Resistance to Virus Infection during the Hyporeactive State of Interferon Induction

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Interferon inducers are more active prophylactically than when given after infection. Potentially, therefore, in many clinical situations interferon inducers would have to be given repeatedly in order to maintain a continuous state of protection from virus infection. The known hyporeactivity of the serum interferon response (Ho, Postic & Ke, 1968; De Clercq & Merigan, 1970) is apparently a serious limitation to this approach. The hyporeactivity of the interferon response to Poly I:C has recently been carefully examined (C. E. Buckler, H. G. Du Buy, M. L. Johnson & S. Baron, personal communication; Du Buy et al. 1970). It was concluded that the ability to obtain high, sustained levels of interferon is hampered by hyporesponsiveness to restimulation. Adjustment of the dosage schedule, however, resulted in sustained moderate levels of serum interferon production for a period of 7 days or more.

In the experiments described here where protection was considered, hyporeactivity was not observed in mice challenged with encephalomyocarditis virus. The interferon inducer was double-stranded RNA, produced and purified at Beecham Research Laboratories from a Pencilliurn culture which contained a large number of virus-like particles. This is part of a joint programme with Professor Sir Ernst Chain and his colleagues at Imperial College, London, to explore the therapeutic and prophylactic potential of double-stranded RNAs. The highly purified double-stranded RNA that was used for these experiments was negative in the Shwartzman reaction at 600 μg./site in the rabbit, thus showing the absence of endotoxin. Doses of endotoxin as low as 5 μg./site gave a positive response. It had a sedimentation coefficient of approximately 12S, and exhibited a marked hyperchromicity and melting profile when heated. In isotonic salts it was relatively resistant to ribonucleases.

No hyporeactivity of protection against virus infection was demonstrated with this inducer. For instance, mice given 10 μg./dose (0.5 mg./kg.) intraperitoneally twice daily for four days immediately before infection showed protection equal to that of a group given a single injection of 10 μg./mouse 24 hr before intraperitoneal infection with encephalomyocarditis virus. We showed previously, with this dose of double-stranded RNA obtained from virus particles from Penicilliurn stoloniferum, that protection following a single dose lasts only 3 days (Planterose et al. 1970) so that the resistance observed with the eight-dose treatment was not in response to the first dose. In another experiment, mice given 50 μg./mouse/day on days -1, -10, -9, -8, -7, -4, -3, -2, -1 relative to infection on day 0 were as well-protected as mice given a single dose of 50 μg. 24 hr before infection.

In further experiments serum interferon concentrations were measured. Groups of mice were given 10 μg. (0.5 mg./kg. intraperitoneally) of double-stranded RNA once daily for 5 days and bled by cardiac puncture at 3, 5 and 24 hr after each dose. Serum interferon was assayed using primary mouse cell monolayers which were challenged with approximately 100 p.f.u. of encephalomyocarditis virus per culture. Equivalent groups of mice were challenged daily with 100 and with 1000 LD50 of encephalomyocarditis virus (intraperitoneally) 5 hr after each dose of inducer. Using these doses of virus there were no survivors in the untreated controls. The well-known hyporeactive state of serum interferon induction
was obtained, no stimulation of interferon being found with the third dose of inducer (Fig. 1). However, equal or better protection against virus challenge was shown by the second or subsequent doses of inducer. Thus mice were equally protected by either a single dose of inducer (giving a high serum interferon concentration) or by the last of a series of doses of the inducer (giving no detectable serum interferon). The absence of hyporeactivity of the protection response was not due to a low threshold of serum interferon giving maximum protection. Animals in the hyporeactive state with a low serum interferon level were more protected than animals with more serum interferon generated by a single dose of double-stranded RNA.

![Graph showing comparison of serum interferon levels with protection](image)

**Fig. 1.** Comparison of serum interferon levels with protection. The time at which double-stranded RNA was given is shown by arrows. Serum interferon levels (---) are expressed as the dilution of serum required to depress the plaque count by 50% (PDD50). Protection against 100 LD50s of encephalomyocarditis virus is shown as % of animals surviving in each group ——.</p>

Similar experiments were made using Sendai virus as the interferon inducer; but the inducer was given intravenously, virus by the intraperitoneal route. Thus any local effect, e.g. stimulation of peritoneal macrophages, can be ruled out. As in the experiments with double-stranded RNA approximately equal protection against encephalomyocarditis virus was observed during this hyporeactivity of serum interferon induction. Furthermore, we have shown that repeated intranasal dosing with inducer gave protection equal to a single dose 24 hr before intranasal challenge with influenza virus.

There appear to be few data in the literature concerning the hyporeactivity of the protection response. However, De Clercq, Nuwer & Merigan (1970) showed parallelism between the hyporeactivity to serum interferon production and hyporeactivity to antivirus protection against intranasal challenge with vesicular stomatitis virus. Here hyporeactivity was induced by repeated doses of the pyran copolymer or by endotoxin followed by Poly I:C and not by repeated doses of double-stranded RNA as was used in our experiments.

Serum interferon concentrations have frequently been used as a measure of antivirus activity of interferon inducers, for instance in the work on the evaluation of statolon, helenine and Poly I:C. It appears that serum interferon is not always a good measure of resistance to a virus challenge. There are some data supporting this view, for example
polyacrylic acid gives protection and induces interferon in mice, whereas *in vitro* protection is observed but no interferon (De Somer *et al.* 1968a, b). The lack of correlation of serum interferon concentrations and protection that has been observed in the special circumstances of the experiments quoted here can be explained in several ways. First, it can be argued that serum interferon is due to an overspill of interferon, interferon having to be retained or taken up by cells to make them resistant to virus challenge. It follows that protection is always observed when serum interferon is demonstrated, but that protection is also observed in the absence of serum interferon. Secondly, a mechanism not involving interferon induction may be involved. Potentiation of immune responses have been reported for Poly I:C (Turner, Chan & Chirigos, 1970) and for other polyanions (Braun *et al.* 1970). Our results could be explained in this way.

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