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Commentary

Interleukin 12 in host defense against microbial pathogens

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Interest in microbial pathogenesis has increasingly focused on the interactions of organisms with the innate immune system. Several investigators using different systems have demonstrated the critical role played by cytokines released during the initial contact of pathogens with host macrophages in shaping the subsequent cognate immune response orchestrated by T cells. Recent commentaries have called attention to these developments (1, 2), but two manuscripts (3, 4) appearing in these *Proceedings* within the past few months have prompted consideration of the role of a relatively recently described cytokine, interleukin 12 (IL-12), in this response.

A number of intracellular pathogens have been shown to induce T-cell responses characterized by the generation of interferon γ (IFN-γ), an appropriate response necessary for the activation of host macrophages to a microbicidal state. Interestingly, most of these also induce T-cell-independent production of IFN-γ from natural killer (NK) cells (5). This system has been best studied by using *Listeria monocytogenes*, a Gram-positive bacterium that replicates intracellularly and requires a cellular immune response for successful resolution. Ongoing investigations by E. R. Unanue and coworkers had established the critical role for IFN-γ and extended these observations to infections in severe combined immunodeficiency (SCID) mice that lack functional T cells and B cells but have NK cells. These mice control *Listeria*, although the organism is not eliminated as in immunocompetent animals (for review, see ref. 5). Earlier studies by this group had shown that tumor necrosis factor α (TNF-α) produced by macrophages was critical in the IFN-γ response, but that TNF-α was insufficient to generate the response *in vitro*. Conditioned medium from macrophages exposed to several different organisms was capable of restoring the IFN-γ response (6). As shown in the communication in the April 15 issue of the *Proceedings*, this group has now demonstrated that the critical element supplied by conditioned medium is IL-12 (3).

These investigators used SCID splenocytes to eliminate contributions by T cells and then added in various cytokine and anticytokine combinations in the presence or absence of IL-2 while monitoring IFN-γ production. The results were relatively clear-cut. Heat-killed *Listeria* induced the release of TNF-α, IL-12, and IL-10 from macrophages. IL-12 was necessary for the subsequent production of IFN-γ by SCID splenocytes (NK cells), as demonstrated using neutralizing antibodies, but was alone inefficient in the absence of TNF-α or IL-2. TNF-α was markedly synergistic with IL-12, but TNF-α alone could not induce IFN-γ release, even at high concentrations or with IL-2. IL-2 was synergistic with IL-12 or IL-12 plus TNF-α but was incapable of inducing IFN-γ release alone. Finally, the IFN-γ response could be down-regulated in a dose-dependent fashion by IL-10, and this inhibitory effect could be abrogated by IL-2.

In this issue of the *Proceedings* (July 13), A. Sher and colleagues report their investigations of IFN-γ production by NK cells (4). This communication extends the earlier observations to an intracellular protozoan, *Toxoplasma gondii*, using both SCID splenocytes and isolated bone-marrow-derived NK cells. As in the case with *Listeria*, live or killed extracts of *T. gondii* induced IL-12 and TNF-α from macrophages and induced IL-12, TNF-α, and IFN-γ from SCID splenocytes. Production of IFN-γ was abrogated by antibodies to either IL-12 or TNF-α. A role for IL-2 was also suggested by the finding that NK cells purified in exogenous IL-2 were responsive to IL-12 in the presence of antibody to TNF-α. These investigators then demonstrated the capacity of exogenous IL-12 to extend the life of SCID mice infected with cysts from the ME-49 strain of *T. gondii*. The protective effect was presumably due to enhanced production of IFN-γ in the absence of IL-4, as recently shown in experiments describing a therapeutic effect for exogenous IL-12 in disease due to another intracellular protozoan, *Leishmania* (7).

The emerging model for NK activation in response to intracellular organisms might be summarized as follows (Fig. 1). Macrophages phagocytose or are actively invaded by organisms or, in some cases, may interact with secreted pathogen-derived molecules (such as endotoxin for the Gram-negative bacteria). TNF-α and IL-12 are induced relatively rapidly, with a peak around 4–8 hr, and synergistically induce the generation of IFN-γ by NK cells. IFN-γ, in turn, serves to activate macrophage microbicidal systems, primarily associated with induction of nitrate synthase (for review, see ref. 8), that enable macrophages to restrict growth of the invading organism. IL-10 is also induced in macrophages by infectious agents or their products, but this induction occurs later, peaking between 24–48 hr after stimulation (9). This cytokine serves to down-modulate the IFN-γ production by effects on both the macrophage (inhibition of TNF-α and IL-12 production) and the NK cell (interfering with the stimulatory effects of IL-12/TNF-α). This down-modulation would occur around the time that cognate T-cell responses begin to contribute their repertoire of cytokines to further modulate the immune response. IL-2, in particular, markedly potentiates the activities of IL-12 and TNF-α in stimulation of NK-derived IFN-γ. In the SCID mice, this IL-2 signal never arrives due to the T-cell deficiency, and the animals are unable to clear *Listeria* or restrict *Toxoplasma* replication. The ability of high doses of exogenous IL-12 to slow *Toxoplasma* replication or cure *Leishmania* in susceptible mice demonstrates some ability to overcome this loss of IL-2 in immunodeficient hosts and, as suggested by A. Sher and colleagues, raises the consideration of using IL-12 therapeutically in immunodeficient humans, including patients with AIDS.

But what of the T-cell response? The immune system has evolved to generate marvelously coordinated responses to pathogens, and so it is of no surprise that IL-12 has also been implicated in polarizing the maturation of T cells to the Th1 phenotype that centrally orchestrates the cellular immune response through the release of IFN-γ, IL-2, and lymphotoxin. Elegant studies from a collaborative effort between A. O'Garra's laboratory at DNAX Research Institute and K. M. Murphy's laboratory at Washington Uni-

Abbreviations: IL-2, -4, -10, and -12, interleukins 2, 4, 10, and 12, respectively; IFN-γ, interferon γ; TNF-α, tumor necrosis factor α; SCID, severe combined immunodeficiency; NK, natural killer.

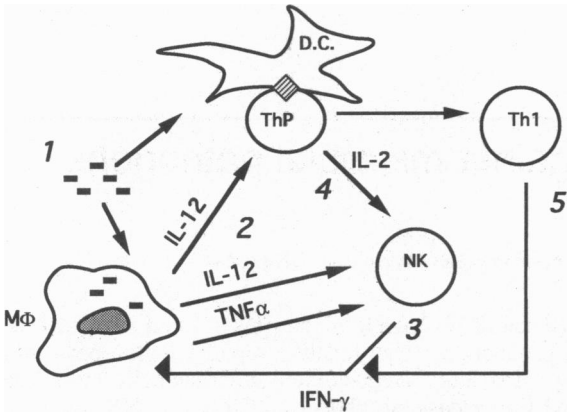

FIG. 1. Development of the cellular immune response. Arrows: 1, microbial pathogens interact with macrophages (Mϕ) and dendritic cells (D.C.); 2, Mϕ are stimulated to release IL-12 and TNF-α; 3, IL-12 and TNF-α stimulate NK cells to produce IFN-γ, which activates Mϕ to a microbicidal state, restricting spread of the organisms. IL-12 also conditions naive helper T cells (ThP) to differentiate to the Th1 pathway; 4, IL-2 produced by the activated T cells synergistically activates NK cells to augment IFN-γ production; 5, differentiated Th1 effector cells generate IFN-γ to maximize macrophage microbicidal responses.

versity, St. Louis, are particularly informative (10, 11). Using naive T cells derived from a major histocompatibility complex class II-restricted, ovalbumin-specific, transgenic T-cell receptor mouse, these investigators could examine the capacity of various cytokines to modulate the induction of Th1 or Th2 effector cells when included during the primary stimulation with matched antigen-presenting cells and ovalbumin *in vitro*. In the absence of additions, such a stimulation resulted in $CD4^+$ T cells of the Th0 phenotype that produced relatively low levels of both IFN-γ and IL-4. The addition of macrophages conditioned with heat-killed *Listeria*, however, resulted in marked polarization to the Th1 phenotype, a phenomenon that could be reproduced with recombinant IL-12. Importantly, the macrophages could be major histocompatibility complex class II mismatched, indicating that cytokines supplied by neighboring cell populations have major effects on T cells during their stimulation by cells more capable of activating naive T cells—e.g., dendritic cells. Four other aspects of these studies deserve mention. (*i*) As with NK cells, IL-10 could negatively regulate the IFN-inducing activity of IL-12 in an antigen-presenting cell dependent manner. (*ii*) Unlike NK cells, TNF-α was not required for the capacity of IL-12 to mediate Th1 development. (*iii*) Although the small amounts of IL-4 produced in the primary immune response resulted in a Th0 phenotype, neutralization of this endogenous IL-4 resulted in striking subsequent polarization to Th1 cells. (*iv*) Exogenous IL-4 added to the primary stimulation resulted in complete polarization to Th2 cells, even in the presence of IL-12. This is in agreement with a number of investigators who have identified the critical role of IL-4 in the maturation of Th2 effector cells (12–14).

Thus, in an immunocompetent animal infected with *Listeria* or *Toxoplasma* (and probably most intracellular pathogens), microbial peptides are presented by dendritic cells to naive T cells at the same time as neighboring macrophages are releasing TNF-α and IL-12 in response to microbial products (Fig. 1). The TNF-α and IL-12 synergistically induce IFN-γ release by NK cells, which serves to limit growth and spread of the organism through activation of macrophages to a microbicidal state. The naive T cells (designated ThP for T helper precursors) contribute IL-2 during stimulation that augments IFN-γ release from NK cells, and the large amounts of IFN-γ serve to limit the amounts of counter-regulatory IL-10 released (15) some hours later. The T cells are simultaneously conditioned in the presence of IL-12, resulting in their maturation to the Th1 phenotype. Thus, more IFN-γ is contributed by specific T cells, resulting in arrest of the infection. As specific peptide disappears with resolution of the organism, IFN-γ levels fall and IL-10 is left unimpeded to down-regulate the inflammatory response to minimize tissue damage.

The identification of IL-12 as a cytokine that serves to link the innate and cognate cellular immune systems together brings substantial clarification to our understanding, although a number of fascinating questions remain. What is the nature of these microbial products that generate the release of TNF-α and IL-12 from macrophages? Such products would be predicted to be powerful adjuvants for the selection of Th1 maturation when given with a discrete antigen. What underlies the dominant effects of IL-4 during Th2 development, even in the presence of IL-12? Whereas IL-12 functions as a rheostat, controlling IFN-γ production, IL-4 functions as a powerful on–off switch for Th2 development. The answer to this question will require more understanding of the receptors for these cytokines, elucidation of their signaling pathways, and characterization of DNA-binding proteins mediating their effects in T cells. Or is another cytokine, IL-__, lurking in the future? The likelihood of interactions along one or more of these pathways might be predicted on the basis of what is known to date. Lastly, the use of infectious disease models in these various contributions should not be understated. These organisms have interacted with the immune system throughout its evolution and continue to provide us with roadmaps identifying the key elements involved.

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