Study of the in vivo Priming Effect of Interferon in Mice

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(Accepted 8 August 1986)

SUMMARY

Intramuscular injection of mice with 2000 IU/g partially purified murine interferon (IFN) alpha/beta 3 h before the induction of IFN by intraperitoneally administered 3 μg/g poly rI:rC enhanced early IFN production. The differences between the serum IFN levels of IFN-pretreated and control animals were about 10-fold during the first 2 to 3 h of in vivo IFN production. In later stages these differences tended to decrease, and from 8 h post-induction they disappeared. IFN exerted its in vivo priming activity equally after intravenous, intraperitoneal or intramuscular injection. A similar enhancement of IFN production was observed when it was induced by poly rI:rC administered either intraperitoneally or intramuscularly. The duration of IFN pretreatment influenced the establishment of the in vivo primed state. Following administration of 2000 IU/g murine IFN alpha/beta intramuscularly, maximal priming developed after 3 h, and no primed IFN response was detected when the inoculation of IFN preceded inducer administration by 12 h or more. The manifestation of in vivo priming was optimal when 1500 to 3000 IU/g pretreating doses of IFN were applied. Reduction of the amount of injected IFN below this level markedly decreased priming, indicating a time- and dose-dependent induction of priming in vivo.

INTRODUCTION

Treatment of cells with interferon (IFN) before their exposure to an inducer can augment the subsequent formation of IFN (Isaacs & Burke, 1958). Stewart et al. (1973) and Ito & Kobayashi (1974) demonstrated that priming activity co-purifies with the antiviral activity in different IFN preparations, suggesting that IFN per se exerts these seemingly unrelated effects on cells. Priming of cells is frequently used for the enhancement of IFN yields in large-scale conventional IFN-producing systems (Cantell et al., 1974; Clark & Hirtenstein, 1981; Edy et al., 1982).

Priming of IFN production can also be regarded as a model for the regulation of protein synthesis. This recognition has prompted extensive studies with the aim of elucidating its mechanism of action (Stewart et al., 1971; Ito et al., 1975; Rosztóczy 1977; Abreu et al., 1979; Fujita & Kohno, 1981; Nir et al., 1985). Though all these studies were conducted under in vitro conditions, priming in vivo may also be important. IFN-mediated host defences against virus infections might act more efficiently if IFN produced during the primary cycles of an infecting virus primes subsequent target cells. There are data which can be interpreted as evidence for the occurrence of priming in vivo: polyinosinic-polycytidylic acid (poly rI:rC)-pretreated rabbits produced more IFN after secondary induction with endotoxin than animals subjected to a single induction with endotoxin (Ho et al., 1970).

Nagano & Maehara (1972) reported that primary induction with Newcastle disease virus resulted in the enhancement of endotoxin-induced IFN in rabbits. Yershov et al. (1976) found that mice pretreated with poly rI:rC responded to secondary induction with heat-inactivated Venezuelan equine encephalitis virus with increased IFN production. Hashimoto et al. (1978) suggested that cells from IFN-pretreated rabbits show a markedly enhanced endotoxin-induced IFN production as compared with the IFN production of cells from control animals. In the
present study we set out to investigate further the priming effect in vivo in mice. The results obtained favour the conclusion that murine IFN (MuIFN) can also exert its priming activity under in vivo conditions.

METHODS

Preparation of polyinosinic-polycytidylic acid complex. The potassium salts of polyinosinic and polycytidylic acids with $s_{25}$ values of 7.4 to 10 and 3.5 to 4.0, respectively, were products of the REANAL Pharmaceutical Works, Hungary. The homopolymers were dissolved in 0.15 M-saline at a concentration of 2000 μg/ml. The poly rI:rC complex was freshly prepared before each experiment and further diluted in phosphate-buffered saline (PBS) to give appropriate concentrations for inoculations.

Interferon. This was produced in our laboratory by Sendai virus-infected mouse L929 cells. Partial purification was achieved by means of two cycles of controlled pore glass chromatography and a subsequent Sephacryl S-200 gel filtration. Fractions containing peak activities were pooled and concentrated on Amicon YM 10 membranes. The preparation used in the present experiments had a specific activity of $1.2 \times 10^{7}$ IU/mg protein. It was characterized by neutralization with MuIFN-alpha/beta antiserum (G024 501 568) from the Antiviral Substances Program, National Institutes of Health, Bethesda, Md., U.S.A. The antiserum had a neutralizing titre of $1:2.4 \times 10^{5}$ against 10 IU/ml of this IFN.

IFN assay. Tittrations were carried out in mouse L929 cells with vesicular stomatitis virus as a challenge virus against a mouse IFN standard (G 002 904 511) also from the Antiviral Substances Program, and the results are given in reference units (Rosztóczy, 1976). In titrations of mouse serum samples, dilutions started from 1:16, as the presence of higher concentrations of mouse serum affected the morphology of the L929 cells.

Animals. Male CFLP mice were purchased from the LATI Laboratory Animal Breeding Farm, Gödöllő, Hungary. One hour before the experiments, animals weighing 20 ± 1 g were selected; they were allowed to drink water ad libitum, but no food was provided.

In vivo IFN and poly rI:rC treatment. Each injection was administered intraperitoneally (i.p.), intravenously (i.v.) intramuscularly (i.m.) in 0.1 ml. Controls for the IFN-treated group received appropriate dilutions of a mock IFN preparation prepared from culture media of uninduced L929 cells. Animals not receiving poly rI:rC were inoculated with 0.1 ml PBS by the same route as for the poly rI:rC. The amount of poly rI:rC used for IFN induction was 3 μg/g body weight throughout the present experiments. I.m. inoculations of poly rI:rC were administered into the left thigh muscle and those of IFN into the right one.

In vivo sampling. At the indicated intervals, groups of five to seven mice were bled to death. Their blood was collected individually and the sera were also titrated individually for IFN content, and the means were calculated.

RESULTS

Pharmacokinetics of L cell IFN in mice

It has been suggested (Stewart et al., 1971) that the development of priming is a time- and dose-dependent phenomenon similar to the establishment of the antiviral state evoked by IFN. For the in vivo experiments we planned to apply pretreatments which, on the basis of in vitro experience, could provide serum IFN levels sufficient for priming. After considering the results of previous pharmacokinetic studies (Edy et al., 1976; Billiau et al., 1981) and since the preparation used in the present study was a MuIFN-alpha/beta, we administered 2000 IU/g of IFN i.v., i.p. or i.m. into mice. This dose of IFN resulted in serum IFN levels exceeding 16 IU/ml for at least 6 h, regardless of the inoculation route (Fig. 1). The results of i.p. and i.m. inoculations were very similar, whereas i.v. administration of IFN resulted in somewhat lower serum IFN concentrations after a short early period when the serum IFN level was very high.

Kinetics of primed and unprimed IFN production

The pharmacokinetics of 2000 IU/g L cell IFN indicated that this dose of IFN would produce sufficient circulating IFN levels for priming in vivo if this in vivo effect of IFN did not differ to a great extent from that observed in vitro for L cells. We inoculated IFN i.m. into 36 mice 3 h before the i.p. administration of poly rI:rC. Two control groups of mice were included in this experiment. One of them received mock IFN, while the other had no pretreatment before the poly rI:rC. Just before the injection of the inducer, six mice from each group were sacrificed for determination of the residual IFN levels. Further samples were taken from six animals per group at 0-5, 2, 3-5, 8 and 16 h post-induction. Means for two independent experiments showed that the IFN titre for the primed group exceeded that for the mock IFN control group by 10-fold
at 2 h and by sixfold at 3.5 h post-induction (Fig. 2). The difference observed at 0.5 h could be attributed to the presence of residual IFN in the sera of the IFN-treated animals. Interestingly, the IFN titres in the primed animals started to decline sooner, but both eventually declined at the same rate and no substantial difference could be observed between controls and IFN-treated groups at 8 and 16 h post-induction.

**Effect of route of administration of the pretreating IFN**

Since the results in Fig. 1 indicated no major differences between the pharmacokinetics of i.v., i.p. and i.m. administered IFN, it seemed likely that IFN inoculated by any route could induce a primed IFN response to poly rI : rC. This assumption was tested in three groups of animals consisting of 24 mice each. Twelve mice from each group received 2000 IU/g L cell IFN and 12 mock IFN. Three h later, six IFN-treated and six mock IFN-treated animals received poly rI : rC administered i.p., while the others received 0.1 ml of PBS i.p. At 2 h after induction the animals were sacrificed and their sera were titrated for IFN content. The experiment was repeated once and the results in Fig. 3 are the means for the two experiments. The i.v., i.p. and i.m. IFN treatments resulted in comparable levels of priming, as they caused 7.7, 8.5- and 9.3-fold enhancement, respectively.

**Effect of priming on IFN production induced by poly rI : rC administered i.p. and i.m.**

We found that the kinetics of IFN production induced by poly rI : rC injected i.p. or i.m. were very similar. It has not been determined whether the IFN-producing cells are the same or not when different routes of inoculation were used. IFN pretreatment can modulate various target cells differently, which could be reflected in their response to the inducer. Groups of 36 mice were pretreated i.m. with 2000 IU/g L cells IFN or with the appropriate dilution of the mock
IFN. Three h later one half of each group was injected i.p. and the other half i.m. with 3 μg/g poly rI : rC. In this experiment, samples were collected from six animals at 2, 3 and 4 h post-induction for IFN titration. As can be seen in Fig. 4, the effect of priming was the same, independent of the route of inoculation of the inducer.

Kinetics of development of priming

In the in vitro IFN-producing systems, 2 to 6 h pretreatments with optimal IFN concentrations were usually required for the induction of maximal priming (Stewart et al., 1971; Rosztóczy, 1976). To analyse the duration of in vivo IFN treatments necessary for the establishment of the primed state, in the following experiments we induced IFN at different times after the injection of IFN. Thirty-six mice were inoculated i.m. with 2000 IU/g IFN, and groups of six animals received poly rI : rC i.p. at different times after the IFN administration. Another six mice which were inoculated with mock IFN i.m. 3 h before the inducer treatment served as controls. All samples were taken at 2 h post-induction. The efficiency of priming increased up to the 3 h pretreatment period, and was still high after 6 h pretreatment; 12 and 24 h pretreatments were not effective under these experimental conditions (Table 1).

Dose-response to IFN

Antiviral and 'trace' priming titres of IFN have been shown to be similar (Stewart et al., 1973). It has also been reported that the maximally primed state of cells can be achieved with relatively moderate concentrations of IFN (Stewart et al., 1971; Ito & Kobayashi, 1974). These authors and De Maeyer-Guignard et al. (1980) found that increasing the pretreatment dose of IFN above the optimal level did not cause any further change in priming. In the following experiments our aim was to determine the dose-response relationship of in vivo priming. Groups of six mice were pretreated for 3 h with increasing amounts of IFN injected i.m. Poly rI : rC was
Table 1. Effect of duration of IFN treatment on the development of priming

<table>
<thead>
<tr>
<th>IFN pretreatment* (h)</th>
<th>IFN titre (IU/ml)</th>
<th>Enhancement ratio</th>
</tr>
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<tbody>
<tr>
<td>0.1</td>
<td>400</td>
<td>1.6</td>
</tr>
<tr>
<td>1</td>
<td>800</td>
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<td>3</td>
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<td>9.6</td>
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<tr>
<td>6</td>
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<tr>
<td>12</td>
<td>300</td>
<td>1.2</td>
</tr>
<tr>
<td>24</td>
<td>200</td>
<td>0.8</td>
</tr>
<tr>
<td>3†</td>
<td>250</td>
<td>1</td>
</tr>
</tbody>
</table>

* 2000 IU/g i.m.
† Mock IFN.

administered i.p. and all samples including those from mock IFN-treated animals were taken at 2.5 h after induction. The effectiveness of priming increased with the pretreating dose of IFN (Fig. 5). The data obtained indicated that 1500 to 3000 IU/g IFN was optimal for \textit{in vivo} priming, and lowering the amount of pretreating IFN markedly affected the efficiency of priming.

Neutralization of \textit{in vivo} priming activity by antiserum to mouse L cell IFN

To provide evidence for the assumption that IFN present in the preparation used for pretreatment of mice was the substance responsible for the priming observed, we performed the following experiment. An L cell IFN preparation in which approximately 95% of the original antiviral activity was neutralized by the mouse L cell IFN antiserum (G 024 501 568) was prepared. Three groups of six mice were used. The animals in the first group were pretreated for 3 h with 2000 IU/g L cell IFN administered i.m. The second group received the L cell IFN plus
IFN–antiserum mixture diluted to a concentration that would have provided 2000 IU/g IFN for mice in a 0.1 ml injection volume in the absence of IFN antibodies. This preparation was injected i.m., similarly to the mock IFN which was the treatment for the third group. IFN production was induced with poly rI : rC administered i.p., and samples were taken for IFN titration at 2.5 h post-induction. Means of serum IFN titres at this time were 2100, 284, and 230 IU/ml for the first, second and third groups, respectively. This experiment demonstrated that the ability of our IFN preparation to prime in vivo IFN production in mice was neutralized by specific antibody to mouse L cell IFN.

DISCUSSION

Despite extensive studies on in vitro priming, the enhancement of in vivo IFN production by IFN pretreatment has not been investigated in detail. Our studies were conducted to determine whether treatment of mice with IFN can produce a primed state which will be manifested in the poly rI : rC-induced IFN response. The results presented here and studies cited previously (Ho et al., 1970; Nagano & Maehara, 1972; Yershov et al., 1976; Hashimoto et al., 1978) provide further evidence of priming in vivo.

The existence of priming in vivo suggests a role in the host defence mechanism by acceleration of the IFN response to invading viruses or other agents with IFN-inducing capacity whose multiplication is inhibited by IFN. Earlier activation of macrophages (Degré & Rollag, 1979; Huang, 1982) and the enhancement of the production of several lymphokines and other leukocyte products (Dinh et al., 1980; Blomgren & Einhorn, 1981; Arenzana-Seisdedos & Virelizier, 1983; Vlček et al., 1983; Wallach et al., 1983) represent other possible avenues in this network.

The IFN induced by poly rI : rC in mice in vivo has been shown to be MuIFN-alpha/beta (Machida et al., 1984; Brehm & Kirchner, 1986). Priming of IFN-gamma production by other types of IFNs has also been demonstrated in vitro (Wiranowska-Stewart et al., 1980; Lefkowitz & Luna, 1984; Endrész et al., 1985). If this is the case in vivo, it would have further implications for in vivo priming because of the broad spectrum of IFN-gamma activities. With the elucidation of this possibility in mind, we plan further investigations of the MuIFN-alpha/beta effect on in vivo MuIFN-gamma production.

The author thanks Susi Czapff for her skilled technical assistance.

REFERENCES

Priming effect of interferon in vivo


(Received 22 May 1986)