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Foxp3$^+$ regulatory T cells, immune stimulation and host defence against infection

Jared H. Rowe, James M. Ertelt and Sing Sing Way

Departments of Pediatrics and Microbiology, University of Minnesota School of Medicine, Center for Infectious Disease and Microbiology Translational Research, Minneapolis, MN, USA

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Correspondence: Dr S. S. Way, Departments of Pediatrics and Microbiology, University of Minnesota School of Medicine, 2001 6th Street SE, Minneapolis, MN 55455, USA.
Email: singsing@umn.edu
Senior author: Sing Sing Way

Summary

The immune system is intricately regulated allowing potent effectors to expand and become rapidly mobilized after infection, while simultaneously silencing potentially detrimental responses that averts immune-mediated damage to host tissues. This relies in large part on the delicate interplay between immune suppressive regulatory CD4$^+$ T (Treg) cells and immune effectors that without active suppression by Treg cells cause systemic and organ-specific autoimmunity. Although these beneficial roles have been classically described as counterbalanced by impaired host defence against infection, newfound protective roles for Treg cells against specific viral pathogens (e.g. herpes simplex virus 2, lymphocytic choriomeningitis virus, West Nile virus) have been uncovered using transgenic mice that allow *in vivo* Treg-cell ablation based on Foxp3 expression. In turn, Foxp3$^+$ Treg cells also provide protection against some parasitic (*Plasmodium* sp., *Toxoplasma gondii*) and fungal (*Candida albicans*) pathogens. By contrast, for bacterial and mycobacterial infections (e.g. *Listeria monocytogenes*, *Salmonella enterica*, *Mycobacterium tuberculosis*), experimental manipulation of Foxp3$^+$ cells continues to indicate detrimental roles for Treg cells in host defence. This variance is probably related to functional plasticity in Treg cell suppression that shifts discordantly following infection with different types of pathogens. Furthermore, the efficiency whereby Treg cells silence immune activation coupled with the plasticity in Foxp3$^+$ cell activity suggest that overriding Treg-mediated suppression represents a prerequisite 'signal zero' that together with other stimulation signals [T-cell receptor (signal 1), co-stimulation (signal 2), inflammatory cytokines (signal 3)] are essential for T-cell activation *in vivo*. Herein, the importance of Foxp3$^+$ Treg cells in host defence against infection, and the significance of infection-induced shifts in Treg-cell suppression are summarized.

Keywords: infection; regulatory T cells; T-cell activation; tolerance; vaccination

Introduction

The fluid balance between immune activation required for optimal host defence against infection and immune suppression that maintains tolerance by averting autoimmunity is stringently regulated. This allows immune effectors with the potential to cause catastrophic damage to host tissues to be actively silenced during homeostasis, but also rapidly unleashed in response to infection. Accordingly, the cell-associated and cytokine signals that stimulate the activation of immune effectors have been intensely investigated for developing new therapeutic strategies for boosting desired immune responses during infection or immunization. On the other hand, understanding how ubiquitous immune suppression signals are selectively silenced during immune activation, and the extent to which they limit optimal host defence against infection has lagged behind. This bottleneck has been overcome with the identification of a distinct CD4$^+$ T-cell subset with immune suppressive properties called regulatory T (Treg) cells.[1–3] Although Treg cells were initially identified as the CD4$^+$ T-cell subset that constitutively express the interleu-

kin-2 (IL-2) receptor, CD25, subsequent landmark studies have since established that the lineage-defining and master regulator for Treg cells is dictated by expression of the forkhead box P3 transcription factor, Foxp3.[4–6]

Infants who develop a fatal rare constellation of clinical features that includes refractory eczema, diabetes, thyroiditis, colitis, infection susceptibility and generalized wasting called the immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome have mutations in either the *foxp3* promoter or coding sequence resulting in defective Treg cells.[7–9] Similarly, mice with naturally occurring or targeted defects in *foxp3* develop similar clinical features (lymphoproliferation, colitis, weight loss, diabetes and ruffled hair) associated with systemic autoimmunity, and become moribund within 20–25 days of birth.[6,8,10] Accordingly, Foxp3$^+$ Treg cells are essential for maintaining peripheral immune tolerance in humans and mice, and these parallels in clinical features with Treg deficiency illustrate the usefulness of mouse models to investigate how Treg cells may control other facets of the immune response.

In this regard, shortly after their identification as a distinct CD4$^+$ T-cell lineage, numerous studies using various representative mouse models of parasitic, viral and bacterial infections have described an important role for CD25$^+$ CD4 T cells in compromising host defence by suppressing the activation of protective immune components.[11–20] As the ablation of CD25-expressing cells almost uniformly augmented resistance with reduced recoverable *in vivo* pathogen burden, Treg cells were appropriately described as '*a dangerous necessity*' based on their detrimental roles in host defence and essential roles in sustaining immune tolerance.[21] However, with the subsequent identification of Foxp3 as the lineage-defining marker for Treg cells, and the up-regulation of CD25 expression on activated T cells that occurs after infection, the conclusions of initial studies using CD25 expression as a surrogate marker for Treg cells deserve critical re-evaluation using experimental strategies that identify and manipulate these cells based on Foxp3 expression.

This review will summarize the recent literature describing infection outcomes and the immune response to infection using approaches that manipulate Treg cells based on Foxp3 expression, and frame these conclusions in the context of previous studies evaluating the importance of CD25$^+$ CD4$^+$ Treg cells and the epidemiology of human infection. Although an over-simplification, this analysis will be subdivided for pathogens that primarily cause acute versus persistent infection. For each type of infection, the impacts resulting from the manipulation of Foxp3$^+$ cells in infection outcomes, relevance of Foxp3$^+$ Treg-cell antigen specificity and individual Foxp3$^+$ cell intrinsic molecules in mediating immune suppression are discussed (Table 1). Lastly, how shifts in Treg-cell suppression impact infection outcomes and our more basic understanding for how T cells are activated *in vivo* are also summarized.

Host defence against acute infection

Pathogens that cause acute infection stimulate the activation of protective immune components almost immediately after infection. When the pathogen dose or initial rate of pathogen replication are below a preset threshold (lethal dose), innate immune components keep the infection at bay until pathogen-specific adaptive immune effectors that more efficiently mediate pathogen eradication are expanded and mobilized. On the other hand, with higher inocula, these normally protective responses are overwhelmed and the host succumbs to infection. It is in this latter context that initial studies using Foxp3^{DTR} transgenic mice that co-express the high-affinity human diphtheria toxin (DT) receptor with Foxp3, allowing Foxp3$^+$ Treg cells to be selectively ablated with low-dose DT, first uncovered somewhat paradoxical protective roles for these cells in host defence. For example, the ablation of Foxp3$^+$ cells before intra-vaginal infection with herpes simplex virus 2 caused accelerated mortality that was associated with delayed recruitment of protective immune cells into the vaginal tract and draining lymph nodes, and more recoverable virus at the site of infection.[22] These protective effects were not limited to mucosal infection with this pathogen because mice that had undergone Foxp3$^+$ cell ablation also contained increased titres of lymphocytic choriomeningitis virus after systemic infection that was associated with reduced lymph node chemokine levels.[22] Similarly, Foxp3$^+$ Treg-cell ablation before West Nile virus infection in mice caused increased mortality, worse clinical disease scores, and accelerated weight loss that were each associated with higher viral loads in the brain and spinal cord.[23] These results also parallel the lower frequency of Treg cells in humans with symptomatic West Nile virus infection, and an increased ratio of Treg cells to effector T cells in patients with mild compared with severe Dengue virus infection.[23,24] Accordingly, these first studies investigating infection susceptibility using Foxp3^{DTR} mice to ablate Treg cells based on Foxp3 expression established protective roles for these cells in host defence against specific viral pathogens.

In this regard, although Treg-cell ablation using anti-CD25 antibody had been reported to exacerbate inflammatory lesions in herpes simplex virus 1-induced stromal keratitis, manipulating Treg cells in this manner also accelerated the eradication of this virus.[13,14] Therefore, despite the potential for other inherent differences in these more recent studies where Treg cells were ablated based on Foxp3 expression compared with

Table 1. Impacts of Foxp3$^+$ cell manipulation on host defence

Class	Pathogen	Impacts of Foxp3$^+$ cell manipulation on host defence		References
Virus	Herpes simplex virus 2	Protective	Foxp3$^+$ cell ablation accelerates mortality and increases viral load	22
	Lymphocytic choriomeningitis virus	Protective	Foxp3$^+$ cell ablation increases viral load	22
	West Nile virus	Protective	Foxp3$^+$ cell ablation increases viral load and mortality	23
Parasite	*Plasmodium berghei*	Protective	Foxp3$^+$ cell expansion protects against severe disease and reduces pathogen burden, which are each reversed by Foxp3$^+$ cell ablation	25
	Plasmodium berghei	No effect	Foxp3$^+$ cell ablation from baseline levels has no impact on survival or pathogen burden	25,31
	Toxoplasma gondii	Protective	Natural collapse of Foxp3$^+$ Treg cells following infection results in fatal infection that is reversed by Treg-cell stabilization using interleukin-2 cytokine–antibody complexes	32
	Heligmosomoides polygyrus	No effect	No changes in pathogen burden with Foxp3$^+$ cell ablation	68
Fungi	*Candida albicans*	Protective	Tregs co-transferred with effector CD4$^+$ T cells enhance fungal clearance	33
Bacteria	*Listeria monocytogenes*	Detrimental	Foxp3$^+$ cell expansion results in increased pathogen burden that is reversed by Foxp3$^+$ cell ablation	36
	Salmonella enterica	Detrimental	Foxp3$^+$ cell expansion during pregnancy results in increased pathogen burden that is reversed by Foxp3$^+$ cell ablation	36
	Salmonella enterica	Detrimental	Foxp3$^+$ cell ablation accelerates bacterial clearance and effector T-cell activation	59
Mycobacteria	*Mycobacterium tuberculosis*	Detrimental	Selective depletion of Foxp3$^+$ cells in mixed chimera mice reduces pathogen burden	58
	Mycobacterium tuberculosis	Detrimental	Adoptive transfer of pathogen-specific Foxp3$^+$ cells blunts effector cell expansion and increased pathogen burden	70

CD25 expression, these findings suggest that differences in how Treg cells are manipulated can lead to discordant conclusions. In particular, because CD25 expression is up-regulated by effector T cells upon activation, experimental approaches that exclusively identify and manipulate Treg cells based on this surrogate marker do not discriminate between activated effector T cells stimulated by infection and bona fide Treg cells. Therefore, initial conclusions regarding the role of Treg cells in host defence for each specific pathogen using strategies that manipulate these cells based on CD25 expression should be interpreted with caution, and re-investigated using Foxp3-specific reagents for experimentally manipulating Treg cells.

Consistent with these newfound beneficial roles for Foxp3$^+$ Treg cells in host defence after viral infection, similar protective roles for Foxp3$^+$ cells have also been described for other types of pathogens. For example, after infection with *Plasmodium berghei* in a mouse model of cerebral malaria, the expansion of Treg cells using IL-2 cytokine antibody complexes confers protection against severe disease that is associated with reduced parasite burden.[25] These protective effects were the result of expanded Foxp3$^+$ cells because their ablation in infected mice where Treg cells are susceptible to DT-induced ablation eliminated the impacts of IL-2 cytokine antibody complex treatment.[25] On the other hand for malaria infection in humans, Treg-cell frequency appears to correlate more directly with parasite biomass and disease severity, which illustrates more complex roles for Treg cells, immune stimulation and immune-related disease sequelae for human malaria infection in humans that are not recapitulated in mice.[26–30] Interestingly, despite the increasingly established importance of Treg cells in *Plasmodium* infection, the experimental ablation of Treg cells from baseline levels using Foxp3-specific reagents did not significantly impact infection susceptibility.[25,31] These findings illustrate that the potential importance of Treg cells in host defence for some infections is better appreciated using gain-of-function experimental approaches. Similarly, Treg-cell expansion with IL-2 cytokine antibody complexes also averts the natural collapse in Foxp3$^+$ cells after *Toxoplasma gondii* infection and rescues mice from fatal immune pathology triggered by this infection.[32] Furthermore, Foxp3$^+$ Treg cells also synergize with T helper type 17 (Th17) effector CD4$^+$ T cells in eradicating *Candida albicans* after oral infection.[33] Taken

together, these findings indicate Foxp3$^+$ Treg cells play more generalizable protective roles that extend to host defence against parasitic and fungal pathogens.

On the other hand, using similar gain-of-function and loss-of-function experimental approaches for *in vivo* manipulation of these cells, Foxp3$^+$ Treg cells have consistently been shown to impede host defence following infection with bacterial pathogens. This is best illustrated in the context of pregnancy-associated infection susceptibility where the physiological expansion of maternal Treg cells required for sustaining tolerance to paternally derived allo-antigens expressed by the developing fetus occurs.[34,35] In particular, following allogeneic mating using defined strains of inbred mice that more closely recapitulates the magnitude of maternal Treg-cell expansion found in human pregnancy, mice with expanded maternal Treg cells are markedly more susceptible to infection with intracellular bacterial pathogens like *Listeria monocytogenes* and *Salmonella enterica*, each with a natural predisposition for prenatal infection.[36–39] Reciprocally, pregnancy-associated susceptibility to these pathogens was eliminated with maternal Foxp3$^+$ cell ablation when allogeneic pregnancies were established in Foxp3^{DTR} female mice followed by the initiation of DT treatment beginning mid-gestation.[36] However, given the necessity for sustained fetal tolerance maintained by expanded maternal Treg cells, the ablation of these cells although beneficial for host defence also triggers fetal resorption and pregnancy loss.[34–36] In a similar fashion, the expansion of Foxp3$^+$ Treg cells within the first 3 days after intranasal *Francisella tularensis* infection has been described to blunt early innate host defence that may represent a unique immune evasion strategy for this pathogen.[40]

The significance of expanded immune suppressive Foxp3$^+$ Treg cells in compromising host defence against prenatal infection is further supported by increased susceptibility to *Listeria* for non-pregnant mice with expanded Foxp3$^+$ Treg cells that express a constitutively active isoform of signal transducer and activator of transcription (STAT)-5b, and reduced susceptibility with the ablation of expanded Treg cells in these and control mice with baseline levels of Treg cells.[36,41] Therefore, while intracellular bacterial pathogens like *Listeria* and *Salmonella* are capable of *in utero* fetal invasion,[39,42,43] infection susceptibility during pregnancy is not simply the result of the presence of fetal tissue that is susceptible to direct invasion, and instead more likely reflects systemic defects in host defence dictated by expanded maternal Treg cells. These findings with experimental *Listeria* infection in mice are also consistent with the epidemiological features of this infection in humans where a significant portion of disseminated maternal infection cases occur without evidence of fetal direct invasion.[38] Hence, the physiological expansion of maternal Foxp3$^+$ Treg cells during pregnancy compromises host defence, and these immune defects are exploited by pathogens like *Listeria* and *Salmonella* with a predisposition for prenatal infection. Importantly, since the expansion of maternal Treg cells is blunted during syngeneic pregnancy, where the only potential sources of antigen heterogeneity between maternal and fetal antigens are those encoded on the Y chromosome, the importance of expanded maternal Treg cells in host defence for other prenatal pathogens may have been overlooked in previous studies, and deserve re-investigation using allogeneic pregnancy.

The impacts on host defence dictated by the physiological expansion of immune suppressive Treg cells also have broader implications beyond this instance of prenatal infection susceptibility. For example, the progressive expansion of Treg cells among peripheral CD4$^+$ T cells occurs with aging throughout the lifespan of humans and mice.[44–47] In particular, individuals over 60 years have a threefold increased proportion of Treg cells compared with individuals less than 40 years.[44,45] In turn, when pregnancy-associated cases are excluded, individuals over 60 years are also markedly more susceptible to disseminated *Listeria* infection compared with those < 60 years.[48] Reciprocally following natural West Nile virus infection, symptomatic infection is more common in younger than older individuals, and these findings are consistent with the protective role provided by Treg cells in this infection.[23,49] However, the expansion of Treg cells with aging alone does not explain other epidemiological data for this infection where individuals over 70 years compared with those aged 20–69 years have fivefold increased mortality with West Nile virus infection.[50] Together, these findings suggest that other physiological changes with aging play more significant roles in infection-induced mortality despite the expansion of protective Treg cells. Nevertheless, these results illustrate how physiological shifts in Treg cells probably dictate naturally occurring variations in susceptibility to specific pathogens among individuals.

Although these results may suggest that susceptibility to some infections, and bacterial pathogens in particular, are unavoidable consequences of pregnancy and aging, the increasingly established heterogeneity and functional specialization among Foxp3$^+$ cells also opens up the exciting possibility of therapeutically dissociating the Treg-cell-mediated detrimental impacts on infection susceptibility against some pathogens from their protective roles in other types of infections and their beneficial roles in maintaining immune tolerance.[51–54] For example, Treg cells are enriched for cytotoxic T-lymphocyte antigen 4 (CTLA-4) expression, and the sustained ablation of CTLA-4 exclusively in Foxp3$^+$ cells throughout development results in non-specific T-cell activation and systemic autoimmunity.[55,56] Importantly, whereas CTLA-4 ablation in Foxp3$^+$ cells reproduces some features of Treg-cell deficiency, it does not recapitulate the more rapid onset of

fatal systemic autoimmunity in mice with naturally occurring or targeted defects in all Treg cells because of defects in Foxp3.[4,6] In contrast, sustained ablation of IL-10 in Foxp3$^+$ cells throughout development results in minimal systemic autoimmunity, but instead causes inflammation limited to sites with contact to the external environment such as the skin, lung and intestine.[57] This discordance in phenotype with sustained ablation of defined molecules in Foxp3$^+$ cells illustrates non-overlapping and specialized context-specific roles for individual Treg-cell intrinsic molecules in immune tolerance. However, the ablation of each Treg-cell intrinsic molecule throughout development using this approach precludes the investigation into how each molecule impacts host defence against infection, which ideally requires the synchronized and coordinated ablation of each molecule in all Foxp3$^+$ cells in adult mice. Using adoptively transferred Treg cells containing targeted defects in individual Treg-cell intrinsic molecules to reconstitute Foxp3$^+$ cell ablated mice overcomes this technical barrier for systemically interrogating the importance of each Treg-cell intrinsic molecule in host defence against acute infection. Our initial studies using this approach illustrate that Treg-cell intrinsic IL-10, but not CTLA-4, participates in compromising host defence against *Listeria monocytogenes*.[36] Therefore, establishing the Foxp3$^+$ cell intrinsic molecules that compromise or augment host defence, and dissociating these from the Treg-cell intrinsic molecules required for sustaining immune tolerance represent pivotally important next steps in this exciting area with enormous translational implications.

Host defence against persistent infection

In contrast to acute infection, where the expansion of pathogen-specific adaptive immune effectors generally coincides with pathogen eradication, pathogens that cause persistent infection have developed strategies to evade and co-exist with pathogen-specific immune components. On the other hand, allowing pathogen persistence by dampening immune activation may also be beneficial when immune-mediated collateral damage to the host outweighs injury caused by pathogen persistence. In this regard, Treg cells play important roles in counterbalancing immune effectors during persistent infection. This was first described 10 years ago for *Leishmania major* infection, where immune suppression by CD25$^+$ CD4$^+$ Treg cells was found to promote pathogen persistence in the skin after intra-dermal infection.[11]

More recently, these findings have been recapitulated for other persistent infections using more refined strategies that allow Treg-cell manipulation based on Foxp3 expression. For example, the ablation of Foxp3$^+$ cells based on selective expression of the Thy1.1 congenic marker in mixed bone marrow chimera mice before pulmonary infection with *Mycobacterium tuberculosis* stimulates more robust effector CD4$^+$ T-cell interferon-γ production and reduced pathogen burden at the site of infection.[58] Similarly, Foxp3$^+$ Treg cells provide a similar protective role in a model of typhoid fever caused by persistent *Salmonella* infection in *Nramp1*-resistant mice.[59] At early time-points following infection when the activation of effector T cells is blunted and progressively increasing *Salmonella* bacterial burden occurs, Treg-cell ablation in Foxp3^{DTR} mice accelerates the activation of effector T cells with significant reductions in recoverable bacteria.[59] In turn, at later time-points during persistent *Salmonella* infection when effector T cells are already activated and progressive reductions in pathogen burden naturally occur, the impacts of Foxp3$^+$ cell ablation are marginalized with only modest incremental augmentation of effector T-cell activation and no significant changes in pathogen burden.[59] Hence, Foxp3$^+$ Treg cells blunt effector T-cell activation that impedes pathogen eradication, and the significance of Treg-cell-mediated immune suppression can shift and dictate the tempo of some persistent infections. Although these results suggest that Treg cells play detrimental roles in host defence by preventing pathogen eradication, the reduced susceptibility against secondary infection related to low-level pathogen persistence for other pathogens (e.g. *Leishmania* and *Plasmodium*) illustrates that Treg cells may in fact provide protection against more severe disseminated infection with potentially fatal consequences.[30,60,61] It will be interesting to investigate if these Treg-cell-mediated protective activities against secondary infection are more broadly applicable for other pathogens that cause persistent infection.

The co-expression of many effector CD4$^+$ T-cell lineage-promoting transcription factors by Foxp3$^+$ Treg cells that allows functionally distinct Treg-cell subsets to expand in parallel with effector T cells has been recently established. For example, Treg cells that express the Th1 lineage defining transcription factor T-bet expand with Th1 effector CD4$^+$ T cells following Th1 stimulation conditions, whereas the ablation of T-bet specifically in Foxp3$^+$ cells results in uncontrolled Th1 inflammation and autoimmunity.[62] Similarly, Foxp3$^+$ cell expression of the transcription factors signal transducers and activators of transcription (STAT)-3, interferon regulatory factor (IRF)-4, B cell lymphoma protein (BCL)-6 and GATA-3 have each been shown to suppress other specialized effector CD4$^+$ T-cell subsets that would otherwise cause unchecked self-reactive inflammation.[63–67] Importantly, the specialization and dynamic regulation among these various Treg-cell subsets also play important roles in coordinating and fine-tuning immune responses after infection. For example, under Th1 inflammatory conditions triggered by *M. tuberculosis*, T-bet-expressing Treg cells and effector T cells both expand and are recruited into the sites of infection creating a balanced response that facilitates pathogen control, but not eradication.[62] On the other hand, under

Th2 inflammatory conditions triggered by pulmonary thymic stromal lymphopoietin or intestinal *Heligmosomoides polygyrus* infection, T-bet$^+$ Treg cells fail to accumulate and are instead replaced by Treg cells enriched for the Th2 promoting transcription factor GATA-3.[62,67] Interestingly, although the ablation of Foxp3$^+$ Treg cells early after *H. polygyrus* infection augments parasite-specific effector Th2 responses and intestinal inflammation, no significant impacts of pathogen burden or fitness were identified.[68]

Specialization among Treg cells during persistent infection is not limited to expression of CD4$^+$ T-cell lineage-defining transcription factors, but also extends to individual cell intrinsic molecules that probably mediate immune suppression. Foxp3$^+$ Treg cells recovered from the pulmonary lymph node and lung selectively up-regulate expression of inducible T-cell co-stimulator (ICOS) and programmed death (PD)-1 at relatively early and late time point respectively, after aerosol *M. tuberculosis* infection whereas these shifts do not occur for Treg cells in lymph nodes that do not drain the site of infection.[58] Similarly when the impacts of Treg-cell ablation are progressively reduced from early to late time-points after systemic *Salmonella* infection, Foxp3$^+$ Treg cells in the spleen progressively lose CTLA-4 expression that is replaced by increased glucocorticoid-induced tumor necrosis factor receptor (GITR) expression.[59] Hence, functionally distinct Treg-cell subsets that express unique combinations of cell intrinsic molecules accumulate and shift throughout the course of persistent infection. Establishing the role of individual Treg-cell intrinsic molecules in dictating the progression of persistent infection, and investigating how cell intrinsic shifts in expression of each effector CD4$^+$ T-cell lineage defining transcription factors controls Treg-cell suppression represent important next steps in further unravelling the dynamic interplay between Treg cells and effector T cells in host defence during persistent infection.

In addition to the parallel accumulation of lineage-specific Treg cells and effector T cells, the co-expansion of Foxp3$^+$ and Foxp3$^-$ CD4$^+$ T cells exhibiting the same specificity for pathogen-associated antigens also occurs during some persistent infections. For example, Treg cells and effector T cells with specificity to the same pathogen-expressed antigen expand in parallel following intradermal *Leishmania*, pulmonary *M. tuberculosis*, systemic *Salmonella*, or intracerebral coronavirus infections.[59,69–71] By contrast, for other infections including those caused by *Listeria monocytogenes* in immune-competent mice and persistent Friend retrovirus in B-cell-deficient and CD8$^+$ T-cell-deficient mice, only the selective expansion of pathogen-specific Foxp3$^-$ effector CD4$^+$ T cells occur.[72,73] However, for persistent infections that prime the expansion of pathogen-specific Treg cells, these cells are likely to play pivotally important roles in pathogen persistence because augmenting the absolute numbers of these cells in *M. tuberculosis*-infected mice results in dose-dependent increased pathogen burden and delayed expansion of pathogen-specific effector T cells.[70] Similarly, Foxp3$^+$ Treg cells with specificity to defined species of enteric commensal bacteria are found in intestinal tissues, and these cells selectively avert intestinal inflammation in colonized mice.[74] Hence, with the identification of more microbe-specific MHC class II peptide antigens and the development of enrichment tools to track very small populations of antigen-specific CD4$^+$ T cells,[75] microbe-specific Foxp3$^+$ Treg cells will undoubtedly be shown to play more significant roles in regulating both host defence and immune homeostasis. In this regard, interrogating the differentiation stability for pathogen-specific Treg cells, and investigating if the functional plasticity described for Treg cells with specificity for self-antigen is applicable for infection-induced Treg cells represent important areas for further investigation.[71,76,77]

Plasticity in Treg-cell suppression

Given the active immune suppression by Treg cells that occurs *in vivo*, counter-regulatory mechanisms that override Treg-cell suppression must be engaged when immune activation occurs naturally during infection or immunization. In this regard, several infection response pathways have been shown to bypass the impacts of Treg-cell suppression. For example, stimulation of antigen-presenting cell (APCs) with highly conserved microbial ligands (e.g. lipopolysaccharide or CpG DNA) through Toll-like receptors (TLRs) drives effector T-cell proliferation despite the presence of Treg cells.[78] This is in large part mediated by IL-6 production by activated APCs because effector T-cell proliferation is reduced when IL-6-deficient APCs or this cytokine are neutralized in co-culture.[78] Similarly, other purified TLR agonists and inflammatory cytokines that induce the maturation of dendritic cells and augment expression of cell surface molecules that promote T-cell stimulation (e.g. CD80, CD86 and MHC) have also been reported to override Treg-cell suppression through IL-6-independent pathways.[79–81] Even in the absence of APCs, cell-intrinsic stimulation through defined TLRs can also trigger shifts in Treg-cell suppression. For example, purified TLR2 agonists stimulate reductions in suppressive potency for mouse Treg cells, and TLR8 agonists trigger similar reductions in potency for human Treg cells.[82–84] On the other hand, microbial ligands can also augment Treg-suppressive potency. Mouse CD25$^+$ Treg cells selectively express TLR4, and lipopolysaccharide stimulation augments their suppressive potency;[85] whereas flagellin stimulation via TLR5 augments the suppressive potency of human Treg cells.[86] Taken together, these *in vitro* studies illustrate the enormous potential whereby microbes and the response to infection can influence immune activation through shifts in Treg-cell suppression.

The cumulative impacts whereby pathogens that express multiple TLR ligands and the ensuing immune response on shifts in Treg-suppressive potency have also been characterized for green fluorescent protein-positive (GFP$^+$) cells recovered from Foxp3^{GFP} reporter mice directly *ex vivo* following infection.[87] For example, at relatively early time-points during persistent *Salmonella* infection, when the activation of effector T cells is blunted and the pathogen burden is progressively increasing, the suppressive potency for GFP$^+$ Treg cells is augmented.[59] Conversely, at later infection time-points when effector T cells are highly activated and progressive reductions in pathogen burden occur, the suppressive potency for Foxp3$^+$ cells is reduced. Together with the waning impacts of Foxp3$^+$ cell ablation with infection progression, these results illustrate how shifts in Treg-cell suppression can dictate the tempo of persistent infection.[59] Similarly, following acute *Listeria* infection, reductions in suppressive potency are found for GFP$^+$ Treg cells that immediately precede the expansion of pathogen-specific effector T cells.[88] The expansion of circulating Treg cells with increased suppressive potency is associated with increased parasite burdens for patients with severe malaria infection.[26] However, no significant changes in suppressive potency were found for Foxp3$^+$ Treg cells isolated directly *ex vivo* after *Plasmodium berghei* infection in mice.[31] Nevertheless, these findings illustrate how infection-induced shifts in Foxp3$^+$ Treg-cell suppressive potency may play important and increasingly appreciated roles in infection outcomes.

Importantly, the plasticity in Treg-cell suppression can also be exploited for boosting the immune response of vaccines against pathogen-specific or tumour-associated antigens.[76,89,90] In this regard, reduced Treg-cell suppression after stimulation with various purified microbial ligands suggests that classical vaccine adjuvants derived from crude microbial preparations may simulate immune activation by overriding Treg-mediated immune suppression. Indeed, the transient ablation of Foxp3$^+$ cells alone during stimulation with purified peptide is sufficient to trigger the robust activation, expansion and formation of memory CD8$^+$ T cells, which confers protection against subsequent *Listeria* infection in an antigen-specific fashion.[88] Similarly, Foxp3$^+$ cell ablation augments the expansion and activation of antigen-specific CD8$^+$ T-cells primed by the live attenuated viral vector modified vaccinia virus Ankara.[91] These findings are consistent with the enhanced vaccine-induced immunogenicity that occurs with Treg-cell ablation using anti-CD25 antibody treatment, and the sustained priming of protective CD8$^+$ T cells by attenuated *Listeria* even in mice lacking all known signal 3 inflammatory cytokines.[92–97] Hence, overriding immune suppression by Treg cells probably plays pivotally important roles in stimulating protective T-cell responses *in vivo*. However, while immune adjuvants and vaccine vectors have traditionally been evaluated for their ability to activate T cells indirectly through stimulation of professional APCs that in turn elaborate defined stimulation signals [T-cell receptor (signal 1), co-stimulation (signal 2), and inflammatory cytokines (signal 3)],[95,97,98] overriding active suppression by Treg cells probably represents a more fundamental prerequisite 'signal zero' essential for stimulating effector T-cell activation *in vivo*. Although this term has recently been used to describe the activation of innate immunity or chemokine gradients that each also participate in T-cell activation,[99,100] we propose that this descriptor is more appropriate for overriding the impacts of suppression mediated by Treg cells and other immune suppressive cells, which actively restrains T-cell activation (Fig. 1).

Concluding remarks

Since the identification of Treg cells as a separate and defined lineage of CD4$^+$ T cells, there has been an

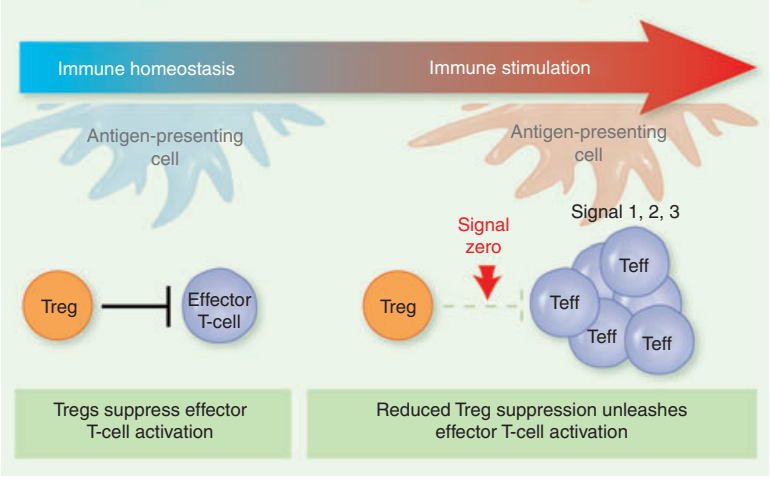

Figure 1. Model whereby overriding Foxp3$^+$ regulatory T (Treg) cell-mediated immune suppression represents a prerequisite 'signal zero' for effector T (Teff) cell activation *in vivo*. During immune homeostasis, Treg cells actively suppress effector cell activation. Following immune stimulation, Treg-cell suppression is blunted allowing effector T-cell activation through previously described cell intrinsic stimulation signals [T-cell receptor (signal 1), co-stimulation (signal 2), inflammatory cytokines (signal 3)].

explosion of studies describing the role these cells play in almost every aspect of the immune response. With the establishment of Foxp3 expression as the lineage-specific marker for Treg cells and the development of transgenic mouse tools for manipulating Foxp3$^+$ cells *in vivo*, new-found protective roles for these cells in host defence against some infections have been uncovered. In turn, for other infections, the detrimental roles played by Foxp3$^+$ cells in host defence have been reinforced. Along the way, an amazing degree of heterogeneity and functional specialization for Foxp3$^+$ Treg cells in terms of antigen specificity, paralleled differentiation along effector CD4$^+$ T-cell lineages, and use of individual cell intrinsic molecules to mediate context-specific immune suppression have each been established. Coupled with increasing refined approaches for expanding human regulatory T cells or manipulating the suppressive potency of these cells using purified adjuvants,[89,90,101,102] these multiple layers of heterogeneity in regulatory T cells reveal many exciting opportunities for therapeutically dissociating the detrimental and beneficial impacts that these cells play in host defence against infection and immune homeostasis.

In concluding the seven-volume *Chronicles of Narnia* series, C.S. Lewis described their adventures as only 'the cover and title page'. In this regard, given the enormous latent potential and arsenal of immune effectors uncovered with the identification of immune suppressive Treg cells together with the ongoing disproportionate burden of infection-related diseases that negatively impact human health, more potent and efficacious immune-mediated therapies for infectious disease treatment and prevention are poised for development. With the identification of Treg cells and the tremendous translational potential associated with therapeutically manipulating newly established facets of the dynamic interplay between Treg cells and immune effectors, chapter one of a great story related to reduced burden of infectious diseases is ready to be written.

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References

1 Sakaguchi S. Naturally arising CD4$^+$ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu Rev Immunol* 2004; **22**:531–62.

2 Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; **155**:1151–64.

3 Suri-Payer E, Amar AZ, Thornton AM, Shevach EM. CD4$^+$ CD25$^+$ T cells inhibit both the induction and effector function of autoreactive T cells and represent a unique lineage of immunoregulatory cells. *J Immunol* 1998; **160**:1212–8.

4 Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4$^+$ CD25$^+$ regulatory T cells. *Nat Immunol* 2003; **4**:330–6.

5 Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* (New York, NY) 2003; **299**:1057–61.

6 Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4$^+$ CD25$^+$ T regulatory cells. *Nat Immunol* 2003; **4**:337–42.

7 Bennett CL, Christie J, Ramsdell F *et al.* The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 2001; **27**:20–1.

8 Wildin RS, Ramsdell F, Peake J *et al.* X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat Genet* 2001; **27**:18–20.

9 Ochs HD, Gambineri E, Torgerson TR. IPEX, FOXP3 and regulatory T-cells: a model for autoimmunity. *Immunol Res* 2007; **38**:112–21.

10 Brunkow ME, Jeffery EW, Hjerrild KA *et al.* Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 2001; **27**:68–73.

11 Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, Sacks DL. CD4$^+$ CD25$^+$ regulatory T cells control *Leishmania major* persistence and immunity. *Nature* 2002; **420**:502–7.

12 Kursar M, Koch M, Mittrucker HW, Nouailles G, Bonhagen K, Kamradt T, Kaufmann SH. Cutting edge: regulatory T cells prevent efficient clearance of *Mycobacterium tuberculosis*. *J Immunol* 2007; **178**:2661–5.

13 Suvas S, Kumaraguru U, Pack CD, Lee S, Rouse BT. CD4$^+$ CD25$^+$ T cells regulate virus-specific primary and memory CD8$^+$ T cell responses. *J Exp Med* 2003; **198**:889–901.

14 Suvas S, Azkur AK, Kim BS, Kumaraguru U, Rouse BT. CD4$^+$ CD25$^+$ regulatory T cells control the severity of viral immunoinflammatory lesions. *J Immunol* 2004; **172**:4123–32.

15 Fulton RB, Meyerholz DK, Varga SM. Foxp3$^+$ CD4 regulatory T cells limit pulmonary immunopathology by modulating the CD8 T cell response during respiratory syncytial virus infection. *J Immunol* 2010; **185**:2382–92.

16 Kaparakis M, Laurie KL, Wijburg O, Pedersen J, Pearse M, van Driel IR, Gleeson PA, Strugnell RA. CD4$^+$ CD25$^+$ regulatory T cells modulate the T-cell and antibody responses in *Helicobacter*-infected BALB/c mice. *Infect Immun* 2006; **74**:3519–29.

17 Netea MG, Sutmuller R, Hermann C *et al.* Toll-like receptor 2 suppresses immunity against *Candida albicans* through induction of IL-10 and regulatory T cells. *J Immunol* 2004; **172**:3712–8.

18 Taylor JJ, Mohrs M, Pearce EJ. Regulatory T cell responses develop in parallel to Th responses and control the magnitude and phenotype of the Th effector population. *J Immunol* 2006; **176**:5839–47.

19 Taylor MD, Harris A, Babayan SA, Bain O, Culshaw A, Allen JE, Maizels RM. CTLA-4 and CD4$^+$ CD25$^+$ regulatory T cells inhibit protective immunity to filarial parasites *in vivo*. *J Immunol* 2007; **179**:4626–34.

20 Zelinskyy G, Kraft AR, Schimmer S, Arndt T, Dittmer U. Kinetics of CD8$^+$ effector T cell responses and induced CD4$^+$ regulatory T cell responses during Friend retrovirus infection. *Eur J Immunol* 2006; **36**:2658–70.

21 Belkaid Y. Regulatory T cells and infection: a dangerous necessity. *Nat Rev* 2007; **7**:875–88.

22 Lund JM, Hsing L, Pham TT, Rudensky AY. Coordination of early protective immunity to viral infection by regulatory T cells. *Science* (New York, NY) 2008; **320**:1220–4.

23 Lanteri MC, O'Brien KM, Purtha WE *et al.* Tregs control the development of symptomatic West Nile virus infection in humans and mice. *J Clin Investig* 2009; **119**:3266–77.

24 Luhn K, Simmons CP, Moran E *et al.* Increased frequencies of CD4$^+$ CD25^{high} regulatory T cells in acute dengue infection. *J Exp Med* 2007; **204**:979–85.

25 Haque A, Best SE, Amante FH *et al.* CD4$^+$ natural regulatory T cells prevent experimental cerebral malaria via CTLA-4 when expanded *in vivo*. *PLoS Pathog* 2010; **6**:e1001221.

26 Minigo G, Woodberry T, Piera KA *et al.* Parasite-dependent expansion of TNF receptor II-positive regulatory T cells with enhanced suppressive activity in adults with severe malaria. *PLoS Pathog* 2009; **5**:e1000402.

27 Walther M, Jeffries D, Finney OC *et al.* Distinct roles for FOXP3 and FOXP3 CD4 T cells in regulating cellular immunity to uncomplicated and severe *Plasmodium falciparum* malaria. *PLoS Pathog* 2009; **5**:e1000364.

28 Walther M, Tongren JE, Andrews L *et al.* Upregulation of TGF-beta, FOXP3, and CD4$^+$ CD25$^+$ regulatory T cells correlates with more rapid parasite growth in human malaria infection. *Immunity* 2005; **23**:287–96.

29 Torcia MG, Santarlasci V, Cosmi L *et al.* Functional deficit of T regulatory cells in Fulani, an ethnic group with low susceptibility to *Plasmodium falciparum* malaria. *Proc Natl Acad Sci U S A* 2008; **105**:646–51.

30 Hansen DS, Schofield L. Natural regulatory T cells in malaria: host or parasite allies? *PLoS Pathog* 2010; **6**:e1000771.

31 Steeg C, Adler G, Sparwasser T, Fleischer B, Jacobs T. Limited role of CD4$^+$ Foxp3$^+$ regulatory T cells in the control of experimental cerebral malaria. *J Immunol* 2009; **183**:7014–22.

32 Oldenhove G, Bouladoux N, Wohlfert EA *et al.* Decrease of Foxp3$^+$ Treg cell number and acquisition of effector cell phenotype during lethal infection. *Immunity* 2009; **31**:772–86.

33 Pandiyan P, Conti HR, Zheng L *et al.* CD4$^+$ CD25$^+$ Foxp3$^+$ regulatory T cells promote Th17 cells *in vitro* and enhance host resistance in mouse *Candida albicans* Th17 cell infection model. *Immunity* 2011; **34**:422–34.

34 Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. *Nat Immunol* 2004; **5**:266–71.

35 Kahn DA, Baltimore D. Pregnancy induces a fetal antigen-specific maternal T regulatory cell response that contributes to tolerance. *Proc Natl Acad Sci U S A* 2010; **107**:9299–304.

36 Rowe JH, Ertelt JM, Aguilera MN, Farrar MA, Way SS. Foxp3$^+$ regulatory T cell expansion required for sustaining pregnancy compromises host defense against prenatal bacterial pathogens. *Cell Host Microbe* 2011; **10**:54–64.

37 Gellin BG, Broome CV, Bibb WF, Weaver RE, Gaventa S, Mascola L. The epidemiology of listeriosis in the United States—1986. Listeriosis Study Group. *Am J Epidemiol* 1991; **133**:392–401.

38 Mylonakis E, Paliou M, Hohmann EL, Calderwood SB, Wing EJ. Listeriosis during pregnancy: a case series and review of 222 cases. *Medicine* 2002; **81**:260–9.

39 Pejcic-Karapetrovic B, Gurnani K, Russell MS, Finlay BB, Sad S, Krishnan L. Pregnancy impairs the innate immune resistance to *Salmonella typhimurium* leading to rapid fatal infection. *J Immunol* 2007; **179**:6088–96.

40 Periasamy S, Singh A, Sahay B, Rahman T, Feustel PJ, Pham GH, Gosselin EJ, Sellati TJ. Development of tolerogenic dendritic cells and regulatory T cells favors exponential bacterial growth and survival during early respiratory tularemia. *J Leukoc Biol* 2011; **90**:493–507.

41 Burchill MA, Goetz CA, Prlic M *et al.* Distinct effects of STAT5 activation on CD4$^+$ and CD8$^+$ T cell homeostasis: development of CD4$^+$ CD25$^+$ regulatory T cells versus CD8$^+$ memory T cells. *J Immunol* 2003; **171**:5853–64.

42 Bakardjiev AI, Theriot JA, Portnoy DA. *Listeria monocytogenes* traffics from maternal organs to the placenta and back. *PLoS Pathog* 2006; **2**:e66.

43 Le Monnier A, Autret N, Join-Lambert OF, Jaubert F, Charbit A, Berche P, Kayal S. ActA is required for crossing of the fetoplacental barrier by *Listeria monocytogenes*. *Infect Immun* 2007; **75**:950–7.

44 Gregg R, Smith CM, Clark FJ, Dunnion D, Khan N, Chakraverty R, Nayak L, Moss PA. The number of human peripheral blood CD4$^+$ CD25^{high} regulatory T cells increases with age. *Clin Exp Immunol* 2005; **140**:540–6.

45 Gottenberg JE, Lavie F, Abbed K, Gasnault J, Le Nevot E, Delfraissy JF, Taoufik Y, Mariette X. CD4 CD25^{high} regulatory T cells are not impaired in patients with primary Sjögren's syndrome. *J Autoimmun* 2005; **24**:235–42.

46 Sharma S, Dominguez AL, Lustgarten J. High accumulation of T regulatory cells prevents the activation of immune responses in aged animals. *J Immunol* 2006; **177**:8348–55.

47 Lages CS, Suffia I, Velilla PA, Huang B, Warshaw G, Hildeman DA, Belkaid Y, Chougnet C. Functional regulatory T cells accumulate in aged hosts and promote chronic infectious disease reactivation. *J Immunol* 2008; **181**:1835–48.

48 Gillespie IA, McLauchlin J, Grant KA, Little CL, Mithani V, Penman C, Lane C, Regan M. Changing pattern of human listeriosis, England and Wales, 2001–2004. *Emerg Infect Dis* 2006; **12**:1361–6.

49 Brown JA, Factor DL, Tkachenko N *et al.* West Nile viremic blood donors and risk factors for subsequent West Nile fever. *Vector Borne Zoonotic Dis* 2007; **7**:479–88.

50 O'Leary DR, Marfin AA, Montgomery SP *et al.* The epidemic of West Nile virus in the United States, 2002. *Vector Borne Zoonotic Dis* 2004; **4**:61–70.

51 Sakaguchi S, Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T. Regulatory T cells: how do they suppress immune responses? *Int Immunol* 2009; **21**:1105–11.

52 Shevach EM. Mechanisms of foxp3$^+$ T regulatory cell-mediated suppression. *Immunity* 2009; **30**:636–45.

53 Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. *Nat Rev* 2008; **8**:523–32.

54 Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nat Immunol* 2010; **11**:7–13.

55 Friedline RH, Brown DS, Nguyen H *et al.* CD4$^+$ regulatory T cells require CTLA-4 for the maintenance of systemic tolerance. *J Exp Med* 2009; **206**:421–34.

56 Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T, Sakaguchi S. CTLA-4 control over Foxp3$^+$ regulatory T cell function. *Science* (New York, NY) 2008; **322**:271–5.

57 Rubtsov YP, Rasmussen JP, Chi EY *et al.* Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* 2008; **28**:546–58.

58 Scott-Browne JP, Shafiani S, Tucker-Heard G, Ishida-Tsubota K, Fontenot JD, Rudensky AY, Bevan MJ, Urdahl KB. Expansion and function of Foxp3-expressing T regulatory cells during tuberculosis. *J Exp Med* 2007; **204**:2159–69.

59 Johanns TM, Ertelt JM, Rowe JH, Way SS. Regulatory T cell suppressive potency dictates the balance between bacterial proliferation and clearance during persistent *Salmonella* infection. *PLoS Pathog* 2010; **6**:e1001043.

60 Mendez S, Reckling SK, Piccirillo CA, Sacks D, Belkaid Y. Role for CD4$^+$ CD25$^+$ regulatory T cells in reactivation of persistent leishmaniasis and control of concomitant immunity. *J Exp Med* 2004; **200**:201–10.

61 Uzonna JE, Wei G, Yurkowski D, Bretscher P. Immune elimination of *Leishmania major* in mice: implications for immune memory, vaccination, and reactivation disease. *J Immunol* 2001; **167**:6967–74.

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

63 Zheng Y, Chaudhry A, Kas A *et al.* Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T_H2 responses. *Nature* 2009; **458**:351–6.

64 Chaudhry A, Rudra D, Treuting P, Samstein RM, Liang Y, Kas A, Rudensky AY. CD4$^+$ regulatory T cells control TH17 responses in a Stat3-dependent manner. *Science* (New York, NY) 2009; **326**:986–91.

65 Linterman MA, Pierson W, Lee SK *et al.* Foxp3$^+$ follicular regulatory T cells control the germinal center response. *Nat Med* 2011; **17**:975–82.

66 Chung Y, Tanaka S, Chu F *et al.* Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat Med* 2011; **17**:983–8.

67 Wohlfert EA, Grainger JR, Bouladoux N *et al.* GATA3 controls Foxp3$^+$ regulatory T cell fate during inflammation in mice. *J Clin Investig* 2011; **121**:4503–15.

68 Rausch S, Huehn J, Loddenkemper C *et al.* Establishment of nematode infection despite increased Th2 responses and immunopathology after selective depletion of Foxp3$^+$ cells. *Eur J Immunol* 2009; **39**:3066–77.

69 Suffia IJ, Reckling SK, Piccirillo CA, Goldszmid RS, Belkaid Y. Infected site-restricted Foxp3$^+$ natural regulatory T cells are specific for microbial antigens. *J Exp Med* 2006; **203**:777–88.

70 Shafiani S, Tucker-Heard G, Kariyone A, Takatsu K, Urdahl KB. Pathogen-specific regulatory T cells delay the arrival of effector T cells in the lung during early tuberculosis. *J Exp Med* 2010; **207**:1409–20.

71 Zhao J, Zhao J, Fett C, Trandem K, Fleming E, Perlman S. IFN-γ- and IL-10-expressing virus epitope-specific Foxp3$^+$ T reg cells in the central nervous system during encephalomyelitis. *J Exp Med* 2011; **208**:1571–7.

72 Ertelt JM, Rowe JH, Johanns TM, Lai JC, McLachlan JB, Way SS. Selective priming and expansion of antigen-specific Foxp3$^-$ CD4$^+$ T cells during *Listeria monocytogenes* infection. *J Immunol* 2009; **182**:3032–8.

73 Antunes I, Tolaini M, Kissenpfennig A, Iwashiro M, Kuribayashi K, Malissen B, Hasenkrug K, Kassiotis G. Retrovirus-specificity of regulatory T cells is neither present nor required in preventing retrovirus-induced bone marrow immune pathology. *Immunity* 2008; **29**:782–94.

74 Lathrop SK, Bloom SM, Rao SM *et al.* Peripheral education of the immune system by colonic commensal microbiota. *Nature* 2011; **478**:250–4.

75 Moon JJ, Chu HH, Hataye J, Pagan AJ, Pepper M, McLachlan JB, Zell T, Jenkins MK. Tracking epitope-specific T cells. *Nat Protoc* 2009; **4**:565–81.

76 Zhou X, Bailey-Bucktrout S, Jeker LT, Bluestone JA. Plasticity of CD4$^+$ FoxP3$^+$ T cells. *Curr Opin Immunol* 2009; **21**:281–5.

77 Zhou X, Bailey-Bucktrout SL, Jeker LT *et al.* Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells *in vivo*. *Nat Immunol* 2009; **10**:1000–7.

78 Pasare C, Medzhitov R. Toll pathway-dependent blockade of CD4$^+$ CD25$^+$ T cell-mediated suppression by dendritic cells. *Science* (New York, NY) 2003; **299**:1033–6.

79 Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, Shimizu J, Sakaguchi S. Immunologic self-tolerance maintained by CD25$^+$ CD4$^+$ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int Immunol* 1998; **10**:1969–80.

80 Thornton AM, Shevach EM. CD4$^+$ CD25$^+$ immunoregulatory T cells suppress polyclonal T cell activation *in vitro* by inhibiting interleukin 2 production. *J Exp Med* 1998; **188**:287–96.

81 Kubo T, Hatton RD, Oliver J, Liu X, Elson CO, Weaver CT. Regulatory T cell suppression and anergy are differentially regulated by proinflammatory cytokines produced by TLR-activated dendritic cells. *J Immunol* 2004; **173**:7249–58.

82 Liu H, Komai-Koma M, Xu D, Liew FY. Toll-like receptor 2 signaling modulates the functions of CD4$^+$ CD25$^+$ regulatory T cells. *Proc Natl Acad Sci U S A* 2006; **103**:7048–53.

83 Sutmuller RP, den Brok MH, Kramer M *et al.* Toll-like receptor 2 controls expansion and function of regulatory T cells. *J Clin Investig* 2006; **116**:485–94.

84 Peng G, Guo Z, Kiniwa Y *et al.* Toll-like receptor 8-mediated reversal of CD4$^+$ regulatory T cell function. *Science* (New York, NY) 2005; **309**:1380–4.

85 Caramalho I, Lopes-Carvalho T, Ostler D, Zelenay S, Haury M, Demengeot J. Regulatory T cells selectively express toll-like receptors and are activated by lipopolysaccharide. *J Exp Med* 2003; **197**:403–11.

86 Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, Levings MK. Human $CD4^+$ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in $CD4^+$ $CD25^+$ T regulatory cells. *J Immunol* 2005; **175**:8051–9.

87 Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor foxp3. *Immunity* 2005; **22**:329–41.

88 Ertelt JM, Rowe JH, Mysz MA, Singh C, Roychowdhury M, Aguilera MN, Way SS. $Foxp3^+$ regulatory T cells impede the priming of protective $CD8^+$ T cells. *J Immunol* 2011; **187**:2569–77.

89 Wang RF, Peng G, Wang HY. Regulatory T cells and Toll-like receptors in tumor immunity. *Semin Immunol* 2006; **18**:136–42.

90 van Duin D, Medzhitov R, Shaw AC. Triggering TLR signaling in vaccination. *Trends Immunol* 2006; **27**:49–55.

91 Kastenmuller W, Gasteiger G, Subramanian N, Sparwasser T, Busch DH, Belkaid Y, Drexler I, Germain RN. Regulatory T cells selectively control $CD8^+$ T cell effector pool size via IL-2 restriction. *J Immunol* 2011; **187**:3186–97.

92 Kursar M, Bonhagen K, Fensterle J, Kohler A, Hurwitz R, Kamradt T, Kaufmann SH, Mittrucker HW. Regulatory $CD4^+$ $CD25^+$ T cells restrict memory $CD8^+$ T cell responses. *J Exp Med* 2002; **196**:1585–92.

93 Moore AC, Gallimore A, Draper SJ, Watkins KR, Gilbert SC, Hill AV. Anti-CD25 antibody enhancement of vaccine-induced immunogenicity: increased durable cellular immunity with reduced immunodominance. *J Immunol* 2005; **175**:7264–73.

94 Toka FN, Suvas S, Rouse BT. $CD4^+$ $CD25^+$ T cells regulate vaccine-generated primary and memory $CD8^+$ T-cell responses against herpes simplex virus type 1. *J Virol* 2004; **78**:13082–9.

95 Curtsinger JM, Mescher MF. Inflammatory cytokines as a third signal for T cell activation. *Curr Opin Immunol* 2010; **22**:333–40.

96 Ertelt JM, Johanns TM, Rowe JH, Way SS. Interleukin (IL)-21-independent pathogen-specific $CD8^+$ T-cell expansion, and IL-21-dependent suppression of $CD4^+$ T-cell IL-17 production. *Immunology* 2010; **131**:183–91.

97 Haring JS, Badovinac VP, Harty JT. Inflaming the $CD8^+$ T cell response. *Immunity* 2006; **25**:19–29.

98 Kastenmuller K, Wille-Reece U, Lindsay RW *et al.* Protective T cell immunity in mice following protein-TLR7/8 agonist-conjugate immunization requires aggregation, type I IFN, and multiple DC subsets. *J Clin Investig* 2011; **121**:1782–96.

99 Bousso P, Albert ML. Signal 0 for guided priming of CTLs: NKT cells do it too. *Nat Immunol* 2010; **11**:284–6.

100 Mays LE, Wilson JM. The complex and evolving story of T cell activation to AAV vector-encoded transgene products. *Mol Ther* 2011; **19**:16–27.

101 Brunstein CG, Miller JS, Cao Q *et al.* Infusion of *ex vivo* expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. *Blood* 2011; **117**:1061–70.

102 Hippen KL, Merkel SC, Schirm DK *et al.* Massive *ex vivo* expansion of human natural regulatory T cells (Tregs) with minimal loss of *in vivo* functional activity. *Sci Transl Med* 2011; **3**:83ra41.