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Contents

Interferons and Cancer: How the Promise is Being Kept

Ernest C. Borden

The Toxic Effects of Interferon in Man

G. M. Scott

Interferon Induction by Viruses: One Molecule of dsRNA as the Threshold for Interferon Induction

Philip I. Marcus

More About Interferon Cloning

Rik Derynck

Genetic and Functional Diversity of Interferons in Man

Michel Revel

Interferon Receptors

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I INTRODUCTION

The indirect evidence accumulated over the past decade suggesting the existence of an interferon receptor system has already been reviewed (Chany, 1976; Stewart, 1979a; Friedman, 1979) and is only briefly dealt with in this article; more detailed attention is paid to the recent reports on direct ligand binding studies. The aim of this article is to emphasize new biological aspects and related questions that arise from these recent advances.

We have tried to draw models for the initial mechanisms of interferon action, i.e. those steps that bring about specific communication between the sites of interferon production and the target of interferon action. Current

research on the interferon receptor system has been stimulated by the pioneer work in endocrinology of the early 1970s, so the models presented are considered in the context of recent progress in the large field of research on ligand receptor interactions.

Hormones bind to cell surface receptors with high affinity. This interaction is specific since it is saturable and is a prerequisite for the induction of a specific biological response (for review see Cuatrecasas and Hollenberg, 1976). A proportion of receptors may be "spare", i.e. their occupation does not result in an increase of the response. Ligand binding can intervene in modulation of receptor expression: receptors can be "down-regulated" upon binding. Generally, ligand–receptor complexes are internalized and degraded according to pathways associated with specific morphological substrates, such as coated pits and specialized vesicles (see e.g. Pastan and Willingham, 1981a, b; Steinman *et al.*, 1983). Recently, antibodies to receptors that mimic specific hormone action have been described (Karlsson *et al.*, 1979; Valente *et al.*, 1982; Schreiber *et al.*, 1981, 1983). This led to the thesis that hormone action is limited to a receptor triggering step, leaving the activated receptor to generate the specific biological response.

On comparing the interferon receptor system with various endocrine systems, similarities and common mechanisms appear. It was therefore of particular interest to try to define specific properties of the interferon system. Although this field is largely unexplored and many results are still preliminary, we have tried not only to consider fundamental aspects but to discuss also potential clinical implications.

To facilitate reading, the term "interferon" includes the interferon classes alpha and beta, whereas interferon gamma is always referred to explicitly.

II EVIDENCE FOR A RECEPTOR SYSTEM

Until Friedman (1967) observed that trypsin treatment of cells previously exposed to interferon at 4°C abolished the induction of an antiviral state upon further incubation at 37°C, there was no convincing information to suggest a direct interaction of interferon with a receptor. However, an alternative mode of action as a working hypothesis was hardly conceivable, since it was known early on that inhibition of virus replication requires exposure of the cells to interferon prior to virus infection (Lindenmann *et al.*, 1957) and is thus an induced biological effect depending on active cellular metabolism (Taylor, 1964; Lockart, 1964). The requirement for interferon production of mRNA synthesis (Heller, 1963; Gifford and Heller, 1963) and the translation of interferon mRNA in heterologous cells (DeMaeyer *et al.*, 1972), as well as the sensitivity of interferon to proteolytic enzymes (Lindenmann *et al.*, 1957; Lockart, 1973) indicated the peptide

nature of interferon. Thus, the action of peptide hormones was a tempting model for the initial mechanisms of interferon action: insulin had been shown to induce its biological effects through interaction with specific high-affinity cell membrane receptors (Cuatrecasas, 1971).

The high specific activity of interferon (Ng and Vilček, 1972) and the observation that only a small fraction was consumed upon interferon treatment of cells *in vitro* (Buckler *et al.*, 1966; Friedman, 1967) were consistent with binding to high-affinity receptors, but, the verification of this hypothesis had to await the availability of pure interferon.

In addition to the experiments by Friedman (1967) evidence for the existence of interferon receptors accumulated with the finding that interferon activity could be recovered from homologous, but not heterologous, cells exposed to interferon (Stewart *et al.*, 1972; Berman and Vilček, 1974). Similarly, Gresser *et al.* (1974) reported that interferon eluted upon incubation from sensitive mouse leukaemia L1210 cells exposed to interferon, whereas none was recovered from similarly treated interferon resistant L1210 cells.

Several observations suggested a possible role for membrane gangliosides in interferon binding. The reversible inhibition of interferon action by pretreatment of target cells with phytohaemagglutinin (Besançon and Ankel, 1974a) was interpreted as blocking of interferon receptors, presumably carbohydrate-containing molecules. Purified gangliosides neutralize interferon activity (Besançon and Ankel, 1974b), presumably through reversible binding to their carbohydrate moiety (Besançon *et al.*, 1976). The biological relevance of gangliosides for binding and action of interferon was suggested by the finding that pretreatment of ganglioside-deficient cells with gangliosides could increase their interferon sensitivity (Vengris *et al.*, 1976). On the basis that the beta-subunits of choleratoxin (Holmgren, 1981) and thyrotropin (Mullin *et al.*, 1976) interacted with membrane gangliosides, these ligands were used as probes for putative interferon-binding sites. Friedman and Kohn (1976) reported that choleratoxin neutralized interferon activity. Furthermore binding of [^{125}I]-labelled choleratoxin and thyrotropin to membranes of mouse L-cells and human KB-3 cells was affected by the addition of mouse or human interferon (Kohn *et al.*, 1976). This finding was at variance with the observations on recovery of interferon from treated homologous cells mentioned above and was interpreted as an indication for the existence of at least two distinct sites on the interferon molecule, a species-nonspecific receptor-binding site and a species-specific activation site. Similar observations led to the concept that a glycoprotein component of the putative interferon receptor was responsible for binding of choleratoxin, thyrotropin and interferon, whereas the interferon-specific signal transmission required a ganglioside component (Grollman *et al.*, 1978). This is a substantial body of evidence, yet it does not correlate with direct binding

studies. In a recent direct binding analysis mouse interferon alpha/beta and choleratoxin were clearly shown to bind to distinct unrelated sites, since no competition occurred between these two ligands (Aguet *et al.*, 1982). The current view on the nature of interferon receptors is further discussed in Section X.

Although the conclusive localization of the human interferon genes on chromosome 9 was demonstrated only recently by use of recombinant DNA techniques (Owerbach *et al.*, 1981) it was already recognized from experiments with heterologous cell hybrids that the sensitivity to interferon was somehow linked to chromosome 21 in the human system (Tan *et al.*, 1973, 1974). It had first been thought that this chromosome carried information for the synthesis of interferon-induced antiviral proteins (Tan *et al.*, 1973). However, antibodies raised against somatic mouse–human cell hybrids retaining only the human chromosome 21 inhibited the response of human cells to interferon (Revel *et al.*, 1976). This observation, which was recently confirmed with monoclonal antibodies (Kamarck *et al.*, 1981), suggested that the gene product(s) for sensitivity were interferon receptors (Revel *et al.*, 1976). Similar observations on the linkage between retained chromosomes and the expression of species specific interferon sensitivity in monkey–mouse cell hybrids gave rise to a receptor model postulating that species specificity of interferon action was determined by specific cell surface receptors (Chany, 1976).

Mogensen *et al.* (1982) recently reported that peripheral lymphocytes from trisomy 21 patients bind more interferon than normal lymphocytes. Similarly, Epstein *et al.* (1982) described quantitative differences in interferon binding to human fibroblasts monosomic, disomic or trisomic for chromosome 21, suggesting a gene dosage effect. These findings clearly suggest that human gene(s) located on chromosome 21 code for the cell surface receptor specific for interferon.

With the use of hamster–mouse (Cox *et al.*, 1980) or mouse–human cell hybrids (Lin *et al.*, 1980), the gene(s) coding for interferon sensitivity in the mouse system could be assigned to chromosome 16. The syntenic association of the genes coding for interferon sensitivity and for cytoplasmic superoxide dismutase in both man (Epstein and Epstein, 1976; Sinet *et al.*, 1976) and mouse (Cox *et al.*, 1980; Lin *et al.*, 1980) strongly support a parallelism between human chromosome 21 and mouse chromosome 16 with regard to the interferon receptor system.

III DIRECT ANALYSIS OF INTERFERON BINDING

The investigation of ligand binding to cellular receptor sites relies *a priori* on the notion of specificity, which implies first that such binding sites exist in a

definite number and can be saturated and secondly that a given ligand receptor interaction cannot be inhibited by unrelated substances. Accordingly, ligand binding to various biological receptor systems has widely been documented through saturation curves and specific competitive binding inhibition. The relevant experimental procedures and the various mathematical approaches to interpretation have been extensively reviewed (Cuatrecasas and Hollenberg, 1976), but some aspects concerning Scatchard analysis, the most commonly used method to represent data on ligand binding, are worth re-emphasizing. Scatchard graphs are based on the mass action law transformed to a linear function. The ratio between the concentrations of specifically bound and free ligand on the ordinate is plotted against the concentration of specifically bound ligand on the abscissa. The slope of the resulting straight line gives the affinity constant (the reciprocal of the dissociation constant K_d), whereas the concentration and the resulting total number of binding sites are determined by extrapolation to the intercept on the abscissa. As this extrapolation is to infinite ligand concentration, some independent method is needed for scanning binding data to see just how close to saturation they do in fact come (Klotz, 1982). Linearity of Scatchard plots suggests non-cooperative ligand binding to homogeneous binding sites.

Limitations of the Scatchard method arise principally from application of the mass action law. For the extrapolated binding constant and the number of receptor sites to represent reliable values, the ligand receptor interaction has to be reversible, purely bimolecular, and the respective concentrations must be measured at equilibrium, so experimental conditions for binding studies on cells have to be chosen in such a way that the fraction of occupied receptors becomes independent of the cell density (Cuatrecasas and Hollenberg, 1976; Aguet and Blanchard, 1981).

Biological responses to interferon are usually measured at 37°C. Receptor binding on viable cells is best measured at 4°C, a temperature at which reaching equilibrium is a practical possibility. If receptor occupation is to be linked to cellular function, one has to accept the probability that none of the reactions followed will ever reach a true stable equilibrium. Receptor turnover and/or recycling, receptor "down-regulation", internalization and degradation processes have to be considered in all experiments carried out with viable cells at 37°C. Not only are such mechanisms incompatible with a simple application of mass action law, they are often inter-related and therefore difficult to investigate separately. Scatchard slopes and intercepts no longer correspond to the parameters of a simple bimolecular reaction (there is a certain amount of blind faith involved in accepting that they do at 4°C): binding is static; activity, dynamic. This is not to imply that Scatchards are useless, merely that what they measure is relative to changes within the system studied.

Ligand-binding analyses and corresponding Scatchard graphs are best used to investigate relationships between mechanisms at the receptor level and the induction of a biological response. It should be emphasized that the terms "binding site" and "receptor" are not synonymous: the word "receptor" implies a function. As long as a correlation between "specific" binding of a ligand and the induction of a specific biological response is not substantiated, the relevance of seemingly specific binding remains questionable (Cuatrecasas and Hollenberg, 1976).

Direct binding studies rely on the availability of highly purified ligand labelled without loss of biological activity. Accordingly, specific binding of interferon to cellular binding sites was demonstrated for the first time when a highly purified mouse interferon preparation became available (DeMaeyer-Guignard *et al.*, 1978). This interferon consisted of three major molecular weight species (Aguet, 1980), identified serologically as alpha and beta classes (Kawade *et al.*, 1982). Binding experiments using a [^{125}I]-labelled preparation revealed saturable, displaceable, high-affinity binding to interferon sensitive mouse L1210 cells (Aguet, 1980), whereas specific binding was observed neither on interferon resistant L1210 cells (Gresser *et al.*, 1974) nor on heterologous fibroblasts insensitive to mouse interferon. Correlation between specific binding and biological response thus characterized these binding sites as probable interferon receptors. Similarly, purified virus-induced human interferon alpha was shown to bind to specific receptors on cells of various human lymphoid cell lines (Mogensen *et al.*, 1981a), peripheral blood lymphocytes (Mogensen *et al.*, 1982; Yonehara *et al.*, 1983) and bovine cells crossreactive with human interferon alpha (Zoon *et al.*, 1982). Several binding studies using [^{125}I]-labelled human recombinant DNA interferon alpha have been reported (Branca and Baglioni, 1981; Baglioni *et al.*, 1982; Epstein *et al.*, 1982), and recently specific high-affinity binding has also been documented with human interferon gamma (Anderson *et al.*, 1982a). The various results are summarized in Table I.

These data underline the similarity between interferon and some peptide hormone systems (Kaplan, 1981). For example, the binding constants for insulin (Cuatrecasas and Hollenberg, 1976) and epidermal growth factor (Carpenter and Cohen, 1976) are of the same order of magnitude. Hitherto the estimated number of receptor sites appears particularly low in the interferon system, possibly 10 to 100 times lower than for insulin and epidermal growth factor. This is a slightly suspicious state of affairs and sooner or later we shall have to consider seriously whether such a low receptor concentration can in fact accommodate all the different effects of interferon.

Nevertheless, the first step in interferon action, i.e. the specific recognition by cellular binding sites, whose primary role it is to increase a low concentration of ligand at the target cell surface, has been well documented.

TABLE I Interferon Receptors: Binding Constants and Number of Receptor Sites

Ligand	Target cell	Apparent binding constant	Approximative number of receptor sites per cell	References
Mouse IFN alpha/beta	Mouse L1210 cells	10^{-10} M (4°C) 2×10^{-11} M (37°C)	10^3 (4°C)	Aguet and Blanchard (1981)
Mouse IFN alpha/beta	Mouse embryonic carcinoma cells (PCD3, PCC4)	10^{-10} M (4°C)	1 to 2×10^4 (4°C)	Aguet *et al.* (1981)
Human IFN alpha (Namalva cells, peripheral leukocytes)	Daudi cells	2×10^{-10} M (4°C) 10^{-11} M to 2×10^{-10} M (37°C)	4×10^3 (4°C)	Mogensen *et al.* (1981a)
	P3HRI cells	3×10^{-10} M (4°C) 9×10^{-11} M to 10^{-9} M (37°C)	$>6 \times 10^3$ (4°C)	
	Raji cells	5×10^{-10} M (4°C) 3×10^{-11} M (37°C)	10^3 (4°C)	
Human IFN alpha (Namalva cells)	Human fibroblasts	4×10^{-10} M (21°C)	1 to 2×10^3 (21°C)	Yonehara *et al.* (1983)
	Bovine cells (MDBK)	$6{\cdot}6 \times 10^{-10}$ M (21°C)	10^3 (21°C)	
Human IFN alpha (Namalva cells)	Bovine cells (MDBK)	6×10^{-11} M (4°C)	650 (4°C)	Zoon *et al.* (1982)
Human IFN alpha-2	Daudi cells	$1{\cdot}5 \times 10^{-10}$ M (37°C)	5×10^3 (37°C)	Branca and Baglioni (1981)
Human IFN alpha-2	Human fibroblasts	2×10^{-10} M (37°C)	350 to 550 (37°C)	Epstein *et al.* (1982)
Human IFN gamma	Human fibroblasts	$1{\cdot}5 \times 10^{-10}$ M (37°C)	2400 (37°C)	Anderson *et al.* (1982a)

IV CORRELATION OF BINDING WITH CROSS-REACTIVITY ON HETEROLOGOUS CELLS

Since the original reports, cross-species activity has exerted a fascination for those engaged in interferon research (Desmyter *et al.*, 1968; Levy-Koenig *et al.*, 1970). The attraction is obvious: certain elements involved in the action of interferon can be isolated experimentally on cells of a different species. With a well-defined third component – neutralizing antibodies (Paucker *et al.*, 1975), cross-species cell hybrids (Cassingena *et al.*, 1971; Chany, 1976), and later, individual species of interferon and even hybrids thereof (Streuli *et al.*, 1981; Weck *et al.*, 1981; Rehberg *et al.*, 1982) – there is enough variation for fine analysis. None of the models generated is satisfied by a simple two-component interaction. Thus interferon either has multiple active sites (Paucker *et al.*, 1975; Streuli *et al.*, 1981), or receptors are composed of two sites for binding and activation (Chany, 1976), or cells have subtype-specific receptors (Rehberg *et al.*, 1982).

Of course interferon could have different effects on different cells: bovine cells, for example, might be primed by human interferon to produce bovine interferon upon virus challenge. However, assuming that the cross-species activity of a human interferon on bovine cells is really like the activity it shows on cells of its own species, we should find a similar receptor binding on both human and bovine cells. Fortunately one line of bovine cells shows satisfactory binding curves with labelled interferon (Zoon *et al.*, 1982), saturable, displaceable and with a K_d in the approved range (see Table I). Actually, the "approved" K_d range for experimental K_d values turns out to be rather narrow (10^{-12} to 10^{-10} M), limited by the number of receptors per cell as determined under the experimental conditions commonly used. This is illustrated by the particular binding properties of human interferon alpha-1. Whereas the K_d of human interferon alpha-1 for bovine MDBK cells is also about 10^{-10} M, saturable displaceable binding was not found on human cells (Aguet *et al.*, 1983a). Displacement of human interferon alpha-2 with interferon alpha-1 indicates that the K_d of human interferon alpha-1 for human cells is approximately 10^{-8} M. Of the various interferons tested so far, human interferon alpha-1 has the lowest binding affinity for homologous receptors. Thus, for the number of receptors expressed in the various systems described (Tables I and II), specific receptor binding with K_d values greater than 10^{-9} M is below the detection threshold.

V NEUTRALIZING ANTIBODIES THAT INHIBIT BINDING

Neutralizing antibodies that inhibit interferon are usually assayed for convenience against a low fixed dose of interferon, just enough to protect the