ROLE OF INTERFERON IN THE PATHOGENESIS OF VIRUS DISEASES IN MICE AS DEMONSTRATED BY THE USE OF ANTI-INTERFERON SERUM

II. Studies with Herpes Simplex, Moloney Sarcoma, Vesicular Stomatitis, Newcastle Disease, and Influenza Viruses*

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As reported in the preceding article, administration of sheep anti-mouse interferon globulin to mice infected with encephalomyocarditis (EMC)[1] virus resulted in the multiplication of virus to high titers in visceral organs, rapid onset of disease, and early death. These results demonstrated the importance of the early production of mouse interferon in the response to this experimental virus disease. It was considered of interest to extend these studies to other viruses exhibiting different pathogeneses. We describe herein the results of experiments undertaken with herpes simplex virus (HSV), Moloney sarcoma virus (MSV), vesicular stomatitis virus (VSV), Newcastle disease virus (NDV), and influenza virus type A.

Materials and Methods

Mice. Unless otherwise stated, 1-mo-old male Swiss mice from a pathogen-free colony at the Institut du Cancer were used in these experiments. For one experiment BALB/c mice were obtained from a mouse colony of the Hôpital Cochin, Paris, France.

Viruses. The source of viruses used is described in Table I. Techniques of intranasal (i.n.) infection of mice with VSV or influenza virus have been previously described (4).

Sheep Anti-Mouse Interferon Globulin. The techniques of preparation, semipurification, and assay of sheep anti-mouse interferon globulin (and normal sheep serum globulin) were described in the preceding article. In the experiments to be described both globulin preparations were diluted 1:3 in phosphate-buffered saline. Animals were inoculated with viruses and immediately injected with 0.1 ml of anti-interferon globulin (or 0.14 ml normal sheep serum globulin) intravenously (i.v.) or as indicated.

Results

Effect of Sheep Anti-Interferon Globulin on Infection of Mice with HSV Type I. In a total of seven experiments, i.v. administration of anti-mouse interferon

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[1] *Abbreviations used in this paper:* EMC, encephalomyocarditis; HSV, herpes simplex virus; i.n., intranasal; MSV, Moloney sarcoma virus; NDV, Newcastle disease virus; VSV, vesicular stomatitis virus.

TABLE I

Virus	Strain	Obtained from:	Passaged in
HSV type I	F (1)	Dr. P. Sheldrick	Rabbit skin fibroblasts
HSV type I	B-12 (2)	Dr. P. Sheldrick	Rabbit skin fibroblasts
MSV	– (3)	Dr. J. P. Lévy	BALB/c mice
VSV	Indiana	Our laboratory	Mouse L cells
NDV	Herts	Our laboratory	Allantoic cavity
Influenza A	PR 8	Dr. C. Hannoun	Embryonated egg

globulin (titer, 4×10^{-5}) resulted in the early appearance of disease and death, and increased the overall mortality due to HSV injected either intraperitoneally (i.p.) or subcutaneously (s.c.) (Figs. 1 and 2). Thus, when mice were inoculated i.p. with the F strain and then injected with normal serum globulin, the LD_{50} was $10^{-2.8}$/0.2 ml compared to an LD_{50} of $10^{-5.6}$/0.2 ml in mice treated with anti-interferon globulin (Fig. 1). Comparable results were obtained with strain B-12.

This enhancing effect was even more striking when HSV was inoculated s.c. (Fig. 2). In three experiments only 1 mouse died of 19 mice treated with normal serum globulin and injected with a 10^{-1} dilution of strain B-12 (none of 20 mice died after injection of a 10^{-2} dilution of virus). In contrast, the LD_{50}/0.2 ml in mice treated with anti-interferon globulin in these three experiments was $10^{-2.7}$, $10^{-2.5}$, and $10^{-1.1}$, respectively.

25 mice having survived HSV inoculation (s.c. or i.p.) were injected with anti-interferon globulin 18 days after viral inoculation. In no instance did this treatment induce signs of HSV disease.

Effect of Anti-Interferon Globulin on Infection of Mice with MSV. 6-wk-old BALB/c mice were injected intramuscularly (i.m.) with 0.1 ml of 10-fold dilutions of MSV in the hind leg. Administration of anti-interferon globulin increased the number of animals bearing tumors (at each dilution of virus tested) and shortened the latent period before appearance (Fig. 3 A). The tumor size was estimated by daily palpation for each mouse and scored on a scale of 0, ½, 1, etc., to a maximum of 3. There was a striking difference between the mean tumor score of tumor-bearing mice treated with anti-interferon globulin and the two groups of control mice (Fig. 3 B). Furthermore the overall tumor-inducing dose of 50/0.2 ml of MSV was $10^{-3.1}$ for anti-interferon globulin-treated mice compared to $10^{-1.1}$ and $10^{-0.8}$ for the two groups of control mice. Despite a second injection of antiserum at 2 wk, regression of tumor occurred in all mice in all three groups and no deaths were observed.

Effect of Anti-Interferon Globulin on Infection of Mice with VSV. In four experiments 1-mo-old mice were inoculated i.n. with VSV and the globulin preparations inoculated either i.v. (0.1 ml) or intracerebrally (i.c.) (0.03 ml) several hours later. Administration of anti-interferon globulin i.v. resulted in an acceleration by approximately 3 days in the onset of symptoms and death (Fig. 4 A), whereas a single injection of this globulin i.c. resulted in a difference of one day (Fig. 4 B). Neither route of administration altered the LD_{50} of VSV. VSV injected i.p. did not cause any apparent disease in untreated or anti-interferon globulin-treated mice.

Effect of Anti-Interferon Globulin on Infection of Newborn Mice with

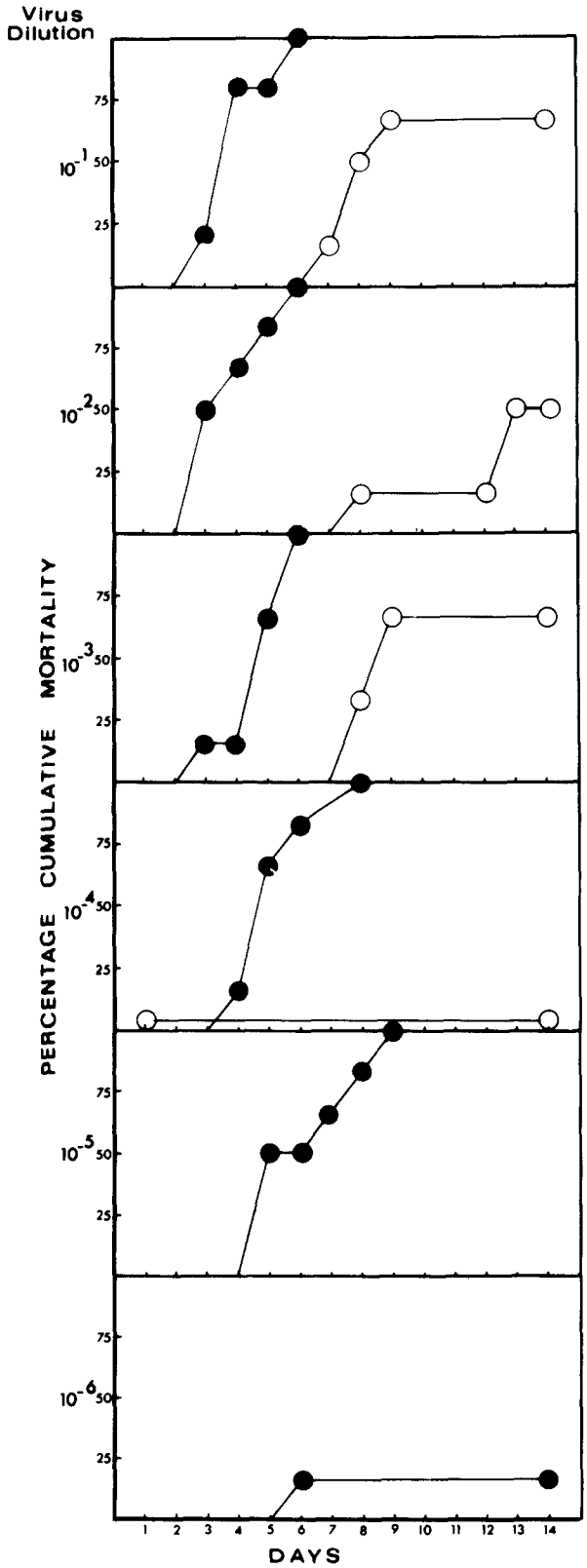

FIG. 1. 1-mo-old Swiss mice were inoculated i.p. with 10-fold dilutions of the F strain of HSV type I, and treated (i.v.) with sheep anti-mouse interferon globulin (titer, 4×10^{-5}), (●); or normal sheep globulin, (○).

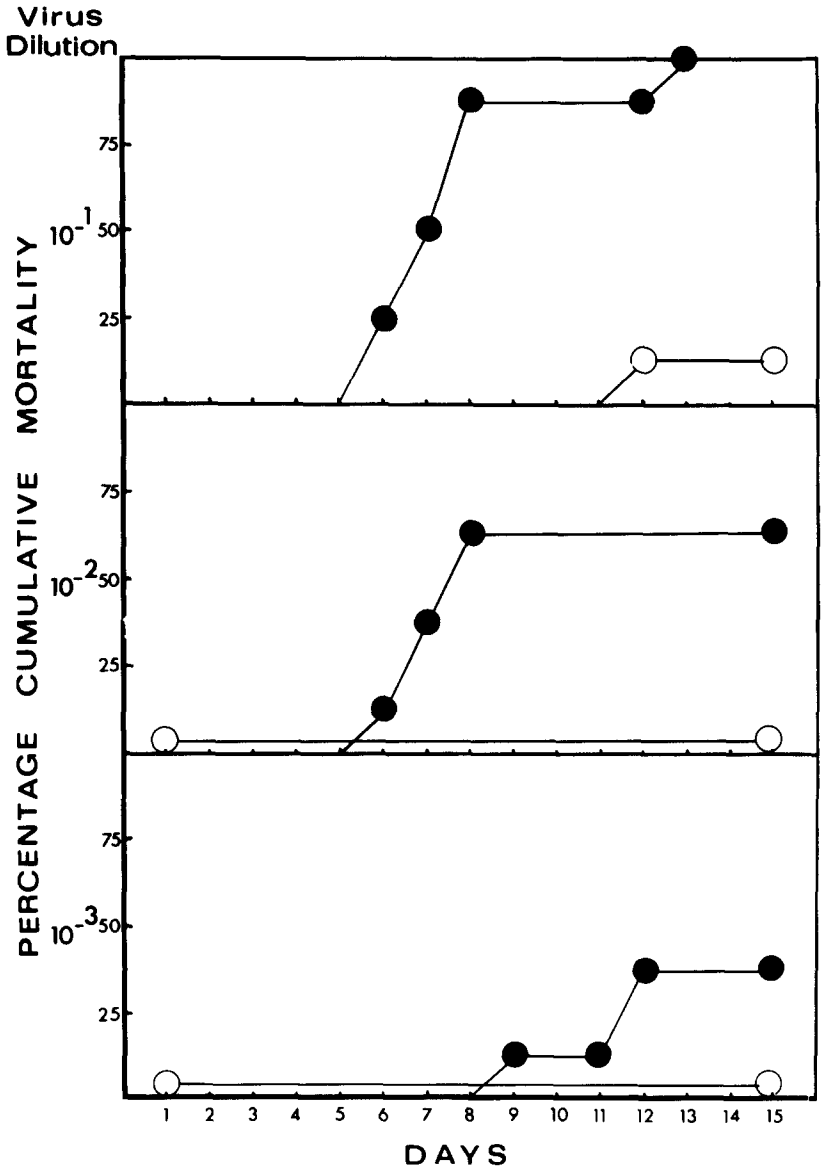

FIG. 2. 1-mo-old Swiss mice were inoculated s.c. with 10-fold dilutions of B-12 strain of HSV type I, and treated (i.v.) with sheep anti-mouse interferon globulin (titer, 4×10^{-5}), (●); or normal sheep globulin, (○).

NDV. NDV inoculated into newborn mice induces the production of interferon (titers of 1:320 to 1:960 per 10% homogenate of newborn mouse). In two experiments newborn mice were inoculated s.c. with approximately 10^6 mean tissue culture infective doses ($TCID_{50}$) of NDV and then injected s.c. with normal serum globulin or anti-interferon globulin. The mortality in the two experiments in NDV-infected newborn mice treated with normal serum globulin was 60% (19/32 mice) and 66% (20/30 mice) with a median day of death of 9 days,

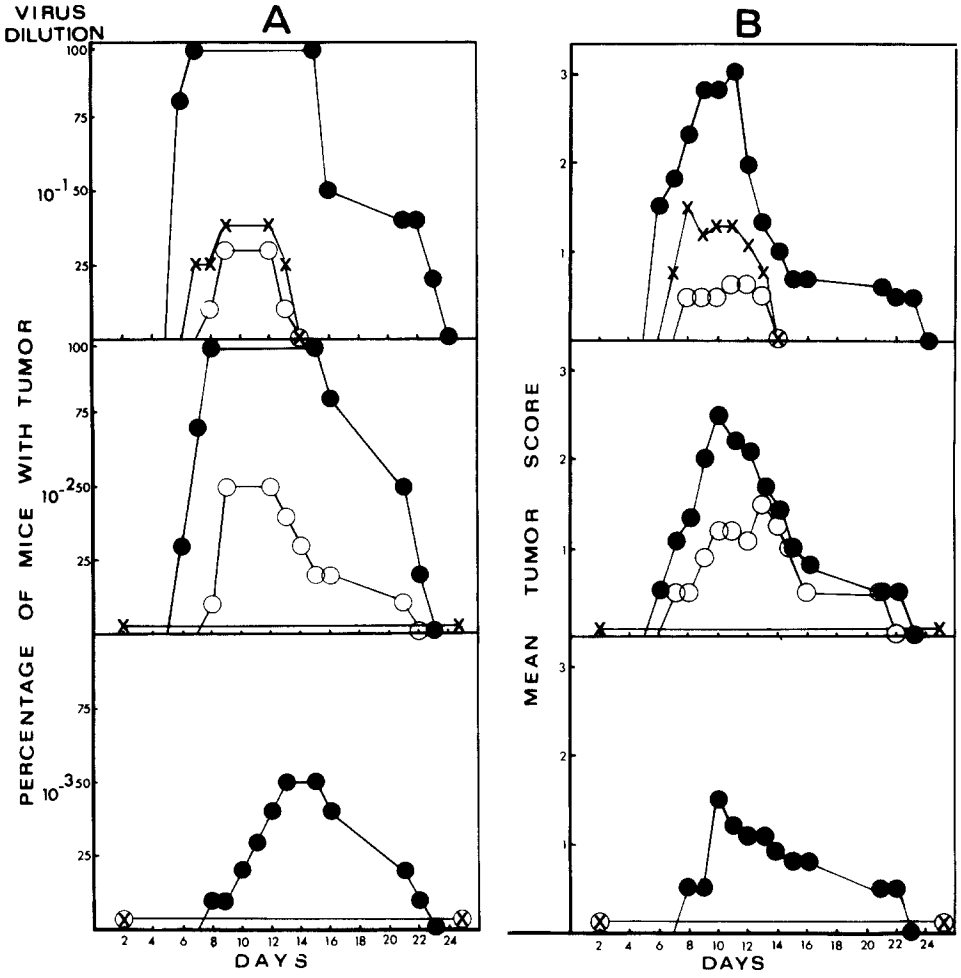

FIG. 3. 6-wk-old BALB/c mice were injected i.m. with 10-fold dilutions of MSV and treated (i.v.) with sheep anti-mouse interferon globulin (titer, 4×10^{-5}), (●); or normal sheep globulin, (○); or left untreated, (X).

whereas 100% (33/33 and 31/31 mice) of mice treated with anti-interferon globulin died with a median day of death of 5 days. Three mice in each group were sacrificed on the 5th day and the virus titers ($TCID_{50}$/0.2 ml of 10% homogenate of whole mouse as assayed on chick monolayer fibroblasts) were $10^{-4.2}$, $10^{-3.7}$, and $10^{-3.7}$ for mice treated with normal serum globulin; and $10^{-5.5}$, $10^{-5.2}$, and $10^{-5.2}$ for mice treated with anti-interferon globulin.

Effect of Anti-Interferon Globulin on Infection of Mice with Influenza A Virus. Swiss mice were infected i.n. with 0.1 ml of 10-fold dilutions of virus and approximately 4 h later were injected i.v. with 0.1 ml of anti-interferon globulin. The LD_{50} of influenza virus was $10^{-3.7}$ for untreated mice, 10^{-4} for mice injected with normal serum globulin, and $10^{-4.2}$ for mice treated with anti-interferon globulin.

In a second experiment, mice were first inoculated i.n. with virus and then

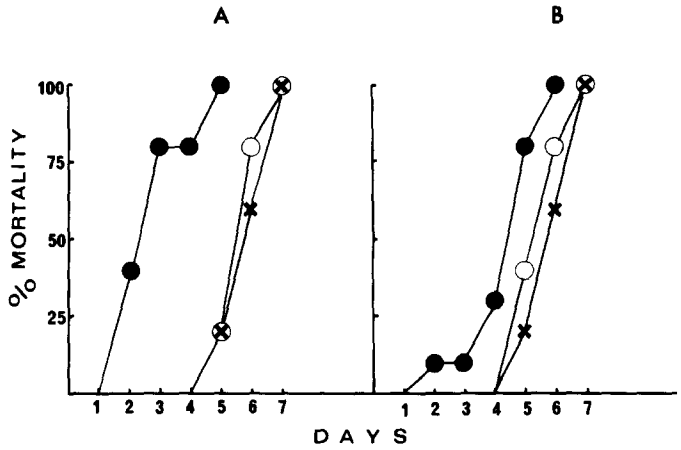

FIG. 4. 1-mo-old Swiss mice were inoculated i.n. with approximately 1,000 LD_{50} of VSV. Mice were treated i.v. (A) or i.c. (B) with sheep anti-mouse interferon globulin (titer, 4×10^{-5}), (●); normal sheep globulin, (○); or left untreated, (X).

several hours later the globulin preparations were also administered i.n. Again no significant difference was observed between the different groups.

Discussion

In the preceding paper we analyzed in some detail the effect of sheep anti-mouse interferon globulin on EMC virus infection in mice. In this article we have been concerned with its effect on HSV type I, MSV, VSV, NDV, and influenza virus infections in mice.

Inoculation of mice with anti-interferon globulin was associated with a very marked effect on the evolution of HSV disease, as shown by an accelerated appearance of signs of disease and death. Furthermore there was a several hundredfold increase in the overall LD_{50} (both by i.p. and s.c. routes). In fact, the B-12 strain of herpes simplex was only rarely lethal (5% mortality) when inoculated s.c., but proved highly lethal (100% mortality) in mice injected with anti-interferon globulin. The studies of Johnson (5, 6) indicate that the relative resistance of adult mice to extraneural inoculation of HSV is due to an age-related macrophage barrier. Although macrophages from adult mice became infected in vitro, HSV did not spread to neighboring macrophages (6). Johnson found no evidence to indicate that interferon was the factor responsible for limiting virus dissemination (6). Nevertheless, our studies with the use of anti-interferon serum suggest that the early interferon response is clearly important in the pathogenesis of herpes simplex in mice.

There have been conflicting reports as to the efficacy of exogenous interferon in delaying the appearance of tumors in weanling mice infected with MSV (7, 8). We are not aware of any reports demonstrating the presence of interferon in MSV-infected tissues and it has even been reported that infection of cells in vitro with MSV inhibits interferon action (9). However, our results suggest that MSV probably induces the synthesis of interferon initially since in anti-interferon globulin-treated mice, tumors appeared earlier, were much larger, and were

present longer than in the two groups of control mice. In addition the overall tumor-inducing dose, 50, of the virus preparation was 100-fold greater in the anti-interferon globulin-treated mice. However in this system, interferon does not play a role in tumor regression since tumors regressed in all mice despite reinjection of anti-interferon globulin.

When VSV is inoculated i.n. it is thought to reach the brain via the olfactory nerves (10). Administration of anti-interferon globulin clearly decreased the latent period before onset of symptoms and death, although the site of production of interferon is unknown, i.e. whether it occurs in nasal mucosa, olfactory nerves, or the central nervous system. Anti-interferon globulin did not influence the course of disease in influenza virus-infected mice (even when the globulin was injected i.n.). Perhaps the absence of effect in influenza infection may be related to the inability of the globulin to diffuse to the tracheobronchial epithelium, and does not necessarily imply that interferon is unimportant in the pathogenesis of influenza virus infection.

The interferon response was also demonstrated to be of importance in newborn mice as exemplified by the rapid onset of disease and increased mortality in NDV-infected mice treated with anti-interferon globulin.

In conclusion, the use of sheep anti-mouse interferon serum in several different experimental virus diseases of mice exhibiting totally dissimilar pathogeneses, [i.e. an acute systemic viral disease exemplified by HSV, a local oncogenic virus disease (MSV), and an acute viral encephalitis (VSV)] indicates the general importance of the early interferon response in host resistance to virus infection.

Summary

The effect of potent sheep anti-mouse interferon globulin was investigated in several different experimental virus diseases of mice. In anti-interferon globulin-treated mice infected intraperitoneally with herpes simplex virus (HSV) type I, the latent period was shortened, and the overall LD_{50} was increased several hundredfold compared to virus-infected control mice. When HSV was inoculated subcutaneously all anti-interferon globulin-treated mice died, whereas only 5% of virus-infected control mice died. Subsequent treatment with anti-interferon globulin of previously HSV-infected mice did not result in reactivation of HSV. Treatment of adult mice with anti-interferon globulin resulted in an earlier appearance of MSV-induced tumors, a greater number of mice bearing tumors, an increase in tumor size, and an increase in the duration of tumors. All tumors eventually regressed despite reinjection of anti-interferon globulin. Anti-interferon globulin treatment resulted in a rapid onset of disease and death in adult mice inoculated (intranasal) with VSV and in newborn mice infected with NDV. Anti-interferon globulin exerted no effect on the course of influenza virus infection of mice. We conclude that the early production of interferon is an important element in the response of the mouse to several viruses exhibiting different pathogeneses.

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