Regulatory T Cells and Infectious Disease

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ABSTRACT

Various cell types that express regulatory function may influence the pathogenesis of most and perhaps all infections. Some regulatory cells are present at the time of infection whereas others are induced or activated in response to infection. The actual mechanisms by which different types of infections signal regulatory cell responses remain poorly understood. However a most likely mechanism is the creation of a microenvironment that permits the conversion of conventional T cells into cells with the same antigen specificity that have regulatory function. Some possible means by which this can occur are discussed. The relationship between regulatory cells and infections is complex especially with chronic situations. The outcome can either be of benefit to the host or damage the disease control process or in rare instances appears to be a component of a finely balanced relationship between the host and the infecting agent. Manipulating the regulatory cell responses to achieve a favorable outcome of infection remains an unfulfilled objective of therapeutic immunology. **(Immune Network 2007;7(4):167-172)**

Key Words: Regulatory T cells, infection

Introduction

The concept that positive host responses to self and foreign antigens was subject to inhibition by dedicated suppressor cells was rejuvenated in the mid 90s when phenotypic markers were identified that could distinguish cells that functioned as negative regulators. As observed by Sakaguchi, one such marker was the high affinity IL-2R alpha chain (CD25) that was expressed on 5～10% of CD4+ T cells in naive animals (1). These cells were thymus-derived and largely self-reactive and since their removal resulted in autoimmunity, it became evident that their function in healthy animals was to prevent the occurrence of autoimmune diseases (1). Several additional phenotypic markers were found useful to distinguish these so-called natural Tregulators (nTreg) from conventional T cells, but expression of the Forkhead box transcription factor 3 (Foxp3) turned out to be the most reliable identifier (2). Indeed, so called scurfy mice with a mutation in the gene for Foxp3 developed multiple autoimmune lesions (3) as do humans with several types of Foxp3 gene mutations which develop the immune dysfunction polyendocrinopathy/enteropaty X linked syndrome (4). Expression of the transcription factor Foxp3 is a canonical marker of mouse regulatory T cells but it is less reliable for cells with same function in humans (5).

Although most Foxp3$^+$ T cells are usually considered as autoreactive and are involved in preventing and constraining autoimmunity, cells of the same phenotype may also participate in responses to foreign antigens and tumors. Indeed, regulatory cells may function beneficially to control tissue damage caused by autoreactivity, allergies and allotransplants, as well as responses to many tissue damaging pathogens (6). Circumstances also exist where the Treg response is considered as detrimental to the host. These include blunting the protective immune response to tumors (6) as well as impairing protective immune response to several infectious agents (7). This may result in pathogen persistence and chronic disease as discussed subsequently. Under some circumstances Treg may form part of a balanced system wherein they prevent effector responses from relegating the infectious agent or its ability to cause tissue damage (8). This is a valuable

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circumstance in some parasitic infections where persistence of the agent appears to be necessary to impart immunity to re-infection.

Types of interactions between regulatory T cells and infectious agents

Although most articles on the role of regulatory T cells during infectious disease have focused on Foxp3$^+$ Tregs there are in fact multiple types of cells that can have regulatory functions (see Table I) Basically, these fall into two groups: those present at the time of infection which include the largely autoreactive nTreg as well as some less well investigated cell phenotypes. Pathogens may also cause the induction and or activation of regulatory cells from pathogen specific conventional precursors (7). These are often called adaptive Treg. Some of these also express the Foxp3 transcription factor, but others do not. The potential means by which infections can result in conversion of non regulatory cells to Foxp3 regulators is further discussed in a later section.

Since the pathogenesis of most infectious diseases is complex and not fully understood, it is likely that many types of regulatory cells may influence events and these may act at different stages of the infectious process. Moreover, the differential outcome of an infection in individual patients might be influenced by the magnitude and nature of the type regulatory T cell response that is brought into play. In many cases of natural and experimental infection, the Treg response that occurs appears to be counterproductive (7). Accordingly, Treg may inhibit the magnitude or delay the induction of several components responsible for protective immunity. Inhibition of the effector functions of both T cells and NK cells against infected target cells has also been reported (9,10). The outcome of such immune blunting may be that the agent is more able to persist which in some infections can result in chronic tissue damage (reviewed in 7,11). Examples of immune blunting by one or another types of Treg can be observed with all categories of infectious agents, but this type of relationship appears to be more relevant with those that cause chronic infections. In fact, it could be that if an individual makes a prompt and strong Treg response to an infection they may be more likely to develop chronic disease. This has been advocated to occur with both hepatitis C and hepatitis B infections in humans (12,13). A strong Treg response might also account for the rapid onset of disease after HIV infection of humans (14), as well as whether or not individuals can effectively control malaria infections (15). Incriminating a strong Treg response as detrimental as the explanation for a poor outcome of infections in humans is difficult to substantiate. Thus, rarely if ever have longitudinal studies been done that quantify and characterize regulatory T cells the Treg response at different times after the onset of infection and relating such measurements to the eventual outcome.

In experimental infections in animal systems, it has proven easier to demonstrate that regulatory T cell response can be detrimental, since convenient means are available to deplete or block the function of Treg before or during the course of infection. In our own studies, for example, we have shown that animals depleted of Treg develop better acute and memory immune responses to herpes simplex virus (HSV) (16). Depleted animals may also clear virus more rapidly and are less likely to develop herpetic encephalitis (16). The effect of Treg depletion may be even more apparent in neonatal animals (17). Indeed, it seems that neonates of many species have more Treg than adults which may help explain why young animals show greater susceptibility to many infections (18).

Interference by a Treg response has also been advocated as an explanation for inferior immune responses to some vaccines (19,20). Recently, for example, vaccine responses to a recombinant poxvirus vector expressing influenza antigens were enhanced if Tregs were depleted with a fusion protein of interleukin 2 and diphtheria toxin that targets Tregs (21). A more

Table I. Types of regulatory cells that can influence responses to infections

- Those present at the time of infection
  - Foxp3$^+$ CD4$^+$ T cells
  - NK-T cells, $\gamma\ \delta$ CD4$^+$ T cells
- Those induced or expanded as a consequence of infection
  - Foxp3$^-$ IL-10 producing (Tr1 cells)
  - CD4$^+$ TGFβ producing
  - CD8$^+$ T cells
  - Highly polarized Th1 cells producing IL-10
  - Conversion of Ag-specific Foxp3$^-$ cells to become Foxp3$^+$ regulators

detailed account of the apparent detrimental effects of Treg responses on the outcome of infection was published recently in an excellent review by Belkaid (11).

Perhaps more commonly, especially during chronic infections, regulatory Tcell responses benefit the host by reducing the collateral tissue damage that usually accompanies immune responses to infections and which in some circumstances are fully responsible for any lesions observed. An example of the later situation occurs with infections of the cornea of the eye with HSV (22). Here, the chronic blinding inflammatory reaction appears to be caused by an immunopathological response to the pathogen. We have shown that the severity of the lesion is influenced beneficially by $Foxp3^+$ Treg since animals depleted of such cells develop more severe disease and are susceptible to lower doses of infection (22).

In Human immunodeficiency virus (HIV) infection too, some have advocated that Treg largely play a beneficial role (23). This is because the regulatory cells may suppress the HIV induced hyperactivity that leads to the AIDS syndrome (24). A similar conclusion was arrived at from results of experimental SIV infection in monkeys. In this model, African Green monkeys (AGM) failed to progress to AIDS and remained asymptomatic (25). However, similarly infected macaques developed disease and usually died from infection. The difference appears to be that the AGM develop a rapid and effective Treg response which serves to blunt CD4 T cell hyperactivity. Such a response failed to occur or only developed later in macaques with animals developing generalized T cell activation and ultimately dying with AIDS (25).The value of Treg responses in controlling tissue damaging inflammatory reactions has also been described in several other human infections as well as in numerous model infection systems in mice. That topic has been recently reviewed (7,11,26).

Treg responses can exert Bystander regulatory effects

Studies of nTreg activities *in vitro* have revealed that once cells are activated as a consequence of receptor triggering, they may exert their regulatory effects non specifically. This nonspecific activity is particularly true for cells whose regulatory function is dependent on the production of an abundance of cytokines such as IL 10 and TGF-β (27). Accordingly, one anticipates that when Tregs are in the act of mediating their regulatory effects *in vivo* they are likely capable of bystander regulatory activity. This might translate into effects on other ongoing inflammatory diseases, such as autoimmunity and allergic disease, as well as inflammatory responses to coinfecting pathogens. Conceivably bystander function could have negative implications for anti tumor immunity. Reports which support most of these ideas have already been published (28-30) but more investigation is needed especially if Treg manipulation is to become a common therapeutic modality.

How do pathogens signal regulatory T cells

This remains an important but still poorly understood issue (see Table II). Initially, it was thought that $Foxp3^+$ nTregs were all self- reactive but this idea is now questioned (31) and it is evident that some $Foxp3^+$ cells can react with foreign antigens, which include those antigens expressed by pathogens (11). It is not clear, however, if pathogen specific nTreg already exist in meaningful numbers in naive animals at the time of initial infection, and whether or not it is this minority of cells alone that are signaled by the pathogen to play a regulatory role. It seems more likely, however, the pathogen would signal the response polyclonally of preexisting Treg by non antigen specific means such as by their toll-like receptors ligand activity or by their induction of cytokines and other mediators that could activate Treg. Other possibilities could include instances of cross reactivity with self antigens and in rare cases, as occur with some retroviruses, of infection of Treg resulting in their activation (32).

Table II. How do pathogens signal regulatory cells?

- Still poorly understood and controversial
- nTreg repertoire includes epitopes from some pathogens
- Direct infection expands and activates Treg-some retroviruses
- Expansion and activation by non-T cell receptor binding activity such as Toll-like receptor ligand stimulation
- Creation of a microenvironment that induces Treg-
  - e.g. abundant TGFβ, IL-10 and minimal IL-12, IL-6, IL-21
  - minimal DC maturation and presentation by specialized types of DCs and perhaps macrophages

A far more likely mechanism by which pathogens could induce and activate Treg is by creating a microenvironment which permits the generation of mainly pathogen specific regulatory cells from non-regulatory precursors. This has been a "hot topic" recently in the Treg field since it is particularly relevant in terms of devising useful ways of manipulating the response therapeutically. We have known for some time that the administration of TGF-β along with antigen can result in Foxp3$^+$ Tregs induction *in vivo* (33). In addition repeated exposure to low doses of antigen presentation by certain types of dendritic cells (DCs) may result in Foxp3$^+$ Treg induction *in vivo* (34). More recently methods have been refined that succeed in converting naïve antigen specific non-regulatory T cells into Foxp3$^+$ regulatory cells of the same specificity. This was achieved by TCR stimulation in the presence of TGF-β and IL-2 (35). Curiously, adding inflammatory cytokines such as IL-6 resulted in the induction of T cells that were IL-17 producing. The cytokine IL-6 as well as others such as IL-12 and IL-21 are advocated to inhibit function of Tregs *in vitro* as well as *in vivo* (36,37).

Additional means of achieving Foxp3$^+$ Treg conversion are appearing on the scene. Most recently a mechanistic explanation became evident for the observation that oral antigen administration can result in tolerance and the fact that many gut tissues have an abundance of Foxp3$^+$ Tregs (38). Thus a numbers of groups observed that DCs isolated from the gut express enzymes such as retinal dehydrogenase that permit the conversion of vitamin A into retinoic acid (39, 40). Furthermore, retinoic acid along with TGF-β act as a factor that drives the conversion of TCR stimulated Foxp3$^-$ conventional T cells into Foxp3$^+$ regulatory cells. Retinoic acid may also drive the conversion of human cells although in this instance the co-addition of TBF-β is not required (41).

There is likely to be additional means of converting conventional T cells into regulators some of which may prove useful for therapeutic use. Our group has investigated one such candidate. Accordingly, we have observed that the TCR stimulation of T cells in the presence of the fungal metabolite FTY720 and IL-2 results in Treg conversion (42). This conversion does not require the addition of TGF-β to cultures although the conversion process appears to be TGF-β dependant. More interestingly, exposure of animals to Ag along with FTY720 treatment caused Ag-specific Foxp3$^+$ T cell induction. Such cells were capable of inhibiting inflammatory lesions caused by ocular infection by HSV (see Fig. 1). We anticipate that FTY720 treatment *in vivo*, which has several additional anti-inflammatory activities, could represent a convenient means of expanding pathogen specific regulatory T cells. This approach may prove useful to control some chronic infections. Such studies are being pursued in the authors' laboratory.

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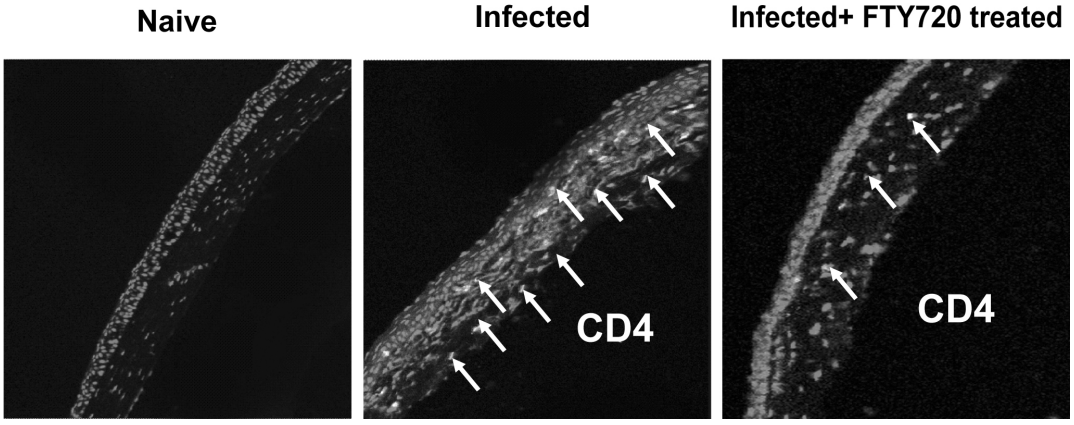

Figure 1. FTY720 treatment diminishes the severity of stromal keratitis induced by HSV ocular infection in BALB/c animals. The results shows diminished lesion severity, angiogenesis and incidence in mice treated with FTY720. The picture shows histological sections of corneas from naïve, HSV infected untreated and FTY720 treated animals 12 days post infection. Arrows indicate CD4$^+$ T cells.

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