The Effects of Interferon and Double-stranded RNA upon the Virus–Host Interaction: Studies with Togavirus Strains in Mice

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SUMMARY

Partially purified fibroblast interferon and double-stranded RNA (dsRNA) of fungal origin were administered as single graded doses to A2G and Balb/c mice shortly before intraperitoneal infection by specified virulent or avirulent strains of representative togaviruses (Semliki Forest virus, Venezuelan equine encephalomyelitis virus and yellow fever virus). Changes of efficiency of infection arising from interference at the level of clearance or replication and changes of the expression of virulence or protective immunity, were compared for different initial doses of interferon or dsRNA in relation to different levels of infection by defined virus strains. Interferon and dsRNA, although acting through quantitatively different mechanisms, both reduced effective dose of virus and influenced only the extent of primary replication and host stimulation. Neither agent changed in any way the outcome of infection in terms of expression of virulence (regulatory immunity) or protective immunity. These results are discussed in terms of the control of virus infections at stages before or after immune stimulation can be effective.

INTRODUCTION

The initiation by interferon and its inducers of the blocking of virus replication has been documented for many in vitro and in vivo systems. The activity of these agents in in vivo systems is greatest when they are applied to infection sites as early as possible or, ideally, shortly before virus infection (Perkins & Regamey, 1970; Finter, 1973; Tyrrell, 1976; Anon, 1977).

Interferon and its inducers have been associated with other effects which depend upon many specific and non-specific mechanisms of host defence (Hilleman, 1970; Baron, 1973; Johnson & Baron, 1976; Sonnenfeld et al., 1977; Gresser, 1977). Thus, interferons have been considered as alternatives to immunization and as factors in the control of virus virulence or enhancement of protection against virulent challenge. The clarification of such roles of endogenous or exogenous interferons demands close quantitative control in view of the variation of stimulation and regulatory immunity (expression of virulence) with the primary infecting dose and strain, and the dependence of protective immunity upon the time, dose and strain of the secondary virus challenge.

In the present studies with several defined strains of togaviruses, an optimum prophylaxis with one dose of interferon or inducer has been adopted to define its influences upon the several phases of the virus–host interaction.

METHODS

Virus strains. The strains of Semliki Forest virus (SFV) have been described by Bradish et al. (1971, 1972, 1975a). The avirulent A774 strain has been used after three passages in

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suspensions of primary chick embryo cells (A774.C3). The virulent L10 strain of SFV and the virulent (P2023) or avirulent (TC83, 21t) strains of Venezuelan equine encephalomyelitis virus (VEEV; Walder & Bradish, 1975, 1979) have all been used after only one passage in suspensions of primary chick embryo cells. The virulent strains were usually lethal for mice within 6 to 7 days of intraperitoneal (i.p.) infection.

Assays of virus infectivity. These were by plaque counting in agar suspensions of primary chick embryo cells (Bradish et al., 1971; Fitzgeorge & Bradish, 1980) prepared at the time of inoculation of the same sample dilutions into mice. The standard diluent was Parker's medium 199 with 10% foetal calf serum, pH 7.4.

Assay of interferon activity and of neutralizing activity in serum. Interferon and antibody activities were estimated through the reductions of the numbers of plaques formed by infective virus in agar suspensions of mouse L-cells or primary chick embryo cells (Bradish & Allner, 1970, 1972). Alternative assays were made by zone inhibition of plaque formation in the same agar suspensions of mouse L-cells or chick embryo cells.

Interferon assays were standardized by the use of the reference research standard (Bradish & Allner, 1970). Antibody assays were standardized by the use of reference antisera in all tests: the activity of antiserum in the neutralization of virus infectivity is expressed through the serum neutralization index (SNI) as the logarithm of the antibody activity or concentration (Fitzgeorge & Bradish, 1973).

Preparations of mouse interferon and double-stranded (ds)RNA. The mouse L-cell interferon (sp. act. about $10^6$ units/mg protein) was prepared and standardized as quoted by Bradish & Allner (1970). The dsRNA of fungal origin (BRL 5907) was very kindly supplied by Dr D. N. Planterose and M. Boyd of Beecham Research Laboratories, Brockham Park, Betchworth, Surrey, U.K.: this was distributed as a dry powder and its dosage for mice was standardized by weight and dilution. This preparation has been described by Buck et al. (1971) and by Planterose (1977).

Procedures in mice and administration of interferon or dsRNA. Inbred Balb/c and limited-outbred A2G mice 60 to 100 days old were supplied by Allington Farm, Porton, Wilts., U.K. The required doses of interferon and dsRNA were applied prophylactically by i.p. inoculation of 0.025 ml at times from 4 to 24 h before virus infection. Control tests in A2G and Balb/c mice treated with 10 to 30 ~g dsRNA showed a peak of induced interferon activity in serum of about $10^4$ units/ml 4 to 8 h later. This activity fell to less than 1% by 18 h and was undetectable in most mice within 2 days of treatment with dsRNA: this is shown by line A of Fig. 3 (Baron et al., 1970). On this basis, dsRNA was administered at 4 h before virus injection. Other tests showed a maximum effect for interferon administered about 1 day before virus and little or no effect following administration after virus or more than 2 days before (Bradish et al., 1975b).

Usually about 50 or 60 mice received each dose of dsRNA or interferon and these treated mice, with normal mice, were then distributed as groups of 10 or 12 for use as described.

Observations of early responses in mice. Mice were bled from the retro-orbital plexus at 2 to 5 and 10 to 14 days after injection to provide sequential, individual samples for assay of interferon activity in serum, viraemia and virus-neutralizing activity in serum (Bradish & Allner, 1972; Bradish et al., 1975a, b). The detection of any one of these activities confirms infection and a progressive replication which then follows a course which characterizes the virus–host interaction in that individual. Thus, in the normal mouse infected i.p. by strains of SFV at doses of 10 p.f.u. or more, viraemia and interferon activity in blood rise together to a maximum level at 36 to 48 h after i.p. infection and then fall together to negligible levels by the 4th or 5th day. Antibody activity is first detected at 2 to 3 days following infection and achieves a maximum and maintained level within about 5 days. Any departures from this combined pattern of responses to infection by 10 p.f.u. or more indicates a modification of the critical early course of the virus–host interaction.
Action of interferon and dsRNA in mice

Fig. 1. Depression of infectivity and efficiency of infection (see text) in A2G mice for various strains of virus administered i.p. A, Fibroblast interferon administered 1 day before infection by the avirulent A774.C3 (▲) or virulent L10.C1 (■) strains of SFV. B, dsRNA administered 4 h before infection by the avirulent A774.C3 (▲) or virulent L10.C1 (■) of SFV. C, dsRNA administered 4 h before infection by the avirulent 2lt.C1 (Ο) or virulent P2023.C1 (●) strains of VEEV.

Distribution of host responses to infection. Regardless of the actual efficiency of infection (Walder & Bradish, 1975, 1979) or its impairment in the treated host (Bradish et al., 1975b) the outcome of infection may range from death (ID₅₀ = LD₅₀), through intermediate responses, to subclinical infection with protection against later challenge (ID₅₀ = PD₅₀).

Protective immunity was normally tested by the challenge infection of all survivors at the 14th or 21st day with a lethal dose (10⁵ p.f.u., i.p.) of the L10 strain of SFV or P2023 strain of VEEV. The mixed responses of some deaths following primary infection (D%), some protected against later challenge (P%), and some uninfected and susceptible to challenge (S%), did not permit calculation of conventional LD₅₀ or PD₅₀ values: in these important cases the actual distributions of responses (D + P + S = 100%) are given for the various conditions of virus dose and treatment of host (Bradish et al., 1975a).

RESULTS

Dose factors in the effects of interferon and dsRNA

Groups of 10 A2G mice were treated with a range of standard unit doses of mouse interferon at 1 day before infection, or with standard weight dose of dsRNA at 4 h before infection. These groups were then used for the i.p. titration of the infectivity of stock preparations of the A774 and L10 strains of SFV or of the 2lt and P2023 strains of VEEV. Mice were confirmed as infected, or not, through the detection of viraemia, serum-neutralizing activity or interferon activity. All infected mice remaining at 21 days were found to be protected against virulent challenge. The various tests confirmed that the mice had been subclinically infected or infected by minimal and non-lethal doses of usually virulent strains of virus.

The presentation in Fig. 1 of the effects of interferon using a linear scale of dose units, and the effects of dsRNA using a logarithmic scale of dose weights, shows that distinct mechanisms of depression of infectivity are involved. Surprisingly, both for interferon and for dsRNA, the depressions of infectivity for the distinct avirulent (A774) and virulent (L10) strains of SFV fall on the same lines.

The similar results obtained for the depression of the infectivity for the virulent (P2023) and avirulent (2lt) strains of VEEV show that these are less sensitive to the effects of dsRNA than the strains of SFV. This difference of about threefold in sensitivity to 10 μg or less of dsRNA
became much more pronounced at higher doses of dsRNA and virus: this is shown further in Fig. 2.

The scale on the right-hand side of Fig. 1 shows the observed depressions of infectivity as the progressive impairments of the efficiency of infection (ID₅₀/p.f.u.) in the interferon- or dsRNA-treated host. Thus, for mice treated with 30 μg dsRNA before infection with either strain of SFV, only about 1 virion in 50000 achieves productive infection compared with about 1 in 10 for the normal host. This applies to both avirulent and virulent strains of virus despite the outcome of infection being subclinical in the first case and lethal in the second.

**Distributions of responses to infection in mice treated with dsRNA**

In view of the importance of the distinctions between efficiency of infection (Fig. 1) and outcome of infection, and the fact that normal mice infected by low doses of ‘virulent’ strains of virus may not die, more detailed observations were made in mice treated with various doses of interferon or dsRNA.

Fig. 2 shows the responses of groups of A2G mice to the administration of 10³ p.f.u. of different virus strains at 4 h following i.p. treatment with a range of doses of dsRNA up to 30 μg. For the avirulent A774 strain of SFV (line A in Fig. 2) there was a gradual decline in the proportion showing subclinical infection and subsequent protection against virulent challenge: there was an exactly corresponding gradual increase in the proportion of mice showing no evidence of infection (see Methods).

For the mice receiving 10³ p.f.u. of the ‘virulent’ L10 strains of SFV (line D in Fig. 2) there was a very sharp decline at 0.3 to 1 μg dsRNA in the proportion of mice dying at 6 to 8 days following infection. This decline in primary mortality was not due to the blocking of infection since up to 25% of mice receiving 10 μg dsRNA showed subclinical infection with subsequent protection against virulent challenge (line P). It was as if the virulent strain of SFV had become avirulent. Doses of dsRNA much greater than 100 μg would be required to ensure a blocking of all infections by 1000 infective units of strains of SFV.

The controls on the responses of normal and dsRNA-treated mice to minimally infective doses of virus are shown in detail in Table 1. These groups of 30 A2G or Balb/c mice received 10 or 1000 p.f.u. of the A774 and L10 strains of SFV at 4 h following treatment with dsRNA as shown. A2G and Balb/c mice showed similar responses and their distributions have been
Table 1. Grouped responses of A2G and Balb/c mice to dsRNA at 4 h before i.p. infection by virulent (L10.C1) or avirulent (A774.C3) strains of SFV, with the incidence of antibody conversions before and after confirmation of protection against virulent challenge

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>L10.C1</th>
<th>A774.C3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antibody conversion % in survivors (S + P)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-chall.</td>
<td>Post-chall.</td>
<td>Pre-chall.</td>
</tr>
<tr>
<td>D*</td>
<td>P†</td>
<td>S‡</td>
</tr>
<tr>
<td>(i)</td>
<td>0</td>
<td>10³</td>
</tr>
<tr>
<td>(ii)</td>
<td>0</td>
<td>10²</td>
</tr>
<tr>
<td>(iii)</td>
<td>0</td>
<td>10³</td>
</tr>
<tr>
<td>(iv) Mean of (ii) + (iii)</td>
<td>10³</td>
<td>22</td>
</tr>
</tbody>
</table>

* D, Percentage of mice in groups of about 60 dying after primary infection.
† P, Percentage of mice subclinically infected and then protected against virulent challenge 3 weeks later.
‡ S, Percentage of mice not primarily infected and dying after virulent challenge.
§ Pre-challenge, percentage of mice showing antibody conversion at 15 days after primary infection and before confirmation of protection against virulent challenge.
‖ Post-challenge, percentage of mice showing antibody conversion at 15 days after confirmation of protection against virulent challenge.

Pooled in Table 1. The lines (ii) and (iii) of Table 1 show that mice respond similarly when infected either by 10 to 30 p.f.u. directly or by 1000 p.f.u. together with dsRNA. This comparison embraces the subclinical, protective response to low doses of the virulent L10 strain of SFV and also the incidence of antibody conversions before or after the secondary virulent challenge. There was no indication of a change in the outcome of infection in the few mice now infected.

The comparative results in Fig. 2 (line V) for the virulent P2023 strain of VEEV show that in mice treated with 30 μg dsRNA, a dose of 10³ to 10³.5 p.f.u. was required for the LD₅₀. All surviving mice remained uninfected (S) and there were no subclinical protective infections (line C) of the type considered above for the L10 strain of SFV. Furthermore, the P2023 strain of VEEV required a dose of dsRNA in mice which was 50 times greater than that shown for SFV to reduce 10³ to 10³.5 p.f.u. of virus to 1 LD₅₀. Thus, despite the similar impairments by dsRNA treatment of the efficiencies of infection shown by different strains of one virus (Fig. 1), different viruses may show very different sensitivities in dsRNA-treated mice.

Distributions of responses to SFV infection in mice treated with interferon

Mice treated with various doses of fibroblast interferon at 1 day before virus infection (Fig. 1) showed changes in the efficiency of infection which were not accompanied by any significant changes in the outcome of infection. These results will not be presented in detail since they follow closely those above for dsRNA-treated mice. Regardless of the impairment by interferon of the efficiency of infection by the avirulent A774 strain (Fig. 1), all infected mice responded subclinically and were protected against subsequent lethal challenge. By contrast, mice infected by 10³ to 10³.5 p.f.u. of the L10 strain of SFV showed a decline in primary mortality as the interferon dose was increased. This was accompanied by an increase in the proportion of mice subclinically infected and protected against subsequent lethal challenge, as shown above for dsRNA-treated mice. Thus, for mice treated with 5000 units of interferon and then infected by the now elevated ID₅₀ dose of 10³ to 10³.5 p.f.u. the
Table 2. Influence of dsRNA (28 μg) administered 4 h before primary infection upon the efficiency of infection, protection and antibody conversion by several vaccine strains of togaviruses

<table>
<thead>
<tr>
<th>Virus strain</th>
<th>Control</th>
<th>With dsRNA</th>
<th>Control</th>
<th>With dsRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFV, A774.C3</td>
<td>0-5</td>
<td>4-4</td>
<td>~1-0</td>
<td>5-3</td>
</tr>
<tr>
<td>VEEV, TC83.C1</td>
<td>2-9</td>
<td>&gt;7-0</td>
<td>5-0</td>
<td>&gt;7-0</td>
</tr>
<tr>
<td>VEEV, 21t.C1</td>
<td>0-4</td>
<td>3-3</td>
<td>0-4</td>
<td>3-4</td>
</tr>
<tr>
<td>YFV, 17D.C1</td>
<td>4-0</td>
<td>&gt;6-0</td>
<td>5-5</td>
<td>&gt;6-0</td>
</tr>
</tbody>
</table>

* A2G mice 92 days old were bled on the 9th day following primary infection for assessment of the neutralizing activity of their antisera, and were challenged with secondary virulent virus on the 19th day; for assessment of protection see Methods.

† After primary infection i.p. by this human vaccine strain mice were later challenged by an intracerebral inoculation of 1000 p.f.u. of the same strain (Fitzgeorge & Bradish, 1980).

The distribution of responses was 50% non-infection (S), 25% primary death (D) and 25% benign protection (P). This distribution of responses corresponds with that in normal mice receiving about 1 ID$_{50}$ or 10 p.f.u. (Table 1).

**Influence of dsRNA treatment upon responses to infection by 'vaccine' strains of togaviruses**

To extend observations of the effects of dsRNA treatment, the efficiency of infection and the stimulation of antibody synthesis (regulatory immunity) and protective immunity were assessed in mice treated with 28 μg dsRNA at 4 h before the titration of the vaccine strains shown in Table 2.

The virus strains tested included two with normally high efficiency of infection in mice (A774.C3 and 21t.C1: 1 p.f.u. infective per 3 to 30 inoculated) and two with very low efficiency of infection (TC83.C1 and 17D.C1: 1 p.f.u. infective per 10$^3$ or more inoculated). For all of these avirulent strains the efficiency of infection (ID$_{50}$/p.f.u.) and protection (PD$_{50}$/p.f.u.) were depressed by $>$ 1000-fold in mice treated with dsRNA. Furthermore, antibody conversion following primary infection was impaired even further so that virus doses for 50% antibody conversion were about $>$ 1000-fold higher in dsRNA-treated mice than in normal mice.

The differences between the p.f.u. per PD$_{50}$ and per 50% antibody conversion for normal and dsRNA-treated mice confirm that stimulations of regulatory and protective immunity occur without detectable antibody conversions in minimally infected mice. The further and independent impairments by dsRNA treatment of already very low efficiencies of infection (virus strains TC83.C1 and 17D.C1) confirm that mechanisms occurring later than normal clearance and reception of virus are involved. In all cases the effect of dsRNA treatment is to reduce the infecting dose of virus but not to modify the normal outcome of infection and immunogenic stimulation.

**Recovery from treatment with dsRNA and its resulting impairment of efficiency of infection**

To follow the recovery of the several indicators of host infection and response, groups of 15 Balb/c mice were treated with 10 μg dsRNA and then infected from 4 h to 13 days later with 10$^3$ p.f.u. of the A774 strain of SFV. Interferon activity, infectivity and neutralizing activity in blood were measured. All mice were bled for assay of antibody activity before the virulent challenge and its test of protective immunity at 20 days after the primary infection.
The results at D and E in Fig. 3 show that when the avirulent strain A774 of SFV was administered 4 h after 10 μg dsRNA there was no detectable viraemia or interferon synthesis at 2 days following infection. Despite this, 30% (3 out of 10) showed antibody conversion when tested at 20 days and at least 73% (8 out of 11) were protected against virulent challenge. Thus, by 20 days minimum replication and stimulation had occurred.

Interferon activity and viraemia in blood at 2 days after infection showed a recovery to normal levels in 50% of mice when these were infected at 60 to 100 h after administration of dsRNA (D and E in Fig. 3). When the mice were not infected until the 6th day following the administration of dsRNA, all showed a complete recovery of these critical early responses.

Recovery from the comparable impairments by administered interferon was almost twice as fast (Bradish et al., 1975a) as that shown here for dsRNA. Furthermore, the effects of dsRNA are long lasting despite the very rapid elimination of the induced interferon activity in serum (line A of Fig. 3).

**DISCUSSION**

We have considered two aspects of the prophylactic actions of interferon or dsRNA in the modulation of the togavirus–host interaction. The actions of these agents have been compared quantitatively in terms of their doses in relation to the dose and strain of virus. Also, estimates have been made of the sensitivity to interferon or dsRNA of the distinct phases of the virus–host interaction. The optimum regimen and modes of action of any modulating drug can only be defined in terms of the cellular compartments and phases of host response that may be influenced differentially (Hilleman, 1970; Nathanson & Cole, 1971).

Regardless of the fundamental differences between their dose responses and associated rates of host recovery, the effects of interferon and of dsRNA may be contrasted quantitatively at a standard level through the indication (Fig. 1) that a change in efficiency of infection for SFV in mice from 1/10 to 1/100 requires a twofold increase of interferon dose (2800 to 5600 units) but an 18-fold increase of dsRNA dose (0.018 μg to 0.32 μg). For the narrower range *in vitro* effects a number of systems have shown that a change in percentage...
inhibition from 25 to 75% requires about a fivefold increase of interferon dose but about a 30-fold increase of dsRNA dose (Gifford & Toy, 1970). This much shallower response to dsRNA and its brief synthesis of circulating interferon (Fig. 3; Baron et al., 1970; Sharpe et al., 1971), suggest that the longer lasting effects of dsRNA may be unrelated to interferon induction, or are associated with specific interferon induction in cryptic target tissues which are little influenced by the bulk administration of exogenous interferons: levels of interferon activity in serum may be poor indicators of in vivo activity against virus invasion and disease.

The depression by dsRNA or interferon of the dose of virus that can be effective in replication and subsequent immunostimulation has been shown through the depression of the efficiency of infection and immunogenicity for several pathogenic and vaccine strains of viruses. Additionally, as shown by type II interferons, the immunoresponsiveness of the host may be depressed (Kadish et al., 1980). The demonstration here that interferon and dsRNA do not influence events subsequent to the limitation of the initiating dose of virus, suggests that the place of these agents in chemotherapy is to limit the incidence of infection and to reduce clinical signs and so give more time for other combinations of treatments in those still infected.

The present studies in mice have shown that the efficiency of primary infection, an intrinsic quality of a virus strain (Walder & Bradish, 1975, 1979), may be further and independently impaired by the actions of interferon or dsRNA. The outcome of virus infection is to produce a range of host responses appropriate to the quantitatively reduced dose of virus. Parallel studies on the effects of immunomodifying drugs have shown that immunosuppression (cyclophosphamide, Myocrisin) and immunoenhancement (l-asparaginase) may be distinguished and that each may occur with (Myocrisin, l-asparaginase) or without (cyclophosphamide) enhancement of the efficiency of infection (Bradish et al., 1975a, b). Since the administration of interferon or dsRNA produced none of these effects, it appears that these agents block virus replication and early immunostimulation through unique intermediate mechanisms and without modification of earlier phases of clearance and reception or later phases of immunoregulation and expression of virulence.

The clinical trials summarized by Merigan et al. (1975), Tyrrell (1976), Dunnick & Galasso (1979) and Scott & Tyrrell (1980), show the effects of prophylaxis and treatment with interferons or inducers to be a general reduction of the incidence or grade of illness but usually not of the incidence of persistence in established infections. Clearly, dosages of agents and viruses were difficult to compare or standardize and the relationship of host response and status in immunity to effective virus dose is uncertain. The critical relationships in prophylaxis or treatment between dose of dsRNA and effective dose of virus have been emphasized by Stephen et al. (1977) and Levy et al. (1976) in their tests in rhesus monkeys infected by the viruses of simian haemorrhagic fever or yellow fever: their results suggest that dsRNA impaired infection and replication but did not modify the course of disease in those still infected by minimal doses of virus. Similar conclusions emerge from the studies by Harrington et al. (1977) on the treatment with poly(I).poly(C) of rhesus monkeys infected with Japanese encephalitis virus. The studies by Bowen et al. (1978) on Ebola virus infections in interferon-treated rhesus monkeys showed a delay in viraemia without change in the ultimate course or severity of disease. In all of these cases, two essential controls appear to be the observation of the responses of the normal host to a range of minimal doses of virus (as Table 1) and the assessment of protective immunity against actual secondary infection (as Table 2). The difficulty and cost of such controls argues for the most detailed reporting of dose relationships and of individual responses in each phase of the virus–host interaction.

The temporal distinctions between 'protection' against primary infection by virus (efficiency of infection), protection against disease if infected (virus generalization and regulatory immunity) and protection against subsequent virulent challenge (protective immunity) are of
fundamental importance to the definition of the activities of interferons or dsRNA (Hilleman, 1970; Friedman, 1977), and in terms of the prophylactic-therapeutic strategies which may be appropriate to life-threatening situations (Anon. 1977; WHO. 1978; Bowen et al., 1978).

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REFERENCES


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