Hyporesponsiveness to Dog Interferon Induction in vitro

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SUMMARY

Sera from dogs that were hyporesponsive to interferon (IFN) induction blocked virus induction of IFN in spleen cells in vitro but not IFN action in dog kidney cells. Similarly, macrophages or their culture fluids were found to block IFN induction in lymphoid cells in vitro. Lymphoid cells deprived of phagocytic cells secreted IFN continuously for 4 days, whereas lymphoid cells in the presence of phagocytic cells produced IFN for only 1 day. It was speculated that the 'hyporeactivity factor' in the sera from hyporesponsive dogs was macrophage-derived.

INTRODUCTION

Interferon (IFN) is a small protein with potent antiviral and anti-tumour cell activity. Research for the therapeutic use of IFN has been approached in two ways: (i) production of pure exogenous IFN for inoculation, and (ii) development of potent non-toxic IFN inducers. Although production of exogenous IFN has been greatly improved by genetic engineering (Derynck et al., 1980; Goeddel et al., 1980; Hitzeman et al., 1981; Weissbach et al., 1981), it would be more desirable to have a non-toxic IFN inducer which would induce substantial amounts of natural IFN in vivo. One of the major barriers in using an IFN inducer is the development of a refractory state or 'hyporesponsiveness' to IFN induction, which prevents repeated induction of IFN within a short period of time (Breinig et al., 1975; Ho & Kono, 1963; Ho et al., 1965; Youngner & Stinebring, 1965; Stringfellow & Glasgow, 1974; Stringfellow et al., 1977; Tsai & Appel, 1979; Tsai, 1980).

Hyporesponsiveness to IFN induction has been demonstrated in dogs. When dogs were inoculated with virus or synthetic IFN inducers, IFN was released in the serum within 24 h, and not thereafter. When the inoculation was repeated 2 days after the first, IFN production was impaired or not detected. This hyporesponsive condition was not inducer-specific and lasted for almost 2 weeks after the first inoculation (Tsai & Appel, 1979). The 'hyporeactivity factor' has been reported to be an in vivo phenomenon, and previous attempts at in vitro study of the cellular origin of this factor have been unsuccessful (Ho & Postic, 1967; Ho et al., 1976; Tarr et al., 1978).

In the present study, hyporesponsiveness to IFN induction was examined in vitro in dog lymphoid cells. Serum from hyporesponsive dogs and macrophages and their secretions were found to block the induction of dog IFN.

METHODS

Preparation of lymphoid cells. The lymphoid cells used to produce IFN were obtained from spleen and peripheral blood. Preparation of the cells has been described elsewhere (Tsai & Appel, 1983). The spleens were aseptically removed from 2- to 6-week-old specific pathogen-free (SPF) pups, minced to 1 mm³ size, and suspended in Medium 199 with Hanks' balanced salt solution (HBSS, Gibco), 0.5% lactalbumin hydrolysate and antibiotics (100 U penicillin/ml and 50 μg streptomycin/ml). Suspensions were stirred at room temperature for 1-5 to 2 h, then filtered through gauze and centrifuged at 400 g for 20 min. Cell pellets were washed once with the same medium, then resuspended in RPMI 1640 culture medium (Gibco) supplemented with 20% foetal calf serum (FCS).

For the preparation of blood lymphoid cells, 10 to 20 ml blood was drawn from the jugular vein of an SPF dog and defibrinated in 125 ml Erlenmeyer flasks by swirling with 10 to 15 glass beads at 200 rev/min on a Model G-2 shakerplate (New Brunswick Scientific, New Brunswick, N.J., U.S.A.) for 10 min. Defibrinated blood was
RESULTS

Effect of a serum hyporeactivity factor on IFN induction in dog spleen cells

The sera collected from dogs 31 h after IFN induction with poly(IC)LC greatly reduced IFN production in dog spleen cells when compared with IFN production in cells exposed to normal
Hyporesponsiveness to dog interferon induction

Table 1. Effect of a hyporeactivity factor derived from dog serum on NDV-induced* IFN production in dog spleen cells

<table>
<thead>
<tr>
<th>Treatment of dog spleen cells</th>
<th>IFN titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Dog serum no. 1†</td>
<td>20 ± 2‡</td>
</tr>
<tr>
<td>50% Dog serum no. 2†</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>50% Normal dog serum</td>
<td>413 ± 6</td>
</tr>
<tr>
<td>20% FCS</td>
<td>447 ± 21</td>
</tr>
</tbody>
</table>

* NDV was used at an m.o.i. of 1.
† Sera collected from dogs 31 h after intravenous injection with 0·25 mg poly(IC)LC/kg body weight.
‡ Results are the average of data from two experiments. IFN titres are in units per 0·3 ml ± standard error of the means.

Table 2. Effect of a hyporeactivity factor derived from dog serum on IFN activity in DK cells

<table>
<thead>
<tr>
<th>Treatment of DK cells</th>
<th>First incubation (24 h)</th>
<th>Second incubation (24 h)</th>
<th>IFN titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog serum no. 1†</td>
<td>IFN</td>
<td></td>
<td>1532 ± 220‡</td>
</tr>
<tr>
<td>Normal dog serum</td>
<td>IFN</td>
<td></td>
<td>1488 ± 282</td>
</tr>
<tr>
<td>IFN‡</td>
<td>Dog serum no. 1</td>
<td></td>
<td>1122 ± 48</td>
</tr>
<tr>
<td>IFN</td>
<td>Normal dog serum</td>
<td></td>
<td>1444 ± 52</td>
</tr>
</tbody>
</table>

* Sera collected from dogs 31 h after intravenous injection with 0·25 mg poly(IC)LC/kg body weight.
† Results are the average of data from two experiments. IFN titres are in units per 0·3 ml ± standard error of the means.
‡ IFN in sera from dogs collected 8 h after intravenous injection with 1·5 mg poly(IC)LC/kg body weight.

dog serum or 20% FCS (Table 1). Sera containing a hyporeactivity factor were not toxic to spleen cells when tested by dye exclusion, and did not contain IFN.

Effect of a serum hyporeactivity factor on IFN activity

The hyporeactivity factor in dog serum that affected IFN production did not influence IFN activity greatly. Interferon titres expressed in DK cells that were treated with the hyporeactivity factor for 24 h either before or after IFN exposure differed only slightly from IFN titres produced in DK cells that were treated with normal dog serum (Table 2).

Influence of macrophages on IFN production

The secretion of NDV-induced IFN from dog spleen cells without phagocytic cells continued after 24 h and reached a peak 4 days after incubation (Fig. 1), whereas IFN production from spleen cells with phagocytic cells ceased and the titre decreased slightly after 24 h. At day 4, the IFN titres produced by cells without phagocytic cells were 2843 ± 47 units per 0·3 ml and 1925 ± 212 units per 0·3 ml in two individual experiments. IFN produced by spleen cells containing phagocytic cells were 864 ± 34 units per 0·3 ml and 835 ± 40 units per 0·3 ml respectively. Like spleen cells, peripheral lymphocytes deprived of phagocytic monocytes produced significantly (P < 0·01) higher IFN titres than cells with phagocytic monocytes, when both cell populations were adjusted to the same concentration (Table 3).

Effect of SCMP on IFN production

SCMP in culture produced little, or no IFN upon induction with NDV. In contrast, peripheral blood lymphocytes produced substantial amounts of IFN after NDV stimulation (Table 4). When peripheral lymphocytes were cocultivated with SCMP at a ratio of 5 : 1 and infected with NDV, IFN production never began.

Culture fluids from SCMP were tested for their effect on IFN induction by NDV in peripheral blood lymphocytes and spleen cells. IFN production was greatly impaired in cells
Fig. 1. Effect of phagocytic cells on NDV-induced IFN production in dog spleen cells. NDV, B-1 strain, was used at an m.o.i. of 1; dog spleen cells were used at a concentration of 1 × 10⁷ cells/ml. Experiment 1, with phagocytic cells (●−●); experiment 2, with phagocytic cells (○−○); experiment 1, without phagocytic cells (●−●); experiment 2, without phagocytic cells (○−○).

Table 3. Effect of culture fluids from SCMP on NDV-induced IFN production by lymphoid cells*

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Phagocytic monocytes</th>
<th>Culture fluid</th>
<th>Amount of IFN induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBL</td>
<td>+</td>
<td>RPMI 1640 + 20% FCS</td>
<td>404 ± 48</td>
</tr>
<tr>
<td>PBL</td>
<td>+</td>
<td>NDV-SCMP†</td>
<td>0</td>
</tr>
<tr>
<td>PBL</td>
<td>−</td>
<td>RPMI 1640 + 20% FCS</td>
<td>1671 ± 200</td>
</tr>
<tr>
<td>PBL</td>
<td>−</td>
<td>NDV-SCMP</td>
<td>427 ± 16</td>
</tr>
<tr>
<td>PBL</td>
<td>−</td>
<td>Control-SCMP†</td>
<td>400 ± 79</td>
</tr>
<tr>
<td>Spleen cells</td>
<td>+</td>
<td>RPMI 1640 + 20% FCS</td>
<td>1124 ± 61</td>
</tr>
<tr>
<td>Spleen cells</td>
<td>+</td>
<td>NDV-SCMP</td>
<td>373 ± 29</td>
</tr>
<tr>
<td>Spleen cells</td>
<td>−</td>
<td>RPMI 1640 + 20% FCS</td>
<td>2475 ± 131</td>
</tr>
<tr>
<td>Spleen cells</td>
<td>−</td>
<td>Control-SCMP</td>
<td>822 ± 101</td>
</tr>
</tbody>
</table>

* Final cell concentrations were adjusted to 1 × 10⁷ cells per vial for cultures with or without phagocytic monocytes. NDV was used at an m.o.i. of 1. IFN was tested 24 h after induction.† Culture fluids from NDV-infected SCMP (NDV-SCMP) or uninfected SCMP (control-SCMP).‡ Results are the average of data from two experiments. IFN titres are in units per 0.3 ml ± standard error of the means.

Table 4. Production of IFN by SCMP and peripheral lymphocytes in dogs

<table>
<thead>
<tr>
<th>Cell source</th>
<th>NDV*</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCMP†</td>
<td>2 ± 0†</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral lymphocytes§</td>
<td>264 ± 70</td>
<td>3 ± 0</td>
</tr>
<tr>
<td>Combined</td>
<td>2 ± 0</td>
<td>6</td>
</tr>
</tbody>
</table>

* NDV, B-1 strain, was used at an m.o.i. of 1.
† 1 × 10⁶ cells/culture.
‡ Results are the average of data from two experiments. IFN titres in units per 0.3 ml ± standard error of the means.
§ 5 × 10⁶ cells/culture.
incubated in culture fluids from SCMP, infected or uninfected with NDV, as compared to IFN production in cells incubated in control medium RPMI 1640 with 20% FCS (Table 3).

**DISCUSSION**

It appears that macrophages are the cells responsible for early termination of IFN production by dog lymphocytes. When phagocytic cells were removed from spleen cells, the production of IFN continued over a period of 4 days (Fig. 1). This concept was further supported by direct *in vitro* cocultivation of SCMP with peripheral blood lymphocytes. The production of IFN from lymphocytes was completely blocked by the cocultivation (Table 4). The interference probably was mediated by soluble factors secreted from macrophages rather than cell-to-cell contact because culture fluid from macrophages alone impaired the IFN production of lymphocytes either from spleen or peripheral blood (Table 3). Since IFN was not detected in macrophage culture fluids, the previous possibility that IFN itself may be involved in hyporesponsiveness induction could be eliminated. Culture fluids from macrophages also impaired IFN production in DK cells, indicating that the action was not limited to lymphoid cells. Culture fluids from both NDV-infected and uninfected SCMP had the same modulating effect on IFN production. Activation of macrophages may be required in order to secrete the modulating factor. The macrophages used in these experiments were obtained from implanting coverslips subcutaneously and had been activated.

The production of a 'hyporeactivity factor' by canine macrophages appears to be in contrast with reports of IFN production in macrophages in other species (Smith & Wagner, 1967; Smith et al., 1973; Rudenko & Smorodintsev, 1969). There may really be species differences, or it might be that different macrophage populations in various stages of activation or maturation may respond in different ways. Further search for the 'hyporeactivity factor' in macrophages from other species may be warranted, therefore.

It remains unresolved whether the 'hyporeactivity factor' in the serum from hyporesponsive dogs was macrophage-derived. However, the data obtained *in vitro* with macrophages and their products make it highly probable.

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**REFERENCES**


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