CD103$^+$ GALT DCs promote Foxp3$^+$ regulatory T cells

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There is evidence that Foxp3$^+$ regulatory T (T_R) cells contribute to intestinal homeostasis and that deficiencies in this population can lead to chronic intestinal inflammation. Here, we review recent studies that demonstrate that the gut is a site of peripheral generation of T_R cells. Functionally specialized gut dendritic cell populations promote T_R cells through a transforming growth factor-β and retinoic acid-dependent mechanism. Gut-driven T_R cells may represent a tissue-specific mechanism to broaden the repertoire of T_R cells focussed on the gut.

INTRODUCTION

The immune system contains effector T cells capable of mediating host protective immunity, as well as negative regulatory populations that control these responses and thus prevent autoimmunity and chronic immune pathologies. Recent studies indicate that naturally arising CD4$^+$CD25$^+$Foxp3$^+$ regulatory T (T_R) cells play a nonredundant role in immune homeostasis as mutations in the *foxp3* gene result in a lethal inflammatory disease in rodents, and a similar condition in humans termed immune polyendocrinopathy X-linked.[1] In the latter, affected individuals develop type 1 diabetes, allergic skin disease, and intestinal inflammation. These results indicate that Foxp3-dependent pathways control organ-specific autoimmune diseases, as well as chronic inflammatory responses at environmental interfaces. Importantly, transfer of naturally arising CD4$^+$CD25$^+$Foxp3$^+$ T_R cells not only prevent but also reverse established inflammation in various experimental models, including colitis, suggesting the therapeutic utility of enhancing T_R cell-mediated pathways.[2]

A large proportion of the Foxp3$^+$ T_R cells present in the periphery are thought to acquire their function in the thymus.[3] In addition, it is evident that T_R cells can also be generated in peripheral tissue from the naive T-cell pool, and that transforming growth factor (TGF)-β plays a key role in this process.[4] Although these induced T_R cells share many phenotypic and functional features with thymic-derived naturally arising CD4$^+$CD25$^+$Foxp3$^+$ T_R cells, their contribution to immune homeostasis is not yet well established. Furthermore, the physiological pathways involved in their generation are poorly defined. Here, we review recent studies from our own laboratory and others that reveal a role for the gut in the generation of induced T_R cells and identify a functionally distinct population of intestinal dendritic cells (DCs) that contribute to this process.

DCs AND INTESTINAL IMMUNITY

The immune response in the intestine is tightly regulated to ensure sustained effector responses to pathogens in the absence of potentially deleterious inflammatory responses to commensal bacteria or food antigens. Indeed, it is well established that feeding of soluble antigens leads to a subsequent state of antigen-specific hyporesponsiveness.[5] This phenomenon termed oral tolerance presumably represents a physiological mechanism to prevent immune hyperreactivity in the gut.

The balance between tolerance and immunity in the intestine is in part dictated by antigen presenting cell populations present in the gut. DCs are unique in their ability to activate naive T cells and are present in the gut-associated lymphoid tissues (GALT), such as the Peyer's patches and mesenteric lymph nodes (MLN), scattered throughout the lamina propria (LP) and located within the epithelium itself.[6] They are strategically positioned to sample luminal antigens and following maturation can process and present these to T cells.[7] Gut-derived DC populations play a pivotal role in initiating host protective responses to pathogens and may also drive immune pathological responses in the gut.[8]

Under homeostatic conditions, however, gut DCs seem to promote noninflammatory T-cell responses, and their expansion in the gut is linked to tolerance induction.[9] This has been attributed to their differential production of immune suppressive cytokines. Thus, DCs from the Peyer's patches, but not spleen, produce high levels of interleukin (IL)-10 and TGF-β, leading

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to the production of IL-10 and IL-4 by T cells.[10] Similarly, DCs from the small intestine (SI) LP, in contrast to their splenic counterparts, have been shown to constitutively express IL-10 but not IL-12 mRNA, even after lipopolysaccharide maturation.[11] Other distinct functional properties of gut DCs include the ability to induce T cell-independent IgA secretion by naive B cells[12] and the imprinting of gut-homing receptors CCR9 and $\alpha_4\beta_7$ on activated T and B cells, endowing effector cells with the capacity to home to the intestine.[13–15]

Gut DCs are a dynamic population. Even in the absence of inflammation, they rapidly turnover,[16] and constantly traffic from the LP to the MLN in a CCR7-dependent manner.[17] Migration from the gut to the MLN may be a crucial part of tolerance induction, whereas initiation of oral tolerance is impeded by mesenteric lymphadenectomy and is completely abrogated in CCR7-deficient mice.[18] DCs in the afferent lymph have also been shown to carry apoptotic bodies derived from intestinal epithelial cells, suggesting that migratory DCs may induce tolerance to both luminal and self antigens.[19]

Multiple mechanisms have been shown to be involved in oral tolerance including clonal deletion, anergy, and induction of TGF-β-secreting Th3 cells.[5] There is also evidence that oral administration of antigen leads to the induction of antigen-specific immune suppressive $CD4^+CD25^+$ T_R cells in the MLN.[20,21] Studies from our laboratory and others also identify the intestine and MLN as an extrathymic site for the *de novo* differentiation of $Foxp3^+$ T_R cells.[22,23] Thus, adoptive transfer of naive DO11.10 SCID $CD4^+$ T cells to ovalbumin-fed Balb/c mice led to the emergence of $CD4^+Foxp3^+$ cells that were present at higher numbers in the MLN compared with the spleen.[23] Together, these data indicate that the intestine is a distinct microenvironment that favors T_R-cell generation and points to a role for GALT DCs in this process.

$CD103^+$ MLN DCs PROMOTE T_R-CELL DEVELOPMENT THROUGH A TGF-β AND RETINOIC ACID-DEPENDENT MECHANISM

A confounding issue in the further characterization of gut-derived migratory DCs has been the lack of markers to distinguish these cells from the lymphoid tissue-resident DCs within the MLN. However, recent evidence suggests that the integrin α chain CD103 (α_E) marks intestinal derived-DCs. CD103 is expressed by the majority of DCs in the LP of the colon and SI, by a proportion of DCs in the MLN and by only a small percentage of splenic DCs.[24,25] Consistent with their LP origin, the number of $CD103^+$ DCs in the MLN of CCR7-deficient mice was markedly lower than in wild-type mice, suggesting that $CD103^+$ DCs constitutively migrate to the MLN under homeostatic conditions.[25] Their intestinal origin is further supported by recent findings from our group and others that similar to SI LP DCs, MLN $CD103^+$ DCs, but not their $CD103^-$ counterparts, induce responding T cells to express the gut-homing receptor, CCR9.[24,25] Imprinting of gut-homing receptors by GALT DCs is dependent on the vitamin A metabolite retinoic acid (RA), and GALT DCs as opposed to splenic DCs seem to be specialized in this function as a result of their expression of the retinol-metabolizing enzyme, retinal dehydrogenase.[26] Imprinting of CCR9 on T cells by $CD103^+$ DCs was also RA-dependent,[27] and $CD103^+$ DCs were found to express significantly higher levels of retinal dehydrogenase *aldh1a2* mRNA than the $CD103^-$ DCs, suggesting that they too have the capacity to metabolize vitamin A.[23]

In earlier studies, we found that $CD4^+CD25^+$ T_R cells were unable to prevent T cell-mediated colitis in immune-deficient recipients that lacked CD103, suggesting an important role for $CD103^+$ DCs in maintaining intestinal immune homeostasis.[24] In an effort to find a cellular link between CD103 and the regulation of intestinal immune responses, we investigated the ability of $CD103^+$ DCs to drive T_R-cell development *in vitro*. Our results showed that $CD103^+$, but not $CD103^-$, DCs supported the antigen-induced spontaneous differentiation of $Foxp3^+$ T_R cells from naive precursors, and were more efficient at maintaining a preexisting population of $Foxp3^+$ T cells in culture. Furthermore, $CD103^+$ DCs isolated from the MLN of ovalbumin-fed mice, could activate and drive naive DO11.10 $CD4^+$ T cells to express Foxp3.[23] These findings demonstrate that not only can $CD103^+$ DCs acquire antigen from the gut and drive *de novo* T_R-cell development in the intestine, but they can also support conventional T_R cells, and in doing so maintain intestinal homeostasis.

Mechanistic analysis indicated a key role for TGF-β in $CD103^+$ DC-induced T_R-cell differentiation *in vitro*. Compared with $CD103^-$ DCs, the $CD103^+$ subset expressed higher mRNA levels of *tgfb2*, *plat* (tissue plasminogen activator) and *ltbp3* (latent TGF-β binding protein 3), two enzymes involved in the conversion of latent to active TGF-β. This suggested that the differential ability of $CD103^+$ DCs to drive T_R-cell differentiation was because of the production of endogenous TGF-β. However, the addition of exogenous TGF-β was not sufficient to enable the $CD103^-$ DCs to generate $Foxp3^+$ T_R cells, suggesting that this DC population was lacking an additional cofactor required for T_R-cell generation. This was shown to be RA as inclusion of all-trans RA together with TGF-β enabled the $CD103^-$ DCs to drive T_R-cell differentiation, producing similar proportions and numbers of $Foxp3^+$ T cells as occurred in cultures containing $CD103^+$ DCs.[23] As discussed above, $CD103^+$ DCs express retinol-metabolizing enzymes, and the induction of $Foxp3^+$ T_R cells by the $CD103^+$ DCs was inhibited by a RA receptor antagonist.[23] This indicated that the enhanced ability of the $CD103^+$ DCs to drive T_R-cell generation was because of their capacity to metabolize vitamin A. Similar to MLN $CD103^+$ DCs, SI LP DCs also induced high levels of T_R-cell conversion, which was TGF-β and RA-dependent.[22] Furthermore, these retinoid-induced T_R cells expressed $\alpha_4\beta_7$ and CCR9, and *in vivo* preferentially migrated to the SI.[28,29] Several other groups have also shown that RA enhanced Foxp3 expression and concomitantly inhibited Th17 cell differentiation.[30–33] Together, these studies newly identify RA as a cofactor to augment TGF-β-mediated Foxp3 induction, and this pathway may play a role in regulating the balance between protective and anti-inflammatory immune responses in the gut.

The mechanism by which RA functions is currently the subject of intense investigation. It is known that RA suppresses

inflammatory Th1 responses[34] and that vitamin A deficiency exacerbates disease in an experimental model of colitis,[35] but the specific receptors and signaling pathways involved are poorly defined. Two families of retinoid receptors exist, the RA receptor isotypes (α, β, and γ) and the retinoid X receptor isotypes (α, β, and γ),[36] and recently the RA receptor-α isotype has been identified as mediating the effects of all-trans RA in Foxp3 induction.[30] Furthermore, there is evidence to suggest that RA enhances Foxp3 expression by attenuating inhibitory signals generated in the presence of high levels of costimulation,[29] which could potentially facilitate the induction of Foxp3$^+$ T_R cells in the presence of inflammation.

By contrast with the CD103$^+$ DCs, the CD103$^-$ MLN DC population promoted interferon-γ production by responding T cells.[24] Following CD40 or Toll-like receptor (TLR) stimulation CD103$^-$ DCs produced inflammatory cytokines, such as tumor necrosis factor-α and IL-6.[24] Upon CD40 ligation the CD103$^-$ DCs also expressed substantially higher levels of IL-23p19 mRNA compared with the CD103$^+$ DCs,[23] a cytokine crucially involved in the mucosal pathogenesis of colitis.[37,38] Therefore, we hypothesize that the CD103$^-$ DCs represent a functionally distinct DC population, generating effector T cells capable of mediating inflammatory responses.

These studies raise a question of how two distinct DC populations in the MLN of normal mice can differ so dramatically in their functional properties? It is not yet clear, whether this reflects the differentiation of two separate DC precursor lineages with differing intrinsic programmes, or whether the DCs arise from the same precursor population, but conditioning by the specialized microenvironment of the intestine alters CD103 expression and DC function. There is evidence to support both possibilities[39,40] but the majority of the attention has focused on the role of intestinal epithelial cells in educating gut DCs. It is likely that CD103, through an interaction with its ligand, E-cadherin, on intestinal epithelial cells may facilitate DC retention within the intestine. During homeostatic conditions, intestinal epithelial cells constitutively secrete the cytokine thymic stromal lymphopoietin, which drives DCs to secrete IL-10 and IL-6 but not IL-12.[41] Exposure to RA,[42,43] microbial antigens,[44] and to immunomodulatory cytokines, such as IL-10[45] and TGF-β,[46] which are present in the gut, have all been shown to modulate DC function. This suggests that an array of signals from the intestinal microenvironment may contribute to the differentiation of the functionally unique CD103$^+$ DCs. In contrast, the CD103$^-$ DCs may have arrived in the MLN directly from the blood and thus escaped gut conditioning. This could allow the CD103$^-$ DCs to efficiently drive the differentiation of an inflammatory T-cell response, which if inappropriately regulated, could induce immune pathology.

CONCLUSION

The intestine is a unique microenvironment that uses stringent tissue-specific inflammatory and regulatory pathways to respond appropriately to exogenous antigens. Recent studies indicate that the intestine, through the presence of functionally specialized CD103$^+$ DCs, is a site of peripheral generation of Foxp3$^+$ T_R

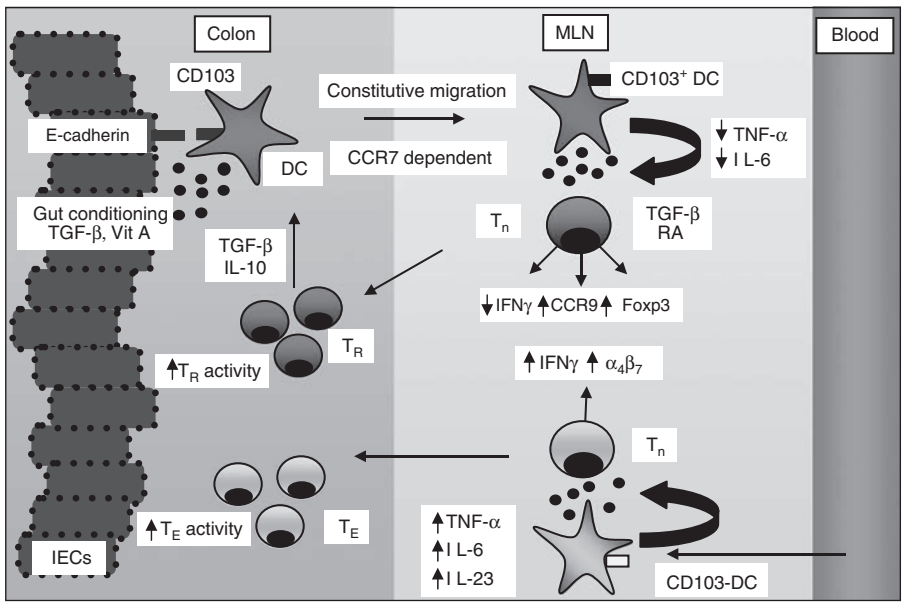

Figure 1 CD103$^+$ DCs in the intestinal LP are conditioned by IEC-derived factors, such as RA and TSLP. The DCs migrate to the MLN, where they favor the development of Foxp3$^+$ T_R cells, which preferentially migrate back to the colon and prevent intestinal inflammation, possibly by secreting immunosuppressive cytokines such as TGF-β and IL-10. CD103$^-$ DCs may enter the MLN directly from the blood, escaping gut conditioning, which leaves them poised to respond to pathogen entry. Upon exposure to danger signals, the CD103$^-$ DCs release pro-inflammatory cytokines and drive the differentiation of effector T cells, which may be able to traffic back to the site of pathogen entry through an $\alpha_4\beta_7$-dependent mechanism.
DCs, dendritic cells; IEC, intestinal epithelial cell; IL, interleukin; LP, lamina propria; MLN, mesenteric lymph nodes; RA, retinoic acid; TGF, transforming growth factor; T_R, regulatory T cells; TSLP, thymic stromal lymphopoietin.

cells. Intestinal DCs are subject to tissue-specific conditioning that endows them with the ability to produce or activate TGF-β and metabolize vitamin A into active RA. The presence of TGF-β and RA during T-cell activation inhibits the development of Th17 cells and favors the development of Foxp3$^+$ T_R cells with a gut seeking phenotype (**Figure 1**). We speculate that this is a mechanism to broaden the repertoire of T_R cells in the gut and that this gut-driven population may play an important role in intestinal homeostasis. Further understanding of T_R cells and regulatory DCs, and of their interactions within the complex immune system will be of vital importance for future therapeutic intervention of hyperinflammatory disorders such as inflammatory bowel disease.

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DISCLOSURE

The authors have declared no financial interests.

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