

## Th2 Cells: Help for Helminths

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Landmark studies by Mosmann and Coffman in the mid-to-late 1980s established the division of a number of murine CD4<sup>+</sup> T cells clones into distinct subsets, designated Th1 and Th2 cells, that mediate their effector functions through the elaboration of distinct groups of cytokines (1). Th1 cells uniquely generate IFN- $\gamma$ , lymphotoxin, and IL-2 after stimulation, whereas Th2 cells uniquely generate IL-4, IL-5, IL-9, IL-10, and IL-13. Both subsets produce additional cytokines, such as IL-3 and GM-CSF. Not surprisingly, based on the known effects of these cytokines, Th1 cells are adept at macrophage activation and immunoglobulin selection for isotypes (IgG2a and IgG3) that mediate antibody-dependent cellular cytotoxicity and complement activation. Such cells have been demonstrated in numerous infectious disease models to activate appropriate host defense against facultative and obligate intracellular microbes, including viruses, bacteria, yeast, and protozoa. In some of these infectious disease systems, Th2 cells have proven deleterious, largely through the ability of certain cytokines, particularly IL-4, IL-10, and IL-13, to downregulate macrophage activation, even in the presence of IFN- $\gamma$  (2).

Aside from those activities which might be important in moderating macrophage-mediated inflammatory capacity, cytokines released by Th2 cells favor the production and activation of mast cells and eosinophils, and stimulation of B cell growth, differentiation, and isotype switching to cells producing IgE and IgG1. These host responses have long been noted to characterize those seen during infection by intestinal helminths. Substantial controversy has arisen over whether such responses are helpful or harmful. Are these infestations examples of chronic infections that evade effective host defense, such that the body must downregulate the immune response to minimize tissue damage, or do these Th2 responses limit the extent of parasitization? Examples supporting either hypothesis occur in both human and rodent studies (3), but the report by Finkelman et al. in this issue of *The Journal of Experimental Medicine* (4) clearly demonstrates not only the protective role of Th2 responses in helminth infection, but also the deleterious effect of interventions (e.g., IL-12) that promote Th1 responses.

The question of Th1/Th2 bias in infectious disease has become of more than academic interest because of recent understanding regarding the priming and differentiation of Th1 and Th2 effector cells that permits the manipulation of this process. The best available data, derived both from limiting dilution analysis (5) and studies in T cell receptor transgenic mice (6, 7), is that naive CD4<sup>+</sup> T cells have the capacity

to differentiate into either Th1 or Th2 cells, and that this process is markedly influenced by the cytokine milieu during initial priming by specific antigen. Naive T cells generate IL-2 when activated by the appropriate MHC-peptide complex and costimulatory signals, and progress rapidly through a multipotential cell that generates a mixed spectrum of cytokines, including IL-2, IL-4, and IFN- $\gamma$ . Although many immune responses probably retain this mixed phenotype, designated the Th0 subset, infectious agents frequently cause polarization of responses to either the Th1 or Th2 types, presumably because they alter the cytokine milieu during priming. Organisms that induce Th1 responses, such as *Listeria*, *Toxoplasma*, and the tissue forms of *Leishmania*, contain antigens that trigger IL-12 release from macrophages (8–10). IL-12, in turn, directly primes CD4<sup>+</sup> cells towards a Th1 phenotype (8). Schistosome eggs constitute the predominant antigens that polarize the immune response towards Th2 cells in the murine model of schistosomiasis. Recently, an egg-derived oligosaccharide was demonstrated to induce IL-10 production from B cells (11). IL-10, in turn, has been demonstrated to promote Th2 development of naive CD4<sup>+</sup> T cells (6). Further study of additional organisms will be required to confirm whether these examples will be extrapolated to all polarized responses, but the important point is that by knowing the signals, investigators have begun to manipulate responses in ways that reveal the underlying contributions by individual components of a multifaceted immune response.

Such studies have been put to the test in three murine models of helminth infection and, in each, the results are a resounding vote of confidence for the protective role of the Th2 response in intestinal infestation. Each system has sufficiently different nuances worth examining for the non-worm biologist. *Trichuris muris*, or murine whipworm, has an entirely enteric life cycle, maturing in the intestinal mucosa to the egg-laying adults that reside in the cecum and large bowel. Work from the Grencis laboratory has identified resistant (BALB/k) mice that expulse adult worms by 35 d and demonstrate immunity to rechallenge, and susceptible (AKR) mice that maintain persistent infection (12). These disparate outcomes are correlated with Th2 or Th1 cytokine production by mesenteric lymph node cells in the resistant or susceptible strains, respectively. Neutralization of IFN- $\gamma$  allowed susceptible strains to expulse worms, whereas blocking IL-4 using anti-IL-4R antibody resulted in persistent infection in resistant mice. Further, administration of IL-4 (in the form of IL-4/anti-IL-4 complexes [13]) conferred a resistant phenotype (worm ex-

pulsion) on the susceptible AKR strain. Thus, in this system, not only was IL-4 demonstrated to be beneficial, but IFN- $\gamma$  was demonstrated to be detrimental.

*Heligmosomoides polygyrus* also leads an enteric life, invading the mucosa to molt several times before emerging as egg-laying adults that reside in the upper small bowel where the worms reside for months. Infected mice are resistant to additional infestations if rechallenged, however, which is an example of concomitant immunity. The latter can be quantitated experimentally by drug-curing infected mice and demonstrating that rechallenge inocula are rapidly expelled over 2–3 wk. Such concomitant immunity was completely blocked by administration of anti-IL-4 and anti-IL-4R antibodies, thus implicating IL-4 in mediating host defense (14). Immunity was also absent when IL-4 gene knockout mice were infected, drug-cured, and secondarily challenged with *H. polygyrus* (Le Gros, G., personal communication). Further, administration of IL-4 complexes enhanced expulsion during primary infection.

*Nippostrongylus brasiliensis* is a rat parasite that has been adapted to the mouse through repeated passage. Larvae are inoculated subcutaneously, migrate to the vascular space, through the lungs, and up the trachea before being swallowed to gain access to the intestinal lumen. There, organisms begin laying eggs, although production is short-lived, and adults are expelled by approximately 10 d. Secondary challenges are rebuffed more quickly. Infection generates tremendous Th2 responses, permitting interventions designed to test the components of normal immunity. Studies performed by Finkelman, Urban, Gause, and their colleagues have provided a number of insights. First, as in the *T. muris* system, IFN- $\gamma$  or IFN- $\alpha$  increased the duration of egg-laying and significantly delayed expulsion of the worms (15). When administered during antigen priming, these cytokines have been demonstrated to bias T cells towards the Th1 phenotype. Second, as demonstrated in this issue, IL-12 had similar but more profound effects, and these effects were correlated with the capacity of IL-12 to enhance endogenous IFN- $\gamma$  production while abrogating production of the Th2 cytokines IL-4, IL-5, and IL-9 (4). As predicted, such effects blocked intestinal mast cell hypertrophy, eosinophilia, and IgE production. Co-administration of anti-IFN- $\gamma$  could abolish most of these effects,

although eosinophilia remained curiously IFN- $\gamma$  independent. Third, administration of IL-12 during secondary rechallenge had little effect on worm persistence, egg-laying, or on the production of IL-4, IL-5, or IL-9 by mesenteric lymph node cells. Even in the primary infection, IL-12 had to be administered within the first 4–6 d in order to achieve maximum effects, suggesting that committed Th2 cells, as suggested by additional studies elsewhere (16), may not express IL-12 receptors like naive, Th0, or Th1 cells. Lastly, these studies raise some caution in considerations of the therapeutic role of IL-12 in boosting cellular immunity, since immunity against these intestinal helminths was clearly compromised because of the inhibition of Th2 cell development.

One of the more fascinating aspects of these systems remains the mechanism(s) by which worms are actually expelled. Although depletion of CD4<sup>+</sup> cells blocks the normal expulsion of *N. brasiliensis*, antibodies to IL-3, IL-4, IL-5, and IL-9 have no effect on the course of infection in normal mice, despite the demonstrable abrogation of mast cell hyperplasia, eosinophilia, and IgE production (4, 17, 18). It is surprising that administration of IL-4 complexes to SCID mice that are normally unable to expulse *N. brasiliensis* caused expulsion of the worms over a 6-d period (Finkelman, F., personal communication). The efficacy of IL-4 and the ineffectiveness of anti-IL-4 antibodies in modulating the disease in normal mice raises the possibility that IL-13, a cytokine closely related to IL-4 in structure and function, may mediate these IL-4-independent effects (19, 20). Indeed, IL-13 has been shown to bind to components shared by the IL-4 receptor (21). Further, these studies point out that the traditional effectors of helminth biology—eosinophils, mast cells, and IgE—are not required for host defense in these systems. A search for IL-4-inducible molecules involved in inflammation, such as 15-lipoxygenase expressed in human monocytes (22) or pancreatic lipase expressed in murine cytotoxic T cells (23), might be an instructive way to connect the immune system to intestinal physiology. Perhaps the final lesson from these studies is in pointing out that the ultimate judgment regarding the appropriate or inappropriate nature of an immune response can only be defined within the context in which it occurs.

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The author thanks S. Reiner, R. Coffman, F. Finkelman, and A. Sher for helpful discussions, and F. Finkelman and G. Le Gros for sharing unpublished information.

This work was supported by grants AI-30663 and AI-26918 from the National Institutes of Health. R. Locksley is a Burroughs Wellcome Fund Scholar in Molecular Parasitology.

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