blockade of the high-affinity sites by interferon alpha or beta. J. exp. Med. *167* (1988) 1171–1185.
102 Zav'yalov, V. P., and Denesyuk, A. I., Possible conformations of interferons: a prediction based on amino acid composition and sequence. Immun. lett. *4* (1982) 7–14.
103 Zoon, K. C., Karasaki, Y., zur Nedden, D. L., Hu, R. Q., and Arnheiter, H., Modulation of epidermal growth factor receptors by human alpha interferon. Proc. natl Acad. Sci. USA *83* (1986) 8226–8230.
104 Zoon, K., zur Nedden, D., Hu, R., and Arnheiter, H., Analysis of the steady state binding, internalization and degradation of human interferon-alpha 2. J. biol. Chem. *261* (1986) 4993–4996.

0014-4754/89/060500-09$1.50 + 0.20/0

Interferon receptors and their role in interferon action

S. E. Grossberg, J. L. Taylor and V. M. Kushnaryov

Department of Microbiology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee (Wisconsin 53226, USA)

Summary. Interferon (IFN) proteins interact with cells through specific cell surface receptors, some of which have been purified and cloned. The alpha-IFNs and beta-IFN bind to a common receptor (type I), whereas gamma-IFN binds to a separate receptor (type II). Both types of high-affinity receptors have been demonstrated on a variety of different kinds of cells but in relatively low numbers (10^2–10^4/cell). The relationship between IFN binding to receptors and the ways in which IFNs may affect cellular physiology and gene expression is discussed.
Key words. Interferons; cell receptors; nuclear receptors; membrane effects.

Introduction

Interferons (IFNs) may be considered as polypeptide hormones. Although produced by many different cell types and not specifically in endocrine organs, the interferons act as intercellular chemical messengers, bind to specific cell receptors, have stimulatory as well as inhibitory effects on cells, and oppose as well as enhance the activities of other such factors[17, 23]. The multiple effects of IFNs on the phenotype of vertebrate cells include the establishment of antiviral resistance, a reduction in the rate of cell proliferation, and the inhibition or stimulation of the expression of many differentiated cellular functions and properties[53]. The primary actions of the interferons and the cellular loci of their actions to obtain these very different effects remain uncertain. The biological activity of interferons is very high on a molar basis. Specific activities of about 10^8 International Units (IU) of antiviral activity/mg protein make them among the most potent, biologically active compounds known: they show antiviral activity in vitro at about 10^{-14} M, whereas prostaglandins are active at about 10^{-9} M, and peptide hormones (e.g., insulin) at 10^{-9}–10^{-12} M.

The three major types of IFN, IFN-alpha, -beta and -gamma, are classified according to their structure, i.e. their amino acid sequences, and antigenicity[18, 44]. Different systems of nomenclature have been used to identify interferons. The terms 'leukocyte' and 'lymphoblastoid' interferons (primarily IFN-α) and 'fibroblast' interferon (primarily IFN-β) refer to preparations named for the cells from which they were derived; they are all type I interferons which appear to bind to a common receptor. Type II, or 'immune', interferon designates IFN-γ preparations produced by T-lymphocytes; this IFN binds to a different receptor from that for type I IFNs. Whereas there are many subtypes (more than 15) of human (Hu) IFN-α, differing by as much as 30% in their amino acid sequences as well as in some biological characteristics, there is only one type of HuIFN-β and one type of HuIFN-γ.

Like some hormones, IFNs appear to bind to cells by specific receptors. Their biological activities as well as some of their individual physicochemical features may relate to their receptor-binding capacity and possibly to subsequent cellular processing. The major types of interferons can give different dose-response curves in bioassays. Some human interferon-α subtypes produce a far greater antiviral effect in cells from heterologous animal species than do comparable doses of human interferon-β or -γ. Further, different IFN types bind to various substrates, e.g. lectins, albumin, or metallic-ion ligands with different affinities[18]. The reader is referred to several excellent comprehensive reviews that provide a detailed analysis of the literature on IFN receptors[6, 9, 31, 44, 46, 54]. The nature of the interactions between IFNs and their receptors as well as of the process leading to the multiple actions of the IFNs is only beginning to be understood.

Characterization of IFN receptors

For the most part, interferons must first bind to specific cellular receptors to be active. The receptors for interferons -α and -β (the type I receptors) appear to be distinct from those for interferon-γ (the type II receptor). Purified interferon-α and -γ fail to compete reciprocally in receptor-binding studies, as is mostly true for interferon-β and

-gamma, although some exceptions may exist in the latter case[45]. There are many indications that the receptors for IFNs -α and -β may be the same whereas those for IFN-γ are different. Evidence for this comes from (i) the results of competitive binding assays, (ii) the different sizes of the covalent complexes with their ligands, (iii) the segregation of interferon sensitivity in cell mutants or somatic cell hybrids, (iv) the reports of synergistic action (or occasionally antagonism) of interferons-α or -β in combination with interferon-γ, as well as (v) their genetic mapping. It would be expected that the relative species specificity of interferon activity should correlate with the degree of binding to the respective cellular receptors. Different recombinant HuIFN-α subtypes compete for a common receptor on human and bovine cells; indeed, bovine cells are as sensitive as human cells to interferon-α, if not more so. On the other hand, HuIFN-β, which on the basis of mutually exclusive competition studies appears to bind to the same type I receptor on human cells, does not bind well to bovine cells, nor is it active on them. IFN-β binding to human cells could be increased by a factor of 20 or more if the IFN was pretreated with lanthanide ions[50], which correlated with enhanced antiviral activity.

The gene for the interferon type I receptor is located on chromosome 21 and that for the type II receptor on chromosome 6q[31]. The genes for the IFN receptor proteins are not linked to the structural genes for human interferons-α and -β, which are located on the short arm of chromosome 9, or to the gene for interferon-γ, which is on the long arm of chromosome 12[44]. The type II receptor most probably requires the contribution of a product from the human chromosome 21 locus for expression of its activity, since antibodies to cell surface components coded for on chromosome 21 inhibit interferon-γ action. In the mouse the type I receptor is located on chromosome 16 and the type II receptor on chromosome 10[31]. The degree of interferon-sensitivity appears to relate to the number of duplications of chromosome 21 in human cells and of chromosome 16 in mouse cells[12], the same chromosomes in which trisomy occurs in Down's Syndrome in human beings and in its counterpart in the mouse (a possible happenstance among the hundreds of gene loci involved).

The use of interferons labeled with radioactive substituents, a development made possible by the high degree of purity that has been achieved with both natural and recombinant interferons, has provided a great deal of information about binding to specific receptors. Such assays provide considerable precision and provide data from which calculation of receptor-ligand association constants and estimates of cell receptor numbers can be made. However, these techniques are susceptible to considerable bias and several pitfalls, as has been helpfully evaluated critically in detail by Branca[6]; to his elegant analysis should be added the caveat that such experiments necessarily depend upon the interaction between large populations of cells and ligand molecules that may be partially denatured and often of relatively low radiolabeled specificity. Nevertheless, such studies have revealed that a variety of cell types have a relatively low number of IFN receptors (in the range of 200–10 000) with high affinity (K_d of 1×10^{-9} to 1×10^{-11} M)[6,31]. The binding curves do not necessarily show true saturation; and the data may produce curvilinear or two-component Scatchard plots. Such data have been interpreted as meaning that, in addition to high-affinity receptors, either there are large numbers of low-affinity receptors or there is non-specific binding. Cultured cell lines appear to have larger numbers of receptors than peripheral blood leukocytes or other naturally occurring primary cells[32]. Since the number or affinity of receptors can change with the state of growth or differentiation, such circumstances can contribute to the different sensitivity of cells to interferon.

The degree of interferon effect, especially on transcription of interferon-inducible genes, appears to relate to the number of interferon receptor sites occupied[20]. The incubation of cells in vitro at physiological temperatures with low concentrations of interferons -α or -β appears to lead to a decrease in the number of cell surface receptors, as has been observed in other receptor-ligand systems. This down-regulation induced by ligand binding has also been reported to occur on peripheral blood cells from patients treated with HuIFN-α[32,36]. Following this apparent reduction in receptor numbers, receptors increase following the removal of HuIFN-α or after the therapy is stopped. It has been difficult to correlate binding measurements with the degree of cellular activation or of biological effects. Biological activities can be measured after treatment with interferon concentrations that correspond to a low percentage of occupancy of receptors by interferon. Furthermore, measurements of the biological activity of interferon in the antiviral assay may not necessarily correlate with assays using inhibition of cell proliferation or activation of natural killer cells[41]. Correlations were observed in mouse L1210 cells between the concentrations of MuIFN-α needed to inhibit growth and the amount of receptor occupancy; however, the concentrations required for antiviral activity were several orders of magnitude lower[1]. Langer and Pestka[31] point out that parallel sigmoidal dose-response curves, relating interferon-α concentration to biological response and the cellular binding of IFN, but separated by several orders of magnitude in concentration, do not indicate that binding and activation are necessarily related as to cause and effect.

Purification and cloning of interferon receptors

The type I receptor has been isolated from lymphoblastoid cells (especially Daudi and Namalwa lines), giving a range of sizes of interferon:receptor complexes, including 130, 220, 230, and 320 kDa. The type II human inter-

feron receptor has been purified from foreskin fibroblasts to give a range of molecular weights of 60, 80, and 95 kDa and interferon: receptor complexes of 105–125 kDa. The IFN-γ receptor isolated from Raji lymphoblastoid cell membranes consisted of proteins of 90 and 50 kDa[6, 31, 44]. There may indeed be subtypes of the type II receptor; the type II receptor isolated from human monocytes differed from that of WISH cells, a fibroblastic line of human amnion cells, and from that of HeLa cells[40].

The recent large-scale purification of the human IFN-γ receptor from solubilized placental membranes by means of receptor-specific monoclonal antibodies and wheat germ agglutinin showed that the receptor is composed of (1) a 65 kDa polypeptide core, (2) at least 23 kDa of N-linked carbohydrate, and (3) a small amount of O-linked carbohydrate. The receptor further appears to contain a 55 kDa extracellular domain that carries the ligand-binding site and N-linked carbohydrates; the intracellular domain may consist of a 35 kDa moiety[8, 49]. These studies also showed that the IFN-γ receptors on different cell types appear to be antigenically and structurally similar; this finding is at variance with the observations of Orchansky et al.[40].

Both types I and II receptors have been cloned by gene transfer. Following transfection of the human α/β receptor gene into mouse cells, the cells responded to human IFN-β by induction of 2′-5′oligoadenylate synthetase. The receptor is a 110 kDa protein[51]. The gene for the IFN-γ (type II) receptor cloned from human Raji cells could be expressed in human B-cells and mouse L 929 cells and detected by binding of IFN-γ to these cells. However, biological activity in transfected cells, as shown by an increase of Class I MHC antigen expression, could not be demonstrated following HuIFN-γ treatment, suggesting the need for species-specific factors in receptor function[2, 4].

Receptors and the mechanism of interferon action

The conceptual connection between the binding of IFN at the cell surface and the events associated with the derepression as well as repression of host genes remains elusive. Thus, without the needed evidentiary proof, the controversy continues as to whether the IFNs and/or their receptors achieve their actions directly or indirectly.

IFN Binding to cell surface receptors. Cell surface binding occurs at 0 °C, but the demonstration of antiviral resistance requires subsequent incubation at physiological temperatures[18]. Some circumstantial evidence suggests that interferons need not enter cells to act. IFN covalently bound to agarose beads could confer cellular antiviral resistance[9], but the possibility of localized, hydrolytic release of the IFN from the beads has been raised as a theoretical objection to this intriguing observation. Microinjection of IFN into the cytoplasm or nuclei of cells failed to induce antiviral resistance[21, 22], but IFN was not shown to be present inside the cells; further, microinjection can disrupt normal cell architecture enough to make negative experiments difficult to interpret. Of interest is the finding that anti-idiotypic antibodies (i.e., antibodies produced against anti-IFN immunoglobulin) not only bind to interferon receptors but can elicit a biological response, through the activation of types I and II interferon receptors on human cells[39, 42]. This observation may be interpreted to mean either that receptor binding at the surface is necessary or that the resultant complex is susceptible to internalization and further cellular processing.

Membrane effects and second messengers. Various approaches have been taken to try to demonstrate transmembrane signalling, similar to that seen with hormones[10], following interferon treatment of cells. It is difficult to conclude from current evidence that interferon activity results from rapid alterations in (1) phosphotidylinositiol metabolism, (2) cytoplasmic alkalinization, (3) cytoplasmic concentrations of free calcium, (4) cyclic AMP or cyclic GMP levels, (5) diacylglycerol, or (6) ATP-dependent protein kinase activity[31]. Although one or another of these physiological reactions to treatment of cells by interferons may relate to direct membrane effects, it is difficult to obtain convincing evidence of a direct relationship to the transcriptional activation of several genes, which can begin to occur as soon as 5–10 min after interferon treatment[14, 45].

IFN Internalization and cellular processing. Several findings do not support the concept that IFNs must simply bind to cell surface receptors to induce all the subsequent antiviral and other cellular activities. After binding, IFN molecules are internalized[28, 54, 55]. With the thought that IFN receptors, alone or in combination with IFN ligand, might act as their own messengers, different lines of evidence have been obtained to show that IFN molecules are transported into the nucleus within a few minutes by facilitated receptor-mediated endocytosis[25, 35]. By immunocytochemical techniques employing immunoferritin or colloidal gold-protein A conjugates with anti-interferon antibody, it is possible to demonstrate that IFNs bind first to coated areas of the plasma membrane or coated pits[27, 30]. These structures are specialized cell membrane formations involved in a highly selective and efficient mechanism of receptor-mediated endocytosis of ligands, such as hormones, serum lipoproteins, antibodies, toxins, and viruses[5, 43]. Several investigators have noted that the addition of ^{125}I-labeled -α, -β or- γ interferons to mass populations of cells was followed by internalization of the ^{125}I label[6, 31]; however, the fate of the internalized interferon molecules cannot be unequivocally determined by such methods. The use of an electron microscopic technique employing post-embedding immunodetection of IFN[25, 26] demonstrated unequivocally that natural and recombinant interferon molecules entered cells, were processed within the cytoplasm and moved towards the nucleus, which they entered through

nuclear pores and passed into the dense chromatin. Recombinant IFN-γ molecules appear to be more slowly internalized than the natural product[29].

Further, the use of isolated, purified nuclei treated with highly purified IFN -β or -γ demonstrated the presence of specific high-affinity receptors on the nuclear envelope of L-cells[28,35]. Scatchard analysis of murine (Mu) IFN-γ binding provided an estimate of 24000 high-affinity binding sites per nucleus, which had a K_a of 2×10^{-10} M, with some low affinity sites as well. The number of MuIFN-β receptors on the nuclear membrane was 14000 with a K_a of 1.4×10^{-10} M. Brief treatment of the isolated nuclei with trypsin reduced the binding of ^{125}I-MuIFN-β and -γ, indicating that these nuclear receptors contain protein. The lack of reciprocal competition for nuclear binding of MuIFN-β and -γ suggested that the nuclear membranes contain separate type I and II receptors, a distinction noted for plasma membrane receptors, as described above. To demonstrate that IFN can have a direct physiological effect on the cell nucleus, presumably mediated by nuclear membrane receptors, it was shown that treatments of isolated L 929 cell nuclei with MuIFN-β reduced the energy-dependent efflux of ^{3}H-RNA from these nuclei in a dose-dependent manner[34], an effect opposite to that noted with insulin treatment[16]. It is interesting that relatively large amounts of IFNs -α and -γ can directly alter the activity in vitro of nuclear DNA polymerases -α and -β of heterologous as well as homologous species, presumably through binding of IFN to the enzyme-substrate complex[52].

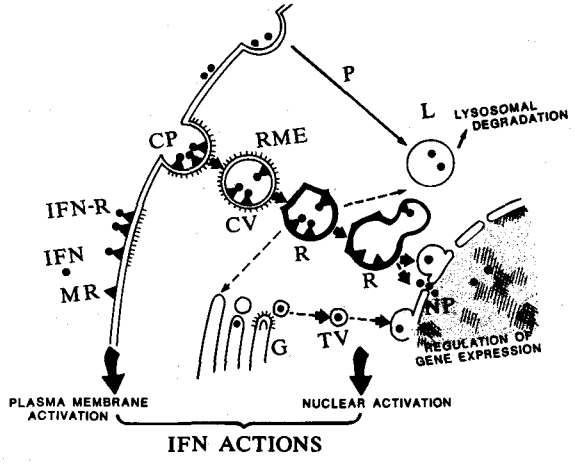
Experiments employing a photoactive, crosslinking reagent in intact cells have demonstrated an association between interferon molecules and nuclear proteins or receptors intracellularly[19]. Two complexes were observed in the nuclear fraction, one of 80 kDa and the other of about 235 kDa, whereas in the cytoplasmic fraction there was a single moiety of 110 kDa, the latter of a size repeatedly reported for cell surface receptors[6,31]. IFN-binding proteins from nuclear extracts eluted from a MuIFN-β affinity column could be crosslinked in vitro to IFN to provide a complex of about 235 kDa (MacDonald et al., unpublished), confirming the observations made in intact cells. An analysis of the distribution of ^{125}I-MuIFN-β between nuclear and cytoplasmic fractions of L-cells incubated at either 4 °C or 37 °C for 15 min showed that the nuclear portion of cells incubated at 37 °C contained 16 times more radioactive counts than the nuclear fraction of cells kept at 4 °C after IFN treatment (MacDonald, et al., unpublished data), suggesting the existence of a very active system for delivery to the nucleus. These results, made on large cell populations with radiolabelled interferon, strongly support the observations made by electron microscopy on individual cells. The possible role of interferon-binding nuclear proteins in IFN action is evocative of, but clearly different from, the circumstances with steroid and thyroid hormone gene activation[17]. The association of polypeptide hormones and growth factors with the nuclei of target cells has recently been reviewed[7].

Other observations support the possible physiological activity of intracellular IFN molecules, which may bypass surface receptors. Mouse cells transformed with a truncated human DNA coding for HuIFN-γ, but without the signal peptide residues, allowed for the accumulation of intracellular HuIFN-γ without release of the interferon; the increased levels of interferon-induced enzymes and the demonstrated antiviral resistance in these cells provide strong evidence for a vital role for intracellular processing of interferon[48]. Sakaguchi et al.[47] have shown in heterokaryons treated with IFN that a functioning nucleus of the same animal species as that of the IFN is required for the induction of antiviral activity, indicating that the nucleus plays a vital role in the selective response to interferon. The microinjection into cytoplasm and nucleus of IFN fragments and anti-IFN polyclonal antibodies to IFN prior to IFN treatment inhibit the induction of antiviral resistance[54]; however, as noted above, microinjection can disrupt normal cellular processing channels, a circumstance that might make the interpretation of such negative results difficult. Antiviral resistance has been induced in cells exposed to interferons encapsulated in liposomes, which presumably would bypass interaction with cell surface receptors[13]; however, it may be difficult to exclude the presence of IFN on the exterior of the liposomes.

There is increasing evidence that interferon-induced nuclear factors that bind promoter elements can be correlated with both positive and negative transcriptional controls[11,15,33,37]. With the availability of antibodies to interferon receptors for -α, -β and -γ[3,38], perhaps in combination with the ultrastructural immunocytochemical techniques of Kushnaryov et al.[25,26] it should be possible to determine whether these factors binding to promoter elements are related to the interferon-binding proteins in the nucleus[19] (and MacDonald et al., unpublished studies) or to cell surface receptor molecules.

Conclusion

It can be postulated that interferons can enter cells by two mechanisms: specific receptor-mediated endocytosis and/or bulk pinocytosis[26] (fig.). The pinocytotic pathway leading to engulfment into lysosomes would permit the reutilization of plasma membrane receptors following degradation of the ligand and recycling of the receptor to the cell surface. The possibly more specific processing pathway through endocytosis can provide for the transcytoplasmic delivery of interferon with or without its receptor to the nucleus. Nuclear IFN-binding proteins may facilitate transit through nuclear pores into the dense chromatin for transcriptional activation of IFN-inducible genes. To these processes can be added the direct effects of interferon molecules on cellular mem-



Hypothetical schema of MuIFN-β internalization and cellular processing by murine L 929 fibroblasts. IFN (●) binds to plasma membrane receptors (MR, ▲) which are located in coated pits (CP). IFN-MR complexes are transported in coated vesicles (CV) into receptosomes (R). IFN-MR complexes or separated IFN molecules and receptors are carried in vesicles to the nuclear envelope for transport through nuclear pores (NP), possibly first through the Golgi (G) complex for translocation in transport vesicles (TV) to the cell nucleus. Nuclear effects may include changes in nuclear macromolecular (RNA) transport[34] as well as the induction or repression of gene expression[14,45]. Broken lines designate transport stages for which evidence is still to be obtained but which have been implicated in other systems. L, lysosome; P, bulk pinocytosis. Reproduced with permission from Kushnaryov et al.[26].

branes (with or without still undiscovered second messengers), that may contribute to the multiple effects of interferons on eukaryotic cells of different types and states of differentiation.

Acknowledgment. We acknowledge the support of the National Institutes of Health (#R 01-EY 06990), the Food and Drug Administration, Triton Biosciences, and the Walter Schroeder Foundation.

1 Aguet, M., and Blanchard, B., High affinity binding of ^{125}-I-labeled mouse interferon to a specific cell surface receptor. Virology *115* (1981) 249–261.
2 Aguet, M., Dembic, Z., and Merlin, G., Molecular cloning and expression of the human interferon-gamma receptor, p. 16 (Abstr.). 1988 Annual Meeting of the International Society for Interferon Research, Kyoto, Japan 1988a.
3 Aguet, M., and Merlin, G., Production of a polyclonal antiserum against affinity purified human interferon-gamma receptors. J. Interferon Res. *7* (1987) 683.
4 Aguet, M., Dembic, Z., and Merlin, G., Molecular cloning and expression of the human interferon-gamma receptor. Cell *55* (1988b) 273–280.
5 Anderson, R. G. W., and Kaplan, J., Receptor-mediated endocytosis, in: Modern Cell Biology, vol. 1, pp. 1–52. Alan R. Liss Inc., New York 1983.
6 Branca, A. A., Interferon receptors. In Vitro cell. devl Biol. *24* (1988) 155–165.
7 Burwen, S. J., and Jones, A. L., The association of polypeptide hormones and growth factors with the nuclei of target cells. TIBC *12* (1987) 159–162.
8 Caldron, J., Sheehan, K. C. F., Chance, C., Thomas, M. L., and Schreiber, R. D., Purification and characterization of the human interferon-gamma receptor from placenta. Proc. natl Acad. Sci. USA *85* (1988) 4837–4841.
9 Chany, C., Interferon receptors and interferon binding, in: Interferon, vol. 3: Mechanisms of production and action, pp. 11–32. Ed. A. B. Friedman. Elsevier Science Publishers B. V., Amsterdam 1984.
10 Cuatrecasas, P., Hormone Receptors, Membrane Phospholipids, and Protein Kinases. The Harvey Lectures, Series 80, (1986) pp. 89–128.
11 Engel, D. A., Snoddy, J., Toniato, E., and Lengyel, P., Interferons as gene activators: Close linkage of two interferon-activatable murine genes. Virology *166* (1988) 24–29.
12 Epstein, C. J., and Epstein, L. B., Genetic control of the response to interferon. Tex. Rep. Biol. Med. *41* (1982) 324–331.
13 Fidler, I. J., Fogler, W. E., Kleinerman, E. S., and Saiki, I., Abrogation of species specificity for activation of tumorcidal properties in macrophages by recombinant mouse or human interferon-gamma encapsulated in liposomes. J. Immun. *135* (1985) 4289–4296.
14 Friedman, R. L., and Stark, G. R., α-Interferon-induced transcription of HLA and metallothionein genes containing homologous upstream sequences. Nature *314* (1985) 637–639.
15 Fujita, T., Sakakibara, J., Sudo, Y., Miyamoto, M., Kimura, Y., and Taniguchi, T., Evidence for a nuclear factor(s), IRF-1, mediating induction and silencing properties to human IFN-β regulatory elements. EMBO J. (1988) 3397–3405.
16 Goldfine, I. D., Clawson, E. A., Purrello, F., and Vigneri, R., Action of insulin at the nuclear envelope. Molec. cell. Biochem. *48* (1982) 3–14.
17 Gorski, J., The nature and development of steroid hormone receptors. Experientia *42* (1986) 744–750.
18 Grossberg, S. E., Interferons: An overview–general biological and biochemical properties, in: Mechanisms of Interferon Actions, pp. 1–32. Ed. L. M. Pfeffer. CRC Press, New York 1987.
19 Grossberg, S. E., Kushnaryov, V. M., MacDonald, H. S., and Sedmak, J. J., Nuclear localization of internalized interferons-beta and -gamma: A role for nuclear receptors, in: The Biology of the Interferon System, pp. 53–56. Eds K. Cantell and H. Schellekens. Martinus Nijhoff Publishers, Dordrecht 1987.
20 Hannigan, G., and Williams, B. R. G., Transcriptional regulation of interferon-responsive genes is closely linked to interferon receptor occupancy. EMBO J. *5* (1986) 1607–1613.
21 Higashi, Y., and Sokawa, Y., Microinjection of interferon and 2′5′-oligoadenylate into mouse L-cells and their effects on virus growth. J. Biochem. *91* (1982) 2021–2028.
22 Huez, G., Silhol, M., and Lebleu, B., Microinjected interferon does not promote an antiviral response in HeLa cells. Biochem. biophys. Res. Commun. *110* (1983) 155–160.
23 Inglot, A. D., Interferons and growth factors viewed as two families of hormones with opposing actions. Tex. Rep. Biol. Med. *41* (1982) 402–410.
24 Kushnaryov, V., DeBruin, J., Sedmak, J. J., MacDonald, H. S., Kushnaryov, T., and Grossberg, S. E., Quantitation of low numbers of interferon molecules by immunogold staining of section of Lowicryl K 4 M-embedded cells. J. Electron Microsc. *35* Suppl. (1986a) 2309–2310.
25 Kushnaryov, V. M., MacDonald, H. S., DeBruin, J., LeMense, G. P., Sedmak, J. J., and Grossberg, S. E., Internalization and transport of mouse beta-interferon into the cell nucleus. J. Interferon Res. *6* (1986b) 241–245.
26 Kushnaryov, V. M., MacDonald, H. S., LeMense, G., DeBruin, J., Sedmak, J. J., and Grossberg, S. E., Quantitative analysis of mouse interferon-beta receptor-mediated endocytosis and nuclear entry. Cytobios *53* (1988) 185–197.
27 Kushnaryov, V. M., MacDonald, H. S., Sedmak, J. J., and Grossberg, S. E., Ultrastructural distribution of interferon receptor sites on mouse L cells grown in suspension: Ganglioside blockade of ligand binding. Infect. Immun. *40* (1983) 320–329.
28 Kushnaryov, V. M., MacDonald, H. S., Sedmak, J. J., and Grossberg, S. E., Murine interferon-beta receptor-mediated endocytosis and nuclear membrane binding. Proc. natl Acad. Sci. USA *82* (1985) 3281–3285.
29 Kushnaryov, V. M., MacDonald, H. S., Sedmak, J. J., and Grossberg, S. E., The cellular internalization of recombinant gamma interferon differs from that of natural interferon-gamma. Biochem. biophys. Res. Commun. *157* (1988) 109–114.
30 Kushnaryov, V. M., Sedmak, J. J., Bendler, J. W. III, and Grossberg, S. E., Ultrastructural localization of interferon receptors on the surfaces of cultured cells and erythrocytes. Infect. Immun. *36* (1982) 811–821.
31 Langer, J. A., and Pestka, S., Interferon receptors. Immun. Today *9* (1988) 393–400.
32 Lau, A. S., Hannigan, G. E., Freedman, M. H., and Williams, B. R. G., Regulation of interferon receptors expression in human blood lymphocytes in vitro and during IFN therapy. J. clin. Invest *77* (1986) 1632–1638.
33 Levy, D. E., Kessler, D. S., Pine, R., Reich, N., and Darnell, J. E. Jr, Interferon-induced nuclear factors that bind a shared promoter ele-

ment correlate with positive and negative transcriptional control. Genes Devl. *2* (1988) 383–393.
34 MacDonald, H. S., Kushnaryov, V. M., Hevner, R., Sedmak, J. J., and Grossberg, S. E., Mouse beta-interferon reduces RNA efflux from isolated nuclei, J. Interferon Res. *6* (1986a) 247–250.
35 MacDonald, H. S., Kushnaryov, V. M., Sedmak, J. J., and Grossberg, S. E., Transport of gamma-interferon into the cell nucleus may be mediated by nuclear membrane receptors. Biochem. biophys. Res. Commun. *138* (1986b) 254–260.
36 Maxwell, B. L., Talpaz, M., and Gutterman, J. U., Down regulation of peripheral blood cell interferon receptors in chronic myelogenous leukemic patients undergoing human IFN (HuIFN-α) therapy. Int. J. Cancer *36* (1987) 23–28.
37 Miyamoto, M., Fujita, T., Kimura, Y., Maruyama, M., Harada, H., Sudo, Y., Miyata, T., and Taniguchi, T., Regulated expression of a gene encoding a nuclear factor, IRF-1, that specifically binds to IFN-β gene regulatory elements. Cell *54* (1988) 903–913.
38 Novick, D., Orchansky, P., Revel, M., and Rubinstein, M., The human interferon-gamma receptor. J. biol. Chem. *262* (1987) 8483–8487.
39 Novick, D., Rubinstein, M., and Fischer, D. G., Anti-idiotypic antibodies to the idiotypes of anti-interferon-gamma, alpha and beta antibodies as probes to study the interferon receptor. J. Interferon Res. *7* (1987) 769.
40 Orchansky, P., Rubinstein, M., and Fischer, D. G., The interferon-gamma receptor in human monocytes is different from the one in nonhematopoietic cells. J. Immun. *136* (1986) 169–173.
41 Ortaldo, J. R., Mason, A., Rehberg, E., Kelder, B., Harvey, D., Osheroff, P., Pestka, S., and Herberman, R. B., Augmentation of NK activity with recombinant and hybrid recombinant human leukocyte interferons, in: The Biology of the Interferon System, pp. 353–358. Eds E. DeMaeyer and H. Schellekens. Elsevier Science Publishers, Amsterdam 1983.
42 Osheroff, P. L., Chiang, T., and Manousos, D., Interferon-like activity in an anti-interferon anti-idiotypic hybridoma antibody. J. Immun. *135* (1985) 306–313.
43 Pastan, I. H., and Willingham, M. C., Receptor-mediated endocytosis: Coated pits, receptosomes and the Golgi. TIBC *8* (1983) 250–254.
44 Pestka, S., Langer, J. A., Zoon, K. C., and Samuel, C. E., Interferons and their actions. A. Rev. Biochem. *56* (1987) 727–777.
45 Revel, M., and Chebath, J., Interferon-activated genes. TIBC *11* (1986) 166–170.
46 Rubinstein, M., and Orchansky, P., The interferon receptors. CRC Crit. Rev. Biochem. *21* (1986) 249–275.
47 Sakaguchi, A. Y., Stevenson, D., and Gordon, I., Species specificity of interferon action: A functioning homospecific nucleus is required for induction of antiviral activity in heterokaryons. Virology *116* (1982) 441–453.
48 Sanceau, J., Sondermeyer, P., Beranger, F., Falcoff, R., and Vaquero, C., Intracellular human gamma-interferon triggers an antiviral state in transformed murine L cells. Proc. natl Acad. Sci. USA *84* (1987) 2906–2910.
49 Schreiber, R. D., Calderon, J., Sheehan, K. C., and Khurana, N., Purification and characterization of the human IFN-gamma receptor. 1988 Annual Meeting of the International Society for Interferon Research, p. 16 (Abstr.) Kyoto, Japan 1988.
50 Sedmak, J. J., MacDonald, H. S., and Kushnaryov, V. M., Lanthanide ion enhancement of interferon binding to cells. Biochem. biophys. Res. Commun. *137* (1986) 480–485.
51 Shulman, L., Fellous, M., Goldberg, M., and Revel, M., Molecular cloning of the human IFN-α/β receptor cDNA. 1988 Annual Meeting of the International Society for Interferon Research, p. 17 (Abstr.) Kyoto, Japan 1988.
52 Tanaka, M., Kimura, K., and Yoshida, S., Inhibition of mammalian DNA polymerases by recombinant α-interferon and gamma-interferon. Cancer Res. *47* (1987) 5971–5974.
53 Taylor, J. L., Sabran, J. L., and Grossberg, S. E., The cellular effects of interferons, in: Interferons and Their Applications, Handbook of Experimental Pharmacology, vol. 71, pp. 169–204. Eds P. E. Came and W. A. Carter. Springer-Verlag, New York 1984.
54 Zoon, K. C., and Arnheiter, H., Studies of the interferon receptors. Pharmac. Ther. *24* (1984) 259–278.
55 Zoon, K. C., Arnheiter, H., Zur Needen, D., Fitzgerald, D. J. P., and Willingham, M. C., Human interferon alpha enters cells by receptor-mediated endocytosis. Virology *310* (1983) 195–203.

0014-4754/89/060508-06$1.50 + 0.20/0

Interaction of interferon with other cytokines

G. Opdenakker, Y. Cabeza-Arvelaiz and J. Van Damme

Rega Institute for Medical Research, University of Leuven, Minderbroedersstraat 10, B-3000 Leuven (Belgium)

Summary. Interferons interact with other cytokines to exert their antiviral, cell growth regulatory and immunomodulatory activities. Growth factors, tumor necrosis factors, colony stimulating factors, interleukins and interferons have pleiotropic effects and form a parallel network of intercellular signals. These signals are transduced at the cell surface through specific receptors with intrinsic enzymatic activity or with the capacity to regulate intracellular enzymes through interactive effects with G-proteins. This leads to regulated gene transcription of intracellular and secreted, functional and structural proteins. Although much is known about the interaction of cytokines with their receptors and about the regulation of transcription at the genomic level the various steps linking these two phenomena deserve further research.
Key words. Cytokines; interferons; interleukins; colony stimulating factors; growth factors; inflammation; macrophages; cytokine receptors.

Introduction

Interferon was the term originally given to secreted (glyco) proteins capable of inducing an antiviral state in cells[45, 54]. The purification of interferons and the generation of antibodies directed against interferons made it possible to classify these substances on the basis of their seroreactivity into interferon-α, interferon-β, and interferon-γ[18]. In addition interferon-α and interferon-β, originating mainly from leukocytes and fibroblasts respectively, were clearly distinguished by biochemical and biological characteristics from interferon-γ, which is pro-