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Article

Decrease of Foxp3$^+$ Treg Cell Number and Acquisition of Effector Cell Phenotype during Lethal Infection

Guillaume Oldenhove,[1] Nicolas Bouladoux,[1] Elizabeth A. Wohlfert,[1] Jason A. Hall,[1,4] David Chou,[1] Liliane Dos santos,[1] Shaun O'Brien,[4] Rebecca Blank,[1] Erika Lamb,[3] Sundar Natarajan,[3] Robin Kastenmayer,[2] Christopher Hunter,[5] Michael E. Grigg,[3] and Yasmine Belkaid[1,*]

[1]Mucosal Immunology Unit, Laboratory of Parasitic Diseases
[2]Comparative Medicine Branch, Bill Young Center for Biodefense and Emerging Infectious Diseases
[3]Molecular Parasitology Unit
National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[4]Immunology Graduate Group
[5]Department of Pathobiology, School of Veterinary Medicine
University of Pennsylvania, Philadelphia, PA 19104, USA
*Correspondence: ybelkaid@niaid.nih.gov
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SUMMARY

Using a model of lethal oral infection with *Toxoplasma gondii*, we examined the fate of both induced and natural regulatory T (Treg) cells in the face of strong inflammatory responses occurring in a tolerogenic-prone environment. We found that during highly T helper 1 (Th1) cell-polarized mucosal immune responses, Treg cell numbers collapsed via multiple pathways, including blockade of Treg cell induction and disruption of endogenous Treg cell homeostasis. In particular, shutdown of interleukin 2 (IL-2) in the highly Th1 cell-polarized environment triggered by infection directly contributes to Treg cell incapacity to suppress effector responses and eventually leads to immunopathogenesis. Furthermore, we found that environmental cues provided by both local dendritic cells and effector T cells can induce the expression of T-bet transcription factor and IFN-γ by Treg cells. These data reveal a mechanism for Th1 cell pathogenicity that extends beyond their proinflammatory program to limit Treg cell survival.

INTRODUCTION

Failure to properly control immune responses in the face of infections or at sites of high antigenic exposure leads to pathologic consequences. To preserve tissue integrity, complementary strategies are in place, including specialized lymphocytes and antigen-presenting cell populations. Foxp3-expressing regulatory T (Treg) cells represent one of the major arms of this regulatory network by controlling both innate and adaptive immune responses (Sakaguchi et al., 2001).

In some microenvironments, such as the gastrointestinal (GI) tract, in which immune reactivity against intestinal flora or dietary antigen poses a substantial risk to the host, regulatory elements are constitutively represented (Izcue et al., 2006). For instance, in addition to various populations of Treg cells, the GI tract is home to dendritic cells (DCs) displaying regulatory functions via their capacity to release cytokines or to induce Treg cells (Coombes and Powrie, 2008). We and others have shown that in the gut environment, a substantial fraction of naive T cells can also become Foxp3$^+$ Treg cells after oral exposure to antigen (Coombes et al., 2007; Mucida et al., 2005; Sun et al., 2007). This process is associated with the capacity of gut-associated lymphoid tissue (GALT) antigen presenting cells (APC) to generate Treg cells via a mechanism that, in addition to TGF-β, is dependent on the Vitamin A metabolite retinoic acid (RA) (Coombes et al., 2007; Denning et al., 2007; Sun et al., 2007).

Nevertheless, even in highly regulated sites, immune responses need to occur to allow proper control of microbial expansion. This implies that regulatory mechanisms have to be temporally neutralized or overcome. Several lines of evidence suggest that Treg cells themselves are subject to regulation. Such control can be direct, via triggering Toll-like receptor ligands (Wei et al., 2009), or indirect, via enhanced activation of APC or effector T cells (Pasare and Medzhitov, 2003). Another means by which Treg cells could be controlled is associated with a loss of function or enhanced apoptosis (Tang et al., 2008). Recent evidence also supports the idea that Treg cells can become unstable after exposure to defined stimulatory signals or in lymphopenic environments (Degauque et al., 2008; Duarte et al., 2009; Wei et al., 2009; Xu et al., 2007). In contrast, excessive limitation of regulatory pathways can have detrimental consequences. Indeed, any failure to maintain a tight control of the equilibrium between regulation and immunity is at the core of pathologic processes from autoimmune disorders to pathogen-driven diseases. However, in most circumstances, and in particular during infection, critical environmental cues limiting the induction and function of Treg cells are poorly understood.

Treg cells control a large array of immune responses both in the context of highly polarized settings and in various microenvironments. This implies that maintenance of peripheral homeostasis also relies on the capacity of these cells to appropriately adapt to the environment they have to regulate. Recent evidence

suggests that acquisition of additional transcription factors by Treg cells is required for proper control of defined environments. For instance, expression of the transcription factor IRF4 by Treg cells contributes to their capacity to control Th2 cells responses (Zheng et al., 2009). In contrast, a recent report demonstrated that expression of T-bet by Treg cells can favor their homing to Th1 cell environments and is required for the homeostasis and function of Treg cells during type 1 inflammation (Koch et al., 2009). How, in pathogenic situations, expression of these transcription factors could also induce the expression of an effector program by Treg cells and potentially contribute to pathogenesis has not been addressed.

One of the first models to reveal the immunologic paradox of microbial control associated with host death is murine infection with *Toxoplasma gondii* (*T. gondii*) (Gazzinelli et al., 1996). This parasite manipulates innate cells in a way that induces the development of a highly polarized Th1 cell response (Gazzinelli et al., 1994). In certain strains of mice, oral infection with *T. gondii* induces a severe form of intestinal inflammation referred to as the lethal ileitis model (Liesenfeld, 2002; Liesenfeld et al., 1996; Mennechet et al., 2002). When insufficiently controlled, this pathological process that is $CD4^+$ T cell dependent leads to the death of the infected host (Liesenfeld, 2002; Mennechet et al., 2002). Using murine *T. gondii* infection, we explored the interplay between Treg and effector T cells in this tolerance-prone environment following challenge with a virulent pathogen. In particular, we addressed the factors controlling the induction of Treg cells and the fate of endogenous Treg cells in a situation in which regulation is clearly overwhelmed.

Using this model, we found that cues emerging from tissue resident DCs and effector T cells control the size of Treg cell populations via multiple pathways ranging from blockade of Treg cell induction to limitation of endogenous Treg cell proliferation. In particular, shut down of IL-2 by effector T cells played a major role in the pathogenesis of this infection by limiting bioavailability of Treg cell survival factors. The strong Th1 environment triggered by *T. gondii* infection also induced T-bet and IFN-γ expression on Treg cells. Together, our data support the idea that Th1 cells can subvert regulatory networks and become pathogenic through not only their capacity to produce inflammatory cytokines, but also their reduced capacity to produce a major survival factor for Treg cells.

RESULTS

Acute Infection by *T. gondii* Is Associated with Collapse of Treg Cell Numbers and Frequencies

In order to evaluate the fate of $Foxp3^+$ Treg cells in highly inflammatory settings, C57BL/6 mice were infected orally with 40 cysts of the type II *T. gondii* strain, ME-49. After oral infection, the parasite was primarily detected in the small intestine, as well as in distal tissues (Figure 1A and data not shown). ME-49 in C57BL/6 mice induced severe small intestine inflammation with loss of epithelial architecture in the ileum and the jejunum, shortened villi, massive influx of inflammatory cells, and scattered patches of necrosis, particularly in Peyer's patches (Figure 1B; Figure S1A available online) (Liesenfeld, 2002; Mennechet et al., 2002). This infection also induced severe necrosis of the liver, leading to hepatic dysfunction, as indicated by increased concentrations of serum alanine and aspartate aminotransferase (Figure 1C). Although mice controlled parasite expansion, they eventually succumbed to immunopathology (Figures 1A and 1D). At various time points after infection, Treg cell frequencies and absolute numbers were evaluated in different tissues. Gut pathology peaked between days 8 and 10 and coincided with a strong reduction of $Foxp3^+$ Treg cell frequencies both at the primary site (LP) and systemically (Figures 1E and 1F). Scattered foci of parasites were present along the small intestine, characterized by a massive influx of $CD4^+$ T cells (Figure 1G). At these sites, $Foxp3^+$ Treg cells were virtually absent (Figure 1G). Of note, the absolute numbers of $Foxp3^+$ T cells were also significantly reduced compared to naive control mice (Figure 1H). In contrast, the absolute number of $CD4^+Foxp3^-$ T cells was sustained in all compartments with the exception of the MLN (data not shown). Thus, after infection with a lethal dose of *T. gondii*, $Foxp3^+$ Treg cell numbers and frequencies were dramatically reduced at the site of infection and systemically.

Failure to Sustain Treg Cell Conversion after *T. gondii* Infection

We and others demonstrated that in the GALT, oral exposure to antigen can induce conversion of naive T cells into Treg cells (Coombes et al., 2007; Mucida et al., 2005; Sun et al., 2007). To evaluate if reduced frequencies of Treg cells during *T. gondii* infection could be associated with reduced Treg cell conversion, we adoptively transferred $eGFP^-$, $CD45.2^+$ T cells from OTII × $Foxp3^{eGFP}$ mice into $CD45.1^+$ recipients that were infected or not infected with *T. gondii*. These recipient mice were then fed ovalbumin (OVA) antigen dissolved in their drinking water. After 5 days of OVA administration, $CD45.2^+$ OVA-specific T cells had expanded and were readily detectable in the GALT, spleen, and in distal lymph nodes (LNs) of both infected and noninfected hosts (Figure 2A and data not shown). Although the frequency of transferred cells was lower in infected hosts, OVA-specific cells proliferated robustly in these animals (data not shown and Figure 2B). Thus, antigen presentation was not impaired during *T. gondii* infection. As we previously described in naive control mice (Sun et al., 2007), $Foxp3^+$ OVA-specific T cells were only appreciably detected in the GALT, including the LP of naive hosts (Figure 2C). In contrast, after infection with *T. gondii*, the frequency of cells expressing Foxp3 de novo was dramatically reduced. Rather, transferred $CD45.2^+$ cells efficiently polarized toward a Th1 cell phenotype after oral feeding with OVA in the presence of *T. gondii* (Figure 2D).

Previous studies have demonstrated that inflammatory mediators such as IFN-γ can limit the conversion of naive $CD4^+$ T cells into $Foxp3^+$ T cells (Wei et al., 2007). Indeed, via their capacity to induce IFN-γ production by T cells, LpDCs purified from day-6-infected mice were significantly less efficient at inducing $Foxp3^+$ T cells compared to LpDCs from naive mice (Figure S2). Similar data were obtained when naive LpDCs exposed to STAg (soluble toxoplasma antigen) were utilized in a Treg cell conversion assay (Figure S2). Collectively, these data demonstrate that *T. gondii* establishes an environment that is unfavorable for the constitutive generation of Treg cells in the GI tract via manipulation of the APC status of activation and induction of effector responses.