

Marking the 50th Anniversary of *Immunology*

Special regulatory T-cell review: regulatory T cells and the intestinal tract – patrolling the frontier

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Summary

Tolerance to self and harmless antigens is one of the central features of the immune system, and it is obtained through a combination of multiple mechanisms. Discriminating between pathogens and non-pathogenic antigens is especially important in the intestine, which constitutes the main contact surface between the body and the outside environment. Recently, the role of Foxp3⁺ regulatory T cells (Treg) in the establishment and maintenance of tolerance has been the focus of numerous studies. In this review, we briefly discuss the historical background leading to the identification of Foxp3⁺ Treg and give an overview of their role in controlling systemic and mucosal immune responses.

Keywords: Foxp3; intestine; IL-10; regulatory T cells

Characterization of Treg

Evidence for active T-cell mediated regulatory mechanisms was found in the 1970s with the discovery of 'suppressor T cells'.¹ However, the cellular and molecular basis of this phenomenon was not clearly established and studies in this area were largely abandoned in the 1980s.

In spite of this, a few groups continued to investigate dominant T-cell mediated immunoregulatory pathways. In these models, induction of lymphopenia through irradiation and/or neonatal thymectomy led not only to impaired immune responses, but paradoxically also to autoimmunity.² Importantly, transfer of normal lymphocytes could prevent disease.³ Development of monoclonal antibodies allowed further characterization of the cell populations that could mediate suppression with evidence in T cell transfer experiments that both pathology and protection from disease were mediated by T cells.^{4,5} Importantly, naïve CD4⁺ T cells isolated from unmanipulated healthy animals also induced disease when transferred into immunodeficient recipients, showing that pathogenic cells are present in the normal T-cell repertoire.^{6,7} Self-reactive T cells were further identified in

healthy humans.⁸ Despite these findings, autoimmune disease is relatively rare, indicating that regulatory mechanisms normally control this pathogenic activity.⁹ In complementary experiments, T cells with the ability to inhibit pathogenic responses were isolated from unmanipulated rodents.^{5,6,10,11}

Further fractionation of the CD4⁺ T-cell subset showed that immune suppressive activity was enriched within the Lyt-1⁺ subset in mice and in the antigen-experienced CD45RB^{low} fraction in rats.^{6,12} Encounter with the antigen in the periphery, however, did not seem to be essential for acquiring regulatory activity, as *in vivo* tolerance could also be achieved by the transfer of CD4 single positive thymocytes.^{11,13} *In vivo* subset analysis by Sakaguchi *et al.*¹⁴ showed that the regulatory activity was enriched within the CD25⁺ population; this population was then found in humans and shown to suppress *in vitro* T-cell responses.^{15–17} While CD25 remains the most widely used regulatory T cell (Treg) surface marker, it is neither Treg exclusive nor expressed by all Treg. The CD25⁺ T cell population still includes activated T cells with pathogenic rather than regulatory potential. Furthermore, the CD45RB^{low} CD25[–] population still possesses some regulatory activity. A more definitive Treg marker was needed.

The answer came from the studies of a naturally occurring mouse mutation called scurfy. Scurfy mice have a mutation in the *Foxp3* gene on the X chromosome.¹⁸ Affected males suffer from a severe autoimmune syndrome, similar to the human IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, which is also caused by mutations in *FOXP3*.^{19,20} Interestingly, the mouse disease is mediated by T cells and has some similar features to the disease induced by transfer of Treg-depleted subsets. In 2003, three independent groups found that the transcription factor Foxp3 is highly expressed in the CD4⁺ CD25⁺ T cell subset.^{21–23} Moreover, enforced Foxp3 expression in naïve T cells endowed them with regulatory activity *in vitro* and *in vivo*. Further analyses have confirmed *Foxp3* as a key gene for Treg generation and maintenance, and it is now the most widely used marker for Treg, despite some reports indicating that it can be transiently expressed by non-Treg in humans.^{24,25}

The identification of the link between Foxp3 and Treg has been instrumental for many recent studies on regulation, allowing identification of Treg by flow cytometry and immunohistochemistry, and not solely on the basis of their activity.

Treg and intestinal responses

While IPEX can affect many organs, one of the most common features is intestinal inflammation.²⁶ The gastrointestinal tract represents the main surface by which the organism encounters exogenous antigens. In addition to its diverse dietary antigens, it is home to a vast number of commensal bacteria. These foreign antigens however do not induce inflammation under normal conditions, pointing to a system to downregulate inappropriate immune responses in the intestine. Still, lack of intestinal inflammation does not mean absence of immune responses, as shown by the fact that immunodeficient individuals often get opportunistic infections by members of the normal commensal flora.^{27–29} On the other hand, a dysregulated, over-exuberant response to the intestinal flora is believed to play a role in chronic intestinal pathologies such as inflammatory bowel disease.³⁰ Thus, the immune response in the intestine has to be finely tuned to avoid infection while remaining tolerant to food antigens and resident bacteria.

Foxp3⁺ Treg are known to play an important role in intestinal homeostasis. Besides the evidence from IPEX patients, transfer of Treg inhibits experimental colitis induced when naïve T cells are injected into immunodeficient mice and react to the intestinal flora.^{7,31–33} Strikingly, Treg can also cure established colitis and this is associated with their proliferation in the intestine.³⁴ Although most studies concerning Treg and intestinal homeostasis have focused on thymically imprinted natural

Treg, there is also evidence that the intestine with its associated lymphoid tissue is a site for induction of Foxp3⁺ Treg from naïve precursors. Dendritic cells (DC) are essential in antigen presentation, and they seem to play an important role in Treg generation.³⁵ Functionally specialized intestinal DC that express the integrin CD103 have been linked to Treg development. CD103⁺ DC are enriched in the colon and in the mesenteric lymph nodes (MLN), and CD103⁺, but not CD103[−], MLN DC can induce gut-homing receptors on naïve T cells.^{36,37} Importantly, CD103⁺ DC can also induce Foxp3⁺ Treg in an antigen-specific manner, through a mechanism depending on transforming growth factor- β (TGF- β) and retinoic acid.^{38,39} This could represent a mechanism to generate specific regulation to local, non-thymically expressed antigens. It does not however necessarily mean that only peripherally induced Foxp3⁺ cells can control intestinal inflammation. Indeed, CD4⁺ CD25⁺ Treg isolated from the spleen or thymus can prevent intestinal inflammation in T-cell transfer colitis.⁴⁰ Clearly much remains to be learnt about the roles of thymically arisen and peripherally induced Treg in tolerance towards foreign antigens.

Mechanisms of intestinal immune regulation

Several factors have been identified to be critical for Treg function, including IL-10, IL-2, TGF- β and cytotoxic T lymphocyte antigen-4 (CTLA-4).^{14,41–44} While mice deficient for the latter exhibit multiorgan autoimmune disease, which is rapidly fatal in the case of TGF- β or CTLA-4 knockout (KO), IL-10-deficient mice reach adulthood without signs of severe disease.^{45–47} However, in the presence of bacteria such as *Helicobacter hepaticus*, which is common in animal facilities, the mice develop spontaneous intestinal inflammation, indicating a major role for IL-10 in gut homeostasis.⁴⁸ Indeed, Foxp3⁺ Treg isolated from spleen or MLN produce very little IL-10, whereas it is produced by a significant proportion of Foxp3⁺ cells isolated from the colonic lamina propria, and also by some Foxp3[−] cells in the intestine.^{34,49,50} Interestingly, IL-10 is not required for the prevention of colitis induced by naïve T-cell transfer into immunodeficient recipients.⁵¹ However, it is necessary to abrogate established intestinal inflammation, as CD25⁺ Treg cannot cure T-cell transfer-induced colitis if the IL-10 signalling pathway is blocked, and Treg-produced IL-10 plays a significant role in protection.³⁴ Similarly, IL-10 production is essential for Treg-mediated prevention of colitis caused by antigen-experienced IL-10 KO cells isolated from the MLN.⁵¹ These data are consistent with a specific role for IL-10 in controlling intestinal inflammation, while being dispensable for the control of immune responses initiated systemically.

The targets of IL-10 in the intestine have not been fully characterized yet. IL-10 is an immunoregulatory cytokine which can act on T cells and other components of the immune system.⁵² IL-10 is required for Treg to prevent colitis in an innate model of intestinal inflammation, suggesting that IL-10 not only controls pathogenic T cells, but can act on other immune cells in the intestine.⁵³ Complementing these data, conditional knockouts lacking signal transducer and activator of transcription-3 (STAT3), an essential mediator of IL-10 signalling, specifically in macrophages and neutrophils develop colitis.⁵⁴ The relative importance of IL-10 in controlling myeloid and T-cell responses during intestinal inflammation in intact mice remains to be ascertained.

The fact that Treg-derived IL-10 is essential for the control of certain pathologies, such as colitis, and dispensable to regulate other disorders is intriguing, but not at all unique. Other Treg-associated molecules, such as CTLA-4 and TGF- β , show similar results. For example, CTLA-4-deficient Treg can still prevent disease *in vivo*, but not if the effector cells also lack CTLA-4.⁵⁵ Similarly, TGF- β 1 production by Treg appears to be essential in one report and dispensable in another one.^{56,57} These discrepancies are compatible with a model where Foxp3⁺ Treg can regulate via several independent mechanisms that have partially overlapping functions. The particular mechanism required in each case will depend on the nature of the inflammatory stimulus, the inflammatory cells that have to be controlled and the location in which the response is taking place.

While it remains to be determined to what extent Treg-derived IL-10 is also necessary to control inflammation in other organs apart from the gut, it is not surprising that systemic and tissue-specific inflammation are controlled by partly different mechanisms. Indeed, different inflammatory pathways have been shown to mediate systemic wasting disease and colitis in an experimental model.⁵⁸ As a result of the special challenges the intestinal immune system has to confront, it is not impossible that its reactivity is controlled in different ways to the central immune system. Similarly, other tissues are also likely to have different regulatory requirements to the central lymphoid organs.

Perspectives

The past decade has seen the field of dominant tolerance blossom. While we now know more about Foxp3⁺ Treg, there are still many remaining questions about how they are generated, how they are controlled and how they control immune responses. Furthermore, other Foxp3⁺ regulatory cell subsets have been described but are not yet well characterized.^{59,60} On the other hand, it is becoming increasingly clear that different pathways of inflammation and tolerance are predominant in specific tissues. Identifying

the main players in immune regulation and learning how to control them will open a great range of possibilities in the treatment not only of autoimmune disease, but also of infectious diseases and maybe even cancer. However, systemic manipulations of the immune system could have deleterious side effects. Tissue-specific approaches could allow for better targeted treatments while reducing unwanted side effects.

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