Protection of Mice Against Infection with Wild-type Mengo Virus and an Interferon Sensitive Mutant (IS-1) by Polynucleotides and Interferons

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SUMMARY

Single-stranded polynucleotide preparations [tRNA, poly(rI) plus poly(ho6C)-copolymer] which protect mice against picornavirus infections without inducing interferon, protected mice equally against infection with an interferon-sensitive mutant (IS-I) of Mengo virus and with wild-type virus (IS+). Poly(rI).poly(rC) and mouse macrophage interferon [i.e. serum from mice treated with poly(rI).poly(rC)] protected mice equally against infections with the two viruses, but fibroblast interferon protected better against infection with the interferon-sensitive mutant than with the wild-type virus. These and other results indicate that: Mengo virus has a genetic locus affecting sensitivity to fibroblast but not macrophage interferon; these two types of interferon have different mechanisms of action against Mengo virus infections in mice; Mengo virus genes controlling sensitivity to fibroblast interferon may modulate disease since infection in vivo induces only fibroblast interferon; the antiviral activity of the single-stranded polynucleotides is unlikely to be mediated by induction of either macrophage or fibroblast interferon.

Polyriboinosinic acid [poly(rI)] and polyribocytidylic acid [poly(rC)] protect mice against encephalomyocarditis (EMC) virus infection, but not apparently because of induction of interferon (Stebbing et al. 1976a). Transfer RNA preparations, particularly of bacterial origin, are also antiviral without giving rise to detectable serum interferon (Stebbing et al. 1976b). The interferon-independent antiviral activity of poly(rI) and poly(rC) is greatly enhanced when they are both administered to mice under conditions preventing formation of double-stranded structures; poly(rI) and poly(rC) can be administered sequentially 4 h apart (Stebbing & Grantham, 1976), or poly(I) can be administered together with a derivative of poly(rC) to which it does not anneal (Stebbing et al. 1977). There are a number of other features which distinguish antiviral protection resulting from single-stranded polynucleotides and from those double-stranded polynucleotides which do induce interferon (Stebbing & Grantham, 1976; Stebbing et al. 1976a, b, 1977). There are now extensive data supporting the notion that single-stranded polynucleotides can protect against various virus infections in the absence of interferon induction, particularly where the polynucleotides have been shown to inhibit virus enzymes (Pitha, 1973; Stebbing, 1979). However, for the above cited polynucleotides it can still be argued that interferon plays a role in their antiviral effects. For example, it can be argued that the effective treatments with single-stranded polynucleotides induce interferon which does not circulate and has unusual properties. This possibility can be studied by examining the protective effect of these polynucleotides against infection by an interferon-sensitive mutant virus such as the mutants of Mengo virus isolated by Simon et al. (1976). The present work demonstrates that treatment with single-stranded polynucleotides or macrophage interferon protects mice equally against an interferon-sensitive Mengo virus mutant and the parent wild-type virus, but interferon from fibroblasts is more protective against the mutant.
The most effective of the single-stranded polynucleotide treatments cited above were tRNA from *Escherichia coli* and mixtures of poly(rI) and a copolymer containing principally 5-hydroxycytidylic acid residues [poly(hoC)-copolymer]. These polynucleotides were therefore studied in mice infected with IS-1, the most fully characterized of a series of interferon-sensitive Mengo virus mutants, and the parent wild-type virus IS+ (Simon *et al.* 1976), both obtained from Dr Edward Simon, Purdue University, Indiana, U.S.A.

When the viruses were received they were thawed, mixed with an equal volume of glycerol and stored at -20 °C. Their titre (LD<sub>50</sub>) in mice varied very little during the period of the present experiments. Female BK:W mice 6 to 10 weeks old, obtained from Bantin & Kingman Ltd., Hull, U.K., were maintained at 22 °C. Virus and polynucleotides were prepared in 0.89% (w/v) NaCl, 10 mM-Hepes, pH 7.5, and administered intraperitoneally (i.p.) in 0.1 ml. Poly(rI) and poly(rC) and the double-stranded complex between these two polynucleotides [poly(rI).poly(rC)] were obtained from P-L Biochemicals, Milwaukee, U.S.A. Poly(rC) was converted to a copolymer, containing 81% 5-hydroxycytidylic acid residues and 19% 5-bromocytidylic acid residues as described elsewhere (Stebbing *et al.* 1977), which is termed poly(hoC)-copolymer. Transfer RNA (tRNA) from *E. coli* was obtained from British Drug Houses Ltd., Poole, Dorset, U.K. Crude mouse (macrophage) interferon was serum from mice bled out 2 h after i.p. administration of 60 μg poly(rI).poly(rC) per mouse, and contained over 5000 units/ml interferon (see below). Lyophilized mouse fibroblast interferon, obtained from Bio-technics Laboratory Products Inc., Rockville, Maryland, U.S.A., was reconstituted with sterile distilled water to 10<sup>6</sup> units/ml before use. Interferons were titrated by a plaque-reduction assay in which a mouse reference interferon was included (see Stebbing *et al.* 1978): interferon doses are given in terms of reference interferon units.

To assess the effects of treatments of virus-infected mice, the survival time in hours (t) was obtained from records prepared twice daily. Mean values of 10<sup>2</sup>/t were calculated for each group of mice (including surviving mice), as described previously (Stebbing *et al.* 1977, 1978). To assess the significance of differences in the survival times of different groups of mice, χ<sup>2</sup> values, with one degree of freedom, were calculated by the log-rank method as described by Peto & Pike (1973). Significance levels are indicated by asterisks as follows: ***, P < 0.001; **, P < 0.01; *, P < 0.05. No asterisk indicates P > 0.05 and is taken as not significant. For convenience of presentation, where results are shown in the form of mortality curves, deaths occurring in any one day are shown at one time only.

To compare the antiviral effects of polynucleotides in mice infected with the wild-type and mutant virus, both viruses were administered intraperitoneally at doses causing the same percentage deaths in mice. The LD<sub>50</sub> dose of both viruses was found to be 0.1 ml of 10<sup>-6</sup> dilutions of the stocks; 100 LD<sub>50</sub> killed between 75 and 95% of mice. This latter dose was used in most experiments in order to give a sensitive and statistically efficient test system for antiviral activity. It was found that dilutions of 10<sup>-3</sup> or 10<sup>-2</sup> killed all mice in the case of the wild-type virus, but there were always surviving mice using these dilutions of the IS-1 mutant virus.

Table 1 shows the effect of i.p. administered tRNA or a mixture of poly(rI) and poly(hoC)-copolymer in mice infected with 100 LD<sub>50</sub> of the mutant (IS-1) or wild type (IS+) Mengo virus. In expt. 1 both viruses killed 19 out of 20 control mice and the polynucleotide treatments conferred highly significant protection. With treatments at 24 h before infection (expt. 2, Table 1) 15 or 16 mice out of 20 were killed in the untreated groups. The mixture of poly(rI) and poly(hoC)-copolymer conferred significant protection against both viruses, but tRNA protected significantly only against the wild-type virus. Essentially the same result was obtained with a 1 LD<sub>50</sub> dose of the two viruses in experiments with 40 mice per group and polynucleotide doses ranging from 50 μg/mouse to 800 μg/mouse.
Table 1. Comparison of the antiviral effects of tRNA, a mixture of equal weights of poly(I) and poly(hoSC)-copolymer and macrophage interferon against wild-type and IS-1 mutant Mengo virus infection of mice

<table>
<thead>
<tr>
<th>Treatment of mice</th>
<th>IS-1 virus</th>
<th>IS+ wild type virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt.</td>
<td>Substance</td>
<td>i.p. dose/mouse</td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tRNA</td>
<td>400 µg</td>
</tr>
<tr>
<td></td>
<td>‘Mixture’</td>
<td>400 µg</td>
</tr>
<tr>
<td></td>
<td>Interferon</td>
<td>500 units</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tRNA</td>
<td>400 µg</td>
</tr>
<tr>
<td></td>
<td>‘Mixture’</td>
<td>400 µg</td>
</tr>
<tr>
<td></td>
<td>Interferon</td>
<td>500 units</td>
</tr>
</tbody>
</table>

† N = number of mice surviving per group of 20.
‡ χ² = Log-rank comparisons between treated groups and infected control (no treatment). These showed no significant differences between groups receiving the same treatments but infected with the mutant or wild-type virus.

The protective effect of poly(rI).poly(rC), which induces interferon in macrophages of mice (Jullien et al. 1974), was also found to be the same against the wild-type and mutant virus when used in doses between 10 µg/mouse and 100 µg/mouse.

It should be noted that there was no significant difference between groups of mice infected with the wild-type and mutant virus at identical doses (see Table 1). This was found to be the case in many experiments using the same dilutions of the two viruses between 0·1 and 240 LD₅₀; with higher virus doses the mutant virus was relatively less lethal than the wild-type.

The similar protective effect of poly(rI).poly(rC) in mice infected with either wild-type or the interferon-sensitive mutant of Mengo virus suggests that the mutant phenotype was not expressed during infection of mice. This possibility was further investigated: Table 1 shows that crude interferon induced with poly(rI).poly(rC) (i.e. macrophage interferon) also protected mice equally against wild-type and the interferon-sensitive mutant. However, the mutant virus was originally detected by its sensitivity to fibroblast interferon induced in L cells by Newcastle disease virus (Simon et al. 1976). The sensitivity of the viruses to fibroblast interferon was therefore examined.

The results in Fig. 1(a) show that infection of mice with the wild-type Mengo virus was greatly diminished by as little as 250 units of macrophage interferon (χ² against infected control = 29·11*** ) but that 1000 units of fibroblast interferon had no protective effect. In contrast these doses of macrophage and fibroblast interferon both significantly protect mice against the mutant (χ² values against the infected control = 16·16*** and 8·12**, respectively). In this experiment poly(rI).poly(rC) at 60 µg/mouse had a protective effect essentially identical to that of the macrophage interferon (data not shown). Higher doses of the interferon preparations behaved correspondingly: macrophage interferon was again equally effective against both viruses; with fibroblast interferon the mean time of death was delayed and the number of deaths was lower in mice infected with the IS-1 mutant compared with the wild-type virus.

Macrophage interferon has a greater antiviral effect than fibroblast interferon against wild-type Mengo virus, just as against EMC virus in infections of mice (Stebbing et al. 1978). However, the interferon-sensitive Mengo mutant, IS-1, is more sensitive than the wild-type virus to the antiviral effect of fibroblast interferon, which indicates that the mutant
phenotype is expressed during infection of mice. This observation, combined with the similar sensitivity of the wild-type and IS-I mutant to macrophage interferon, indicates that the two forms of interferon have different mechanisms of action. Since the interferon induced in mice as a result of an EMC virus infection appears to arise in fibroblasts (Jullien et al. 1974), one might anticipate that this induced interferon might limit infection of mice with high virus doses. Thus, the failure of high doses of the interferon-sensitive mutant of Mengo virus to kill all mice may be a result of the mutant's sensitivity to fibroblast interferon. It therefore seems possible that lethal effects of a picornavirus infection may in
general result from insensitivity of the virus to the interferon which is formed during its replication in vivo, namely fibroblast interferon.

Simon et al. (1976) isolated Mengo virus mutants with increased and with decreased sensitivity to fibroblast interferon, and a genetic locus regulating sensitivity to this type of interferon may be general for picornaviruses. However, in the case of the IS-I mutant at least, an increase in sensitivity to fibroblast interferon is not accompanied by a change in sensitivity to macrophage interferon. Whether there are virus genetic loci affecting sensitivity to macrophage interferon remains to be seen, but quite possibly this will not prove to be the case. Since macrophage interferon is not produced naturally during infections in vivo, selection pressure for generating macrophage interferon insensitivity in the virus genome may not have existed. If genetic loci in the virus genome of picornaviruses generally regulate sensitivity to fibroblast but not macrophage interferon, the implications of these observations for interferon therapy of these viruses at least are clear: macrophage interferon is inherently more effective than fibroblast interferon and mutants may readily arise with altered sensitivity to fibroblast interferon but this may not be the case for macrophage interferon.

The antiviral activities of the single-stranded polynucleotide treatments reported here are similar to those observed with other picornavirus infections (Stebbing et al. 1976a, b, c, 1977). Induction of fibroblast interferon by these polynucleotides seems unlikely since the wild-type and IS-I mutant respond unequally to this form of interferon whereas the polynucleotide treatments confer equal protection. It cannot be excluded that the single-stranded polynucleotides are protective because they induce macrophage interferon. However, this is most unlikely since the amounts of macrophage interferon necessary to protect mice against wild-type Mengo and EMC viruses (Stebbing et al. 1978) would have been readily detected in the assay. The mechanisms of action of the single-stranded polynucleotides have been investigated but remain obscure although there is no evidence against the notion that they act by mimicking the poly(C) tract of picornaviruses (Stebbing et al. 1976a, c) or the tRNA-like region in the genome (Stebbing et al. 1976b, c).

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REFERENCES


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