Production of Antibodies Against Mouse Immune T (Type II) Interferon and their Neutralizing Properties

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SUMMARY

An antiserum to immune T-type mouse interferon was prepared in rabbits by repeated injections of interferon, induced by phytohaemagglutinin (PHA) in mouse spleen cells and purified to $1 \times 10^4$ units/mg protein. The antiserum neutralized mouse interferons synthesized \textit{in vitro} in response to several T mitogens and Brucella organisms, and also the type II interferon present in the serum of BCG-sensitized mice after an injection of the specific antigen, tuberculin. All these interferons are thus antigenically related; they are also all unstable at pH 2. In contrast, the antiserum did not neutralize interferons induced by West Nile virus (WNV) and Newcastle disease virus (NDV) \textit{in vitro}, or by lipopolysaccharide and Brucella organisms \textit{in vivo}; all these interferons are stable at pH 2. It also failed to neutralize a non-glycosylated virus interferon prepared in the presence of tunicamycin.

Stimuli other than viruses can also trigger interferon synthesis, particularly in immunocompetent cells. Thus, interferon can be induced in response to a specific antigen by injecting it into primed animals (Youngner & Salvin 1973) or by incubating it \textit{in vitro} with primed lymphocytes (Valle \textit{et al.} 1975; Sonnenfeld \textit{et al.} 1977). T- and B-cell mitogens can also induce synthesis of interferon by non-sensitized spleen cells (Wallen \textit{et al.} 1973; Johnson & Baron, 1976; Wietzerbin \textit{et al.} 1977a). It has been shown that the interferons induced in mice in response to T mitogens (T-type interferon) and B mitogens (B-type interferon) are different molecular species (Johnson & Baron, 1976, Wietzerbin \textit{et al.} 1977a). B-type interferon is similar to virus interferon in its antigenic properties and pH stability, whereas T-type interferon is unstable at acid pH and is antigenically unrelated to virus-induced interferon (Youngner & Salvin, 1973; Johnson & Baron, 1976; Wietzerbin \textit{et al.} 1977a, b). By the same criteria T-type interferon seems similar to the interferon produced in BCG-sensitized mice after injection of the specific antigen, tuberculin (the type II interferon of Youngner & Salvin, 1973). During the last few years, knowledge of the antigenic relationship of different virus-induced interferons has greatly improved (Berg \textit{et al.} 1975; Havell \textit{et al.} 1975; Paucker, 1977), largely due to the availability of various specific antisera (Sipe \textit{et al.} 1973; Paucker, 1977; De Maeyer-Guignard \textit{et al.} 1978). However, the antigenic relationship between type II and T mitogen-induced interferons is still not known, because specific antisera have not been available. The main reasons for the lack of such antisera were the difficulties connected with their production and purification. We recently reported a method for making PHA-induced interferon in relatively large amounts and for its partial purification (Wietzerbin \textit{et al.} 1978b, 1979). This enabled us to produce an antiserum to interferon in rabbits and the neutralizing properties of this serum against different kinds of murine interferons are described in this paper.

Newcastle disease virus (NDV)-L cell interferon and interferon induced by West Nile virus (WNV) in the brains of Balb/c mice were prepared as described elsewhere (Falcoff \textit{et al.} 1968, 1973). A non-glycosylated virus interferon was prepared in mouse L cell cultures infected with NDV in the presence of tunicamycin (Fujisawa \textit{et al.} 1978).
Phytohaemagglutinin (PHA)-induced interferon was prepared as already reported (Wietzerbin et al. 1978b). Briefly, spleen cells from heterozygous nude mice (CNRS, Orleans) were suspended at a concentration of $1 \times 10^7$ cells/ml in RPMI 1640 medium (Flow), supplemented with 5% foetal calf serum, 2 mm-glutamine and 0.004% gentamicin, and incubated at 37 °C with 3 μg/ml of purified PHA (Wellcome Laboratories, Beckenham, Kent) in Petri dishes in a humidified incubator (5% CO₂). After 24 h, the cells were removed by centrifugation and the supernatant was precipitated with 42% saturated ammonium sulphate at 4 °C. The precipitate was discarded and the supernatant concentrated (five- to tenfold) under vacuum. The resulting interferon, which will be referred to as crude PHA interferon, was purified by chromatography on Blue-Sepharose CL-6B, as specified in the legend to Fig. 1. We have already reported the affinity of PHA interferon for this sorbent (Wietzerbin et al. 1979). Under the conditions described here, 85% of the antiviral activity was recovered in one peak with a specific activity of $1 \times 10^5$ units/mg protein.

Interferons respectively induced by concanavalin A (Con A) and succinyl-concanavalin A (Suc-Con A) were prepared by incubating Balb/c mouse spleen cells ($5 \times 10^8$ cells/ml) with 5 μg of Con A or 30 μg of Suc-Con A for 24 h at 37 °C (5% CO₂) under the culture conditions used for preparing PHA interferon. Lipopolysaccharide (LPS) interferon was obtained in the serum of Swiss mice 2 h after an intravenous injection of 0.15 mg of Escherichia coli LPS.

Brucella-induced interferons were prepared as described previously (Bousquet et al. 1978; Bousquet-Ucla et al. 1980) and were a gift from Dr C. Bousquet-Ucla. BCG (tuberculin)-induced interferon (type II) was prepared according to Youngner & Salvin (1973) and kindly provided by Dr K. Huygen. A rabbit antiserum against virus interferon (NDV-L cell) was prepared as described by Wietzerbin et al. (1978a).

Interferons were assayed by a cytopathogenic inhibition test in L cell culture microplates, with vesicular stomatitis virus as challenge. Titres are expressed in International reference units (one reference unit = 0.3 laboratory unit).
### Table 1. Neutralization properties of antisera to mouse NDV-L cell (virus-induced) interferon and mouse PHA-induced (T-type) interferon

<table>
<thead>
<tr>
<th>Interferon prepared*</th>
<th>Neutralization titres against 10 units of interferon</th>
<th>Stability at pH 2†</th>
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<tr>
<td></td>
<td>In vitro</td>
<td>In vivo</td>
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<tr>
<td>PHA (nude homo-zygous spleen cells)</td>
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<tr>
<td>PHA (nude hetero-zygous spleen cells)</td>
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<tr>
<td>PHA (Balb/c spleen cells)</td>
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<td>Con A (Balb/c spleen cells)</td>
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<td>Suc-Con A (Balb/c spleen cells)</td>
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<tr>
<td>NDV-L cell</td>
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<tr>
<td>Non-glycosylated NDV-L cell</td>
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<tr>
<td>West Nile virus</td>
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<td>LPS (Swiss mice)</td>
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<tr>
<td>BCG-tuberculin (Swiss mice)</td>
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<td>Brucella</td>
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<td>Brucella</td>
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* See text for abbreviations and details. † +, Stable; −, unstable.

The anti-interferon neutralization titre of a serum was determined by mixing 10 units of each interferon preparation with an equal volume of a series of twofold dilution of the serum and incubating the mixtures at 37 °C for 60 min. A sample from each mixture was then assayed for interferon activity. The reciprocal of the dilution of antiserum that permitted the development of 50% of the c.p.e. was taken as the antiserum titre.

Antibodies against T-type interferon were prepared by repeated inoculation of rabbits (Roux de Bourgogne) with PHA interferon. These animals were first given nine monthly injections of 30,000 units of crude PHA interferon emulsified in Freund's complete adjuvant. As this procedure failed to yield detectable levels of neutralizing antibodies, we decided to continue the immunization by injecting, at weekly intervals, 60,000 units of PHA interferon purified on Blue-Sepharose CL-6B with adjuvant. After a further period of 3 months, T interferon neutralizing activity was detectable in the serum at a titre of about 1000. Further weekly injections of purified PHA interferon were given for several months more, and the antiserum titres rose to 2000, but then remained stable despite the repeated injections.

In order to study the neutralizing properties of this anti-T interferon serum, we tested it against several mouse interferons induced by WNV and NDV, and by several mitogens; PHA, Con A, Suc-Con A and LPS. Concurrently, we determined the pH 2 stability of each interferon studied, to see if there was any correlation between pH 2 stability and antigenic make up. As shown in Table 1, the antiserum neutralized not only the PHA interferon used as the antigen in preparing this antiserum, but also the PHA interferon induced in Balb/c and nude homozygous mouse spleen cell cultures. These results provide additional evidence in support of our previous observations concerning the production of T-type interferon in athymic mouse spleen cell culture (Wietzerbin et al. 1978a).

The antiserum raised against PHA interferon also inhibited the antiviral activity of the interferons produced in response to the two other T-cell mitogens, Con A and Suc-
Con A (Table 1), indicating that all three T mitogens induced antigenically related interferons. On the other hand, the antibodies directed against T interferon failed to neutralize interferons induced either in vitro by NDV on L cell cultures or in vivo by WNV. To find out whether structural differences between virus and T-type interferon might be linked to differences in the glycan moiety, we prepared a non-glycosylated virus interferon in L cell cultures induced with NDV in the presence of tunicamycin. This antibiotic inhibits glycosylation by preventing the formation of the N-acetylglucosamine–lipid intermediate involved in N-glycosidic linking of core oligosaccharide to proteins (Fujisawa et al. 1978). As shown in Table 1, the non-glycosylated virus interferon was not neutralized by the antibodies against T-type interferon, despite the absence of the glycan moiety. It is, however, interesting to note that the non-glycosylated virus interferon was still neutralized by antiviral interferon serum to the same extent as conventionally prepared virus interferon (Table 1). These results indicate that the sugar moiety does not play an important part in antibody recognition.

We examined the neutralizing capacity of the antiserum against type II interferon induced in vivo by injecting the specific antigen, tuberculin, into BCG-infected mice. This interferon is unstable at pH 2 and, as reported earlier by Youngner & Salvin (1973), cannot be neutralized by an antisera directed against virus-induced interferon. However, as shown in Table 1, anti-T interferon serum was able to inhibit the antiviral activity of BCG (tuberculin)-induced interferon, which shows that these interferons are antigenically related. Bousquet et al. (1978) and Bousquet-Ucla et al. (1980) have shown that Brucella interferons produced in vivo or in vitro do not have the same properties. The interferon induced in vivo and present in mouse serum is stable at pH 2 and antigenically related to virus interferon. Nevertheless, adherent cells, mostly macrophages, obtained from mice 45 min after a Brucella injection, are able to trigger the synthesis of interferon when cultured together with spleen cells obtained from syngenic mice. This in vitro Brucella-induced interferon is acid unstable and cannot be neutralized by an antiserum against virus interferon.

It was of interest to determine whether the antibodies directed against T interferon were able to discriminate between both types of Brucella-induced interferon. The results in Table 1 show that this is in fact the case. Thus, the anti-T interferon serum neutralized Brucella interferon produced in vitro but not that produced in vivo. As already reported (Wietzerbin et al. 1977a; Bousquet-Ucla et al. 1980), the latter was neutralized by the antiviral interferon serum.

The findings reported in this paper show that the serum directed against T interferon was able to neutralize several T mitogen-induced (T-type) interferons as well as an antigen-induced type II interferon and an interferon induced in vitro with Brucella. These interferons, which must thus be antigenically related, have another common property, namely their instability at pH 2.

In addition, virus-induced interferons, LPS-induced interferon (B-type) and interferons induced in vivo with Brucella, which were all stable at acid pH, could not be neutralized by the anti-T interferon serum. Results with similar implications have been obtained by others (L. C. Osborne et al. 1979, personal communication).

To sum up, our results confirm previous observations that there are differences in the antigenic properties of T mitogen- and antigen-induced interferons on the one hand and B mitogen- and virus-induced interferons on the other. They also show a direct correlation between the pH 2 lability of a mouse interferon and its neutralization by our anti-T interferon serum.

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REFERENCES


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