Commentary

Multiple Pathways for the Initiation of T Helper 2 (Th2) Responses

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Since the initial descriptions of $CD4^+$ T cell subsets with distinct functions and cytokine production profiles, the question of how these cells originate during an immune response has been asked. Both Th1 and Th2 cells can develop from naive, peripheral $CD4^+$ T cell (Thp) populations, and individual Thp cells appear capable of differentiating into either Th subset (reviewed in reference 1). The differentiation process is initiated by ligation of the TCR, but additional signals are required for maturation into cells capable of producing high levels of cytokines upon restimulation. The most clearly defined differentiation inducers are themselves cytokines: IFN-γ and IL-12 for Th1, and IL-4 for Th2 induction. Thus, understanding the cellular origin and control of production of these cytokines during a primary immune response is central to understanding the genesis of Th1 and Th2 responses.

The principal early events that lead to Th1 differentiation in mice infected with any of a number of intracellular pathogens are reasonably well understood. These begin with the production of IL-12 by macrophages responding either to microbial products or to direct infection and the subsequent induction, by IL-12, of IFN-γ production by NK cells (reviewed in reference 2). This response usually occurs within 24 h of infection and constitutes the most effective form of innate immunity for this class of pathogens. Naive T cells initiating a specific response to the pathogen in a microenvironment dominated by IFN-γ and IL-12 develop preferentially into Th1 cells. Thus, the effector functions of the innate response that are most appropriate for the control of these pathogens are conserved in the subsequent antigen-specific T cell response.

The sources and modes of regulation of the initial IL-4 needed to induce Th2 development have, in contrast, not been so clearly defined. Candidate sources of IL-4 include atypical subsets of T cells not restricted by class II MHC (including $NK1^+$ $CD4^+$ T cells), conventional $CD4^+$ memory T cells, eosinophils, and cells of the mast cell/basophil lineage. In this issue, a paper by Rincón et al. (3) now suggests that naive T cells themselves can be the source of IL-4 that leads to their own Th2 development and that IL-6 is a potent inducer of this IL-4. The basic experimental system used in this work consisted of purified mouse $CD4^+$ T cells stimulated in vitro for 4 d. The cells were then harvested, washed, and restimulated, and the supernatants were assayed for IL-4 and IFN-γ as measures of Th2 or Th1 differentiation, respectively. Most experiments employed the polyclonal stimulators Concanavalin A or anti-CD3, but a few experiments were performed with T cells from a TCR-transgenic mouse stimulated with the appropriate peptide epitope. The addition of either IL-6 or IL-4 to the primary cultures led to a severalfold increase in IL-4 production and a concomitant decrease in IFN-γ production by the cells when restimulated. Significant IL-4 was produced upon restimulation even when no cytokines were added to the primary culture, and this IL-4 was eliminated either with anti-IL-6 antibodies or by using APC from $IL\text{-}6^{-/-}$ mice. Importantly, the ability of IL-6 to induce Th2 differentiation was blocked by antibodies to IL-4, implying that IL-6 acted by inducing IL-4 in the primary cultures, which in turn was the direct inducer of Th2 differentiation. In contrast, IL-4 was fully active on $CD4^+$ T cells from $IL\text{-}6^{-/-}$ mice, demonstrating that Th2 induction by IL-4 did not require IL-6. The evidence that IL-6 induces IL-4 production directly by the responding naive Th cells derives from experiments showing the induction of Th2 development in purified naive $CD4^+$ T cells by IL-6. These results suggest that IL-6 can rapidly induce sufficient IL-4 from these cells to lead to stable Th2 differentiation, although this was not directly demonstrated. It should also be noted that the cultures of naive T cells included whole splenocytes as the APC, and it was not ruled out that these were the source of IL-4.

IL-6 is a prominent component of inflammatory and acute-phase responses but has not previously been implicated in the preferential development of either Th2 or Th1 responses (reviewed in reference 4). Rincón et al. (3) suggest the interesting possibility that IL-6 is a key component in a link between innate immunity and Th2 responses, which parallels the connection between the macrophage/NK response to intracellular pathogens and Th1 induction. Not yet clear, however, are the specific conditions or pathogens that would favor Th2 over Th1 induction by this pathway. Furthermore, the relatively few published experiments with $IL\text{-}6^{-/-}$ mice do not give clear evidence of a defect in Th2 responses (5, 6).

Several other recent studies have addressed the question of which cell population provides the early source of IL-4 in a Th2 response, and it is becoming evident that there are multiple answers to the question. The key cell involved in Th2 induction may depend on the route of immunization or infection or on the nature and concentration of the antigen.

The cells that have drawn the most attention are $NK1^+$ T cells (7, 8), an unusual T cell population that is mostly $CD4^+$, expresses several markers characteristic of NK cells, has a highly restricted TCR Vα and Vβ usage, and is re-

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stricted by nonclassical class I MHC molecules, including CD1 (9) and TL (10). The most striking feature of $NK1^+$ T cells, in terms of potential function, is that they can very rapidly produce IL-4 upon activation in vivo with anti-CD3 antibodies (11). Thus, $NK1^+$ T cells have the potential to provide IL-4 at the onset of Th2 responses. A number of recent reports, however, fail to support a role for these cells in the induction of Th2 responses to a variety of pathogens and protein antigens.

The nonprotective Th2 response to *Leishmania major* in BALB/c mice was quite normal in mice rendered deficient in $NK1^+$ T cells by backcrossing a mutated β2-microglobulin (β2m) gene onto the BALB/c background (12, 13). Lack of a requirement for $NK1^+$ T cells in the BALB/c *L. major* response was confirmed independently by backcrossing the NK1.1 allele onto the BALB/c background and depleting mice with an anti-NK1.1 monoclonal antibody (12). Consistent with the lack of effect of these depletions on Th2 development, neither the earliest IL-4 response to infection nor the basal IL-4 mRNA level in lymph nodes was reduced by the elimination of $NK1^+$ T cells (12). Responses to the helminth parasites *Schistosoma mansoni* and *Nippostrongylus brasiliensis* (13) as well as to protein antigens continuously delivered by osmotic pumps (14) or injected subcutaneously (13) or intraperitoneally (15) with adjuvant were similarly not impaired in $\beta2m^{-/-}$ mice as compared with their wild-type controls. It appears, therefore, that IL-4 originating from $NK1^+$ T cells is not required for the initiation of Th2 responses against antigens administered locally or against localized chronic infection. Indeed, only the IL-4 response or the IL-4–dependent IgE response to polyclonal stimuli such as systemic anti-CD3 and anti-IgD have been shown to be dependent on $NK1^+$ T cells (16–18).

Evidence remains that conventional $CD4^+$ T cells (meaning $CD4^+$ T cells that are class II MHC–restricted and lack NK markers) can act as the sources of the initial IL-4 for Th2 induction. Transient, partial depletion of $CD4^+$ T cells from BALB/c mice with anti-CD4 antibodies at the time of *L. major* infection changed these mice from Th2 to Th1 responders (19). This experiment could be interpreted as showing that the early IL-4 source in this model is a $CD4^+$ T cell, an interpretation supported by direct demonstrations that the basal IL-4 level and the earliest IL-4 induced by *L. major* could be abrogated by anti-CD4 treatment (12, 20). Likewise, Th2 development of naive $CD4^+$ T cells ($CD62L^+$) stimulated in vitro with immobilized anti-CD3 can be driven by IL-4 originating from $CD4^+$ T cells that had been previously activated, based on their low levels of CD62L expression (21). In addition to the current paper from Rincón et al. (3), a few other studies have suggested the possibility that the early source of IL-4 may originate from naive-responding $CD4^+$ T cell, possibly during a transient multipotential precursor stage of $CD4^+$ T cell differentiation (22, 23).

A different view of the initiation of a Th2 response, at least for the BALB/c response to *L. major*, comes from study of the strong response to the leishmania antigen LACK (24). *L. major* infected BALB/c mice make a strong early (6 d) Th2 response to LACK, although the response of the same mice to several other *L. major* antigens is predominantly Th1-like (25). A role for LACK-specific T cells in the development of a dominant Th2 response to infection is suggested by observation that BALB/c mice expressing a LACK transgene, and therefore unresponsive to LACK, develop a dominant Th1 response to *L. major* infection. Several interesting questions about this early response to LACK remain. Does this response originate entirely from naive Th cells, or do LACK-reactive Th2 cells preexist, possibly primed by cross-reactive antigens from other environmental antigens? If the LACK-specific Th2 response comes from naive Th cells, does LACK induce unusually strong or rapid IL-4 production, perhaps in a manner similar to that suggested by Rincón et al.?

Mast cells, basophils, and eosinophils can produce significant amounts of cytokines, including IL-4, upon activation (26, 27) and can express MHC class II molecules and present peptides to $CD4^+$ T cells (28, 29). The ability of mast cells and eosinophils to produce IL-4 and present Ag, combined with their presence in the peritoneal cavity and at mucosal sites, makes them candidates to play a role in initiating Th2 differentiation, especially to challenges at these sites. A recent paper by Sabin et al. (30) provides perhaps the clearest evidence to date of the role of a non–T cell source of IL-4 in the initiation of a Th2 response. Studying the initial response to intraperitoneal injection of *S. mansoni* eggs (a strong Th2 stimulus), they have shown that the earliest IL-4 originates from eosinophils and that mast cells indirectly play an important role by secreting IL-5 and recruiting eosinophils to the site of egg injection. Thus, non–T cells may play a crucial role in the initiation of Th2 responses against some pathogens in specific tissues.

In conclusion, there appear to be multiple pathways by which Th2 responses can develop and multiple cellular sources for the IL-4 responsible for the initial differentiation of Th2 cells from naive precursors. Much remains to be answered, however, about the specific properties of the antigens, pathogens, or anatomical sites that are critical to such Th2 induction.

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