Interferon in Mice Acutely Infected with M-P Virus

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M-P virus infection of adult mice exerts an anti-tumour effect on certain murine carcinomas and lymphatic leukaemia (Molomut & Padnos, 1965). However, adult mice made tolerant to M-P virus by inoculation as neonates are deficient in the anti-tumour activity (Padnos, Molomut & Ferris, 1967). Attempts have been made to elucidate the mechanism of the anti-tumour activity of M-P virus and it has been suggested that the immune defence mechanisms of the host may assist in the destruction of the tumour cells. An immunological reaction need not be the only mode of anti-tumour action exerted by M-P virus however, since there is greater tumour inhibition during the early acute viraemic period than during the post-viraemic period when virus antibody levels are highest (Molomut et al. 1964; M. Padnos, N. Molomut & P. Ferris, unpublished results). Furthermore, the entire role of the reticuloendothelial system in the host reaction to neoplasias is not completely understood but is known to be very important (Old et al. 1961).

Recent work (Gresser & Bourali, 1970; Wheelock & Larke, 1967; Regelson, 1967; Levy, Law & Rabson, 1969; Zeleznich & Bhuyan, 1969; Came & Moore, 1971) has shown that tumours may be repressed either by administration of exogenous interferon or by stimulation of endogenous interferon. Since the complete mechanisms of anti-tumour activity of M-P virus are not known, the agent has been assessed as an inducer of interferon in this study. The results show that serum from mice acutely infected with M-P virus contains interferon. We have also correlated the titre of circulating interferon with virus titre and other variables.

Swiss/Innes mice were obtained in 1962 from Dr Innes of the Brookhaven National Laboratories as a closed colony and have been inbred at the Waldemar Medical Research Foundation. Mice were used at 6 to 9 weeks.

M-P virus was propagated in HeLa cells grown in Eagle's basal medium in Hanks's salt solution supplemented with 10% foetal calf serum and antibiotics. Supernatants of infected cultures harvested on the fifth day after inoculation yielded $10^8$ to $10^{10}$ ID$_{50}$/ml. when assayed in Swiss mice. Supernatants were filtered (0.22 μm filter) and stored at $-84^\circ$.

Mouse interferon was titrated using the plaque inhibition test described by Wagner (1960) employing L929 cells and vesicular stomatitis virus (VSV). Interferon titres were expressed as the reciprocals of the dilution of 10 ml. of serum which inhibited 50% of VSV plaques.

Mouse serum was separated from blood obtained following intraperitoneal injection of M-P virus. Serum was acidified to pH 2, allowed to remain at 5$^\circ$ overnight, adjusted to pH 7.0 to 7.3 and filtered (0.45 μm. filter). A sample of mouse serum interferon obtained from the Research Reagents Branch of the National Institutes of Health, diluted to contain 600 units, was found to contain between 400 and 800 units by the methods employed here.

Fig. 1 presents the data obtained from an experiment in which mice were inoculated intraperitoneally with $10^8$ ID$_{50}$ of M-P virus and at various intervals following inoculation were bled and sacrificed in groups of 10 to 20 mice. It can be seen that the circulating interferon rises from 12 hr after infection and to a maximum at 72 hr. At 96 hr there was little detectable interferon. The inhibitory substance in the serum was shown to have the properties generally identified with interferon in that it was destroyed by trypsin, was non-sedimentable at 105,000 g for 2 hr., its action was blocked by actinomycin D and it was not
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active in chick embryo fibroblasts. The kinetics of appearance of circulating virus in a typical experiment is expressed in Fig. 2, as is the total white blood cell count and lymphocyte count. It may be noteworthy that serum interferon titre is greatest (3 days after infection, Fig. 1) at a time near maximum lymphocyte depression (3 to 4 days after infection, Fig. 2).

Adult mice made tolerant by neonatal injection with M-P virus have a persistent circulating virus titre of approximately $4 \times 10^6$ ID 50/ml of serum, the haemogram reveals normal WBC and lymphocyte counts, and there is no detectable neutralizing antibody. Interferon was not detectable in serum from such mice before super-infection with $10^4$ ID 50 of M-P virus or at 3 days following super-infection. However, when MP-virus-tolerant mice are injected intravenously with u.v.-irradiated Newcastle disease virus ($2 \times 10^8$ p.f.u. before irradiation), 2,000 to 4,000 units of circulating interferon can be detected 8½ hr after challenge. This titre is equal to that induced in control mice.

![Fig. 1](image1.png)

**Fig. 1.** Circulating interferon in mice acutely infected with 100 ID 50 of M-P virus.

![Fig. 2](image2.png)

**Fig. 2.** Circulating virus concentrations (■—■)*, total white blood cell counts (○—○)† and lymphocyte count † in mice acutely infected with M-P virus. *Each point represents the mean value of sera from 12 mice. † Each point represents average counts obtained from 6 mice.

The data presented here show that inoculation of M-P virus into 'normal' adult mice results in an acute viraemia and in the appearance of circulating interferon.

Tolerant mice do not produce detectable interferon when superinfected with M-P virus propagated in HeLa cells. Mice persistently infected with M-P virus, however, are capable of producing circulating interferon when challenged with an unrelated virus (Newcastle disease virus (NDV)).

The M-P virus has not yet been completely characterized but it has some common, and some differing characteristics with lymphocytic choriomeningitis (LCM) virus (Molomut & Padnos, 1965). Earlier reports on mice acutely and persistently infected with LCM virus have indicated an absence of interferon (Wagner & Snyder, 1962; Volkert, Laisey & Pfau, 1964; Mims & Subrahmanyan, 1966; Lehmann-Grube, 1967). The detection of circulating interferon following acute infection with M-P virus prompted recent studies on well-characterized strains of LCM virus (SC and LM plaque-type mutants of the we strain of LCM obtained from Dr J. Hotchin) and have revealed circulating interferon kinetics similar to M-P virus, with peak titres approximating those seen with M-P virus developing from 2 to 4 days after infection (P. E. Came, E. T. Sikora & J. Hotchin, unpublished results).

The absence of interferon response in M-P virus-tolerant mice to homologous virus and
the capability of interferon response to heterologous virus is similar to the findings in LCM carrier mice reported by Mims & Subrahmanyan (1966), who described interferon induction after challenge with West Nile virus. Holterman & Havell (1970) have shown that there is a lesser interferon response to NDV, in mice made tolerant to LCM, than in normal control mice.

Although the exact relationship between the presence of circulating interferon and anti-tumour activity of M-P virus is not the subject of this report, it has been well-documented that exogenous interferon may exert an anti-tumour effect. The finding of interferon in the circulation of mice inoculated with M-P virus could suggest that the interferon is, at least in part, responsible for the regression of certain tumours. Such an interpretation would not be unique and indeed the work of numerous investigators demonstrates the anti-tumour effect of exogenous as well as endogenous interferon on murine tumours.

The coincidence of lymphopenia and circulating interferon in mice acutely infected with M-P virus appears to link lymphocyte number inversely with interferon levels. Further studies in acutely and tolerantly infected mice may explain the mechanism of this observation and also serve to reveal the role of interferons in the inhibition of certain tumours.

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REFERENCES


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