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Inheritable defects in interleukin-12- and interferon-gamma-mediated immunity and the TH₁/TH₂ paradigm in man

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TH₁ cells were defined by their coordinated production of a well-defined set of cytokines (including interleukin [IL]-2, tumor necrosis factor-beta [TNF-β], and the signature cytokine interferon-gamma [IFN-γ]), and have been thought to play an essential role in host defense against intracellular pathogens (1–10). In contrast, TH₂ cells were defined by their secretion of a different set of cytokines (including IL-5, IL-6, and the signature cytokine IL-4), and were thought to be essential for protective immunity against extracellular pathogens. The TH₁/TH₂ paradigm also implies that TH₁ cells downregulate TH₂ responses, and vice versa. Although its existence is widely accepted by immunologists, the extent to which this paradigm holds is still debated. Some studies have suggested that the dichotomy applies to all T-cell types (α/β cytotoxic, γ/δ) (6, 11), or have assigned a growing number of functions to each TH cell subset (exacerbated TH₁ and autoimmunity, exacerbated TH₂ and allergy) (2, 3, 6, 9, 12). In contrast, other studies have questioned the existence of discrete TH cell subsets, as defined by coordinated cytokine production (13, 14), or the specific role of each TH cell subset in protective immunity against extracellular or intracellular infectious agents (15).

Recently, recessive null mutations have been identified in the IFN-γ receptor ligand-binding chain (IFN_γR1) (16–22), IFN-γ receptor signaling chain (IFN_γR2) (23), IL-12 p40 subunit (IL-12 p40) (24), and IL-12 receptor β1 chain (IL-12Rβ1) (25, 26) genes of a number of patients. The clinical phenotype of affected individuals is unusual, because they have selective vulnerability to severe mycobacterial infections. Delayed-type hypersensitivity to mycobacterial antigens is normal, but mycobacterial granuloma formation is abnormal. These conditions share a common pathogenic mechanism, as all impair IFN-γ-mediated immunity, either by preventing IFN-γ from signaling through its receptor

(IFN- γ R1 and IFN- γ R2 deficiencies) or by markedly reducing the production of the IFN- γ cytokine itself (IL-12 p40 and IL-12R β 1 deficiencies). Signaling by the TH1 signature cytokine (IFN- γ) or its major cytokine inducer (IL-12) is abolished, so these experiments of nature provide a unique opportunity for investigating the functional relevance of the TH1/TH2 paradigm in man. This paper briefly reviews the genetic defects and compares the clinical phenotypes to those expected from the paradigm. Other features of this recently described group of inheritable disorders have been reviewed elsewhere (27–32).

Inheritable defects in IL-12- and IFN- γ -mediated immunity

Mutations impairing IFN- γ signaling were identified first, and 18 cases of complete IFN- γ R1 deficiency have been reported (16–21). The *IFNGR1* mutations are recessive, because only homozygous or compound heterozygous individuals are affected, and null, because they prevent the receptor from being produced or functioning at the cell surface. A child with complete IFN- γ R2 deficiency was also identified (23). A small frameshift deletion, which was homozygous and null, was found in the *IFNGR2* coding region. No signaling via the IFN- γ receptor complex was possible, despite the normal production and sequence of IFN- γ R1. IFN- γ is a major macrophage-activating cytokine secreted by natural killer (NK) and T cells, but its receptor is ubiquitous; therefore, it is unclear which cell types are responsible for the phenotype. Mutations impairing IFN- γ secretion were subsequently identified. A child homozygous for a large deletion in the gene encoding the p40 subunit of IL-12, a potent IFN- γ -inducing heterodimeric cytokine (p70) secreted by macrophages and dendritic cells, has been reported (24). Neither p40 nor p70 was detected in the supernatant of activated phagocytes or dendritic cells of the patient. His lymphocytes secreted much less IFN- γ than normal after the stimulation of peripheral blood mononuclear cells *in vitro*. The impaired secretion of IFN- γ was complemented, however, by exogenous recombinant IL-12 in a dose-dependent manner, implying that IFN- γ deficiency is not a primary event, but a consequence of inherited IL-12 deficiency. Recessive mutations in the gene encoding the β 1 subunit of the IL-12 receptor have been identified in seven other patients (25, 26). These mutations preclude cell surface expression of IL-12R β 1, and neither NK cells nor T cells are responsive to IL-12 stimulation *in vitro*.

Impairment of IFN- γ secretion by NK cells and T cells was also demonstrated.

Impaired TH1 immunity?

Clinically, TH1 responses are generally thought to play a major role in protective immunity to intracellular pathogens, including all viruses, most fungi, a number of protozoans, some bacteria, and a few helminths if any. In all patients with complete IL-12, IL-12R, or IFN- γ R deficiency, infections with weakly pathogenic mycobacteria, such as attenuated bacillus Calmette-Guérin or environmental nontuberculous mycobacteria, were diagnosed. Infection with *M. smegmatis*, one of the least virulent mycobacterial species, never previously identified as a cause of disseminated disease in man, was observed (18). One child with partial IFN- γ R1 deficiency had clinical tuberculosis (22). This is consistent with the paradigm, according to which protective immunity to mycobacteria is characteristic of TH1 responses. Along these lines, the patients suffered no infections due to extracellular microorganisms, and other intracellular bacteria were occasionally found to be pathogenic. *Salmonella* other than *S. typhi* and *S. paratyphi* were documented in fewer than half of the patients; *Listeria monocytogenes* (21) and *Legionella pneumophila* (22) in one patient each. Clinical disease due to environmental fungi was not observed, however, and the course of common viral diseases was unremarkable. No parasitic infections were reported either, but the parasites to which the patients were exposed are generally unknown. The investigation of more patients, possibly from various geographic origins, and a systematic screen of titers of their antibodies against intracellular pathogens, is required to assess resistance to the diversity of potential pathogens. Overall, however, the patients were resistant to the vast majority of intracellular pathogens encountered, and the spectrum of infections in children with impaired IL-12- or IFN- γ -mediated immunity was certainly narrower than was predicted by the TH1/TH2 paradigm. Patients were vulnerable to only a small minority of intracellular organisms, mostly *Mycobacterium* and, to a lesser extent, *Salmonella*.

Exacerbated TH2 immunity?

It is generally accepted that TH2 responses are down-regulated by TH1 cytokines, such as IFN- γ . It has also been suggested that TH2 responses are responsible for atopy (2, 3,

6, 9, 12). An exacerbated TH₂ response, leading to biologic indicators and clinical signs of atopy, would therefore be expected in patients with genetically abrogated IFN- γ - or IL-12-mediated responses. However, the patients showed no disorders of severe atopy, such as asthma or eczema, or of mild allergy, such as rhinitis and conjunctivitis. The patients had high IgG and IgA levels, but IgE levels were within the normal range. Hypergammaglobulinemia may simply reflect chronic antigenic stimulation by mycobacteria. Neither blood eosinophilia nor tissue mastocytosis was reported. Further studies are required to define clearly the allergic status of such patients. In particular, the quantification of serum IgE reactive to common allergens may be a useful indicator of atopy. It would also be of value to assess the role of genes, such as those encoding IFN γ R1, IFN γ R2, IL12 p40, and IL12R β 1, in the susceptibility to atopy in the general population. Association and linkage studies should be used to investigate this question. The clinical information available suggests that patients with IL-12 or IFN- γ deficiency have no exacerbated TH₂ response, or at least have no overt biologic and clinical signs of allergy. Thus, these natural mutations suggest that TH₁ signature cytokines have no major regulatory effect on TH₂ cells, and that allergen-specific T cells may be downregulated in the absence of IFN- γ or IL-12.

Concluding remarks

When we consider the TH₁/TH₂ paradigm at the physiologic level, two conclusions can be drawn from this study

of patients with inheritable defects of IL-12- or IFN- γ -mediated immunity. Firstly, IFN- γ and IL-12 have a more limited function in human host defense (immunity to mycobacteria) than was initially thought, on the basis of the paradigm (immunity to intracellular pathogens). The diversity of infectious agents probably requires a high diversity of recognition and effector mechanisms, and mycobacteria may preferentially trigger IL-12 secretion, leading to protective IFN- γ -mediated immunity. Secondly, atopy does not develop in the absence of IL-12 or IFN- γ , and the role of these cytokines in downregulating allergen-specific T cells is less important than was predicted from the paradigm. Other, as yet unknown, mechanisms probably downregulate the expansion of the population of allergen-specific T cells. When considering the TH₁/TH₂ paradigm at the cellular level, the disorders studied here provide interesting prospects for the investigation of helper T-cell differentiation in man. Further studies are required to define the cytokine profile of helper T cells of the patients.

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