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Plasticity of T_{reg} at infected sites

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Regulatory T cells (T_{reg}) control an array of immune responses both in the context of various polarized settings as well as in distinct microenvironments. This implies that maintenance of peripheral homeostasis relies on the capacity of T_{reg} to appropriately adapt to these defined settings while sustaining a regulatory program in the face of inflammation. Adaptation of T_{reg} is particularly critical in tissues constantly exposed to microbes such as the gut or the skin or in the context of exposure to pathogenic microbes. Recent evidence supports the idea that the capacity of T_{reg} to control defined polarized settings can be associated with the acquisition of specific transcription factors previously associated with effector T cell lineages. In this commentary we will discuss how such adaptation of T_{reg} can play a major role in the control of host microbe interaction.

A role for T-bet in the control of T_{reg} function at Th1 sites

One required feature of tissue regulation relies on the proper accumulation of the regulatory cells to the inflamed tissue. Until recently it was unclear how T_{reg} responded to environmental cues and targeted defined sites. A recent report by Koch et al.[1] supports the idea that T-bet expression by T_{reg} may be instrumental in the capacity of T_{reg} to accumulate at Th1 polarized sites. Using various experimental settings and in particular *Mycobacterium tuberculosis* infection, this group demonstrated that acquisition of T-bet via its capacity to induce CXCR3, favors the homing of T_{reg} to Th1 sites of inflammation. In competitive bone marrow chimeras *Tbx1*−/− T_{reg} cells were out competed by WT T_{reg} during Th1 inflammation suggesting an additional role for T-bet in their survival/proliferation. The induction of T-bet in T_{reg} was found to be IFN-γ dependent yet did not require expression of IL-12Rβ[1]. Similarly, during *T. gondii* infection, T-bet expression correlated with expression of CXCR3[2]. When isolated from the primary site of *T. gondii* infection, small intestine lamina propria DCs readily induced T-bet expression by T_{reg} due, in part, to their capacity to induce IFN-γ by T cells. As LpDCs gained the capacity to produce IL-12 in this environment, T-bet expression was associated with acquisition of responsiveness to IL-12 via enhanced Stat4 phosphorylation[2]. Other factors—such as IL-27 highly expressed in LpDCs from infected mice—are also likely to contribute to this imprinting. At the population level, the expression of T-bet did not interfere with the capacity of T_{reg} to suppress proliferation of effector T cells *in vitro* [1, 2]. Thus, the appropriation of T-bet appears to provide a fitness advantage to T_{reg} in the context of Th1 polarized infections. Such control can be associated with an enhanced homing property as well as acquisition of responsiveness to defined growth factors such as IL-12 present in Th1 polarized microenvironments.

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Adaptation of T_{reg} to sites constitutively exposed to microbes

At steady state, the gut is home to a large number of lymphocytes that have the capacity to produce cytokines such as IL-17, IFN-γ as well as IL-4[3, 4]. This constitutive production of cytokines is tightly controlled by the flora as germ-free mice show extensive deficiencies in intestinal immune system development and basal cytokine production[3, 5]. Based on the aforementioned findings one can speculate that in order to control immune responses at mucosal sites T_{reg} may express transcriptional programs analogous to tissue resident effector T cells. Furthermore, such barrier surfaces may require more proficient T_{reg} to maintain homeostasis. In support of this theory, previous studies have identified the requirement of two other transcription factors, IRF4[6] and Stat3[7], associated with effector function and responsiveness, to be required for the capacity of T_{reg} to control Th2 and Th17 inflammation, respectively. Zheng et al. first demonstrated that IRF4 expression by T_{reg} was required to control Th2 pathology as mice with IRF4$^{-/-}$ T_{reg} succumb to disease directed at multiple barrier sites including the lungs, stomach and pancreas by 3–4 months of age. Similarly, Chaudry et al. showed that selective deletion of Stat3 in Foxp3^{+} cells resulted in the development of an uncontrolled and lethal Th17 inflammation in the gut. Examination of the requirements for these transcription factors in polarized infectious settings will provide a more complete understanding of the capacity of T_{reg} to adapt to defined microenvironments.

Excessive adaptation as a potential trigger of immunopathology

Initial studies in T helper differentiation described a unidirectional pathway to effector lineage commitment and cytokine production. However, recent evidence showed that lymphocytes maintain a certain degree of plasticity with respect to their capacity to produce cytokines [8, 9]. Cells expressing both Foxp3 and IL-17 can be found in mucosal tissue or *in vitro* cultures [8, 10, 11]. Genome wide mapping of H3K4me3 and H3K27me3 performed in ex vivo T_{reg} revealed markers of both repression and induction at the tbx21 locus. On the other side, Ifnγ locus did not show any sign of induction or repression [9] suggesting that it is poised for transcriptional activation. A previous report demonstrated that regulatory T cells expressing both Foxp3 and T-bet were induced by CD8α^{+}DCs and could protect against airway hyperactivity [12]. A role for IFN-γ in mediating T_{reg} function has been reported in a model of graft transplants [13] and recent evidence demonstrates that, *in vitro*, T_{reg} can acquire expression of this cytokine [9]; Following oral infection with *T. gondii* under conditions associated with high immunopathology and eventual death of the infected host T_{reg} can produce IFN-γ, a cytokine responsible for both effector and pathogenic responses during this infection. When isolated from infected animals, T_{reg} were capable to exert effector functions as evidenced by their capacity to activate macrophages and induce parasite killing[2]. Such an aberrant fate for T_{reg} appears to be associated with, or arise as a consequence of pathology. Indeed, IFN-γ production by T_{reg} was only detected in situations leading to death of the infected host. This would suggest that in the presence of high levels of inflammatory mediators, T-bet expression may reach a threshold that could lead to T_{reg} destabilization. Notably, in *T. gondii* infected mice the level of T-bet in T_{reg} was much higher than that observed in T_{reg} during *M. tuberculosis* infection[1, 2]. Both *M. tuberculosis* and *T. gondii* are strong Th1 inducing infections however under certain conditions, *T. gondii* triggers a cytokine storm, with very high levels of inflammatory cytokines such as of IL-6, IL-27 and IL-12. This in conjunction with severely decreased levels of IL-2, which was also seen during this infection, may act on T_{reg} to imprint an effector phenotype. Indeed, when isolated from the primary site of infection, Lamina propria DCs from *T. gondii* infected mice can only induce IFN-γ production by T_{reg} in the presence of high levels of IL-12. This implies that acquisition of IFN-γ by these cells requires an amplification loop provided by enhanced IL-12 production, a response not normally seen in the gut environment[2].

Another example of adaptation of T_{reg} to microbes was observed in the context of exposure to fungal products. T_{reg} exposed to DCs that had been incubated with curdlan, a β-glucan, co-express RORγt [14]. A similar phenotype had been previously described on T_{reg} residing in the intestinal mucosa [15]. The physiological relevance of RORγt expression for T_{reg} function remains to be addressed but the observation that microbial products or exposure to sites exposed to microbes favor this phenotype suggests that, as for T-bet, RORγt may represent a positive adaptation of T_{reg} cells in defined settings.

On the other hand, as observed with pathogenic levels of infection with *T. gondii*, high doses of curdlan led to the production of IL-17 by $Foxp3^{+}ROR\gamma t^{+}$ T_{reg} that was dependent on IL-23 production by DCs. Similarly, T_{reg} resident in mucosal tissues of both mice and human can produce IL-17 [16, 15]. The roles of IFN-γ or IL-17-producing T_{reg} remain difficult to assess. However, given their high degree of T_{reg} self-reactivity, it is plausible that if armed with effector cytokines these cells can contribute to tissue damage or lose suppressive capacity. Indeed, a recent report highlighted that Foxp3 instability and acquisition of IFN-γ can favor the development of autoimmune diabetes [17].

Control of T_{reg} conversion by defined environment

In addition to the regulation provided by thymically derived T_{reg}, recent findings support the idea that the GI tract represents a privileged site for the induction of T_{reg} from naïve $CD4^{+}$ T cells. Previous work demonstrated that *in vitro* T_{reg} conversion was abolished in the presence of Th1 or Th2 associated effector cytokines [18–21]. Additionally, IL-6 required for polarization towards Th17 can down-modulate Foxp3 expression. Accordingly, conversion in the highly Th1 response to *T. gondii* is halted[2]. Interestingly, converted T_{reg} although reduced in number during this infection, still adapt to the Th1 environment by expressing T-bet[2] suggesting that plasticity is not the sole prerogative of naturally occurring T_{reg} cells. Previous reports examining both gut and lung inflammation support the idea that restricted or defective T_{reg} conversion can enhance immunopathology [22, 23]. The relative contribution of blockade of T_{reg} conversion to the pathology induced by *T. gondii* remains difficult to evaluate but is likely to play a role in the overall decrease of T_{reg} during this infection. These findings also raise the possibility that exposure to antigen at a time of acute infection may impair the acquisition of tolerance against innocuous antigens (e.g flora or food antigens) that could, in turn, further contribute to the pathologic process.

As highlighted by the studies discussed, plasticity of T_{reg} during infection may play a positive role in their capacity to target defined sites, control polarized settings and survive in a competitive fashion with the cells they have to regulate. On the other hand, acquisition of additional transcription factors may lead to T_{reg} destabilization with acquisition of effect cytokines and in some cases, loss of Foxp3. Another important point to consider is that in most cases strict polarization of immune responses is a rare event in tissues. How T_{reg} integrate these complexes and in some cases antagonistic signals to adapt appropriately remains to be addressed. A further examination of T_{reg} in tissue infected with microbes that induce different classes of immune responses and various levels of pathology will be a powerful tool to define the factors controlling the fate of T_{reg} cells.

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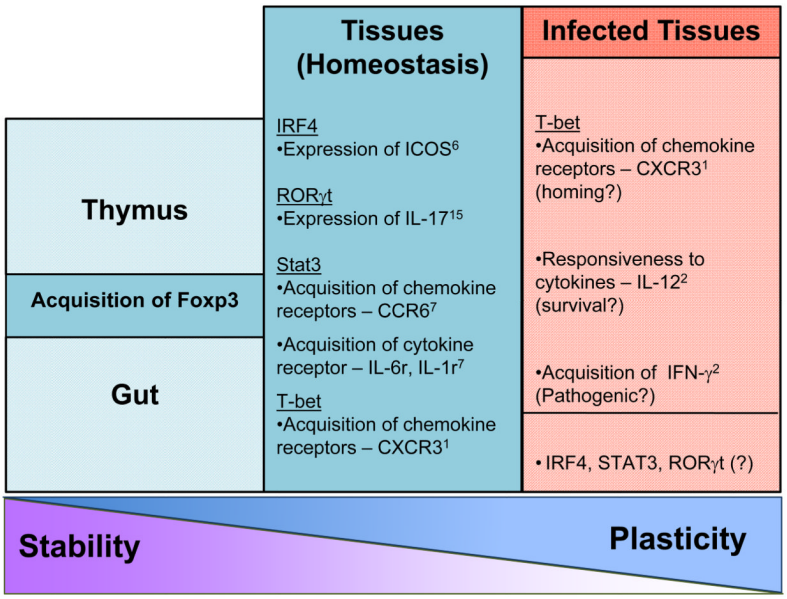

Figure 1.

Features of tissue-resident regulatory T cells (Treg) during homeostasis and infection. The schematic depicts the stable expression of Foxp3 during thymic generation (and potentially de novo generation in the gut). As Treg enter the periphery, especially of tertiary tissues, the level of plasticity of Treg increases during homeostasis and especially infection.