The Reaction of the Anti-interferon-α Monoclonal Antibody, NK2, with Different Interferons

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SUMMARY

Analysis of the neutralization of human interferon-α by the monoclonal antibody NK2 showed, by three different methods, that the avidity constant was about $10^{10} \text{M}^{-1}$. A small decrease in the avidity constant (and in the antibody titre) with increasing interferon concentrations was observed, suggesting that the antibody–interferon complex had a little biological activity. The antibody neutralizes the interferon by preventing the binding of interferon to susceptible cells. It does not react with human-γ, mouse-α, monkey, bovine or rabbit interferons.

Secher & Burke (1980) first reported the isolation of a monoclonal antibody to human interferon-α (IFN-α). In contrast to other monoclonal antibodies which have been isolated more recently (Montagnier et al., 1980; Staehelin et al., 1981), Secher & Burke selected their antibody, called NK2, by screening for neutralization of the antiviral effect of human IFN-α, while other investigators have usually used radiolabelling methods. Recently, Novick et al. (1983) have used a new screening procedure based on immune precipitation with a second antibody and recovery of interferon activity from the immune precipitate. The NK2 antibody is thus, because of the selection procedure used, a neutralizing antibody, and since it was screened against the mixture of IFN species secreted by induced lymphoblastoid cells, it is not surprising that it neutralizes most of the IFN-α species found in that preparation. Recently, Allen et al. (1982) have shown that NK2 binds six out of the eight IFN-α molecules found in a lymphoblastoid interferon. The monoclonal antibody NK2 has been used for large-scale purification of IFN (Secher & Burke, 1980) and also as the basis for an immunoradiometric assay for IFN (Secher, 1981). It does not react with human IFN-β (Morser et al., 1981). We have now examined its reaction with human IFN-α in more detail, and also looked for any reaction with other vertebrate interferons.

The methods have been described in earlier publications (Secher & Burke, 1980; Morser et al., 1981; Allen et al., 1982). Authentic IFN-α2 was a kind gift from Genentech Ltd., San Francisco, U.S.A. Monkey IFN was prepared by the method of Yakobson et al. (1979). Mouse IFN-α was a kind gift of Dr Y. Kawade, Institute for Virus Research, Kyoto University, Kyoto 606, Japan. IFN-α2 with an additional 18 amino acids at the N-terminus was prepared by the method of Slocombe et al. (1982) in which Escherichia coli is infected with the phage M13 mp7 containing an IFN-α2 gene, followed by purification on NK2-Sepharose. All human IFN titres are given in international units, except for the subtypes of IFN-α for which there are as yet no international units, although the same correction has been applied, and IFN-γ where the results are reported in laboratory units.

Kawade (1980) has shown that use of an IFN antibody with a low affinity to neutralize IFN will generate a series of parallel dose–response curves when various concentrations of IFN are mixed with different antibody dilutions. A similar series of assays using the monoclonal antibody NK2 and human lymphoblastoid IFN gave such a family of parallel dose–response curves (Fig. 1a). The IFN was assayed in this experiment by the RNA reduction method (Atherton & Burke, 1975) but similar results were obtained if a c.p.e. inhibition assay, a yield reduction assay or a plaque inhibition assay was used (data not shown). These parallel dose–response curves suggested that NK2 did not have a very high affinity for some or all of the
Fig. 1. Neutralization of human IFN-α by NK2. (a) Lymphoblastoid IFN dilutions were mixed with an equal volume of NK2 in ascites fluid diluted $10^{-1}$ (●), $10^{-2}$ (■), $10^{-3}$ (▲) or with medium (○), incubated for 1 h at 37 °C before assay of the residual IFN on human foreskin fibroblasts by the RNA reduction assay. (b) IFN-α2 at $5 \times 10^4$ units (●), $5 \times 10^3$ units (■), $5 \times 10^2$ units (▲) or $5 \times 10^1$ units (○) per ml was mixed with an equal volume of various dilutions of NK2 IgG (originally at 2 mg/ml) and incubated for 1 h at 37 °C before assay of the residual IFN on EBTr cells by the RNA reduction assay.

Table 1. Antibody titres of NK2 against human interferon-α*

<table>
<thead>
<tr>
<th>Antibody source and dilution</th>
<th>Antibody titres using Human lymphoblastoid IFN</th>
<th>Antibody titres using IFN-α2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascites fluid 10^{-1}</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>10^{-2}</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>10^{-3}</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>IgG 10^{-3}</td>
<td>8710</td>
<td></td>
</tr>
<tr>
<td>10^{-4}</td>
<td>12880</td>
<td></td>
</tr>
<tr>
<td>10^{-5}</td>
<td>51310</td>
<td></td>
</tr>
</tbody>
</table>

* NK2 antibody (either as ascites fluid or as NK2 IgG at 2 mg/ml) was diluted as shown and incubated with different amounts of human IFN-α (using either human lymphoblastoid IFN, a mixture of the α species, or pure IFN-α2) and the antibody titre calculated by the method of Kawade (1980) for each antibody dilution. The amount of IFN used was increased as the amount of antibody was increased in order to obtain equivalence.

components of the human IFN-α mixture. Calculations of the antibody titre [defined by Kawade (1980) as the antibody dilution that reduces the IFN titre from 10 to 1] showed that the values were low, and became smaller as the amount of IFN used for the neutralization increased (Table 1). The low antibody titre reflects the fact that NK2 neutralizes only six out of the eight components present in lymphoblastoid IFN (Allen et al., 1982), so that there is always some biologically active IFN remaining in the mixture after neutralization. Dr Y. Kawade (personal communication) also found low values using NK2 and leukocyte IFN, which also contains components not neutralized by NK2. The fall in antibody titre suggested either that there was an inhibitor present in the ascites fluid used as a source of NK2, in this experiment, or that the antigen–antibody complex had some biological activity. In order to simplify interpretation, all subsequent experiments were carried out with an NK2 IgG preparation and a cloned IFN-α, either authentic α2 or the IFN-α2 prepared by the method of Slocombe et al. (1982), which
contains an additional 18 amino acids at the N-terminus, but is otherwise indistinguishable from authentic IFN-α2 (King et al., 1983).

A direct determination of the avidity constant was made by determining the amount of NK2 required to neutralize high concentrations of IFN-α2. Various amounts of the purified IFN-α2 were incubated with different dilutions of NK2 IgG before the mixture was assayed for residual IFN activity by the RNA reduction assay (Atherton & Burke, 1975). In this way, the concentration of NK2 IgG that would reduce the effective IFN concentration to 1 unit/ml was determined.

The mass-action equation: $[\text{IFN} - \text{NK2}] / ([\text{IFN}] \times [\text{NK2}]) = k_a$, where $[\text{IFN}] = \text{interferon concentration at equilibrium}$ and $[\text{IFN} - \text{NK2}] = \text{interferon-antibody complex concentration}$ can be reduced to: $(\text{[initial IFN units]} - 1) / [\text{NK2}] = K_a$ since $[\text{initial IFN units}] = [\text{IFN} - \text{NK2}] + [\text{unbound IFN}]$, and since the titration determines the concentration of antibody that reduces the [unbound IFN] to 1 this becomes $[\text{initial IFN units}] - 1 = [\text{IFN} - \text{NK2}]$ and the bottom line similarly becomes $[\text{NK2}]$.

This derivation assumes that a 1:1 IFN:NK2 complex is formed, that the IFN bound is totally inactive and the NK2 is in large excess, the last being easily confirmed by comparing the number of molecules of each substance present. Using three different concentrations of the IFN-α2 containing an additional 18 amino acids (Fig. 1b), and assuming a molecular weight for NK2 IgG of 160,000, values of $1.4 \times 10^{10} \text{ M}^{-1}$ (for 50 units IFN/ml), $1.2 \times 10^{10} \text{ M}^{-1}$ (for 500 units IFN/ml) and $0.7 \times 10^{10} \text{ M}^{-1}$ (for 5000 units IFN/ml) were found for the avidity constant, giving a mean of $1.1 \times 10^{10} \text{ M}^{-1}$. This small change in the avidity constant with increasing interferon concentration again suggested that the antibody–antigen complex might have some biological activity, although other explanations cannot be ruled out, e.g. IgG itself might have some inhibitory effect on the neutralization. Calculation of the antibody titres by the method of Kawade (1980) using purified IFN-α2 again showed that the antibody titre fell as the amount of IFN in the neutralization reaction was increased and correspondingly more antibody was used (Table 1). Whatever the explanation, it is clear that the change in the neutralization titre, an effect not seen before, is a property of the interaction between a purified IgG fraction and pure IFN. Similar results were obtained when the residual interferon was titrated on embryonic bovine tracheal (EBTr) or monkey (Vero) cells using the RNA reduction assay, values of $1.1(\pm 0.4) \times 10^{10} \text{ M}^{-1}$ and $1.0 \times 10^{10} \text{ M}^{-1}$ being obtained. A similar value of $1.0 \times 10^{10} \text{ M}^{-1}$ was obtained with authentic IFN-α2, the value again decreasing with increasing IFN concentrations. By the use of NK2 bound to Sepharose beads and assaying the residual IFN activity, D. S. Secher & R. M. Hawkins (personal communication) obtained a value of $10^9 \text{ M}^{-1}$ for the binding of NK2 to IFN-α. The difference between the two may be accounted for by the use of different IFN-α species, because not all the IFN bound to antibody is neutralized or because of the difficulty in assessing the NK2 concentration on the beads.

The mechanism of neutralization of IFN-α by NK2 was further investigated by measuring the effect of addition of NK2 IgG on the binding of $^{125}$I-labelled IFN-α2 to Daudi cells. The interferon was iodinated by the use of chloramine-T, and using the method of Branca & Baglioni (1981), we found an apparent dissociation constant ($K_d$) for the binding of interferon to its receptor of $5 \times 10^{-11} \text{ M}$, threefold lower than that given by these authors for IFN-α-A, and a receptor number of 2000 per cell. A $10^{-2}$ dilution of NK2 IgG (originally 2 mg/ml) completely abolished the specific binding of up to 1000 units of IFN/ml, showing that the monoclonal antibody effected neutralization by inhibition of binding.

Human IFN is active on a number of non-human cells, and it has been suggested that these different activities are due to different epitopes on the IFN molecule (Paucker et al., 1975). If this was so, then the amount of IFN neutralized might be different when the residual IFN was assayed on different cells, or there might even be no neutralization when assayed on some cells, the appropriate epitope not being masked. However, when human IFN-α2 was incubated with NK2 and then assayed on human (HFF), bovine (EBTr), monkey (Vero) or mouse (L929) cells there was no difference in the extent of neutralization, suggesting that different epitopes are not involved in the activity on different cells (Table 2).

We have already shown that NK2 does not react with IFN-β (Morser et al., 1981) and a
Table 2. Neutralization of human IFN-α2 by NK2 IgG in human, bovine, murine or monkey cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Antibody dilution</th>
<th>Titre (log10 units)</th>
<th>Neut. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human (HFF)</td>
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<td>3.00</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10^-2*</td>
<td>6.00</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10^-3</td>
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</tr>
<tr>
<td></td>
<td>10^-4</td>
<td>4.30</td>
<td>99.9</td>
</tr>
<tr>
<td></td>
<td>10^-5</td>
<td>4.70</td>
<td>98.0</td>
</tr>
<tr>
<td>Bovine (EBTr)</td>
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<td>Murine (L929)</td>
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<td>4.70</td>
<td>90</td>
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<td></td>
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<tr>
<td></td>
<td>10^-5</td>
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<td>4.00</td>
</tr>
<tr>
<td>Monkey (Vero)</td>
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<tr>
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</table>

*10^-2 is equivalent to 0.02 mg IgG/ml. † Neut., Neutralization.

similar result was found with IFN-γ, the titre of a human IFN-γ preparation falling from log10 3.75 to log10 3.45 on passage through the column, a difference within the experimental error of the assay. Kawade et al. (1981) have demonstrated some antigenic cross-reaction between the α types of human and mouse IFN. This cross-reaction was due to cross-reactivity by a small proportion of the human antibodies, and it was therefore possible that NK2 might also neutralize or interact with mouse IFN-α. However, NK2 failed to neutralize a purified preparation of mouse IFN-α nor did a column of NK2-Sepharose retain any mouse IFN-α. Similarly, no monkey, bovine or rabbit interferons were retained by a column of NK2-Sepharose. It was concluded that there was no detectable reaction between NK2 and human IFN-γ, mouse IFN-α, bovine IFN, rabbit IFN or monkey IFN, and that the antibody was specific for human IFN-α.

Three different methods have been used to measure the avidity constant of NK2 IgG for human IFN-α2, and all gave similar values. It is thus possible to obtain a valid value for the avidity constant of an antibody without the use of a purified antigen if it is biologically active, and this may be a useful general method. The mean value of 10^10 M^-1 is an intermediate value for monoclonal antibodies, and this may be the reason why NK2 has been such a useful reagent for the purification of IFN by immunochromatography (Secher & Burke, 1980), since the IFN is bound firmly enough to be retained from the crude solution but loosely enough for the antigen–antibody bond to be broken. The small change in avidity constant with increasing concentrations of antibody or IFN suggests that the antibody–antigen has small but real biological activity. This effect is not large enough to prevent NK2 being an efficient neutralizing antibody (see Table 2), but it does suggest that the epitope recognized by the antibody is not identical to that recognized by the cell. Certainly recognition is quite specific, more than that of the cell-binding site for IFN, since mouse-α, human-α and -β, rabbit and bovine IFNs are active on human cells and are, therefore, recognized by the cell surface receptor, whereas NK2 does not interact with any of the non-human IFNs nor with human IFN-β.

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REFERENCES


Short communication


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