125I-labelled Human Interferons Alpha, Beta and Gamma: Comparative Receptor-binding Data

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SUMMARY

Binding of 125I-labelled human recombinant DNA interferons (IFNs) alpha-2, beta and gamma was compared on various human lymphoid cells and embryonic fibroblasts. While binding constants were within an order of magnitude for all three interferons (10^-10 to 10^-9 M), no competition was observed between IFN-γ on the one hand and IFN-α2 and IFN-β on the other. However, consistent with previous reports, IFN-α2 and IFN-β competed for presumably common receptors. Depending on the cell type, binding sites for IFN-γ were expressed in different numbers compared to those for IFN-α2 and IFN-β. These direct comparative binding studies support the hypothesis that the receptor system for IFN-γ is unrelated to the IFN-α/β system.

It is commonly accepted that the initial event in interferon (IFN) action is its binding to specific cell-surface receptors (for review see Aguet & Mogensen, 1984). IFNs comprise three types, alpha, beta and gamma, and the alpha type is further subdivided into at least eight subtypes (Weissmann et al., 1982). Human IFN-α subtypes cross-react with presumably common receptors, although their binding affinities vary considerably (Aguet et al., 1984). IFN-β also competes for the same binding sites as IFN-α both in man and mouse (Branca & Baglioni, 1981; Aguet & Blanchard, 1981). Absence of mutual competition strongly suggests that IFN-γ binds to receptors distinct from those for IFN-α (Branca & Baglioni, 1981; Aguet et al., 1982; Anderson et al., 1982; Orchansky et al., 1984; Sarkar & Gupta, 1984). IFN-γ receptors, however, were reported to cross-react to some extent with human IFN-β (Anderson et al., 1982). Differences in the binding affinity of two ligands may account for the absence of competition; on the other hand, competition for apparently common binding sites may be due to steric hindrance or non-specific interactions. To provide unequivocal information on the cross-reactivity of the various interferons at the receptor level, it was important to compare their binding properties directly with pure labelled ligands.

Pure human recombinant DNA IFN-α2 (Biogen; 10^6 U/mg), IFN-β (Cetus; 2 x 10^8 U/mg) and IFN-γ (Biogen; 2 x 10^7 U/mg) were labelled with 125I to a specific radioactivity of about 40 to 80 μCi/μg protein using a chloramine T method described by Aguet et al. (1984). The recovery of biological activity was better than 50% as determined by titration of antiviral activity on human T98G cells for IFN-α and -β (Aguet et al., 1984) and WISH cells for IFN-γ (Wietzerbin et al., 1984) challenged with vesicular stomatitis virus. The binding properties of these labelled interferons were first compared on human Daudi cells which have been well characterized for IFN-α2 binding (Aguet et al., 1984; Branca & Baglioni, 1981; Eid & Mogensen, 1983). The saturation curve of labelled IFN-β as determined at 4°C revealed a binding constant of about 10^-10 M and approximately 400 binding sites per cell (Fig. 1, Table 1) which is consistent with the binding properties of labelled IFN-α2 (Branca & Baglioni, 1981; Mogensen et al., 1981). While the binding affinity of labelled IFN-γ was of the same order of magnitude, the number of binding sites extrapolated from the Scatchard plots (3500 to 7000) was markedly higher than for IFN-α2 and IFN-β (Fig. 1, Table 1). Identical experiments were carried out with the various cells listed in Table 1, confirming the observation that the numbers of binding sites for IFN-α2

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Fig. 1. Specific saturable binding of recombinant 125I-labelled IFN-β (a) and IFN-γ (b) to 2 × 10⁶ Daudi cells. The cells were grown as described in Table 1 and the binding experiments carried out as previously described (Aguet & Blanchard, 1981; Aguet, 1985) at 4°C for 2 h at a density of 2 × 10⁶ cells/ml in triplicate. The total cell-bound radioactivity was determined (A) and non-specific linear binding determined by addition of 2 × 10⁴ U/ml unlabelled IFN (O). Each point represents the mean value of three experiments (the standard deviation did not usually exceed 10%). Insets represent Scatchard plots calculated from the specific binding data (Aguet & Blanchard, 1981; Aguet et al., 1981) (abscissa: U/ml bound IFN; ordinate: ratio of bound IFN to free IFN expressed as a percentage).

Fig. 2. Competition between various IFNs for binding to Daudi cells. Binding experiments were carried out as described in Fig. 1 except that constant amounts of labelled IFN [(a) 500 U/ml 125I-IFN-α2; (b) 500 U/ml 125I-IFN-β; (c) 100 U/ml 125I-IFN-γ] were added together with increasing amounts of unlabelled IFN-α2 (O), IFN-β (●) or IFN-γ (▲) as indicated on the abscissa. Consistent with the saturation curves shown in Fig. 1, non-specific binding of 125I-labelled IFN-β and IFN-γ (b, c) was more pronounced as compared to 125I-labelled IFN-α2 (a).

and IFN-β are similar, while they may differ from those for IFN-γ. Saturable binding was observed for all cells tested and the extrapolated binding constants were consistently of the same order of magnitude.

To investigate cross-reactivity, competition of labelled and unlabelled interferons was investigated. As exemplified with Daudi cells, IFN-α2 and IFN-β competed for highly cross-reactive, presumably common binding sites (Fig. 2a, b). Unlabelled IFN-γ, however, did not compete with labelled IFN-α2 and IFN-β (Fig. 2a, b), nor was binding of labelled IFN-γ
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Table 1. Receptor-binding data of human interferon (IFN) alpha-2, beta and gamma

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Target cell*</th>
<th>Apparent binding constant (M) per cell</th>
<th>Approximative number of receptor sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-α2</td>
<td>HEp-2</td>
<td>2 × 10^{-10}</td>
<td>1000</td>
</tr>
<tr>
<td>IFN-β</td>
<td>HEp-2</td>
<td>7 × 10^{-10}</td>
<td>1000</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>HEp-2</td>
<td>10^{-9}</td>
<td>7500–15000</td>
</tr>
<tr>
<td>IFN-α2</td>
<td>A549</td>
<td>4 × 10^{-10}</td>
<td>1000</td>
</tr>
<tr>
<td>IFN-β</td>
<td>A549</td>
<td>3 × 10^{-11}</td>
<td>400</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>A549</td>
<td>1 × 10^{-10}</td>
<td>4000</td>
</tr>
<tr>
<td>IFN-α2</td>
<td>Daudi</td>
<td>2 × 10^{-10}</td>
<td>500–1000</td>
</tr>
<tr>
<td>IFN-β</td>
<td>Daudi</td>
<td>10^{-10}</td>
<td>400</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Daudi</td>
<td>5 × 10^{-10}–10^{-9}</td>
<td>3500–7000</td>
</tr>
<tr>
<td>IFN-α2</td>
<td>Lymphocytes†</td>
<td>3 × 10^{-10}–9 × 10^{-10}</td>
<td>100 ± 60</td>
</tr>
<tr>
<td>IFN-β</td>
<td>Lymphocytes†</td>
<td>3 × 10^{-10}–5 × 10^{-11}</td>
<td>60 ± 30</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Lymphocytes†</td>
<td>0.5 × 10^{-10}–2 × 10^{-10}</td>
<td>200 ± 100</td>
</tr>
</tbody>
</table>

* HEp-2 cells were obtained from Flow laboratories and A549 cells were obtained from Dr R. Beaupain. These cells were grown as monolayer cultures in Dulbecco’s modified MEM (Gibco Biocult) supplemented with 10% foetal calf serum (FCS; KC Biological) and passaged by trypsinization. Daudi cells (a human Burkitt cell line) were provided by Dr K. E. Mogensen and grown in non-agitated suspension cultures in RPMI 1640 medium (Flow Laboratories) supplemented with 10% FCS. Binding experiments were carried out at 4°C as described for monolayer cell cultures (Aguet et al., 1981; Aguet, 1985) and for cell growth in suspension (Aguet & Blanchard, 1981; Aguet, 1985). Human peripheral blood leukocytes were isolated from heparinized blood samples by centrifugation on Ficoll-Paque (Pharmacia) as described by the manufacturer. Binding data from saturation curves were subjected to Scatchard analysis (Aguet & Blanchard, 1981; Aguet et al., 1981).

† Human peripheral blood lymphocytes (mean of eight donors).

inhibited by unlabelled IFN-α2 and IFN-β (Fig. 2c). In terms of antiviral units per ml, glycosylated IFN-β (Miles-Yeda, Rehovot, Israel) and IFN-γ (kindly provided by Dr W. Fiers) were as effective as their unglycosylated counterparts in competing with labelled recombinant DNA interferons (data not shown). Identical results were obtained with the cells listed in Table 1.

The notion of distinct receptors for IFN-γ versus IFN-α/β was stimulated by observations of different effects of these interferon families: mouse L1210 cells resistant to the action of IFN-α/β lack relevant receptors (Aguet, 1980) but are sensitive to mouse IFN-γ (Ankel et al., 1980; Besançon et al., 1983) which binds specifically to these cells (J. Wietzerbin, personal communication). Recently, human IFN-γ was reported to induce a set of additional proteins not seen in cells treated with IFN-α (Weil et al., 1983). Conversely, mouse IFN-γ lacks the capacity of inducing the protein Mx which is specifically induced by IFN-α/β (Staehli et al., 1984). Although subtypes of IFN-α may display different specific activities (Weck et al., 1981; Rehberg et al., 1982; Fish et al., 1983; Bell et al., 1983) and binding affinities (Aguet et al., 1984; Yonehara et al., 1983), to our knowledge differential qualitative action has not been observed within the IFN-α/β family. The experiments presented herein confirm and support the notion that distinct independent receptor systems mediate the action of IFN-γ and IFN-α/β. On the other hand, competition between IFN-α and IFN-β favours the hypothesis of a homogeneous IFN-α/β receptor system.

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REFERENCES


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