Multi-author Review

Recent developments in interferon research

The Editors wish to thank Dr H. K. Hochkeppel for coordinating this review.

Introduction

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This collection of articles is not intended to review or update the whole interferon field; many reviews for this purpose have appeared in recent and past years. The following chapters should, rather, give the reader an insight into very recent developments in this field and discuss new, even at times controversial aspects concerning the mechanism of interferon action, and its role as a member of the super-cytokine family. The interferons were identified and defined by their ability to induce cells to inhibit viral replication and to make new proteins. In addition, it was found that interferons exert antiproliferative as well as cell regulatory activities. Because interferons influence cells of the immune system, they have also been assigned an additional, immunoregulatory role. Interferons are a heterogeneous family of proteins (interferon alpha and interferon beta or type I interferon, and interferon gamma or type II interferon). They are synthesized and secreted by a variety of cell types in response to several classes of inducers, and exert their actions through specific receptors. In this respect, they are analogous to the polypeptide hormones, the big difference, however, being that interferons are produced locally and released into microenvironments while hormones are transported to and act on distant parts of the body. In this multi-author review two chapters (K. E. Mogensen et al. and S. E. Grossberg et al.), devoted to interferon receptors, attempt from somewhat different angles to shed light on the complex process of interferon interactions with their receptors as well as on the role of these receptors in interferon actions. As the reader will see, these complicated processes are just beginning to be understood, and are still open to different interpretations and speculation. No less intriguing are the interactions with other cytokines (described by G. Opdenakker et al.), and, within the cytokine network, the actions of interferon and interacting cytokines on B cells (A. Jurado et al.). Gene expression of interferons in normal and diseased tissue of man are subsequently discussed by M. G. Tovey. Specific effects of interferons or other cytokines on certain enzymes involved in defense mechanisms against pathogens (J. M. Carlin et al.) or associated with autoimmune diseases (S. Masure and G. Opdenakker) are described, followed by a discussion of the very complex activities of interleukin 6 (alias interferon beta 2) in infection and inflammation (M. Revel). The collection is concluded by a chapter on recent trends in animal models in interferon research (H. Schellekens) showing us, among other things, how little of our knowledge gained from in vitro experiments can be transfered to the in vivo situation.

The cellular receptor of the alpha-beta interferons

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Summary. This is a selective review of recent trends in research on the cellular receptor for the alpha-beta interferons. It deals mainly with work published in the last three years (1985–88), and therefore mainly with receptors for the human interferons. The binding characteristics of several human alpha interferons are examined, and the importance of in vitro experimental models for establishing the relationship between receptor binding and the cellular response is emphasized.

Key words. Interferon receptor; polypeptide receptor; multiple ligands; cellular differentiation; binding response relationship.

The subject has been reviewed at regular intervals; most recently by Branca [13], who cites all the original work and the previous reviews, and also by the DeMaeyers [25]. Technical details of the radiolabelling of interferons and the measurement of receptors are contained in sections V and VI of volume 119 of Methods in Enzymology (edited by S. Pestka, Academic Press, New York, London 1986). This volume also gives details of the amino acid sequences of the various interferons and their nomenclature. We shall deal mainly with the recent trends in receptor research, and not cite the earlier literature unless there is a particular reason to do so. We shall not review work on the gamma interferon receptor unless it has a particular bearing on the behaviour of the alpha-beta receptor. As a type, we shall call the molecules that bind to receptors: ligands. Free ligands are then those in solution outside the cell, and bound ligands those attached to a binding site on or in a cell. How one defines one's receptor depends pretty much on what kind of experiments are being done. We shall say that a binding site contains at least one ligand-binding protein.

In vivo and in vitro

Receptor binding experiments can be done with whole cells, with membrane fragments and with detergent extracts of membrane receptors. They are essentially in vitro experiments. For practical reasons it is difficult to work with concentrations of interferon much greater than 1 nM or much less than 1 pM. Fortunately, most of the response ranges can be measured at these concentrations, interferon binding to a relatively small number of sites with a relatively high affinity. The amount taken up, even by whole cells, turns out to be a small fraction of the total ligand added.Consequently, binding in vitro takes place at a more or less constant concentration of free ligand. The local concentration of interferon in vivo may be subject to enormous variations and a cursory examination of the pharmacokinetics of injected interferon shows that concentrations in the extravascular fluid are unlikely ever to be constant [68]. As interferon produced in response to viral infection generally appears as a discrete burst, it would seem that in vivo activity must occur in the presence of changing concentrations of interferon. The constitutive production of interferon in vivo is dealt with in the review by Tovey in this issue.

Most, but by no means all, of the work on receptors for human interferon has been done with just two cell lines; one of these was derived from a cow, the other from a human lymphoma. It so happens that each line has its particular uses; this is reasonable enough, but we should be careful of regarding either of them as typical.

Generalities

The general aspects of the structure and behaviour of cell surface receptors for humoral protein ligands involved in the stimulation or inhibition of cellular functions were established using insulin, certain tissue-specific mitogenic factors and the low density lipoprotein carrier for cholesterol. A recent review is that of Hollenberg [48]. These conclusions were more or less complete before any direct measurement of receptors using radiolabelled interferon had been made [3]. Interferon has been found to conform to the pattern.

The ligand binds to its receptor at the cell surface; complexes aggregate and cluster, often causing the binding to decrease in amount and increase in affinity; the complexes are engulfed and enter the cell in vesicles lined with the protein clathrin; if there is fusion of vesicles and lysosomes, both ligand and receptor may be destroyed. It can be shown that almost any stage of the process, which can be blocked or mimicked, will elicit some particular response from the cell. Right from the very first binding to the final elimination of the degraded fragments some physiological response can be found to correlate with the concentration of the free ligand (ignoring for the moment those instances where the cell is resistant to the ligand). A variety of transduction mechanisms (e.g. with the formation of 'second messengers') have been proposed or established, including the binding of undamaged ligand to receptors on the nuclear membrane [40, 54]. In the sense that results can still be controversial this is one of the areas in which research is active [48, 52].

Clathrin, which is a component of the pits and vesicles that are responsible for the endocytosis of protein ligands, has been shown to be necessary for eukaryotic cell growth [57]. Endocytosis and receptor cycling appear to be a universal response of cells to extracellular protein ligands [16]. This is a dynamic response and, although the receptor and its ligand may make a tight fit, it is a more comprehensive idea than the earlier one of lock and key. The extent of this response is remarkable; much more remarkable than a generalized diagram would indicate. The flavour is found in the titles of the research articles. The table lists just a few from articles dealing, on the one hand, with the mitogenic response of cells to growth factors and, on the other, with the inhibitory effects of interferon.

Resistance to interferon and the clinical response

In the case of responsive cells the correlation between interferon binding and response has been shown to be precise for the very earliest reactions right through to the final gross effects [27, 37, 42, 46, 68, 99, 100]. There are, however, many cell lines which respond poorly or not at all to interferon. Most of them have receptors and in many cases interferon binding can be quite substantial at concentrations of free ligand that produce no obvious effect [3]. There have been several suggestions that resistant cells express reduced levels of receptor. Such studies are often unsatisfactory; mainly, we suspect, because no one wishes to study in detail the effects of interferon on an

A comparison of titles from published works on ligand-receptor dynamics of mitogenic growth factors and of interferon.

Reference	Title
80	Early signals in the mitogenic response.
42	Early plasma membrane depolarisation by alpha interferon: biologic correlation with antiproliferative signal.
8	Down-regulation of the epidermal growth factor in KB cells is due to receptor internalization and subsequent degradation in lysosomes.
30	Kinetics of internalization and degradation of surface-bound interferon in human lymphoblastoid cells.
59	Protein kinase C phosphorylation at thr 654 of the unoccupied EGF receptor and EGF binding regulate functional receptor loss by independent mechanisms.
83	Interferon receptor interaction: internalization of interferon alpha 2 and modulation of its receptor on human cells.
94	Stabilized complexes of epidermal growth factor and its receptor on the cell surface stimulate RNA synthesis but not mitogenesis.
100	Cell surface receptor-mediated internalization of interferon: its relation to the antiviral activity of interferon.
97	Only high-affinity receptors for interleukin 2 mediate internalization of ligand.
34	Single high affinity binding of interferon alpha 2 to receptors on human lymphoblastoid cells: internalization and inactivation of receptors.
85	Epidermal growth factor: biological activity requires persistent occupation of high-affinity cell surface receptors.
28	Interferon removes its own receptors as it blocks the division of Daudi cells.
71	Binding, internalisation and intracellular processing of protein ligands. Derivation of rate constants by computer modelling.
104	Analysis of the steady state binding, internalization and degradation of human interferon-alpha 2.
40	Cellular uptake and nuclear binding of insulin to human cultured lymphocytes.
53	Quantitative analysis of mouse interferon-beta receptor-mediated endocytosis and nuclear entry.

unresponsive cell. Resistant strains selected from a well-characterized 'wild-type' sensitive line are more promising. The cell line Daudi, isolated from a Burkitt's lymphoma, is a favourite for studying the effects of human interferon both at the level of receptor binding and of inhibition of serum stimulated cell division. The resistant strains all carry receptors, but the dynamic changes that lead to increases in binding affinity and a decrease in binding capacity in the sensitive parental line are absent or much reduced in the resistant lines[21, 27, 45].

These aspects of receptor binding have attracted the attention of clinicians looking for a means of monitoring and, better still, predicting the response of tumour patients to interferon therapy. Binding studies on neoplastic tissue are really only possible in the lymphoproliferative diseases, some of which do respond to interferon therapy. At least one patient with a hairy cell leukaemia, unresponsive to interferon, has been found to have tumour cells without receptors for human alpha interferon[11]. Otherwise the cells of patients resistant to interferon therapy carry receptors similar, in density and behaviour, to those of patients that respond well[12, 24, 36, 64, 79]. Peripheral blood leukocytes from patients undergoing interferon therapy bind less interferon than those taken from untreated individuals[12]. This induced decrease in binding occurs in vitro as well[55]. It has been examined as a possible marker for responsiveness to interferon both in a positive and a negative sense. This phenomenon, which has acquired the meaningless name of 'down-regulation', correlates with neither responsiveness nor resistance to interferon in the patient and seems to be a marker for no more than the presence of circulating interferon[12, 55, 64]. Receptors, whether on the normal cells of the host or on the tumour cells themselves, may be necessary but are certainly not sufficient for interferon to produce a response.

Particularities

The human alpha interferons constitute a family of proteins which, together with beta interferon, bind to the same receptor to produce the same sort of effects[2, 20, 66, 67, 73, 77, 81, 87, 91, 92, 96]. The reason for this multiplicity of ligands is not known, It is not even known if there is a reason or whether, indeed, it makes sense to ask. However, neither the binding affinities of the interferons, nor their behaviour once bound, are identical and it might make sense to examine the consequences of this. The most striking and the best documented difference is that of the specific activity of the two major interferon alpha species -1 and -2 (respectively -D and -A in the alternative nomenclature) as measured on human cells[2, 91, 92]. A synergistic action has been proposed[73]. Mostly binding has been measured directly with radiolabelled interferon alpha-2 and the binding of the other interferons has been inferred from competition, that is, with unlabelled interferons competing with labelled alpha-2. It is possible to label interferon alpha-1 and measure its binding directly on human cells. This allows us to measure the kinetic contribution to affinity and we have proposed that the slower actions of alpha-1 might buffer the faster ones of alpha-2[92]. It is likely, though, that an in vitro experimental system is inadequate to reveal all the kinetic consequences of having two distinct binding affinities[68]. Direct binding studies with radiolabelled interferon alpha-8 (type -B) have revealed another aspect that was obscured in one-way competitive binding, namely that receptors bound more interferon alpha-8, for a given response, than they did alpha-2. This case is in contradistinction to that of alpha-1 where the affinity of the receptor for the ligand is less but the binding: effect ratios are as for alpha-2[91]. As far as we have been able to establish the alpha interferons in a mixture bind independently so that the proportion of these three interferons in the bound phase must be quite different from that free in the supernatant.

Finally there are the reports of the cross-binding of human alpha-interferon and sheep blastocyst antiluteolytic protein to membrane receptors from the endometrium of ewes[86], of sequence homologies between the alpha interferons and an ovine trophoblastic protein[19, 50]; and the reports of interferon-like substances in human amniotic fluid[56] and in foetal blood[18]. These have extended the

physiological range that needs to be taken into account for the interferons. In particular the fact that alpha interferon induces prostaglandin secretion in ovine embryos[82] may have some bearing on the toxic side effects seen in patients receiving interferon. It is notable that the human alpha interferons cross-react well on cells derived from ungulates and that the distinction between interferons alpha-1 and alpha-2 disappears on bovine cells[66]. This aspect is, of course, not really a particularity of interferon; a great many humoral factors have been found in related forms stimulating different responses in tissues, organs and species other than those in which they were first described[7].

Differentiation models for interferon

It is possible that the inhibitory effects of interferon on the multiplication of cells in culture may actually have a function in vivo in bringing to an end the proliferative phases that precede a differentiated state[14, 29, 41, 70, 78]. The Burkitt lymphoma-derived cell line Daudi is an excellent model for interferon activity as the antiproliferative response goes to completion. Once quiescence is established it cannot be reversed by removing interferon and replacing it with fresh culture medium. Exley et al.[31] have shown that growth inhibition of the Daudi cell line is accompanied by surface changes characteristic of plasmacytoid differentiation. It can be shown that inhibition of proliferation by interferon is equivalent to the blocking of growth stimulation[9, 10, 28, 29, 33, 65, 74, 78, 88, 103]. It has also been shown that the induced quiescence goes hand in hand with the disappearance of interferon receptors from these cells[28].

There are several indications that interferon blocks the activity of different growth factors independently[47], i.e. there is not a particular target in the cell cycle. Several phases are prolonged[22]; cells pile up at the end of a cycle, competence to enter another having been blocked[60]. This rather suggests that the block might occur where pathways for the different growth factors either converge or, at least, share certain features that are not specific.

The effects of interferon on macrophage activation also provide an interesting model. We said at the beginning that we would ignore gamma interferon in this review because its receptors are different from those of alpha-beta interferon, i.e. binding of radiolabelled gamma interferon is not displaced by alpha-beta interferon and vice versa[67] (though there was always a small question mark about beta interferon[5, 90]).However, in this case it is precisely the effects of alpha-beta interferon on the action of gamma interferon that are of interest. Synergistic effects, antagonistic at low concentrations, agonistic at high, of alpha-beta interferons on the activity of gamma interferon have been noted by several authors[23, 32, 35, 38, 49, 61, 101].They are not peculiar to macrophages. An effect of interferon gamma on the action of interferon alpha has also been described with pre-treatment by the former reducing the binding capacity of the cells for the latter[44]. The interest of the macrophage activation model lies in the fact that alpha-beta interferon blocks the effects of gamma interferon on membrane antigens and receptors as well as affecting the biological activities induced[32, 61]. Maturation of macrophages is associated with a single class of gamma interferon receptors evolving into two populations one of which is of demonstrably higher affinity. Yoshida et al.[101] have shown that the antagonistic effects of alpha-beta interferons occur at the higher affinity binding site and at concentrations that are considerably lower (fM as against pM) than those of the interferon gamma whose binding was being measured.

Binding at the receptor

It can be shown that interferon comes into intimate contact with a surface membrane protein as it binds to cells[28, 43, 83, 91]. It is possible to establish a covalent chemical link between a protein ligand and a ligand-binding protein by reacting the complex with a bifunctional reagent, containing a reactive group at either end of a linear spacer of given chain length. If the reaction is successful and the product is distinct, the complex of a labelled ligand and its binding protein can be separated and characterized with respect to its molecular properties. If the ligand itself is already well characterized the properties of the binding protein may be inferred. Commonly spacer lengths of about 10 Å are used. This means that the orientation of the ligand with respect to the binding protein must be such that the chance of two reactive amino acid residues being within this distance of each other is much greater for this particular combination of proteins than for any other. Larger combinations are possible, though one would expect them to be minor[76]. This is a long-winded description of a common enough technique, but it is almost the sole basis upon which the receptor is defined.

For alpha interferon binding to human lymphoblastoid cells, purification and radiolabelling of such cross-linked complexes followed by scission of the chemical link yields a ligand binding protein (or perhaps proteins) which migrates as a doublet, of Mr between 95 000 and 100 000, on electrophoresis in the presence of the denaturing detergent sodium dodecyl sulphate (Eid, unpublished results). From a comparison with receptors isolated in a milder detergent than sodium dodecyl sulphate, it would appear that the site to which interferon actually binds may be several times the size of the binding protein isolated by cross-linking (Eid, unpublished results). During binding of alpha interferons to Daudi cells there are obvious changes in apparent affinity of these sites and in their solubility; changes that probably involve a re-organization of the receptors; changes that are necessary to inhibit the proliferation of the culture[28, 43]. The 'receptor' for which concentration and affinity are determined as con-

stants is not necessarily the same as the binding protein that is isolated and purified as 'receptor'.

In respect of receptor studies the term affinity is borrowed from the chemical notion of a reversible bimolecular reaction which has reached equilibrium. The popular method of calculating the affinity from experimental measurements is to take the slope of a Scatchard plot (bound ligand/free ligand against bound ligand) as being the affinity constant (with units M^{-1}). For technical reasons, the more precise notion of equilibrium is replaced by that of steady state where it is assumed that the combined terms for the forward velocities equal those of the reverse. In terms of binding experiments this means that, at a given concentration of free ligand, bound ligand should achieve a certain level (not necessarily a maximum), and then stay there. This, for example, is how interferon binds to the resistant strains of Daudi cells[27]. Steady-state analysis of ligand binding is capable of considerable refinement[71,98]. Fundamentally though, the theory is mal-adapted and, though we often pay lip service to its constraints, in practice we ignore them. The physical meanings that became attached to the Scatchard plot were developed largely from the study of the binding of small molecules to proteins. As the requirements for cellular receptors differ, so these meanings may no longer be relevant. For receptor work an affinity 'constant' often has a particular value merely because we choose to work in a particular range of ligand concentration.

It is largely a matter of convenience whether we regard the affinity being independent of the ligand concentration (linear Scatchard plot) as the basic relationship from which all others deviate, or as a special case of a more general relationship where the affinity varies with the ligand concentration. The choice, however, is important because, in the latter case, it becomes possible to relax the constraints of the steady state formulation. The two aspects of the Scatchard plot that are important are the rotation (for change of affinity read change of slope) and the translation (for change of receptor concentration read change of intercept on the abscissa). If the slopes are taken as tangents at a point, they can be treated as derivatives of the binding function. We are interested in these transformations during the induction of biological effects. It is, of course, convenient to call them changes in affinity and changes in receptor concentration and we shall probably continue to do so; but it seems confusing and unnecessarily difficult to try and build on such foundations. We may take a case in point from our own work. We noted that the initial binding of interferon to Daudi cells did not correlate with the subsequent antiproliferative effects but that it later evolved to give 'high affinity sites' whose occupation by interferon was proportional to the effect. The transformation was coherent, being described as well by kinetic as by fixed interval experiments, and it was concluded that affinity appeared to increase because ligand dissociation, as measured by displacement, was slower. It was subsequently found[28] that dissociation of interferon from its binding site was unchanged but that at later times a substantial part of the bound ligand was being 'displaced' into the cell rather than into the supernatant. The change in affinity was illusory (which a proper steady-state formulation of the problem would show); but the transformation of the Scatchard plot nevertheless showed up the exact dependence upon ligand concentration of the binding and the response (which a steady-state formulation could not have done).

Mechanisms at the receptor

Given the reservations outlined above, we can look at some of the mechanisms involved. The important point that had to be established was the congruence between the specific activity of an interferon and its binding affinity in a model system (the qualification is important not just because resistant cells carry receptors but because even among those of intermediate sensitivity there may be quite large variations in receptor density). After the production of individual interferons by recombinant DNA technology had been perfected, it was possible to recognize and then exploit the differences in specific activity between interferon alpha-1 and the other alpha interferons[77,87,96]. It became possible to use monoclonal antibodies for epitope mapping[4,6,17,62,89]. In particular the notion of neutralization could be made a little more precise; a monoclonal antibody that neutralized at mol-to-mol ratios would probably be directed against an important determinant. The determinant could be localized by constructing hybrid interferon molecules based on the restriction sites in the recombinant DNA that coded for the interferons[66,87,96]. Structural predictions could be made on the basis of the known sequences of the interferons[102] and point mutations could be introduced in the regions of interest[15,93,95]. Where they have been tested the differences and changes in specific activity have correlated quite nicely with the receptor binding[63,66]. If we should summarize these results we would say that the interaction between interferon and its receptor on human cells depends totally on a ligand conformation which places the cysteine at position 29, close enough to the cysteine at 138 for the two to form a disulphide bridge[69]. There are sensitive residues close to both these cysteines[15,72,84]. The interferon alpha-1 determinant probably lies on the N-terminal side of the bond[15,63,66]. The extreme C-terminal segment is apparently exposed when interferon is receptor-bound (at least at 4 °C), as monoclonal antibodies bind to it[6]. There are several clusters of conserved sequences among the alpha-beta interferons of which two include the Cys 29-Cys 138 bond. In the cluster at 47–50 various substitutions can be made by site-directed mutagenesis in the gene without unduly altering the biological activity of the expressed protein[93]. There are several sections which are obviously alpha-helical in both the N-terminal and the C-terminal halves of the model[102]. There is one helix from ca 55–70 whose sur-

face residues are hydrophobic on one side and hydrophilic on the other, and that probably helps to maintain the structure of the molecule[26, 102].

Studies of this type have revealed quite significant differences in the binding of interferon analogues to human and bovine cells[63]. The results with bovine cells may well be fairly typical for all the cross-species activity that is shown by human alpha interferon. We have not attempted to review these results in any detail because the picture has changed completely with the discovery of the homology between the ovine pregnancy proteins and human alpha interferon[19, 50, 82, 86].

In the nucleotide sequence of the genes coding for alpha interferons there are restriction sites corresponding to amino acids at positions 61 and 90 of the protein[66]. Hybrid molecules containing this section derived from the human interferon alpha-8 (or B) contain a unique cluster from amino acids 84–90. The change from Lys-84 to give Glu-84 is unique to interferon alpha-8, so is the change Thr-87 to give Ile-87. At residue 90, alpha-8 has Asp instead of the common Tyr. These are major changes in charge, hydrophobicity and steric size and they are probably responsible for the characteristic receptor binding shown by alpha-8[91]. An analysis of the binding of parental and hybrid interferons on membrane fragments from Daudi cells showed that the receptor-complexed forms of interferons alpha-1 and alpha-2 were subject to an electrostatic interaction that reduced the total quantity bound under physiological conditions and that this effect was absent from the binding of interferon alpha-8. Because the effect was unrelated to the specific activity and the affinity of binding but altered the evolution of binding, it was described as an interaction between receptor-bound interferons[91]. DeGrado et al.[26] showed how amino acid homologies between the alpha-beta and the gamma interferons could be revealed by postulating a similar secondary structure and re-aligning the sequences with respect to it. A better-than-random homology with interferon gamma was found for the sequence corresponding to 63–104 of interferon alpha-2. Homologies with gamma were even better in the sequence 59–106 of beta interferon. It may be that the antagonistic effects of the alpha-beta interferons with respect to gamma interferon are generated by the later interactions between ligand receptor complexes.

We have said nothing about human chromosome 21, which appears to carry a gene for a protein essential for the proper functioning of the alpha-beta interferon receptor[39]. There is a considerable literature on the subject[13, 25] but the identity of the protein is not yet established. However, the binding protein for gamma interferon has been cloned[1] and assigned to human chromosome 6[1, 75]. The inclusion in hybrid cells of the human chromosome 6 was enough to permit ligand binding but was insufficient to establish responsiveness to gamma interferon. The additional presence of chromosome 21 was required for that[51].

We have also been silent on the relationship between receptor binding and the enzymic activities induced by interferon[58]. This is because on the one hand the published work on these activities is too extensive to review here, and on the other hand there is practically nothing known about the order of the events that follow receptor binding. It is, for example, fairly straightforward to establish a correlation between the concentration of interferon and the removal of interferon receptors, and the enhancement of 2',5'-oligo A synthetase activity, and the cessation of cellular proliferation. However, in a culture of exponentially dividing Daudi cells treated with interferon, the maxima of these three ligand-induced effects are contemporary rather than sequential.

In vivo again

There is a need for good in vitro quantitative models capable of giving useful qualitative descriptions of ligand effects in vivo. To answer questions like: does it happen? where does it happen? when does it happen? is it enough or too much? or even, is it better or worse? There must be many in vitro results that have no counterparts in vivo; there should be ways of recognizing them.

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

Interferon receptors and their role in interferon action

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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