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THE MOLECULAR CELL BIOLOGY OF INTERFERON-γ AND ITS RECEPTOR

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Abstract

The last ten years have seen an explosive growth in our understanding of IFNγ. The cloning of the cDNAs for IFNγ and its receptor have made available large amounts of highly purified recombinant IFNγ and soluble IFNγ receptor. In addition, highly specific neutralizing monoclonal antibodies have been generated to both of these proteins. Using these reagents, IFNγ and the IFNγ receptor have been characterized on a molecular basis. Structure-function studies carried out on these proteins have identified key molecular regions that are required for biologic activity. Moreover, a great deal is now known concerning the physiologic role that IFNγ plays in vivo. In this review we focus on the new developments in the areas of IFNγ biochemistry and biology and pay particular attention to the IFNγ receptor and three aspects of IFNγ biology that are of special interest to immunologists: host defense, inflammation, and autoimmunity.

INTRODUCTION

Interferon-gamma (IFNγ) was first recognized nearly 30 years ago on the basis of its antiviral activity (1). During the ensuing years, a great deal of information has accumulated which unequivocally establishes that this pleotropic cytokine plays an important role in modulating nearly all phases of immune and inflammatory responses. During the past 10 years, the genes for IFNγ and its receptor have been cloned, and the structures of

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the corresponding proteins determined. Large amounts of highly purified recombinant IFNγ and IFNγ receptor are now available as well as neutralizing monoclonal antibodies specific for either the cytokine or its receptor. The availability of these reagents has facilitated many studies aimed at elucidating IFNγ's mechanism of action at the molecular level and defining its physiologic role in vivo. Ironically, the information derived from 30 years of IFNγ research now points to the relatively minor physiologic importance that IFNγ has as a direct antiviral agent. In the current review we focus on some of the more recent developments in the areas of IFNγ biochemistry and biology, and we place particular emphasis on work concerning the IFNγ receptor.

THE RELATIONSHIP BETWEEN IFNγ AND OTHER INTERFERONS

IFNγ belongs to a family of proteins related by their ability to protect cells from viral infection. Based on several criteria, the interferons have been divided into three distinct classes termed interferon-α, -β, and -γ (Table 1). IFNα (originally known as Type I IFN or Leukocyte IFN because it was produced by peripheral blood mononuclear cells) and IFNβ (also originally known as Type I IFN or Fibroblast IFN because of its cell of origin) are classical interferons induced in response to viral infection of cells (2, 3). Twenty six IFNα genes (including several pseudogenes) have been identified (4). The genes have common structures, i.e. they lack introns and appear to derive from a common ancestral precursor (5). The IFNα gene cluster resides on human chromosome 9 and murine chromosome 4 (6). This gene family encodes at least 22 distinct proteins which are single polypeptide chains of 165–166 amino acids with molecular weights of approximately 20 kDa (7). The reasons for the complexity of the IFNα gene system remain unclear, but recent studies suggest that the different IFNα species effect distinct arrays of biological responses in different cells (7). There is only a single form of IFNβ. It is encoded by a distinct gene located next to the IFNα locus in both human and mouse. Based on the general organization of their genes and proteins, IFNα and IFNβ are thought to have evolved from a common ancestral precursor. However, IFNβ shares only limited antigenic relatedness to the IFNα family, and the proteins display only 15–30% amino acid sequence homology (3). Nevertheless, both forms of Type I IFN bind to the same receptor on the surface of susceptible target cells, indicating that at least some of the functionally important regions of the molecules have been conserved. Although an earlier report suggested the existence of a second IFNβ species, designated IFNβ2 (8), subsequent studies revealed that the

Table 1 Properties of the interferons

Property	IFNα	IFNβ	IFNγ
NOMENCLATURE	Type I Leukocyte	Type I Fibroblast	Type II Immune
MAJOR INDUCERS	Virus	Virus, LPS ds-poly RNA	Antigens Mitogens
PHYSICAL PROPERTIES			
M.W. (kDa)			
Predicted/mature	20/20	20/20–25	17/34–50
Amino acids	165–166	166	143
N-linked glycosylation	Some species	+	2 sites
Subunit composition	Single polypeptide	Single polypeptide	Noncovalent homodimer
pH stability	Stable	Stable	Labile
GENE STRUCTURE			
Number of genes	26	1	1
Chromosomal location			
Murine	4	4	10
Human	9	9	12
Presence of introns	None	None	3
CELLULAR SOURCE	T cells, B cells and macrophages	Fibroblasts and epithelial cells	T cells and NK cells
CELLULAR RECEPTOR			
M.W. (kDa)			
predicted/mature	60.5/95–100		52.6/85–95
Amino acids	530		472
Domain structure			
Extracellular	409 a.a.		228 a.a.
Transmembrane	21 a.a.		23 a.a.
Intracellular	100 a.a.		221 a.a.
Glycosylation sites	12		5
Chromosomal location			
Murine	16	16	10
Human	21	21	6

observed activity was ascribable to IL–6 which elicited antiviral responses in certain in vitro assays in an indirect manner (9, 10)

IFNγ (also termed Type II IFN or Immune IFN) is unrelated to the Type I interferons at both the genetic and the protein levels (11–13). Moreover, IFNγ is induced by a unique set of stimuli and is produced only by T lymphocytes and natural killer (NK) cells. Importantly, viral infection of these cells does not directly induce IFNγ production. Although IFNγ displays most of the biologic activities that have been ascribed to the other interferons, it has a 10–100 fold lower specific antiviral activity than either

IFNα or IFNβ. On the other hand, IFNγ is 100–10,000 times more active as an immunomodulator than are other classes of interferons (14). This observation has lead to the concept that whereas IFNα/β are primarily antiviral agents which display some immunomodulatory activity, IFNγ is primarily an immunomodulator that also can exert some antiviral activity (15).

MOLECULAR CHARACTERISTICS OF HUMAN AND MURINE IFNγ

The cDNAs for human and murine IFNγ were first cloned in 1982 by Gray & Goeddel (11–13), and today a great deal is known about the structure of the IFNγ genes and the corresponding proteins they encode. Both humans and mice contain only a single IFNγ gene. The IFNγ gene is considerably more complex than the genes for either IFNα or IFNβ. The human and murine genes are 6 kb in size, and each contains four exons and three introns. Using in situ hybridization techniques, the genes for human and murine IFNγ have been localized to human chromosome 12 (12q24.1) and murine chromosome 10, respectively (6, 16, 17). The regulatory elements in the IFNγ gene have been only partially characterized. A tissue-nonspecific enhancer element has been detected in a 220 bp sequence within the first intron of the human gene (18). Positive and negative *cis*-acting promoter elements have been identified in a region 700 bp immediately 5′ to the transcription start site (18, 19). The sequences in the promoter responsible for tissue-specific gene expression remain unknown. Interestingly, when DNA containing the entire human IFNγ gene and 2.3 and 1.0 kb of 5′ and 3′ flanking genomic sequence, respectively, was introduced into the murine germ line, the resulting transgenic mice displayed inducible expression of human IFNγ in a tissue appropriate manner (20). In contrast, when the human gene was introduced into a variety of different cultured murine cell lines (such as T cells and fibroblasts), the tissue specificity of expression was lost (21, 22). Thus, it has been suggested that regulation of IFNγ gene expression must also depend on as yet undefined developmental factors.

Activation of the human gene leads to the generation of a 1.2 kb mRNA that encodes a 166 amino acid polypeptide (11, 23). The amino terminal 23 residues of the human protein constitute a typical hydrophobic signal sequence which, when proteolytically removed, gives rise to a mature 143 residue positively charged polypeptide with a predicted molecular mass of 17 kDa (Figure 1). Although the amino terminus of the mature polypeptide was originally predicted to be Cys-Tyr-Cys (11), amino terminal sequence analysis performed on natural forms of human IFNγ demonstrated that

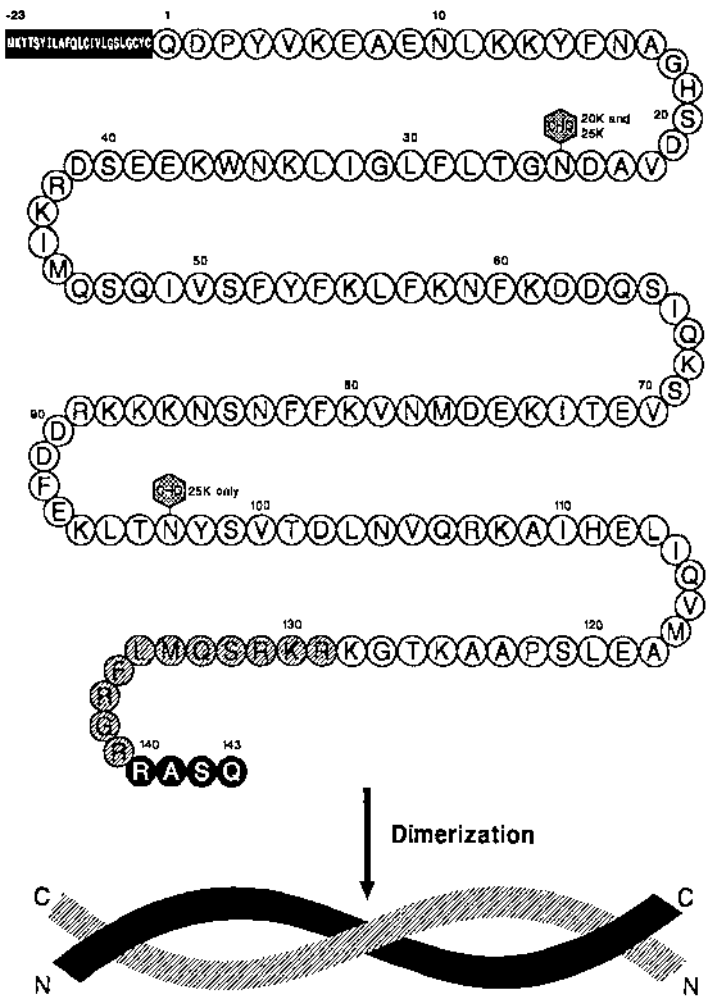


Figure 1 Schematic representation of the human IFNγ molecule. IFNγ is synthesized as a 166 amino acid polypeptide that contains a 23 amino acid signal sequence (designated in the shaded box). The mature protein contains 143 amino acids. The location of the two N-linked glycosylation sites is noted. A functionally important region of the molecule has been mapped to the amino terminal 39 residues. In addition, a second important region has been localized to the carboxy terminal 15 residues. Two IFNγ polypeptides self-associate to form a noncovalent homodimer.

the amino terminus was actually pyroglutamic acid (Figure 1) (24). The carboxy terminus of the molecule is susceptible to post-translational enzymatic degradation (24). At least six different carboxy termini have been detected on natural and recombinant forms of human IFNγ. Since this portion of the molecule contains a large number of positively charged residues, the various truncations contribute to the charge heterogeneity seen in the fully mature molecule. Two polypeptides self-associate to form a homodimer with an apparent molecular weight of 34 kDa (25–30). At physiologic concentrations, little if any monomer is detectable. Only the dimer can display IFNγ biologic activity, possibly because it is the only form of the molecule that can effect IFNγ receptor dimerization (31, 31a, 32). Since the mature human IFNγ polypeptide is devoid of cysteine, the homodimer is held together entirely by noncovalent forces. The quaternary structure of the molecule explains its characteristic sensitivity to extremes of heat (the protein is denatured at temperatures above 56°C), and pH (activity is rapidly lost at pH values less than 4.0 and greater than 9.0) (33–37)

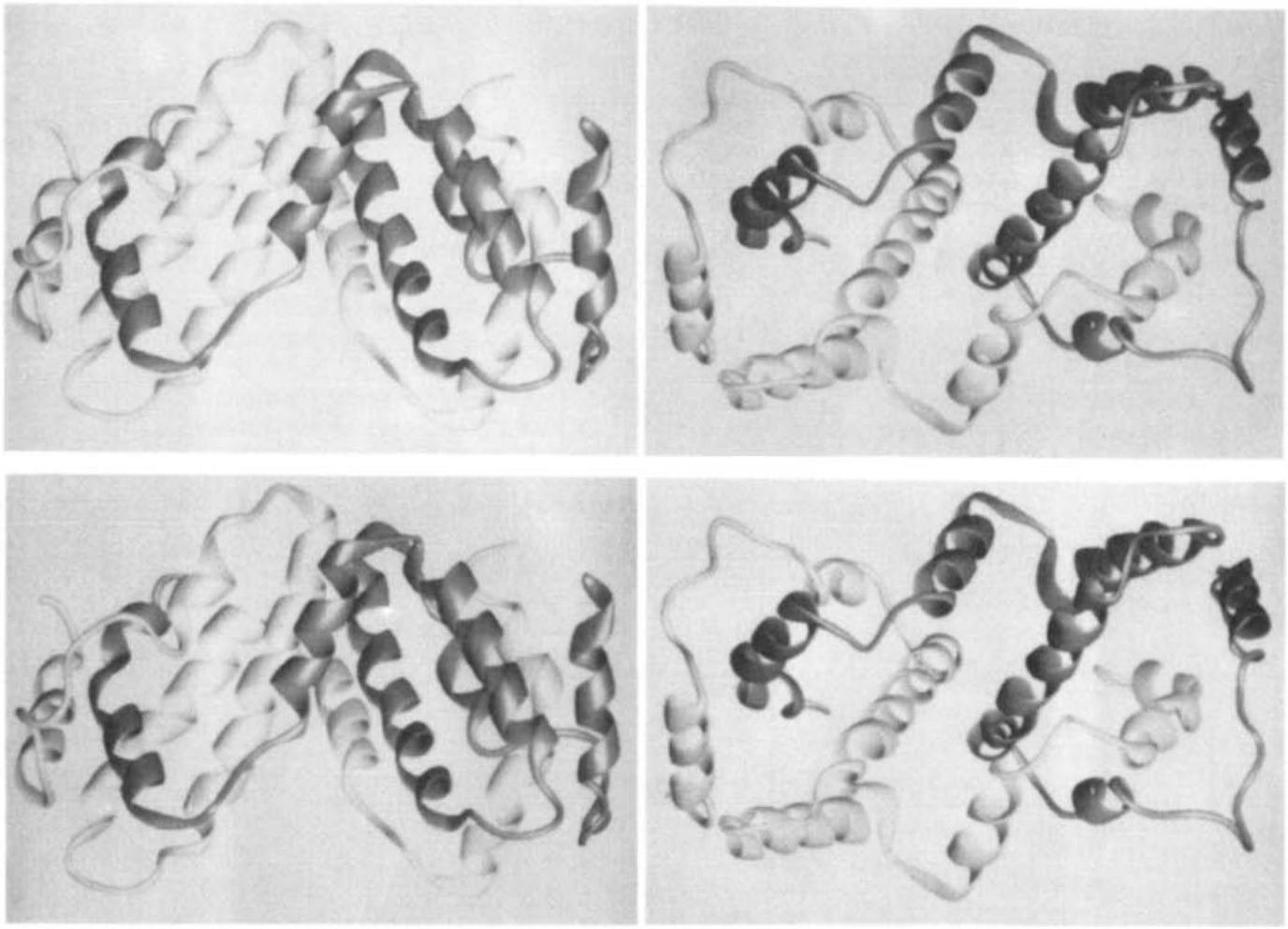
The murine gene gives rise to a 1.2 kb mRNA that encodes a mature 134 amino acid polypeptide with a predicted molecular mass of 15.4 kDa (13). Like its human counterpart, murine IFNγ exists exclusively as a noncovalent homodimer. Human and murine IFNγ display only modest homology at either the cDNA or amino acid levels (60% and 40%, respectively). This low level of sequence homology explains why the human and murine proteins display a strict species specificity in their ability to bind to and activate human and murine cells.

The individual human and murine polypeptide chains contain two N-linked glycosylation sites (residues 25 and 97 in mature human IFNγ and residues 16 and 69 in the mature form of murine IFNγ) that are independently and differentially glycosylated, thereby giving rise to subunits of differing molecular weights. Natural human IFNγ is composed of polypeptides that display molecular masses of 17, 20, and 25 kDa, which correspond to molecules with 0, 1, or both glycosylation sites occupied (38, 39). This differential glycosylation accounts for much of the observed molecular weight heterogeneity in the fully mature homodimeric molecules (i.e. natural human and murine IFNγ display molecular weights that range from 30–50 kDa). Whereas glycosylation is not important for expression of IFNγ activity, it appears to influence the circulatory half-life of the molecule (38, 40, 41).

The functionally important regions of the IFNγ molecule are now being elucidated. Current data indicate that both the amino and carboxy terminal regions play critical roles in maintaining an active conformation of the protein. The importance of the amino terminal region has been docu-

mented by several independent studies. Monoclonal antibodies specific for amino terminal portions of human and murine IFNγ block binding of IFNγ to cellular receptors and neutralize IFNγ's ability to induce antiviral activity in fibroblasts and activate nonspecific cytocidal activity in macrophages (42–46). In addition, a synthetic peptide with a sequence that corresponds to the amino terminal 39 amino acids of human IFNγ has been shown to block binding of IFNγ to cell surfaces when supplied in high concentration (44, 45). Finally, human and murine IFNγ molecules lacking the first 10 amino terminal residues (produced either by enzymatic digestion of the molecule with *Staphylococcus aureus* V–8 protease—47, 48—or using molecular genetic approaches—49, 49a) are devoid of biologic activity. Other studies have provided evidence for an important role of the carboxy terminal portion of the molecule. Although removal of the carboxy terminal 9 amino acids from human IFNγ results in little or no reduction in biologic activity, enzymatic removal of residues 129–143 of the protein with various endoproteases (such as clostripain or submaxillaris protease—50, trypsin or pronase—28, 50), results in a 10–100 fold reduction in IFNγ's specific activity (as determined by monitoring IFNγ-dependent induction of antiviral activity in fibroblasts and Fc receptor expression on mononuclear phagocytes). In addition, the enzymatically truncated form of IFNγ displays a 1000-fold reduction in receptor binding affinity (50). A truncated form of human IFNγ, generated by placing a stop codon after residue 125, also showed a similar reduction in the capacity to bind to IFNγ receptors and induce IFNγ-dependent cellular responses (51, 52). Finally, certain monoclonal antibodies reactive with carboxy terminal peptides of human (53) and murine (54) IFNγ neutralize the protein's antiviral activity.

Recently, the x-ray crystallographic structure of human IFNγ was solved to 3.5 Å (55) (Figure 2). This study confirmed the dimeric nature of the mature protein. The individual subunits have a flattened prolate elliptical shape. However, the overall structure of the dimer is compact and globular. The molecule appears to be primarily helical (62%) and lacks β sheet structure. Each subunit consists of six α helices held together by short nonhelical regions. The dimer is formed by a unique intertwining of the helices across the subunit face which provides an opportunity for multiple interactions along each subunit. This type of intimate subunit interaction is extremely unusual and has been seen in only a few other proteins. The model predicts that the subunits associate in an antiparallel fashion, thereby leading to a juxtaposition of the amino and carboxy terminal portions of the opposing polypeptide chains. Whereas the amino termini coordinates have been firmly established, the carboxy termini do not adopt a rigid conformation in solution, as determined using either x-



ray crystallographic or nuclear magnetic resonance approaches (56, 56a). Nevertheless, the model suggests that each IFNγ dimer may be able to bind two IFNγ receptors. Experimental data recently obtained support this possibility (31, 31a, 32)

IFNγ BIOSYNTHESIS

In the normal host, the T lymphocyte represents the major cellular source of IFNγ. All $CD8^+$ T cell populations and certain subsets of $CD4^+$ T cells can produce the protein (57, 58) (Figure 3). IFNγ synthesis has been demonstrated in the T_H1 helper T cell subset, and data has also been obtained indicating that it may be produced by a less differentiated/activated type of $CD4^+$ T cell designated T_H0 (59–61). The external stimuli that induce IFNγ production by T cells are similar to those that induce other T cell–derived cytokines (57, 58, 62). The primary physiologic stimulus is antigen in the context of either major histocompatibility (MHC) class II (for $CD4^+$ T cells) or MHC class I antigens (for $CD8^+$ T cells). Experimentally, IFNγ can also be induced by either (1) direct stimulation of the T cell receptor/CD3 complex with antibodies such as anti-CD3, (2) T cell mitogens (such as concanavalin A or phytohemagglutinin) or (3) pharmacologic stimuli (such as the combination of phorbol myristate acetate and calcium ionophore) (63). In addition, T cell–dependent production of IFNγ is enhanced by products of activated T cells and macrophages such as IL–2, hydrogen peroxide, and leukotrienes LTB4, LTC4, and LTD4 (64–69). Stimulation of T cells results in the induction of IFNγ mRNA which is first detectable at 6–8 hours, peaks by 12–24 hours, and slowly declines thereafter. The protein is secreted immediately after synthesis. It can first be detected in the extracellular environment 8–12 hours after stimulation and reaches peak levels after 18–24 hours. IFNγ produced as a result of experimental in vitro T cell stimulation (such as during the mixed leukocyte reaction) is not significantly consumed by the cells of the culture and therefore can be detected in the medium long after the T cell activation response has ended (70). In contrast, IFNγ is rarely seen in the circulation of humans or mice undergoing immunologic stimulation. This apparent discrepancy between in vitro and in vivo levels of

Figure 2 Stereo views of the human IFNγ homodimer as derived from coordinates obtained from the x-ray crystallographic analysis of the protein. The crystal structure has been resolved to 3.5 angstroms. The ribbon drawings are based on the Cα positions. Panel A: the view is approximately parallel to the dimer two-fold axis. Panel B: The view is approximately perpendicular to the dimer two-fold axis. The precise position of the carboxy terminus has not been established. (Reprinted with permission from Ealick et al (55).)

fluid phase IFNγ is most likely due to the rapid removal of IFNγ from the circulation by IFNγ receptors that are ubiquitously expressed on nearly all cells (71)

Two newly described cytokines are noteworthy in their respective abilities to regulate IFNγ production in either a positive or negative manner. IL–12 (formerly called NK stimulatory factor, NKSF) is a product of B cells and macrophages and induces IFNγ gene expression in T cells and NK cells in a manner that is at least partially distinct from the conventional pathway of T cell activation (72, 73). IL–12 dependent IFNγ induction is insensitive to cyclosporin A and is synergistic with phytohemagglutinin, phorbol esters, anti-CD3, IL–2, and allogeneic antigens but not Ca^{++} ionophores (74). The precise stimuli that lead to IL–12 induction in vivo are not yet characterized. On the other hand, another newly described cytokine, IL–10, *inhibits* IFNγ production by T cells (75, 76). The inhibitory effects of IL–10 on IFNγ production are more profound than effects on the production of other T_H1 cytokines such as IL–2. IL–10 is produced by the T_H2 $CD4^+$ T cell subset, as well as B cells and macrophages (77). The mechanism by which IL–10 exerts its inhibitory effects on IFNγ

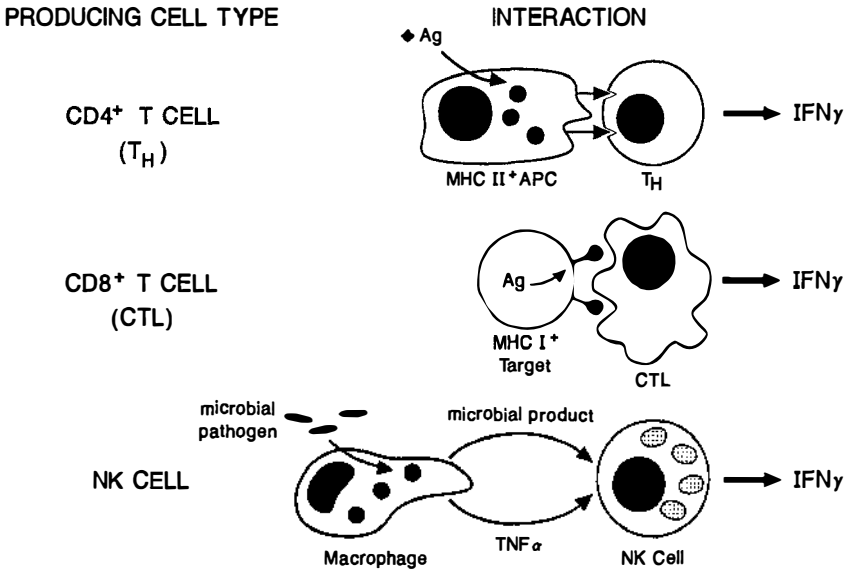


Figure 3 Cellular sources of IFNγ. IFNγ can be produced either by $CD4^+$ T cells in response to antigen presented in the context of MHC class II molecules or by cytotoxic T lymphocytes following recognition of antigen associated with MHC class I. In addition, NK cells elaborate IFNγ after exposure to TNFα and microbial products.

production is only now being elucidated. However, the target of IL–10 action clearly is the antigen presenting cell and not the T cell (78). The current hypothesis is that IL–10 may inhibit the expression of APC-derived accessory molecules required to induce full activation of the T cell for IFNγ production.

More recent studies have demonstrated that IFNγ can also be produced by natural killer cells (NK) (79–83). Mitogens can induce IFNγ from populations of human and murine NK cells expanded in vitro with IL–2. However, of far greater importance is the observation that bacteria and/or microbial products can rapidly stimulate IFNγ production from naive NK cell populations either in vitro or in vivo (80, 81, 83). This conclusion is based on experiments performed using either normal mice or immunodeficient C.B–17 mice expressing the scid mutation (SCID). SCID mice lack the ability to generate rearranged T cell receptor and immunoglobulin genes and therefore are completely devoid of functional T and B cells. Initial experiments indicated that SCID mice were capable of elaborating activated, MHC class II–positive macrophages during infection with *Listeria monocytogenes*, and they displayed partial resistance to the infection, responses that have been shown to be obligatorily dependent on IFNγ. The ability of SCID mice to produce IFNγ was unequivocally established using a neutralizing monoclonal antibody specific for murine IFNγ. SCID mice pretreated in vivo with anti-IFNγ failed to elaborate activated, MHC class II–positive macrophages, and they died following injection with a sublethal dose of live *Listeria*. In addition, IFNγ could be immunochemically identified in culture supernatants of SCID splenocytes stimulated in vitro with heat killed *Listeria* (HKLM). Treatment of SCID splenocytes with anti-asialo GM1 and complement ablated their ability to produce IFNγ when exposed to HKLM, thereby indicating that the IFNγ producing cell was an NK cell (81, 84).

Subsequent studies identified TNFα and macrophages as two of the cofactors required for the generation of IFNγ by NK cells (81–83). In vitro stimulation of SCID splenocytes with HKLM was found to effect production of *both* TNFα and IFNγ but with different kinetics. Peak levels of TNFα were observed 18 hr after stimulation and always preceded production of IFNγ which peaked only after 48 hr. Whereas the addition of neutralizing monoclonal antibody to TNFα prevented elaboration of IFNγ, control antibodies (including neutralizing antibodies specific for murine IL–1) did not. Moreover, injection of neutralizing anti-TNFα into either SCID or normal mice blocked the ability of the animals to produce IFNγ in vivo and to mount an anti-*Listeria* response. Macrophages from antibody-treated, infected mice did not show increased levels of MHC class II, and the mice failed to clear the bacteria from the spleen and liver

(81). These results thus show that generation of IFNγ by stimulated SCID spleen cells required the production of TNFα.

Cell depletion experiments revealed that macrophages were the cellular source of TNFα. Moreover, cell mixing experiments using pure populations of SCID NK cells derived by in vitro propagation of cells in IL–2 showed that IFNγ production occurred only in the presence of macrophages and bacteria or in the presence of purified TNFα and soluble bacterial products (82, 83). In the latter case, TNFα alone was not sufficient to induce IFNγ production from NK cells. Thus, NK cell activation to produce IFNγ requires two components: TNFα produced physiologically upon exposure of a macrophage to a microbial pathogen and a second stimulus that can be a bacterial product. Taken together, these results indicate that the IFNγ produced by NK cells represents the host's first line of defense against microbial pathogens that are susceptible to killing by activated macrophages.

THE IFNγ RECEPTOR AND MECHANISMS OF SIGNAL TRANSDUCTION

IFNγ exerts its pleiotropic effects on cells through an interaction with a specific receptor expressed at the cell surface. On the basis of immunochemical, radioligand binding, and molecular genetic analyses, there appears to be only a single type of IFNγ receptor that is ubiquitously expressed on all cells (except the erythrocyte) (85, 103, 86–95). Even platelets express IFNγ receptors at a level of 300 receptors/cell (96). Considering the large number of platelets in the circulation (3×10^8/ml), it is possible that this cell plays an important role in transporting IFNγ through the circulatory system. It is noteworthy that when receptor expression in different tissues is analyzed at either the mRNA or protein levels, the highest expression is observed in tissues not generally considered to have primary immunologic functions (95; M. Luquette, J. Calderon, R. D. Schreiber, unpublished results). Specifically, skin, nerve, and syncytial trophoblasts of the placenta express levels of IFNγ receptor that are often 10–100 times that observed in spleen or on hematopoietic cells. The receptor binds ligand with high affinity ($Ka = 10^9 - 10^{10}\ M^{-1}$) and is expressed on most cells only at modest levels (200–25,000 sites/cell). Human and murine IFNγ receptors display strict species specificity in their ability to interact with human and murine IFNγ.

IFNγ RECEPTOR SYNTHESIS AND RECYCLING

Using radioligand binding and immunoprecipitation techniques, the lifecycle of human and murine IFNγ receptors have been partially elucidated

(97–100). The receptor is synthesized in the endoplasmic reticulum and is glycosylated as it moves from the ER through the golgi. At least three glycosylation intermediates with molecular masses of 65, 70, and 75 kDa have been identified. Only fully glycosylated receptors are expressed at the cell surface. Carbohydrate analysis indicates that most if not all of the carbohydrates are N-linked. However, O-linked glycosylation of receptors in some cells has not been stringently ruled out. Fully mature receptors expressed on the plasma membrane display molecular masses that vary between 80 and 95 kDa. This modest molecular weight heterogeneity is a result of cell-specific differences in glycosylation. Following interaction with ligand at the cell surface, the intracellular domain of the receptor is phosphorylated on serine and threonine residues (101, 102). Although the functional significance of this phosphorylation remains unclear, the rate and extent of phosphorylation correlates perfectly with the magnitude of the biologic response induced. The phosphorylated receptor-ligand complex is internalized, enters an acidified endosomal compartment, and dissociates. Free IFNγ eventually traffics to the lysosome where it is ultimately degraded. In many cells, such as fibroblasts, the uncoupled receptor enters a large intracellular pool of mature receptor and eventually recycles back to the cell surface. The size of the intracellular receptor pool is generally 2–4 times greater than the pool of receptors expressed at the cell surface (103–105). The ability of the IFNγ receptor to recycle on macrophages remains controversial. Whereas some reports indicate that recycling does indeed occur on either primary or cultured mononuclear phagocytes (97, 103, 105), at least one other group has reported that internalized receptors are degraded on primary monocytes (106).

Receptor Structure

Recently, human and murine IFNγ receptors have been purified to homogeneity (89–92) and partially characterized. The complete characterization of the human receptor structure was made possible by the cloning of its cDNA, first accomplished by Aguet and colleagues in 1988 (107). Subsequently, several laboratories cloned and expressed the murine homologue (108–112). The genes for the human and murine receptors have been localized to chromosomes 6 (q16-q22) and 10, respectively (113–115). The human and murine IFNγ receptor genes are approximately 30 kb in size (116). Each consists of 7 exons and, upon activation, gives rise to a single 2.3 kb mRNA transcript. The resulting human and murine proteins are organized in a similar manner (Table 2, Figure 4). The mature proteins consist of 472 and 451 amino acids, respectively, and have predicted molecular masses of 52.5 and 48.5 kDa. Both proteins are symmetrically oriented around single 23 amino acid transmembrane domains. Each pos-

Table 2 Comparison of human and murine IFNγ receptors

Property	IFNγ receptor	
	Human	Murine
Primary sequence		
Signal peptide	17aa	26aa
Mature form	472aa	451aa
Homology	52%	
Domain structure		
Extracellular	228aa	228aa
Transmembrane	23aa	23aa
Intracellular	221aa	200aa
Potential N-linked		
Glycosylation sites	5	5
Predicted molecular weight	52,563	49,819
Molecular weight mature molecule	90,000	90,000
Serine/threonine content		
Whole molecule	17.6%	20.0%
Extracellular domain	11.8%	15.8%
Intracellular domain	23.3%	24.6%

sesses a 228 amino acid extracellular domain that contains 10 cysteine residues and 5 potential N-linked glycosylation sites. Based on biosynthetic labeling experiments, all 5 glycosylation sites appear to be occupied (98, 99, 117), and N-linked oligosaccharides contribute approximately 25 kDa to the apparent molecular weight of the fully mature protein. Soluble forms of the extracellular domain of the human and murine IFNγ receptors have been produced either in bacteria or eukaryotic cells (31, 118, 119). The availability of these reagents has led to the demonstration that the extracellular domain is sufficient to account for high affinity ligand binding and that receptor glycosylation is not critical for ligand binding activity. A structure-function analysis of this domain has been initiated. Proteolytic digestion of the receptor's soluble extracellular domain demonstrated that the amino terminal six residues are not required for ligand binding activity. However, the smallest proteolytic fragment of the *E. coli*–derived soluble receptor capable of expressing full ligand binding activity was a 25 kDa component encompassing residues 6–227, which is nearly the entire extracellular domain (120). Obviously, more work is needed before the structural elements of the receptor that are involved in ligand binding are defined.

Based on primary sequence comparisons, the IFNγ receptor bears little

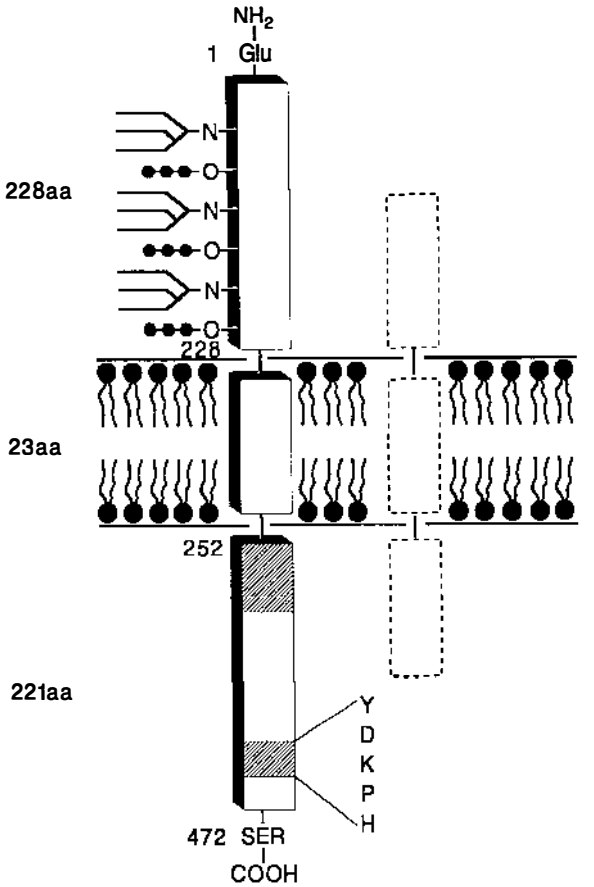


Figure 4 Model of the human IFNγ receptor. The ligand binding chain is a glycoprotein composed of 472 amino acids and is symmetrically oriented around a single 23 amino acid transmembrane domain. The extracellular and intracellular domains are composed of 228 and 221 amino acids, respectively. The protein contains N-linked oligosaccharides and possibly O-linked sugars on some cells. The intracellular domain is rich in serine and threonine residues, some of which are phosphorylated upon interaction of the receptor with ligand in intact cells. Two functionally important regions in the intracellular domain have been identified (shaded area). The membrane proximal region (residues 256–303) is need for both receptor-mediated internalization of ligand and induction of biologic responses. The carboxy terminal region (residues 434–472) is needed only for biologic response induction. The three critical amino acids within the latter region (Y–440, D–441, and H–444) are identified. Functionally active receptors require the presence of a second, as yet undefined, accessory molecule that must be species matched to the IFNγ receptor (shown as the component on the right side of the figure). The suggestion that this component is a membrane protein is based on experiments using human:murine chimeric receptors.

identity to any other known proteins. Specifically, it is neither a member of the Type I cytokine (or hematopoietic) receptor family (characterized by the position of 4 conserved cysteine residues and a membrane proximal WSXWS motif) nor the immunoglobulin superfamily (121). In fact, the sequence identity between the human and murine IFNγ receptors themselves is only 52% (50% identity between the extracellular domains and 55% identity between the intracellular domains). More refined structural analyses of the IFNγ receptor's extracellular domain using predictive algorithms have indicated that the human and murine IFNγ receptors belong to a new family of cytokine receptors (termed the Type II cytokine receptor family) whose members include the receptor for IFNα and tissue factor. The members of this protein family share a similarly organized 210 amino acid binding domain which contains conserved cysteine pairs at both amino and carboxy termini. The Type II receptor family appears to be only distantly related structurally to the Type I family (121)

The intracellular domains of the human and murine IFNγ receptors are 221 and 200 residues, respectively. Both are particularly rich in serine and threonine residues. These two amino acids constitute approximately 25% of all the residues within this domain. Moreover, the intracellular domains of the human and murine proteins contain 6 and 5 tyrosine residues, respectively, five of which are conserved. This observation is particularly noteworthy because of the low overall sequence identity within the human and murine intracellular domains. The receptors' intracellular domains show no significant sequence or structural homologies to any other known receptor polypeptides. Moreover, they do not possess any identifiable kinase, phosphatase, SH2, or SH3 domain characteristics. Therefore, the primary structure analysis of the IFNγ receptor has not provided insights into the mechanism of action of this protein

Functional IFNγ Receptors Require a Species Specific Accessory Component

Expression of the human and murine receptor cDNAs across species boundaries (i.e. in murine and human cells, respectively) confirmed that the cloned cDNAs encoded proteins that bound ligand in the appropriate species-specific manner and internalized it with kinetics that were indistinguishable from natural receptors expressed on homologous cells (107–112). However, the receptors expressed across species lines were unable to induce a functional response in the transfected cells. This result suggested that one or more additional species matched components were required to form a functionally active IFNγ receptor. It also supported the conclusions reached in the seminal experiments of Pestka and colleagues, who in 1987 used murine: human somatic cell hybrids to investigate the minimal

requirements to form a functionally active human IFNγ receptor in murine cells. Fusion of murine and human fibroblasts was known to generate stable cell hybrids that contained the full complement of murine chromosomes but only a random assortment of human chromosomes. All hybrids that contained human chromosome 6, bound human IFNγ. However, responsiveness to the human ligand was observed only in hybrids that contained both human chromosomes 6 and 21. This obligate requirement for two (or more) distinct species-matched gene products led to the hypothesis that functionally active human receptors were composed of at least two distinct polypeptides: the IFNγ receptor itself, responsible for the binding of ligand, and a species matched undefined protein needed for development of functional responses in cells (122). During the past few years, the validity of this hypothesis has been significantly enhanced by the demonstration that expression of the human IFNγ receptor cDNA in murine cells containing only human chromosome 21 leads to formation of a functionally active human IFNγ receptor capable of inducing most if not all IFNγ–dependent biologic responses in the transfected cells (122–124).

Since this accessory factor(s) has not yet been identified, little is known about its structure or function. Using human-murine chimeric IFNγ receptors produced by interchanging the extracellular, transmembrane, and intracellular domains of the human and murine proteins, the site of the species-specific interaction between the receptor and the human accessory protein has been localized to the receptor's extracellular domain (125–127). This result suggests but does not prove that the accessory molecule is expressed at the plasma membrane. In contrast, more is known about the chromosomal location of the gene(s) encoding the human receptor accessory component. Using murine cells containing human chromosome 21 fragments, the accessory component has been localized to a one megabase area on human chromosome 21 in the 21q22 region. Interestingly, this region also contains the gene for the human IFNα receptor (128) (although the IFNα receptor is known not to be the accessory component of the IFNγ receptor). Subsequent experiments utilizing hamster:mouse somatic cell hybrids have indicated that the gene(s) for the murine IFNγ receptor accessory component is located on murine chromosome 16 (129).

Structure-Function Relationships within the IFNγ Receptor

In contrast, considerably more information is available concerning the structure and function of the human and murine IFNγ receptor polypeptides themselves. The ability to reconstitute a functional human IFNγ receptor in murine fibroblasts that contain human chromosome 21 has been used to map the functionally important regions of the human IFNγ

receptor's intracellular domain (123, 130, 133). Full length or truncated (i.e. a receptor lacking all but 3 amino acids of the intracellular domain) receptors were stably expressed in murine fibroblasts containing a single copy of human chromosome 21. The cells expressing the full-length human receptor bound and internalized human IFNγ and responded to it by upregulating expression of MHC class I molecules. In contrast, cells expressing the truncated human receptor bound human IFNγ but neither internalized it nor responded to it. Subsequent experiments that used receptor deletion mutants identified two distinct regions of the intracellular domain which were obligatorily required for receptor function (Figure 4). The first encompassed the 48 amino acids closest to the membrane (termed region I, residues 256–303) and was required for both receptor mediated ligand internalization and degradation and induction of biological responses. The second consisted of the 39 amino acids at the carboxy terminus (termed region IV, residues 434–472) and was required exclusively for biologic response induction (123). Although region I has only been partially characterized to date, a leucine-isoleucine sequence (residues 270–271) has been identified within this region that is involved in effecting receptor-mediated ligand internalization/degradation (130). This sequence matches a motif found in the intracellular domains of the γ and δ chains of the T cell receptor complex and in the cation-dependent mannose–6-phosphate receptor which has been shown to direct the trafficking of these proteins to lysosomes (131, 132). The finding that IFNγ receptor mutants, in which this sequence has been deleted or replaced by alanines, show decreased degradation of bound ligand suggests a similar role for this sequence in directing the IFNγ receptor-ligand complex to a late endosomal or lysosomal compartment

Region IV has been more extensively characterized (133). A point mutational analysis of this region (in which each residue was individually changed to alanine) demonstrated that only three residues are functionally important. These are tyrosine at position 440, aspartic acid at position 441, and histidine at position 444. Alteration of any one of these residues to alanine produced a receptor which was unable to induce a variety of IFNγ-dependent biological responses in murine fibroblasts that contained human chromosome 21. These included induction of IRF–1 (an IFN-inducible transcription factor), MHC class I protein, and nitric oxide. The particular functional importance of tyrosine–440 was confirmed by two additional observations. First, substitution of phenylalanine for tyrosine–440 also resulted in generation of a functionally inactive receptor. This result suggests that the hydroxyl group present on the tyrosine's side chain plays an important role in the signaling process either by (i) forming a structurally critical intramolecular hydrogen bond, (ii) contributing to

intermolecular protein-protein interactions, or (iii) serving as the target for protein tyrosine kinase induced phosphorylation. Second, mutation or deletion of any of the other tyrosine residues within the receptor's intracellular domain did not ablate receptor activity. Additional support for the functional importance of region IV is derived from microinjection experiments in which a monoclonal antibody specific for an overlapping sequence to this region (residues 388–449) inhibited cellular response to IFNγ (134). Microinjection of antibodies that reacted with the middle portions of the intracellular domain (regions II and III) did not inhibit cellular responsiveness. Thus, taken together these studies have identified functionally critical regions within the intracellular domain of the IFNγ receptor. It is expected that the exact roles of these two intracellular domain regions in the signaling/internalization process will soon be elucidated.

Mechanisms of Signal Transduction

Recently, the important observation has been made that IFNγ induces dimerization of its receptor. Using radioligand binding techniques, sucrose density gradient ultracentrifugation, and HPLC gel filtration chromatography, the soluble human IFNγ receptor extracellular domain (ECD) was shown to form a complex with ligand that contained two moles of ECD and only one mole of IFNγ homodimer. The stoichiometry of the complex was dependent on the relative proportions of ligand and receptor added to the reaction mixture. At limiting inputs of IFNγ, a 2:1 (ECD:IFNγ) complex was formed. When ligand was added in vast overabundance, it was possible to demonstrate a 1:1 complex, although even under these conditions the 2:1 complex seemed to be preferred. On the basis of this data, it is likely that the receptor:ligand complex formed physiologically at the cell surface is composed of two receptors bound to one IFNγ homodimer (31, 31a). Support for this concept is derived from recent cross-linking studies, which have shown that under the appropriate conditions, a 2:1 receptor:ligand complex can be immunoprecipitated from human cells treated with physiologic levels of human IFNγ (D. Pennica, D. V. Goeddel, personal communication). A previous report suggested that IFNγ was only capable of producing a 1:1 complex with its receptor (135). However, the recombinant soluble receptor used in those studies was engineered to contain 5 carboxy terminal histidine residues, and it is likely that the presence of these amino acids interfered with the generation of the 2:1 complex (31a).

The physiologic relevance of ligand-induced receptor dimerization is also strongly supported by the finding that functionally inactive receptors act as a dominant negative mutant when overexpressed in homologous cells (32). These studies utilized murine L cells that overexpressed mutant

murine IFNγ receptors lacking either (i) the entire intracellular domain, (ii) the carboxy terminal 39 amino acids (encompassing functional region IV), or (iii) a receptor point mutant in which alanine was substituted for the murine tyrosine residue (residue 420) corresponding to the functionally important human tyrosine-440. Cell lines in which nonfunctional receptors were expressed at levels 100-fold higher than the endogenous receptors no longer responded to murine IFNγ when analyzed in a variety of assays (IRF-1 induction, MHC class I enhancement, nitric oxide induction, and development of antiviral activity). In contrast, comparable overexpression of the wild type murine receptor did not produce a dominant negative effect. Inactivation of IFNγ responsiveness was dependent on the ratio of mutant:endogenous receptors. At 6:1 ratios no inhibition was observed. However, a profound inhibition was seen at ratios of 25:1, and complete inactivation was achieved at a 100:1 ratio. This effect was not due simply to competition for ligand by inactive receptor, because the cells remained unresponsive to ligand even when exposed to IFNγ concentrations 3×10^5 times higher than that normally required to induce a maximal response in wild type cells. Overexpression of inactive receptor did not alter expression of the endogenous receptor. Thus, ligand induced dimerization of the IFNγ receptor, and in particular the formation of a dimeric form of the receptor's intracellular domain may be a critical first step in IFNγ receptor mediated signal transduction.

Subsequent events in the signal transduction pathway are less clearly defined. Whereas some reports indicate that the receptor acts through signal transduction pathways involving protein kinases (136–140), receptor phosphorylation (101, 102), and/or ion fluxes (141), other investigators have suggested that the receptor is primarily responsible for transporting ligand into the cell and propose that intracellular IFNγ somehow induces a cellular response (142–144). Based on the structure-function experiments described above, there is little doubt that receptor-mediated ligand internalization is *not sufficient* to induce biologic responses in cells. This conclusion is based on two observations. First, whereas human IFNγ receptors expressed in normal murine cells effect ligand internalization and degradation with kinetics that are indistinguishable from that of functionally active receptors, no biologic response is observed. Second, mutant human receptors lacking the carboxy terminal 39 amino acids (region IV), when expressed in murine cells containing human chromosome 21, are also functionally inactive despite their ability to mediate ligand internalization. Thus, the molecular explanation for the reports indicating a requirement for ligand internalization remain unclear. It is likely that this uncertainty will soon be resolved when the receptor-related accessory component's identity and function are elucidated and when the mapping of the recep-

tor's intracellular domain region (region I) required for both internalization and function is completed. Finally, the recent exciting identification of key components of the IFNα signaling pathway (145–147) and the observation that the signal transduction pathways utilized by IFNγ and IFNα overlap (148, 149) may provide additional insights that will help define the downstream signalling events and identify the functional branching points that characterize the pleotropic activities of IFNγ.

IFNγ BIOLOGY

Work performed in many laboratories during the past 12 years has unequivocally established that IFNγ is an extremely pleiotropic cytokine that has unarguable physiologic importance in regulating immune and inflammatory processes. To a large extent, this research was made possible by the large scale availability of highly purified recombinant human and murine IFNγ and the generation of neutralizing IFNγ-specific monoclonal antibodies. Most recently, two new and exciting models of genetic IFNγ deficiency in mice have been derived by ablating either IFNγ receptor or IFNγ gene expression using homologous recombination and embryonic stem cell technologies (150, 151). It is expected that these mice will provide new and exciting insights into IFNγ biology.

There is currently a vast amount of information available concerning IFNγ's biologic activities. It is impossible to cover this area in its entirety in this review article. Therefore we have decided to focus on some of the major biologic activities of this cytokine and describe them in the context of three systems which are of prime interest to immunologists: host defense, inflammation, and autoimmunity.

IFNγ'S ROLE IN HOST DEFENSE

IFNγ as a Regulator of Response Induction

Clearly one of the major physiologic roles of IFNγ is its ability to regulate MHC class I and class II protein expression on a variety of immunologically important cell types. These include mononuclear phagocytes, endothelial cells, and epithelial cells, to name a few (57, 62, 152–154). Interestingly, although IFNγ acts to increase class I and class II expression on most cells, it inhibits class II expression on B cells (155). Whereas IFNα and IFNβ can also upregulate class I expression on cells, they are not inducers of MHC class II proteins. IFNγ's ability to perform these functions was first appreciated in in vitro experiments in which purified recombinant IFNγ was added to primary cells or cultured cell lines and MHC protein expression monitored using immunochemical techniques (152).

Subsequently, this observation was confirmed using two types of in vivo approaches. In the first, IFNγ was injected directly into the host and MHC antigen expression monitored. These experiments showed that IFNγ could upregulate MHC class I and class II proteins both locally at the injection site and systemically. In the second type of experiment, the host was injected with IFNγ-specific, neutralizing monoclonal antibodies and then exposed to an immunologic challenge such as a parasitic infection. Whereas the tissues and cells of control animals displayed significant induction of MHC class II antigens on their surface, cells from anti-IFNγ treated animals did not. The latter experiment was particularly informative because it documented that endogenously produced IFNγ plays an important physiologic role in regulating MHC protein expression (81, 156). More detailed analyses have shown that IFNγ *enhances* MHC class I expression (2–4 fold) on cells that constitutively express class I and *induces* class II expression on cells that are normally devoid of these proteins (157). At the molecular level, IFNγ has been shown to exert its activity by regulating MHC gene transcription. MHC class I and class II genes contain *cis*-acting elements in their promoter regions that bind to IFNγ-induced *trans*-acting factors. The molecular nature of these elements and factors are currently being elucidated (158, 159). At the functional level, IFNγ-dependent upregulation of MHC gene expression is an important step in promoting antigen presentation during the inductive phase of immune responses (160–164). On most cells, IFNγ is sufficient to upregulate MHC molecules. However, its action is often synergistically enhanced by additional endogenous stimuli such as TNF or exogenous agents such as bacterial or microbial products. Importantly, some cells such as pancreatic islets display an obligatory requirement for the combined actions of IFNγ and TNF (165–167).

The monocyte/macrophage is a prime cellular target for IFNγ under physiologic conditions. Work from several laboratories has indicated that IFNγ is one of the major cytokines responsible for activating or otherwise regulating the differentiation and function of mononuclear phagocytes (58, 168). IFNγ has been shown to effect the differentiation of immature myeloid precursors into mature monocytes. It promotes antigen presenting activity in macrophages, not only through the induction of MHC class II expression, but also by increasing levels of several intracellular enzymes that may be important for antigen processing (169, 170). In addition, IFNγ augments expression of macrophage cell surface proteins such as ICAM–1 that enhance the functional consequences of the interaction between macrophages and T cells during antigen presentation (171–173)

IFNγ also exerts its effects on other cells of the immune system. It regulates immunoglobulin isotype switching on B cells (174) and anta-

gonizes the ability of IL-4 to induce MHC class II expression on murine B cells (175). These responses result from the direct effect of IFNγ on the B cell. B cell responses are also influenced indirectly by IFNγ's ability to regulate the development of specific subsets of $CD4^+$ T cells. IFNγ has a profound antiproliferative effect on the T_H2 subset of murine $CD4^+$ T cells but not on T_H1 (61, 63). The ability to regulate $CD4^+$ T cell activation/differentiation thereby establishes IFNγ as a key component in determining the type of immune effector function that eventually develops during the course of an immune response (Figure 5). The opposing effects of IL-10 and IFNγ thereby serve to cross-regulate the development of specific immune responses. IL-10 inhibits IFNγ production by T cells and NK cells and thereby diverts the response to the humoral pole. In contrast, IFNγ inhibits the expansion of T_H2-like T cells, thereby eliminating a key cellular source of IL-10. This event then diverts the response to the cell-mediated immunity pole. The suggestion has been made that IFNγ serves a positive role in the generation of $CD8^+$ cytolytic T cells (CTL) (176). However, this hypothesis has not yet been stringently demonstrated and, in fact, mice with genetic IFNγ-unresponsiveness produce more CTL activity during a mixed leukocyte reaction than their normal counterparts (150, 151). This result indicates that IFNγ may actually exert an antiproliferative

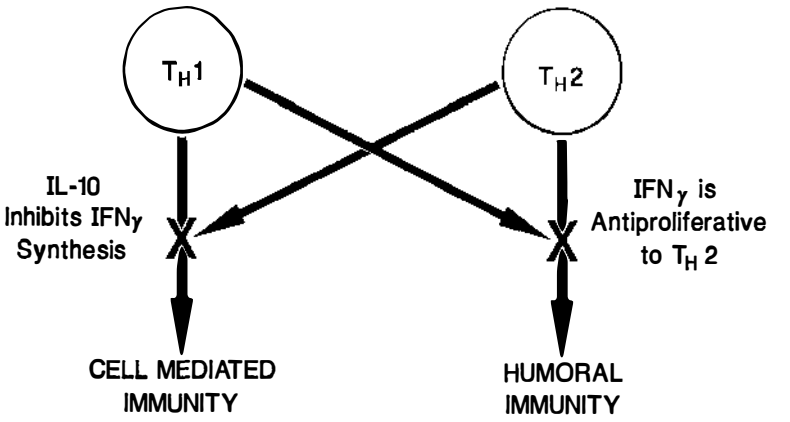


Figure 5 IFNγ and IL-10 cross-regulate the development of specific arms of immune effector functions. IL-10 inhibits IFNγ production by TH1 $CD4^+$ T cells and NK cells and thereby inhibits development of cell-mediated immune responses. At the same time IL-10's actions promote development of humoral immunity. In contrast, IFNγ inhibits the proliferation of TH2 $CD4^+$ T cells thereby inhibiting production of TH2 derived cytokines including IL-10. This regulatory action results in the preferential development of cell mediated immunity and the depression of humoral immunity. The identity of the signals that lead to the predominance of IL-10 versus IFNγ effects remain undefined.

effect on the $CD8^+$ T cell population, as well as the $CD4^+$ T_H2 cell population

IFNγ as a Regulator of Effector Mechanisms

There can be little doubt that IFNγ is the major physiologic macrophage activating factor (MAF) and therefore is the primary cytokine responsible for inducing nonspecific cell mediated mechanisms of host defense. Work from several laboratories has unequivocally established IFNγ's ability to activate nonspecific cytocidal activity in macrophages toward a variety of intracellular and extracellular parasites and neoplastic cells (54, 80, 177). IFNγ (i) induces the expression of, as yet undefined, structures on macrophages that recognize target cells and (ii) promotes the elaboration of macrophage-derived cytocidal compounds such as reactive oxygen– and reactive nitrogen–intermediates and TNFα (178). IFNγ also reduces the susceptibility of macrophage populations to microbial infection (81, 179). The importance of IFNγ in the clearance of microbial pathogens has been amply demonstrated using animal models. Mice pretreated with neutralizing monoclonal antibodies to IFNγ lose their capacity to resolve infection initiated with a sublethal dose of a variety of microbial pathogens such as *Listeria monocytogenes* (80, 81, 156), *Toxoplasma gondii* (180) or *Leishmania major* (181). These experiments thus document the capacity of endogenously produced IFNγ to activate macrophages under physiologic in vivo conditions

Currently a great deal of attention is being focused on IFNγ's ability to induce nitric oxide (NO) production in cells. NO is a cellular product that appears to play an important role in effecting intracellular killing of microbial pathogens in the mouse. Nitric oxide is generated as a result of the enzymatic conversion of L-arginine to L-citrulline (182). This reaction is catalyzed by a family of enzymes known as nitric oxide synthase (NOS). At least 3 forms of the enzyme have been identified (183). Two are expressed constitutively in a tissue-specific manner (endothelium and nervous tissue) and produce low levels of NO that function to effect cell-cell communication. In contrast, the third is an inducible form of the enzyme whose expression is controlled by two stimuli: IFNγ and a second signal. The signals that trigger NO production in IFNγ primed cells are a diverse group of endogenous and exogenous substances such as TNFα, IL–1, LPS, and whole bacteria (184, 185). The array of components that trigger NO production in cells varies depending upon the nature of the NOS producing cell. For example, in macrophages NOS is induced by IFNγ and TNF but not IL–1, while in certain fibroblasts or pancreatic islets NOS is induced by IFNγ and either TNF or IL–1 (185, K. C. F. Sheehan, R. D. Schreiber,

unpublished observations). The inducible macrophage NOS has recently been cloned and characterized (186–188). Whether the inducible NO synthase expressed in other cell types is identical to the macrophage enzyme needs to be established.

IFNγ-dependent formation of nitric oxide appears to be a major mechanism in the mouse for the macrophage-mediated killing of intracellular pathogens. Much of this information has been gleaned from the study of murine models of infection with *Leishmania* or *Listeria*. Macrophages exposed in vitro to IFNγ and infected with either *Leishmania* amastigotes or *Listeria* develop the capacity to kill the intracellular pathogens. Killing is completely inhibited when the macrophages are treated with competitive inhibitors of NOS such as the L-arginine analogs, N-monomethyl-L-arginine (L-NMMA) (181) or aminoguanidine (189). In vivo, the important role of IFNγ-dependent induction of NO in murine models of microbial immunity has been indicated by at least three types of experiments. First, mice undergoing active infection produce NO as detected by the presence in the urine of the stable (NO) oxidation product nitrite (NO_2). NO_2 production was blocked when the mice were treated with neutralizing monoclonal antibodies specific for either IFNγ or TNF (181). Second, killing of live *Leishmania* in vivo at a local site (footpad) was significantly decreased when the animals were treated with the NOS inhibitor NMMA (190). Third, and perhaps most significant, is the recent observation that mice treated with aminoguanidine succumb to infection with a sublethal dose of *Listeria monocytogenes*, much like mice treated with anti-IFNγ (K. Beckerman, H. Rogers, C. Tripp, J. Corbett, R. Schreiber, M. McDaniel, E. Unanue, manuscript in preparation). Interestingly, human mononuclear phagocytes have not been shown to produce nitric oxide or NOS thus far (although human islets and hepatocytes can indeed produce NO). Whereas this latter observation may indicate that species specific differences exist in IFNγ-dependent macrophage mediated cytocidal responses, the possibility must also be considered that the human mononuclear phagocyte requires an additional signal to induce NOS.

In addition to enhancing *nonspecific* cell mediated cytocidal activities, IFNγ also enhances the ability of the macrophage to participate in other immune response effector functions. It increases expression of high affinity Fc receptors on monocytes/macrophages (FcγRI) and thereby enhances the capacity of these cells to participate in antibody dependent cellular cytotoxicity (ADCC) reactions (191). IFNγ also enhances the biosynthesis of a variety of complement proteins (such as C2, C4, and Factor B) by macrophages and fibroblasts (192) and regulates the expression of complement receptors on the mononuclear phagocyte plasma membrane

thereby promoting humoral immunity through enhancement of complement activity.

IFNγ IN THE INFLAMMATORY RESPONSE

A substantial amount of data is now available that supports the concept that cytokines play a major role in promoting inflammatory responses. IFNγ also participates in this process largely through its ability to enhance TNF production and/or activity. The focus of this section is therefore to highlight the important synergistic action of IFNγ and TNFα in inflammation.

IFNγ Regulation of TNFα Production

It is now well established that LPS stimulated macrophages produce increased amounts of TNFα when concomitantly treated with IFNγ (193–196). A few early studies indicated that IFNγ could directly induce TNFα production in macrophages. However, this result was not substantiated in other laboratories, and it is likely that the induction was a result of increased sensitivity of IFNγ-treated cells to low levels of LPS present in the medium. IFNγ can cause the upregulation of LPS-induced TNFα production in a variety of different murine macrophage populations, including resident and elicited peritoneal exudate macrophages and bone marrow derived macrophages grown in vitro. Thus the ability of IFNγ to enhance LPS-induced TNFα production is not strongly dependent on the activation state of the macrophage. Thioglycollate-elicited macrophages concomitantly stimulated with a mixture of LPS and IFNγ displayed 6–8 times higher steady-state levels of TNFα specific mRNA than cells treated with LPS alone. Maximal levels of TNFα mRNA were achieved 2–4 hours after exposure to either LPS alone or to the LPS/IFNγ mixture (193, 194). IFNγ also enhanced the ability of LPS to induce the TNFα protein (as measured 16 hr after stimulation). Enhancement of TNFα production was most evident in cells pretreated for 4 hr with IFNγ but was still detectable even when IFNγ was added 6 hr after LPS stimulation (193). IFNγ can also correct the genetic defect in macrophages derived from the LPS unresponsive C3H/HeJ mouse. C3H/HeJ macrophages do not produce TNFα when stimulated either in vivo or in vitro with moderate concentrations (1 μg/ml) of LPS. However, thioglycollate-elicited macrophages from these mice treated with physiologic concentrations of purified recombinant murine IFNγ, produce both TNFα mRNA and protein when stimulated with LPS at 1 μg/ml (193).

Several studies indicate that the enhancement of LPS-induced TNFα production by IFNγ is a result, at least in part, of increased transcription

of the TNFα gene (193–195, 197, 198). However, the suggestion has also been made that, in this system, IFNγ may also increase TNFα mRNA stability. In part, this latter hypothesis is based on the demonstration that TNFα mRNA contains an octomeric sequence (UUAUUUAU) within its 3′ untranslated region that is a point of attack for a selective nucleolytic activity capable of hydrolyzing mRNA. Similar sequences have also been found in the 3′ untranslated regions of other short-lived cytokine mRNAs such as those that encode IL–1 and GM-CSF. Independent studies have shown that the half-life of TNFα mRNA is increased in cycloheximide-treated cells stimulated with LPS. Therefore it seems likely that IFNγ may exert some of its enhancing effects on TNFα production by inhibiting the generation of this short-lived repressor activity (199).

IFNγ has also been shown to enhance expression of TNFα receptors on a variety of different cell types (200–202). Treatment of cells with IFNγ does not alter their ligand binding affinity but increases receptor expression 3–5 fold. Both types of TNFα receptors (p55 and p75) can be increased by IFNγ. Enhanced receptor expression appears to be due to increased protein synthesis and not to translocation of receptors from an intracellular pool since IFNγ's effects can be blocked by treatment of the cells with actinomycin D. However, the biologic significance of this effect remains to be established, because no correlation has yet been found between the level of TNFα receptor expression and the magnitude of the cellular response induced (200, 201, 203)

Cellular Recruitment

During an inflammatory response, cells leave the circulation and migrate to the point of infection. During this process they must first bind to and then extravasate through the vascular endothelium. IFNγ and TNFα can promote the expression of overlapping sets of cell-surface molecules that play an important role in this process (171). Studies using cultured human umbilical vein endothelial cells (HUVEC) demonstrated that in vitro treatment with IFNγ induces a significant but moderate increase in ICAM–1 (5-fold) and MHC class I molecules (11-fold) but no induction of ELAM–1 (171, 204). In the same setting, TNFα provoked a dramatic enhancement of ICAM–1 expression (40-fold) as well as moderate increases in ELAM–1 and MHC class I proteins. However, when HUVEC were cultured in the presence of both IFNγ and TNFα, the expression of all three molecules was enhanced in a synergistic manner with ICAM–1 and class I showing 60- and 24-fold increases, respectively. In addition, the combination of IFNγ and TNFα induced ELAM–1 expression on a significantly higher percentage of cells than TNFα alone. In the presence of both cytokines ELAM–1 also displayed a longer half-life at the cell surface. This latter

effect may result in a prolongation of the endothelial cell's ability to recruit circulating lymphocytes (204). Thus, the ability of IFNγ and TNFα to enhance expression of cell surface adhesion molecules may serve to expand and amplify the overall inflammatory response.

The cooperative ability of IFNγ and TNFα to modulate cell migration was confirmed in in vivo experiments (205). Skin biopsies, taken from baboons treated intracutaneously with both IFNγ and TNFα were found to contain twice the numbers of monocytes compared to animals injected with either cytokine alone. Expression of ELAM–1, ICAM–1, and MHC class I molecules was also synergistically enhanced in animals treated with both cytokines.

Shock

TNFα has long been known to mediate many of the toxic effects of LPS (206–208) and is a key mediator in the Shwartzman reaction (a model of LPS-mediated tissue damage) (209, 210). In the classical Shwartzman reaction, animals are initially treated with a local, sensitizing dose of LPS (5 μg), followed 24 hr later with a provocative intravenous dose of LPS (100 μg). The physiologic responses evoked by this protocol mimic those seen clinically in septic shock and disseminated intravascular coagulation and include hemorrhagic necrosis, fibrin- and platelet mediated vascular occlusion and accumulation of neutrophils at the local site (206, 207, 211). Using the Shwartzman reaction as a model to investigate the mechanism(s) involved in LPS-induced disease, several investigators have shown that IFNγ plays a crucial role in the progression of this inflammatory response (209, 212–217). Treatment of animals with IFNγ prior to sensitization with LPS leads to enhanced production of TNFα and increased mortality (212, 213, 218). Conversely, treatment of animals with neutralizing IFNγ-specific monoclonal antibodies prior to injection of the sensitizing dose of LPS, protects them from the pathologic effects of the provocative dose (215). It is possible that the IFNγ produced in this reaction is derived from NK cells in a manner resembling that described above for the SCID mouse. The sensitizing dose of LPS may stimulate resting macrophages to produce TNFα. TNFα and LPS then may stimulate NK cells to produce and secrete IFNγ. NK cell–derived IFNγ then should prime other macrophages in the vicinity to produce copious amounts of TNFα (and other inflammatory mediators such as IL–1 and IL–8) when exposed to the provocative dose of LPS. Once produced, the large amounts of TNFα then would induce a cascade of reactions that have immunopathologic consequences. Thus, in this model, IFNγ serves a crucial intermediary role in activation of effector macrophage populations. A similar role for IFNγ was noted using a model of endotoxin shock (218). Mice pretreated with recombinant murine IFNγ

18 hr prior to intravenous injection with an LD_{50} dose of LPS showed significantly increased mortality (92% dead at 72 hours). Conversely, mice treated with neutralizing IFNγ-specific antibodies prior to administration of an LD_{90} dose of LPS were protected (218) from LPS induced lethal shock. Thus, LPS-induced IFNγ appears to be a key mediator in the development of the immunopathologic consequences of inflammatory responses.

IFNγ IN AUTOIMMUNITY

Although IFNγ appears to play a participatory role in the development of some autoimmune processes, different experimental models have yielded conflicting interpretations as to its immunopathologic versus protective roles. One model in which IFNγ has been implicated as a causative agent is the development of autoimmune nephritis in the (NZB × NZW)F1 mouse, a syndrome which produces a pathology similar to that seen in the human disease, systemic lupus erythematosis. Administration of exogenous IFNγ to (NZB × NZW)F1 mice accelerated the progression of spontaneous glomerulonephritis (50% survival = 9.5 months for control animals and 7.5 months for IFNγ treated mice) (219–221). Conversely, animals treated with IFNγ-specific monoclonal antibodies displayed significant remission and increased survival (220). Additional support for IFNγ's role in producing tissue specific damage that leads to autoimmune responses has been obtained from experiments utilizing transgenic mice expressing the IFNγ gene under the control of the rat insulin promoter (222). These mice develop a severe insulitis and become hypoglycemic and thereby develop a syndrome that appears very much like insulin dependent diabetes mellitus, which is commonly thought to be an autoimmune disease.

In contrast, IFNγ plays a protective role in certain murine models of experimental autoimmune encephalomyelitis (EAE). This demyelinating disease can be generated either by active immunization with myelin basic protein (MBP) in adjuvant or by the adoptive transfer of encephalogenic MBP-specific $CD4^+$ T cell clones (223–227). Immunization of C57BL/6 or SJL/J mice with MBP results in moderate or lethal forms of the disease, respectively (227). Treatment of C57BL/6 (moderate disease) mice with anti-IFNγ exacerbates disease and leads to increased mortality. Conversely, treatment of SJL/J mice (which normally exhibit 100% mortality) with IFNγ led to enhanced survival. Thus, using the active immunization protocol, IFNγ appears to downregulate the development of EAE. This protective role is not observed when EAE is induced using the passive T cell transfer protocol. Moreover, administration of IFNγ to human

subjects with active multiple sclerosis (the natural human disease that resembles murine EAE) leads to an exacerbation of the disease (228). Together, these results demonstrate that IFNγ can play different roles in the development of various autoimmune responses and may act through a variety of different mechanisms. Further experimentation is required to determine the molecular mechanism(s) responsible for the seemingly contradictory roles of IFNγ in autoimmune disease.

CONCLUDING REMARKS

In this chapter we have reviewed some of the recent developments that have significantly added to our understanding of the biochemistry and biology of IFNγ. To a large extent, this progress was made possible by the technological advances that have occurred within recent years in the fields of molecular genetics, protein chemistry, and cell biology. We now know a great deal about the structure of the IFNγ molecule and its receptor, and we have begun to identify which of the functional activities of the protein are physiologically relevant. Nevertheless the molecular mechanisms that underlie IFNγ's pleotropic activity remain undefined, and we still have not yet found a means to fully develop the protein's therapeutic potential. It is likely that these subjects will be the focus of future IFNγ research.

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