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The Role of Natural Regulatory T cells in Infection

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Abstract

Naturally occurring regulatory T cells (T_{Reg}) suppress multiple cell types of the immune system to maintain dominant tolerance to protect from autoimmunity, down-modulate anti-tumor immunity and restrain allergic diseases. In addition to these functions, T_{Reg} can alter effector responses to invading pathogens, leading to a variety of outcomes affecting both the host and infecting microorganisms. Here, we review how T_{Reg} can influence the immune responses to chronic infections where pathogen-specific T_{Reg} can contribute to pathogen persistence and, in some cases, concomitant immunity, as well as control immunopathology associated with robust immune responses. We also review the data on T_{Reg} during acute infection, focusing on the questions these studies raise regarding the most appropriate model(s) to examine T_{Reg} during infection. Finally, we discuss ways in which the T_{Reg} function can be altered by invading pathogens and how these can be exploited to develop methods therapeutically to influence disease and vaccine outcomes.

Keywords

Regulatory T cells; infection; immunopathology; Foxp3

Natural regulatory T cells

More than 100 years ago, Paul Ehrlich speculated that the immune system has the potential for “horror autotoxicus” and that mechanisms must exist to avoid this deleterious fate [1]. We now know that lymphocytes of the adaptive immune system have the unique ability to discriminate between foreign and self-antigens to successfully defend against pathogens while maintaining self-tolerance. The delicate balance between autoimmunity and pathogen defense is maintained by several processes that act in both cell-intrinsic and cell-extrinsic manners. Central tolerance ensures that self-reactive T cells are deleted in the thymus in response to a high-affinity major histocompatibility complex (MHC)/self-peptide and T-cell receptor (TCR) interaction [2]. Despite this process, a small number of self-reactive T cells enter the periphery and are controlled by three main mechanisms: deletion, induction of anergy and suppression [3]. Regulatory T cells (T_{Reg}) are devoted to the active suppression of the immune system to maintain self-tolerance and immune homeostasis. For the purpose of this review, we will be focusing on naturally occurring $CD4^+CD25^+$ T_{Reg} that develop in the thymus as a unique lineage of $CD4^+$ T cells (for a review of induced T_{Reg} see [4]).

T_{Reg} develop in the thymus as a subset of $CD4^+$ T cells that acquire their suppressive ability as the result of a unique microenvironment. While all of the signals required for this microenvironment are unknown, studies using TCR transgenic mice that co-express their cognate antigen have suggested that T_{Reg} development in the thymus requires high affinity

TCR stimulation [5–7] and is critically dependent on B7 costimulation [8, 9]. It is believed that these and potentially other signals lead to the expression of the transcription factor forkhead box p3 (Foxp3), which is required for and defines the T_{Reg} lineage [10, 11]. In addition to the expression of Foxp3, T_{Reg} are characterized by the high level expression of the IL-2 receptor α chain (CD25) [10, 12], cytotoxic T lymphocyte antigen-4 (CTLA-4) [13, 14] and glucocorticoid-induced TNFR (GITR) [15, 16]. Because these molecules can also be found on activated non-T_{Reg}, Foxp3 is considered the most specific marker for T_{Reg} in both mice [10, 11] and humans [17, 18]. Once T_{Reg} develop in the thymus, they exit into the periphery where they represent 5–10% and 2–4% of the CD4⁺ T cell population in healthy mice and humans, respectively [19].

Once T_{Reg} exit into the periphery, they perform a variety of functions. Early studies of T_{Reg} focused on their role in dominant tolerance—the active, trans-acting suppression of the immune system. These studies initiated when it was shown that thymectomy in neonatal mice three days after birth resulted in the development of organ-specific autoimmunity that could be prevented by transferring syngeneic T cells from the spleen or thymus [20]. Further work by Sakaguchi *et al.* demonstrated that the cell type responsible for preventing autoimmune disease in this model was CD4⁺ CD25⁺ T_{Reg} [10, 12]. Later studies further classified these cells as Foxp3⁺ [10, 11, 21].

The importance of T_{Reg} for the maintenance of tolerance is exemplified in studies where genetic mutations or other manipulations that functionally delete T_{Reg} results in aggressive and fatal autoimmune disease. Mice with the spontaneous scurfy mutation (*sf*), which renders Foxp3 nonfunctional, develop fatal autoimmune disease characterized by severe lymphoproliferative and lymphocytic organ-infiltration ultimately leading to death three to four weeks after birth [22, 23]. In humans, an analogous mutation leads to a condition termed ‘immune dysregulation, polyendocrinopathy, enteropathy, X-linked’ (IPEX), where patients display symptoms similar to those seen in *sf* mice [24–26]. Disregulation of T_{Reg} has also been associated with other autoimmune diseases including multiple sclerosis, type 1 diabetes and rheumatoid arthritis [27–29]. In addition to autoimmune diseases, T_{Reg} have been implicated in the development of allergic diseases such as asthma [30], in the suppression of anti-tumor immunity [31] and during pathogen infection, which will be discussed at length here.

The diverse functions of T_{Reg} are a result of their ability to suppress multiple cells of the immune system, including non-T_{Reg} CD4⁺ T cells, CD8⁺ T cells, dendritic cells (DC), B cells, natural killer (NK) cells, NK T cells, macrophage and mast cells [8, 32–38]. Currently, the exact mechanisms by which T_{Reg} suppress all these cell types are not fully elucidated, and many mechanisms remain controversial. Current evidence suggests that *in vivo* suppression can be mediated by anti-inflammatory cytokines, such as interleukin 10 (IL-10), IL-35 and transforming growth factor β (TGF- β) and via cell-cell contact potentially through the inhibitory receptor CTLA-4 [39, 40]. Additional studies have suggested a role for granzyme or perforin-dependent killing [41–43] and adenosine and cyclic AMP in T_{Reg} suppression [44, 45]. Studies using intravital two-photon laser scanning microscopy (TPLSM) have attempted to further understand the dynamics of T_{Reg} suppression *in vivo*. These studies have demonstrated that T_{Reg} suppress the immune response by preventing stable contact between DCs and non-T_{Reg} [46, 47] suggesting that *in vivo* T_{Reg} may modulate antigen presenting cells (APC) function to suppress T cells. Further studies are required to more fully appreciate how suppression occurs *in vivo* and what mechanisms of suppression are utilized during pathogen invasion.

Regulatory T cells in chronic infection

While initial studies with T_{Reg} focused on their role in dominant tolerance, accumulating evidence indicates an important role for T_{Reg} in the control of immune responses to pathogens [48, 49]. One of the earliest implications for this role came from a model of chronic infection with *Leishmania major* when it was demonstrated that IL-10, which is known to be produced by T_{Reg}, contributes to pathogen persistence [50]. Subsequent studies demonstrated that Leishmania-specific T_{Reg} found at the sites of infection suppress T cells, by both IL-10-dependent and –independent mechanisms, to prevent complete pathogen clearance, leading to the development of concomitant immunity [51, 52]. Additional studies in both experimental animal models and humans have shown that T_{Reg} can accumulate following multiple types of infections and modulate the immune response in a variety of ways that can be beneficial for both the host and the pathogen.

Studies in humans have demonstrated that T_{Reg} can expand following infection and accumulate at the sites of infection. For example, T_{Reg} have been found in patients with parasitic infections (i.e. *Leishmania braziliensis* [53]), viral infections (i.e. hepatitis B [54]), fungal infection (i.e. *Paracoccidioides brasiliensis* [55]) and bacterial infection (i.e. *Helicobacter pylori* [56]). Studies to examine the effect of T_{Reg} during human infection are generally limited to an *in vitro* suppression assay whereby responder cells (non-T_{Reg}) are cultured with T_{Reg} in the presence of a stimulus for activation (i.e. APC + anti-CD3 antibody or agonist peptide). When these cells are cultured together, T_{Reg} will suppress the proliferation of non-T_{Reg}, which can be experimentally measured. Using this method, the suppression of pathogen-specific effector cells by human T_{Reg} has been demonstrated in a variety of disease models including *Plasmodium falciparum* where the removal of T_{Reg} enhances *in vitro* immune responses against the pathogen [57]. While *in vivo* evidence for T_{Reg} function during infection in humans is lacking, one study comparing an ethnic group with a naturally lower susceptibility to *Plasmodium falciparum* malaria than sympatric ethnic groups has shed some light. It was found that the group's resistance was not associated with classic malaria-resistance genes, but rather may be related to the fact that members of this group have a propensity for functionally deficient T_{Reg} characterized by reduced RNA for genes associated with T_{Reg} function, as well as lower serum levels of TGF- β [57]. These data suggest a strong role for T_{Reg} in malaria resistance and provide a unique human system to further understand how T_{Reg} can affect the outcome of pathogen infection.

To more fully understand the role of T_{Reg} during infection than can be garnered with human studies, murine models of chronic parasitic, bacterial, viral and fungal infections have been utilized. These experiments have shown that the presence of T_{Reg} can suppress effector cells, preventing the immune system from mounting an effective immune response, which can lead to pathogen persistence. In some extreme cases, this can be detrimental to host survival, such as infection with a lethal strain of *Plasmodium yoelii* where T_{Reg} vigorously block an effective immune response, and depletion of T_{Reg} can actually improve host survival [58]. In what appears to be a similar mechanism, during infection with a hypervirulent, lethal strain of *Mycobacterium tuberculosis* as the infection proceeds, the number of effector T cells is reduced, and this is associated with an increase in the presence of T_{Reg} [59]. Further studies are required to confirm that the T_{Reg} are responsible for the decrease in effector cells and whether this is related to the lethality of this strain.

Multiple other studies of chronic infection have demonstrated that, while the presence of T_{Reg} does not contribute to the pathogen's lethality, depletion of T_{Reg} leads to increased effector responses, supporting pathogen clearance. For example, in a murine model of ocular infection with herpes simplex virus 1 (HSV1), depletion of T_{Reg} leads to enhanced CD4 and

CD8 expansion, effector function and infiltration into the cornea [60, 61]. Similar enhancement of effector function has been observed in multiple other models of parasitic, bacterial, fungal and viral infection (for review see [62]). However, not all studies have demonstrated that T_{Reg} function to block effector activation. With *Mycobacterium tuberculosis* (Mtb) infection, depletion of Foxp3⁺ T_{Reg} prior to infection results in significantly reduced bacterial burden in the lungs, but this reduction was not associated with an increase in pathogen-specific non-T_{Reg} effector function. This seemingly paradoxical effect may be related to the low levels of antigen available for T cell activation that occurs following T_{Reg} depletion in this model [63]. However, using the same model of infection, Quinn *et al.* demonstrated that depleting T_{Reg} with an anti-CD25 antibody increased effector IFN- γ production but did not alter bacterial load [64] suggesting that the effects observed during T_{Reg} depletion may be related to the method of depletion utilized, which may be critical in this particular model because pathogen load is highly limiting to T cell expansion due to the inefficiency of antigen presentation.

While the presence of T_{Reg} may lead to pathogen persistence, this can actually be beneficial for the host as it is becoming increasingly clear that this can be critical to develop protective immunity. For example, with *L. major* infection the downregulation of the effector response by T_{Reg} leads to concomitant immunity, which is lost by removal of T_{Reg} [52]. This has also been demonstrated with a murine model of infection with the fungus *Candida albicans* where the presence of T_{Reg} was required for resistance to reinfection [65]. Additionally, similar results have been observed in infection with a lose-dose of HSV1 [60].

In addition to allowing for the development of protective immunity, T_{Reg} can benefit the host by reducing immune-mediated pathology that can occur as collateral damage associated with a strong anti-pathogen immune response. This role for T_{Reg} in protecting against immune-mediated damage has been demonstrated in models of pulmonary inflammation caused by *Pneumocystis pneumonia*, inflammatory eye lesions with HSV, liver pathology in *Schistosoma mansoni* infection and stomach pathology with *Candida albicans* where depletion of T_{Reg} leads to enhanced pathology [60, 65–68]. However, not all chronic models of infection support a role for T_{Reg} in the prevention of immunopathology. Paradoxically, in a model of *Plasmodium yoelii* infection, removal of T_{Reg} actually resulted in reduced immunopathology in the brain [69]. This result was thought to occur because the depletion of T_{Reg} significantly reduced both parasite burden and the recruitment of CD8⁺ T cells in the brain [69]. In this model of experimental cerebral malaria (ECM) it appears as if the high parasite burden localized in the brain, as well as the potential exclusion of T_{Reg} from the brain during ECM [70], may make immunopathology an unavoidable consequence to combating *P. yoelii* infection.

Collectively, these results indicate there is a delicate balance between T_{Reg} and non-T_{Reg}, and any changes to this equilibrium can alter the outcome of a chronic infection. If T_{Reg} suppression is too vigorous, this can lead to poor pathogen clearance and possibly even host death. On the other end of the spectrum, without adequate suppression, non-T_{Reg} can cause collateral tissue damage due to an overzealous immune response. However, if non-T_{Reg} and T_{Reg} are balanced then this can lead to protective immunity without excessive immunopathology. Studies are underway to determine whether this balance can be exploited to modulate vaccine or disease outcomes.

Regulatory T cells in acute infection

Compared to studies with chronic infection, to date very few studies have been conducted to understand the function of T_{Reg} during acute infection. The results of these studies have painted contradictory roles for T_{Reg} during acute infection and highlight how different

models of T_{Reg} depletion can lead to opposing results. Utilizing an anti-CD25 antibody to deplete T_{Reg}, it was shown that T_{Reg} do not affect the immune response or the disease outcome in *Pseudomonas aeruginosa* lung infection in mice [71]. Using a similar method of depletion, two independent studies examined T_{Reg} during acute *Trypanosoma cruzi* infection in mice and found no role in one of the studies and a limited role for T_{Reg} in the other study [72, 73]. The impact of T_{Reg} was largely dependent on the dose of *T. cruzi* where mice receiving a lower dose displayed reduced parasitemia and mortality following T_{Reg} depletion, but these effects were no longer observed when higher numbers of parasites were used for infection [73]. The discrepancies between these two studies and between the different parasite doses may be related to the relative inefficiency of T_{Reg} depletion by anti-CD25 antibodies observed during infection and/or the depletion of effector T cells, which upregulate CD25 following activation [74, 75]. These pitfalls of depletion may be more apparent in models of acute infection because effector cells can express higher levels of CD25 during acute compared to chronic infection [75]. Because of these caveats with CD25 antibody depletion, newer mouse models that selectively deplete Foxp3⁺ T_{Reg} may provide a better method to study the role of T_{Reg} during infection.

To overcome the inherent problems of using anti-CD25 antibodies to study T_{Reg} during infection, Foxp3^{DTR} mice, which express the human diphtheria toxin receptor (DTR) under control of the Foxp3 promoter, have been used to study T_{Reg} during acute genital infection with HSV-2 [76]. In these mice, treatment with DT allows for the rapid and efficient depletion of Foxp3-expressing cells [77]. The results from these experiments were quite unexpected; while T_{Reg}-depleted mice displayed increased activation of effector cells in the draining lymph nodes, they were unable to control genital HSV-2 infection [76]. This led to severe lesions and faster viral dissemination into the spinal cord resulting in hindlimb paralysis, and as a result, DT-treated Foxp3^{DTR} mice succumbed to disease much earlier than T_{Reg}-sufficient mice [76]. This apparently paradoxical effect was related to T_{Reg} modulating chemokine levels to ensure proper recruitment of immune cells to the site of infection [76]. In T_{Reg}-depleted mice the arrival of DCs, NK cells and T cells was delayed at the site of infection compared to T_{Reg}-sufficient mice [76]. This is not the first report that has implicated a role for T_{Reg} in cellular migration; in a model of respiratory syncytial virus infection, T_{Reg} were shown to alter CD8⁺ T cell trafficking to the lung following infection [78].

While using Foxp3-DTR mice is a much more specific method to study the role of T_{Reg} during infection, they must be carefully controlled to ensure that they are properly interpreted. Depletion of T_{Reg} using this method leads to increased activation of T cells, an increase in the number and activation of DC and the production of inflammatory cytokines and chemokines even in the absence of infection [76, 77]. These effects may be a direct result of increased activation of autoreactive T cells, which are normally controlled by T_{Reg}, but may also reflect direct or indirect effects of T_{Reg} on other cells of the immune system, such as NK cells or DC. These changes highlight the catastrophic changes that occur in the immune system when T_{Reg} are ablated and suggest that a more fine-tuned approach may be necessary to study T_{Reg} function during an immune response to ensure that the effects observed are not related to induced autoimmunity.

These studies also raise the question as to whether suppression during infection is antigen-specific or whether it occurs as the result of a bystander effect of T_{Reg} responding to self-antigens. This is critical to determine whether depletion of the polyclonal T_{Reg} population is the best method to study T_{Reg} during infection. Previous work to classify the T_{Reg} TCR repertoire has suggested some overlap between the TCRs of T_{Reg} and non-T_{Reg} suggesting that T_{Reg} could recognize more than just self-antigens [79, 80]. Indeed, work by Suffia *et al.* demonstrated that T_{Reg} found at the sites of *L. major* infection proliferate in a pathogen-

specific manner and that suppression *in vitro* is antigen-specific [51]. Additional studies with both mice and humans in other models of infection support these observations [81, 82]. While the antigen-specificity of suppression *in vivo* has been demonstrated in our laboratory in an autoimmune setting [83], further studies are necessary to demonstrate that the *in vivo* suppression observed during infection is antigen-specific. Additionally, studies to examine whether non-T_{Reg} and T_{Reg} recognize the same the immunodominant epitopes as this may provide a mechanism to discriminate between the two cell types in vaccine development.

Taken together, it is clear that additional studies with acute infection are necessary as multiple additional questions remain. First, it is still not entirely clear whether T_{Reg} play a role during acute infection or perhaps in just select acute infection models. These questions can be addressed utilizing different models of acute infection coupled with different approaches to deplete T_{Reg} perhaps identifying systems that do not remove the polyclonal population but rather study pathogen-specific T_{Reg}. Additionally, work will need to be done to further clarify how T_{Reg} modulate the outcome acute infection, for example, whether they simply modulate migration as with genital HSV-2 infection or influence effector function and/or immunopathology as observed in chronic infection models. Finally, studies using acute infection models can be used to determine what happens to activated T_{Reg} following the resolution of an acute infection.

Manipulating regulatory T cells to regulate disease or vaccine outcome

Because of the important function of T_{Reg} during infection, researchers are studying how to manipulate them to adjust either disease or vaccine outcome. While therapeutic modulation of T_{Reg} is a viable research area, it appears as if microorganisms have already devised mechanisms to alter T_{Reg} function to promote their survival. One mechanism for modulation by pathogens may be through recognition of pathogens by innate pathogen pattern receptors such as Toll-like receptors (TLRs) (for review see [84]). While it has been demonstrated that T_{Reg} express various TLRs, how direct TLR signaling on T_{Reg} has not been fully elucidated and remains debated. Some studies have suggested that TLR2 or 8 stimulation abrogates the suppressive capacity of T_{Reg}, while others have shown that TLR4 and 5 can act to enhance suppression [84]. These studies are not without controversy, however, as it was recently suggested that TLR2 stimulation of T_{Reg} results in increased survival, but does not affect suppressive capacity [85]. Because many of these studies have been conducted *in vitro*, additional studies are necessary to fully tease apart the intricacies of TLR signaling on T_{Reg} function *in vivo*. In addition to direct TLR stimulation on T_{Reg}, TLRs can also affect T_{Reg} through an indirect mechanism via DCs to block their ability to suppress via an IL-6-dependent mechanism [86].

Another mechanism by which pathogens may alter T_{Reg} is through the creation of chemokine microenvironments that support T_{Reg} retention at the site of infection. There is evidence that chemokine receptor and integrin expression on T_{Reg} are critical for their function [87–89]. Expression of the integrin $\alpha_E\beta_7$ (CD103), which primarily binds to E-cadherin, can be used to separate T_{Reg} with “effector/memory” phenotypes that have enhanced suppressive capabilities and distinct homing properties [90, 91]. Studies have demonstrated a role for CD103⁺ T_{Reg} during infection. Work with *L. major* has demonstrated that CD103 is required for retention of T_{Reg} at the sites of infection and that *L. major*-infected DC can stimulate CD103 expression in T_{Reg} [92] suggesting that the pathogen manipulates DCs to support T_{Reg} retention. Further work with *L. major* infection has shown that the chemokine receptor CCR5 is critical for T_{Reg} migration into the skin. [93]. Similar results with CCR5 have also been shown with the dimorphic fungi *Paracoccidioides brasiliensis* and *Histoplasma capsulatum* [94, 95]. These data suggest unique chemokine and integrin signals are utilized by T_{Reg} to migrate to sites of infection and perhaps that the expression of these

receptors can be modulated by pathogens. Additional work is necessary to explore other chemokines and/or integrins that affect T_{Reg} migration during infection and the ways in which pathogens can manipulate the expression of these molecules.

By understanding the ways in which microbes modulate T_{Reg}, as well as the ways in which T_{Reg} suppress, therapies can be developed to target T_{Reg} to manipulate disease or vaccine outcome to control microorganisms, block immunopathology or support the development of protective memory. With any of these potential therapies, care will need to be taken to maintain the balance between immunity to pathogens and autoimmunity as ablation of T_{Reg} can have deleterious effects [77]. As with studies in infection, perhaps therapies aimed a pathogen-specific T_{Reg} may be an effective and specific method that could avoid potentially damaging side effects.

Concluding remarks

Our knowledge about the functions of T_{Reg} in the immune system has grown tremendously since the days of the ill-fated “suppressor cell.” This expanding knowledge has revealed unique roles for T_{Reg} during pathogen invasion, especially in models of chronic infection. To further understand how T_{Reg} modulate infection outcomes, novel model systems of T_{Reg} depletion or addition will need to be carried out, particularly with acute infections. Elucidating these roles more fully, as well as the ways in which pathogens modulate T_{Reg} function is critical to harness their potential for vaccine development or therapeutics to treat infection. Overall, research examining T_{Reg} during infection has suggested that the relationship between T_{Reg}, invading microorganisms and the resulting immune response is one filled with various ententes, which will need to be considered in any therapeutic intervention.

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