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Pillars of Immunology

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The Colon as a Major Site of Immunoregulation by CD4$^+$ T Cell Subsets in the Steady State

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The late 1980s and 1990s were the golden age of CD4$^+$ T cell subset immunobiology. The pioneering work of Bob Coffman and Tim Mosmann published in *The Journal of Immunology* in 1986 established that murine CD4$^+$ T cells exist in at least two different subpopulations (Th1 and Th2) based on their cytokine secretion patterns and distinct effector functions. Further work with outside collaborators and colleagues at the DNAX Research Institute established that CD4$^+$ T cells, and in particular, those belonging to the Th2 subset, can exert cross-regulatory activity through the production of inhibitory cytokines, such as IL-10 and IL-4. These findings raised a host of interesting questions that stimulated the field during the next decade. A methodologic challenge was that, unlike subsets of other immune cells, murine Th1 and Th2 lymphocytes had no discernable cell surface markers and could only be distinguished by their cytokine secretion patterns and immunobiological functions.

This was not the case in the rat, in which Don Mason and colleagues at Oxford had demonstrated two major subsets of CD4$^+$ lymphocytes that could be discriminated based on their expression of isoforms of CD45, the common leukocyte Ag (1, 2). As a graduate student with Mason, Fiona Powrie made the exciting discovery that athymic rats develop a multiorgan wasting disease following adoptive transfer of naive CD4$^+$ T cells expressing high levels of CD45RC (CD45RC^{hi}), whereas rats receiving purified CD45RC^{lo} CD4$^+$ T cells or unfractionated CD4$^+$ T cells remained normal (3). These two rat CD4$^+$ T subsets possessed distinct cytokine production patterns reminiscent of murine Th1 and Th2 cells. What did the two T cell populations correspond to in the mouse? What was their antigenic specificity? How did they exert their effector and regulatory functions? In 1990, Dr. Powrie, newly graduated from Oxford, joined the laboratory of Bob Coffman at DNAX, then a citadel of T cell subset research, to tackle these fascinating questions.

Powrie began by generating a mouse version of the rat adoptive transfer model using the mouse homolog of CD45CR, CD45RB, as the subset defining marker. CD45RB^{lo} CD4$^+$ T cells had been recently defined by Kim Bottomly and Ellen Puré as distinct populations (4, 5). Whereas Bottomly had proposed that, based on their cytokine secretion patterns, CD45RB^{hi} cells corresponded to Th1 cells and CD45RB^{lo} cells corresponded to Th2 effector cells, Puré's work suggested that they shared a lineage relationship (6). When Powrie injected purified naive donor cells of each population into congenic C.B-17 *Scid* recipients, she observed an outcome that recapitulated her earlier findings in the rat (7); animals reconstituted with CD45RB^{hi} cells developed a wasting disease, as also noted in a study by a different group (8), whereas recipients of either CD45RB^{lo} or unfractionated CD4$^+$ T cells gained weight. Importantly, mixing of isolated CD45RB^{lo} cells with CD45RB^{hi} cells in ratios as low as 1:8 was able to suppress the pathology induced by the latter population.

The most striking outcome of these experiments, however, was the severe colonic pathology seen in recipients of CD45RB^{hi} but not CD45RB^{lo} or unfractionated CD4$^+$ T cells. This colitic tissue response was characterized by an infiltration of monocyte/macrophages, CD4$^+$ T cells, and a small number of neutrophils. Prominent epithelial hyperplasia, a loss of goblet cells, the development of crypt abscesses, and, occasionally, epithelial ulceration were also observed. These findings were reminiscent of Crohn disease in humans. Importantly, in contrast to the earlier findings of Powrie and Mason in the rat adoptive transfer model, only minor microscopic lesions were observed in other tissues of the CD45RB^{hi} T cell–transferred mice, including the small intestine, suggesting specific targeting of the response to the colon.

Powrie, Coffman, and their DNAX colleagues also investigated the nature of the lymphokine responses occurring at the colonic site of inflammation in the reconstituted *Scid* recipients by means of semiquantitative PCR (7). Colon tissue from recipients of CD45RB^{hi} cells expressed high levels of both IFN-γ and TNF, and these responses were suppressed by cotransfer of CD45RB^{lo} cells. In contrast, mice receiving CD45RB^{lo} cells only failed to express IFN-γ but nevertheless produced substantial levels of TNF, arguing against an effector role for TNF in inducing pathology in the absence of pathogenic T cells. However, later experiments showed that systemic Abs against TNF-α or IFN-γ, as well as recombinant IL-10, could inhibit disease development (9). Interestingly, the mice with cotransferred CD45RB^{lo} cells also failed to show an elevated IL-4 response, suggesting that the functionally active donor CD45RB^{lo} cells were not cross-regulating Th2 cells. The latter

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Abbreviation used in this article: Treg, regulatory T cell.

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hypothesis was confirmed in subsequent studies by Powrie et al. (10), who showed that TGF-β and IL-10, but not IL-4, were necessary for their suppressive function. The discovery of $CD25^+CD4^+$ regulatory T cells (Treg) by Sakaguchi et al. (11) during the same period suggested an alternative identity for these cells. Pursuing this after returning to Oxford to build her own group, Powrie and her new colleagues (12) formally showed that the cells within the $CD45RB^{lo}$ population responsible for inhibiting pathology were $CD25^+$ and thus represented Tregs. Consistent with this conclusion was an earlier study describing a spontaneous colitis-like disease similar to that occurring in *Scid* recipients of $C45RB^{hi}$ cells in IL-2–deficient, T cell–sufficient mice (13), now known to be Treg deficient.

A particularly intriguing question raised by Powrie et al. (7) in this *Pillars of Immunology* article (https://doi.org/10.1093/intimm/5.11.1461) concerned the antigenic target of the $CD45B^{hi}$ cells that trigger pathology. Although leaving open the possibility of autoimmune recognition of colonic epithelium and arguing against targeting of a specific pathogen emerging in the lymphocyte-deficient recipients, they proposed the gut flora as the likely target of the Treg population. They based their argument, in part, on the localization of inflammation in the colon, which harbors the highest density of commensal bacteria in the intestinal tract. Their prediction turned out to be on the mark, as it was later shown that germ-free recipients of $CD45RB^{hi}$ cells failed to develop colonic pathology (14) and that adoptive transfer of Th1 cytokine-producing $CD4^+$ T cell clones specific for colon-resident bacteria induces pathology that mimics that observed in the original $CDRB^{hi}$ transfer model (15). Taken together, these pioneering studies made major contributions to our understanding of the microbiome as a primary target of steady-state T cell responses, along with the importance of Tregs in suppressing this activity to protect the host against gut pathology.

In addition to its important immunobiological implications, the Powrie-Coffman *International Immunology* article stands as a major contribution in its description of an in vivo model that has been extensively used in studies of the induction and function of pathogenic T cells and on the basic biology of Treg generation and effector mechanisms involved in maintaining host immune homeostasis (16, 17). These studies have contributed to our understanding of IFN-γ, TNF-α, and IL-17 as pathogenic T cell effector mechanisms; implicated IL-10 and TGF-β in Treg function; and provided important data regarding the role of myeloid cell populations and their production of key cytokines, such as IL-23, IL-6, TGF-β, and retinoic acid in controlling the balance of T cell effector and peripheral Treg induction. Furthermore, although the role of Tregs in the pathogenesis of human inflammatory bowel disease has yet to be formally shown, studies using this model have contributed to the preclinical development of current therapeutic interventions for inflammatory bowel disease. At a fundamental level, this *Pillars of Immunology* article illustrates the major impact of $CD4^+$ T cell subset research on our understanding of both immune homeostasis and disease.

Disclosures

The authors have no financial conflicts of interest.

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