Interferons

I. Their Origin and Actions

Karen L. Jacobsen, DVM, MS, and Gail A. Rockwood, MS

THE PHENOMENON of viral interference, first described in 1935,[1] is the ability of one virus to interfere with the replication of another (challenge) virus. Thus, the quest was underway for the mediator of viral interference for over 20 years before Isaacs and Lindenmann assigned the name interferon (IFN) to it in 1957.[2] Their discovery of a soluble antiviral factor released from chick chorioallantoic membranes after exposure to a heat-inactivated influenza virus was the beginning of IFN research. IFNs are now recognized to be low molecular weight proteins and glycoproteins that affect a variety of functions in animal cells, including virus replication,[2] cell growth,[3,4] and the immune response.[5] It is likely that the cells of all vertebrate animals are capable of producing IFNs.[6] To qualify as an IFN, a viral inhibitor must have virus-nonspecific antiviral activity in at least homologous cells through cellular metabolic processes that involve the synthesis of both RNA and protein.[7]

This discussion, the first part of a two-part series, reviews the sources of IFNs, IFN induction processes, and the antiviral, antiproliferative, and immunomodulatory properties of IFNs. The second part will review the results of antiviral and antitumor clinical trials with IFNs. This will be followed by a discussion of pharmacokinetics, doses, and side effects of IFN therapy. Last, the IFN systems of domestic animal species and present and future clinical applications of IFN in veterinary medicine will be discussed. These articles are not intended to be a complete review of the IFN literature, which would be inappropriate for a clinical journal. Instead, they are intended to be concise summaries, aimed at the veterinary internist, with emphasis on clinically relevant topics. Specific articles and more extensive reviews are referenced for the interested reader.

From the Departments of Large Animal Medicine and Medical Microbiology, College of Veterinary Medicine, University of Georgia, Athens, Georgia.

Reprint requests: Karen L. Jacobsen, DVM, MS, Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602.

(Journal of Veterinary Internal Medicine 1988; 2:47–53)

Classification of IFNs

Alpha, beta, and gamma (also known as leukocyte, fibroblast, and immune, respectively) are the three known species of IFNs. Alpha and beta IFNs are collectively called Type I IFNs, and can be produced by virtually all nucleated cells.[8] In *in vitro* IFN production, alpha IFN is the major species released from stimulated leukocyte or lymphoblastoid cultures, whereas beta IFN is usually produced by fibroblasts or epithelial cells. Viruses, synthetic polynucleotides, bacteria, bacterial products, foreign nucleic acids, and certain polymeric chemicals can be used to stimulate production of alpha and beta IFNs.[9] Gamma IFN, also called Type II IFN, is a true lymphokine because it is released from T-lymphocytes after stimulation with mitogens, antigens, or interleukin-2 (IL-2).[10,11] Alpha, beta, and gamma IFNs differ in their antigenic, biologic, and physicochemical properties. As a general rule, alpha and beta IFNs are acid stable, whereas gamma IFNs are acid labile.[12,13] However, there are some acid labile alpha IFNs.[14] IFNs may be either proteins or glycoproteins, depending on their species of origin and whether they are produced naturally or recombinantly. Human alpha IFNs are most likely proteins (vs. glycoproteins), since no carbohydrate was detected on analysis of ten homogeneous natural human alpha IFNs and there was no N-glycosylation site on the molecules.[15,16] However, O-glycosylation cannot be excluded.[15] It appears, however, that mouse, rat, cow, and rabbit alpha IFNs are glycoproteins.[11,17,18] Human and bovine beta and human gamma IFNs are glycoproteins, but bovine gamma IFNs are not glycosylated.[17] Although IFNs produced bacterially lack carbohydrate moieties, the oligosaccharide side chains are probably not essential for biological activity.[19,20]

The human genome contains at least 15 to 17 different alpha IFN genes, two or more beta IFN genes, and two gamma IFN genes.[21–28] These genes code for the production of structurally distinct polypeptides, and are the basis for the diversity of subspecies of IFNs. However, whether all of the different genes are actually expressed (i.e., transcribed and translated) during physiologic encounters between viruses and cells has not yet been determined. Among IFNs from domestic animal

species, the bovine IFNs constitute the group about which the most is known presently. Bovine alpha IFN genes have been grouped into the following two homologous, but distinct classes: (1) class 1 (containing ten to 12 members) and (2) class 2 (containing 15 to 20 members).[29] The bovine beta IFN gene family consists of at least five members,[30] whereas only one bovine gamma IFN gene has been identified.[31] Four of the bovine alpha genes, three of the beta genes, and the gamma gene have been cloned and expressed in *Escherichia coli*.[29-31] Leung and coworkers explored the beta IFN gene families of several animal species recently and found that whereas the human, mouse, and cat genomes contain single beta IFN genes, those of the cow, horse, and pig have multigene families for beta IFN.[30] Genes have been identified for four subspecies of equine alpha IFN and one equine beta IFN.[32] Two equine alpha IFN genes and one equine beta IFN gene have been cloned and expressed in *E coli*.[32] Differences in the biological activity of subspecies of veterinary IFNs have not been investigated. Human alpha IFN subspecies can differ quantitatively in their antiviral activities up to 200-fold,[33] and in the target cells of their cross-species activities.[34-36]

Sources of IFNs

Large-scale production of IFNs can be accomplished both by natural methods and through the use of recombinant DNA technology.[37-39] Naturally derived ("native") IFNs are produced by stimulating various cells in culture, and purifying the IFN from the culture supernatants subsequently.[40] Recombinant IFNs can be produced with high specific activities (expressed as units per milligram of protein) and high degrees of purity. Natural IFNs are less concentrated usually and may contain a mixture of IFN types and other lymphokines,[41] which can be more or less desirable, depending on the intended therapeutic use.

Although human IFNs from both sources are available in ample supply for human research use and clinical trials,[42,43] this is not true for most veterinary IFNs. Several animal (bovine, porcine, and equine) leukocyte IFNs have been produced in small amounts,[44-46] and methods have been developed recently for large-scale production of bovine leukocyte IFN.[47] In addition, bovine beta IFNs[46,48] and gamma IFNs[46,49] and equine beta and gamma IFNs[50] have been produced in small amounts by natural methods. Techniques have been developed for recombinant production of bovine alpha,[29,51] beta,[30] and gamma[31] IFNs and equine alpha IFNs.[32] However, bovine and equine recombinantly derived IFNs have had limited availability for veterinary research use, and none are approved for clinical use presently.

Viruses have been used commonly to induce cells to produce natural IFNs. The ability of double-stranded RNA viruses to trigger IFN synthesis is well-recognized. However, some single-stranded RNA viruses are also good IFN inducers.[37] Cells infected with these viruses contain double-stranded RNA segments in the form of replicative and transcriptive intermediates.[52] Viruses irradiated with ultraviolet (UV) light can also induce IFN production, which has been demonstrated by the induction of IFN in bovine tissues *in vitro* by UV-irradiated infectious bovine rhinotracheitis virus.[53] The exact pathway for IFN induction by viruses has not yet been elucidated, and IFN inducers may share an ability to alter cellular metabolism in such a way as to trigger IFN synthesis or there may be several pathways of IFN induction. To induce production of gamma IFNs, mitogen stimulation of T-lymphocytes has been used most commonly.[12]

A technique referred to as "superinduction" has been used to increase yields of beta IFN in fibroblasts induced with the polynucleotide known as polyriboinosinic-polyribocytidylic acid (poly-I poly-C).[54] The addition of dactinomycin (an RNA synthesis inhibitor) and cyclohexamide (a protein synthesis inhibitor) to fibroblast cultures following IFN induction results in increased beta IFN yields, presumably by inhibiting production of mRNA for a repressor protein responsible for terminating the translation of IFN proteins.[54] Priming is another technique used in natural IFN production to increase IFN yields. Small amounts of IFN or virus are added to cells prior to induction, thereby "gearing up" cells for IFN production.[55] Although the molecular mechanism of priming is not known, primed cells produce more mRNA faster than unprimed cells. In addition, primed leukocytes produce IFN faster than unprimed leukocytes.[56]

Assays of IFNs

The assay used most commonly for IFN activity is the microtiter cytopathic effect (CPE) inhibition assay.[57] Two other common types of antiviral assays are plaque reduction assays[58] and virus yield reduction assays.[59] Antiviral assays used less commonly are hemadsorption inhibition, immunofluorescent cell counting, cytochemical assays, and agar diffusion assays.[60] In the CPE inhibition assay, an IFN sample is tested for its ability to prevent lysis of tissue culture cells by a virus because it produces visible CPE in a wide range of cell lines, is particularly sensitive to IFN, and is a poor inducer of IFN (and thus will induce a negligible amount of IFN in the assay cell system). The end point of a CPE inhibition assay is commonly accepted to be the culture well where 50% of the cells have been protected against viral CPE by IFN. The IFN titer (expressed in units) is considered to be the reciprocal of the IFN dilution in the end point well. Because IFN assays vary widely from laboratory to laboratory, the World Health Organization has adopted international reference standards for human, mouse,

rabbit, and chicken IFNs. Since no reference standards exist for other animal IFNs of veterinary interest, caution must be exercised when comparing titers from different laboratories. In addition, there are no accepted standards for the non-antiviral activities of IFN.

Antiviral Actions of IFNs

Every family of mammalian viruses has its own unique strategy of replication, and different families of viruses appear to be affected by IFN through different mechanisms.[61] In general, when virus infection of a cell occurs, the virus first attaches to and penetrates the cell. Next, the virus is uncoated (the protein coat is disrupted and shed, and the inner nucleic acid is released). The protein and nucleic acid moieties replicate independently and reassemble to form a new virus particle. They are then released from the cell. One or more aspects of the virus replication process are interpreted by the genetic apparatus of the cell as an activation signal for IFN production, and the cell then elaborates specific mRNA for IFN. The IFN mRNA is translated into an IFN protein at the ribosomes and IFN is then released into the extracellular fluid. Although the exact site of glycosylation of IFN proteins is not known, it has been suggested that the addition of carbohydrate moieties to IFN proteins takes place within membraneous structures prior to excretion.[62] IFNs are produced by cells very early in the course of viral infection. Thus, they are available much earlier than antibodies.[63] Also, in contrast to antibodies, IFNs have antiviral activity against a wide range of virus families.[64] However, viral families differ in their susceptibility to the antiviral effects of IFN. In addition, different families of viruses may respond differently to a given IFN in the same cell line, and this spectrum of activity differs among different cell lines from a given animal species.[64] Some cell lines are resistant to IFN with any kind of virus,[65] whereas other cell lines are resistant to IFN only with certain virus classes.[66]

IFNs do not interact directly with virus particles, but exert their antiviral effects on the host cells by rendering them unable to support virus replication. A series of changes in intracellular enzyme levels occur, some enzymes being induced and others being inhibited, giving rise to the antiviral state.[67] Among the enzymes induced are oligo A synthetase (2-5A synthetase), endoribonuclease (RNase L), and protein kinase. Oligo A synthetase results in activation of endoribonuclease with subsequent destruction of cellular mRNA and rRNA.[68] Protein kinase inhibits viral and cellular protein synthesis by inactivating (through phosphorylation) the peptide chain initiation factor known as eIF-2.[69,70] Phosphodiesterase prevents elongation of viral proteins. Glycosyltransferase results in decreased posttranslational processing of viral proteins. Thus, rather than a single "translational inhibitory protein," numerous IFN-induced enzymes mediate the antiviral effects of IFN. A comparison of the antiviral mechanisms of action of IFN with other antiviral drugs is given in Table 1.

TABLE 1. Mechanisms of Action of Antiviral Chemotherapeutics*

Mechanism†	Drug
Penetration	Diarylamidines Virus-specific oligopeptides
Uncoating	Arildone Amantadine, rimantadine
1° transcription	Specific oligonucleotides Ribavirin Diarylamidines
mRNA processing	Ara-A, ara-AMP Ribavirin DHPA IFNs
Translation	
mRNA degradation	IFNs
eIF-2 inactivation	IFNs
Viral DNA replication	Ara-A, ara-AMP Acyclovir BVDU FIAC Phosphonoformic acid Ribavirin DHPA Trifluorothymidine Idoxuridine
Viral RNA replication	Enviroxime
Viral protein processing (cleavage)	Diarylamidines

ara-A: adenine arabinoside; ara-AMP: adenine arabinoside monophosphate; DHPA: (S)-9-(2,3-dihydroxypropyl) adenine; BVDU: (E)-5-(2-bromovinyl)-2′-deoxyuridine; FIAC: 2′-fluoro-5-iodoaracytosine.

* Reprinted in modified form with permission from Table 11-2 of White DO, Fenner FJ. Medical Virology, 3rd ed. Orlando, FL: Academic Press, 1986; 306–307.

† Target site in viral replication cycle.

The IFN molecule interacts with a cell by binding to its surface.[71] Alpha and beta (but not gamma) IFNs appear to share a common receptor.[72] When IFNs bind to receptors, the fluidity of the cell membrane decreases and the electrophoretic mobility of the cell is altered.[73,74] IFN-induced changes in the cell membrane can affect virion attachment, virus release, or maturation at the cell membrane, as is the case with oncornaviruses.[75] Only relatively short treatment with IFN is necessary to elicit antiviral activity in cultured cells.[76] In addition, the antiviral effect cannot be immediately reversed by removing IFN from the cell culture media.[77] Whether IFN must be internalized to initiate the antiviral process is not agreed upon.[74] IFN species and even individual IFN subspecies differ quantitatively, and possibly qualitatively, in their antiviral activities *in vitro*.[35,78]

IFNs and Cell Growth Inhibition

Alpha, beta, and gamma IFNs significantly reduce the rate of division of normal and tumor cells.[72,79] It has been suggested that rapidly growing cells are affected by IFN to a greater extent,[80] thus forming the basis for

exploration of IFNs as antineoplastic agents. In some studies, recombinant IFNs did not inhibit cell growth to the same extent as natural IFNs.[81] Individual species and subspecies of IFN can have quantitatively different antiproliferative activities.[32,71,78] Natural gamma IFN preparations have superior antiproliferative effects compared with natural alpha or beta IFNs, bind to different cell membrane receptors, and accentuate the antiproliferative effects of either alpha or beta IFN.[71] In contrast to the antiviral effects of IFN, the antiproliferative effects are refractory and are maintained only by constant exposure to IFN.[82,83]

The antiproliferative effects result from the extension of three phases of the cell growth cycle. These three phases are G_1, S, and G_2.[84] Early events in the cell cycle, corresponding to G_1, are inhibited by IFN more than the S-phase of DNA synthesis or the G_2-phase of protein synthesis.[84] Delay of entry into the S-phase is mediated by 2-5 oligoadenylate, which is stimulated by 2-5A synthetase, an IFN-induced enzyme.[85]

Cross-Species Activities of IFNs

Although originally thought to be species-specific substances, IFNs are now known to have defined host ranges of cross-species activities.[86] All three species of IFN (alpha, beta, and gamma) have now been shown to have cross-species activities, although the host range can vary for the different IFN species.[87,88] The degree of cross-reactivity observed also may vary with the type of cells (i.e., epithelial vs. fibroblastic feline cells) and challenge virus used.[89,90] The phylogenetic relationship has little bearing on cross-species activity, as many IFNs are more active on cells of distantly related animals than on those of closely related animals (i.e., human leukocyte IFN is more active on bovine and feline cells than on monkey cells).[88,89] *In vitro* cross-species antiviral activity has been demonstrated for human IFNs on monkey, rabbit, hamster, cow, mouse, rat, cat, and pig cells.[87,89,91-94] Various bovine IFNs have activity on human, monkey, rabbit, pig, sheep, horse, and dog cells.[87,88,91] Porcine IFN has activity on bovine cells, canine IFN has activity on bovine and rabbit cells, and feline IFN has activity on canine cells.[89,91] Cross-species activities have also been documented *in vivo*[95] and are not limited to the antiviral actions of IFNs. Cross-species activity has also been observed for cell growth inhibitory, priming, and immunomodulatory activities of IFNs.[55,87,96]

IFNs and the Immune Response

Reports appearing in the early 1970s indicated that IFN-containing preparations were capable of stimulating phagocytosis[97] and influencing antibody responses.[98] However, it was not until the late 1970s that much attention was given to the role of IFNs as major regulators of immune responses. In addition, early investigations were made with impure preparations of IFN, so that questions remained as to whether the effects on the immune system were due to IFN itself. Nevertheless, it now appears that most of the effects noted initially are probably true effects of IFN. This field has expanded so rapidly that a complete review is not possible in an article of this nature. Instead, a summary of recent concepts is presented here, and the reader is referred to additional articles and reviews (many of them entire texts) now devoted to the effects of IFNs on the immune system.

Antibody Production

The effects of IFNs on antibody synthesis may differ between Type I and II IFNs. Gamma IFN appears to be more potent in regulating antibody synthesis than alpha or beta IFNs on an antiviral unit basis.[99] Exposure time and IFN dose are important.[100] IFNs appear to suppress antibody production when added to cells prior to or with antigen, but stimulate antibody production when added after antigen or when used in low doses.[5,100] When natural alpha IFN was injected into patients at the time of immunization, high doses reduced antibody production and low doses enhanced it.[98] The mechanisms of the effects of IFNs on antibody responses are complex and poorly understood. The effects have been reproduced *in vitro* using purified B-cells, with neither T-cells nor macrophages present.[101] However, interaction with T-helper or T-suppressor cells does appear to play a role in augmenting antibody suppression by alpha and beta IFNs.

Lymphocyte Blastogenic Responses

Phytohemagglutanin (PHA), Concanavalin A (ConA), and pokeweed mitogen (PWM) provoke nonspecific blastogenesis of T-lymphocytes (PHA and ConA) or both, T- and B-lymphocytes (PWM). Treatment of mitogen-stimulated lymphocyte cultures with any of the three species of IFN results in decreased lymphoproliferative responses.[5,85] The same responses have been variably observed in clinical trials.[102,103] The inhibition of lymphocyte blastogenic responses observed with IFNs is most likely a reflection of their antiproliferative action.

Mixed Lymphocyte Reaction

A mixed lymphocyte reaction (MLR) is the induction of blastogenesis in a responder population of T-cells after the recognition of alloantigens of foreign lymphocytes. Gamma IFN is a natural product of all MLRs. In a recent study using recombinant human IFNs, alpha and beta suppressed MLR responses over a wide range of doses, whereas gamma suppressed the MLR at low doses and either enhanced or had no effect at high doses.[5] Cytotoxic T-lymphocytes (CTLs) are generated in an MLR and their specific cytotoxicity is enhanced by IFN.

ADCC and NK Cells

In antibody-dependent cell-mediated cytotoxicity (ADCC), macrophages, neutrophils, or killer (K) cells lyse antibody-coated target cells. In contrast, natural killer (NK) cells lyse tumor cells and virus-infected cells in the absence of antibody. Both K cells and NK cells are non-B, non-T large granular lymphocytes. ADCC is augmented by alpha and beta IFNs, and the cytotoxicity of NK cells is augmented by all three types of IFN.[104,105] In normal patients and those with tumors, increased NK cell activity usually does not occur immediately after initiation of IFN treatment, and may not become evident for several days.[106]

Macrophage Function

IFNs have been shown to be major regulators of macrophage differentiation.[107] IFNs modulate both nonspecific and receptor-mediated phagocytosis by macrophages.[108-110] In mice with Friend virus-induced leukemia, depressed phagocytic and migratory activities of macrophages are returned to normal by IFN treatment.[11] IFN also renders macrophages cytotoxic for leukemic cells, and at least in some cases, virus-infected cells.[112,113] In addition, gamma IFN and the lymphokine known as macrophage activating factor (MAF) derived from T-cells appear to be identical molecules.

IFNs, IL-2, and IL-1

Upon mitogenic stimulation, T-lymphocytes produce both IL-2 and gamma IFN. IL-2 was previously known as T-cell growth factor (TCGF). IL-2 may be involved in the regulation of gamma IFN production by T-cells, and gamma IFN may be required for IL-2 induction of active T-cells. Pretreatment of macrophages with alpha or beta IFNs enhances the endotoxin-induced production of IL-1. IL-1 increases IL-2 production by T-cells and promotes T- and B-cell proliferation and functional capacity. Reviews of the role of IFNs in the rapidly expanding field of lymphokine immunology may be found in several recent texts.[114,115]

References

1. Hoskins MA. A protective action of neurotropic against viscerotropic yellow fever virus in *Macacus rhesis*. Am J Trop Med Hyg 1935; 15:675-680.
2. Issacs A, Lindenmann J. Virus interference. I. The interferon. Proc R Soc Lond [Biol] 1957; 147:258-267.
3. Gresser I, Bandu M, Tovey M, et al. Interferon and cell division VIII. Inhibitory effect of highly purified interferon on the multiplication of leukemia L 1210 cells. Proc Soc Exp Biol Med 1973; 142:7-10.
4. Stewart WE II, Dresser I, Tovey MG, et al. Identification of the cell multiplication inhibitory factors in interferon preparations as interferons. Nature 1976; 262:300-302.
5. Shalaby MR, Weck PK, Rinderknecht E, et al. Effects of bacteria-produced human alpha, beta, and gamma interferons in *in vitro* immune functions. Cell Immunol 1984; 84:380-392.
6. Wilson V, Jeffreys AJ, Barrie PA, et al. A comparison of vertebrate interferon gene families detected by hybridization with human interferon DNA. J Mol Biol 1983; 166:457-475.
7. Stewart WE II, Blalock JE, Burke DC, et al. Letters to the editor. Interferon nomenclature. J Immunol 1980; 125:2353.
8. Krim M. Towards tumor therapy with interferons, part I. Interferons: Production and properties. Blood 1980; 545(5):711-721.
9. Epstein LB. The comparative biology of immune and classical interferons. In: Cohen S, Pick E, Oppenheim JJ, eds. Biology of lymphokines. New York: Read Press, 1979; 433-514.
10. Epstein LB. Induction and production of human immune interferon by mitogen- and antigen-stimulated purified lymphocytes cultured in the presence of macrophages. Methods Enzymol 1981; 78:147-153.
11. Dorner F, Scriba M, Weil R. Interferon: Evidence for its glycoprotein nature. Proc Natl Acad Sci USA 1973; 70:1981-1985.
12. Langford MP, Georgiades JA, Stanton GJ, et al. Large-scale production and physiochemical characterizations of human immune interferon. Infect Immun 1979; 26:36-41.
13. Stewart WE II. The interferon system, 2nd ed. New York: Springer-Verlag, 1981; 145-149.
14. Preble OT, Black RJ, Klippel JH, et al. Interferon in systemic lupus erythematosis. In: Merigan TC, Friedman RM, eds. Interferons. New York: Academic Press, 1982; 219-231.
15. Allen G, Fantes KH. A family of structural genes for human lymphoblastoid (leukocyte-type) interferon. Nature 1980; 287:408-411.
16. Rubinstein M, Levy WP, Moschera JA, et al. Human leukocyte IFN: Isolation and characterization of several molecular forms. Arch Biochem Biophys 1981; 210:307-318.
17. Kijkmans R, Billiau A. An introduction to the genes of the interferon system. In: Taylor-Papadimtrion J, ed. Interferons. Their impact in biology and medicine. New York: Oxford University Press, 1985; 1-18.
18. Bielefeldt Ohmann H, Lawman MJP, Babiuk LA. Bovine interferon: Its biology and application in veterinary medicine. Antiviral Res 1987; 7:187-210.
19. Havell EA, Carter WA. Effects of tunicamycin on the physical properties and antiviral activities of murine L cell interferon. Virology 1981; 108:80-86.
20. Knight E Jr, Fahey D. Human interferon-beta: Effects of deglycosylation. J Interferon Res 1982; 2:421-429.
21. Allen G, Fantes KH. A family of structural genes for human lymphoblastoid (leukocyte-type) interferon. Nature 1980; 287:408-411.
22. Levy WP, Rubinstein M, Shively J, et al. Amino acid sequence of a human leukocyte interferon. Proc Natl Acad Sci USA 1981; 78:6186-6190.
23. Mantei N, Schwarzstein M, Streuli M, et al. The nucleotide sequence of cloned human leukocyte interferon cDNA. Gene 1980; 10:1-10.
24. Streuli M, Nagata S, Weissmann C. At least three human type alpha interferons: Structure of alpha2. Science 1980; 209:1343-1347.
25. Zoon KC. Purification, sequencing, and properties of human lymphoblastoid and leukocyte interferon. In: DeMaeyer E, Galasso G, Schellekens H, eds. The biology of the interferon system. Amsterdam: Elsevier/North Holland, 1981; 47-55.
26. Goeddel DV, Leung DW, Dull TJ, et al. The structure of eight distinct cloned human leukocyte interferon cDNAs. Nature 1981; 290:20-26.
27. Weissman C. The cloning of interferon and other mistakes. In: Gresser I, ed. Interferon 3. London: Academic Press, 1981; 101-134.
28. Knight E Jr. The molecular structure of interferons. In: Billiau A, ed. Interferon, vol 1: General and applied aspects. Amsterdam: Elsevier Science Publishers, 1984; 61-78.
29. Capon DJ, Shepard HM, Goeddel DV. Two distinct families of human and bovine interferon-alpha genes are coordinately expressed and encode functional polypeptides. Mol Cell Biol 1985; 5(4):768-779.
30. Leung DW, Capon DJ, Goeddel DV. The structure and bacterial expression of three distinct bovine interferon-beta genes. Biotechnology 1984; 2:458-464.

31. Cerretti DP, McKereghan K, Larsen A, et al. Cloning, sequence, and expression of bovine interferon-gamma. J Immunol 1986; 136(12):4561–4564.
32. Himmler A, Hauptmann R, Gunther RA, et al. Molecular cloning and expression in *Escherichia coli* of equine type I interferons. DNA 1986; 5(5):345–356.
33. Evinger M, Rubinstein M, Pestka S. Antiproliferative and antiviral activities of human leukocyte interferons. Arch Biochem Biophys 1981; 210:319–329.
34. Weck P, Apperson S, Stebbing N. Comparison of the antiviral activities of various cloned human interferon-subtypes in mammalian cell cultures. J Gen Virol 1981; 57:233–237.
35. Kramer MJ, Dennin R, Kramer C, et al. Cell and virus sensitivity studies with recombinant human alpha interferons. J Interferon Res 1983; 3(4):425–435.
36. Streuli M, Hall A, Boll W, et al. Target cell specificity of two species of human interferon-alpha produced in *Escherichia coli* and of hybrid molecules derived from them. Proc Natl Acad Sci USA 1981; 78:2848–2852.
37. Cantell K, Hirvonen S. Large-scale production of human leukocyte interferon containing 10^8 units per ml. J Gen Virol 1978; 39:541–543.
38. Derynck R, Remaut E, Saman E, et al. Expression of human fibroblast interferon gene in *Escherichia coli.* Nature 1980; 287:193–197.
39. Goeddel DV, Yelverton E, Ullrich A. Human leukocyte interferon produced by *E. coli* is biologically active. Nature 1980; 287:411–416.
40. Cantell K, Hirvonen S, Koistinen V. Partial purification of human leukocyte interferon on a large scale. Methods Enzymol 1981; 78:499–505.
41. Vilchek J (chairman), Burke D, Chany C, et al. Interferon nomenclature recommendations of the interferon nomenclature committee. J Interferon Res 1983; 3(4):377–378.
42. Powledge TB. Interferon on trial. Biotechnology 1984; 2(3):214–222.
43. Berneman LP. Personal communication. Stafford, TX: Immunomodulators Laboratories.
44. Carter WA, Johnson FH. Induction and production of interferon with porcine, bovine, and equine leukocytes. Methods Enzymol 1981; 78:48–54.
45. Fulton RW, Rosenquist BD. *In vitro* interferon production by bovine tissues: Induction with infectious bovine rhinotracheitis vaccine. Am J Vet Res 1976; 37(12):1497–1502.
46. Luna VE, Luk AD, Tyring SK, et al. Properties of bovine interferons. Experientia 1984; 40:1410–1412.
47. Jacobsen KL, Rockwood GA, Abolhassani M, et al. Bovine leukocyte interferon: Characterization and large-scale production. J Interferon Res (in press).
48. Rossi CR, Kiesel GK. Factors affecting the production of bovine type I interferon on bovine embryonic lung cells by polyriboinoisnic-polyribocytidylic acid. Am J Vet Res 1980; 41(4):557–560.
49. Babiuk LA, Rouse BT. Immune interferon production by lymphoid cells: Role in the inhibition of herpes viruses. Infect Immun 1976; 13:1567–1578.
50. Yilma T, McGuire TC, Perryman LE. Preliminary characterization of equine interferons and their antiviral activities on bovine, ovine, and human cells. J Interferon Res 1982; 2(3):363–370.
51. Velan B, Cohen S, Grosfeld H, et al. Bovine interferon alpha genes. J Biol Chem 1985; 260:5498–5504.
52. Portner A, Kingsbury DW. Identification of transcriptive and replicative intermediates in sendai virus-infected cells. Virology 1972; 47:711–725.
53. Fulton RW, Rosenquist BD. *In vitro* interferon production by bovine tissues: Effects of hydrocortisone. Am J Vet Res 1976; 37(12):1493–1495.
54. DeMaeyer E, Demaeyer-Guignard J. Interferons. Comp Virol 1979; 15:205–284.
55. Stewart WE II, Gosser LB, Lockart RZ Jr. Priming: A non-antiviral function of interferon. J Virol 1971; 7:792–801.
56. Lefkowitz SS, Reyes Luna VE. Priming of bovine interferons. Intervirology 1984; 21:221–223.
57. Familletti PC, Rubinstein S, Pestka S. A convenient and rapid cytopathic effect inhibition assay for interferon. Methods Enzymol 1981; 78:387–394.
58. Green JA, Stanton GJ, Goode J, et al. Vesicular stomatitis virus plaque production in monolayer cultures with liquid overlay medium: Description and adaptation to a one-day, human interferon-plaque reduction assay. J Clin Microbiol 1987; 4:479–485.
59. Weigen DA, Stanton GJ, Langford MP, et al. Virus yield-reduction assay for interferon by titration of infectious virus. Methods Enzymol 1981; 78:346–351.
60. Grossberg SE, Jameson PM, Sedmak JJ. Assay of interferon. Handbook Exper Pharmacol 1984; 71:28–29.
61. Friedman RM. Antiviral activity of interferon. Bacteriological Reviews 1977; 41:543–567.
62. Ng MH, Berman B, Vilcek J. Membrane-bound intracellular interferon in rabbit kidney cell cultures. Virology 1972; 49:322–325.
63. Gerber JD, Marron AE, Kucera CJ. Local and systemic cellular and antibody immune responses of cattle to infectious bovine rhinotracheitis virus vaccines administered intranasally or intramuscularly. Am J Vet Res 1978; 39:753–760.
64. Stewart WE II. The interferon system, 2nd ed. New York: Springer–Verlag, 1981; 200–206.
65. Chen HY, Sato T, Fuse A, et al. Resistance to interferon of a human adenocarcinoma cell line, HEC-1, and its sensitivity to natural killer cell action, J Gen Virol 1981; 52:177–181.
66. Nilsen TW, Wood DL, Baglioni C. Virus-specific effects of interferon in embryonal carcinoma cells. Nature 1980; 286:178–180.
67. White DO. Antiviral chemotherapy, interferons and vaccines. Monographs in Virology 1984; 16:1–112.
68. Nilsen TW, Baglioni C. Mechanism for discrimination between viral and host mRNA in interferon-treated cells. Proc Natl Acad Sci USA 1979; 76:2600–2604.
69. Chernajovsky Y, Kimchi A, Schmidt A, et al. Differential effects of two interferon induced translational inhibitors on initiation of protein synthesis. Eur J Biochem 1979; 96:35–41.
70. Gupta SL, Holmes SL. Antiviral mechanism of interferon: Activation of interferon-induced protein kinase in mouse L929 cells by reovirus infection. J Cell Biochem [Suppl] 1982; 6:98.
71. Aguet M. High-affinity binding of ^{125}I-labeled mouse interferon to a specific cell surface receptor. Nature 1980; 284:459–461.
72. Czarniecki CW, Fennie CW, Powers DB, et al. Synergistic antiviral and antiproliferative activities of *E. coli*-derived human alpha, beta, and gamma interferons. J Virol 1984; 49(2):490–496.
73. Besancon F, Ankel H. Bindings of interferon to gangliosides. Nature 1974; 52:478–480.
74. Knight E Jr, Korant BD. A cell surface alteration in mouse L cells induced by interferon. Biochem Biophys Res Commun 1977; 74(2):707–713.
75. Billiau A, Herremans H, Allen PT, et al. Trapping of oncornavirus particles at the surface of interferon-treated cells. Virology 1976; 73:537–542.
76. Dianzani F, Levy HB, Berg S, et al. Kinetics of the rapid action of interferon. Proc Soc Exp Biol Med 1976; 152:593–596.
77. Paucker K, Cantell K. Quantitative studies on viral interference in suspended L-cells. V. Persistence of protection in growing cultures. Virology 1963; 21:22–29.
78. Fish EN, Banerjee K, Stebbing N. Human leukocyte interferon subtypes have different antiproliferative and antiviral activities on human cells. Biochem Biophys Res Commun 1983; 112(2):537–546.
79. Dahl H, Degre M. Human interferon and cell growth inhibition I. Inhibitory effect of human interferon on the growth rate of cultured human cells. Acta Pathol Microbiol Immunol Scand [B] 1976; 84:285–292.
80. Strander H, Einhorn S. Effect of human leucocyte interferon on the growth of human osteosarcoma cells in tissue culture. Int J Cancer 1977; 19:468–473.
81. Stebbing N, Weck PK, Fenno JT, et al. Comparison of the biological properties of natural and recombinant DNA derived human interferons. In: DeMaeyer G, Galasso G, Schelekens

H, eds. The biology of the interferon system. New York: Elsevier/North Holland Biomedical Press, 1981; 25–33.
82. Dahl H. Human interferon and cell growth inhibition. VII. Reversibility of interferon activities. J Interferon Res 1983; 3(4):387–393.
83. Krueger LJ, Terry RW, Sussman AJ, et al. Interferon-induced growth modulation: Low dose maintenance of the antiproliferative response. J Interferon Res 1984; 4(1):29–49.
84. Balkwill FR, Taylor-Papadimitriou J. Interferon affects both G_1 and S + G_2 in cells stimulated from quiescence to growth. Nature 1978; 274:798–799.
85. Kimchi A, Shure H, Revel M. Regulation of lymphocyte mitogenesis by (2'-5') oligoisoadenylate. Nature 1979; 282:849–853.
86. Stewart WE II. The interferon system, 2nd ed. New York: Springer–Verlag, 1981; 134–135.
87. Babiuk LA, Rouse BT. Bovine type II interferon: Activity in heterologous cells. Intervirology 1977; 8(4):250–256.
88. Desmyter J, Stewart WE II. Molecular modification of interferon: Attainment of human interferon in a conformation active on cat cells but inactive on human cells. Virology 1976; 70:451–458.
89. Ahl R, Rump A. Assays of bovine interferons in cultures of the porcine cell line IB-RS-2. Infect Immun 1976; 14(3):604–606.
90. Gresser I, Bandu M, Brouty-Boye D, et al. Pronounced antiviral activity of human interferon on bovine and porcine cells. Nature 1974; 251:543–545.
91. Pollikoff R, Donikian MA, Padron A, et al. Tissue specificity of interferon prepared in various tissue cultures. Proc Soc Exp Biol Med 1962; 110:232–234.
92. Levy-Koenig RR, Golgher RR, Paucker K. Immunology of interferons. II. Heterospecific activities of human interferons and their neutralization by antibody. J Immunol 1970a; 104:791–797.
93. Duc-Goiran P, Galliot B, Chany C. Studies on virus-induced interferons produced by the human amniotic membrane and white blood cells. Arch Geschwulstforsch 1971; 34:232–243.
94. Borecky L, Fuchsberger N, Hajnicka V. Electrophoretic profiles and activities of human interferon in heterologous cells. Intervirology 1974; 3:369–377.
95. Roney CS, Rossi CR, Smith PC, et al. Effect of human leukocyte A interferon on prevention of infectious bovine rhinotracheitis virus infection of cattle. Am J Vet Res 1985; 46(6):1251–1255.
96. Greenberg PL, Mosny SA. Cytotoxic effects of interferon *in vitro* on granulocytic progenitor cells. Cancer Res 1977; 37:1794–1799.
97. Huang KY, Donahoe RM, Gordon FB, et al. Enhancement of phagocytosis by interferon-containing preparations. Infect Immun 1971; 4:582–588.
98. Braun W, Levy HB. Interferon preparations as modifiers of immune responses. Proc Soc Exp Biol Med 1972; 141:769.
99. Johnson HM, Torres BA. Recombinant mouse interferon-gamma regulation of antibody production. Infect Immun 1983; 41:546–548.
100. Gisler RH, Lindahl P, Gresser I. Effects of interferon on antibody synthesis *in vitro*. J Immunol 1974; 113:438–444.
101. Harfast B, Huddlestone JR, Casali P, et al. Interferon acts directly on human B lymphocytes to modulate immunoglobulin synthesis. J Immunol 1982; 127(5):2146–2150.
102. Maluish AE, Ortaldo JR, Sherwin SA, et al. Changes in immune function in patients receiving natural leukocyte interferon. J Biol Response Mod 1983; 2:418–427.
103. Einhorn S, Blomgren H, Jarstrand C, et al. Influence of interferon-alpha therapy on functions of the human immune system. In: DeMaeyer E, Schellekens H, eds. The biology of the interferon system. New York: Elsevier Scientific Publishers, 1983; 347–352.
104. Droller MJ, Borg H, Perlman P. *In vitro* enhancement of natural and cytotoxicity against tumor target cells by interferon. Cell Immunol 1979; 47:248–260.
105. Ortaldo JR, Mantovani A, Hobbs D, et al. Effects of several species of human leukocyte interferon on cytotoxic activity of NK cells and monocytes. Int J Cancer 1983; 31:285–289.
106. Breinig M, Ho M, White L, et al. Effect of prolonged administration of interferon-alpha on pharmokinetics, fever, lymphocyte proliferative response, and NK cell activity. J Interferon Res 1982; 2:195–207.
107. Vogel SN, Friedman RM. Interferon and macrophages: Activation and cell surface changes. In: Vilcek J, DeMaeyer E, eds. Interferon, vol 2: Interferons and the immune system. Amsterdam: Elsevier Science Publishers, 1984; 35–59.
108. Gresser I, Bourali C, Chouroulinkov I, et al. Treatment of neoplasia in mice with interferon preparations. Ann NY Acad Sci 1970; 173:694–707.
109. Rollag H, Degre M. Effect of interferon preparations on the uptake of non-opsonized *Escherichia coli* by mouse peritoneal macrophages. Acta Pathol Microbiol Immunol Scand [B] 1981; 89:153.
110. Vogel SN, English KE, Fertsch D, et al. Differential modulation of macrophage membrane markers by interferon: Analysis of Fc and C3b receptors, Mac-1 and Ia antigen expression. J Interferon Res 1983a; 3:153.
111. Levy MH, Wheelock EF. Impaired macrophage function in Friend virus leukemia: Restoration by statolon. J Immunol 1975; 114:962–965.
112. Schultz RM, Papamatheakis JD, Chirigos MA. Interferon: An inducer of macrophage activation by polyanions. Science 1977; 197:674–676.
113. Stanwick TL, Campbell DE, Nahmias AJ. Cells infected with herpes simplex virus induce human monocyte-macrophages to produce interferon. Immunobiology 1981; 158:207–212.
114. Balkwill FR. The regulatory role of interferons in the human immune response. In: Taylor-Papdimition J, ed. Interferons: Their impact in biology and medicine. New York: Oxford University Press, 1985; 74–75.
115. Farrar JJ, Benjamin WR, Steeg PS. Regulatory interactions between the interferons and the interleukins. In: Vilcek J, DeMaeyer E, eds. Interferon, vol 2: Interferons and the immune system. Amsterdam: Elsevier Science Publishers, 1984; 101–111.