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THE PHARMACOLOGY AND TOXICOLOGY OF THE INTERFERONS: An Overview[1]

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INTRODUCTION[2]

Early virologists observed that a patient or an experimental animal, once recovered from a virus infection usually resisted reinfection from the same or similar virus. Vaccination was one of the early applications of this phenomenon, which was termed *viral interference*. As immunology evolved, investigators recognized that the infected body produces specific antiviral antibodies which appear in the circulation and on mucous surfaces attacked by viruses (humoral immunity), or that sensitized cells (principally lymphocytes) appear which can attack both virus particles and cells infected by viruses (cell-mediated immunity). The requirement of specific antibodies for specific viruses limited the general clinical usefulness of humoral and cell-mediated immunity.

As experience with cell cultures evolved, it became apparent with several experimental models that infection of cells with one virus protected against reinfection with an antigenically unrelated virus. The groundwork for the discovery of interferon was laid by Henle & Henle (6) in 1943 when they demonstrated interference between inactive and active influenza viruses in the developing chick embryo, but the mechanism of this type of interference was not understood until Isaacs & Lindenmann (7) performed an unconventional experiment. They incubated small pieces of chicken chorioallantoic membrane

[1]The vast literature that has accumulated during the past decade has made it necessary in many cases to cite reviews here rather than individual publications, particularly in the tables.

[2]For reviews see 1–5.

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with heat-inactivated influenza virus, recovered the cell- and virus-free medium, and incubated it with fresh membranes inoculated with live virus. The live virus did not grow. Interference had been transferred to the fresh membrane by something that had been produced in the first membrane in response to the heat-killed virus. A virus-inhibiting substance was also reported in 1958 by Nagano & Kojima (8).

Isaacs & Lindenmann called their substance *interferon*. The name was "coined as a convenient laboratory shorthand and not as a result of a deliberate taxonomic exercise" (4). Interferon research has persisted for three decades only because of the stubborn efforts of a small cadre of "interferonologists" who continued to believe in the reality and importance of interferon while enduring widespread skepticism and occasional ridicule. Only recently has the question evolved from "Interferon, does it exist?" to "Interferons, how many?"

Isaacs & Lindenmann (9) went on to demonstrate that interferon is a protein, stable within pH extremes of 2 and 10, stable when heated at 60°C for 1 hr, and not affected by incubation with antiserum prepared against the virus that induced its production. Their observation that incubation of a virus with interferon had no effect on viral infection led them to assert the novel concept that interferon exerted its interference by rendering cells incapable of supporting viral replication rather than by interacting directly with a virus particle. The concept of species specificity was introduced shortly thereafter when Tyrrell (10) showed that interferon prepared in chickens had no activity in calf cells. Interferon has been induced in cultured cells from a large variety of mammals and other animals, including fish, amphibia, reptiles and birds. Interferon-like factors have also been reported in plants (11).

Interferon is not the only inhibitor of virus. According to Lockhart (12) and Stewart (5), the following criteria must be met before a viral inhibitor can be accepted as an interferon: (*a*) It must be a protein. (*b*) Its antiviral effect must not result from a nonspecific toxic effect on cells. (*c*) It must be active against a wide range of unrelated viruses. (*d*) It must inhibit virus replication through an intracellular effect which must involve synthesis of both RNA and protein by the cells. (*e*) It must exhibit activity on a defined host range of cells. (*f*) It must induce the following non-antiviral alterations in cells: priming, blocking, enhancement of double-stranded RNA toxicity, and inhibition of cell-multiplication, always in constant ratios.

Had interferon not been discovered by virologists, it certainly would have been revealed through one or more of its other effects and named accordingly. In fact, γ-interferon is classified as a lymphokine as well as an interferon. Before the recent purification of natural interferon and the production of recombinant interferons, crude preparations for laboratory and clinical studies were employed that were little more than protein mixtures contaminated with small amounts of interferon. It is remarkable that so much was learned about

interferon before its purification. However, the many non-antiviral effects produced in a variety of biological models by these crude preparations were ignored or discounted largely because virologists, obsessed with the conviction that antiviral activity was the single *raison d'être* for interferon, assumed that the other effects must be due to impurities. Recent studies with pure interferons, however, show that crude preparations produce their multiple effects by virtue of their interferon content. Interferon can no longer be defined simply as a cellular protein that confers antiviral activity to cells. In fact, certain of the non-antiviral effects of interferons may prove to be of equal or greater importance than the antiviral activity.

CLASSIFICATION OF INTERFERONS[3]

Two of the three major classes of interferons were recognized quite early as originating in leukocytes or fibroblasts (Table 1). The discovery of γ-interferon was delayed considerably by a definition that required interferons to be stable at pH 2, relatively heat stable, and inducible by virus or double-stranded RNA. None of these criteria apply to γ-interferon. An international committee of scientists decided recently that differences in antigenicity could form a basis for classification of leukocyte, fibroblast, and immune interferons with respective designations of IFN-α, IFN-β, and IFN-γ. This classification is not addressed to molecular, or even biological, characteristics of interferons; but it has been generally accepted for its convenience. Such a scheme may be the best that can be expected at this time because the amino acid sequencing of at least 15 human interferons has not revealed homologous sequences or secondary or tertiary structures that allow chemical classification. There is a fair amount of homology of amino acid sequences of human α- and β-interferons, but almost none between γ-interferon and α/β-interferons.

Until recently, glycosylation was considered to be a feature of all interferons. Many believed it determined species and receptor specificities. However, pure natural and recombinant human IFN-αs are not glycosylated, yet they exhibit both species specificity and individual patterns of biological activity.

The rapid progress made in the isolation and characterization of a host of subspecies of IFN-αs has given rise to impromptu classification of subspecies of IFN-αs. Some publications designate human interferon with an Hu (IFN-α becomes HuIFN-α). Subtypes of IFN-α have been designated a_1, a_2, etc. Pestka & Baron (13) propose that recombinant interferon be differentiated from natural interferon by use of a lower case "r" (HuIFNr-α or HuIFLr-α).

The creation of recombinant hybrid interferons will undoubtedly generate still other subclassifications. Several recombinant hybrid interferons have

[3]For reviews see 13–17.

Table 1 Classification and properties of human interferons[a]

Type	Other designations	Source	Induced by	Specific activity (U/mg)	Number of subspecies	Molecular weight	Functional unit	Chromosome location	pH stability	Glycosylation[b]
α (IFN-α)	leukocyte IF type I, IFNα, IFL, LeIF, LIF, ifnLe	β and null lymphocytes and macrophages; recombinant	virus; double-stranded RNA	2–4 × 10^8	≥ 15	16,500–25,000	monomer	(a, . . . a >18) chr 9	+	−
β (IFN-β)	fibroblast IF, type I, FIF, INF, ifnF	Fibroblasts, lymphoblasts; epithelial cells; recombinant	virus; double-stranded RNA	2–4 × 10^8	1	20,000	dimer	2,5,9	+	+
γ (IFN-γ)	immune, type II, IFI, ImIF	T lymphocytes; recombinant	foreign antigens: mitogens	>10^8	1–2	17,000	trimer	12	−	+

[a]Assembled from publications by Pestka & Baron (13), Sikora (14), Pestka (15), and Knight (16).
[b]Refers to natural interferon; recombinant interferons are not glycoslated.

already been constructed and others will certainly be engineered with additions, deletions, substitutions, and overlaps. Species specificity and function of individual interferons can be altered by hybridization; for example, HuIFNr-A and HuIFNr-D produce an antiviral state in human but not in murine cell lines. However, HuIFNr-AD, one of four hybrids formed from these two recombinant interferons, is antiviral in murine cells (18, 19). Hybridization offers the hope that interferons can be tailored to elicit desired cellular activities without producing many of the undesired effects.

CELLULAR SOURCES OF INTERFERON[4]

Although interferon appears in the lungs, liver, brains, and other tissues of animals treated with appropriate inducing agents, the major production of the three main types of interferon occurs in a few cell types: IFN-α in B and null lymphocytes and macrophages, IFN-β in epithelial and fibroblast cells, and IFN-γ in T lymphocytes with the support of macrophages. There are exceptions to this generalization; for example, IFN-γ can be induced in bone marrow T cells with T-cell mitogens, and some IFN-β can be induced in macrophages. With the appropriate inducer, large granular lymphocytes may produce all three major types of interferon. Relatively large amounts of crude IFN-α have been produced for clinical trials by the Finnish Red Cross from interferon-primed, Sendai virus–induced human leukocytes obtained from the buffy coat of donor transfusion blood (23). Clinical trials have been conducted with IFN-α and IFN-β isolated from Sendai virus–induced Namalwa cells (24) and polyriboinosinic acid·polyribocytidylic acid (poly IC)-induced human foreskin fibroblasts (25), respectively.

INDUCTION OF α/β INTERFERONS[5]

Three milestones mark the development of the induction of α/β-interferons. The first was the previously cited observation of Isaacs & Lindenmann (7) that led to the discovery of interferon. The second was the realization that interferon can be induced by substances other than viruses. The third was the discovery that microgram quantities of the synthetic double-stranded polyribonucleotide, polyriboinosinic·polyribocytidylic acid (poly IC), induce large amounts of interferon in rabbits and rabbit kidney cells (30).

Interferon inducers can be classified according to source, potency, molecular size, chemical structure, mechanism of induction, types of interferon induced, and the cells and animal species they induce. Baron et al (17) categorize

[4]For reviews see 17, 20–22.

[5]For reviews see 17, 23, 26–29.

interferon inducers into two major classes: α/β inducers and γ inducers. Class A α/β inducers are relatively potent; they include the RNA viruses of animal, plant, insect, fungal, or bacterial origin, DNA viruses, and synthetic double-stranded RNA polymers. Most, but not all, Class B α/β inducers are relatively weak; they include microbes, microbial products, and synthetic chemicals (nonRNA polymers and low-molecular-weight compounds). Inducers of IFN-γ (in immunocompetent cells) are comprised of antigens (in sensitized immunocytes), antibody to OKT3 antigen on mature T lymphocytes, and a variety of mitogens.

Five types of interferon inducers are considered in this section: viruses, microbes and microbial products, synthetic RNA polymers, synthetic nonRNA high-molecular-weight polymers, and synthetic low-molecular-weight chemicals.

Induction by Viruses

Perhaps the most general characteristic of viruses is that with few exceptions they induce interferon in vertebrates and cultures of vertebrate cells. Ho (29) lists 87 viruses representing 15 families and 5 unclassified viruses that have been shown to induce interferon in animals (mammals, birds, reptiles, or fish), cultured cells, or both. The amount of interferon detected depends on (*a*) the virus inoculum (species, type and strain of virus, the particle composition and concentration, and the presence of contaminating substances), (*b*) conditions for production in animal hosts (species, strain, age, sex, route of inoculation, time after inoculation, tissue sampled for assay, temperature of housing, and previous exposure to virus or other inducing agent), and (*c*) conditions for production in cell cultures (species, type of tissues, passage level, "ageing," and contamination by mycoplasma and other agents, whether the cell population is pure or mixed). Descriptions of viruses as "good" or "poor" inducers are not always applicable; manipulation of conditions may convert a "poor" inducer to a "good" inducer or vice versa. Nevertheless, double-stranded RNA viruses are considered to be the best viral inducers. The best single-stranded RNA virus inducers of interferon are the paramyxoviruses, especially Newcastle disease virus or Sendai virus.

A virus need not be infective or (in many cases) even viable to be a good inducer of interferon. Blue-tongue virus, an orbivirus, is pathogenic for sheep but not for man. Both active and heat-killed blue-tongue virus produce high titers of interferon in a large number of cell and animal systems, and therapeutic uses of the virus have been proposed.

A large number of viruses from fungi, bacteria, and plants induce interferon in animals. Many of these nonanimal viruses have double-stranded RNA as their genome. Some are potent inducers.

Induction by Microbes and Microbial Products

A great variety of bacteria, chlamydia, rickettsia, mycoplasma, and protozoa are interferon inducers. Unicellular inducers are commonly intracellular parasites. Their mechanisms of induction are not clear. In the case of gram-negative bacteria, released endotoxin is believed to play a role. Double-stranded RNA can also be a microbial product responsible for interferon induction; for example, the antiviral properties of extracts of *Penicillium funiculosum* were shown to be due to double-stranded RNA found in the fungus. Mannan, a polysaccharide from *Candida albicans*, induces circulating interferon (probably IFN-γ) in mice. Other microbial interferon inducers include a capsular polysaccharide from *K. pneumoniae*, a B-cell mitogen from *Nocardia*, glycoprotein from Sendai virus, and the cell wall and ribosomal fractions of mycobacterium tuberculosis strain BCG.

Induction by Synthetic RNA Polymers

The synthetic RNA polymers rival the viruses as potent inducers of interferon. Although many of these polymers have been synthesized and tested, the molecular requirements for activity have not been completely defined (31–33). A double helix of high molecular weight (10^5daltons) is important. Sequences of the primary bases may be critical, but the 2'OH position on the ribose is crucial. Substitution at the purine N-7 and the pyrimidine C-5 positions diminishes activity. A high melting point is also important. Poly IC is an excellent inducer of interferon in laboratory rodents but a poor inducer in monkeys, chimpanzees, and humans because they possess high levels of serum ribonuclease. High titers of interferon are induced with poly ICLC, a hydrophilic complex of poly IC with poly-L-lysine and carboxymethylcellulose, because it resists hydrolysis by ribonuclease (34, 35).

The toxicity and refractiveness of the double-stranded RNA polymers have restricted their clinical application. Many of the toxic manifestations (leukopenia, fever, headache, nausea, lethargy, insomnia, and changes in hematopoietic and liver functions) are those that occur after administration of interferon (36, 37). However, Ts'o and colleagues (38, 39) have synthesized "mismatched" analogs of poly IC that induce high interferon titers with relatively low toxicity. They found that uracil or guanine interspersed into the poly (C) strand retained the ability to induce interferon if the frequency of random insertions did not exceed 1 residue in 12, a condition that preserves the 0.5–1 helical structure required for the triggering of interferon synthesis. Poly (I)-poly ($C_{12}U$) is undergoing clinical trials (39).

Induction by nonRNA Polymers[6]

Polycarboxylic polymers (pyran, polyacrylic acid, polymethacrylic acid), polysulfates (polyvinyl sulfate), and polyphosphates (polyphosphorylated polysaccharides) induce interferon in mice. The most studied of these polymers is pyran, a polymer of maleic anhydride and divinyl ether with a molecular weight of about 17,000. Its antiviral activity is much greater than can be explained by the small amount of interferon it induces. It is not degraded or excreted and it is prohibitively toxic. The diverse effects of these polymers, including the induction of interferon and its disproportionate antiviral activity relative to interferon induction, may result from the activation of macrophages.

Induction by Low-Molecular-Weight Chemicals[7]

Low-molecular-weight interferon inducers are an odd assortment of chemically diverse agents. Except for the antimetabolites (cycloheximide, streptovitacin A, streptimidone, and tenauzonic acid), little is known about their mode of action. Cyclohexane and other compounds that inhibit RNA synthesis are believed to induce interferon by reducing the critical concentration of a repressor that normally prevents the expression of an interferon gene. Many of the structurally unrelated chemical inducers are active in cell cultures, usually of macrophages or lymphocytes.

Tilorone is the best known of 800 bis-basic low-molecular-weight compounds synthesized in an unsuccessful attempt to create a clinically useful interferon inducer (43). High serum interferon titers were observed after the oral administration of tilorone to mice; a lesser inductive effect was seen in rats, and very little effect was seen in rabbits, hamsters, ferrets, cats, dogs, and unfortunately, humans. It is highly reactive in immunoregulation, but toxic. Tilorone intercalcates into DNA (44), an observation that could lead to an understanding of its mechanism of action. Tilorone is not effective intravenously. Other tricyclic compounds that have received considerable attention as interferon inducers are quinoline derivatives [e.g. BL-20803, 1,3-dimethyl-4-(3-dimethylaminopropylamino)-1H-pyrazolo-(3,4-b)-quinoline], anthraquinone derivatives e.g. 1-5-bis[(2-(diethylamino)ethyl)amino]-anthraquinone, and cationic dyes (e.g. toluidine blue, methylene blue, trypaflavine, and acridine orange). 10-Carboxymethyl-9-acridanone is a potent inducer of interferon that can be administered parenterally or orally.

Of substituted pyrimidines tested, 7 proved to be relatively good inducers of interferon in mice when given either parenterally or orally. Of these, 2-amino-5-bromo-6-methyl-4-pyrimidine (U-25;166) offered some promise as a therapeutic agent.

[6]See reviews in 17, 40–42.

[7]See reviews in 17, 26, 27, 29, 43.

Nasal applications of the propanediamine CP-20,961 and the xylenediamine CP-28,888 were effective in humans challenged with rhinovirus; interferon was detected in their nasal secretions.

Several radioprotective thiols induce interferon, the most active of which are 5,2-aminoethylisothiouronium (AET) and 3-aminopropylisothiourea.

INDUCTION OF γ-INTERFERON[8]

The target cells for induction, chromosomal affiliation, and the structural and physical nature of IFN-γ differ markedly from those of IFN-α and IFN-β. It is therefore not unexpected that the inducers of IFN-γ should also differ markedly. Far fewer substances are known to induce IFN-γ; they include mitogens, bacterial and viral antigens, allogeneic cells, and antisera directed against surface components.

Early work showed that exposure of animals or lymphocytes from animals that had been sensitized to specific antigens resulted in the production of IFN-γ. Later work showed that the target cells are specific subsets of T cells—e.g. specific cytotoxic T cells. These cells produce IFN-γ in culture when they are derived from animals previously sensitized to a viral or nonviral antigen (e.g. tetanus toxoid, diphtheria toxoid, tubercular PPD). Cell membrane–active substances that induce IFN-γ include phytohemagglutinin, concanavalin A, pokeweed antigen, streptolysin O, bacterial lipopolysaccharides, anti-lymphocyte serum, monoclones against OKT-3, staphylococcal enterotoxin, staphylococcal protein A, phorbol esters, calcium ionophore, and galactase.

"SPONTANEOUS" INDUCTION OF INTERFERON

Interferon has slowly become recognized as a hormone-like messenger that induces metabolic changes in distant cells (46–48). Since the production of interferon involves the switching on of transcription of interferon genes that otherwise remain dormant, the "hormonal" function of interferon must implicate a true induction process.

The lungs and intestines present vast "external" surfaces that come in contact with exogenous interferon inducers such as heterologous proteins, bacteria, viruses, toxins, allergens, and chemicals (49). Although these substances do not generally elicit detectable levels of serum interferon, interferon produced in this way may be taken up by circulating leukocytes and transported to other sites. Low levels of endogenous interferon are not readily detectable in blood; Bocci and associates (50) found antiviral activity in the abdominal and thoracic lymph of untreated rabbits but not in the plasma or lymph collected from the

[8]For reviews see 17, 20, 45.

hind leg. Evidence for the maintenance of low levels of both endogenously and exogenously induced interferon has been published recently by Galabru and associates (51), who measured serum, spleen, and lung interferon levels in untreated conventional and germ-free mice. They also measured normal and interferon-induced levels of spleen (2'5')oligo(A) synthetase (2-5A synthetase) and lung protein kinase. As will be discussed later, 2-5A synthetase and protein kinase are antiviral proteins induced by interferon and activated by double-stranded RNA. The assays of 2-5A synthetase and protein kinase, which have much longer lives than interferon, are convenient indirect ways of assessing interferon production. Serum interferon levels were not measurable in the great majority of either conventional or germ-free mice, but about 10% showed low levels of IFN-α and IFN-β; these mice also possessed the higher tissue levels of 2-5A synthetase and protein kinase. Relatively high levels of the two enzymes were present in the spleens and lungs of conventional mice; they were about doubled after interferon administration. On the other hand, levels of spleen 2-5A synthetase and lung protein kinase in the tissues of germ-free mice were only about 10 and 7%, respectively, of those seen in conventional mice. Moreover, administered interferon raised the levels of 2-5A synthetase and protein kinase activities in germ-free mice to only 15 and 40%, respectively, of those seen in untreated conventional mice. Thus it would appear that low tissue levels of interferon are induced continuously by both internal and external agents. Perhaps more importantly, these studies suggest that external agents may be required for full inductive responses of 2-5A synthetase and protein kinase to interferon.

Low levels of IFN-α have been detected consistently in human amniotic fluid in the absence of viral or other infections (52–54). Lebon and associates (53) reported the presence of interferon in the amniotic fluid of 60 women during their 16th to 38th week of pregnancy. The absence of detectable levels of interferon in the sera of mothers not only tends to exclude a maternal contribution of interferon to amniotic fluid but suggests that amniotic interferon is not transported into the mother's blood.

Relatively high levels of interferon have been detected in the uterus, placenta, and fetus of healthy, pregnant mice (52). Levels of placental interferon increase throughout gestation; the level in the uterus peaks at 13 days and disappears by parturition. Interferon was not detectable in the fetus except at 10 days of gestation when contamination from extra-embryonic tissues is difficult to avoid.

The suggestion has been made that amniotic interferon is more involved in the regulation of fetal development and the immunoregulation of fetal acceptance than as an antiviral agent. The presence of interferons in the placenta and amniotic fluid supports the view that the most important role for some species of interferon could be the regulation of normal (or even abnormal) cellular processes (55).

Circulating interferon has been detected in acquired immune deficiency syndrome (AIDS), systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis in cases where virus does not appear to be the inducer (56). Levels of interferon of 90–159 units/ml of plasma have been found in apparently normal, healthy humans (57).

PRODUCTION OF INTERFERON BY TUMOR CELLS

Tumor cells, like normal cells, produce interferon when exposed to viruses and other interferon inducers (58–60). In fact, lymphoblastoid cells induced with Sendai virus are used commercially for the large-scale production of interferon (61). Of 19 lymphoblastoid cell lines tested, 11 were found to produce interferon spontaneously; of the remaining 8, 4 did not produce interferon in response to Sendai virus (62). The spontaneously produced interferons were shown to differ from virus-induced interferons in molecular weight and antigenicity. β-Lymphoblasts and other leukocyte cell lines also generate interferon spontaneously. While the mechanism of spontaneous induction of interferon in tumor cells is not known, one might postulate that many of the genes that remain dormant in normal cells, including interferon genes, are derepressed in the poorly differentiated tumor cell. Alternatively, tumor cells may produce interferon inducers. That this may be the case is suggested by the observation of Trinchieri and associates (63) that peripheral blood lymphocytes produce interferon when cultured with tumor cells. Although several investigators have suggested that natural killer (NK) cells are responsible for tumor-induced interferon, Weigent et al (64) showed that enriched human B-cells, cultured with xenogeneic or allogeneic tumor cells, produced 1,000–10,000 units of interferon per milliliter. Lackovic and co-workers (64a) identified IFN-α in the peritoneal cavities of mice inoculated with allogeneic Ehrlich ascites cells. They suggested that the interferon was produced by macrophages. Djeu and associates (65) showed that conventional or nude mice inoculated with syngeneic or allogeneic tumor cells rapidly develop serum interferon levels that peak within 24 hr. Timonen and associates (66) conclude that NK cells are responsible for the interferon induced in human lymphocytes by tumor cell contact.

Relatively high levels of interferon in the plasma of malignancy patients have been reported recently (57).

MECHANISM OF INDUCTION OF IFN-α AND IFN-β BY VIRUSES AND POLYRIBONUCLEOTIDES[9]

Isaacs (72) postulated in 1963 that a "foreign nucleic acid" was responsible for interferon induction. Four years later, Field et al (73) discovered that synthetic

[9]For reviews see 67–71.

polyribonucleotides were comparable to viruses in their ability to induce interferon. It is now generally accepted that viruses and polyribonucleotides induce interferon by the same mechanism. While there are still many unanswered questions regarding the mechanism, it is firmly established that interferon synthesis results from the switching on of transcription that occurs when dormant interferon genes are derepressed.

Space restrictions do not allow a discussion of the several hypothetical models that have been proposed through the years for the induction of interferon. The recent working model (Figure 1) of Marcus (71) was selected for this overview because it accommodates many of the contradictions that have pervaded earlier hypotheses and because it is likely to stimulate research that will challenge its many speculative features. The major divergence of this model from its predecessors is the assignment of a role of inducer-receptor to (2'5')oligo(A) synthetase (2-5A synthetase) and protein kinase, the two enzymes directly involved in the impairment of viral replication.

Credibility of the working model depends on several observations and speculations:

1. Induction requires new cellular RNA and protein synthesis.

2. Double-stranded RNA of viral or synthetic origin introduced to or formed in the cell, is the proximal inducer.

3. A single molecule of double-stranded RNA provides the threshold for the induction of a quantum (finite) yield of interferon. This important observation was made by Marcus and associates in carefully conducted and interpreted dose (virus)-response (interferon production) studies. These dose-response curves showed that the simultaneous introduction of a second virus particle suppressed the interferon-inducing activity of the first virus particle. This would explain why cells become refractive to interferon induction by viruses and other inducers, why a large amount of exogenous interferon can prevent induction, and why some double-stranded viruses do not induce measurable amounts of interferon (they induce too much interferon too rapidly).

4. An interferon inducer-receptor is formed when double-stranded RNA interacts reversibly with 2-5A synthetase or protein kinase. The interferon inducer-receptors are the actual inducers; they derepress interferon genes. The synthetase and kinase interferon receptors may function specifically for IFN-α and IFN-β gene banks, respectively.

5. The cell must harbor basal levels of 2-5A synthetase and protein kinase. Helical regions of heterologous nuclear (hn) RNA may induce low levels of "endogenous" interferon, thereby maintaining basal levels of the two receptor enzymes. Low basal levels of 2-5A synthetase and 2-5A per se have been observed in normal and regenerating livers of non-induced rats. A radioimmune assay has been used to show the presence of 2-5A in the liver, kidney, and spleen of untreated and germ-free mice (51). Basal levels of interferon inducer-

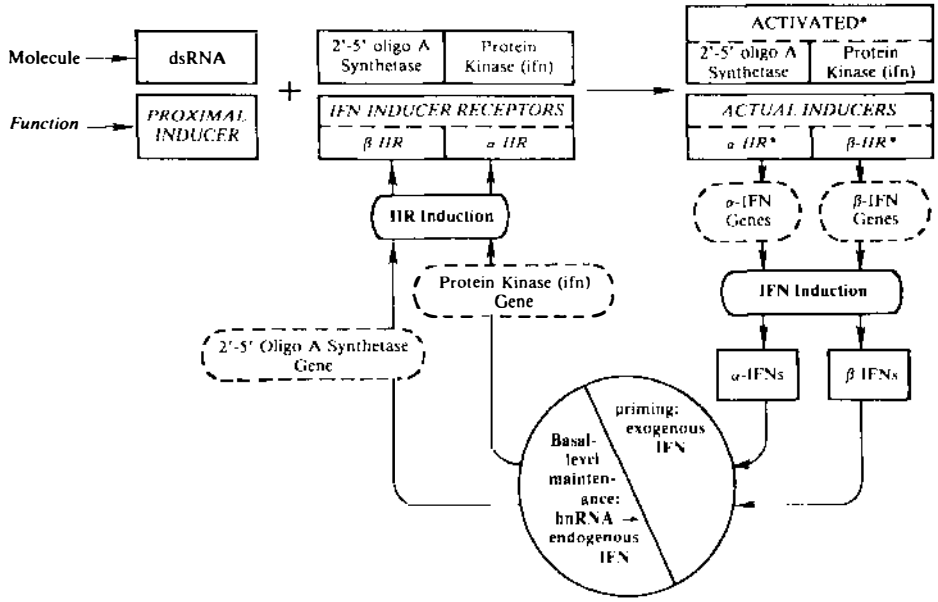

Figure 1 A schematic representation of the working model proposed for the mechanism of interferon induction by viruses (double-stranded RNA). The various elements of the model and their postulated functions are described in the text. Reprinted with permission from (71).

receptors and turnover rates must be high enough that induction occurs even in the presence of only one molecule of double-stranded RNA per cell.

When cells are treated with small amounts of interferon prior to exposure to viruses, induction occurs earlier and much larger amounts of interferon are produced. The procedure, known as "priming," has been used to increase the amounts of interferon produced by human leukocytes for use in clinical trials. Priming is attributed to a shortened induction phase because transcription of mRNA occurs earlier than in control cells. The concentrations of both 2-5A and protein kinase are increased in cells primed with interferon. In accordance with the model (Figure 1), the consequences of this would be an accelerated production of activated 2-5A synthetase and protein kinase and therefore a shortened lag phase for interferon induction.

MECHANISM OF INDUCTION OF γ-INTERFERON[10]

The mechanism of induction of IFN-γ is not known. Perhaps the molecules that induce IFN-γ (mitogens, antigens, etc) substitute for double-stranded RNA as

[10]For review see 45.

the proximate inducers in systems similar to those involved in the induction of IFN-α and IFN-β.

INTERFERON RECEPTORS[11]

When molecules of interferon leave a cell they attach to receptors in the adjacent cells or they enter the circulation and combine with receptors of distant cells. Receptors are localized randomly on the cell surface (75) or on coated pits (76). Estimates of the number of receptors range from 650 to 20,000 per cell (77). There is conflicting evidence about whether interferon must be internalized to act, but the consensus is that even if this does occur it is not a requirement. Interferon may bind directly or produce aggregates of receptors. Mouse IFN-α and IFN-β share and compete for the same receptors, but IFN-γ occupies different receptors. Nevertheless, genes for the receptors for all three major types of human interferon are located on the distal segment of the long arm of chromosome 21 (45, 77). The degree of sensitivity to interferon relates to the number of duplications of chromosome 21; thus children with Down's syndrome (trisomy 21) have an exaggerated sensitivity to the effects of interferon (77).

The role of gangliosides in interferon binding is not established but is suggested by the observations that Sepharose-bound interferon loses its antiviral properties when preincubated with gangliosides and that the addition of gangliosides to ganglioside-deficient mouse cells prior to treatment with interferon increases antiviral response (78). Inhibition of the antiviral properties of Sepharose-bound interferon is reversed by sialyl lactose, which indicates that interferon does not bind to the ceramide portion of gangliosides but to the polysaccharide end residues containing sialic acid and lactose. Gangliosides may function as low-affinity discriminators that may be involved in some interferon functions and not in others.

Interferon increases the concentration of cAMP in cells, but not in every cell type in which interferon is induced (79, 80). This suggests that the elevation of cAMP content is a consequence rather than a component of induction.

MECHANISMS OF ANTIVIRAL ACTIVITY OF INTERFERON[12]

Almost everything known about the biochemical mechanisms of the antiviral action of interferons has been derived from experiments with cell extracts. One

[11]For reviews see 17, 55, 74.

[12]For reviews see 68, 70, 81–84.

of the most provocative of these studies was made by Friedman and associates (85, 86), who suggested that interferon may create a latent antiviral state in cells that can be triggered by virus infection. These studies were relevant to the earlier observation by Kerr et al (87) that cell-free extracts from interferon-treated cells were extremely sensitive to inhibition of protein synthesis by double-stranded RNA. Since double-stranded RNA circumvented the need of viral infection for an interferon-induced translational blockade, it raised speculation that it might signal the antiviral state. Roberts et al (88) reported that a low-molecular-weight inhibitor of protein synthesis was generated when extracts of interferon-treated mouse L cells were incubated with ATP and double-stranded RNA. Kerr & Brown (89) determined the low-molecular-weight inhibitor to be 2-5A, a member of a new class of 2'5' isoadenylates that have novel 2'5' phosphodiester linkages between the riboses of the adenylic acid rather than the usual 3'5' linkages. This class of oligonucleotides promises to have regulatory functions that extend far beyond its involvement in antiviral activity. The 2-5A-dependent endonucleases also appear to be a ubiquitous component of mammalian cells, whether cultured or obtained directly from the animal. A variety of chemicals and glucocorticoids that do not induce interferon increase levels of 2-5A synthetase (e.g. butyrate, DMSO, phenobarbital, hydrocortisone, dexamethasone, and cortisol).

2-5A-Dependent antiviral activity involves at least three enzymes: 2-5A synthetase, 2' phosphodiesterase and endonuclease (Figure 2). Two activation steps occur; double-stranded RNA-activation of 2-5A synthetase and activation of inactive endonuclease by the 2-5A released by the activated synthetase. Two synthetases have been identified, one in the cytoplasm, the other in the nucleus; conceivably, they may regulate different events, depending on whether the activating double-stranded RNA is nuclear or cytoplasmic. Phosphodiesterase is present in nonlimiting concentrations relative to 2-5A but it is also induced by interferon in some systems.

One of the first questions raised by the discovery of the 2-5A system was whether it degrades viral RNA specifically. Some reports support one side, some the other, and still others support both sides of this question. However, while the question is not resolved, the preponderance of available evidence suggests that the 2-5A-dependent endonuclease cleaves both host and viral RNAs. If interferon can affect both nucleic acid and protein synthesis, why does it not kill cells as well as prevent virus multiplication? Burke (90) offers three explanations that do not require a specific biochemical action of the 2-5A system: (*a*) Virus replication is an exponential process; therefore a small effect on an early event can have a magnified effect on subsequent stages; (*b*) Interferon is induced in large amounts only in cells that are infected with virus; these cells may in fact die because the synthesis of host proteins is impaired, but most cells are not infected with virus and will not die. Interferon-inflicted cell

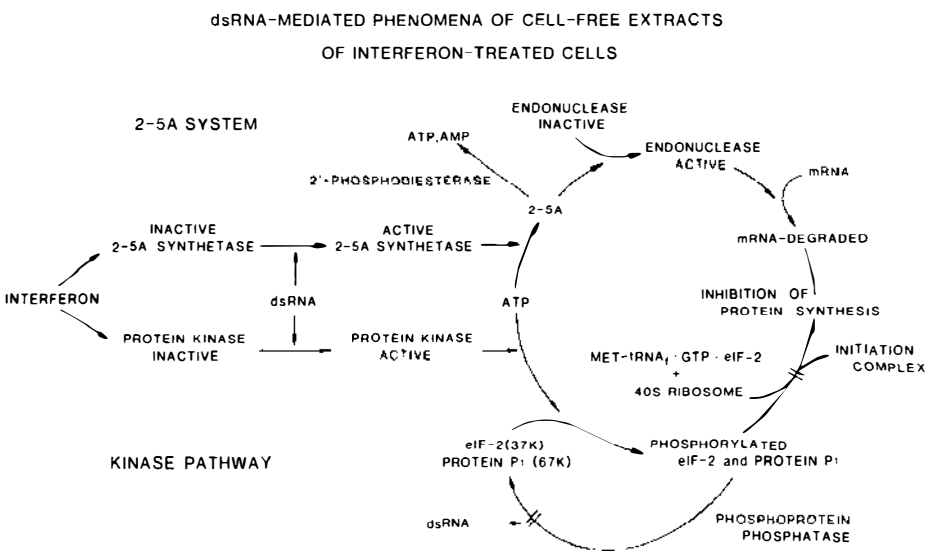

Figure 2 The role of induced enzymes in the inhibition of translation caused by double-stranded RNA. Reprinted with permission from (84).

death may in fact be a first line of defense against the spread of a virus infection; (*c*) Viral mRNAs may have a higher affinity than cellular RNAs for components of the protein-synthesis machinery of the cell and may therefore be more strongly affected by interferon treatment. To this may be added the comment that the RNAs involved in an exponentially expanding process are turning over more rapidly than those involved in processes that have achieved a steady-state status (normal cellular processes) and therefore might be expected to be more involved in the antiviral process than cellular macromolecules. This could also explain putative differential effects of interferon on exponentially replicating tumor and fetal cells in vivo.

Discovery of a second double-stranded mediated inhibitor of protein synthesis occurred about the same time as that of the 2-5A system (69, 81, 84). Lysates from control or interferon-treated rabbit reticulocytes were incubated with or without double-stranded RNA and ATP. Enhanced phosphorylation of two proteins was seen only in lysates that had been exposed to both interferon and double-stranded RNA. The smaller (37K) of the two was identified in several laboratories as the smallest subunit (α) of initiation factor eIF2. The larger (67K) proved to be a cAMP-independent protein kinase. As shown in Figure 2, initiation factor eIF2 is phosphorylated when protein kinase is activated by double-stranded RNA. This impairs the participation of eIF2 in the formation of the 40S initiation complex, and protein initiation synthesis is inhibited.

All three major classes of interferon induce the kinase. Kinase activity is enhanced in the liver, spleen, and plasma of mice injected with Newcastle disease virus, EMC virus, poly IC or polyriboadenylic acid·polyribouridylic acid (poly AU). The enhancement of kinase activity by poly AU is of interest because this polyribonucleotide does not induce interferon (91, 92).

Enzymes other than 2-5A and protein kinase may be involved in antiviral and other effects of interferon. One- and two-dimensional gel electrophoreses have revealed a number of unidentified polypeptides in cells that appear simultaneously with the antiviral state (84).

An antagonist of 2-5A such as p5'A2'p5'a2'p5'A might be useful in the treatment of what have been termed "interferon-induced diseases" if it were able to penetrate the eukaryotic cell and not be rapidly degraded by the 2'5' diesterase. If antagonists of this type can be chemically modified to overcome these problems, they must also be able to bind to the endonuclease.

PHARMACOKINETICS[13]

The fate of endogenous or exogenous interferons is determined largely by the type of interferon, the site of its induction or administration, and the amount. The very small amounts of interferon produced more or less continuously in organs such as the lung probably do not enter the circulation unless perhaps, they are transported by leukocytes. Interferon is probably diffused through extracellular fluid (paracrine excretion) to neighboring cells and catabolized. Some of this interferon appears to enter the lymph system. Viral infections induce large amounts of interferon which flood most of the body fluid pools. This interferon has a short half-life in the circulation owing to its binding to cellular receptors throughout the body and its catabolism by the kidney and liver. The immediate delivery of interferon to target cells must be paracrine in nature.

Intravenously administered IFN-α disappears rapidly from the plasma, with less than 0.1% of large doses remaining in the circulation after 24 hr. The relatively small size of the interferon molecule allows it to be filtered by the renal glomerulus; it is reabsorbed almost quantitatively by the tubules, where it is destroyed by proteolysis (97). Bocci (96) estimates that the kidneys eliminate at least 2% of the plasma IFN-α/min, thus clearing the plasma pool in less than an hour. Megadoses of interferon can overcome this rapid loss, but high plasma levels produce severe central nervous system toxicity. Moreover, high interferon levels suppress the immune system, which in some cases may compromise therapy. Intramuscular administration has therefore been the preferred route for IFN-α in clinical trials. Because intramuscular IFN-α enters the

[13]For reviews see 49, 93–96.

circulation primarily through capillaries, plasma levels are less transient and catabolism is less rapid than with intravenous interferon. Peak plasma levels occur in 1–6 hr, remain fairly stable for 6–12 hr and slowly disappear between 18 and 36 hr (96).

The pharmacokinetics of IFN-β and IFN-γ differ markedly from that of IFN-α. IFN-β and IFN-γ do not appear in significant concentrations in the plasma after intramuscular injection, and they are catabolized mainly by the liver rather than by the kidney (98–104). These interferons appear to reach target organs despite their apparent lack of transport by the circulatory system. Thus natural killer (NK) cells are activated as readily by intramuscular IFN-β and IFN-γ as by intramuscular IFN-α (105). This could mean that these interferons are removed slowly from the injection site by the lymphoid system or that circulating NK cells are activated while passing through the injection site. Antiviral activities measured in spleen and lung are similar after the intramuscular injection of IFN-α and IFN-β, despite a 10- to 30-fold difference in their serum levels (98).

The pharmacokinetics of IFN-β and IFN-γ were once believed to differ from that of IFN-α because IFN-β and IFN-γ are glycosylated and therefore attracted to tissues whereas IFN-α is not glycosylated and is therefore more capable of moving into the plasma pool (104). However, it has been shown in rats that the pharmacokinetics of recombinant HuIFNr-β and HuIFNr-γ, which are not glycosylated, do not differ from that of their natural glycosylated counterparts (106). However, in very thorough studies with the rabbit, Satoh and collaborators (103) observed differences in the pharmacokinetic parameters of recombinant and fibroblast-derived human IFN-β which they attributed to glycosylation. Differences in the pharmacokinetics of interferons are probably related to their hydrophobicity (106). All three IFN types of interferon are secretory proteins and have a hydrophobic signal peptide 20–23 amino acids long (107–109), but IFN-α is less hydrophobic than either IFN-β or IFN-γ.

Bocci (96) favors a strategy of delivering interferons via the lymph pool. This would minimize direct transfer of interferon into the blood and thereby maintain more constant tissue levels. The result would approximate that seen with continuous infusion. An estimated half of subcutaneously injected, and even more of intraperitoneally administered interferon would enter the lymph pool.

Only traces of serum interferon reach the central nervous system. Human IFN-β injected intrathecally in monkeys diffused throughout the cerebrospinal canal and reached the serum compartment (98). Some interferon was recovered from the pia mater surrounding the brain hemispheres, but none was found in the deeper layers of the brain. IFN-β injected into patients intrathecally or intraventricularly did not evoke central nervous system toxicity (110–111).

Intraperitoneal injection of partially purified mouse IFN-α + IFN-β produced concentrations in the spleen, liver, and lungs of mice that were about

100-fold greater than could be expected from the amount of serum in these organs (112). The recent availability of large amounts of human interferons has allowed the determination of conventional pharmacokinetic parameters of a variety of interferons administered intravenously to laboratory animals (108, 113). Results are compatible with earlier observations showing that interferon leaves the circulation rapidly, is cleared rapidly by the kidney, and is readily taken up by tissues.

One third of a dose of human recombinant IFN-α (HuIFNr-αA) labeled with ^{125}I was found in the kidney of mice 5 min after its intravenous administration; liver and stomach contained 5.5% and 1.4% of the dose, respectively (114). Sixty minutes after the injection, the kidney had lost three fourths and the liver about half of its interferon. The distribution of ^{125}I-labeled HuIFNr-A/D, a recombinant intramolecular hybrid of HuIFNr-αA and HuIFNr-αD was quite different from that of HuIFNr-αA; e.g., the amount in the liver (24%) was greater than in the kidney (15%). Moreover, not only was the initial concentration of HuIFNrα-A/D in the stomach several fold higher than that of HuIFNr-αA, but unlike that of HuIFNr-αA, it increased with time. This study raises hopes that it may be possible to tailor recombinant interferons to seek out target organs with some selectivity.

The therapeutic usefulness of interferon would be improved considerably if its clearance from the serum could be delayed. This has been accomplished recently by Rosenblum and associates (115) through the use of a murine interferon-specific monoclonal antibody that binds to HuIFNr-αA without altering its in vitro antiproliferative or antiviral properties. The interferon-antibody complex disappeared from the plasma of the intact rat three times more slowly than the free interferon.

NON-ANTIVIRAL EFFECTS[14]

Interferons modify a multitude of seemingly unrelated biological functions both in cultured cells and *in vivo* (Table 2). Some of these effects may be mediated through indiscriminating actions of the antiviral enzymes, 2-5A synthetase and protein kinase, whereas other functions may be elicited by still unidentified mechanisms, possibly triggered by binding to receptors not associated with antiviral activity. These functions may be enhanced or depressed by interferon depending on the biological model, temporal factors, or the concentration of interferon. Enhanced activity need not exclude the participation of the antiviral enzymes. Steady-state levels of enzymes are determined by the net activities of synthetic and degradative enzymes; an apparent increase in synthesis could be due to an interferon-induced depression of degradative processes.

[14]For reviews see 17, 20, 116–127.

Consideration of all, or even many, of the pleiotropic effects of interferon listed in Table 2 is not possible in this overview. The effects of interferon on cell proliferation, cell differentiation, activation of certain leukocytes, and drug metabolism are selected for brief discussion because of their relevance to prevalent therapeutic uses of interferon.

Cell Proliferation[15]

Six years after the discovery of interferon, Paucker and associates (231) described the first non-antiviral effect of interferon when they showed that the growth of mouse fibroblasts was inhibited by crude preparations of mouse interferon. Their observation was greeted with some skepticism because impurities in the crude preparation could have produced the effect. However, when highly purified interferon became available, it was demonstrated in several laboratories that interferon molecules inhibit the proliferation of a wide variety of cell types. Gresser & Tovey (232) were the first to demonstrate that chemically induced, transplantable, and spontaneous tumors of nonviral etiology were inhibited by interferon.

Cell lines have been used for most of the studies of the antiproliferative effects of interferon. Since they are derived from different sources and many have undergone changes during their continuous culture, it is not unexpected that they should exhibit widely different sensitivities to interferon. The concentrations of interferon required to inhibit their proliferation ranges between 0.2 and 10,000 units/ml (roughly 0.002 and 50 ng/ml) (117).

Few controlled studies have been made of the comparative antiproliferative effectiveness of the three major types of interferon. Based on units derived from their antiviral activity, IFN-γ has a greater antiproliferative effect than IFN-β, but this comparison can only be meaningful if IFN-β and IFN-γ have the same antiviral activity in the cells in which they were tested (117). The observation that IFN-γ synergizes the antitumor activity of IFN-α and IFN-β may have clinical significance (233). Several cloned human IFN-α subtypes differ in both their antiproliferative and antiviral activities (15). Interferon has an antiproliferative effect on normal human diploid fibroblasts, human mammary epithelium, and hematopoietic cells of laboratory animals; however, comparisons have not been made of the selective sensitivities of these cells to interferon with those of tumor cells taken from the same human or animal (117).

Most of the studies using asynchronously growing mouse or human tumor cell lines show that interferon lengthens all phases of the cell cycle, with G1 and G2 usually extended more than S. Quiescent cells stimulated to grow by serum or mitogenic factors show extended G1 and G2 or a slowing of entry into S from G1 (117).

[15]For reviews see 116, 117, 127.

Interferon inhibits the synthesis of enzymes and other proteins (Table 2). Induced systems may be particularly affected; for example, steroid- or DMSO-induced hemoglobin synthesis and steroid-induced enzyme synthesis in Friend cells. The inhibition by interferon of ornithine decarboxylase, an enzyme induced by mitogens, growth factors, and tumor promoters, may suggest that interferons can depress the growth and proliferation of cells and modify some of the more complex functions listed in Table 2 by inhibiting the synthesis of proteins and enzymes induced by growth factors (155). This could also contribute to interferon-induced modification of cell differentiation.

Gresser and associates (234) showed that the proliferation of interferon-resistant L1210 cells (later shown to lack IFN-α and IFN-β receptors) was inhibited by interferon in vivo but not in vitro. This was an important observation not only because it revealed that factors other than a direct action of interferon can be involved in the in vivo depression of cell proliferation but also because it led to the demonstration that interferon markedly potentiates natural and immunization-dependent lymphocyte cytotoxicity (128, 235, 236). This experiment and a similar study that used another interferon-resistant cell line (118) suggested that the antiproliferative effect of interferon in vivo was primarily, if not entirely, host-mediated. However, support for a direct action of interferon on cell proliferation came from the observation that interferon affects human tumors transplanted in athymic nude mice, which lack the immunoresponsive cells involved in the antiproliferative process. This experiment does not exclude the possibility that interferon may act primarily by some yet unidentified mechanism (118).

Since a major contribution of the antiproliferative effect of interferon in vivo is host-mediated, the in vitro testing of the antiproliferative effect of interferon on human tumor cells may not contribute much to therapeutic predictability.

Although the antiviral enzymes 2-5A synthetase and protein kinase may depress replication of viruses and cellular proteins indiscriminately, the destruction of viruses may be favored simply because they are replicating more rapidly than macromolecules of nonreplicating or slowly replicating cells. By analogy, tumor cells, which grow and proliferate more rapidly than most normal cells in vivo, would be affected preferentially by interferon. Although interferon has been shown to have an antiproliferative effect on normal cells in vitro, its depression of proliferation in vivo when administered to adult animals for relatively short periods has not been demonstrated. However, newborn mice injected for 6 days with large doses of interferon died of diffuse liver cell necrosis (217, 218). Liver damage did not occur if interferon injections were begun when the mice were 8 days old. The liver of the newborn mouse is comprised largely of hematopoietic cells, which are replaced by rapidly proliferating hepatocytes during the first week after parturition. The inhibition of liver regeneration in partially hepatectomized adult rats is another example of a preferential effect of interferon on rapidly dividing cells (132).

Table 2 Effects of interferon and interferon inducers[a]

Effect upon:	Change in vivo	Change in vitro	References
A. Cell Physiology			
Cell growth		↓	127–29
Induction of cell differentiation		↑ ↓	125, 127
Bone marrow cell proliferation		↓	130
Hematopoietic colony formation	↓	↓	131
Mitotic response to partial hepatectomy	↓		132
Expression of malignant phenotype		↓	133
Expression of carcinoembryonic antigen		↑	134
Cell locomotion		↓	135, 136
Pinocytosis		↓	136
Negative electrophoretic mobility		↑	137
Beat frequency of myocardial cells		↑ ↓	138, 139
Excitability of cultured neurones		↑	140
Thymidine uptake		↓	141
Iodine uptake in thyroid cells		↑	142
Saturation of 18-carbon fatty acids		↑	143, 144
Cholesterol and phosphatidylcholine synthesis		↑	145
Lipid synthesis		↓	146
Phospholipid synthesis	↓		147
Prostaglandin synthesis		↑	148, 149
HLA synthesis in melanoma cells		↑	150
Melanogenesis in melanoma cells		↓	151
Priming for interferon synthesis		↑	129, 152
Synthesis of new proteins		↑	153
"Overall" protein synthesis		↓	153
DNA synthesis		↓	154, 155
Globin mRNA		↑	156
Degradation of mRNA		↑	89
Degradation of c-MYC mRNA		↑	157
Release of plasminogen activator		↓	158
Induction of autoimmune disease	↑		159
Radioprotective activity (X or gamma rays)	↑		160, 161
Cytotoxicity of dsRNA	↑	↑	129
B. Enzyme Systems			
Xanthine oxidase	↑		162–164
Aldehyde oxidase	↓		165
2' 5' Oligoadenylate synthetase		↑	89
Poly(ADP-ribose) polymerase		↓	166
Creatine kinase	↑		167
Thymidine kinase	↓		141
Glycerol-3-phosphate dehydrogenase	↓		168
Δ^4_3 Ketosteroid synthesis		↑	80
tRNA methylase	↑		169
Prostaglandin E synthetase		↑	170, 171

Effect upon:	Change in vivo	Change in vitro	References
Steroid-inducible tyrosine amino transferase	↑ ↓		172, 173
Glutamine synthetase		↓	174
Ornithine decarboxylase		↓	155
Guanylate cyclase	↑	↑	175, 176
Adenylate cyclase activity		↑ ↓	79, 177
Indoleamine 2,3,dioxygenase	↑		178, 179
Tryptophan dioxygenase	↑		180
Cytochrome P-450 (uninduced, phenobarbital-induced, 3-methylcholanthrene-induced)		↓	181–183
Cytochrome b_5		↓	180, 181
NADPH cytochrome P-450 reductase		↓	184
Aryl hydrocarbon hydroxylase	↓	↓ ↑	185–187
Aminopyrine N-demethylase	↓	↓ ↑	183, 186, 188
Ethylmorphine N-demethylase	↓		181, 184, 188
Aniline hydroxylase	↓		181, 184, 188
p-Nitrophenetole deethylase	↓		183
Heme oxygenase	↑		180
ALA synthetase	↑		180
Catalase	↓		180
C. Cell Membrane			
Density of plasma membrane		↓	189
Microfilament network in submembrane		↑	135
Rigidity of membrane		↑	135
Association of actin in membrane		↑	136
Number of intermembrane particles		↑	189
Movement of membrane receptors		↓	135
Expression of surface antigens		↑	190, 191
Proportion of saturated acyl side chains in membrane phospholipid		↑	143
Cholera toxin and TSH binding		↓	192
Lectin binding		↑	193
Transport of small ions and molecules		↑	194
Transport of thymidine and uridine across membranes		↓	195, 196
Net negative charge		↑	137
D. Immune Systems			
Fc receptor expression		↑	197, 198
Natural killer cell activity	↑	↑	129, 199, 200
H-2 antigen expression	↑	↑	201
HLA + β_2 antigen expression		↑	202, 203
I-region antigen		↑	204
Antibody formation	↓	↓	129, 201, 205
Delayed-type hyperactivity	↓		206

Table 2 *(continued)*

Effect upon:	Change in vivo	Change in vitro	References
Experimental allergic encephalomyelitis		↓	207
Fc receptor–mediated macrophage phagocytosis	↑	↑	208
Cytotoxicity of sensitized T cells		↑	209
Cytotoxicity of monocytes		↑	210, 211
Cytotoxicity of M. L. R.		↑	212
Graft vs host reaction	↑ ↓		206, 213
Generation of allospecific suppressor T lymphocytes		↓	214
E. Whole Animal			
Embryo toxicity	↑		187, 215, 216
Neonatal mortality	↑		216, 217
Liver glycogen	↓		172
Progressive glomerulonephritis	↑		218, 219
Abnormal tubular profiles in hepatic endoplasmic reticulum	↑		220
Neonatal liver necrosis	↑		216
Mitotic activity of hepatectomized livers	↓		132
Regeneration of hepatectomized liver	↓		221
Steroid dependence	↑		222
Survival time of lethally X- and γ-irradiated mice	↑		160, 161
Hexobarbital sleeping time	↑		184, 223, 224
Globules in kidney tubule	↑		49, 95, 219
Autoimmune disease	↑		159
Ascorbate synthesis	↓		182
Chemical carcinogenesis	↓		225
EMC virus–induced diabetes	↓		226, 227
Morphine addiction	↓		228
Analgesia + catalepsy	↑		229
Amplitude of EEG waves	↑		230

[a]For reviews see 116–27.

Cell Differentiation[16]

The earliest demonstration of the effect of interferon on cell differentiation was made by Chany & Vignal in 1968 (133) when they observed that a mouse cell line transformed by a Maloney strain of Sarcoma virus (MSV) reverted to its normal phenotype after prolonged culture with a crude interferon preparation. These reverted cells no longer form colonies in agarose nor do they induce tumors in mice.

[16]For reviews see 123–127.

Depending on the experimental model, interferon can inhibit or enhance spontaneous or induced differentiation of normal cells or tumor cells (Table 2). In some cases this can be accomplished with very low concentrations of interferon; for example, morphological differentiation of insulin-induced murine 3T3 cells can be inhibited with a concentration of interferon as low as 1 unit/ml (123). Differentiation requires a programmed change in gene expression that produces specific proteins associated with particular, terminally differentiated cell types. An example of this was seen during the conversion of mouse fibroblast 3T3-L1 cells to adipocyte cells (123). Levels of 10 proteins were increased and 2 were decreased; interferon prevented these changes. Total protein synthesis was not affected by interferon. Thus interferon appears to arrest the pattern of increases and decreases in synthesis of specific proteins without affecting total protein synthesis. The effects of certain enzymes and other proteins that are known to increase or decrease during spontaneous or induced differentiation of normal and tumor cells have been reviewed recently by Grossberg & Taylor (123) and Sreevalsan (122).

The detection of interferon in the amniotic fluid, uterus, and placenta of normal individuals raises the suspicion that interferon may play a role in the differentiation of developing fetal cells (53, 54).

Immunoresponsive Cells[17]

Interferon modifies the functions of NK cells, T cells, B cells, and monocytes. Interferon and interferon inducers such as poly IC, bacteria, and alloantigens enhance NK activity by a protein synthesis–dependent mechanism that increases the ability of NK cells to bind to and lyse virus-infected and tumor targets (241). Interferon increases NK activity by recruiting NK precursors and by increasing the lytic rate and recycling capacity of target-binding cells (197–198). This increased human NK cell activity is enhanced by interferon both in vivo and in vitro (199).

Interferon enhances target-specific T-cell cytotoxicity (209) and the cytotoxic phase of the mixed leukocyte reaction (212, 242); the proliferative phase of the reaction is inhibited. Similar antiproliferative but cytotoxicity-enhancing effects of interferon occur in mitogen-stimulated cell cultures (147, 243). Interferon inhibits the generation of T suppressor cells from mixed leukocyte cultures (214) but activates those that act on concanavalin A–stimulated cell cultures, immunoglobulin (Ig) production, and mixed leukocyte cultures (244).

Low doses enhance and high doses suppress Ig synthesis during activation of polyclonal B cells by pokeweed mitogen (245–246). Pretreatment of the lymphocytes with interferon enhances Ig production, but treatment after exposure to the mitogen has the opposite effect (247–249).

Cytotoxic (209, 210, 250) and phagocytic functions (208) of monocytes are

[17]For reviews see 17, 237–240.

enhanced by interferon in normal cells; as with T cells, suppressor function is depressed. Suppression of NK activity by monocytes is highly sensitive to interferon (211). The net effect of in vivo enhancement and suppression of T cells and monocytes remains to be delineated; the outcome may depend on the time and dose of interferon.

The nature and variety of the immunostimulatory and immunoresponsive effects of interferon (Table 2) justify the inclusion of all three major types of interferon as bona fide components of the immune system. In fact, immunomodulation of immunoreceptive cells may be the principal function of IFN-γ, which by definition qualifies both as a lymphokine and an interferon. IFN-γ may in fact be required for the maintenance of the physiological level of NK cell activity (238).

Drug Metabolism[18]

The biotransformation of drugs and other xenobiotics occurs primarily in the liver through oxidation, reduction, hydrolysis and conjugation. The oxidations are mediated principally by the cytochrome P-450 (P-450, monooxygenase; mixed function oxidase; MFO) system, which is comprised of NADPH P-450 reductase, NADH-cytochrome b_5 reductase, cytochrome b_5 and several P-450 isozymes of low substrate selectivity (254). The products formed by P-450-linked oxidations are usually more polar, water soluble, and excretable by the kidney than their antecedents. They are therefore less therapeutically effective or toxic, although in a few cases they may be more active (e.g. increased carcinogenicity of certain polycyclic hydrocarbons). The duration and intensity of action of individual drugs are determined largely by the activity of the P-450 system. A large number of xenobiotics induce their own oxidative biotransformation and that of other xenobiotics. A few others depress the P-450 system via suicidal reactions, through the formation of reactive intermediate metabolites that bind strongly to P-450, by acting as substrate inhibitors, or more relevantly to this overview by responding to immunomodulatory agents. Induction and depression of the P-450 system can alter the therapeutic effectiveness or toxicity of drugs markedly.

The concept of a special mechanism for the detoxification of foreign chemicals developed about the turn of this century at a time when there was a great deal of excitement over the new disciplines of microbiology and immunology. The concept, termed the "chemical defense hypothesis" (255), envisioned a general protective mechanism that dealt with toxic chemicals in a manner comparable to those involved in natural resistance to invading microorganisms and viruses. The hypothesis was challenged when it was found that certain xenobiotics became more toxic through biotransformation. (In this context,

[18]For reviews see 251–253.

note that allergies and autoimmune diseases are adverse immune responses.) Not only does the P-450 system qualify as a "chemical defense" mechanism of sorts, it also responds to a variety of modulators of the immune system (Table 3). In addition to inducing interferon, the immunomodulators listed in Table 3 exhibit a broad spectrum of biological activities, many of which are related to host immunity: (*a*) alteration of reticuloendothelial activity; (*b*) antiviral, antifungal, antibacterial, and antineoplastic activity; (*c*) sensitization of bacterial endotoxin; and (*d*) inhibition of adjuvant-induced arthritis (42). Although all of these agents are interferon inducers, it would seem unlikely that all would depress the P-450 system by the same mechanism. However, it would be a mistake to dismiss interferon as the cause of the depression of drug biotransformation simply on the basis that a given agent is a poor inducer of serum interferon; serum interferon levels are not necessarily reliable indicators of the interferon content of the liver and other organs (256, 301). All of these agents alter drug metabolism when administered in vivo but have no effect when added to microsomal preparations that biotransform drugs.

The first example of the impairment of the elimination of a drug by a host-defense mechanism was provided by Samaras & Deitz (302) in 1953 before P-450 was discovered. They observed that trypan blue, a depressant of the reticuloendothelial system, greatly prolonged the hypnotic effect of pentobarbital. Almost two decades later Munson and associates (223, 224, 280) reported that poly IC, pyran, and zymosan depressed in vivo and in vitro P-450-dependent drug biotransformation. Pyran induced little or no serum interferon and poly IC induced large titers of serum interferon, yet both agents depressed drug biotransformation about equally. This was interpreted to mean that modification of the reticuloendothelial system was indirectly involved in the depression of hepatic drug metabolism rather than a more direct action of interferon. Renton & Mannering (184) and Leeson & associates (303), showed that tilorone, a potent interferon inducer, markedly depressed the P-450 system when administered to rats. The question of whether the depression was due to some unknown property of tilorone or to the ability of tilorone to induce interferon was approached indirectly by studying the effects of a variety of known interferon inducers of widely different structures and molecular weights. All of these agents depressed the hepatic P-450 system (181). The authors suggested that the depression of the P-450 system is a general property of interferon inducers. Immunomodulators that have been shown to affect P-450 systems are listed in Table 3.

Other indirect evidence supports the view that interferon per se is involved in the depression of drug biotransformation. Peak serum levels of interferon appear about 2 hr, 12 hr, and 7 days after the administration of poly IC (304), tilorone (305), and *C. parvum* (306), respectively. The time required for the first indications of depression of drug biotransformation by each of these agents

Table 3 Depression of hepatic cytochrome P-450 systems by immunomodulators

Immunomodulator	Cytochrome or drug biotransformed[a]	Species	Reference
Poly IC	Ethylmorphine, benzo[a]pyrene, aniline, p-nitrophenetole, aminopyrine, hexobarbital S. T., P-450	rat or mouse	180, 181, 183, 187, 188, 223, 224, 251
Poly I and Poly C	ethylmorphine, P-450	mouse	256
Tilorone	ethylmorphine, aniline, aminopyrine, hexobarbital S. T. benzo[a]pyrene, P-450	rat	180, 181, 184, 187, 188
Quinacrine	ethoxyresorufin, ethylmorphine, aniline, P-450	rat	181, 257
Statolon	ethylmorphine, aniline, P-450	rat	181
Endotoxin	ethylmorphine, aniline, benzo[a]pyrene, nitroreductase, aminopyrine, azoreductase, biphenyl, ethoxyresorufin, benzyloxyphenoxazone, hexobarbital S. T., zoxazolamine P. T.	rat or mouse	181, 258, 260–262
CP 20,961	ethylmorphine, aniline, P-450	rat	181
CMA	ethylmorphine, P-450	mouse	unpublished
Maleic anhydride Divinyl ether copolymer (PYRAN)	aminopyrine, aniline, hexobarbital S. T., P-450	mouse	42, 223, 263
Freunds adjuvant *(mycobacterium butyricium)*	benzo[a]pyrene, pentobarbital S. T., ketamine, aminopyrine, paracetamol, P-450	rat, mouse	259, 264–267
Corynebacterium Parvum	p-nitroanisole, hexobarbital, aminopyrine, aniline, antipyrine, benzo[a]pyrene, P-450	mouse or rat	268, 269
Bacillus Calmette-Guerin (BCG)	aminopyrine, aniline, DICD, ethoxycoumarin, benzo[a]pyrene, P-450	rat, mouse	270–272
Bordetella pertussis	ethylmorphine, aniline, aminopyrine, phenytoin, P-450	mouse, rat	181, 273, 274
OK-432	aniline, aminopyrine, pentobarbital S. T., P-450	mouse	275
Lipid A	aminopyrine, aniline	mouse	276
Nocardia cell wall skeleton	aminopyrine, aniline, P-450	rat	277
N-acetylmuramyl-l-analyl-D-isoglutamine (MDP)	aniline, ethoxycoumarin, ethylmorphine, P-450	rat or mouse	278
Peptidoglycan monomer (PGM)	P-450, ethoxycoumarin	mouse	279

Methyl palmitate	hexobarbital S. T.	mouse	280
Zymosan	pentobarbital S. T., hexobarbital S. T.	mouse	280
Dextran sulfate	benzo[a]pyrene, aminopyrine, P-450	mouse	281
Latex	P-450	mouse	252
Colloidal carbon	ethylmorphine, carbon tetrachloride, P-450	rat	282, 283
Schistosoma mansoni	aminopyrine, aniline, benzo[a]pyrene, hexobarbital S. T., zoxazolamine P. T., P-450	mouse	284, 285
Trypanosoma brucei	aniline, p-nitroanisole, anthracene, P-450	mouse	286
Plasmodium berghei	hexobarbital S. T., aniline, p-nitroanisole, P-450	rat	287
Fasciola hepatica	hexobarbital S. T., zoxazolamine P. T., aniline, aminopyrine, P-450	rat	288
Viruses			
Mouse hepatitis virus	aniline, hexobarbital, strychnine, P-450	mouse	289, 290
Mengo virus	ethylmorphine, aniline, P-450	rat	181
Encephalomyocarditis virus (EMC)	aminopyrine, P-450	mouse	291
Newcastle Disease virus (NDV)	aminopyrine, ethylmorphine P-450	mouse	165, 292
Duck hepatitis virus	ethylmorphine	duck	293
Influenza vaccine	benzo[a]pyrene	human, mouse	294
Sindbis virus	tryptophane	mouse	295
Interferons			
Crude mouse Gamma (2000 × 3)	P-450 aminopyrine, diphenylhydantoin	mouse	271, 296
HuIFLr-αA,D or -αAD (50,000)	aminopyrine, P-450	mouse	297, 298
HuIFLr-αA,D or -AD (0.06–48 μg)	zoxazolamine P. T., hexobarbital S. T., ethoxycoumarin, benzphetamine, benzo[a]pyrene	mouse	19
IFN-α crude mouse (50,000)	aminopyrine, benzo[a]pyrene, P-450	mouse	297
IFN-β Calbiochem mouse (50,000)	aminopyrine, benzo[a]pyrene, P-450	mouse	297
IFN-α/β mouse (10,000 × 5 weeks)	ethoxyresorufin, ethoxycoumarin	mouse	299
rIFN-γ mouse (50,000 × 3 days)	p-nitroanisole, P-450	mouse	300
rIFN-γ mouse (5,000 × 3)	ethylmorphine, aminopyrine, P-450	mouse	162

[a]All immunomodulators were administered to animals, and preparations of their livers were assayed for cytochrome P-450 content or drug biotransformation activities, except when indicated by S. T. or P. T., in which cases animals were injected with the immunomodulator and their sleeping time after pentobarbital or hexobarbital, or the paralysis time after zoxazolamine was measured.

corresponds well with the appearance of these peak serum interferon levels (180, 184, 268). A temporal coincidence of interferon production and depressed drug biotransformation was also observed after mice were infected with encephalomyocarditis virus (291). Neither polyriboinosinic acid (poly I) nor polyribocytidylic acid (poly C) induces appreciable amounts of interferon nor do they depress P-450 systems (181). However, when an injection of poly I was followed an hour later with an injection of poly C, as much serum interferon was induced as by poly IC and drug biotransformation was depressed (256, 307). When the order of injection of poly I and poly C was reversed, neither interferon induction nor depression of the P-450 system occurred. Poly IC induces about equal serum interferon titers in C57B1/6J and C3H/HeJ mice, but Newcastle disease virus (NDV) induces only about one tenth as much serum interferon in C3H/HeJ mice as in C57B1/6J mice (308). As anticipated, NDV depressed the P-450 system in C57B1/6J mice but not in C3H/HeJ mice (165, 292).

Proof of the involvement of interferon in the depression of hepatic P-450 awaited the availability of a pure interferon that is active in a laboratory animal. This came in the form of HuIFNr-AD, a recombinant hybrid of HuIFNr-A and HuIFNr-D, which possesses antiviral activity in the mouse (18). HuIFNr-AD depressed the P-450 system in the mouse; HuIFNr-A and HuIFNr-D, which do not induce antiviral activity in the mouse, had little or no effect (19, 297). Pure recombinant mouse IFN-γ also depresses the P-450 systems (162, 300), possibly by a mechanism different from that initiated by IFN-α and IFN-β. When IFN-γ was used, three intraperitoneal injections spaced 3 hr apart were required (162).

The mechanism for the depression of the P-450 system by interferon remains unknown. The involvement of 2-5A synthetase or protein kinase would seem likely. This would require at least a small amount of endogenous double-stranded RNA (Figure 1); but, as discussed earlier, that is not an improbability. P-450 is only one of many examples in a growing list of enzymes and other proteins that are depressed by interferon (Table 2). It is conceivable that the same mechanism may be involved in the depression of most if not all of these proteins. Patterns of biotransformation of substrates and SDS-polyacrylamide gel electrophoresis of apoP-450 show that not all P-450 isozymes are depressed equally by interferon (309) or interferon inducers (310). The turnover rate of P-450 heme was used as an index of the turnover of P-450 to show that poly IC and tilorone depress only the more rapidly turning-over forms of P-450 (311).

Ghezzi and associates have recently proposed a novel mechanism for the depression of the P-450 system by interferon and interferon-inducing agents (163, 164). They observed that these agents increase xanthine oxidase activity in the liver and other tissues several fold. Because xanthine oxidase generates free oxygen radicals and oxygen radicals destroy P-450 (312), they proposed

that interferon depresses the P-450 system because it induces xanthine oxidase activity. Allopurinol, an in vivo inhibitor of xanthine oxidase, partially protected mice against P-450 depression. However, in a more recent study from our laboratory (G. J. Mannering, L. B. Deloria, V. S. Abbott & N. J. Gooderman, unpublished data) the P-450 systems of untreated mice and mice whose hepatic xanthine oxidase activity had been lowered to less than 10% of normal by tungstate treatment responded equally to poly IC.

Studies of the mechanism of depression of the P-450 system are hampered by the lack of an experimental cell model. P-450 is not found in significant concentrations in replicating cells. Moreover, the P-450 present in these cells does not biotransform the xenobiotics of usual interest. This leaves nonreplicating primary hepatocytes as the only practical cell model. Only recently has it been possible to culture hepatocytes for 24 hr or more without great loss of P-450. In an early study, Renton et al (186) reported that a crude interferon preparation induced P-450 in cultured hepatocytes that had lost about 80% of their P-450. In a recent study (S. K. Kuwahara & G. J. Mannering, unpublished data), we showed that poly IC, a partially purified α/β mouse interferon, and recombinant HuIFNrAD did not affect the P-450 system of cultured mouse hepatocytes that had been cultured for 24 hr without appreciable loss of P-450. On the other hand, P-450 (P_1-450) that had been induced by 3-methylcholanthrene while the hepatocytes were being cultured was depressed by mouse interferon and poly IC. The failure of interferon to depress uninduced P-450 in cultured hepatocytes might be explained in two ways.

First, humoral factors not present in hepatocytes but supplied by other cells may be required. Kupffer cells may supply the humoral factor. Dextran sulfate, like pyran and several other insoluble polymers, depresses drug biotransformation (252). The effect is believed to be initiated as a consequence of phagocytosis by macrophages. Peterson & Renton (281) showed that levels of P-450 and aryl hydrocarbon hydroxylase (AHH) activity are not affected when freshly isolated hepatocytes are incubated for 30 min with dextran sulfate. However, when isolated Kupffer cells (sequestered macrophages) were added to the incubation mixture, the P-450 content and AHH activity of the hepatocytes were depressed markedly. The same effect was produced by the supernatant fraction from Kupffer cells that had been incubated with dextran sulfate. The low molecular weight of the humoral factor (less than 12,000 daltons) excluded interferon as the active component of the supernatant fraction. It was not determined whether interferon would have produced the same results in hepatocytes incubated for 30 min (a time increment too short to have had any appreciable effect on the enzymes regulating the steady-state of P-450 in hepatocytes) or whether the humoral factor from macrophages induced interferon in these hepatocytes.

Second, the maintenance of near normal levels of P-450 in hepatocytes may

result from stabilization of the enzyme rather than an enzymically controlled steady state. In this case, the P-450 would not be turning over and its synthesis could not be affected by interferon-induced 2-5A synthetase or protein kinase. The observation that P_1-450 induced by 3-methylcholanthrene during culture was depressed by interferon whereas preexisting P-450 was not (S. K. Kuwahara & G. J. Mannering, unpublished data) supports the suggestion that endogenous P-450 in cultured hepatocytes is not turning over fast enough to be affected by interferon-induced mechanisms that depress protein synthesis.

Evaluation of the effects of interferon and other immunomodulators on drug biotransformation in humans has not received much attention. Interferon has been used clinically for the most part on advanced cancer patients. Under the circumstance, the inclusion of a drug metabolism study during treatment of these patients might seem frivolous. Moreover, the disease itself could complicate interpretation of the results; for example, tumors release "toxohormone" that greatly depresses P-450-dependent drug biotransformation (313–316). However, it is inevitable that chemical antitumor agents will be used in combination with interferon with increasing frequency. These drugs have such notoriously unfavorable therapeutic indexes that even a small depression of their biotransformation could produce toxicity with dire consequences.

Mouse interferon stimulated benzanthracene-induced aryl hydrocarbon hydrolyase activity in fetal mouse secondary cell cultures (185).

Viral and bacterial infections that produce immunomodulatory agents (e.g. endotoxins) are known to depress drug biotransformation (Table 3). It was originally assumed that hepatitis virus depressed P-450 (289, 290) because of associated morphological and biochemical changes in the liver. While this may be the case during advanced stages of the disease, the effect on drug biotransformation seen during early infection is likely due to the interferon induced by the virus. Viruses that do not inflict primary pathological effects also depress drug metabolism (165, 192). There is strong suspicion that much of the considerable day-by-day variation in levels of the P-450 systems seen in laboratory animals may be due to viruses that produce none of the usual overt signs of viral infections. This becomes particularly apparent when unusually low values are not further depressed by poly IC or other immunomodulators.

P-450-dependent benzo[a]pyrene hydroxylase activity in the lungs of mice infected with mouse-adapted influenza virus was only 10% of that of uninfected control mice, whereas no change was seen in the activity of this enzyme in the livers of the infected animals (294). This is of interest not only because it demonstrates that drug metabolism can be affected by an immunomodulator in an organ other than the liver but also because it shows that the effect can be localized.

Viral infections often occur in subjects who are receiving medication for reasons unrelated to the infection or to ameliorate the unpleasant effects of the

infection. Several cases are now on record where virus infection has enhanced drug toxicity. The infecting agents have not always been identified, but the decreased ability to eliminate theophylline appears to be a common occurrence among patients suffering from upper respiratory infections (317). During an epidemic of influenza B infection in Seattle in 1980, theophylline toxicity was observed in 11 children; 6 of these patients had been maintained on the same dose of theophylline prior to the infection without exhibiting theophylline toxicity (318). The half-life of theophylline was increased in 4 young patients suffering from acute upper respiratory influenza A viral illness. Acute theophylline toxicity was observed in one of these patients (319). The half-life of theophylline was more than doubled 24 hr after the vaccination of 4 healthy volunteers with trivalent influenza vaccine (320). The vaccine also impaired the clearance of aminopyrine in healthy human subjects (321). The vaccine had no significant effect on biotransformation of theophylline and warfarin by elderly residents of rest homes (322). Activity of the immune system declines with age. Does this mean that the depressant effect of the vaccine on drug metabolism is inversely related to the activity of the immune system? Is it only a coincidence that activities of the immune system and the P-450 system decline with age (323–325)?

Depression of the P-450 system by interferon may influence chemical carcinogenesis. Many procarcinogenic polycyclic hydrocarbons are converted by the P-450 system to reactive metabolites that initiate carcinogenesis after they bind covalently to cellular macromolecules (326). Kishida et al (327) observed a delay in the development of carcinogenesis in 3-methyl cholanthrene (3-MC)-treated mice. Salerno et al (225) observed that none of the 3-MC-treated mice that received both 3-MC and interferon developed tumors whereas tumors developed in all of the animals that received 3-MC only. How much of the protective effect was due to inhibition of biotransformation of 3-MC and how much was due to an antiproliferative effect of interferon on the early formation of aberrant cells has not been determined.

CLINICAL APPLICATIONS[19]

The promise of a "magic bullet" that would eliminate viral diseases began with the discovery of interferon in 1957. Here was a natural therapeutic agent that would destroy almost all viruses as effectively as penicillin had killed bacteria. Best of all, it would do so without producing undesirable side effects. Enthusiasm, as unrealistic as it was unbounded, was heightened with the discovery that interferon worked against cancer in experimental animals. Now all that was needed was enough human interferon for clinical trials. This opportunity arrived when Cantell (23) produced large (for those days) amounts of crude

[19]For reviews see 328–335.

Table 4 Antiviral activity of exogenous interferon in humans

Disease	Interferon		Response	Reference
	Type	Dose ($\times 10^6$)[a]		
Acute fulminant hepatitis	IFN-α	3 qd	3/6	337
Fulminant hepatitis B	IFN-β	3–6 qd	2/4	338, 339
Hepatitis B	IFN-β	10 qd × 19	2/2	340
	IFN-α	900	4/16	341
Herpes simplex	IFN-α	3 qd	4/4	337
	IFN-α	0.07/kg/5d	14/19	342
Herpes zoster	IFN-α	0.25/kg/4×	1/17	343
	IFN-α	0.5 kg/14×	5/5	344
Ocular dendritic keratitis	IFN-α	0.6 + trifluorothymidine	?/54	345 346
Human warts	IFN-α	1.2	2/2	347
	IFN-β	2.7 qd	0/1	348
Encephalitis	IFN-α	3 qd	2/3	337
Postmeasles dermatitis	IFN-α	3 qd	1/1	337
Cytomegalovirus (renal transplant patients)	IFN-α	3 qd × 15	0/41	349 350
Viral infections (renal transplant patients)	IFN-β	3/2 × 10/90d	0/16	351
Varicella in cancer patients	IFN-α	0.35/6×	13/21	352

[a]Units of interferon; qd = daily; d = number of days; × = number of times.

interferon from induced human leukocytes. Early trials with this material, both as an antiviral agent and as an antitumor agent, were encouraging (336). Genetic engineering, fostered by commercial enterprise, produced several molecular species of human recombinant IFN-α in the abundant quantities required for clinical testing. The results of these clinical trials, summarized in Tables 4 and 5, have had a sobering effect on those who had expected miracles. On the other hand, they have not discouraged those whose expectations had been tempered by their knowledge of interferon experiments with mice and by their experience with chemical antitumor and antiviral agents in general. The results of these initial trials should not be minimized even though they may not fulfill the expectations of the press, the lay public, and some clinicians.

IFN-α was effective in the treatment of several virus pathologies (Table 4), including chronic active hepatitis resulting from hepatitis B virus; life-threatening, virus-induced juvenile laryngeal papillomatosis; herpes zoster and simplex infections; acute epidemic keratoconjuntivitis; and warts. Twelve of 15 patients suffering from life-threatening viral illness (acute fulminant hepatitis, spreading herpes simplex in immunosuppressed patients, encephalitis, juvenile laryngeal papillomatosis, postmeasles dermatitis) recovered after being treated with IFN-α. All of these patients were critically ill, and many were near death

Table 5 Antitumor activity of exogenous interferon in humans

Tumor	Interferon		Route of administration	Response	Reference
	Type	Dose[a]			
Brain	β human	3 qd	i.v., i.c.	2/4	353
	β human	3 qd		4/6	333
Breast	αA Recombinant	50/m^2, tiw × 28d		0/17	354
	α Cantell	3 qd × 28d		5/23	355
	α Cantell	3–9 qd × 28d		8/17	356
	α Lymphoblastoid	2 or 5–18/m^2 qd × 10d		1/27	357
	β Roswell Park	1 qd 34d	i.m., i.v.	1/6	358
	αA Recombinant	200–600/total		0/3	359
	αA Leuk(crude)	1 prior to surgery	i.t.	3/4	360
	rIFN-α2	10 + doxorubicin	i.m. + i.v.	2/2	334
Cervical	HuLeuk (crude)	2 qd in pressary prior to surgery		12/15	361
	rIFN-α2	2/2 × w	topical gel	5/7	362
	rIFN-α2	10 + doxorubicin	i.v. + i.m.	13/13	334
Colon	α Cantell	3 qd × 5		0/19	363
	αA Recombinant	50/m^2 tiw		1/18	364
	α Lymphoblastoid	3/m^2 tiw		0/19	365
	rIFN-α2	3–100 qd	i.m.	0/4	366
	α Cantell	?		2/32	367
	β Roswell Park	1/40 ×		0	358
	β Bioferon	?		0	368
	rIFN-α2	10 + doxorubicin	i.m. + i.v.	1/4	334
Chronic myelogenous leukemia	α Cantell	9–15 qd		22/25	369

(continued)

Table 5 (continued)

Tumor	Interferon		Route of administration	Response	Reference
	Type	Dose[a]			
Hairy cell leukemia	α Cantell	3	i.m.	19/20	370
	rIFN-α2	10/3 × w		7/8	334
	rIFN-α2	2/m² tiw	s.c.	13/13	371
Kaposi's sarcoma	αA Recombinant	36–54 qd × 28		14/34	372
	rIFN-α2	50/5d		8/20	373
	rIFN-α2	1–50/m²		10/11 early stage	334
				12/29 late stage	334
Liver	α Cantell	3 qd × 60d		0/5	374
	IFLr-A	12–850/m²/tiw		0/16	375
Lung (nonsmall cell)	α Cantell	3 qd × 30 d		1/37	376
	rIFL-α2	10 + doxorubicin	i.m. + i.v.	3/3	334
Lymphoma	α Cantell	3–9 qd		6/11	356
	αA Recombinant	50 m² tiw		17/46	377
	α	?		35/51	378
	β Bioferon	4–6/qd		1/7	378
	α Cantell	1 qd/30d	i.m.	6/18	379
Melanoma	α Lymphoblastoid	2.5/m² qd		1/17	380
	αA Recombinant	50/m² tiw		7/31	381
	α Cantell	1,3,9 qd/42d		1/45	382
	α2 Recombinant	10–100 qd × 20		2/16	383
	HuLeuk (crude)	2	i.t.	1/2	360
	α Lymphoblastoid	15/m² every other day		3/33	384
	β Bioferon	?		1/2	368
	α	4–12 + cimentidine	i.m. or i.t.	3/6	385

Myeloma	α Cantell	3–9 qd		6/10	356
	α Cantell	3–6 qd × 6 mo		3/11	386
	rIFN-α2	2–100	i.v., s.c.	20/38	334
	β Bioferon	3–10/29d		0/3	387
	rIFN-α2	3–100/qd	i.m./i.v.	5/16	366
Non-Hodgkin's lymphoma	IFN-α2 (high-grade lymphoma)	50/m^2/5d		4/27	334
	IFN-α2 (low-grade lymphoma)	10/m^2/tiw	s.c.	3/12	334
	IFL-rA	3–118		8/11	24
	β	4.5–9 qd/6 weeks		2/8	368
	α	2.5–5 qd/30	i.m.	4/6	388
Osteogenic sarcoma	α Cantell	3/tiw		16/30	389
Ovary	α Cantell	1–6 qd		1/15	390
	α Lymphoblastoid	2 or 5–18/m^2 qd × 10		1/13	391
	β	3/2 × w	i.p.	0/8	392
	rIFN-α2	5–50/qd × 16	i.p.	7/11	393
	α lymphoblastoid	5/5d × 6		19/28	394
Renal	α Cantell	3 qd		5/19	395
	αA Recombinant	50/m^{2}qd		2/19	382
	αA Recombinant	2/m^2 qd		0/8	396
	αA Recombinant	20/m^{2}qd		2/8	397
	α Cantell	1 qd		0/14	398
	α Cantell	10 qd		3/16	398
	α Lymphoblastoid	3/m^2/tiw		2/21	399

[a]Dose, 10^6 units of interferon; qd = daily; tiw = three times a week; number = injections; response: stable, partial, or complete remission; i.t. = intratumor, i.c. = intracranial.

when interferon therapy was begun (337). Interferon has good potential for the treatment of other viral infections, including rabies (400–402).

The first and longest ongoing trial of interferon as an antitumor agent is that by Strander (389, 403), who has used crude human leukocyte interferon to treat postsurgical osteogenic sarcoma patients. Some of these patients have been followed for more than a decade. Fifty percent of those who received interferon have survived for 5 years as opposed to 25% in the control group. Results of more recent clinical trials, which usually employed huge doses of recombinant interferon, have reached the current literature. As can be seen in Table 5, these results do not encourage unbridled optimism that interferon will prove to be the ultimate antitumor agent. Nevertheless, various degrees of tumor regression have been observed in breast carcinoma, malignant lymphoma, multiple myeloma, acute leukemia, malignant melanoma, Kaposi's sarcoma, chronic leukemias, bladder carcinoma, malignant gliomas, and nasopharyngeal carcinoma. Responses have been recorded as tumor shrinkage, stabilization of tumor size, remissions, survival time, and other measures of antitumor activity but rarely as a "cure". Of 38 patients with breast cancer, multiple myeloma, and lymphoma treated with 3–9 $\times$ 10^6 units of IFN-α daily, 50% had some kind of favorable response, 34% experienced complete or partial remission, and 16% showed limited responses (355, 356). The effect of interferon on hairy cell leukemia is the most dramatic recorded to date; complete remissions were observed in 19 of the 20 patients treated with IFN-α (334, 370, 371).

Ikic and associates (360) have used crude IFN-α as an adjunct to surgical removal of tumors. Eight patients with bladder cancer, four with breast cancer, and two with malignant melanoma, all with poor prognoses, were treated with crude IFN-α injected either into the tumor, into its adjacent tissue, or in some cases, intramuscularly. All tumors showed complete or partial regression for up to 6 months. Two patients with bladder papillomatosis, who were unsuited for conventional therapeutic procedures, were treated with transurethral and intramuscular interferon. The reduction of tumor mass that resulted permitted resection, and complete regression was achieved. Topical application of interferon to tumors in two breast cancer patients led to more than 50% regression of the tumors, which were then removed by surgery. In another study, crude IFN-α produced substantial or total remissions in patients with cancers of the head and neck; 10 of 30 patients were considered completely cured (404). Similar encouraging results were obtained with presurgical injection of crude IFN-α in cervical cancer (361). IFN-α (1×10^6 units) applied in a gel with a vaginal applicator to areas of cervical intraepithelial neoplasm in 7 patients produced complete response in 2 patients and partial responses in 3 others (334).

In retrospect, mouse studies have largely predicted what has been found clinically. Gresser (405) has summarized the mouse studies as follows: (*a*)

Interferon is most effective when injected repeatedly and during the period of tumor growth; (*b*) efficacy is inversely related to the tumor load; (*c*) efficacy is roughly proportional to the dose; (*d*) antitumor effects are maximal when the direct contact between tumor cells and interferon is maximal; and (*e*) in most systems, interferon inhibits tumor growth, but regression of established tumors has not been reported. Although these studies were conducted in highly artificial systems, a great deal that can be used in the treatment of patients can be learned from mice. This is particularly true of pharmacokinetics, which does not differ among animal species as much as most of the other factors that influence treatment. Clearly there is need for recombinant mouse interferons, not only for pharmacokinetic studies but also for studies of the mechanisms of antiproliferative and other pleiotropic actions of interferon, most of which are not understood.

Combinations of interferon with known antitumor agents or modulators of the immune system have not been undertaken in earnest largely because of the need for preliminary evaluation of the effectiveness of interferon in the absence of agents that would complicate interpretation of results. Combination of the antiviral agent adenine arabinoside (Ara A) with interferon was more effective in the treatment of chronic hepatitis B virus infection than with either agent alone (328, 406, 407). This strategy had to be discontinued because the patients developed acute Ara A neurotoxicity. Attempts to treat dendritic keratitis with IFN-α were disappointing. However, results were excellent when interferon was combined with the antiviral agent trifluorothymidine (345, 346).

Cimetidine, a histamine$_2$ antagonist used in the treatment of gastric ulcers, was administered with IFN-α to 6 patients with metastatic melanoma on the basis that it would inhibit suppressor T cells, which bear histamine receptors (385). Since suppressor cells suppress helper T cells, and helper T cells promote the activities of macrophages and NK cells, which are believed to be involved in the antiproliferative effect of interferon, cimetidine should in theory enhance the antiproliferative action of interferon. No tumor regression was observed in these patients during the 3–8 weeks they received interferon alone. In contrast, coadministration of interferon and cimetidine was associated with complete remissions in two patients, partial remission in a third, and stationary disease status in a fourth. In a more recent report, complete regression was seen in an additional 5 of 8 patients (408). Complete remission was achieved in a patient with acute myelogenous leukemia treated with 4×10^6 units of intramuscular IFN-α and 1 g of oral cimetidine daily for 6 weeks (409).

In vitro studies that showed synergistic cytotoxic effects of recombinant IFN-α when used with 8 standard chemotherapeutic agents led to the selection of doxorubicin for Phase I clinical trials (234, 410). Partial responses were seen with several tumor types; complete responses were obtained in 3 cases of ovarian cancer (334).

Transformed cells are less sensitive to interferon than normal cells in vitro

(411–412). Any agent that makes tumor cells as responsive to interferon as normal cells are would be expected to enhance the antitumor activity of interferon. Butyrate is such a substance (413). Arginine butyrate coadministered with IFN-α reduced tumor incidence in mice inoculated with 180 TG Crocker tumor cells. When *C. parvum* was administered with either interferon or arginine butyrate alone, significant protection of the animals was achieved. However, optimal results were obtained when a single injection of *C. parvum* was followed by nine daily alternating injections of arginine butyrate and interferon. This combination of agents has not yet been tested clinically.

The story of the clinical use of the interferons has just begun. Few of the numerous recombinant IFN-αs have been tested; there have been few trials with IFN-β, probably because early results have been less encouraging than those obtained with IFN-α; the testing of IFN-γ has only reached the Phase I level (414). The concept of using recombinant hybrid interferons or interferons with altered amino acid sequences is still on the drawing board. The dose, frequency, and route of administration of interferon have been derived empirically. Serum levels of interferon have proven of little predictive value, particularly when the interferon has been injected intramuscularly. The intraperitoneal route, which Bocci (96) believes may be the preferred mode of administration, has received only preliminary evaluation (360, 385). Few trials have been made with direct injection of interferon into solid tumors. There is still no information on how much of the interferon reaches the tumors.

These early clinical trials predict that the future of interferon as an antineoplastic agent will depend largely on multiple factors: the kind of tumor (whether it is growing or quiescent, its size and age, its access to interferon), the patient (many have been in a profound state of immunodepression as a result of their malignancy or previous chemotherapy), and the kind, amount, frequency, and mode of administration of interferon. The bullet may acquire some magic status when it is the proper type and caliber and is aimed in the right direction at the right target at the right time.

TOXICOLOGY[20]

The early belief that interferon would not elicit untoward side effects was based largely on the philosophy that interferon existed only in response to, and to protect against, viral infections. Accordingly, the pleiotropic effects observed were attributed to impurities in the crude interferon preparations used at that time. When pure interferon was made available, it became apparent that most if not all of the multiple effects observed with the crude preparations were due to their interferon content (129). Moreover, when large amounts of recombinant

[20]For reviews see 94, 415–420.

interferon came into use, some new and more severe toxic manifestations surfaced.

The recording of side effects by clinicians has been remarkably consistent. Similar toxic effects have been reported for all three major types of interferon, although not always to the same extent. Since individual subspecies of IFN-α have different spectra of activities (18), it can be predicted that they will also exhibit different spectra of toxicities. Some of the toxic symptoms are dose related, some are not; tachyphylaxis develops in some patients, but not in others. All of the toxic signs and symptoms disappear when interferon treatment is discontinued or doses are reduced. It is now recognized that the unpleasant flu symptoms experienced with viral diseases are in fact due to the interferon induced by the virus.

Pyrexia, the earliest sign of toxicity observed with commonly used doses of interferon, appears in all patients within 3 hr after injection. Temperatures return to normal spontaneously within 12–24 hr after a single dose of interferon, and tachyphylaxis usually develops after about two weeks of therapy. The effect is not dose related. Headache and myalgia occur in most patients between 8 and 12 hr after injection. These symptoms are largely not dose dependent, and tachyphylaxis is commonly observed.

Reported cardiovascular effects include hypotension, cardiac dysrhythmia, tachycardia, and premature ventricular extrasystole.

Leukopenia has been observed in most patients 24 hr after the initiation of interferon therapy; it is transient and rarely causes the white count to fall below 2000 cells/ml of blood. The degree of leukopenia seems to relate to the degree of therapeutic effectiveness of interferon. Transient declines in platelet counts may accompany leukopenia. Hemoglobin levels are not usually affected, although mild anemia has been reported. Bone marrow depression, reminiscent of that seen with chemical neoplastic agents, has been observed after prolonged treatment. The hair loss seen after extensive interferon therapy is also remindful of that seen with chemical antineoplastic agents. The loss of hair is not uniform; in fact, eyelash growth is increased—in one case, to 6.5 cm (421)!

Although frank liver pathology has not been reported, interferon-induced elevation of serum transaminase levels indicates some hepatic dysfunction.

The effects of interferon on the central nervous system are the most problematical and can be dose limiting. Many patients receiving large doses of interferon complain of fatigue after 4–7 days of treatment. They become extremely lethargic and anorexic and in danger of further complicating a catabolic state that may in many cases already exist. Fatigue, often associated with nausea, can be a dose-limiting toxicity. An impressive number of CNS side effects have been reported: coma, confusion, conceptual disorganization, increased EEC wave activity, malaise, paresthesia, psychomotor slowing, speech stoppage, thought blocking, and visuospatial disorientation (422–24).

Recent evidence for the function of IFN-α as a regulatory polypeptide in both neuroendocrine and immune systems suggests a modus operandi for the neurotoxicity of interferon (228–230, 425–430). Human leukocyte interferon possesses immunological and biological endorphin- and ACTH-like activities (425). The antiviral activity of human IFN-α is neutralized by anti-ACTH, anti-IFN-α, and anti-γ-endorphin antisera; anti-IFN-α serum neutralizes ACTH activity. Neutralization of IFN-α activity by anti-ACTH and anti-γ-endorphin is prevented with ACTH and γ-endorphin, respectively. IFN-β and IFN-γ do not share these antigenic properties with IFN-α. Pro-opiomelanocortin is a polyprotein that generates several hormones, including ACTH and endorphin (431). Structural commonalities suggested by the immunological cross-reactivities between ACTH endorphin and interferon were verified by the revelation that 6 amino acid sequences are common to both pro-opiomelanocortin and IFN-α (432).

The intracerebral injection of HuIFNr-α, γ-endorphin, or morphine produce analgesia, catalepsy, and immobilization in mice (229); naloxone, a morphine antagonist, reverses these effects. Interferon mitigates opiate addiction liability and eliminates the withdrawal phenomenon initiated by naloxone in morphinized rats (228).

Whatever the relationship may be between the neurotoxicity and the opiate-like activities of interferon, at least one inconsistency requires explanation. The antigenic associations and some of the functional similarities that have been made between the interferon and the opiates in mice apply only to IFN-α, not IFN-β or IFN-γ, yet neurotoxicity is produced in humans by all three interferons (102, 358, 422–24).

As described earlier, Gresser and associates (216–220) produced severe liver necrosis in suckling mice when they injected them with large doses of interferon throughout the first 6 days postpartum. When smaller doses were given during the same early neonatal period, livers were damaged but in time they recovered. However, all of these mice died several weeks later from glomerular nephritis. A very similar glomerulonephritis developed in mice that recovered from lymphocytic choriomeningitis (LCM) virus infection. Recognition of the similarity in the interferon- and LCM-induced kidney lesions led Gresser and associates to a classical experiment (417). Some of the mice that were injected with an increased amount of LCM virus were given potent sheep antimouse interferon globulin; other similarly infected mice received control immunoglobulin. All of the control mice showed extensive liver necrosis, but no liver pathology was seen in mice that received the interferon antibody. Antimouse interferon globulin reduced the incidence of mortality from 72% to 27% in one experiment and from 31% to 10% in a second experiment. Whereas all of the control mice rapidly developed severe glomerular lesions, a marked delay was observed in the development of these lesions in the mice that received the

interferon antibody, and the lesions were less severe. The results clearly indicated that the endogenous interferon induced by LCM virus was largely responsible for inhibition of growth, liver cell necrosis, glomerular nephritis, and death.

This experiment suggests that some effects of viral infections in humans may appear long after the infection has subsided, that these effects may be due to the interferon induced by the virus, and that they may not be recognized as such. The question has been raised as to whether autoimmune diseases may have been triggered by interferon induced early in life by viral infections. Elevated titers of interferon have been found in the sera of patients with SLE, rheumatoid arthritis, scleroderma, and Sjögren's syndrome (56, 417). A good correlation was found between interferon titers and disease activity (433). In one report, "high therapeutic effectiveness" resulted from the injection of interferon antibody into patients with autoimmune or allergic diseases. Is the teratogenesis attributed to rubella due to the large amount of interferon known to be induced by this virus during early pregnancy?

Is the induction of interferon by virus in itself a pathological response? If one assumes that low levels of endogenous interferon are required for the regulation of a variety of vital cellular functions, and that this is controlled by delicately balanced levels of endogenous double-stranded RNA, any sudden large influx of double strandedness introduced by a viral infection would seem catastrophic. In short, do cells have a very low tolerance to double-stranded RNA?

SUMMARY

Interferon was discovered three decades ago. The next 20 or more years of research were directed largely toward an understanding of its antiviral activity. The persistent short supply of interferon hampered progress, and the impure preparations available throughout these years clouded interpretation of results. Many of the experiments and clinical studies that interferonologists had dreamed of for 20 or more years became realities when modern technology provided quantities of pure interferon that exceeded expectations. Studies with these pure recombinant interferons removed all doubts that the many pleiotropic effects that had been observed with impure interferon preparations were due to interferon and not to the impurities. The interferons are now acknowledged lymphokines that are involved in many cellular processes. In fact, the antiviral activity of interferon, which led to its discovery, may be an exaggerated adaptive utilization of an interferon-regulated function that plays a more general role in cellular physiology.

The ability to isolate, purify, and produce pure interferon has led to the discovery of multiple species of leukocyte interferon. These interferons display

different patterns of activity when tested by a variety of systems. This suggests that specific leukocyte interferons may be involved in specific physiological functions.

Results of preliminary trials of the interferons as antiviral and antitumor agents have been encouraging and occasionally dramatic. The overall clinical picture is expected to improve when more is learned about the pharmacokinetics of the interferons and which of the interferons are best suited for the treatment of specific tumors and antiviral diseases. There are indications that coadministration of interferons with chemical antineoplastic and antiviral agents may increase the effectiveness of interferon in clinical situations. As might be expected of agents that influence a large number of physiological functions, interferons produce numerous toxic side effects, some of which resemble those inflicted by viral diseases. These side effects are reversible and not life threatening.

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