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Biologic functions of the IFN- γ receptors

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Overview

Interferon-gamma (IFN- γ) is a cytokine that plays an important role in inducing and modulating an array of immune responses. Cellular responses to IFN- γ are mediated by its heterodimeric cell-surface receptor (IFN- γ R), which activates downstream signal transduction cascades, ultimately leading to the regulation of gene expression. In order to study the role of IFN- γ in a number of immune responses and pathways, researchers have generated mice with altered patterns of IFN- γ R gene expression. These studies, together with analyses of naturally occurring mutations of the IFN- γ R in man, have been instrumental in elucidating the diverse functions of IFN- γ , and are the subject of this review.

Introduction

Originally identified 30 years ago as an agent with antiviral activity, IFN- γ has since been characterized as a homodimeric glycoprotein with pleiotropic immunologic functions (1–3). IFN- γ is primarily secreted by activated T cells and natural killer (NK) cells, and can promote macrophage activation, mediate antiviral and antibacterial immunity, enhance antigen presentation, orchestrate activation of the innate immune system, coordinate lymphocyte–endothelium interaction, regulate Th1/Th2 balance, and control cellular proliferation and apoptosis (1, 2). It was not until 20 years after the identification of IFN- γ that its cell-surface receptor was discovered (4–9). The α chain of the IFN- γ R, also known as IFN- γ R1 or CD119, was the first component of the receptor to be identified and cloned (10–14). Although it binds IFN- γ with relatively high affinity, IFN- γ R1 alone is

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unable to mediate the biologic responses to this cytokine (12, 15–17). Subsequent complementation studies led to the identification and cloning of an accessory factor (AF-1), also known as the β receptor chain or IFN- γ R2, as the protein required, in addition to IFN- γ R1, to endow a cell with the ability to respond to IFN- γ (15, 18–21). Specific residues within the cytoplasmic domains of both the α and β chains of the IFN- γ R are critical for transducing the IFN- γ signal from the cell surface to the nucleus through the activation of intracellular signaling pathways (22–25). Mutations in either component of the IFN- γ receptor that impair or alter the ability of cells to respond to this ligand have global consequences for IFN- γ -mediated immunity, and therefore serve as an important tool for analyzing the pleiotropic effects of this cytokine (26, 27).

The biochemical and signaling properties of the IFN- γ R

The receptor complex that mediates the full biologic function of IFN- γ consists of at least two species-matched chains: IFN- γ R1, a 90-kDa glycoprotein (which is 472 amino acids (aa) long in man, and 451aa in mice) encoded on human chromosome 6 and mouse chromosome 10, and IFN- γ R2, a 60–67-kDa glycoprotein (which is 316aa in man and 314aa in mice) encoded on human chromosome 21 and mouse chromosome 16 (4, 18, 20, 28–35). IFN- γ R1 is the major ligand-binding subunit, binding IFN- γ with a K_a of 10^9 – 10^{10} M $^{-1}$ and a receptor-to-ligand ratio of 2 : 1, as inferred from the crystal structure of the occupied receptor and other studies (4, 34, 36, 37). IFN- γ R2 increases the affinity of IFN- γ R1 for its ligand, presumably by enhancing the stability of the complex, but plays only a minor role in direct ligand binding (38). The β chain is, however, obligatory for transducing the IFN- γ signal (21, 38, 39).

Both chains of the IFN- γ receptor are members of the class II family of cytokine receptors that includes tissue factor, the IL-10 ligand-binding component, and both chains of the IFN- α receptor (IFN- α R) (40, 41). Like other family members, the IFN- γ R α and β chains lack intrinsic kinase activity. Signaling through the IFN- γ R is mediated through JAK1 and JAK2, members of the Janus family of protein tyrosine kinases, which are constitutively associated with specific membrane-proximal residues on the cytoplasmic domains of IFN- γ R (25, 34, 39, 42–44). JAK1 binds the $_{266}$ LPKS $_{269}$ motif (also known as the box 1 motif) on IFN- γ R1, while JAK2 binds the $_{263}$ PPSIPLQIEEYL $_{274}$ motif (or the box 1, box 2 motif) on IFN- γ R2 (23, 25, 44–46). Ligand binding leads to

receptor oligomerization, with two IFN- γ R1 chains bound to one IFN- γ homodimer, and the subsequent recruitment of two IFN- γ R2 chains to the complex (25, 37, 38, 45–48). IFN- γ -mediated aggregation of its receptor components brings the inactive JAKs associated with the cytoplasmic tails of the α and β chains into close proximity with one another (Fig. 1). Once clustered, the JAKs are reciprocally activated through sequential auto- and transphosphorylation events (42, 49). Activated JAKs phosphorylate a specific tyrosine residue near the C-terminus of the IFN- γ R1 (Y440 in man) (24, 42, 45, 50) (Fig. 1). This phosphorylated tyrosine residue pair (one on each IFN- γ R1 chain) is embedded within a recognition sequence ($_{440}$ YDKPH $_{444}$) to which STAT1 (a member of the Signal Transducers and Activators of Transcription family of latent cytoplasmic proteins) binds through its SH2 (src homology 2) domain (24, 45, 51, 52). The docking of STAT1 molecules at their target sequences on the IFN- γ R complex is followed by their phosphorylation on tyrosine residue Y701 by the receptor-associated JAKs (53–55) (Fig. 1). Once phosphorylated, two STAT1 proteins homodimerize via reciprocal SH2-phosphotyrosine interactions, forming a protein complex first identified as GAF (gamma-activated factor) (51, 56). The STAT1 homodimer then translocates to the nucleus, where it binds a nine-nucleotide consensus sequence, TTNCNNNAA, known as a GAS (gamma-activated site) element (57–59). This binding site has been identified in the regulatory regions of over 200 genes; therefore, recognition of this element by STAT1 homodimers can modulate the expression of a vast array of genes, thereby mediating the biologic functions of IFN- γ (2).

Mechanistically, it has been suggested that after ligand binding, IFN- γ signaling is initiated by JAK2 autophosphorylation, followed by phosphorylation of JAK1 (34, 60). Activated JAK1 is then thought to phosphorylate IFN- γ R1, providing a docking site for STAT1. After binding to its receptor site, STAT1 is believed to be activated through phosphorylation by JAK2. Studies with kinase-negative JAK1 and JAK2 mutants have demonstrated that while the mutant JAK2 cannot support IFN- γ -stimulated gene transcription, the kinase activity of JAK1 is not fully required for its role in transducing the IFN- γ signal (49).

Signaling through the IFN- γ receptor may be regulated at several points along the pathway. One mechanism is the modulation of receptor expression. Control of IFN- γ R1 levels on the cell surface has been proposed to be a mechanism through which a cell alters its sensitivity to IFN- γ (61–63). Attenuation of the IFN- γ signal by this mechanism has been linked to differences in the biologic

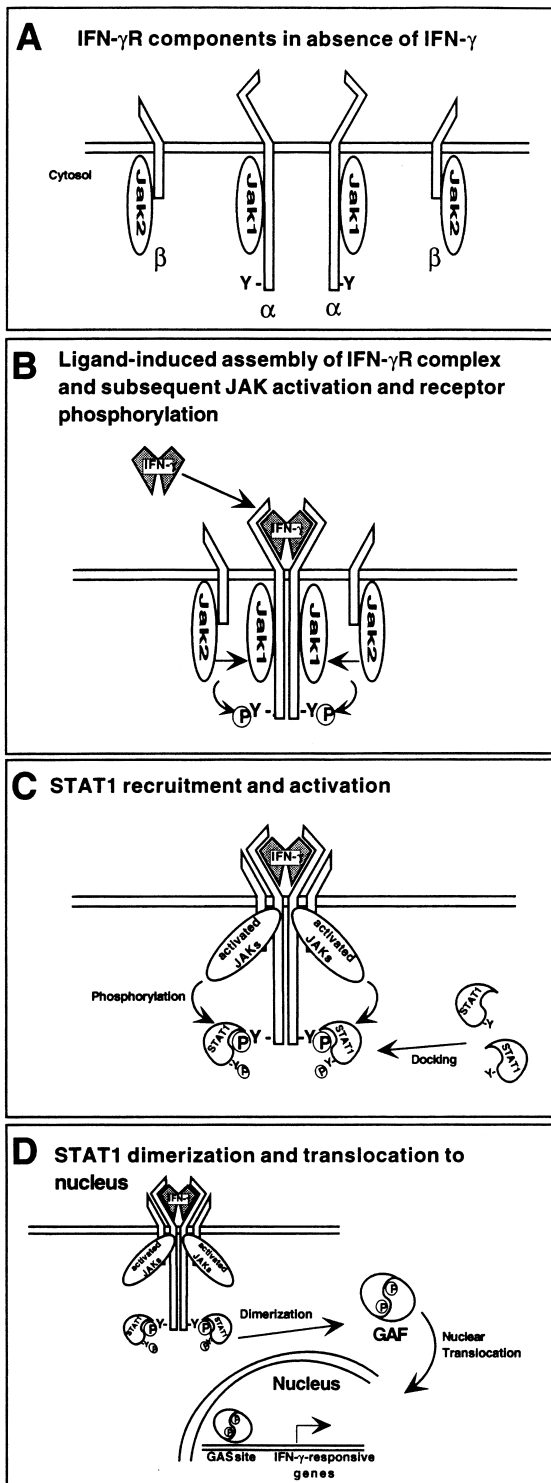


Figure 1. IFN- γ signaling cascade. A) IFN- γ R is composed of α and β chains. JAK1 is constitutively associated with IFN- γ R1 while JAK2 is constitutively associated with IFN- γ R2. B) IFN- γ binding to its receptor leads to aggregation of receptor components. Subsequently, JAKs are activated through auto- and transphosphorylation events. Activated JAKs then phosphorylate tyrosine residue near C-terminus of IFN- γ R1. C) STAT1 molecules dock at phosphorylated receptor, and are then phosphorylated by activated JAKs. D) Phosphorylated STAT1 proteins homodimerize via reciprocal SH2-phosphotyrosine interactions, and translocate to nucleus, where they regulate gene transcription.

responses it elicits in cells expressing different levels of IFN- γ R1. Moreover, downmodulation of IFN- γ R2 expression in IFN- γ -producing T cell subsets renders these cells unresponsive to IFN- γ (64–66). Cells appear to have intracellular mechanisms to regulate the IFN- γ signal as well. Members of the recently identified SOCS (Suppressors Of Cytokine Signaling) family of proteins may negatively regulate cytokine signaling through a number of potential mechanisms (67–69). For example, SOCS1 has been shown to inhibit IFN- γ signaling, presumably by preventing JAK kinase activation (70, 71). In fact, the expression of SOCS-1 is induced by IFN- γ , suggesting that a negative feedback pathway exists in which cells may attenuate their sensitivity to this cytokine after the activation of IFN- γ signaling. In contrast, the transcriptional activity of STAT1 (and therefore the IFN- γ signal) has been shown to be enhanced through serine phosphorylation (72, 73). Additional intracellular mechanisms thought to regulate IFN- γ -mediated signal transduction may include the dephosphorylation of IFN- γ R1, which would prevent STAT1 docking; the dephosphorylation of STAT1, which would prevent its homodimerization; and the ubiquitination of STAT1, which would lead to its degradation by targeting it to the proteasome pathway (42, 51, 74, 75). These mechanisms could limit the availability of activated STAT1, although their importance in the regulation of IFN- γ signaling is unclear.

Disruption of IFN- γ R1 expression in mice

To study the role of IFN- γ R1 in mediating IFN- γ signaling and biologic responses, researchers have disrupted its endogenous locus to generate IFN- γ R1-null ($-/-$) mice (26). These mice have not only proved to be a useful tool to study IFN- γ signal transduction, but also constitute one of the first physiologic systems available to study IFN- γ biology. IFN- γ R1-deficient mice develop normally to adulthood with no phenotypic anomalies. They appear to have normal leukocyte populations in lymphoid organs and normal baseline levels of MHC class I and II molecule expression. However, cells derived from these mice are unable to initiate signaling in response to IFN- γ . They are therefore insensitive to any of the biologic effects of IFN- γ , including its antiviral effects and antitumor activity (26, 76). As described below, these mice have severe defects in their immune responses.

Macrophage function and IL-12 in IFN- γ R1-deficient mice

IFN- γ has long been thought to be the most important activator of macrophages; in fact, it was, for a time, known as macrophage-activating factor (MAF) (77). Nitric oxide (NO) production and MHC class II upregulation are two key effector mechanisms of macrophages, both critically dependent on IFN- γ , and are essential for cell-mediated and Th1-type immune responses (1, 2, 77, 78). Therefore, it was not surprising that the IFN- γ R1 (-/-) mice exhibited severely compromised macrophage functions, such as granuloma formation (79–85). The IFN- γ dependence of protective and self-destructive immune responses mediated, in part, by macrophages was examined in several infectious and autoimmune models that are discussed in later sections of this review.

IL-12 is a cytokine secreted primarily by activated macrophages (2, 86–88). It induces IFN- γ production by NK cells and Th1 cells, while IL-12 production itself is induced by IFN- γ (89). IL-12 also primes naive T cells (Thp) to differentiate along the Th1 pathway (90–94). It is, however, unclear whether the biologic functions of IL-12 are mediated principally by IFN- γ (95). A number of groups utilized IFN- γ R1-deficient mice to study the dependence of the effector functions of IL-12 on IFN- γ .

Systemic administration of IL-12 is known to inhibit hematopoiesis, leading to a decrease in the number of peripheral blood lymphocytes (PBLs) and a diminished bone-marrow cellularity (96). Administration of IL-12 also increases splenic cellularity, secondary to an influx of activated macrophages and NK cells. In contrast, IFN- γ R1 (-/-) mice injected with IL-12 mainly develop a severe, lethal lung disorder characterized by interstitial and perivascular infiltrates and diffuse pulmonary edema. Therefore, it appears that, in the absence of IFN- γ responses, many effects of IL-12 are attenuated. On the other hand, IL-12 appears to induce a different set of immune disorders that are independent of, or normally suppressed by, IFN- γ . Other experiments also suggest that IL-12 may have IFN- γ -independent effects. For example, in one study of collagen-induced arthritis (CIA), the severity of this autoimmune disease was attenuated in IFN- γ R1-deficient mice after treatment with anti-IL-12 neutralizing antibodies, which are thought to suppress inflammatory disease by, in part, indirectly inhibiting IFN- γ secretion (97, 98). Therefore, while many of the effects of IL-12 (such as cross-regulation of type 2 inflammatory responses) are abrogated in IFN- γ R1 (-/-) mice, other effector functions of IL-12 (such as direct

Th1 priming, which may explain the CIA phenotype) may be independent of IFN- γ (90, 97, 99, 100).

Primary CD4+ T-cell responses in IFN- γ R1-deficient mice

Naive CD4+ cells, or Thp cells, are believed to have the potential to develop into either of the two major subsets of CD4+ T helper cells: Th1 and Th2 cells (100, 101) (Fig. 2). Th1 cells are primarily defined by their ability to secrete IFN- γ , IL-2, and tumor necrosis factor (TNF)- β , and are clinically associated with an ability to orchestrate cell-mediated immune responses and organ-specific autoimmunity (102). Differentiation of Thp cells toward the Th1 phenotype is dependent on the presence of IL-12 in their microenvironment during stimulation throughout the T-cell antigen receptor (TCR) (92, 103). Th1-polarizing effects similar to those of IL-12 have been ascribed to IFN- γ (104). On the other hand, Th2 cells arise when IL-4 is present during antigenic stimulation, and produce IL-4, IL-5, IL-10, and IL-13 (105). These cells are clinically associated with phagocyte-independent, antibody-mediated host defense and allergic immune responses (102). Because of the potential role of IFN- γ in generating Th1 cells, mediating their effector function and regulating Th1/Th2 balance, T-cell responses were examined in IFN- γ R1 (-/-) mice (94, 99, 106).

In some experimental models utilizing IFN- γ R1 (-/-) mice, there appeared to be a shift in the Th1/Th2 balance in response to stimuli that normally induce Th1 immunity (Fig. 2). For example, as described above, a predominant Th2 response ensued in response to systemic IL-12 administration in these mice (96). A similar Th2-directed immune disorder was also seen in one model of schistosomiasis, where not only were Th1-dependent immune responses impaired, but also aberrant Th2 responses led to a severe lung disorder (107). These observations support previous work that suggested that, while IFN- γ is normally considered an effector of inflammation, it may have an important inhibitory role in certain immune responses (108). In another study, Th2-mediated pulmonary inflammation was induced by rechallenge of previously sensitized mice with nasally administered OVA (109). This inflammatory lung disease persisted in IFN- γ R1 mutant mice long after it was resolved by wild-type mice. Allergic pulmonary inflammatory reactions are often mediated by a type 2 response characterized by eosinophils, IgE antibodies, and cytokines such as IL-4 and IL-5 (110–112). Therefore, one explanation of the observed lung phenotype in IFN- γ R1 mutant mice is that, in the absence of the cross-regulation

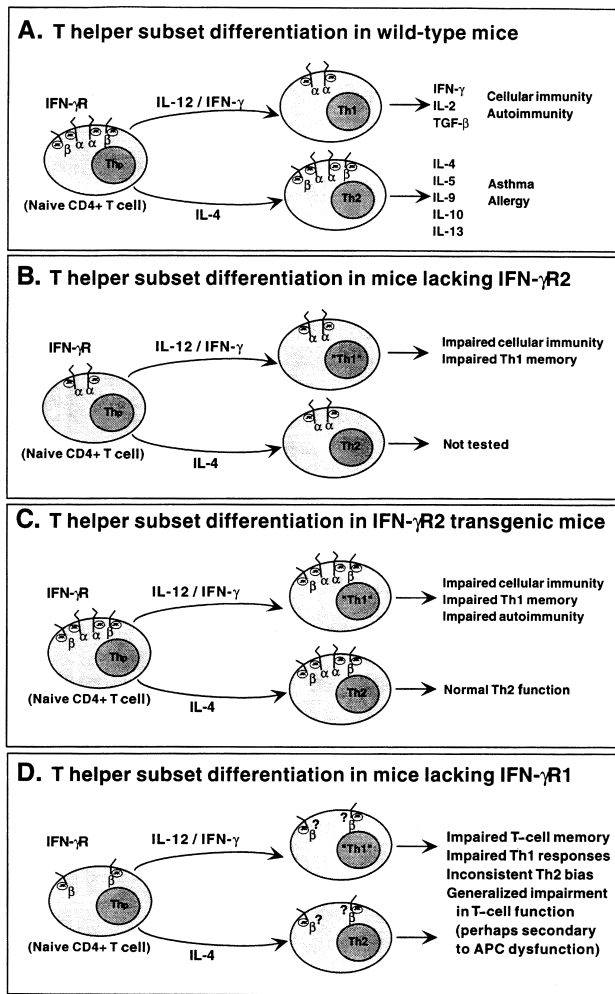


Figure 2. Effects of IFN- γ R on T helper subset differentiation.

normally seen between Th1 and Th2 responses (with Th1-derived IFN- γ presumably suppressing the Th2 response), a Th2-mediated immune response can proceed unhindered (99, 102). Alternatively, there may be other mechanisms by which IFN- γ suppresses inflammation (108, 113).

In another group of studies, the response to the pathogen in mutant mice was normal, without a concomitant Th2-mediated disorder, although abnormally high levels of Th2 cytokines and/or Ig isotypes were detected (see Table 1 for a summary of all disease models in which IFN- γ R1 (-/-) mice were used). For instance, the outcome of mouse mammary tumor virus (MMTV) infection is similar in mutant and control mice, but is characterized by increased IL-4 levels in the former (114). In other models, IFN- γ R1 (-/-) mice elaborated defective Th1 responses with no associated Th2 response. For example, while wild-type mice are resistant to infection by either coronavirus or *Leishmania major*, IFN- γ R1 mutant mice are susceptible to these

pathogens (115, 116). An analysis of the pathologic changes in these mice revealed no evidence of a Th2-type immune response.

In summary, some of these studies uncovered global defects in T-cell responses, others demonstrated a specific Th1 dysfunction, and yet others revealed an altered Th1/Th2 balance in IFN- γ R1 (-/-) mice. However, the extent of the difference in the clinical course of disease between wild-type and mutant mice was found to be highly variable. Nevertheless, these data suggest an interesting trend in T-cell-mediated immunity in IFN- γ R1 (-/-) mice: a Th2-bias with impaired Th1 responses. The observed set of T-cell phenotypes may be the consequence of a host of factors. For instance, IFN- γ -insensitive macrophages and dendritic cells are impaired in their ability to upregulate expression of MHC class II molecules on their cell surface (81). This defect in antigen presentation may alter CD4+ T-cell activation in these mice. IFN- γ R1 (-/-) antigen-presenting cells (APCs) are also unable to secrete normal amounts of IL-12, an important Th1-polarizing stimulus (120). This may lead to a bias toward Th2-type responses. Moreover, it is believed that Th1-derived IFN- γ can cross-regulate Th2 cells (99). Unresponsiveness of Th2 cells to IFN- γ would therefore lead to a bias toward type 2 immune responses. It has also been suggested that in the absence of IFN- γ responses, monocytes can become alternatively activated, and immature dendritic cells can develop into alternatively activated dendritic cells (Dc2) (121–123). Alternatively activated macrophages and Dc2 cells have been shown to support differentiation of Thp cells to the Th2 phenotype. The role of these unusual APCs in promoting the Th2 pathway may, in part, account for the inability of IFN- γ -unresponsive mice to mount type 1 inflammatory processes. Of course, it is also likely that IFN- γ R1 (-/-) T cells have intrinsic differences in their response to antigen. Robust type 2 responses seen in some of these immune models suggest that Th2 cells are less dependent than Th1 cells on antigen presentation by IFN- γ -primed APCs (96, 109, 121, 122). Finally, T cells must be able to migrate to their target sites in order to be effective regulators and mediators of immunity. The expression of adhesion molecules on vascular endothelial cells and on T cells, as well as the production of a number of chemokines, is regulated by IFN- γ (117–119). Therefore, part of the observed T-cell defect may be secondary to impaired migration to target sites.

CD4+ T-cell memory in IFN- γ R1-deficient mice

Like primary T-cell-mediated immunity, immunologic memory, which relies on T helper cell function, may be

Table 1. Summary of phenotype of IFN- γ R1 (-/-) mice in models of infection

Pathogen	Phenotype in normal mice	Phenotype in IFN- γ R1 (-/-) mice
<i>Listeria monocytogenes</i>	Resistant	Highly susceptible
<i>Bordetella pertussis</i>	Contain infection	Lower threshold of lethal dose with atypical disseminated disease
Bacillus Calmette-Guérin	Resistant	Susceptible
<i>Chlamydia trachomatis</i>	Healing response	Severe and prolonged infection relative to normal mice
	Resistant to secondary infection	Susceptible to secondary infection
<i>Yersinia enterocolitica</i>	Intermediate susceptibility on 129/SV background	Highly susceptible on 129/SV background
<i>Staphylococcus aureus</i>	Susceptible to intraperitoneal administration	Earlier mortality than normal mice
<i>E. coli</i>	Peritonitis and NO production	Normal NO synthesis
Pseudorabies virus	Vaccine effective; resistant to rechallenge	Vaccine ineffective; susceptible to rechallenge
Sendai virus	Clear infection	Clear infection
Murine γ -herpesvirus 68	Resistant to large-vessel disease	Develop large-vessel arteritis and splenic disorder
Vaccinia virus	Resistant	Increased susceptibility, but normal CTL response
Vesicular stomatitis virus	Mount CTL response	Normal CTL response
LCMV	Transient immunodeficiency phenomenon	No transient immune deficiency
		Impaired viral clearance with persistent infection in one model
Theiler's virus	Resistant on 129/SV background	Develop chronic disease
Coronavirus	Develop hepatitis	More severe hepatitis with increased mortality
Murine cytomegalovirus	Clear infection	Do not resolve, develops chronic arteritis
MMTV		Same clinical phenotype as normal mice
		Increased Th2 parameters
<i>Leishmania major</i>	Resistant mouse strains clear infection	Lethal in resistant genetic background
<i>Plasmodium chabaudi chabaudi</i>	Resistant	Increased susceptibility
<i>Plasmodium yoeli</i>	Vaccine protective	Vaccine ineffective; prolonged parasitemia in primary challenge
<i>Toxoplasma gondii</i>	Contain pathogen, but develop chronic infection	Pathogen causes greater disorder than in normal mice
<i>Encephalitozoon intestinalis</i>	Resistant	Chronic, nonhealing disease
<i>Schistosoma mansoni</i>	Contain pathogen, but develop chronic infection	Develop greater immune-mediated disorder
		Impaired granuloma formation with increased Th2 parameters
		Same clinical outcome as wild-type mice (in another study)
	Vaccine protective	Vaccine ineffective
<i>Trypanosoma cruzi</i>	Contain pathogen, but develop chronic infection	Succumb to infection
African trypanosome	Chronic disease with anaemia	Increased parasitemia but reduced anemia relative to normal mice
	Severe immunosuppression	Less severe immunosuppression

affected by the absence of a functional IFN- γ system (124–126). To assay the role of IFN- γ responsiveness in secondary T-cell responses, IFN- γ R1 (-/-) mice were used in a number of immunization models. In every instance, mutant mice were unable to respond normally to antigenic rechallenge. Antigenic recall was impaired in these mice in response to rechallenge with *Chlamydia trachomatis*, pseudorabies virus, *Schistosoma mansoni*, and *Plasmodium yoeli* (107, 127–129). In fact, previously sensitized IFN- γ R1 (-/-) mice elaborate inappropriate, and therefore unproductive, T helper cell-mediated responses upon re-exposure to *S. mansoni* (129). While the normal response to rechallenge with this pathogen is characterized by a strong Th1

component, helping contain the infection, the response in mutant mice is Th2 in nature, and it is ineffective in controlling parasitemia. Interestingly, repeated exposure of IFN- γ R1 (-/-) mice to *P. yoeli* led to lasting immunity against this pathogen, thereby somehow bypassing the need for the IFN- γ system (129). It therefore appears that T-cell memory and, perhaps more specifically, Th1-mediated secondary responses are usually dependent on IFN- γ signaling.

CD8+ T-cell function in IFN- γ R1-deficient mice

Like CD4+ T helper cells, CD8+ cytotoxic T lymphocytes (CTLs) are activated by an antigen presented in the context

of an MHC molecule. However, unlike MHC class II, MHC class I molecules (which present intracellularly derived peptides to CD8 cells) are ubiquitously expressed (130, 131). Interestingly, recent evidence suggests that CD8⁺ T cells must first be activated by dendritic cells (that, in this case, encounter and then present extracellularly derived antigen in the context of MHC class I molecules) before leaving lymphoid areas and surveying the periphery (132). Moreover, whereas antigen presentation through the MHC class II pathway is enhanced mainly by IFN- γ , antigen presentation through the MHC class I pathway can be stimulated by either IFN- γ or IFN- α (2, 130, 131, 133, 134). Tc1 cells, which constitute the predominant CD8⁺ T-cell subset, secrete substantial amounts of the IFN- γ that is thought to participate in some aspects of their effector function (135). It was therefore of interest to examine CTL function in IFN- γ R1 (-/-) mice in order to clarify the relevance of IFN- γ sensitivity to the function of these cells.

CTLs derived from IFN- γ R1 mutant mice infected with vaccinia virus lysed target cells infected with this virus normally, suggesting that IFN- γ -insensitive CTLs are capable of elaborating normal cytotoxic effector and memory functions (26). CD8⁺ cells in LCMV-infected IFN- γ R1 (-/-) mice exhibited delayed kinetics of clonal exhaustion as compared to wild-type mice, indicating that they are less susceptible to activation-induced cell death (AICD), perhaps owing to inefficient activation (which may be secondary to either an antigen presentation or an intrinsic T-cell defect) (136).

A number of studies suggest that CD8⁺ T cells are important mediators of the effector phase of contact hypersensitivity (CHS) responses to haptens (137–140). Although tissue swelling in response to oxazolone and TNCB was comparable in IFN- γ R1 (-/-) mice and wild-type mice, mutant mice had reduced dermal mononuclear infiltrates (141). Because of the complexity of this model of immunity, impaired CHS may be caused by defects in either the sensitization phase (dendritic cell function, homing, or antigen presentation) or elicitation phase (chemokine secretion; CD8 T-cell activation or migration) of this response (142–144). Therefore, reduced dermal mononuclear infiltrates in IFN- γ R1-deficient mice may not be caused by an intrinsic CD8⁺ T-cell defect. Although CD8⁺ CTL also participate in graft rejection (145–147), models of allo- and xeno-transplantation failed to uncover differences in the ability of wild-type and IFN- γ R1 mutant mice to reject transplanted grafts (148, 149). These data suggest that, for the most part, intrinsic CTL function appears to be intact; CD8⁺ T cells can elaborate normal

effector and memory functions despite their inability to transduce an IFN- γ signal.

Resistance of IFN- γ R1 (-/-) mice to viral infection

Both IFN- γ R1- and the IFN- α R-deficient mice proved to be valuable model systems in elucidating the specific roles of types I and II interferons (IFN- α/β and IFN- γ , respectively) in fighting viral infection (114, 115, 128, 150–153). *In vitro* studies have revealed that interferons must initiate signaling via their respective cell-surface receptors in order to protect cells against the cytopathic effects of viruses (26, 76). *In vivo* studies, on the other hand, demonstrated that while type I interferons are essential for protection against viral infection, the relative importance of IFN- γ is pathogen-dependent, suggesting that the antiviral actions of type II interferon are partially redundant, as they duplicate those of type I interferons (114, 115, 128, 150–153). IFN- γ R1-deficient mice were found to be resistant to some viruses (such as vesicular stomatitis virus, LCMV, and MMTV) while susceptible to others (such as murine γ -herpesvirus, vaccinia virus, Theiler's virus, murine cytomegalovirus, and coronavirus) (114, 115, 128, 150–153).

Even though IFN- γ R1-deficient mice were able to resolve some viral infections, they developed exacerbated disease in response to these pathogens as compared to control mice. For example, mutant mice could clear an infection with LCMV but exhibited delayed kinetics of viral clearance as compared to wild-type mice (151). LCMV-infected IFN- γ R1 (-/-) mice developed greater organ abnormality and had an increased frequency of latently infected cells relative to control mice. These studies suggest that the immune system elaborates a number of simultaneous and/or sequential, functionally complementary, antiviral responses. Some of these may be dependent on IFN- γ , although not all may be essential for a healing or normal response.

Intracellular bacterial infection in IFN- γ R1 (-/-) mice

Survival and clearance of infection with intracellular bacteria are dependent upon innate and cell-mediated immunity (154, 155). IFN- γ R1-deficient mice uniformly exhibit greater susceptibility to, or increased severity of bacterial infection relative to wild-type mice. Infection of mutant mice with *Chlamydia trachomatis* is characterized by a longer resolution time than in normal mice (156, 157). While mice normally contain and resolve infections with intracellular bacteria such as bacillus Calmette-Guérin (an attenuated form of *Mycobacterium bovis*), *Bordetella*

pertussis, and *Listeria monocytogenes*, these pathogens disseminate, often fatally, in mutant mice (82, 156, 157). As discussed earlier, IFN- γ is an important activating stimulus for macrophages, which are involved in granuloma formation, a mechanism essential for containing and eliminating intracellular bacteria. Activated macrophages also undergo a respiratory burst, producing reactive oxygen species and NO, both of which are important for their bactericidal function (2). Therefore, it is likely that an underlying macrophage defect is the cause of the exquisite sensitivity of IFN- γ R1 (-/-) mice to infection with intracellular bacteria (81).

Resistance of IFN- γ R1 (-/-) mice to protozoan and helminth infection

Control of parasitic infection is dependent upon the development of an appropriate immune response. In general, Th1 responses are associated with healing responses to intracellular parasites, such as *Leishmania major*, whereas Th2 responses are associated with protection from extracellular pathogens, such as *Nippostrongylus brasiliensis* (158, 159). The innate immune system is also important in protection against parasites. The role of IFN- γ in protection against parasitic infection and in the development of Th1- and Th2-type immune responses was explored with IFN- γ R1 (-/-) mice.

Protection against infection with *L. major* depends on normal macrophage and Th1 function (160, 161). Infection of IFN- γ R1 (-/-) mice with *L. major* or trypanosomes is associated with significantly greater mortality than that seen in control mice in the absence of any detectable parameters of Th2-type immunity (80, 83, 116). Interestingly, iNOS (inducible nitric oxide synthase)-deficient mice display a similar phenotype in response to *Trypanosoma cruzi* infection to the IFN- γ R1 (-/-) (80). These studies show that the IFN- γ system is critical for host defense against these intracellular pathogens, and indicate a defect in macrophage function as a key contributor to the observed phenotype. Studies of infection with *Toxoplasma gondii* suggest that the primary defect in antiprotozoan immunity in IFN- γ R1-deficient mice may not be due to a T-cell dysfunction (79). When infected with a low-virulence form of this protozoan, normal mice are able to control infection, but develop chronic toxoplasmosis. On the other hand, IFN- γ R1 (-/-) mice are unable to control infection, developing a necrotizing hepatitis, and ultimately succumbing to the pathogen. *T. gondii*-specific memory T cells derived from wild-type mice are unable to confer immunity

when adoptively transferred to mutant mice. Furthermore, hepatic macrophages in infected IFN- γ R1 (-/-) mice produced lower levels of TNF- α , iNOS, and IL-1- β (79). These observations suggest that the specific impaired response to *T. gondii* infection in IFN- γ R1 (-/-) mice may be secondary to alteration of macrophage function.

Infection of IFN- γ R1 (-/-) mice with *Plasmodium* species recapitulates some of the immune system dysfunction seen in other infections studies (129, 163). Relative to normal mice, the symptoms of infections with a number of *Plasmodium* species of mutant mice range from prolonged convalescence to increased incidence of death. The immune response in IFN- γ R1-deficient mice to some of these pathogens was Th2-biased relative to the anti-*Plasmodium* immunity seen in normal mice. This, again, suggests that in the absence of IFN- γ signaling, the elaboration of Th1 responses is impaired. In sum, mutant mice infected with parasites elaborate impaired or inappropriate immune responses that alter the clinical course of the disease, and at times lead to increased mortality as compared to normal mice.

Autoimmunity in IFN- γ R1 (-/-) diseases

Organ-specific autoimmune diseases are believed to be mediated and regulated by Th1 cells and IFN- γ (102, 113, 164). This paradigm, however, is not all-encompassing since the relationship between IFN- γ and organ-specific autoimmune disorder is complex. IFN- γ has been shown to have differential effects on disease progression based on the mode (local vs systemic) and timing (early or late in disease) of its administration or neutralization (113). To explore the IFN- γ dependence of organ-specific autoimmune diseases, the IFN- γ R1 (-/-) mice have been used in a number of model systems including CIA, nonobese diabetes (NOD), autoimmune lupus nephritis, experimental autoimmune thyroiditis (EAT), experimental autoimmune myasthenia gravis (EAMG), and experimental autoimmune encephalomyelitis (EAE) (165–168). In most cases, the development of organ-specific autoimmune diseases in IFN- γ R1-deficient mice follows this Th1/Th2 paradigm. For example, IFN- γ R1 (-/-) mice are less susceptible to these pathologic processes and exhibit reduced penetrance, delayed onset, or attenuated severity of disorder as compared to normal mice in diseases such as diabetes (both NOD and the BDC2.5 insulino-genic TCR transgenic system), EAMG, and EAT (167, 168). Additionally, administration of soluble IFN- γ R1 (which neutralizes IFN- γ) reduces the severity of NOD in wild-type mice (169). These studies demonstrate the importance of the

IFN- γ system in the development of these organ-specific autoimmune diseases.

CIA is induced in susceptible mouse strains, such as DBA/1, by immunization with type II collagen protein (170, 171). This autoimmune model has been used as a tool for studying the development and treatment of rheumatoid arthritis in man. The development and regulation of CIA in mice has been shown to rely on IL-12 and, less consistently, on IFN- γ (98, 172). Neutralization of IL-12 leads to attenuation of disease, suggesting that it is Th1-mediated, with cellular immunity and IgG2a-type antibodies causing the abnormality. Studies with IFN- γ -deficient and IFN- γ R1 (-/-) mice corroborated this observation; these mice were more resistant to CIA induction (166). However, in other studies of CIA, IFN- γ R (-/-) mice developed a more severe disease than did normal mice, suggesting that, in some cases, IFN- γ may have immunosuppressive functions (173).

IFN- γ also appears to attenuate the severity of disease in the MOG (myelin oligodendrocyte glycoprotein) peptide-induced EAE system (174). Wild-type 129/Sv mice are resistant to EAE, whereas IFN- γ R1 (-/-) are susceptible. This suggests that IFN- γ can not only promote inflammation, but also serve in an inflammation-limiting capacity in certain cases. In fact, mechanistically, immunosuppressive properties have previously been ascribed to IFN- γ secondary to its ability to induce NO production in macrophages, a production which has a downmodulatory effect on T-cell activation and proliferation (2, 108, 162). Furthermore, adoptive transfer of splenocytes from MOG-immunized IFN- γ R1-deficient mice can induce disease in naive wild-type recipients, suggesting the presence of functional memory or effector CD4⁺ T cells or, perhaps, a functional CD8-mediated component in this disease (174).

A number of studies utilized strains of mice that are predisposed to systemic autoimmunity, in order to examine the importance of IFN- γ in the development of autoantibodies and immune complex deposition-induced kidney disease. Mice on the MRL-*Fas*^{lpr} and the NZB X NZW genetic backgrounds lacking IFN- γ R1 expression develop attenuated disease relative to normal mice (175–177). IFN- γ R1 (-/-) mice in these autoimmune models produce fewer antibodies (with Th1- and Th2-dependent Ig isotypes equally affected) and, therefore, develop less severe immune complex-induced nephritis and show reduced incidence of death as compared to control mice. T-cell activation, T:B-cell interaction, or an intrinsic B-cell defect may underlie impaired antibody production in the absence of IFN- γ signaling. Like NOD, the disease phenotype in normal NZB X NZW mice can be attenuated by systemic admin-

istration of soluble IFN- γ R1 (178). Crescentic glomerulonephritis is experimentally induced by administration of anti-glomerular basement membrane (GBM) Abs. IFN- γ R1-deficient mice treated with α GBM Abs developed slightly less severe glomerular disease than wild-type mice, suggesting that antibody production in autoantibody-mediated immune disorder is primarily affected in the absence of an IFN- γ signal, rather than the events that ensue after immune complex deposition (179).

Disrupting IFN- γ signaling with a mutant IFN- γ R1

Tissue-specific transgenic mouse model of IFN- γ insensitivity

To distinguish among the effects of IFN- γ on the various cell types whose function is thought to be particularly affected by this cytokine, it is useful to disrupt IFN- γ signaling in only a subset of cells. Schreiber's group developed a transgenic system in which the expression of a dominant negative IFN- γ R1 is driven by a tissue-specific promoter, thereby conferring unresponsiveness to IFN- γ in one cell type, while leaving all others intact (180). The dominant negative receptor α chain (mgRAIC) lacks the intracellular domain of the wild-type protein; therefore, while it can participate in ligand binding and receptor complex formation, it is unable to transmit the IFN- γ signal (181).

In order to generate mice in which only macrophages are unable to respond to the IFN- γ signal, the human lysozyme promoter (hLP) was used to drive expression of the dominant-negative transgene (hLP-myc-mgRAIC) (180). The dominant negative α chain was indeed specifically expressed on macrophages, and inhibited IFN- γ responses in these cells. As such, macrophages derived from this mouse were unable to produce NO in response to stimuli such as IFN- γ and lipopolysaccharide (LPS) (180, 182). Macrophages derived from IFN- γ R1 (-/-) mice showed diminished responses to LPS as well (82). hLP-myc-mgRAIC transgenic mice were found to be more susceptible to infection with *L. monocytogenes* than littermate controls. Moreover, the authors have found that neither hLP-myc-mgRAIC TG macrophages nor IFN- γ R1 (-/-) macrophages were able to produce IL-12 after treatment with either IFN- γ or heat-killed *L. monocytogenes* (HKLM), whereas control macrophages responded robustly. Macrophages from these two mutant mouse strains were also unable to support HKLM-specific memory responses by wild-type Th1 cells *in vitro*. Therefore, it can be inferred that IFN- γ is pivotal in

activation and function of macrophages as well as in their ability to serve as APCs to CD4+ T cells.

For determination of whether the impaired T-cell function seen in IFN- γ R1 (-/-) mice was secondary to an intrinsic T-cell defect, transgenic mice (lck-myc-mgRAIC) with T-cell-specific expression of this mutant receptor were generated with the *lck* proximal promoter (180). *In vitro*, lck-myc-mgRAIC TG T cells were resistant to the antiproliferative effects of IFN- γ as compared to nontransgenic T cells. Interestingly, the transgene did not affect *in vitro* T helper cell differentiation under Th1-polarizing conditions (in the presence of IL-12), nonpolarizing conditions, or Th2-polarizing conditions (in the presence of IL-12-neutralizing Abs). In contrast, polarization of transgenic T cells toward the Th2 phenotype (driven by IL-4) may have been slightly enhanced, supporting the inhibitory role IFN- γ may play in this differentiative process. This evidence suggests that the *in vitro* generation of Th1 cells can occur independently of an IFN- γ signal in the presence of IL-12.

The IFN- γ R signal and antitumor immunity

IFN- γ has been shown to participate in antitumor immune responses (1, 2). However, the potential differential effects of this cytokine on the tumor, as opposed to the host, have not been explored. The dominant negative transgenic system described above was utilized to evaluate the role of IFN- γ signaling by cancer cells in antitumor immune responses in a normal host. The dominant-negative IFN- γ R1 mutant was introduced into the Meth A fibrosarcoma cell line, rendering these cells unresponsive to IFN- γ (183). Modified cells were resistant to LPS-induced rejection in a normal syngeneic host and exhibited a greater tumorigenic potential than the IFN- γ -responsive parental cell line. Furthermore, IFN- γ -insensitive cells were ineffective in priming their host to reject subsequent tumor grafts, and did not elicit antitumor immunity when grafted into a host previously sensitized by the parental cell line. One interpretation of these observations is that IFN- γ -resistant tumor cells are not inherently more aggressive in lesion formation per se, but are less immunogenic than their IFN- γ -sensitive counterparts. In a more recent study, Lee's group modified two transformed murine cell lines (SKC and K1735) to be unresponsive to IFN- γ , utilizing a similar dominant-negative IFN- γ R1 construct, and subsequently tested their ability to form tumors in mouse hosts (184). IFN- γ -resistant cell lines were more tumorigenic than IFN- γ -responsive parental lines, corroborating previous studies. IFN- γ augments antigen presentation through both the MHC class I and class II pathways, thereby

increasing the immunogenicity of tumor cells and enhancing their detection and killing by immune surveillance and effector mechanisms (131, 185–187). It is also believed that IFN- γ alters the types of peptides that are presented through the MHC class I pathway by inducing expression of alternate LMP and TAP molecules. Therefore, in the absence of IFN- γ signaling, tumor cells may be unable to present peptides that normally activate antitumor immunity, thereby evading detection by surveillance mechanisms.

The effects of disruption of IFN- γ R2 expression in mice

To study the importance of the β chain of the IFN- γ R for mediating the biologic functions of IFN- γ , our laboratory generated mice carrying a targeted mutation in the IFN- γ R2 locus, abrogating its expression (27). These mice developed normally in a pathogen-free facility, with a normal composition of lymphocyte populations in lymphoid organs. IFN- γ signaling, however, was abrogated at all steps of the cascade. Cells derived from IFN- γ R2(-/-) mice were unable to activate JAK1, JAK2, and STAT1 (all expressed at normal levels) or to express IFN- γ -inducible genes in response to IFN- γ treatment. When cultured on anti-CD3 antibody-coated plates, naive CD4+ T cells isolated from spleens of IFN- γ R2 (-/-) were impaired in their ability to differentiate toward the Th1 subset under Th1-polarizing conditions (in the presence of either IFN- γ or IL-12), but exhibited normal Th2 differentiation (Fig. 2). Interestingly, no default toward the Th2 phenotype was observed when these cells were cultured under neutral conditions.

Consistent with these *in vitro* findings, IFN- γ R2 (-/-) mice have profound defects in Th1-mediated immunity, such as Th1 memory responses to rechallenge with protein antigen. The ability of IFN- γ R2 (-/-) B cells to undergo immunoglobulin heavy-chain class switching to IFN- γ -dependent isotypes, such as IgG2a, was also impaired. Additionally, the inhibition of class switching to IL-4-dependent isotypes, such as IgG1 and IgE, normally observed when B cells are cultured in the presence of both IL-4 and IFN- γ , was not seen under these conditions. As in IFN- γ R1 (-/-) mice, impaired macrophage function may be central to the immune disorder in IFN- γ R2 (-/-) mice. β chain-deficient mice are highly susceptible to infection with *L. monocytogenes*, a pathogen that primarily infects macrophages and requires their proper function for a healing response. Additionally, contact hypersensitivity responses were defective in these mice.

These studies not only establish the obligatory role of IFN- γ R2 in transducing the IFN- γ signal and thus its biologic function, but also demonstrate that IFN- γ is critical for the development of functional Th1 cells and Th1-dependent immunity. Though Th1 cells are normally unresponsive to IFN- γ (see below), abrogation of their ability to regulate responsiveness to this cytokine during their development may impair their generation and function. Alternatively, the T-cell defect seen in IFN- γ R2 (-/-) mice may be, in part, secondary to impaired APC function, which may inefficiently activate or polarize CD4+ T cells. Furthermore, IL-12 did not seem to have a Th1-polarizing effect, suggesting that, in this system, this function of IL-12 requires IFN- γ .

In general, mice deficient in either α or β chains of the IFN- γ R had similar defects in their immune systems. Interestingly, while *in vitro* Th1-polarized CD4+ T cells expressing the dominant-negative IFN- γ R1 transgene were able to produce IFN- γ as well as normal CD4+ cells, helper T cells derived from IFN- γ R2-deficient mice could not. The discrepancy between these studies may be due to differences in the type of stimuli used; mgRΔIC TG T cells were stimulated with APC+ peptide whereas IFN- γ R2 (-/-) T cells were stimulated with α CD3. This is consistent with previous studies that have shown that the quality of the activating stimulus can affect T helper subset phenotype acquisition (188). It is also possible that the reason underlying the difference between the *in vitro* differentiation potential of T cells from these two lines of mutant mice is that the dominant-negative IFN- γ R1 construct does not completely abolish the IFN- γ signal in T cells but, instead, attenuates it considerably.

Obligate IFN- γ responsiveness imparted by IFN- γ R2 transgene

Several groups have previously shown that, whereas Th2 cells are responsive to IFN- γ , Th1 cells are unable to activate IFN- γ signaling in response to this cytokine (64–66, 189, 190). These data corroborate earlier studies demonstrating differential growth arrest of Th1 and Th2 clones upon IFN- γ treatment, with only the latter helper T-cell subset susceptible to the antiproliferative effects of this cytokine (191). Further analysis of the components of the IFN- γ response pathway in Th cells revealed that, unlike Th2 cells, Th1 cells do not express mRNA encoding IFN- γ R2 (65) (Fig. 2). Transfecting an IFN- γ R2 construct into Th1 cells rescues their IFN- γ -unresponsive phenotype (65). These data

suggest that during T helper subset differentiation, responsiveness to IFN- γ is regulated by expression of IFN- γ R2.

Analysis of the immune functions of IFN- γ -insensitive mice suggests that IFN- γ promotes Th1 cell development and function. Therefore, Th1 cells must be able to respond to this cytokine during their development, phenotype acquisition, and/or other points during their growth cycle. However, during their differentiation, Th1 cells stop expressing IFN- γ R2 and become unresponsive to IFN- γ . We hypothesized that downmodulation of IFN- γ R2 expression, and therefore the loss of responsiveness to IFN- γ , is a significant event in the process of Th1 phenotype acquisition and may, in fact, be important for normal Th1 cell function. To test this hypothesis, we engineered mice in which expression of IFN- γ R2 cDNA is driven by the human CD2 locus control region (LCR); thus, all CD2+ cells (TNK, and some B cells) would constitutively express this receptor (192). The β chain transgene rescued the IFN- γ -insensitive phenotype of Th1 cells, as Th1 cells cloned from transgenic (TG) mice were responsive to IFN- γ , whereas wild-type Th1 cells were not.

Like IFN- γ R-deficient mice, IFN- γ R2 TG mice exhibited no gross developmental abnormalities and had normal lymphocyte numbers and composition in lymphoid organs; however, when challenged, IFN- γ R2 TG mice were found to have specific immunologic defects. IFN- γ R2 TG mice were impaired in their ability to generate Th1 cells *in vitro* (Fig. 2). These mice were also unable to elaborate Th1-mediated memory responses such as DTH (delayed-type hypersensitivity) *in vivo*, and responses to protein and bacterial antigens (such as keyhole limpet hemocyanin and heat-killed *L. monocytogenes*, respectively) *in vitro* (192, 193).

Unlike T-cell function, antigen presentation in IFN- γ R2 TG appears to be normal. *In vitro*, TG APCs are able to support normal T-cell activation, whereas normal APCs were unable to remedy the T-cell defect in TG T cells (192, 193). Survival of primary listeriosis is dependent on innate immunity including leukocytes such as neutrophils, NK cells, and especially macrophages (154, 155). Naive IFN- γ R2 TG mice withstood challenge with *L. monocytogenes*, indicating that macrophage function is intact in these mice (193). It is possible that the IFN- γ necessary for normal macrophage activation is secreted by NK cells in these mice. While host defense against *L. monocytogenes* depends primarily on the innate immune system, survival of infection with *Leishmania major* also requires normal Th1 function (160). TG mice are susceptible to infection with *L. major*, and develop nonhealing lesions, suggesting,

once again, that Th1 responses in these mice are impaired (192).

CNS pathology in EAE (a model of autoimmune demyelinating disease) is believed to be mediated by Th1 cells (164). We examined the effect of forced responsiveness to IFN- γ in Th1 cells on the induction of this organ-specific autoimmune disease (193). While control mice developed severe and often fatal paralysis, IFN- γ R2 TG mice were generally resistant to EAE induction. The ability to modulate IFN- γ R2 expression in Th cells is therefore required for a broad range of Th1 effector functions including autoimmunity.

Like helper T cells, CD8⁺ T cells can be grouped into an IFN- γ -producing subset (Tc1) and an IL-4-producing subset (Tc2) (194, 195). We have recently examined patterns of IFN- γ responsiveness in Tc subsets and found that, like Th1 cells, Tc1 cells lack IFN- γ R2 and are therefore unable to activate STAT1 or upregulate cell-surface expression of MHC class I molecules in response to IFN- γ (193). Tc1 clones generated from IFN- γ R2 TG mice are responsive to IFN- γ , whereas those generated from littermate controls do not respond to IFN- γ . TG Tc1 clones have profound defects in their cytotoxicity (measured by their ability to lyse target cells) as compared to wild-type CTLs. TG Tc1 clones, however, do not show impaired activation, proliferation, or IFN- γ secretion, indicating that the cytolytic effector functions of these cells are not necessarily dependent on IFN- γ . It is possible that other effector mechanisms of Tc1 cells, such as FasL expression or perforin production, may be impaired. Contact hypersensitivity responses to haptens in IFN- γ R2 TG mice are markedly diminished (192). As discussed earlier, CHS is dependent on normal APC and CD8⁺ T-cell function. Since antigen presentation appears normal in these mice, the defect in this *in vivo* model of CTL function may lie in the CD8⁺ T-cell compartment. Interestingly, earlier studies demonstrated that although exogenous IFN- γ can augment CTL activity and proliferation *in vitro*, this cytokine is not required for their effector activity (196, 197).

Taken together, these studies demonstrate that IFN- γ R2 is obligatory in transducing the IFN- γ signal and in mediating its immunologic function. Furthermore, forced responsiveness to IFN- γ results in severe Th1 and CTL dysfunction that is intrinsic to these cells and is unlikely to be related to impaired antigen presentation. Therefore, modulation of responsiveness to IFN- γ (by regulating expression of the IFN- γ R2 gene) during Th1 and Tc1 differentiation, and probably in the mature Th1 and Tc1 states, is essential for the generation of these cells and their proper function.

Naturally occurring IFN- γ R mutations in man

Man is normally resistant to nontuberculous mycobacteria (NTM) and BCG. Nevertheless, cases of disseminated atypical mycobacterial infection in children have been reported (198–200). The underlying causes in most of these cases were found to be classical immunodeficiencies such as severe combined immunodeficiency (SCID) or chronic granulomatous disease. Genetic mapping and Mendelian analysis of idiopathic cases have revealed mutations in either IFN- γ R1 or IFN- γ R2 that are transmitted in an autosomal recessive pattern. Interestingly, children unresponsive to IFN- γ were found to be resistant to most common viral and microbial infections or fungi, and presented solely with disseminated mycobacterial infection (*Mycobacterium fortuitum*, *M. avium*, *M. bovis*, BCG, *M. chelonae*, *M. smegmatis*, and *M. tuberculosis*), *Salmonella enteritidis*, or *L. monocytogenes* (201–207). A genetically heterogeneous array of alterations (missense, nonsense, insertions, deletions, and splice mutations) in the IFN- γ R1 locus were identified. In contrast, only one case in which the mutation lay in the IFN- γ R2 locus has been reported (202).

These cases of idiopathic, disseminated mycobacterial infection can be grouped into two phenotypic categories by their clinical presentation and outcome: complete and partial IFN- γ insensitivity. Children with complete IFN- γ insensitivity respond poorly to antimycobacterial treatment, either succumbing to the pathogen or developing chronic infection. These patients form poorly differentiated and poorly circumscribed granulomas and are therefore unable to control effectively their mycobacterial infection (204). Mutations underlying complete IFN- γ insensitivity result in a truncated IFN- γ R1 or IFN- γ R2 peptide that is not expressed on the cell surface.

In contrast, the two siblings with partial IFN- γ insensitivity were found to have a missense mutation in the IFN- γ R1 extracellular domain (I187T) (207). Although it is expressed, this IFN- γ R1 mutant binds IFN- γ R with very low affinity, and cells carrying this mutation respond only to very high doses of IFN- γ . *In vitro* analysis of T cells isolated from these kindred and their parents show diminished parameters of antigenic recall such as T-cell proliferative responses and IFN- γ secretion. Granulomas in mycobacteria-infected partially IFN- γ -insensitive individuals are well defined with fully differentiated, multinucleated epithelioid cells, and are circumscribed by lymphocytes. These patients respond to antimycobacterial

treatment and can survive pathogen-free without prophylaxis.

Cases of idiopathic mycobacterial infection arising from mutations in either of the IFN- γ R chains are helpful for defining the subset of nonredundant immunologic function of IFN- γ essential for survival in a natural environment. All such kindred presented with cases of mycobacterial infection either secondary to immunization with BCG or naturally acquired, indicating that IFN- γ plays a critical, nonredundant role in mediating antimycobacterial immunity (208). However, the absence of consistent presentation with other infections does not preclude the participation of IFN- γ in immunity to other pathogens, but does suggest that IFN- γ is redundant in other immune mechanisms. These findings suggest that responsiveness to IFN- γ , and therefore IFN- γ R, is required for host defense against these intracellular pathogens whose clearance is mediated by macrophages. Impaired antigenic recall in these individuals suggests that IFN- γ R is important in the development of Th1-mediated immunologic memory, although this defect may be, in part, caused by APC dysfunction rather than fully by T-cell-intrinsic mechanisms (209).

Commentary

The study of mice and human subjects with altered IFN- γ R expression pattern has told us a great deal about the diverse roles IFN- γ plays in inducing and modulating immune responses. First and foremost, both the α and β chains of IFN- γ R are obligatory in initiating the known IFN- γ signaling pathway through JAK1, JAK2, and STAT1 and in mediating the cellular effects of this cytokine. IFN- γ responsiveness has been shown to be essential to, or at least to participate in, host defense against a wide array of pathogens including

viruses, bacteria, and protozoa. Much of the phenotype in IFN- γ R-deficient animals can be attributed to a defect in macrophage function. IFN- γ responsiveness is critical for macrophage activation, function, and antigen presentation. These cells play a pivotal role in both innate and acquired immune responses. They must be activated for clearance of intracellular pathogens such as *Listeria* and mycobacteria and the development of their phagocytic functions. They are also important in further activating NK cells by secreting IL-12. Neutrophils are also activated by IFN- γ (182). Therefore, in infections such as listeriosis, where the first leukocyte line of defense is mediated by neutrophils, impaired function of these cells undoubtedly enhances the susceptibility of IFN- γ -unresponsive mice to such infections.

Unlike the IFN- γ R (-/-) mice, in which a macrophage defect supersedes the T-cell defect, T-cell function is clearly impaired in IFN- γ R2 TG mice. These animals have allowed us to explore the functional role of IFN- γ -responsiveness and the ability of T cells to modulate it. We have demonstrated that Th1 and Tc1 cells that are constitutively responsive to IFN- γ are unable to elaborate their respective effector functions. It is possible that constitutive signaling through the IFN- γ R (such as in TG Th1 and Tc1 cells which are continuously bathed in an IFN- γ -rich milieu) activates inhibitory pathways, or inactivates an effector mechanism in these cells, thereby causing the observed defect. Therefore, not only must cells be responsive to IFN- γ for normal innate, acquired, and antitumor immunity, but IFN- γ -secreting T cells also must be able to regulate their ability to respond to IFN- γ for their normal function. It appears that for IFN- γ , as for life in general, timing is everything.

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