microbial biotechnology

Microbial Biotechnology (2012) **5**(2), 260–269 doi:10.1111/j.1751-7915.2011.00299.x

# Tregs in infection and vaccinology: heroes or traitors?

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## Summary

**The development of effective vaccines against life-threatening pathogens in human diseases represents one of the biggest challenges in biomedical science. Vaccines traditionally make use of the body's own immune armoury to combat pathogens. Yet, while our immune system is mostly effective in eliminating or controlling a diverse range of microorganisms, its responses are incomplete or somewhat limited in several other cases. How immune responses are restrained during certain infections has been a matter of debate for many years. The discovery of regulatory T cells (Tregs), an immune cell type that plays a central role in maintaining immune homeostasis and controlling appropriate immune responses, has shed light into many questions. Indeed, it has been proposed that while Tregs might be beneficial in preventing excessive tissue damage during infection, they might also favour pathogen persistence by restraining effector immune responses. In addition, Tregs are believed to limit immune responses upon vaccination. Different strategies have been pursued to circumvent Treg activity during immunization, but the lack of specific tools for their study has led sometimes to controversial conclusions. With the advent of novel mouse models that allow specific depletion and/or tracking of Treg populations *in vivo*, novel aspects of Treg biology during infection have been unravelled. In this review, we describe the new advances in understanding Treg biology during infection and evaluate Treg depletion as a novel adjuvant strategy for vaccination.**

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This work was supported by the Deutsche Forschungsgemeinschaft (DFG) SFB900.

## Introduction

The mammalian immune system, which develops from a sterile intra-uterine micro-environment before birth, is soon exposed to a plethora of external microbiota. These microorganisms can cause highly deleterious infections by overpowering the host immune response. To prevent this, the immune system orchestrates a number of mechanisms, which include the innate and adaptive arms of the immune response, specifically directed to providing clearance and resistance to unwanted pathogens. This system requires potent regulatory mechanisms to govern excessive T-cell responses during infections in order to prevent infection-induced tissue damage (Belkaid, 2008; Belkaid and Tarbell, 2009). This includes $CD4^+$ regulatory T cells (Tregs) that play a central role in maintaining peripheral homeostasis and controlling appropriate immune responses against pathogens (Wohlfert and Belkaid, 2008). Tregs were initially identified by their constitutive expression of CD25, the $\alpha$-chain of the IL-2 receptor, and most of the early studies investigating the role of Tregs during infections used antibodies directed against CD25 for Treg depletion. However, CD25 is also expressed on activated conventional T cells and, in addition, some Tregs lack CD25 expression, thus excluding a selective and complete depletion of Tregs with CD25-targeting reagents. More recently, the transcription factor Foxp3, which is of central importance for the Treg suppressive phenotype, was shown to be rather specifically expressed in these immune-regulatory cells (Fontenot *et al.*, 2003; Hori *et al.*, 2003; Khattri *et al.*, 2003). To date, although Foxp3 is the most widely used Treg marker, its intracellular expression precludes its direct usage for depletion or isolation of Tregs. To circumvent this limitation, novel Treg depletion mouse models were generated, which express the diphtheria toxin (DT) receptor under the control of the *Foxp3* locus, thereby allowing the selective depletion of $Foxp3^+$ Tregs at any time during an immune response (Kim *et al.*, 2007; Lahl *et al.*, 2007; Suffner *et al.*, 2010). These mouse models opened a whole new dimension of possibilities to investigate the *in vivo* interactions of Tregs and infectious agents and also provided interesting but also sometimes contradictory

results, suggesting a higher complexity in the biology of Treg cells than previously envisioned. In this review, we highlight the importance of Foxp3$^+$CD4$^+$ Tregs in microbial and parasitic infections and introduce the novel Treg depletion mouse models, which have potential implications for vaccine development. We finally discuss alternative Treg manipulation strategies that may substitute currently available tools with the intention to eliminate unwanted side-effects in the host immune system.

## Tregs in chronic and persistent infections

Foxp3$^+$ Tregs have been shown to mediate a constant equilibrium between pathogens and the host, in order to minimize exuberant immune responses and to maintain tissue integrity (Belkaid and Tarbell, 2009). Tregs suppress immune responses, thereby providing a window of opportunity for a pathogen to persist and prevail in the host. Alternatively, Tregs can also be subjected to regulation themselves as demonstrated in a *Toxoplasma gondii* model (Oldenhove *et al.*, 2009). The underlying mechanisms controlling this behaviour leave room for intense speculation. Current knowledge in the field suggests that Tregs may either directly be activated by Toll-like receptor (TLR) ligands or indirectly via secreted immunochemical cues from antigen presenting cells and T cells generated during infection (Pasare and Medzhitov, 2003). In addition, after infection the microenvironment may also influence the fate of Tregs to determine the outcome of an immune response towards tolerance or immunity (Oldenhove *et al.*, 2009). This paradoxical role of Tregs in host–pathogen interaction remains somewhat enigmatic, and the underlying mechanisms that control Tregs in an infectious setting may need further clarity. In this section, we discuss current knowledge involving interactions between Tregs and different microbial and parasitic pathogens and highlight niches in the role of Tregs during infection that could be subjected to manipulation during vaccine development (Fig. 1).

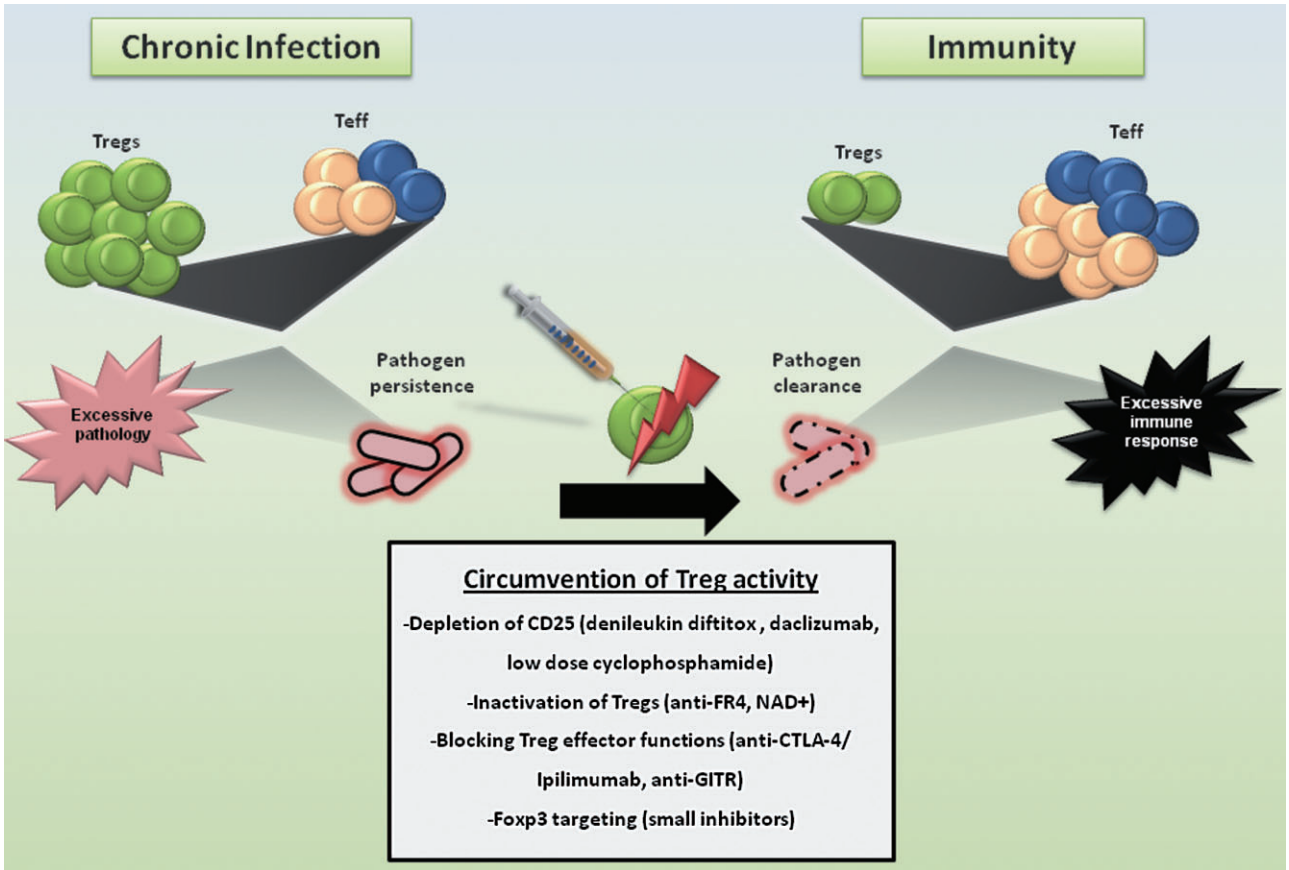


**Fig. 1.** Depletion of Treg suppressive activity as a novel promising approach to induce protective cellular immune responses against infectious agents. Expansion of Tregs during infection can contribute to pathogen persistence and concomitant chronicity, while preventing excessive tissue damage. Similarly, Treg suppressive activity during vaccination may prevent effective immune protection. In both contexts, although therapeutic circumvention of Treg activity would tilt the Treg/Teff balance favouring pathogen clearance, autoimmunity would need to be avoided.

 

**Tregs in viral infections**

Evasion from recognition by effector T cells and memory T cells has been well documented with a number of viral infections in mice and humans. In addition, during viral infections, the capacity of Tregs to dampen virus specific CTL responses in mice has been demonstrated in a number of studies. It has been proposed that Tregs may limit $CD8^+$ T cell-mediated viral destruction by a mechanism dependent on cell-to-cell contact as demonstrated in an *in vitro* Friend's virus infection model (Dittmer *et al*., 2004; Robertson *et al*., 2006; Zelinskyy *et al*., 2006) or by reducing $CD8^+$ T-cell proliferation *in vivo* after HSV-1 ocular infection (Suvas *et al*., 2003; 2004; Belkaid and Rouse, 2005; Sarangi *et al*., 2008). Alternatively, Tregs may control the recruitment of virus-specific $CD8^+$ T cells after RSV-induced lung infection and inhibit their local activation, promoting severe disease due to extensive lung immunopathology (Fulton *et al*., 2010). In neonatal mice, Tregs may also influence $CD4^+$ and $CD8^+$ T-cell responses after *in vivo* subcutaneous infection with HSV-2 (Fernandez *et al*., 2008). Similarly, in humans, HIV-induced increase in Treg numbers in blood and lymphoid organs dampens adaptive immune responses, and ablation of Tregs from these tissues rejuvenates virus-specific immune responses (Aandahl *et al*., 2004; Kinter *et al*., 2004; 2007; Andersson *et al*., 2005). Thus, the suppressive nature of Tregs provides viruses an ideal environment to propagate and hence persist in the host. To circumvent this suppressive activity, recent studies have adopted the use of Treg depletion mouse models like $FoxP3^{DTR}$ (Kim *et al*., 2007) and DEREG mice (Lahl *et al*., 2007) to examine the role of Tregs during viral infections. Studies carried out with the murine Friend's retrovirus suggests that by transiently depleting Tregs, the efficacy and strength of exhausted $CD8^+$ T cells could be renewed giving rise to a reduction of long-term viral loads in infected mice (Dietze *et al*., 2011). Thus, relief from Treg-mediated suppression may not only be important for the *de novo* priming of new antigen-specific $CD8^+$ T cells in chronic viral infections, but may also restore function in exhausted $CD8^+$ T cells, which once exhibited antiviral cytolytic activity. One mechanism of Treg control over CTL responses has been attributed to Treg-mediated modulation of IL-2 bioavailability (McNally *et al*., 2011; Kastenmuller *et al*., 2011). In addition, the cellular microenvironment could also contribute towards the differential behaviour of Tregs after viral infections. A classic example of this phenomenon was demonstrated in two concurrent studies examining the role of Treg depletion in HSV-2 immunity (Fernandez *et al*., 2008; Lund *et al*., 2008). In one study, ablation of Tregs using $FoxP3^{DTR}$ mice in genital HSV-2 infection resulted in accelerated mortality caused by infection and did not reduce viral loads, suggesting a protective role of Tregs in HSV immunity (Lund *et al*., 2008). In contrast, in a subcutaneous model of HSV-2 infection in adult and neonatal mice, ablation of Tregs using anti-CD25 depletion before HSV-2 infection significantly increased HSV-specific CTL responses *in vivo*, represented by increased cell number, activation, IFN-γ responses and granzyme B expression (Fernandez *et al*., 2008). Moreover, depletion of Tregs reduced the viral titre in the draining LN and brain of infected newborn but not adult mice (Fernandez *et al*., 2008). Viewed as a whole, these data suggest that Tregs may respond differently to the same virus infecting two different cellular micro-environments. Furthermore, Zelinskyy *et al*. showed that after infecting DEREG mice with Friend's retrovirus, which efficiently targets lymphoid organs like spleen and bone marrow, virus-specific CTL responses were enhanced in quality and magnitude after selective depletion of $Foxp3^+$ Tregs (Zelinskyy *et al*., 2009). From this study they also postulated that ablation of Tregs would have a greater significance in viral infections targeting cells of lymphoid tissues compared with non-lymphoid tissues (Zelinskyy *et al*., 2009). The principle behind Zelinskyy's study could also be applied to an HIV infection model (Zelinskyy *et al*., 2009), where high infectivity and tissue tropism in the LN seems to be a characteristic feature of the virus and where disease progression and Treg expansion was found to correlate (Andersson *et al*., 2005; Nilsson *et al*., 2006; Kinter *et al*., 2007). Thus, these studies demonstrate the importance of Tregs during Friend's retrovirus infection and provide a strong foundation for translating this concept on the importance of Tregs in more medically relevant lymphotropic viruses like HIV.

**Tregs in bacterial infections**

Similar to viruses, bacteria have evolved mechanisms to circumvent host immune responses. Particularly in chronic infections, the ability of certain bacteria to modulate adaptive immune reactions might be crucial in order to determine microbial persistence. As for viruses, it has been postulated that expansion of Tregs during infection could prevent excessive tissue damage on one side, while favouring pathogen survival on the other (Belkaid and Tarbell, 2009). Thus, although increased Treg numbers are found in many bacterial infections, whether these cells play a critical role on disease outcome is not well understood.

*Mycobacterium tuberculosis* (Mtb) represents one of the most threatening microorganisms for human health and is thus a prime focus of many studies. During Mtb infection, an expansion of Tregs parallel to the expansion of effector T cells has been observed both in mice (Scott-Browne *et al*., 2007) and humans (Guyot-Revol *et al*., 2006; Chen *et al*., 2007). $Foxp3^+$ Tregs have been found not only in the lymphoid organs of Mtb-infected mice, but

also within the lung granulomas, suggesting that Tregs may act locally at the site of infection (Scott-Browne *et al*., 2007). Mtb-specific Treg expansion requires the cognate antigen and occurs almost exclusively from pre-existing Tregs (Shafiani *et al*., 2010), raising the question of whether bacteria and or their components modulate adaptive immune responses by modulating cells of the innate immune system or by acting directly on T cells. Interestingly, a recent report suggests that certain gut commensals can act through TLR2 and MyD88 expressed on Tregs to exert their immunosuppressive functions (Round *et al*., 2011). TLR2-deficient mice are more susceptible to Mtb infection (Reiling *et al*., 2002) and Mtb can release active membrane vesicles that modulate immune responses in a TLR2-dependent fashion (Prados-Rosales *et al*., 2011), but whether the TLR2 pathway is also exploited by mycobacteria to sustain Treg's suppressive activity has not yet been investigated.

Depletion of $CD25^+$ cells by means of anti-CD25 antibodies during Mtb infection did not influence bacterial burden, but increased the capacity of $CD4^+$ effector T cells to produce IFN-$\gamma$ (Quinn *et al*., 2006). In contrast to this finding, specific depletion of $CD4^+Foxp3^+$ Tregs in mixed bone marrow chimeras led to a reduction in bacterial burden directly in the lungs (Scott-Browne *et al*., 2007). Similarly, Kursar and colleagues showed that co-transfer of Tregs into Mtb-infected $Rag^{-/-}$ mice impaired the capacity of $CD4^+$ effector T cells to control bacterial burden, and these effects were not mediated by impaired IFN-$\gamma$ responses or increased IL-10 production (Kursar *et al*., 2007). Thus, although Tregs influence IFN-$\gamma$ responses, this may not directly correlate with disease severity. Recent reports suggest that in human patients, TNF-$\alpha$ single positive $CD4^+$ T cells are better predictors of active tuberculosis (Harari *et al*., 2011). Yet, other mechanisms might also account for the differences observed in bacterial burden upon Treg depletion. In this sense, Scott-Browne *et al*. observed an upregulation of ICOS and PD-1, two molecules that have been extensively implicated in the immunoregulation of effector T cells (Scott-Browne *et al*., 2007). Recently, Urdahl *et al*. proposed that restriction of antigen presentation to DC and infected macrophages might play a role in Treg expansion during Mtb infection (Urdahl *et al*., 2011), which in turn can delay the priming of effector $CD4^+$ and $CD8^+$ T cells in the pLN and their arrival in the lung (Shafiani *et al*., 2010). Altogether, these studies postulate a role for Tregs in regulating bacterial burden during Mtb infection, but the underlying mechanisms need further clarification.

Another well-studied model suggesting the involvement of Tregs during bacterial infections is *Helicobacter pylori* infection. Here, an inverse correlation between increased Foxp3 expression in the gastric mucosa and lower signs of gastritis was observed in children compared with adult infected patients (Harris *et al*., 2008). In the same line, Arnold *et al*. found that infected neonatal mice are protected from the development of gastric preneoplastic immunopathology, and this might be due to induction of Tregs that control overt effector T-cell responses (Arnold *et al*., 2011). Specific depletion of Tregs using the DEREG model led to bacterial clearance but preneoplastic gastric lesions, suggesting that tolerogenic Tregs are necessary to prevent *H. pylori*-induced pathology (Arnold *et al*., 2011). Tregs might suppress Th17 cells, favouring bacterial persistence and counteracting excessive inflammation induced by the host's immune response (Kao *et al*., 2010). Thus, the influence of Tregs on the outcome of an infectious disease might be highly dependent on specific pathogen-to-host interactions, and circumvention of Treg activity as a therapeutic strategy may not always represent an advantage for the host.

### Tregs in parasitic infections

Parasitic infections constitute a large majority of human diseases in the worldwide population, including malaria, trypanosomiasis, leishmaniasis, filariasis and schistosomiasis, all mediated by vector-borne parasites. Similar to viruses, parasites are highly adaptable and can regulate their developmental cycle so as to circumvent detection by the host immune system, thereby prevailing unharmed in the host. Chronic parasitic infections are powerful inducers of Th2-polarized responses (Rausch *et al*., 2009) and also coordinate production of large amounts of anti-inflammatory cytokines like IL-10 and TGF-$\beta$ thereby favouring immunosuppressive responses (Maizels *et al*., 2004). The role of $Foxp3^+$ Tregs in parasite-driven inhibition of host immunity has been implicated in a number of chronic murine and human parasitic infection models (Liu *et al*., 2003; Torcia *et al*., 2008; Grainger *et al*., 2010). One of the most studied diseases, in which Tregs play a central role, is malaria. Malaria is a worldwide epidemic affecting millions of individuals leading to increasing disease-associated mortality. The most common of these parasites affecting humans are *Plasmodium falciparum* (Walther *et al*., 2005) and *Plasmodium vivax*. Blood-derived $Foxp3^+$ Tregs have been shown to play an important role in maintaining a balance between immunity during infection and reduction of immune pathology due to inflammatory responses exerted by the erythrocytic stage of the parasite (Bueno *et al*., 2010). In humans, immunity to infection caused by *P. falciparum* was associated with increased expansion of TGF-$\beta$ induced Tregs, which correlated with increased parasite replication in the infected hosts and ablation of Tregs *in vitro* restored immune responses (Walther *et al*., 2005; Torcia *et al*., 2008). Similarly, in malarial infection caused by *P. vivax*, increased parasitic burden correlated with increased absolute

numbers of circulating IL-10 and TGF-β producing Foxp3$^+$ Tregs and upregulation of surface GITR, intracellular CTLA4 expression and reduced antigen-specific T-cell proliferation (Bueno *et al*., 2010). These data suggest that an increase in the number of activated Tregs contributes to enhanced parasitic load by severely compromising anti-parasitic immune responses (Bueno *et al*., 2010). Mouse models of *Plasmodium yoelli* infection showed, however, controversial results regarding the suppressive effects of Tregs on effector responses (Hisaeda *et al*., 2004; Couper *et al*., 2008). During *Plasmodium berghei* ANKA (PbA) infection, ablation of Tregs using anti-CD25 antibodies contributed to reduced parasite replication and protection against cerebral malaria (Amante *et al*., 2007). However, using the same model, Steeg *et al*. showed that selective removal of Foxp3$^+$ Tregs does not rescue mice from severe disease (Steeg *et al*., 2009). A third study examining the role of Tregs in PbA-induced experimental cerebral malaria (ECM) showed that alternative to depletion of Tregs, expanding their cell numbers *in vivo* by IL-2Jc administration protected mice from ECM disease in a CTLA-4 dependent manner (Haque *et al*., 2010). Thus, the differences observed in these studies may arise from the different strategies used to deplete Tregs cells. Indeed, the resistance to ECM observed after anti-CD25 depletion could be due to depletion of highly activated effector T cells, more than to a reduction in the Treg population, as judged by the reduction in CD8$^+$ T cell sequestered to the brain after anti-CD25 treatment (Amante *et al*., 2007).

Tregs have also been shown to be critical in regulating immune responses generated during helminth infections. In humans, Tregs home to sites of cutaneous *Leishmania braziliensis* infection (Campanelli *et al*., 2006) and rapidly accumulate within the sites of *Leishmania major* infection exerting their suppressive activity on local immune responses so as to favour parasite persistence (Yurchenko *et al*., 2006). The presence of Tregs has also been implicated in parasite host immune evasion as observed during murine *Schistosoma japonicum* infection, where Tregs suppress proliferation of antigen-specific T cells and anti-CD25 mediated depletion of Tregs restored and enhanced Th1 responses (Tang *et al*., 2011). Contrary to this, after *Schistosoma mansoni* infection, the presence of Tregs promoted reduced liver pathology and favoured host survival (Hesse *et al*., 2004; McKee and Pearce, 2004). In some parasitic infections such as intestinal helminth *Heligmosomoides polygyrus* infections, parasite secreted proteins can drive *de novo* FoxP3 expression by converting splenic FoxP3$^-$ cells to FoxP3$^+$ cells (Grainger *et al*., 2010). Induction of these parasite induced Tregs suppressed *in vitro* proliferation of effector cells and *in vivo*, influenced suppression of airway allergic inflammation (Grainger *et al*., 2010). Thus as in the case of viruses, Tregs may play contrasting roles based on the nature of the parasite.

### Role of Tregs in vaccine design

In spite of the success of many vaccines, protection against several threatening pathogens remains an urgent medical requirement. Development of new vaccines not only faces difficulties associated with the pathogens (e.g. genetic variability, life cycle, etc.), but also those related with the establishment of tolerance within the host. In this context, Tregs might represent an obstacle on the way to generate strong cell-mediated immune responses but most importantly, for the establishment of long-lived immunological memory. Indeed, Foxp3$^+$ Tregs have been shown to affect both, T-cell priming as well as their effector phase and depletion of Tregs generally leads to an enhancement in the inflammatory response, as discussed above. Contrary to all predictions, this enhancement in the adaptive immune responses upon Treg depletion does not always correlate with a better disease outcome, suggesting that Treg regulation during infection might be a highly complex and pathogen-dependent mechanism. In addition, the limitation of several studies might reside on the fact that IFN-γ responses, mostly interpreted as a measurement of inflammation, do not always directly correlate with protective responses. Indeed, with the advent of multicolour flow cytometry and thus the possibility to analyse multiple parameters on one single cell, it has become clear that not only the quantity, but also the quality of an immune response is a better predictor of disease outcome (Seder *et al*., 2008). In the case of viral infections, like in HIV, the capacity of T cells to produce multiple cytokines simultaneously has been associated with improved disease control, whereas individuals with high viral loads had increased frequencies of IFN-γ single producer cells (Seder *et al*., 2008). Thus, it would be interesting to determine whether the beneficial effects of Treg depletion are related to an improvement in the quality of the T-cell responses, and thus in the pathogenic outcome.

One important and relevant question that remains to be answered is whether Tregs play a role in the establishment of T-cell memory and/or antibody responses, two critical aspects for the design of new vaccines. Early studies mainly based on anti-CD25 depletion suggest that Tregs can control both the primary and memory CD8 T effector cell response upon vaccination with different peptides and adjuvant costimulation (Heit *et al*., 2008). More recent data suggest that Tregs might control only the pool of short-lived effector cells without affecting the T-cell memory compartment as shown in a Modified Vaccinia Ankara (Kastenmuller *et al*., 2011). In the same model, specific Treg depletion did not affect the antibody response. However, the influence of Tregs on the humoral

arm of the immune response might depend on several factors. In an asthma model for example, depletion of Tregs led to increased IgE levels but only mildly affected IgG responses (Baru *et al*., 2010). Depletion of Tregs in a Th2 context, e.g. vaccines against parasite infections, could therefore represent a benefit. In addition, it has been recently suggested that certain vaccines might be less effective in populations with endemic helminthic infections due to the excessive expansion of Tregs (Elias *et al*., 2008; Wammes *et al*., 2010). Thus, depletion of Tregs could be an adjuvant option when applied concomitant to vaccinations in areas of the world where parasitic infections are endemic.

Strategies to overcome or avoid Treg expansion/induction might include the choice of proper adjuvants that differentially support the expansion of effector T cells over that of Tregs. Understanding the mechanisms, by which activation of the innate immune response through adjuvants, e.g. TLR agonists, can modulate the adaptive immune response is therefore crucial for optimal vaccine development. In a recent study the effect of different adjuvants including zymosan (TLR2/6 agonist), poly(I : C) (TLR3 agonist), LPS (TLR4 agonist), polyU and R848 (TLR7 agonists), or CpG (TLR9 agonist) were tested for their capacity to regulate immune responses in the presence or absence of Tregs (Olivier *et al*., 2011). Here, TLR activation *per se* did not lead to an expansion of Tregs, but concomitant TLR activation and Treg depletion led to enhanced IFN-γ production. With the exception of low levels of TLR2, no other TLRs were expressed on highly purified $CD4^+Foxp3^+$ Tregs (Chen *et al*., 2009; Olivier *et al*., 2011). However, ligation of TLR9 or TLR7 led to an indirect activation of Tregs as indicated by CD69 upregulation. This study is however contradictory with others pointing to a role of TLR7-mediated suppression in a model of asthma (Van *et al*., 2011). In addition, triggering of TLR7 by HIV genomic RNA on pDCs has been shown to induce the differentiation of naive $CD4^+$ T cells into Tregs (Manches *et al*., 2008). Thus, TLR activation on different DC subsets might have a distinctive impact on Treg expansion. Targeting specific DC subsets during vaccination could therefore represent an alternative to generate immune responses in a more controlled manner.

## Therapeutic vaccines

Although the use of mouse models that allow specific depletion of $Foxp3^+$ Tregs represent a useful tool to dissect the immune-regulatory pathways during infection and in vaccinology, the results from these studies cannot be directly translated into the human system because highly selective Treg depletion agents for human $Foxp3^+$ Tregs are still missing. Nevertheless, clinical trials using CD25-targeting agents such as denileukin diftitox (Ontak) and daclizumab or low-dose cyclophosphamide, which has been reported to selectively reduce Treg numbers and to promote effector T-cell differentiation (Sistigu *et al*., 2011), have already been initiated in cancer patients (Dannull *et al*., 2005; Audia *et al*., 2007; Ghiringhelli *et al*., 2007; Mahnke *et al*., 2007; Morse *et al*., 2008; Rech and Vonderheide, 2009). Although the results from these initial studies were promising, the potential of Treg depletion as a therapeutic strategy to improve antigen-specific vaccinations was probably masked by the unwanted effects of these depleting agents on antigen-specific effector T cells. Using DT mediated selective depletion of $FoxP3^+$ Tregs in DEREG mice, Klages *et al*. were able to demonstrate that combination of Treg depletion with vaccination induced regression of B6 melanoma cells and increased proliferation of anti-tumourigenic CD8 T cells in the tumour (Klages *et al*., 2010). Recently, more selective approaches were developed using monoclonal antibodies against folate receptor 4, which is predominantly expressed on Tregs (Yamaguchi *et al*., 2007) and using systemic administration of nicotinamide adenine dinucleotide ($NAD^+$), which reduced $Foxp3^+$ Treg numbers and provoked efficient anti-tumour immunity (Hubert *et al*., 2010; Teng *et al*., 2010). Additionally, vaccination strategies targeting immune response modifiers such as GITR or CTLA-4 have been developed to simultaneously switch off Treg function and boost effector T-cell activation (Ko *et al*., 2005; Dittmer *et al*., 2008; Hoffmann *et al*., 2010; Mitsui *et al*., 2010). Finally, the transcription factor Foxp3 itself was identified as potential target and small peptides, which specifically bind to Foxp3 and block the function of this essential transcription factor, were synthesized (Casares *et al*., 2010). As acetylation of Foxp3 is important for its suppressive function, small molecules disabling specific histone acetyltransferases were tested for their effect on Treg activity (Xiao *et al*., 2010). Although the results from these initial preclinical studies were very promising, more intense research needs to be performed before these novel Treg targeting strategies can be used in the clinics to improve the outcome of pathogen-specific vaccinations (Fig. 1).

## Concluding remarks

The relevance of Tregs in infectious diseases has been disregarded for many years, in part due to the lack of experimental tools for their study. This situation has dramatically changed with the discovery of specific markers expressed on Tregs, and the concomitant development of transgenic mouse models that allow Treg follow up and depletion. Conclusions arising from such studies have not only contributed to the understanding of Treg biology and their dynamics during infections, but also revealed the enormous implications of Tregs for vaccine development.

Indeed, depletion of Treg suppressive activity during vaccination might represent a novel promising approach to induce protective cellular immune responses against infectious agents. This new strategy would, however, need extensive proof of concept as the importance of Tregs might depend on individual infection contexts and manipulating Treg function may carry the risk of considerable side-effects.

**Acknowledgements**

We would like to thank Christian Mayer and Wolfang Kastenmuller for critical reading of the manuscript.

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