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.

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 α-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.

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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; Zelinsky 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 (Aaandahl 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 FoxP3GFP (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 FoxP3GFP 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, Zelinsky 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 (Zelinsky 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 (Zelinsky et al., 2009). The principle behind Zelinsky’s study could also be applied to an HIV infection model (Zelinsky 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 (Mtbc) represents one of the most threatening microorganisms for human health and is thus a prime focus of many studies. During Mtbc 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 Mtbc-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-γ (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-1−/− mice impaired the capacity of CD4+ effector T cells to control bacterial burden, and these effects were not mediated by impaired IFN-γ responses or increased IL-10 production (Kursar et al., 2007). Thus, although Tregs influence IFN-γ responses, this may not directly correlate with disease severity. Recent reports suggest that in human patients, TNF-α 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-β 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-β 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-2-Jc 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

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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; Ghirotchelli et al., 2007; Mahrke 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 (Yamaguichi 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; Mitsu 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.

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References

Aandahl, E.M., Michaelsson, J., Moretto, W.J., Hecht, F.M., and Nixon, D.F. (2004) Human CD4+CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. J Virol 78: 2454–2459.

Amante, F.H., Stanley, A.C., Randall, L.M., Zhou, Y., Haque, A., McSweeney, K., et al. (2007) A role for natural regulatory T cells in the pathogenesis of experimental cerebral malaria. Am J Pathol 171: 548–559.

Andersson, J., Boasso, A., Nilsson, J., Zhang, R., Shire, N.J., Lindback, S., et al. (2005) The prevalence of regulatory T cells in lymphoid tissue is correlated with viral load in HIV-infected patients. J Immunol 174: 3143–3147.

Arnold, I.C., Lee, J.Y., Arnieva, M.R., Roers, A., Flavell, R.A., Sparwasser, T., and Muller, A. (2011) Tolerance rather than immunity protects from Helicobacter pylori-induced gastric preneoplasia. Gastroenterology 140: 199–209.

Audia, S., Nicolas, A., Cathelin, D., Larmonier, N., Ferrand, C., Foucher, P., et al. (2007) Increase of CD4+CD25+ regulatory T cells in the peripheral blood of patients with metastatic carcinoma: a Phase I clinical trial using cyclophosphamide and immunotherapy to eliminate CD4+CD25+ T lymphocytes. Clin Exp Immunol 150: 523–530.

Baru, A.M., Hartl, A., Lahli, K., Krahnswamy, J.K., Fehrenbach, H., Yildirim, A.O., et al. (2010) Selective depletion of Foxp3+ Treg during sensitization phase aggravates experimental allergic airway inflammation. Eur J Immunol 40: 2259–2266.

Belkaid, Y. (2008) Paradoxical roles of Foxp3+ T cells during infection: from regulators to regulators. Cell Host Microbe 3: 341–343.

Belkaid, Y., and Rouse, B.T. (2005) Natural regulatory T cells in infectious disease. Nat Immunol 6: 353–360.

Belkaid, Y., and Tarbell, K. (2009) Regulatory T cells in the control of host-microorganism interactions (*). Annu Rev Immunol 27: 551–589.

Bueno, L.L., Morais, C.G., Araujo, F.F., Gomes, J.A., Correa-Oliveira, R., Soares, I.S., et al. (2010) Plasmodium vivax: induction of CD4+CD25+FoxP3+ regulatory T cells during infection are directly associated with level of circulating parasites. PLoS ONE 5: e9623.

Campanelli, A.P., Roselino, A.M., Cavassani, K.A., Pereira, M.S., Mortara, R.A., Brodsky, C.I., et al. (2006) CD4+CD25+ T cells in skin lesions of patients with cutaneous leishmaniasis exhibit phenotypic and functional characteristics of natural regulatory T cells. J Infect Dis 193: 1313–1322.

Casanova, N., Rudilla, A., Arribillaga, L., Llopiz, D., Riezlo-Boj, J.I., Lozano, T., et al. (2010) A peptide inhibitor of FOXP3 impairs regulatory T cell activity and improves vaccine efficacy in mice. J Immunol 185: 5150–5159.

Chen, Q., Davidson, T.S., Huter, E.N., and Shevach, E.M. (2009) Engagement of TLR2 does not reverse the suppressor function of mouse regulatory T cells, but promotes their survival. J Immunol 183: 4458–4466.

Chen, X., Zhou, B., Li, M., Deng, Q., Wu, X., Le, X., et al. (2007) CD4+CD25+(+)FoxP3(+) regulatory T cells suppress Mycobacterium tuberculosis immunity in patients with active disease. Clin Immunol 123: 50–59.

Couper, K.N., Blount, D.G., Wilson, M.S., Hafalla, J.C., Belkaid, Y., Kamanaka, M., et al. (2008) IL-10 from CD4CD25Foxp3CD127 adaptive regulatory T cells modulates parasite clearance and pathology during malaria infection. PLoS Pathog 4: e1000004.

Dannull, J., Su, Z., Rizzieri, D., Yang, B.K., Coleman, D., Yancey, D., et al. (2005) Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. J Clin Invest 115: 3623–3633.

Dietze, K.K., Zelinsky, G., Gibbert, K., Schimmer, S., Francois, S., Myers, L., et al. (2011) Transient depletion of regulatory T cells in transgenic mice reactivates virus-specific CD8+ T cells and reduces chronic retroviral set points. Proc Natl Acad Sci USA 108: 2420–2425.

Dittmer, U., He, H., Messer, R.J., Schimmer, S., Olbrich, A.R., Ohlen, C., et al. (2004) Functional impairment of CD8(+) T cells by regulatory T cells during persistent retroviral infection. Immunity 20: 293–303.

Dittmer, U., Werner, T., and Kraft, A.R. (2008) Co-immunization of mice with a retroviral DNA vaccine and GITRL-encoding plasmid augments vaccine-induced protection against retrovirus infection. Viral Immunol 21: 459–467.

Elias, D., Britton, S., Aseffa, A., Engers, H., and Akuffo, H. (2008) Poor immunogenicity of BCG in helminth infected children with active disease. J Infect Dis 198: 2382–2392.

Fernandez, M.A., Puttur, F.K., Wang, Y.M., Howden, W., Alexander, S.I., and Jones, C.A. (2008) T regulatory cells contribute to the attenuated primary CD8+ and CD4+ T cell responses to herpes simplex virus type 2 in neonatal mice. J Immunol 180: 1556–1564.

Fontenot, J.D., Gavin, M.A., and Rudensky, A.Y. (2003) Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 4: 330–336.

Fulton, R.B., Meyerholz, D.K., and Varga, S.M. (2010) Foxp3+CD4 regulatory T cells limit pulmonary immunopathology by modulating the CD8 T cell response during respiratory syncytial virus infection. J Immunol 185: 2382–2392.

Ghiringhelli, F., Menard, C., Puig, P.E., Ladoire, S., Roux, S., Martin, F., et al. (2007) Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother 56: 641–648.
An essential role for Scurfin in CD4+ regulatory T cell development by the transcription factor Foxp3 et al. (2010) Extracellular NAD+ shapes the Foxp3+ regulatory T cell compartment through the ART2-P2X7 pathway. J Exp Med 207: 2561–2568.

Kao, J.Y., Zhang, M., Miller, M.J., Mills, J.C., Wang, B., Liu, M., et al. (2010) Helicobacter pylori immune escape is mediated by dendritic cell-induced Treg skewing and Th17 suppression in mice. Gastroenterology 138: 1046–1054.

Kastenmuller, W., Gasteiger, G., Subramanian, N., Sparwasser, T., Busch, D.H., Belkaid, Y., et al. (2011) Regulatory T Cells Selectively Control CD8+ T Cell Effector Pool Size via IL-2 Restriction. J Immunol 187: prepublished online 17 August 2011; jimmunol.1101649/doi:10.4049.

Khatri, R., Cox, T., Yasayko, S.A., and Ramsdell, F. (2003) An essential role for Scurfin in CD4+CD25+ T regulatory cells. Nat Immunol 4: 337–342.

Kim, J.M., Rasmussen, J.P., and Rudensky, A.Y. (2007) Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat Immunol 8: 191–197.

Kinter, A.L., Hennessey, M., Bell, A., Kern, S., Lin, Y., Daucher, M., et al. (2004) CD25(+)CD4(+) regulatory T cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4(+) and CD8(+) HIV-specific T cell immune responses in vitro and are associated with favorable clinical markers of disease status. J Exp Med 200: 331–343.

Kinter, A.L., Horak, R., Sion, M., Riggan, L., McNally, J., Lin, Y., et al. (2007) CD25+ regulatory T cells isolated from HIV-infected individuals suppress the cytolytic and nonlytic antiviral activity of HIV-specific CD8+ T cells in vitro. AIDS Res Hum Retroviruses 23: 438–450.

Kluges, K., Mayer, C.T., Lahl, K., Loddenkemper, C., Teng, M.W., Ngioi, S.F., et al. (2010) Selective depletion of Foxp3+ regulatory T cells improves effective therapeutic vaccination against established melanoma. Cancer Res 70: 7788–7799.

Ko, K., Yamazaki, S., Nakamura, K., Nishioka, T., Hirota, K., Yamaguchi, T., et al. (2005) Treatment of advanced tumors with agonistic anti-GITR mAb and its effects on tumor-infiltrating Foxp3+CD25+CD4+ regulatory T cells. J Exp Med 202: 885–891.

Kursar, M., Koch, M., Mittrucker, H.W., Nouailles, G., Bonhagen, K., Kamradt, T., and Kaufmann, S.H. (2007) Cutting Edge: regulatory T cells prevent efficient clearance of Mycobacterium tuberculosis. J Immunol 178: 2661–2665.

Lahl, K., Loddenkemper, C., Drouin, C., Freyer, J., Arnason, J., Eberl, G., et al. (2007) Selective depletion of Foxp3+ regulatory T cells induces a scurfy-like disease. J Exp Med 204: 57–63.

Liu, H., Hu, B., Xu, D., and Liew, F.Y. (2003) CD4+CD25+ regulatory T cells cure murine colitis: the role of IL-10, TGF-beta, and CTLA4. Cancer Res 63: 491–499.

Heit, A., Gebhardt, F., Lahl, K., Neuenhahn, M., Schmitz, F., Anderl, F., et al. (2008) Circumvention of regulatory CD4(+) T cell activity during cross-priming strongly enhances T cell-mediated immunity. Eur J Immunol 38: 1585–1597.

Hesse, M., Piccirillo, C.A., Belkaid, Y., Prifer, J., Mentink-Kane, M., Leusink, M., et al. (2004) The pathogenesis of schistosomiasis is controlled by cooperating IL-10-producing innate effector and regulatory T cells. J Immunol 172: 3157–3166.

Hisaeda, H., Maekawa, Y., Iwakawa, D., Okada, H., Himeno, K., Kishihara, K., et al. (2004) Escape of malaria parasites from host immunity requires CD4+ CD25+ regulatory T cells. Nat Med 10: 29–30.

Hoffmann, C., Stanke, J., Kaufmann, A.M., Loddenkemper, C., Schneider, A., and Cichon, G. (2010) Combining T-cell vaccination and application of agonistic anti-GITR mAb (DTA-1) induces complete eradication of HPV oncogene-expressing tumors in mice. J Immunother 33: 136–145.

Hori, S., Nomura, T., and Sakaguchi, S. (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science 296: 1057–1061.
Wammes, L.J., Hamid, F., Wiria, A.E., de Gier, B., Sartono, E., Maizels, R.M., et al. (2010) Regulatory T cells in human geohelminth infection suppress immune responses to BCG and \textit{Plasmodium falciparum}. \textit{Eur J Immunol} \textbf{40}: 437–442.

Wohlfert, E., and Belkaid, Y. (2008) Role of endogenous and induced regulatory T cells during infections. \textit{J Clin Immunol} \textbf{28}: 707–715.

Xiao, Y., Li, B., Zhou, Z., Hancock, W.W., Zhang, H., and Greene, M.I. (2010) Histone acetyltransferase mediated regulation of FOXP3 acetylation and Treg function. \textit{Curr Opin Immunol} \textbf{22}: 583–591.

Yamaguchi, T., Hirotta, K., Nagahama, K., Ohkawa, K., Takahashi, T., Nomura, T., and Sakaguchi, S. (2007) Control of immune responses by antigen-specific regulatory T cells expressing the folate receptor. \textit{Immunity} \textbf{27}: 145–159.

Yurchenko, E., Tritt, M., Hay, V., Shevach, E.M., Belkaid, Y., and Piccirillo, C.A. (2006) CCR5-dependent homing of naturally occurring CD4+ regulatory T cells to sites of \textit{Leishmania} major infection favors pathogen persistence. \textit{J Exp Med} \textbf{203}: 2451–2460.

Zelinskyy, G., Kraft, A.R., Schimmer, S., Arndt, T., and Dittmer, U. (2006) Kinetics of CD8+ effector T cell responses and induced CD4+ regulatory T cell responses during Friend retrovirus infection. \textit{Eur J Immunol} \textbf{36}: 2658–2670.

Zelinskyy, G., Dietze, K., Sparwasser, T., and Dittmer, U. (2009) Regulatory T cells suppress antiviral immune responses and increase viral loads during acute infection with a lymphotropic retrovirus. \textit{PLoS Pathog} \textbf{5}: e1000406.