In: 55th Annual Meeting of the American College of Veterinary Pathologists (ACVP) & 39th Annual Meeting of the American Society of Clinical Pathology (ASVCP). ACVP and ASVCP (Eds.) Publisher: American College of Veterinary Pathologists & American Society for Veterinary Clinical Pathology, Middleton WI, USA. Internet Publisher: International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.

T Cell - T Cell Interactions in Regulating the Immune Response (13-Nov-2004)

M. B. Tompkins and W. A. Tompkins

Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA.

Introduction

The immune system is a highly orchestrated, tightly controlled response to foreign antigens. Activation of the response involves antigen processing and presentation followed by engagement of specific cell surface receptors and their ligands, resulting in cytokine production and the expansion of antigen specific effector cells. Equally important as the activation steps are the processes that control the level of the response and "turn it off" once the antigen is cleared. There are a number of these immune-regulation mechanisms including cytokine-induced suppression, antibody feedback, and activation-induced cell death (apoptosis). This discussion will focus on two T cell lineage-specific mechanisms, the B7-CTLA4 signaling pathway and CD4+CD25+ T regulatory cells.

The B7-CTLA4 Signaling Pathway

B7.1 and B7.2 are members of the B7 family of molecules and function as co-stimulatory signals. They are normally found on professional antigen presenting cells (APC), and interact with CD28 and CTLA4 on T cells to provide the necessary second signals for regulating the immune response. B7.1 and B7.2 initially bind CD28 on T cells, leading to sustained IL2 secretion, T cell proliferation, and development of an immune response. If this second signal does not occur following antigen-TCR engagement, the T cell becomes unresponsive or anergic to the antigen and, in the absence of IL2, undergoes apoptosis [1,2]. While CD28 is constitutively expressed on CD4+ T cells and transduces a positive signal, CTLA4 is only expressed after the CD4+ T cell becomes activated (2 - 3 days post APC-TCR engagement) and upon engagement with B7 molecules, transduces a negative signal to T cells, suppressing IL2 transcription and further proliferation, resulting in anergy and apoptosis [3,4]. As the binding affinity of B7.1 and B7.2 for CTLA4 is 40 - 50x greater than for CD28, negative signaling would dominate on activated T cells, thereby terminating the immune response. Thus, CD28 - B7 signaling, leading to clonal expansion, followed by CTLA4 up-regulation, subsequent B7 binding, and clonal deletion, provides a mechanism to maintain a balance between activation and down-regulation of a normal immune response. The importance of B7, CD28, and CTLA4 in regulating immune responses is illustrated by the observations that CD28 knockout mice are unable to develop an immune response to T cell dependent antigens, and CTLA4 knockout mice are unable to negatively regulate immune responses and develop fatal lymphoproliferative disease [1].

Although B7.1 and B7.2 receptors are normally restricted to antigen presenting cells (APC) such as dendritic cells, activated monocyes, and B cells, numerous studies have demonstrated that these molecules are up-regulated on CD8+ and CD4+ T cells activated in vitro, as well as on a small subset of CD4+ and CD8+ cells in vivo under conditions of persistent antigenic stimulation such as autoimmune disease or chronic infections [5-8]. While the functional significance of B7 expression on T cells in the immune response is unclear, these B7+ T cells co-express MHCII molecules [5,8], suggesting to some that these activated T cells are capable of functioning as APC. However, the result of T-T antigen presentation is not co-stimulation and IL2 production, but rather anergy and apoptosis. For example, Taams et al. [9] demonstrated that the anergy associated with T cell presentation of antigens to activated T cells (T-T activation) correlated with failure to produce IL2 and IFNγ, cytokines necessary for a T cell proliferative response. Greenfield et. al. [10] showed that while B7.1 expressed on T cells was capable of binding both CD28 and CTLA4 on co-stimulated T cells, B7.2 was capable of binding only CTLA4 and caused T-cell anergy. Thus, one can predict that the presence of activated LN T cells expressing B7 and/or CTLA4 would result in frequent
T-T interactions mediated by B7.1/B7.2-CTLA4 ligation that would transduce a signal for anergy and apoptosis. Such interactions raise the possibility that B7+ T cells may mediate the normal process of activation-induced cell death that regulates immune responses in germinal centers. In support of this hypothesis is our finding that spontaneous apoptosis occurs in the lymph nodes of normal cats, albeit at a low level (2 - 5% of total lymphocytes) [7]. Using 3-color flow analysis, we were able to determine that the T cell apoptosis was associated with T cells expressing B7.1 and/or B7.2 and CTLA4.

**B7/CTLA4 Signaling and Immunopathology**

The hallmark of HIV and FIV infections is a gradual loss in T-helper cell numbers and function, resulting in immune deficiency and increased susceptibility to secondary pathogens. One of the mechanisms proposed for CD4+ T cell loss is accelerated programmed cell death and is supported by reports of abnormally high frequency of apoptotic T cells in lymph nodes (LN) of HIV-infected individuals [11] and FIV-infected cats [12]. As B7 molecules are up-regulated on T cells under circumstances of persistent antigenic stimulation such as HIV or FIV infection, we explored the possibility that the accelerated apoptosis observed in LN of HIV-infected patients and FIV-infected cats, as well as under other conditions of chronic immune stimulation such as autoimmune diseases could result from anergy signals mediated by activated T cells expressing B7 molecules. Using 3-color flow analysis, we found an increase in the percent lymph node and PBMC T cells in FIV-infected cats that co-expressed B7 and CTLA4 compared to normal cats [7]. Spontaneous apoptosis of T cells from the nodes of FIV-infected cats tended to occur at a higher frequency than in control cats and was largely restricted to T cells expressing B7.1/B7.2 and CTLA4. As noted above, spontaneous apoptosis also occurred in the LN of control cats, although at a much lower frequency than in FIV-infected cats, and was also associated with B7/CTLA4 expression. We (unpublished) and others [5,8,13] have made similar observations in PBMC from HIV-infected patients. These data support the hypothesis that lymph node apoptosis and immune deterioration in FIV-infected cats results from chronic B7.1/B7.2-CTLA4 mediated T-T interactions and that these viruses have conscripted a normal T cell regulatory process.

**CD4+ T Regulatory Cells**

The surface phenotype and function of the activated CD4+ B7+ CTLA4+ T cell described above is similar to a population of T regulatory cells that also express CD25 and act to down-regulate immune responses by inducing T cell anergy and apoptosis. These "professional" CD4+CD25+ regulatory T (Treg) cells have been shown to perform an important anti-autoimmunity function by inhibiting the activation of autoreactive T cells and maintaining peripheral self-tolerance [14-16]. Treg cells have been identified in rodents [14,15], humans [17], and most recently, in cats [18], and comprise 5 - 10% of CD4+ cells in the peripheral blood. At present, the most useful surface marker for their identification is the CD25 (IL2 R α-chain) molecule, although they also express CTLA4, glucocorticoid-induced TNF receptor (GITR) and, when activated, B7 molecules. However, these molecules are not restricted to Treg cells; CD4+CD24- T helper cells can express them when activated, but do not develop Treg function. Recently, it has been reported that a transcription factor gene, *foxp3*, is expressed only in Treg cells and is necessary for their development and function [19,20], and genetic defects in this gene results in autoimmunity in both humans and mice [21,22].

The defining functional feature of CD4+CD25+ Treg cells is their ability to inhibit proliferation and induce anergy/apoptosis of other activated CD4+ or CD8+ T cells in vitro. In vitro suppression requires activation of Treg cells via their TCR, does not involve killing of responder T cells, and is mediated through a cell contact-dependent, cytokine-independent mechanism that transduces a signal for down-regulation of IL2, resulting in anergy [23]. Although activation of CD4+CD25+ cells is antigen-specific, once activated, they suppress CD4+ and CD8+ T cell responses in an antigen nonspecific manner [23,24]. While Treg cells have a partial activation phenotype (CD25+CTLA4+), they are anergic in that they proliferate poorly upon TCR stimulation in vitro and their survival is dependent on exogenous IL2 [25].

Recently, it has been reported that Treg cells express a number of toll-like receptors including TLR-4, and that exposure of CD4+CD25+ T cells to the TLR-4 ligand LPS up-regulated several activation markers, including B7.1, and increased their proliferative response and immunosuppressive activity in vitro and in vivo [26]. These observations support the recent proposal that there may be two populations of Treg cells; the "natural Treg cell" that fully matures in the thymus and whose major role is to keep potentially harmful autoreactive T cells in check; and the "acquired Treg cell" that could be derived from Th cells or natural Treg cells in the periphery in response to exogenous antigen or inflammatory conditions [27-30]. Phenotypically, these acquired Treg cells are indistinguishable from the natural Treg cells and they also express *foxp3*. However, depending on the model studied, these acquired Treg cells differ from natural Trg cells in their cytokine expression, and their suppressor activity is thought to be due to secretion of high levels of IL10 and/or TGFβ [31].

It has been speculated that acquired Treg cells silence or modulate Th immune responses to infectious agents in secondary lymphoid tissue. For example, resistance to *Leishmania* major is dependent on a Th1 immune response. In BALB/c mice,
infection with *L. major* results in an early burst of IL4 production and increased susceptibility to the infection. Aseffa et al. [32] demonstrated that infection of these mice depleted of CD4+CD25+ T cells resulted in even higher levels of IL4 and enhanced disease, suggesting that Treg cells play a role in suppressing the nonprotective Th2 response to the organism. In a *Pneumocystis carinii* model, CD4+CD25+ T cells prevented the development of CD4 CD25+ T cell-mediated pneumonia [29].

**Treg Cells and Immunopathology**

Activation of Treg cells in response to infectious agents can be a double-edged sword. While they can be important in reducing the magnitude of the immune response to pathogens, thus preventing potentially harmful immunopathology, the presence of these cells have also been shown to prevent complete clearance of the pathogen. Th1 responder mice infected with *Leishmania major* were not able to completely clear the organism, but developed a persistent, low-grade infection and a long-lasting immunity to reinfection. However, when these mice were depleted of CD4+CD25+ T cells then challenged with *L. major*, they were able to completely clear the organism, suggesting Treg cells are important in the maintenance of the chronic infection. Interestingly, although the mice were cured of the pathogen, there was also a complete loss of resistance to reinfection [27], indicating a failure to develop a memory T cell response. In a rodent malaria infection model, infection of mice depleted of CD4+CD25+ cells resulted in two waves of parasitemia then eventual complete clearance of the parasites [33]. This clearance was associated with strong anti-malaria T cell responses. In contrast, non-CD25+ cell depleted mice were persistently infected with malaria, and their T cell responses to the organism were severely suppressed [33]. From these data, it is clear that the relative balance of the Th immune response and CD4+CD25+ responses will determine whether the pathogen is eliminated or not.

One of the most interesting and unique immunological features of CD4+ Treg cells is that they are anergic, yet programmed for cell survival, as opposed to apoptosis [16], suggesting they are long-lived in lymphoid tissues. These characteristics could make Treg cells an ideal reservoir for a chronic infection. In this regard, we have recently reported that CD4 CD25+ T cells in the cat support a chronic productive FIV infection as opposed to CD4+CD25- T cells, which are latently infected [34]. Further analysis of these cells from both normal and FIV-infected cats demonstrated that they have the salient characteristics of CD4+ Treg cells in humans and rodents, as they constitute about 5 - 10 percent of the peripheral T cell population, are arrested in the G0/G1 stage of the cell cycle, do not respond to mitogen stimulation, and are relatively resistant to activation-induced programmed cell death [18,34]. When activated in vitro with LPS, CD4+CD25+ T cells from normal cats were able to suppress the proliferative response of Con A-stimulated CD4+CD25- T cells. Interestingly, freshly isolated, unstimulated CD4+CD25+ T cells from FIV-infected cats significantly inhibited proliferation of Con A-stimulated CD4+CD25- T cells, suggesting that these cells are activated as a result of the chronic FIV infection [18]. As activated Treg cells are non-antigen specific in their suppressive function, it is possible that these cells could in turn suppress CD4+ T helper responses a variety of antigens and contribute to the acquired immunodeficiency syndrome (AIDS) that is characteristic of this infection.

**Summary**

While much emphasis in the last two decades has been on APC-T cell interactions regulating the antigen-specific acquired immune response, recent studies have focused on T cell-T cell immune regulatory mechanisms. Engagement of CTLA4 on activated T cells by B7.1 and B7.2 co-receptors expressed on other activated T cells results in suppression of IL2 production, leading to anergy and clonal deletion, and thus termination of immune responses. A second mechanism of T cell anergy is mediated by a distinct subset of CD4+ T cells, CD4+CD25+ T regulatory cells, which also suppress immune responses by down-regulating IL2 production, leading to anergy. It appears there are two types of Treg cells: the natural Treg cells, which are thymus derived, are activated by engagement of their TCR by self-antigen, and once activated, anergize self-reactive T cells by a contact-dependent mechanism; and the acquired Treg cells, which are phenotypically identical but perhaps functionally distinct from natural Treg cells, are activated in the periphery by inflammation/infection, and play a role in regulating Th immune responses to infectious diseases by contact-dependent or cytokine (IL10, TGFβ) dependent mechanisms.
References

27. Belkaid Y, Piccirillo CA, Mendez S, et al. CD4 CD25+ regulatory T cells control Leishmania major persistence and...

All rights reserved. This document is available on-line at www.ivis.org. Document No. P1212.1104. This manuscript is reproduced in the IVIS website with the permission of the ACVP & ASVCP www.acvp.org

American College of Veterinary Pathologists
Future Annual ACVP Meetings: Dec. 4-7, 2005
Dec. 3-6, 2006
Nov. 10-14, 2007
For more information go to http://www.acvp.org