg ablation results in loss of immunity through reduced effectors at site of infection	Lund *et al*. (2008)
Human papillomavirus 16	CD25$^+$ Tregs correlate with persistent infection	Molling *et al*. (2007)
Vaccinia	CD25$^+$ depletion raises CD8$^+$ response	Haeryfar *et al*. (2005)

suppression of antiviral effector cells may allow the establishment and maintenance of chronic viral infection (Li *et al.*, 2008; Mills, 2004).

3.1.1. Retroviral infections and suppression of $CD8^+$ effector function

Retroviral infections in both mice and humans can be influenced by Treg populations (Li *et al.*, 2008; Rouse *et al.*, 2006). In mice, infection with the chronic Friend retrovirus (FV) (Iwashiro *et al.*, 2001) or the LP-BM5 murine leukemia virus mixture (which causes mouse AIDS) stimulated expansion of $CD4^+$ Treg cells co-expressing CD25 or CD38, cell surface markers associated with regulatory cells (Antunes *et al.*, 2008; Beilharz *et al.*, 2004; Robertson *et al.*, 2006; Zelinskyy *et al.*, 2006). Treg expansion was associated with the detection of virus-specific $CD8^+$ T cells that displayed an exhausted phenotype with low levels of effector cytokines and cytotoxic molecules (Dittmer *et al.*, 2004; Zelinskyy *et al.*, 2005).

The protective $CD8^+$ T cell response could be experimentally inhibited by the transfer of virus-specific $CD4^+$ T cells from naive or persistently infected mice to an acutely infected host (Dittmer *et al.*, 2004; He *et al.*, 2004); while both $CD25^+$ and $CD25^-$ populations could mediate this effect, contact-dependent *in vitro* suppression of $CD8^+$ T cell function was associated with the $CD4^+CD25^+$ subset (Robertson *et al.*, 2006). Depletion of $CD25^+$ T cells from persistently infected mice did not consistently improve the ability of $CD8^+$ T cells to control virus loads, but treatment of FV-infected mice with anti-GITR resulted in rescue of $CD8^+$ T cell dysfunction and reversal of retrovirus-induced immunosuppression (Dittmer *et al.*, 2004; He *et al.*, 2004). Similarly, LP-BM5 viral progression was retarded in mice treated with combined anti-CD25, -CTLA-4, and GITR antibodies (Beilharz *et al.*, 2004). These results illustrate the important principle that in ongoing infection, immunity may not be restored simply by removing Tregs, if the resident effector population is anergized or exhausted. Rather, intervention to restimulate effector cells is also required.

The FV system also clearly displays a broader bystander suppression by Tregs, with $CD8^+$ T cell antitumor responsiveness inhibited in mice receiving $CD4^+$ cells from FV-infected mice, with TGF-β and CTLA-4 shown to act *in vitro* (Iwashiro *et al.*, 2001). Normally, FV infection does not elicit immune-related pathology, except in the bone marrow of $RAG1^{-/-}$ mice receiving virus-specific $CD4^+$ T cells. In this setting, pathology is suppressed in an antigen-noncognate manner by both polyclonal TCRβ-transgenic and wild-type $CD4^+CD25^+Foxp3^+$ T cells (Antunes *et al.*, 2008).

When DEREG mice (expressing a BAC-inserted Foxp3-promoter DTR construct) were depleted during the early, acute phase of Friend virus infection, they showed stronger and more multifunctional virus-specific $CD8^+$ T cells, and >10-fold lower viral loads, without evident cost to the host in terms of immunopathology (Zelinskyy *et al.*, 2009a,b).

Interestingly, the transient Treg depletion that is achieved in the DEREG mouse model not only rescued $CD4^+$ effector cells from functional exhaustion, but also had a lasting effect in reducing chronic virus loads (Dietze *et al.*, 2011). Hence, transient depletion of Tregs could be a safe therapy for chronic viral infection.

An important retroviral pathogen is feline immunodeficiency virus (FIV), which expands functionally suppressive $CD25^+$ T cells in chronically-infected cats (Vahlenkamp *et al.*, 2004). In vivo depletion of $CD25^+$ T cells in infected cats resulted in transient increases in both anti-viral and bystander responses (Mikkelsen *et al.*, 2010), although depletion prior to infection did not alter the course of disease (Mikkelsen *et al.*, 2011). In human retrovirus infection, Treg activity is largely inferred from phenotypic analysis of *ex vivo* lymphocytes, with reports showing a positive correlation between viral load and $FOXP3^+$ Treg numbers (Andersson *et al.*, 2005; Nilsson *et al.*, 2006; Tsunemi *et al.*, 2005). However, Tregs may impact on the course of HIV infection not only by impeding protective immunity but conversely by minimizing the pool of activated effector cells which are susceptible to virus infection (Eggena *et al.*, 2005).

In T cells from HIV patients, both HIV-specific and bystander (CMV) *in vitro* responses are enhanced by depletion of $CD4^+CD25^+$ Tregs (Aandahl *et al.*, 2004), and Tregs *in vitro* can suppress cytolytic capacity and cytokine secretion by HIV-specific $CD4^+$ (Weiss *et al.*, 2004) and $CD8^+$ (Kinter *et al.*, 2007a,b) effector T cells *in vitro*. The possibility that Tregs can also act to ameliorate infection was also raised by data showing that Treg inhibition correlated with lower levels of viremia (Kinter *et al.*, 2004), and patients with low Treg numbers had greater peripheral T cell activation, a poor prognostic indicator for disease (Eggena *et al.*, 2005). Later studies showed that molecules of the B7:CD28 family, programmed death-1 (PD-1) and CTLA-4, may maintain virus-specific T cell exhaustion typical of HIV infection (Kaufmann and Walker, 2009), as PD-1 expression correlated with viral load and disease progression in cohorts of HIV^+ untreated patients (Day *et al.*, 2006; Trautmann *et al.*, 2006).

3.1.2. Expansion of Tregs following LCMV infection

Lymphocytic choriomeningitis virus (LCMV) is a natural murine RNA virus transmitted directly from mother to offspring, and different isolates cause infections of varying duration. Chronic, but not acute, infection of mice with LCMV results in a marked expansion of TGF-β-producing $CD4^+CD25^+$ Tregs (Filippi *et al.*, 2009). More precisely, expansion occurs within a TCR Vβ5 $CD4^+Foxp3^+$ population, which derived from pre-existing ''natural'' Tregs, as there was no conversion of GFP^-Foxp3^- T cells transferred to mice immediately prior to infection (Punkosdy *et al.*, 2011). This TCR reacts with the endogenous mouse mammary tumor provirus (MMTV) Mtv9 superantigen, one of several MMTVs

which have segregated during inbreeding of laboratory mouse strains (Cohen and Varmus, 1979), and MMTV superantigen-specific Foxp3$^+$ Tregs have been previously reported (Cabrera *et al*., 2008). The expansion of regulatory T cell mediated by endogenous retroviral superantigens provides a unique mechanism of immune-evasion following chronic LCMV infection.

Interestingly, LCMV is a potent inhibitor of type 1 autoimmune diabetes in mice (Filippi *et al*., 2009), an effect associated with the activity of Tregs (Diana *et al*., 2011; Filippi *et al*., 2011), as discussed below in Section 6.

3.1.3. Tregs and protective immunity to herpes simplex virus

Herpes simplex virus (HSV) is an acute cytolytic virus, immunity to which depends upon a protective CD8$^+$ T cell response. However, HSV-1 infection heightened the suppressive function of CD4$^+$CD25$^+$ Tregs in mice (Suvas *et al*., 2003) and PC61-mediated depletion of CD25$^+$ cells prior to infection amplified the virus-specific CD8$^+$ response, whereas CD25$^+$ Treg transfer had the opposite effect. Moreover, anti-CD25-mediated Treg depletion enhanced memory responses and protective immunity following primary infection with HSV or re-exposure to viral antigen following HSV antigen immunization or primary infection (Toka *et al*., 2004).

Depletion of Tregs using Foxp3^{DTR} knock-in mice in a local HSV-2 infection resulted in increased viral loads in the mucosa and nervous system and fatal infection. Treg depletion, however, attenuated cellular trafficking to the site of infection and reduced inflammatory cytokine levels to a degree that significantly compromised protective immunity (Lund *et al*., 2008). In this instance, and in supporting studies ablating Tregs during LCMV infection of nonlymphoid tissue (Wherry *et al*., 2003), Tregs play an important role in controlling viral load as well as intensifying the cytokine milieu in secondary lymphoid organs.

3.1.4. Tregs benefit both host and pathogen in hepatitis C virus infection

The outcome of human hepatitis C virus (HCV) infection can range from complete control to viral persistence and associated liver disease (Rehermann, 2009); however, the development of therapeutic strategies for treatment have been hampered by difficulties in establishing *in vitro* and *in vivo* models of viral replication, so that currently all data pertain to infection in primate systems.

In HCV-infected patients, frequencies of peripheral CD4$^+$CD25$^+$ and Foxp3$^+$ Tregs are elevated (Cabrera *et al*., 2004; Ebinuma *et al*., 2008; Sugimoto *et al*., 2003) and *in vitro* analysis indicated that CD4$^+$CD25$^+$ cells suppress virus-specific CD4$^+$ and CD8$^+$ responses through IL-10 and TGF-β secretion (Cabrera *et al*., 2004). Although other authors

confirmed the suppressive activity of HCV patient-derived $CD4^+CD25^+$ cells, antibodies to these same mediators did not block the suppression of purified $CD8^+$ effectors, suggesting action through a $CD4^+$ intermediary (Boettler *et al.*, 2005; Rushbrook *et al.*, 2005). Significantly, HCV-associated Tregs were able to suppress influenza virus-specific $CD8^+$ T cell function (Boettler *et al.*, 2005). Comparison with a cohort recovering from acute HCV infection suggested a decline in $CD4^+CD25^+$ regulatory function (Boettler *et al.*, 2005), while longitudinal studies in individual patients more convincingly concluded that spontaneous recovery from HCV infection is associated with the temporal loss of $Foxp3^+$ Treg function (Smyk-Pearson *et al.*, 2008).

Clear evidence has also been provided that HCV-antigen-specific Tregs evolve during infection. $Foxp3^+$ Tregs can be isolated from HCV-positive PBMC stimulated with HCV peptides, with different peptides proving optimal for different patients (Li *et al.*, 2007b), and epigenetic analysis of the Foxp3 locus indicating stable rather than transient commitment to the Treg phenotype (Li *et al.*, 2009). Similarly, HCV peptides were used to expand Tregs from infected patients, some $Foxp3^+$ Tregs reacting with HCV-specific class II-peptide tetramers (Ebinuma *et al.*, 2008).

Although Tregs may impair immunity to HCV, they may also protect the patient from excessive pathology. Thus, liver inflammation is inversely correlated to $CD4^+CD25^+$ T cell numbers in chronic HCV infection (Cabrera *et al.*, 2004). Moreover, $CD25^+$ Tregs from patients with low pathological scores exerted more suppressive effects on HCV-specific $CD4^+$ T cell responses than Tregs from patients with advanced clinical disease (Bolacchi *et al.*, 2006), demonstrating that loss of Treg function can be correlated with organ-specific viral-induced inflammation and pathology.

3.2. Bacteria

Historically, most bacterial immunology focused on the acutely pathogenic species representing the most pressing threat to human health; more recently, research has also encompassed the commensal microbiome, particularly in the gut in which intense interactions occur with the immune system. It is appropriate to consider both pathogens and commensals in terms of Treg activity, in part because they form a biological continuum (with many commensals being opportunistic pathogens), and also because of the shared signaling pathways and specific receptors that are involved in their recognition. We summarize below the data from some of the principal bacterial systems, with additional details listed in Table 3.2.

TABLE 3.2 Tregs in bacterial infections

Mycobacteria		
Mycobacterium bovis BCG in mice	Elevated pulmonary Foxp3$^+$ cells, bacterial load unchanged by anti-CD25 depletion	Quinn *et al.* (2006)
Mycobacterium tuberculosis in humans	Elevated FOXP3$^+$ cells, inversely correlating with immunity, reduced after treatment	Chen *et al.* (2007b); Guyot-Revol *et al.* (2006); Li *et al.* (2007a); Qin *et al.* (2008)
Mycobacterium tuberculosis in mice	Tregs expand, increase bacterial load	Kursar *et al.* (2007); Ordway *et al.* (2007, 2011)
	Tregs delay priming and migration of effectors	Scott-Browne *et al.* (2007)
	Mtb-specific Tregs activated	Shafiani *et al.* (2010)
Mycobacterium vaccae	Induce Tregs, block allergy	Zuany-Amorim *et al.* (2002)
Other intracellular		
Brucella abortus (Gram −)	Tregs block protective immunity	Pasquali *et al.* (2010)
Chlamydia trachomatis	Tregs stimulated but no correlation with disease	Gall *et al.* (2011)
Listeria monocytogenes (Gram +)	Tregs suppress memory CD8$^+$ T cells	Kursar *et al.* (2002)
	No antigen-specific Tregs *in vivo*	Fontenot *et al.* (2005)
Respiratory		
Bordetella pertussis (Gram −)	Tr1 generation through filamentous hemagglutinin	McGuirk *et al.* (2002)
Gastrointestinal pathogens		
Haemophilus ducreyi (Gram −)	Tregs enriched in lesions	Li *et al.* (2010)
Helicobacter pylori (Gram −)	Treg expansion in the mucosa, CD25 depletion reduces bacterial load but generates pathology	Lundgren *et al.* (2005); Rad *et al.* (2006); Raghavan *et al.* (2003)
	Infection-related Tregs suppress airway allergy	Arnold *et al.* (2011)

(*continued*)

TABLE 3.2 (*continued*)

Salmonella enterica (Typhimurium; Gram −)	Treg depletion or anti-CTLA-4 boosts clearance and memory	Johanns *et al*. (2010)
Commensal bacteria		
Bacteroides fragilis (Gram −)	Drives Treg expansion, through PSA binding to TLR2	Round and Mazmanian, (2010); Round *et al*. (2011)
Bifidobacterium infantis (Gram +)	Induction of Tregs, bystander suppression of inflammation following mucosal *S. typhimurium* infection	O'Mahony *et al*. (2008)
Clostridium species (Gram +)	Mediates Treg induction through TGF-β, protects against DSS colitis	Atarashi *et al*. (2011)
Helicobacter hepaticus (Gram −)	Tr1-like IL-10-producing cells block gut inflammation	Kullberg *et al*. (2002)
*Streptococcus pneumonia*e (Gram +)	$CD8^{+}CD28^{+}$ suppressive Tregs producing IL-10 and TGF-β	Mertens *et al*. (2009)

3.2.1. Mycobacteria

Mycobacterium tuberculosis (Mtb) is present in two billion individuals worldwide and remains a major cause of morbidity and mortality around the world (Dye, 2006). Most infectious episodes are effectively resolved, but where elimination of the bacteria does not occur, Th1 immunity is impaired (Jo *et al*., 2003; Lienhardt *et al*., 2002).

In mice, Tregs expand in the lung and associated lymph nodes following Mtb infection (Kursar *et al*., 2007), and bacterial loads are 10-fold lower following depletion of Thy1.1^{+}Foxp3^{+} cells in Thy1.1-wild-type: Thy1.2-Foxp3$^{-/-}$ mixed bone marrow chimeras (Scott-Browne *et al*., 2007). Conversely, co-transfer of CD4^{+}CD25^{+} Tregs neutralizes immunity to infection mediated by effector CD4^{+}CD25^{-} T cells in RAG-1$^{-/-}$ mice (Kursar *et al*., 2007). Treg expansion was particularly rapid in mice infected with a hypervirulent strain of Mtb with the emergence of a CD4^{+}CD25^{+}CD223^{+}Foxp3^{+}IL-10^{+} regulatory T cell population in the lung (Ordway *et al*., 2007). The expansion of Mtb-specific Tregs was followed *in vivo*, using an Mtb-specific TCR transgenic mouse, P25; in a RAG-sufficient background, a subset of P25 Mtb-reactive T cells express Foxp3, possibly representing natural Tregs with dual specificity for this pathogen (Shafiani *et al*., 2010). The Foxp3^{+} pathogen-specific Tregs proliferated faster than the effector populations in the lung, delayed the infiltration of CD4^{+} and CD8^{+} populations, and caused a significant rise in bacterial titer (Shafiani *et al*., 2010).

The saprophytic species *Mycobacterium vaccae* may also have Treg-stimulating activity. While interest in this organism was initially focused as a possible immunogen against Mtb, it was also found to be beneficial in downregulating human atopic dermatitis (Arkwright and David, 2001). Treatment of mice with a heat-killed *M. vaccae* suspension prior to ovalbumin sensitization gave rise to a population of Ova-specific CD4^{+}CD45RB^{lo} regulatory T cells, which mediated inhibition of airway allergy through IL-10 and TGF-β (Zuany-Amorim *et al*., 2002).

In humans, the frequency of FOXP3^{+} cells is substantially higher in TB patients (Guyot-Revol *et al*., 2006; Li *et al*., 2007a) and declines following successful chemotherapy of infection (Chen *et al*., 2007b). In patients, GITR expression is also significantly raised in CD4^{+}CD25^{+} T cells, which functionally suppress effector responses (Li *et al*., 2007a), while depletion of CD4^{+}CD25^{+} Tregs restores *in vitro* responsiveness of peripheral T cells (Ribeiro-Rodrigues *et al*., 2006). Moreover, the number of CD4^{+}CD25^{+}Foxp3^{+} cells present in patients' pleural fluid (PF) inversely correlates with the ability of PF CD4^{+}CD25^{-} T cells to mount a IFN-γ response to Mtb antigens (Chen *et al*., 2007b). CD4^{+}CD25^{+}Foxp3^{+} cells from healthy carriers multiplied *in vitro* in response to heat-killed Mtb, and the active principle shown to be the 19-kDa *M. tuberculosis* lipoprotein

ManLAM (mannose-capped lipoarabinomannan), acting via the mannose receptor of human monocytes (Garg *et al.*, 2008).

An important issue is how Treg activity will be influenced by vaccination. Following infection of BCG-vaccinated mice with naturally virulent strains of *M. tuberculosis,* initial effector responses declined while Treg activity increased, with pathology accentuating over time (Ordway *et al.*, 2011). Whether regulatory T cell populations can be specifically depleted or modified to favor the outcome of mycobacterium vaccination remains to be determined.

3.2.2. *Listeria monocytogenes*

Regulatory T cells can also control the magnitude of a secondary response in infections where $CD8^+$ T cells are important in controlling protective immunity against pathogens such as *Listeria monocytogenes*. In this instance, depletion of $CD4^+$ T cells significantly enhanced the formation of a memory $CD8^+$ T cell response following secondary infection or immunization. Anti-CD25 depletion and transfer experiments demonstrated that this suppressive activity was enriched within the $CD4^+CD25^+$ T cell population from naive or *L. monocytogenes*-infected mice (Kursar *et al.*, 2002). Through the use of transgenic mice where all the T cells recognize Ova presented in the context of $H\text{-}2A^b$ (OT-II), or where Foxp3 was coupled to a GFP reporter (Foxp3–GFP), it was possible to demonstrate that acute infection with *L. monocytogenes* expressing OVA was not associated with the induction of antigen-specific regulatory T cells (Fontenot *et al.*, 2005), suggesting that downstream suppression of immunity was more likely via an interaction with $CD8^+$ T cells than a direct antigen-specific regulation of $CD4^+$ T cell function.

$Foxp3^+$ Tregs also inhibit *Listeria*-specific $CD8^+$ T cell responses *in vivo*; however, $Foxp3^+$ Tregs were found to be less potent at suppressing effector responsiveness, and specific depletion of the Treg population in Foxp3-DTR mice did not alter bacterial clearance or the expansion and activation of virus-specific $CD8^+$ T cells following infection of mice with *L. monocytogenes* (Ertelt *et al.*, 2011). These findings highlight the importance of Tregs in controlling inflammatory responses in the steady state and raise the possibilities of this function being overcome following infection.

Infection with *L. monocytogenes* is more common, and more hazardous, in pregnancy. In this context, a recent study in mice reported that the physiological increase in $Foxp3^+$ Tregs during allogeneic pregnancy was associated with greater susceptibility to *Listeria* (and *Salmonella*) infections, an effect attributable to IL-10 production by these cells (Rowe *et al.*, 2011). Moreover, Treg depletion in pregnant $Foxp3^{DTR}$ mice restored normal levels of resistance to infection while reducing live births by 70%;

hence, the extraordinary balance between infection and reproduction is managed to optimal effect by Foxp3$^+$ Tregs.

3.2.3. *Helicobacter pylori*

Human *Helicobacter pylori* infection correlates with a higher number of regulatory T cells in the gastric mucosa (Lundgren *et al.*, 2005; Rad *et al.*, 2006), which are also found in *H. pylori*-induced gastric adenocarcinoma (Enarsson *et al.*, 2006). The inability of the host to eradicate *H. pylori* infection can therefore be linked to Treg suppression of *H. pylori*-specific effector T cell responses in humans and mice (Lundgren *et al.*, 2003; Raghavan *et al.*, 2003). Accordingly, depletion of CD25$^+$ T cells increased the gastric inflammatory response and reduced bacterial burden in infected mice, but also resulted in development of severe gastritis (Rad *et al.*, 2006; Raghavan *et al.*, 2003), although another laboratory reported no effect of depletion (Kaparakis *et al.*, 2006). Tregs purified from gastric tumors were able to suppress *H. pylori*-specific effector responses *in vitro*, suggesting that antigen-specific regulatory T cells might contribute to tumor progression through bystander suppression, as noted above in Friend virus infection (Enarsson *et al.*, 2006).

Studies on induction of Tregs during *H. pylori* infection have shown that gastric epithelial cells (GECs) exposed to this organism upregulate the PD1 ligand B7-H1, and that increased conversion of naive cells into Tregs is inhibited by anti-B7-H1 antibody (Beswick *et al.*, 2007). In addition, GEC production of TGF-β both acts to induce Foxp3$^+$ Tregs and to inhibit effector T cell responses *in vitro* (Beswick *et al.*, 2011). The systemic impact of *H. pylori* on the generation of Tregs is so strong that infected mice are protected from airway allergic inflammation induced by ovalbumin, and CD4$^+$CD25$^+$ Tregs from infected mice can confer this protection on uninfected, allergen-sensitized animals (Arnold *et al.*, 2011).

3.2.4. *Bordetella pertussis*

Bordetella pertussis infection is associated with a severe and protracted disease, which is often fatal in young children. Although the development of antigen-specific Th1 cells promotes recovery from infection and clearance of bacteria from the respiratory tract, these responses are suppressed in acute infections (McGuirk *et al.*, 1998). One virulence factor implicated in this is filamentous hemagglutinin (FHA), and FHA-specific Tr1 clones have been generated from infected mice, expressing high levels of IL-10 but little IFN-γ (McGuirk *et al.*, 2002).

The major virulence factor of *B. pertussis* is its toxin (PTx), which is widely used to enhance the incidence and severity of disease in murine experimental autoimmune encephalomyelitis (EAE). A single injection is reported to inhibit Tregs and promote Th17 responsiveness

(Chen *et al.*, 2007a). Most recently, however, it has been reported that weekly PTx administration causes expansion and persistence of peripheral $CD4^+CD25^+Foxp3^+$ regulatory T cells and elevations in serum IL-10 and TGF-β (Weber *et al.*, 2010). It will be interesting to ascertain if, in active infection, sustained release of pertussis toxin in fact promotes suppressive Tregs rather than proinflammatory effector responses.

3.2.5. Commensal microbes

The development of germ-free (GF) mice has allowed us to analyze the specific impact of certain commensal bacteria species on the immune system. GF mice appear to have site-specific differences in the phenotype and suppressive capacity of their $CD4^+CD25^+$ regulatory T cell population (Ostman *et al.*, 2006). In particular, GF mice lack $Foxp3^+$ Tregs in the colonic lamina propria, which are induced as a predominantly $Helios^-$ population when animals are colonized with defined commensals (Geuking *et al.*, 2011). However, the generation of $Foxp3^{DTR}$ mice in specific-pathogen free (SPF) and GF conditions demonstrated that the suppressive activity of splenic and lymph node $CD4^+Foxp3^+$ Tregs was equivalent in both mice (Chinen *et al.*, 2010). Treg depletion in either mice also resulted in equivalent systemic inflammatory responses; however, inflammation was much more severe in the small intestine of Treg-depleted SPF mice, reflecting the substantial load of nonself antigen represented by the commensal microbiota, and the critical role of Tregs in subduing reactivity to gut flora.

Earlier work which had established this principle includes the transfer of naive ($CD45RB^{high}$) T cells into T cell-deficient mice, provoking massive gut inflammation, and its suppression by co-transfer of Tregs through IL-10, TGF-β, and CTLA-4 (Maloy *et al.*, 2003). Similarly, transfer of naive T cells from an IL-10-deficient $RAG^{-/-}$ mouse enhanced inflammation induced following *Helicobacter hepaticus* infection, whereas co-transfer of IL-10-sufficient $CD45RB^{low}CD4^+$ T cells, of either $CD25^+$ or $CD25^-$ phenotype, from *H. hepaticus*-infected but not uninfected mice was most able to prevent disease (Kullberg *et al.*, 2002). These studies were key steps toward the concept that regulatory IL-10-producing T cells are essential to prevent bacteria-induced colitis.

Normally asymptomatically resident within the colon, *Bacteroides fragilis* is a Gram-negative bacteria that has been detected within abscesses formed throughout the peritoneal cavity as a result of bowel perforation (Polk and Kasper, 1977). *B. fragilis* was found to protect animals from experimental colitis induced by *H. hepaticus* via a single microbial molecule (polysaccharide A, PSA) (Mazmanian *et al.*, 2008). This molecule induces IL-10 production from T cells, suppresses

proinflammatory IL-17 production, and was further shown to promote the differentiation of $CD4^+Foxp3^+$ regulatory T cells through TLR2 during protection from experimental colitis, as further discussed in Section 4.4 below (Round and Mazmanian, 2010). This microbial polysaccharide and *B. fragilis* were also shown protect against pathology in a mouse model of experimentally induced EAE, where both stimulated $Foxp3^+$ regulatory T cell expansion *in vivo* (Ochoa-Reparaz *et al.*, 2010a,b).

A defined mix of 46 spore-forming *Clostridium* species, prominent and indigenous to the murine gastrointestinal tract, was also found to enhance TGF-β production and expand IL-$10^+Foxp3^+Helios^-$ regulatory T cells in the intestine of previously GF mice (Atarashi *et al.*, 2011). Clostridial enrichment of the neonatal gut flora resulted in resistance to DSS-mediated colitis and reduced polyclonal IgE responsiveness to OVA–alum. A parallel probiotic effect has been found with *Bifidobacterium infantis*, which increases $Foxp3^+$ Tregs and counters inflammation following *Salmonella typhimurium* infection (O'Mahony *et al.*, 2008), while *Lactobacillus reuteri* evokes a similar $Foxp3^+$ Treg expansion and mediates suppression of airway allergy in mice (Karimi *et al.*, 2009).

3.3. Protozoa

Protozoa are single-celled organisms which include parasites of both extracellular and intracellular niches; the major global health problems from protozoal pathogens are caused by *Plasmodium* (malaria) and *Leishmania* species, along with human trypanosomes in South America and trypanosomes of livestock in Africa. These species are highlighted below, with further details given of Tregs in protozoal infections in Table 3.3.

3.3.1. *Leishmania*

One of the founding paradigms of T cell immunology emerged from research into infections of mice with *Leishmania major*, in which the progressive disease in BALB/c mice compared to C57BL/6 is linked to their dominant Th2 response to this parasite (Reiner and Locksley, 1995). C57BL/6 mice resolve infection after several weeks unless their Th1 response is compromised. However, IL-4R-deficient BALB/c are not resistant to all strains of *L. major* (Noben-Trauth *et al.*, 1999), and other *Leishmania* species (which cause cutaneous or visceral forms of disease) elicit little immunity in any strain of mouse. In *Leishmania tropica*, a cutaneous leishmaniasis agent which is equally infective to BALB/c and C57BL/10, only the combined neutralization of TGF-β and IL-10 signaling was able to induce immune clearance of parasites (Anderson *et al.*, 2008). The prominence of these two cytokines is repeated in other species, including the cutaneous *L. major* (Belkaid *et al.*, 2001), and the visceral species *Leishmania donovani* (Murphy *et al.*, 2001; Rodrigues *et al.*, 1998)

TABLE 3.3 Tregs in protozoal infections

Human malaria		
Plasmodium falciparum	Elevated $CD25^+$ and $FOXP3^+$ in infection, correlate with parasite load, and in cord blood of newborns to infected mothers	Brustoski *et al*. (2006); Mackroth *et al*. (2011); Walther *et al*. (2005, 2009)
	$FOXP3^+$ numbers expand in severe malaria, decline following treatment	Minigo *et al*. (2009)
	Human patients have high $FOXP3^+$ Tregs	Goncalves *et al*. (2010) but see Finney *et al*. (2009)
	Bystander FOXP3 (hi) induction in human T cells	Scholzen *et al*. (2009)
	Human placenta Treg induction	Bisseye *et al*. (2009)
P. vivax	Elevated $FOXP3^+$ in infection	Bueno *et al*. (2010); Goncalves *et al*. (2010); Jangpatarapongsa *et al*. (2008)
Murine malaria		
P. berghei	CD25 depletion alleviates cerebral malaria (CM)	Amante *et al*. (2007); Vigario *et al*. (2007); Wu *et al*. (2010)
	$Foxp3^+$ depletion does not alter CM while expansion through IL-2/IL-2R complexes protects from CM	Haque *et al*. (2010); Steeg *et al*. (2009)
P. chabaudi	Foxp3 overexpression compromises protection; Tregs are anti-inflammatory	Berretta *et al*. (2011); Cambos *et al*. (2008)
P. yoelii	Anti-CD25 prevents malaria immune evasion through TLR9 signaling	Hisaeda *et al*. (2004, 2005, 2008)
	IL-10 and anti-CD25 in malaria	Chen *et al*. (2009a); Couper *et al*. (2007, 2008b)
	Early $CD25^+$ Treg expansion in susceptible strain	Wu *et al*. (2007)

Leishmaniasis		
L. braziliensis (cutaneous)	Human lesions have $FOXP3^+$	Campanelli *et al*. (2006)
L. donovani	CD40-low DCs induce Tregs, exacerbate infection	Martin *et al*. (2010)
	Human lesions have high $FOXP3^+$, abated with treatment	Ganguly *et al*. (2010)
L. guyanensis (cutaneous)	Human lesions have high $FOXP3^+$	Bourreau *et al*. (2009a,b)
L. infantum (visceral)	Elevated $Foxp3^+CD103^+$ in infection	Rodrigues *et al*. (2009)
L. major	Tregs maintain low-level infection and protective immunity, require CD103 to access infection site and suppress	Belkaid *et al*. (2002); Suffia *et al*. (2005)
	Treg depletion raises Th2 response and susceptibility; Tregs reactivate infection	Aseffa *et al*. (2002); Mendez *et al*. (2004)
Toxoplasmosis		
Toxoplasma gondii	Tregs reduce parasite-induced abortion in pregnant mice	Ge *et al*. (2008)
Trypanosomiasis		
T. congolense	$Foxp3^+$ Tregs suppress protective $CD8^+$ NKT cells	Wei and Tabel (2008)
T. congolense	Natural Tregs suppress $CD4^+$, $CD8^+$, and macrophage inflammation	Guilliams *et al*. (2007)
T. cruzi	Increased $FOXP3^+$ in human infection, and $CD25^+$ Tregs prolong survival in mice	de Araujo *et al*. (2011); Mariano *et al*. (2008)

and *Leishmania infantum* (Rodrigues *et al*., 2009). In the latter, most recent, study, infection was associated with elevated levels of $CD4^+$ $Foxp3^+$ $CD103^+$ Tregs, which contributed toward a high IL-10 profile (Rodrigues *et al*., 2009).

The role of IL-10 is particularly well documented in *L. major* infection, as for example in the C57BL/6 mouse, in which sterile immunity only takes effect if IL-10 is neutralized (Belkaid *et al*., 2001). Similarly, in IL-4R$\alpha^{-/-}$ BALB/c mice, IL-10R blockade is required for complete parasite elimination (Nagase *et al*., 2007). Importantly, IL-10 is derived primarily from $CD25^-$ T cells, including some co-expressing IFN-γ (Anderson *et al*., 2007), explaining why anti-CD25 depletion is less effective than anti-IL-10R in conferring immunity in IL-4R$\alpha^{-/-}$ mice (Nagase *et al*., 2007).

The modulatory effect of Tregs therefore depends critically on the genetic and immunological status of the host. Thus, anti-CD25 depletion of BALB/c mice resulted in enhanced Th2 responsiveness and greater susceptibility rather than resistance (Aseffa *et al*., 2002). The ability of $CD4^+CD25^+$ T cells to suppress both Th1 and Th2 responses to *L. major* was then shown in co-transfer experiments (Xu *et al*., 2003). Although equal suppression of both Th subsets would have no net effect on protection, it is interesting that BALB/c mice lacking CD103 are resistant to infection (Suffia *et al*., 2005), presumably because T regs cannot access or be retained at the infection site.

In wild-type C57BL/6 mice, $CD25^+$ Tregs down-modulate immunity sufficiently to allow low-level persistence of parasites in the dermal site. In the absence of Tregs, parasites are eliminated but mice also lose their long-term immunity to reinfection (Belkaid *et al*., 2002). Because parasite persistence and reactivation of infection in humans are major issues, it is relevant to note that high-dose reinfection in mice can expand $CD4^+CD25^+$ Tregs thereby allowing latent *L. major* at a distal site to reactivate (Mendez *et al*., 2004). By co-transfer of allotype-marked $CD4^+CD25^+$ and $CD4^+CD25^-$ T cells from naive mice into RAG-$2^{-/-}$ recipients prior to *L. major* infection, it was also established that the infection only stimulates pre-existing ''natural'' Tregs, with little conversion observed from $CD4^+CD25^-$ to $Foxp3^+$ Tregs (Suffia *et al*., 2006); these authors also showed that the $Foxp3^+$ natural Treg population was reactive to *L. major* antigens, and indeed, they were able to propagate parasite-specific Treg clones that maintained this specificity for months *in vitro*.

Each of these factors appears to be at play in human Leishmaniasis. Cutaneous lesions caused by *Leishmania braziliensis* show elevated $Foxp3^+$ Tregs which co-express CTLA-4 and GITR while producing both TGF-β and IL-10 (Campanelli *et al*., 2006), while an independent study on this infection found that IL-10 production (by both Tregs and monocytes) strongly correlated with lesion activity (Salhi *et al*., 2008). Similarly, in a related cutaneous species (*Leishmania guyanensis*), high IL-10 and Foxp3

expression were reported in patients with long-standing lesions who were unresponsive to chemotherapy (Bourreau *et al*., 2009a,b).

3.3.2. Malaria

Malaria, caused by *Plasmodium* species, is one of the world's most prevalent lethal diseases, causing anemia (due to parasitism of erythrocytes) and cerebral inflammation (due to trapping of infected red cells in the vasculature). The complexity of both immunity and inflammation with the parasite is reflected in the dual roles of Tregs as protectors, in different settings, of both the host and the parasite (Finney *et al*., 2010; Scholzen *et al*., 2010), although as with *Leishmania*, IL-10 (Couper *et al*., 2008a,b) and TGF-β (Omer *et al*., 2003) are the critical regulators in malaria. Consistent with the latter study, a human malaria vaccine trial with healthy European volunteers found elevated serum TGF-β in individuals who did not respond to vaccination with inflammatory cytokines (Walther *et al*., 2005).

In endemic humans, many studies have reported elevated $CD25^+FOXP3^+$ cell numbers in *Plasmodium falciparum* malaria (Finney *et al*., 2009; Goncalves *et al*., 2010; Minigo *et al*., 2009; Walther *et al*., 2009) as well as *Plasmodium vivax* (Bueno *et al*., 2010; Goncalves *et al*., 2010; Jangpatarapongsa *et al*., 2008). However, although the frequencies of Tregs can vary significantly between individuals, the ratios of Treg:Th1 may not differ (Finney *et al*., 2009). Of further note is the suggestion that Tregs in humans repress development of malaria-specific T cell memory rather than act on inflammation itself (Walther *et al*., 2009); this study also implicated Tr1 ($IL\text{-}10^+IFN\text{-}\gamma^+$) regulatory cells which do not express FOXP3. Hence, there is currently little compelling evidence that $FOXP3^+$ Tregs suppress immunity to malaria in endemic populations.

A prominent aspect of human malaria is its effect on infants born to infected mothers. In two recent studies, it has been reported that following delivery from infected mothers, cord blood lymphocytes show high IL-10 and low Th1 responsiveness to malaria antigens, which can be reversed by $CD25^+$ T cell depletion (Bisseye *et al*., 2009; Brustoski *et al*., 2006). Prenatal exposure to *P. falciparum* antigens also correlated with greater frequency of $CD4^+CD25^{hi}$ or $CD25^+CD127lo$ Tregs in newborns' cord blood, able to suppress malaria antigen-specific IFN-γ production *in vitro* (Mackroth *et al*., 2011; Walther *et al*., 2009). It is interesting to consider whether these Tregs may persist and so determine the susceptibility of the child to malaria infection and disease.

Murine models of malaria infection reflect a major, but not exclusive, role for Tregs in determining infection outcome. The rodent malaria species *Plasmodium yoelii* is frequently studied in both susceptible (BALB/c) and resistant (DBA/2) mice. Within 3–4 days of infection, the susceptible BALB/c mice raise $CD4^+CD25^+$ Treg frequency and overall IL-10 production, suggesting a functional link with their poor protective

Th1 response (Wu *et al.*, 2007), and supporting an early finding that anti-CD25 depletion generated protective immunity to this parasite (Hisaeda *et al.*, 2004). However, a subsequent study in C57BL/6 mice compared lethal (Py17XL) and nonlethal (Py17X, NL) strains of *P. yoelii* and found that both elicited similar, modest, rises in $Foxp3^+$ Treg numbers and that in neither case did $CD4^+CD25^+$ cell depletion alter the course of infection (Couper *et al.*, 2008b). In contrast, IL-10 from $CD4^+CD25^-Foxp3^-$ T cells following *P. yoelii* infection was the critical factor in impeding parasite clearance and ameliorating liver pathology following infection, with $IL\text{-}10^{-/-}$ mice surviving the otherwise lethal Py17XL infection. Nevertheless, a demonstration that Treg activation can suppress immunity to *P. yoelii* comes from mice co-infected with the helminth *Heligmosomoides polygyrus* (see below, Section 3.4.2); co-infected mice developed more severe malaria infections which were rescued by anti-CD25 antibody treatment (Tetsutani *et al.*, 2009).

The best available mouse model for *P. falciparum*-mediated cerebral malaria (CM) is another rodent species, *Plasmodium berghei* in the C57BL/6 mouse, associated with parasite vascular adhesion and overproduction of Th1 inflammatory mediators within the brain. Perhaps counter-intuitively, anti-CD25 Treg depletion protects mice from CM, reducing parasite sequestration and also $CD8^+$ T cell infiltration (Amante *et al.*, 2007; Randall *et al.*, 2008; Wu *et al.*, 2010). Interestingly, the effect of depletion is time dependent (Vigario *et al.*, 2007) suggesting that the action of Tregs could be to facilitate entry of effector cells into the CNS, as described above (see Section 3.1.3) in HSV-2 infections (Lund *et al.*, 2008). An alternative explanation is that key effector populations for CM express CD25 after infection and are co-depleted by antibody treatment. In support of this, Treg ablation in DEREG mice showed a substantial population of $CD25^+Foxp3^-$ T cells developing after infection, and no amelioration of CM disease (Haque *et al.*, 2010; Steeg *et al.*, 2009). However, when Treg numbers are experimentally boosted with IL-2/anti-IL-2 complexes, mice were fully protected from CM (Haque *et al.*, 2010), arguing again that the action of Tregs depends critically on their proportions and activation state in vivo.

Other mouse strains are more resistant to *P. berghei*-induced CM, but in the BALB/c, anti-CD25-depletion had the opposite effect and accentuated CM symptoms (Nie *et al.*, 2007). However, as in *P. yoelii*, Treg depletion had little effect on overall parasitemias or progression to death from fulminant infections (Wu *et al.*, 2010). Clearly, regulation of the immune response to both human and murine malaria involves multiple cellular components, particularly at the level of tissue infiltration, and is greatly dependent upon dynamic and kinetic factors that have yet to be defined; while Tregs may not be uniquely responsible for susceptibility to

infection, clearly it is essential to strike the appropriate balance with effector mechanisms for a health outcome to this potentially devastating infection (Hansen and Schofield, 2010; Scholzen *et al*., 2010).

3.3.3. Trypanosomes

The trypanosomes encompass two very different groups of parasites, as the African species (e.g., *Trypanosoma brucei*) are extracellular pathogens, which can cause disease in humans and livestock, while the South American species (*Trypanosoma cruzi*) has an intracellular niche in human phagocytes and smooth muscle cells. Immunosuppression has long been a prominent feature in African trypanosomiasis, and active suppressor cell populations were described in mice by the late George Roelants and colleagues (Roelants *et al*., 1979).

C57BL/6 mice, which escape lethality to *Trypanosoma congolense* infection through limitation of an early IFN-γ response, show expansion of IL-10 producing Foxp3$^+$ Tregs. In this ''trypanotolerant'' strain, Tregs were able to downregulate classical activation of macrophages and limit tissue pathology resulting from the inflammatory immune response (Guilliams *et al*., 2007). This role of Tregs in limiting pathology, but allowing increased resistance following trypanosome infection, was also demonstrated following *T. brucei* infection. Treg expansion with the CD28 superagonist resulted in downregulation of inflammatory type 1 cytokines and the development of macrophages into the alternatively activated phenotype (Guilliams *et al*., 2008). Later studies demonstrated that anti-CD25 antibody treatment and effective depletion of natural Foxp3$^+$ Tregs before *T. congolense* infection protects BALB/c mice against this normally lethal disease. Protection was reversed in CD25-depleted mice by administration of a specific inhibitor of inducible nitric oxide synthase (Wei and Tabel, 2008).

T. cruzi is the causative agent of Chagas' disease in South America, and infection has again been associated with immunosuppression of humoral and cell-mediated immunity, in part attributable to the action of IL-10 and TGF-β which disable iNOS-mediated killing by infected macrophages (Gazzinelli *et al*., 1992). In patients, greater CD4$^+$CD25$^+$FOXP3$^+$ Treg numbers are found in both asymptomatic carriers and those developing pathology due to parasites in the myocardium; however, only in healthy patients did Tregs produce IL-10, indicating that cardiomyopathy may results from insufficient production of this cytokine (de Araujo *et al*., 2011). In infected mice, similar phenotype Tregs were found to migrate to the heart, but depleting interventions with anti-CD25 increased mortality, while administration of anti-GITR antibody additionally increased myocarditis and tissue parasitism (Mariano *et al*., 2008).

3.4. Helminths

Helminths are multicellular worms comprised of three broad taxa, the Nematodes (round worms, including the model organism *Caenorhabditis elegans*), Trematodes (flukes), and Cestodes (tapeworms), each separated by approximately 500 million years of evolution. While taxonomically distant, the parasitic species share many immunological features which are likely to have co-evolved under similar selective pressure from the immune system of the host (Allen and Maizels, 2011).

Most helminth infections, in man and livestock, are long-term chronic infestations which are maintained in the population by repeated cycles of reinfection; hence protective immunity is slow to develop, and indeed, most helminth species are associated to some degree with a state of immune suppression. Classic studies demonstrated that peripheral blood T cells from Schistosome and filariasis-infected patients showed parasite antigen-specific hyporesponsiveness, as detailed below, which could be reversed by chemotherapeutic removal of the parasite burden (Cooper *et al*., 2000; Greene *et al*., 1985; Sartono *et al*., 1995).

A marked contrast from infections with microbial agents is seen for the role of IL-10 in helminth infections; while in viral, bacterial, and protozoal infections, IL-10 generally impairs resistance (Couper *et al*., 2008a; Moore *et al*., 2001), the role of IL-10 in Th2-dominated helminth infections is both complex and double-edged (Hoffmann *et al*., 2000a). For example, IL-10 is essential to protect against potentially fatal immunopathology in chronic schistosome infection, but it is equally necessary in the initial stages of infection to establish dominant (and generally protective) Th2 responses by suppressing competing Th1/Th17 activity. Similarly, IL-10 is required for Th2-mediated expulsion of adult *Trichinella spiralis* nematodes from the intestine, and yet acts to block immunity to their offspring, larvae which encyst in tissue musculature (Beiting *et al*., 2007; Helmby and Grencis, 2003). In human helminth infections, IL-10 acts more unequivocally as an immunoregulatory player, perhaps because patients are studied in the chronic, homeostatic phase rather than during the initial events of priming and Th subset selection.

3.4.1. Filarial nematodes

Human filarial nematodes include the causative agents of lymphatic filariasis (*Brugia malayi, Brugia timori*, and *Wuchereria bancrofti*) and onchocerciasis or river blindness (*Onchocerca volvulus*). In these long-lived infections, many infected patients are asymptomatic but carry large numbers of transmission stages (microfilariae, MF) in the blood (for lymphatic filariasis) or skin (in onchocerciasis). Typically, peripheral blood T cells from these patients fail to respond to parasite antigen challenge *in vitro*, and are hence termed hyporesponsive (Piessens *et al*., 1980;

TABLE 3.4 Tregs in helminth infections

Filarial nematodes		
Brugia malayi	Induces Foxp3 expression, including in DO11.10 T cells	McSorley *et al.* (2008)
Brugia pahangi	CD25 depletion raises Th2 response	Gillan and Devaney (2005)
Litomosoides sigmodontis	Tregs maintain infection through CTLA-4 and inhibit allergy	Dittrich *et al.* (2008); Taylor *et al.* (2005, 2007)
Onchocerca volvulus	TGF-β-producing clones from human infection site	Doetze *et al.* (2000)
Wuchereria bancrofti	Raised $FOXP3^+$ T cells in infected patients	Babu *et al.* (2006)
Intestinal nematodes		
Enterobius vermicularis	High FOXP3 expression in uninflamed mucosa of UC patient	Büning *et al.* (2008)
Heligmosomoides polygyrus	*De novo* induction of Tregs; Tregs reduce intestinal pathology, suppress Th2 response and bystander airway allergy	Finney *et al.* (2007); Grainger *et al.* (2010); Rausch *et al.* (2008, 2009); Wilson *et al.* (2005)
Strongyloides ratti	Treg depletion reduces worm burden	Blankenhaus *et al.* (2011)
Strongyloides stercoralis	In HTLV-1 co-infection, excessive $FOXP3^+$ Tregs, suppression of IL-5 and high worm burdens	Montes *et al.* (2009)
Toxocara canis	Tissue-migrating larvae induce Foxp3 in mice	Othman *et al.* (2010)
Trichinella spiralis	$IL\text{-}10^-$ Tregs restrain Th2 responses	Beiting *et al.* (2007)
Trichuris muris	$IL\text{-}10^-$ Tregs restrain Th2 responses	D'Elia *et al.* (2009)

(*continued*)

TABLE 3.4 (*continued*)

Trematodes (flatworms)		
Fasciola hepatica	Infection induces IL-10 and TGF-β from Tr1-like Tregs	Walsh *et al*. (2007)
Schistosoma haematobium	FOXP3^{+} Tregs correlate with infection intensity in children	Nausch *et al*. (2011)
Schistosoma japonicum	Egg antigens stimulate CD25^{+} suppression of airway allergy	Yang *et al*. (2007b)
	Treg induction via TLR2 ligation to HSP60 peptide	Wang *et al*. (2009)
	Anti-CD25 treatment reduces worm load	Tang *et al*. (2011)
Schistosoma mansoni	IL-10^{-} Tregs elevated CD103^{+}, dampen IL-4 responses to eggs	Baumgart *et al*. (2006)
	IL-10^{+} CD25^{+} Tregs control pathology, dampen Th1 allowing Th2 to expand	Hesse *et al*. (2004); McKee and Pearce (2004)
	CD25^{+} Tregs expand through TLR2 to control pathology, upregulating CD103, CTLA4, and many other genes	Layland *et al*. (2007, 2010)
	Tregs induced by eggs, inhibit Th1	Taylor *et al*. (2006)
	Foxp3 expression decreases following chemotherapeutic cure	Watanabe *et al*. (2007)
	Pathology patients have fewer CD25^{high} Tregs	Teixeira-Carvalho *et al*. (2008)
Cestodes (tapeworms)		
Echinococcus multilocularis	Peritoneal T cells express high Foxp3	Mejri *et al*. (2011)

Yazdanbakhsh *et al*., 1993). Treg activity was presaged in this system by Piessens' report on suppressor T cells in hyporesponsive MF+ patients (Piessens *et al*., 1982), and by later work showing that the hyporesponsiveness can be reversed, *in vitro*, with anti-IL-10 and TGF-β antibodies (King *et al*., 1993). Most recently, the link between Tregs and the human filarial infection has been firmly established with elevations of both natural and adaptive Treg numbers (Metenou *et al*., 2010). Moreover, in individuals who are more reactive to parasite infections, with low or zero circulating MF and immunopathological symptoms such as lymphoedema and elephantiasis, Treg activity is deficient (Babu *et al*., 2009b).

Additional evidence for Treg-like cells in human onchocerciasis came from analysis of T cells in the subcutaneous granulomas surrounding adult *O. volvulus* (Doetze *et al*., 2000), with $CD4^+$ T cell clones from this tissue expressing IL-10 and TGF-β (Satoguina *et al*., 2002). At this time, their FOXP3 status was not determined. In lymphatic filariasis, asymptomatic carriers were found to express higher CTLA-4 levels (Steel and Nutman, 2003), with anti-CTLA-4 antibody also raising the cytokine responses of patients' cells *in vitro*. Interestingly, CTLA-4 may act with PD-1 in filariasis patients to block protective Th1 and Th17 responses to tuberculosis (Babu *et al*., 2009a).

A particularly striking feature of human filarial infections is the extremely high levels of IgG4 antibodies, both parasite-specific and total, that are rapidly lost once parasites are removed by chemotherapy (Atmadja *et al*., 1995). Hyporesponsive patients show the maximal IgG4 levels alongside depressed IgE responses (Yazdanbakhsh *et al*., 1993), a relationship which can now be explained by the action of Tregs, as *in vitro* switching of B cells to the IgG4 isotype is promoted by IL-10 (Satoguina *et al*., 2005) as well as TGF-β and GITR ligation, although not CTLA-4 (Satoguina *et al*., 2008). Hence, circulating IgG4 levels in humans could be a marker not only for helminth infection but also for elevated Treg activity.

The conclusion that human filariasis activates Tregs is well supported by studies in animal models; although the mosquito-borne infective larvae of *B. malayi* are tolerated for less than 14 days in mice, the parasites induce a short-lived expansion in $Foxp3^+$ Tregs, as occurs more strongly in mice transplanted with adult worms of the same species (McSorley *et al*., 2008). Dead parasites of either stage did not elicit this response, indicating that the presence or products of live filarial worms were responsible for stimulating Tregs. Moreover, bystander-specificity T cells (carrying the DO11.10 ovalbumin-specific TCR) were induced to express Foxp3 when transferred into BALB/c mice carrying either larval or adult *B. malayi* (McSorley *et al*., 2008).

Because human filariae cannot complete their infection cycle in mice, it is necessary to study related, rodent-compatible, species to ascertain the functional importance of Tregs in the natural context. As such, the model system of *Litomosoides sigmodontis* (Hoffmann *et al.*, 2000b) has proven exceptionally informative. Very soon after infection, there is expansion of natural Tregs, as determined by BrdU uptake *in vivo*, followed by a second wave of inducible Tregs (Taylor *et al.*, 2009), with the initial wave at least essential for parasite establishment. Transfer of cells from infected mice protected allergic recipients from allergic airway hypersensitivity, in a manner inhibited by blockade of TGF-β or anti-CD25 Treg depletion (Dittrich *et al.*, 2008). One consequence of regulatory expansion is silencing of effector cell responses (an interesting parallel to hyporesponsiveness in humans), and the emergence of a $Foxp3^{-}GITR^{+}CTLA4^{+}$ unresponsive $CD4^{+}$ population (Taylor *et al.*, 2005). Most significantly, intervention with depleting antibodies, using anti-CD25 in combination with either anti-GITR (Taylor *et al.*, 2005), or anti-CTLA4 (Taylor *et al.*, 2007) boosted responsiveness and elicited immune killing of worms. These studies were the first to demonstrate that interfering with Treg function (and re-stimulating hyporesponsive effectors through GITR ligation) can reverse susceptibility to a helminth infection.

3.4.2. Intestinal nematodes

Intestinal nematode infections (also termed geohelminths reflecting their fecal-oral transmission) are extraordinarily prevalent in humans in tropical countries, with approximately two billion cases in the world today (Hotez *et al.*, 2008). Studies have not found significant increases in systemic $Foxp3^{+}$ Treg frequencies, but qualitative changes are apparent, which may well be immunologically significant.

In areas hyperendemic for the intestinal helminth infections *Ascaris lumbricoides* and *Trichuris suum*, lymphocytes from infected children constitutively express high levels of IL-10 and TGF-β, while antigen-specific responses are inversely depressed (Turner *et al.*, 2008). For example, $CD4^{+}CTLA4^{+}$ T cells are more numerous in children with intestinal helminths than uninfected subjects (García-Hernández *et al.*, 2009). Functionally, peripheral T cells from geohelminth-infected children show depressed *in vitro* immune responses to malarial and mycobacterial antigens that are rescued by removal of the $CD25^{high}$ cells (Wammes *et al.*, 2010).

In a less common infection, *Strongyloides stercoralis*, patients co-infected with HTLV-1 show exaggerated levels of circulating $Foxp3^{+}$ T cells, reaching ~18% of the total $CD4^{+}$ T cell population (Montes *et al.*, 2009), together with higher worm burdens, while IL-5 and eosinophilia were suppressed.

Research into mouse gastrointestinal parasites has employed several model systems, with the most information to date obtained from *H. polygyrus*, a relative of the human hookworms, which spends its entire parasitic phase within the gastrointestinal tract (Monroy and Enriquez, 1992). This species is particularly associated with immunosuppression, down-modulating responses to allergens, autoantigens, and other infectious organisms (reviewed by Maizels *et al.* (2011)). Early in infection, both the proportion and absolute numbers of $Foxp3^+$ Treg cells expand in the mesenteric lymph nodes (Finney *et al.*, 2007; Rausch *et al.*, 2008), while within the $Foxp3^+$ population, there is also increased expression of CD103, considered to be a marker of Treg activation (Huehn *et al.*, 2004). $CD25^+$ Tregs from *H. polygyrus*-infected mice are suppressive when transferred to uninfected recipients, as shown by inhibition of airway allergic inflammation (Wilson *et al.*, 2005). $CD8^+$ Tregs are also found to expand in the lamina propria (Metwali *et al.*, 2006).

Remarkably, *H. polygyrus* attenuates colitis in IL-10-deficient mice (Elliott *et al.*, 2004), although IL-10 is necessary for this helminth to protect normal mice from chemically induced colitis (Setiawan *et al.*, 2007). In contrast, infection cannot block colitis in mice expressing a T cell-specific kinase-dead TGF-β receptor II (Ince *et al.*, 2009), demonstrating that both IL-10 and TGF-β can be invoked by the regulatory pathways activated by the infection. The importance of TGF-β is emphasized, however, both by the finding that *H. polygyrus* secretes a functional mimic of this cytokine (see Section 4.1 below) and by the successful boosting of immunity to adult worms by *in vivo* administration of an inhibitor of TGF-β receptor kinase I (Grainger *et al.*, 2010).

A recent study reported on *H. polygyrus* infection in DEREG mice, expressing DTR under a BAC transgene (Lahl *et al.*, 2007); in this report, Foxp3-depleted mice showed heightened Th2 responses but similar infection levels (Rausch *et al.*, 2009). It should be noted, however, that intestinal worms were enumerated at an early time point before genetically resistant mice expel most worms (Maizels *et al.*, 2011), and this system will need further investigation.

As with *H. polygyrus,* many (but not all) mouse intestinal nematode infections cause an expansion of $CD4^+Foxp3^+$ Tregs; in the case of *T. spiralis* infections, this occurs in both mice (Beiting *et al.*, 2007) and rats (Gruden-Movsesijan *et al.*, 2010). In mice, anti-CD25 antibody-mediated Treg depletion does not reduce worm numbers, although treatment results in a heightened Th2 and intact IL-10 production by $CD4^+CD25^-$ T cells (Beiting *et al.*, 2007). In a separate study, anti-CTLA-4 antibody administration to infected mice did reduce muscle larval numbers (Furze *et al.*, 2006), indicating that perhaps a $CD25^-$CTLA-4^+IL-10^+ Tr1-like population is in play.

Trichuris muris is, like *H. polygyrus*, a well-studied intestinal model and inhabits the cecum of mice (Cliffe and Grencis, 2004). Interestingly, different

strains of *T. muris* survive for varying times *in vivo*, and the longest-lived isolate elicits the strongest Foxp3$^+$ Treg response (D'Elia *et al.*, 2009). This parasite also elicits a population of intestinal Foxp3$^-$ IL-35-producing suppressive T cells (Tr35 cells), which *in vitro* differentiate under the influence of IL-10 and IL-35 (Collison *et al.*, 2010). An important role in limiting pathology has also been established in these infections, as anti-CD25- and anti-GITR-treated mice develop aggravated pathology, as well as lower worm numbers in the case of anti-GITR treatment (D'Elia *et al.*, 2009).

3.4.3. Schistosomes

Schistosomes are trematode worms causing schistosomiasis (Bilharzia) in some 220 million people worldwide. Like other helminths, they form long-lived, chronic infections which are associated with a degree of parasite-specific immune suppression, deviation (e.g., to IgG4 in humans), and susceptibility to repeated reinfection. As with human filariasis (see Section 3.4.1 above), peripheral T cells from infected patients often fail to respond to parasite antigen challenge *in vitro* (Grogan *et al.*, 1998), and two reports have charted Treg activity in human schistosomiasis. In *Schistosoma mansoni* (in which adult worms live in the mesenteric vasculature), CD4$^+$CD25^{high} T cell frequencies were inversely proportional to effector phenotype (CD25^{mid}HLA-DR$^+$) cells, but not to parasite intensity; however, curative chemotherapy significantly reduced the frequency of Tregs using these markers (Watanabe *et al.*, 2007). More recently, analysis of the urogenital parasite *S. haematobium* found a significant positive correlation between CD4^{dim}CD25^{high}CD127^{low}Foxp3$^+$ T cells and parasite intensity in children at the age at which they are still susceptible to reinfection; interestingly in adults, the reverse was the case (Nausch *et al.*, 2011).

More information is available from mice, for which *S. mansoni* is infective. The infection follows two phases, a Th1-dominated maturation period, during which skin-penetrating cercaria migrate to the lung and then the hepatic portal vasculature; and a later Th2-dominated stage which is provoked by egg release from adult worms (Pearce and MacDonald, 2002). Because eggs become lodged in the liver, this later stage is accompanied by severe granulomatous immunopathology that is moderated by IL-10-producing T cells, both Th2 and Treg, which become numerous at this time (Hesse *et al.*, 2004; McKee and Pearce, 2004). While CD25$^+$Foxp3$^+$ Tregs are not the major contributor of IL-10, they dampen Th2 responses, with anti-CD25 depletion resulting in significantly enhanced IL-4 production (Baumgart *et al.*, 2006). Moreover, anti-CD25 treatment increased egg destruction and aggravated liver pathology around the eggs, demonstrating a beneficial role for Tregs at this stage of the infection (Layland *et al.*, 2007). Subsequent studies confirmed the Foxp3$^+$ phenotype of Tregs surrounding the site of inflammation (Layland *et al.*, 2010), and the interaction was more formally

demonstrated by retroviral expression of Foxp3 in mice resulting in the suppression of liver granuloma formation (Singh *et al.*, 2005).

Schistosome eggs produce a number of immunologically active substances, including an IL-4-inducing protein (IPSE or α-1 (Schramm *et al.*, 2007)) and a ribonuclease, ω-1 (Everts *et al.*, 2009; Steinfelder *et al.*, 2009). While both can drive Th2 responses *in vivo* and *in vitro*, only ω-1 can induce Foxp3 in T cells, requiring the presence of DCs, TGF-β, and retinoic acid (Zaccone *et al.*, 2011). Unrelated to this protein, an HSP60-derived peptide SJMHE1 from *Schistosoma japonicum* was shown to expand $CD4^+CD25^+Foxp3^+$ T cell populations *in vivo* and *in vitro*, with such cells able to inhibit delayed-type hypersensitivity on transfer to mice 1 day prior to allergen sensitization (Wang *et al.*, 2009).

3.5. Fungi

Fungal pathogens are found in a variety of niches and in different developmental forms; in addition, a number are commensals which can adapt opportunistically to immunodeficiency. One such example is oropharyngeal *Candida albicans* infection, immunity to which is compromised by the $CD4^+CD25^+$ IL10-producing Treg population that is deficient in $TLR2^{-/-}$ mice, and which when depleted *in vivo* with anti-CD25 antibody, results in improved resistance to infection (Netea *et al.*, 2004). Interestingly, as observed for *L. major* infection (Belkaid *et al.*, 2002), the $CD4^+CD25^+$ subset was also required to generate normal protective memory responses to infection, establishing a ''protective tolerance'' that restrains pathology while allowing a form of commensalism to persist (Montagnoli *et al.*, 2002). Recent work has elucidated a fascinating dynamic in which, when confronted with an acute infection, Tregs promote Th17 responses to *C. albicans*, while at later time points act to restrain the same effector population from mediating inflammatory bowel disease (Pandiyan *et al.*, 2011); thus early CD25 depletion resulted in diminished Th17 immunity and increased fungal burden, whereas transfer of $CD25^+$ Tregs could prevent colitis in infected RAG mice caused by *in vitro* polarized Th17 cells.

Paracoccidioides brasiliensis is regarded as the most prevalent primary fungal pathogen of Latin America and is the causative agent of a systemic granulomatous disease in the host. $CD4^+CD25^+CD103^+CTLA\text{-}4^+Foxp3^+GITR^+$ Tregs are found in the lesions of infected patients (Cavassani *et al.*, 2006). In a mouse model, adoptive transfer of $CD4^+CD25^+$ but not $CD4^+CD25^-$ T cells from infected mice increased the fungal load in recipients, except in a $CCR5^{-/-}$ setting (Moreira *et al.*, 2008). Mice lacking CCR5 had a reduced number of Tregs in the lungs, and did not exhibit suppressed T cell proliferation *ex vivo* following a more contained infection. $CCR5^{-/-}$ mice may have a generalized defect

TABLE 3.5 Tregs in fungal infections

Aspergillus fumigatus TLR	Inflammation controlled by $CD4^+CD25^+$ Tregs	Montagnoli *et al*. (2006)
Candida albicans	Early Th17 promoted by Tregs, but later immunity suppressed; Tregs neutralized by TLR2 ligation	Montagnoli *et al*. (2002); Netea *et al*. (2004); Pandiyan *et al*. (2011)
Cordyceps sinensis	Increased $Foxp3^+$ Tregs and reduced T1D in NOD mice	Shi *et al*. (2009)
Histoplasma capsulatum	Tregs suppress Th17 at site of infection	Kroetz and Deepe (2011)
Onychomycosis	Higher $CD4^+CD25^+$ cell numbers in patients	Kaya *et al*. (2009)
Paracoccidioides brasiliensis	Tregs control inflammation and limit fungal clearance; migration of $Foxp3^+$ Tregs to lesions	Cavassani *et al*. (2006); Loures *et al*. (2010); Moreira *et al*. (2008)
Pneumocystis carinii	$CD4^+CD25^+$ Tregs suppress inflammation	Hori *et al*. (2002); McKinley *et al*. (2006)

in egress of thymic Tregs, as also demonstrated by their greater resistance to another fungal pathogen, *Histoplasma capsulatum* (Kroetz and Deepe, 2011). The correlation between Treg activity and extent of fungal infection did not hold, however, in TLR4-deficient mice, which showed higher $Foxp3^+$ Treg numbers and yet were able to control *P. brasiliensis* infection more efficiently (Loures *et al.*, 2010); whether this reflects an early stimulatory role by Tregs as observed for *C. albicans* has not yet been tested.

Aspergillus fumigatus is a further fungal pathogen and a causative agent of airway hypersensitivity and allergy. Exposure of mice to *Aspergillus conidia* resulted in the early expansion, activation, and recruitment of $CD4^+CD25^+$ Tregs, which correlated with decreases in inflammation at this time point. Depletion of natural Tregs using cyclophosphamide or anti-CD25 reduced $CD4^+CD25^+$ T cell numbers, exacerbated inflammation, and decreased the survival of infected wild-type mice (Montagnoli *et al.*, 2006). This work also highlighted the role of IDO, as well as IL-10 and CTLA-4, as a mediator feeding back to tolerize DCs and forestall hypersensitivity in the later stages of infection.

4. TREG ACTIVATION—A COMMON IMMUNE-EVASION STRATEGY ACHIEVED THROUGH DIVERSE ROUTES

The evidence from the many and diverse infectious agents reviewed above is that Tregs often suppress protective immunity: examples can be given from the retrovirus (FV) model, through malaria to the helminth worms. However, where pathogens are reliant on Treg activity, this offers a therapeutic route to eliminate infection, which has been reproduced in a number of these same models (Hisaeda *et al.*, 2004). Hence, identifying the pathway(s) for Treg activation (Figure 3.1) is crucial for future intervention strategies.

Treg activation can benefit both host and pathogen however. Most frequently, this is evident at the level of dampening pathology. T cell-mediated responses to HSV in the corneal stroma are a frequent cause of human blindness. Depletion of natural regulatory T cells was shown to enhance lesion formation and keratitis following HSV infection by impairing antiviral immunity and T cell migration to lesion sites (Suvas *et al.*, 2004). Similarly, Tregs restrain intestinal pathology in infections with *T. muris*; in this system, anti-GITR antibody results in lower worm burdens, but incurs more intense gut pathology (D'Elia *et al.*, 2009). In the long term (especially in chronic human infections), the key to a healthy status is the balance between controlling infection and limiting pathology—maintaining a recalibrated homeostasis in chronic infection.

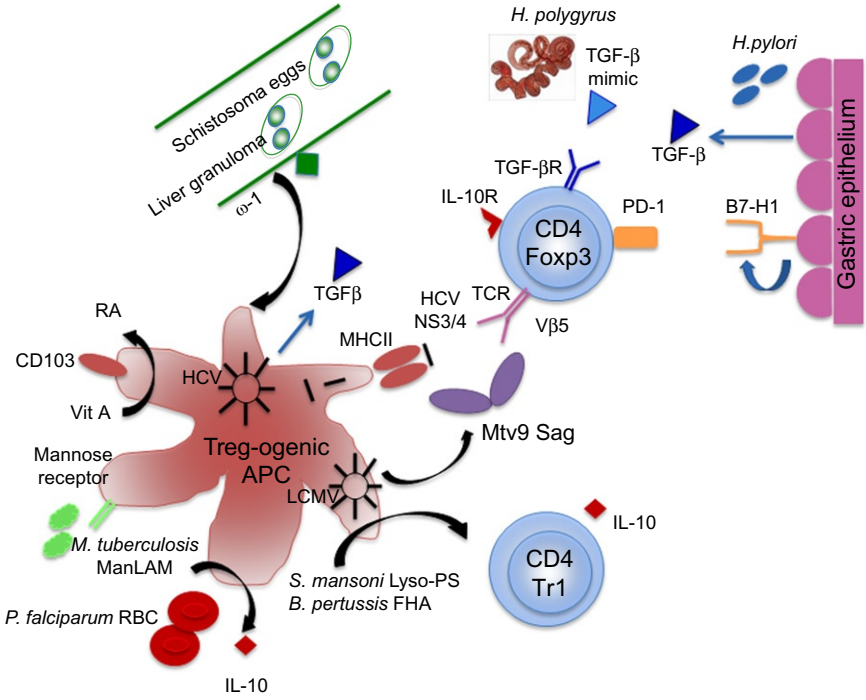

FIGURE 3.1 Pathways of Treg induction and activation in infection.

4.1. Direct conversion of T cells into Tregs

A few examples are now established in which pathogen products directly activate, promote, or induce Tregs; clearly these are important proof-of-principle that the expansion of Tregs *in vivo* is not purely a homeostatic response that accompanies every effector expansion. In these instances, at least, we can surmise that pathogens have evolved to stimulate and exploit the host's down-modulatory Treg populations.

A recent example is the induction of host Tregs by *H. polygyrus*, which secretes a TGF-β-like mimic that activates the TGF-β signaling pathway (Grainger *et al.*, 2010). *H. polygyrus* adult worm excretory-secretory products (HES), when added to naive Foxp3^{-} murine T cells together with TCR ligation, induce *de novo* Foxp3. As this fails to occur in cells expressing the T cell-specific dominant negative TGF-β-RII, the interaction is directly between HES and the T cells in question. Foxp3^{+} T cells, whether induced by HES or mammalian TGF-β, are equally able to suppress airway allergy in recipient mice (Grainger *et al.*, 2010). A similar activity was also found in secreted products of the related sheep parasite *Teladorsagia circumcincta*, indicating that some helminth parasites have evolved

to exploit a key immunosuppressive pathway of their host. The helminth TGF-β mimic may be the biological equivalent of viral cytokine-like molecules, such as the EBV IL-10 homolog and other examples summarized elsewhere (Tortorella *et al.*, 2000).

A very different mechanism is employed by *Streptococcus pneumoniae* which elaborates a zwitterionic polysaccharide able to directly cross-link the TCR of $CD8^+$ T cells and switch them into a regulatory phenotype (Mertens *et al.*, 2009). As with the *H. polygyrus* HES, this is a process which can by-pass any requirement for an APC population.

4.2. Induction of Tregs via DCs

Treg cells, like all T cells, require cognate APC interactions for their activation, and DCs are the major cell type responsible; consequently, it is not surprising that in the majority of systems studied, Treg generation involves the DC population. This is most clearly demonstrated where individual molecular components from pathogens are able to drive Treg differentiation through a DC pathway: for example, the FHA from *B. pertussis* modifies DC interactions with naive antigen-specific T cells, polarizing them to an IL-10-producing phenotype (McGuirk *et al.*, 2002); similarly, the lysophosphatidylserine molecule from *S. mansoni* acts through DCs to induce $IL\text{-}10^+$ Tr1 cells (van der Kleij *et al.*, 2002). In the former case, the FHA binds TLR4 (Higgins *et al.*, 2003) while the lyso-PS ligates TLR2, highlighting the regulatory face of the Toll ligand family as discussed in a following section.

Most recently, the induction of Foxp3 in naive T cells by DCs exposed to the *S. mansoni* egg antigen ω-1 has been described (Zaccone *et al.*, 2011), providing a mechanistic pathway that may be followed *in vivo* when DCs from *S. japonicum*-infected mice promote $Foxp3^+$ and $IL\text{-}10^+$ Treg *in vitro*, inhibiting airway allergy in the process (Liu *et al.*, 2011). Likewise, the Ac-TMP-1 protein released by adult *Ancylostoma caninum* hookworm skews DCs to induce both $CD4^+$ and $CD8^+CD25^+Foxp3^+$ T cells; both subsets expressed IL-10 while the $CD4^+$ Tregs also produced TGF-β (Cuéllar *et al.*, 2009). In addition, *Anisakis simplex* (a nematode from marine mammals), elaborates a homologue of MIF (macrophage migration inhibitory factor) which when injected into mice elicits increased numbers of $Foxp3^+$ T cells (Park *et al.*, 2009).

Many further examples of Treg generation by DCs in infectious settings employ whole organisms, or their secreted products. For example, bone marrow DCs exposed to live *H. pylori* bacteria are able to drive *de novo* induction of Tregs *in vitro* (Zhang *et al.*, 2010), and expand $Foxp3^+$ Tregs when adoptively transferred to mice shortly before infection (Kao *et al.*, 2010). In a different system, secreted products of the nematode *H. polygyrus* were used to pulse DCs which preferentially induced

functional $CD4^+CD25^+IL\text{-}10^+Foxp3^-$ Tregs (Segura *et al.*, 2007). *In vivo*, expansion of pro-regulatory DC subsets occurs, such as the predominant $CD11c^{lo}CD103^-$ DC subset in the mesenteric lymph nodes of *H. polygyrus*-infected mice which are potent inducers of Foxp3 in naive murine T cells (Smith *et al.*, 2011).

In each of these systems, the mechanisms by which DCs induce Tregs are similar to those established in model systems in the absence of infection: TGF-β, IL-10, and retinoic acid are all implicated. For example, the induction of $Foxp3^+$ Tregs by malaria-infected red blood cells is dependent on both TGF-β and IL-10 (Scholzen *et al.*, 2009). Similarly, the Treg-inducing ability of Schistosome egg antigen and its component ω-1 requires target DCs to produce TGF-β (Zaccone *et al.*, 2011).

4.3. Bystander induction by other cell types

Although not involving DCs, the strategy mounted by HCV to induce Tregs is similar to that described for several other pathogens above, except that infected human hepatocytes are induced to express TGF-β, which, using well-characterized pathways, is able to drive expression of Foxp3 and other regulatory markers (including CD25, CTLA-4, and LAP) in human $CD4^+$ T cells (Hall *et al.*, 2010). GECs are similarly stimulated by *H. pylori* to express TGF-β and B7H-1 to evoke $Foxp3^+$ Tregs (Beswick *et al.*, 2011).

In *S. mansoni* infection, regulatory B cells are able to induce $Foxp3^+$ Tregs to infiltrate airways, suppressing allergic inflammation (Amu *et al.*, 2010). B cells are also implicated in *Helicobacter felix* infection, in which TLR2 activation of B cells is required to induce IL-10-producing $CD4^+CD25^+$ regulatory T cells and control immunopathology (Sayi *et al.*, 2011).

4.4. TLRs in Treg activation

Most pathogens are initially recognized by the innate immune system through one or more Toll-like receptors (TLRs), ligating to archetypal molecular species characteristic of particular microbe classes, and expressed by DCs and other sentinels of immunity (Medzhitov, 2007). Adaptive immune lymphocytes, including T cells, may also express TLRs, raising the question of whether in infection Tregs are either stimulated or inhibited by interacting with TLR ligands.

In general, TLR stimulation of DCs and other APCs is considered to be strongly proinflammatory and likely to overcome homeostatic Treg control. However, some pathogen TLR ligands (such as the TLR2-binding *S. haematobium* lysophosphatidylserine (van der Kleij *et al.*, 2002)) drive human DCs to induce IL-10-secreting Tr1 cells, while *S. typhimurium* LPS-driven TLR4 ligation was reported to promote proliferation and suppressive activity of murine $CD4^+CD25^+$ T cells (Caramalho *et al.*, 2003).

Subsequently, numerous studies into pathogen-derived pro-regulatory TLR stimulation have been reported with some clear instances of direct effects on Tregs, rather than pathways routed through APC populations (Himmel *et al.*, 2008; van Maren *et al.*, 2008).

TLR2 emerges as a significant enhancer of Treg activity in the steady state (Sutmuller *et al.*, 2006) as well as in the context of several infections, as indicated by the greater resistance of $TLR2^{-/-}$ mice to *C. albicans* (Bellocchio *et al.*, 2004; Netea *et al.*, 2004) and *Yersinia enterocolitica* (Sing *et al.*, 2002). Likewise, in infections with the helminth *S. mansoni*, $TLR2^{-/-}$ mice, in which $CD4^+CD25^+$ Tregs do not expand, suffer aggravated liver pathology which can be rescued by transfer of wild-type schistosome-primed $CD4^+CD25^+$ T cells (Layland *et al.*, 2007). An increase in Tregs occurred in TLR4- but not in TLR2-deficient mice following administration of the HSP-60-derived *S. japonicum* peptide SJMHE1, as bone marrow-derived macrophage and dendritic cells from $TLR2^{-/-}$ mice, but not wild-type mice, primed *in vitro* or *in vivo* were unable to induce Tregs *in vitro* (Wang *et al.*, 2009).

Treg proliferation can be promoted by TCR signaling and TLR2 ligation with a synthetic agonist; however, in *C. albicans* infection, the effect of TLR2 ligation is to reverse $CD4^+CD25^+$ Treg suppression of anti-fungal responses and allow fungal outgrowth (Netea *et al.*, 2004). Hence, $TLR2^+$ Tregs transferred into $TLR2^{-/-}$ mice promote a 100-fold rise in *C. albicans* infection, which is prevented in the presence of the TLR2 ligand Pam-3-Cys (Sutmuller *et al.*, 2006). It is important to note that later studies indicate that the effects of TLR2 expression on Tregs are largely to promote proliferation independently of APCs (Chen *et al.*, 2009b), and that loss of suppressive activity may be relatively transient (Liu *et al.*, 2006) or circumscribed in effect (Oberg *et al.*, 2010; van Maren *et al.*, 2011). Moreover, other TLR2 ligands can enhance Treg function (Zanin-Zhorov *et al.*, 2006). The apparently contradictory role of TLR2 may be explained by its ability to heterodimerize with different partners (TLR1, TLR6, and TLR10), and the property of some pathogen-derived ligands to selectively stimulate an immunosuppressive, rather than an activating, signal (Depaolo *et al.*, 2008).

Recently, TLR2 activation of B cells has been shown to be critical for microbial Treg induction by the PSA of the commensal bacterium *B. fragilis* (Round *et al.*, 2011). These authors showed that wild-type bacteria, but not PSA-deficient organisms, stimulated IL-10-producing $Foxp3^+$ Tregs in wild-type, but not in TLR2-deficient mice. Since induction was intact in both TLR1- and TLR6-deficient hosts, *B. fragilis* signaling does not appear to be mediated by heterodimers with either of these components, while the TLR2-dependent expression of IL-10 by purified T cells in the absence of APCs argues that the bacterial product acts directly on the T cell without requiring an intermediary population.

Other TLRs show similar involvement in both enhancement and inhibition of Tregs, depending upon the setting. Bacterial flagellin binding through TLR5, for example, was reported to promote human Tregs (Crellin *et al.*, 2005). In other systems, the reversal of Treg suppression by potent synthetic ligands for TLR7 (Hackl *et al.*, 2011; Van *et al.*, 2011) and TLR8 (Peng *et al.*, 2005) does not necessarily reflect the potential for more subtle pathogen-derived molecules to activate Tregs in different ways, and this will clearly be a fertile area for future research.

5. ANTIGEN SPECIFICITY OF NATURAL AND ADAPTIVE TREGS IN INFECTION

Two central issues in regulatory T cell biology are the division between thymic (''natural'') and induced (''adaptive'') Tregs, and the nature of the antigen specificity of Tregs functional in any particular setting (Bluestone and Abbas, 2003; Rudensky, 2011). Although natural Tregs will have been selected in the thymus for self-reactivity, this does not preclude them recognizing exogenous ligands through cross-reactivity, mimicry, or dual specificity; conversely, induced Tregs in infection are not necessarily pathogen-specific, but may carry a third-party specificity having been activated as bystanders in a pro-regulatory cytokine environment. Indeed, molecular analysis of TCR usage among natural Tregs argues that they do not have a self-restricted repertoire (Pacholczyk *et al.*, 2007). In any event, specificity is not essential for suppressive function, as once a regulatory cell has been triggered, their production of downregulatory cytokines and ability to tolerize DCs allow them to modify systemic reactions to bystander antigens, whether of pathogenic or nonpathogenic (e.g., allergen) origin.

A number of studies have addressed whether Foxp3$^+$ T cell responses observed in infection represent stimulation of pre-existing natural/thymic Tregs or conversion of naive/effector T cells into adaptive Tregs in the periphery. *De novo* induction of Tregs did not occur among Foxp3-negative OVA-transgenic (OT-II) T cells adoptively transferred into mice prior to infection with Ova-expressing *L. monocytogenes* (Fontenot *et al.*, 2005). In the *L. major* system, co-transfer of allotype-marked CD25$^+$ and CD25$^-$ T cells demonstrated that Foxp3 expression remained entirely within the CD25$^+$ population, and conversion from naive/effector cells did not occur (Suffia *et al.*, 2006), while in *L. sigmodontis* infections, BrdU labeling showed early natural Treg proliferation in response to infection (Taylor *et al.*, 2009). However, in *H. polygyrus* infections, conversion into induced Tregs was demonstrated in Foxp3-negative T cells from a Foxp3–GFPxDO11.10 F1 mouse when transferred to an infected wild-type recipient. When mice were given oral ovalbumin, up to 50% switched on Foxp3

expression (Grainger *et al.*, 2010). This discrepancy may lie in the specific localization of the pathogen following infection in these models, as peripheral induction of Tregs has been shown to occur most efficiently in gut-associated lymphoid tissue (Sun *et al.*, 2007). This highly regulated site is populated in the steady state by tolerogenic dendritic cell populations producing high levels of TGF-β (Coombes *et al.*, 2007) or by pro-regulatory DCs in helminth infection (Smith *et al.*, 2011).

A closely related issue is whether pathogen persistence depends on either or both thymic and/or induced Tregs. In *L. sigmodontis* infection, prior depletion of existing (thymic and noncognate induced) Tregs (with anti-CD25 antibody) amplified Th2 responses and reduced worm survival (Taylor *et al.*, 2009). Remarkably, the effects of natural Treg depletion were not evident until some 60 days post-infection; this has been attributed to the early rapid proliferation of pre-existing Tregs on infection, which are able then to dominate the course of the ensuing response (Taylor *et al.*, 2009).

An alternative approach is to identify whether Tregs specific for exogenous, pathogen-derived epitopes are generated in infection. For example, it was noted that following chronic LCMV infection, there is selective expansion of a $V\beta5^{+}Foxp3^{+}$ Treg population (Punkosdy *et al.*, 2011). However, these Tregs evolved from a pre-existing pool and are found only in mouse strains carrying an endogenous Mtv9 superantigen-encoding provirus. Hence, LCMV represents a potentially unique case in which a viral Treg epitope is also encoded in the host genome, with specific Treg activation resulting in chronic infection.

A different transgenic TCR system involved the P25 TCR expressed by $CD4^{+}$ T cells specific for an immunodominant *M. tuberculosis* peptide $Ag85B_{240–254}$ presented by the class II molecule $I\text{-}A^{b}$. When P25 transgenic T cells (negative for Foxp3–GFP) were transferred to mice prior to infection, there was no conversion to Foxp3 expression despite an overall increase in the endogenous Treg population (Shafiani *et al.*, 2010); hence the predominant Treg type in this infection could be considered natural Tregs. Interestingly, when donor P25 TCR transgenic mice were analyzed in detail, a population of splenic $P25^{+}Foxp3^{+}$ Tregs was observed, representing ''dual specificity'' natural Tregs. Purified $P25^{+}$ Tregs from uninfected mice were then transferred to naive recipients and shown to delay the priming of effector T cells following Mtb infection (Shafiani *et al.*, 2010). Hence, immunity to infection is impaired by Tregs which are both naturally arising and pathogen-specific. In *L. major* infection, it was previously reported that natural Tregs respond specifically to parasite antigen, as shown by propagation through repeated antigen stimulation (Suffia *et al.*, 2006). As this was a nontransgenic, polyclonal TCR population, this observation implies that the ''dual specificity'' natural Tregs may be found more extensively than in the case of P25 alone.

Despite these illustrations of natural Treg involvement in microbial infections, there are few clear examples of the specificity of peripherally induced Tregs in infection setting. In RSV-infected mice, preferential binding of an MHC class II tetramer containing a defined epitope (M209) was observed to $CD4^+Foxp3^+$ Tregs (Liu *et al.*, 2009); these cells downregulate virus-induced pathology when transferred to infected recipients (Liu *et al.*, 2010), but as they emerge in a polyclonal environment, it is not established whether they represent dual specificity natural Tregs or have been selectively induced by infection. If Helios proves to be an authentic transcription factor for natural/thymic Tregs (Thornton *et al.*, 2010), staining for this molecule would be a valuable adjunct to these analyses.

In addition to specificity, there remains a major unresolved question of whether natural or induced Tregs are more important in the dampening of immunity and control of pathogenesis. While the answer to this question will depend on the infection setting, the tissue site, and the kinetics of the response, it may also underestimate the complexity of immune regulation *in vivo*: in most cases, both types of Treg are likely to be necessary. An interesting perspective has emerged from studies in the filarial nematode *L. sigmodontis* indicating that early natural Tregs act to limit responsiveness (to the detriment of long-term protective immunity), before a second wave of induced Tregs come into play (Taylor *et al.*, 2009).

6. TREGS AND THE HYGIENE HYPOTHESIS

One of the most significant implications of regulatory T cell activation by infectious agents may be the downregulation of immune responsiveness to other coincident antigens. The impact of this modulation may be either beneficial to the host, in suppressing responses to allergens, autoantigens, and commensals (Maizels, 2005), or detrimental, in compromising immunity to life-threatening infections such as malaria (Su *et al.*, 2005).

Robust experimental and epidemiological evidence that infections can protect against allergies and other immunological over-reactions has been established across the board for infectious organisms from helminths (Cooper *et al.*, 2003; Fleming and Cook, 2006; Maizels, 2005; Smits and Yazdanbakhsh, 2007), mycobacteria (Zuany-Amorim *et al.*, 2002), and viruses (Filippi *et al.*, 2009; Richer *et al.*, 2008), extending also to probiotic and commensal bacteria (Feleszko *et al.*, 2007; Karimi *et al.*, 2009; Repa *et al.*, 2003). It should be noted, however, that the same infections can result in poorer responses to childhood vaccination (Cooper *et al.*, 1998, 2001), as well as to co-infections with pathogens such as malaria (Hartgers *et al.*, 2009).

The close link between helminth infections, Tregs, and suppression is one of the most active research areas in this regard (Maizels and Wiedermann, 2009). In mouse models, *H. polygyrus* was originally shown to generate $CD4^+CD25^+$ Tregs which, on transfer to allergen-sensitized recipients, protected them from airway allergy (Wilson *et al.*, 2005). Similarly, *L. sigmodontis* nematode infection has been shown to inhibit airway allergy to a bystander antigen (Dittrich *et al.*, 2008) and block development of autoimmune disease in diabetes-prone NOD mice, alongside expansion of both Th2 and $CD4^+CD25^+Foxp3^+$ Treg cells (Hübner *et al.*, 2009). These findings with helminth infections are remarkably similar to reports of viral infections such as LCMV and Coxsackie virus, involving the expansion of $CD4^+CD25^+$ T cells producing TGF-β and suppression of allergy and diabetes (Diana *et al.*, 2011; Filippi *et al.*, 2011).

Currently, several avenues are being explored for the therapeutic treatment of immunopathological conditions with certain parasite species associated with regulatory effects, such as the porcine intestinal worm *Trichuris suis* which has been reported to benefit patients with inflammatory bowel diseases (Summers *et al.*, 2005, 2006). A further report on the remission of disease in MS patients with adventitious intestinal helminthiases (Correale and Farez, 2007) correlated these benefits of infection with enhanced Treg and TGF-β levels. These findings, together with an intriguing case report of an ulcerative colitis patient (Broadhurst *et al.*, 2010) and reduced diabetes incidence in filariasis patients (Aravindhan *et al.*, 2010), have heightened interest in helminth therapy of severe immunological dysfunction (Fleming and Fabry, 2007). However, not all trials utilizing this strategy have had positive outcomes (Bager *et al.*, 2010), and it remains to be demonstrated that any beneficial effects in humans of helminth therapy are mediated by Tregs rather than parallel regulatory pathways which are likely to be activated by the same infections.

7. CONCLUSION

Any overview of the impact of Tregs across a diverse range of infectious organisms will inevitably highlight the individual features of each system, with unique niches, dynamics, and molecular interactions. Nevertheless, it is clear that Tregs are involved in the outcome of nearly every infectious episode studied, not necessarily in the central role, but invariably modifying the scale and mode of immunity. Moreover, in many cases from viruses to worms, their intervention is pivotal in differentiating healthy from pathogenic outcomes; we can now learn from these examples, and understand the exceptions, to design new strategies for the control of diseases.

Targeting Tregs for the therapy of infectious diseases is an attractive option in settings where the host mounts an immune response of low pathogenic potential, but which normally is muted and cannot attain the level or intensity required to eliminate the pathogen. More caution may be required if Tregs shelter the host from pathogenesis or contribute to immunity in nonintuitive ways such as facilitating tissue access for effector cells or maintaining low-level persistent antigen for immunological memory. As fundamental understanding of Treg function deepens, more precise targeting may become feasible, for example, by preventing *de novo* induction of Tregs in infection, by blocking specific molecular interactions (such as CTLA-4), and by interfering with key molecules required for Treg migration, including CD103, CCR4, and CCR5 (Moreira *et al*., 2008; Sather *et al*., 2007).

A key issue that requires advancing in all the infection systems under consideration, is that of the breadth and antigen specificity of Treg populations in infection. If there is global activation of the natural Treg compartment (e.g., as a physiological response to major inflammatory reactions), then Treg targeting is likely to unleash an unacceptable level of immunopathology if not autoimmunity. However, if the key factor in infection is the activity of a selective subset of pathogen-specific Tregs (or a small subset of natural Tregs which also react to a pathogen determinant), these could be ablated in a more restricted fashion.

Vaccination will, of course, remain our primary strategy to eradicate infectious diseases. Two very interesting perspectives on vaccination emerge from our survey of Tregs in infection. Firstly, the efficacy of vaccines against the major microbial infections is compromised in children harboring common helminth infections, most likely due to the higher level of Tregs in those individuals; hence anthelmintic and/or Treg-reducing interventions may be necessary if current vaccines are to achieve further effects in populations within endemic areas. Secondly, the question is raised of whether vaccines should be fine-tuned to minimize Treg activation. While empirically we have developed adjuvants which stimulate effector immunity, less consideration has been given to whether new vaccines (particularly to parasitic organisms in which vaccines have been notoriously inefficient) should be purged of Treg-stimulating specificities, or even of epitopes which stimulate pathogenic rather than protective effector responses.

Finally, we continue to learn much from those most accomplished immunologists, the successful organisms which can establish themselves in the human body. The complexity of the regulatory network in most infections remains to be defined, but will surely reveal many critically important features of the sequence and hierarchy through which immune suppression is established *in vivo*. In addition, at a molecular level, there is every expectation that new ''drugs from bugs'' will be developed that specifically

enhance Treg development and activity, which should prove invaluable in the treatment of noninfectious immunopathologies such as allergy and autoimmunity.

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