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EFFECT OF ANTI-INTERFERON SERUM OF INFLUENZA VIRUS INFECTION IN MICE

AKINORI HOSHINO[1], HIROSHI TAKENAKA[1], OSAMU MIZUKOSHI[1], JIRO IMANISHI[2,*], TSUNATARO KISHIDA[2] and MICHAEL G. TOVEY[3]

[1] *Department of Otorhinolaryngology,* [2] *Department of Microbiology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamikyoku, Kyoto, Japan and* [3] *Institut de Recherches Scientifiques sur le Cancer, Villejuif, France*

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Mice were infected by an aerosol of influenza virus Type A (0.5 LD_{50}) and subsequently treated with 4 intranasal instillations of anti-interferon antiserum over a period of 72 h. All the mice treated with antiserum died within 7 days post-infection, whilst the mice in the control groups survived. In mice that did not receive the antibody, virus titers in the lung peaked on day 3 and then decreased again. Also, interferon was detectable both in lung homogenates and serum. In mice treated with antiserum, no interferon was detectable and the virus concentrations in the lung increased until death. These results suggest that interferon produced in the respiratory tract plays an important role in the early stages of influenza virus infection.

influenza virus infection; interferon; anti-interferon serum; nebulizer; nasal instillation

INTRODUCTION

Interferon plays an important role in host defense against viral infections [16]. The capability of exogenous interferon to alter the course of virus infections has been demonstrated using numerous experimental models in animals [1,4,8,10–13,18,19]. In the case of influenza virus infection in mice, interferon is effective if given by intramuscular or intravenous injections [2,3]. The important role of endogenous interferon was demonstrated by showing that the administration of antibodies directed to interferon can cause increased mortality and lethality in various experimental virus infections. In influenza virus infections the administration of anti-interferon globulins was not found to alter the course of the disease. One reason for this may be that the globulins were given by the systemic route, whilst the interferon mechanism is operative locally in the respiratory tract. Another reason for ineffectiveness of the antibody may have been that, because relatively high virus doses were given, the lethal effect was difficult to be

* To whom requests for reprints should be addressed.

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increased. In the present study we have tested the effect of locally administered anti-interferon antibody in mice infected with non-lethal doses of influenza virus.

EXPERIMENTAL

Anti-interferon serum was obtained by immunizing a sheep by subcutaneous injections of mouse C_{243} cell interferon titering 5.2–5.9 $\log_{10}$ IU/ml, following the immunization procedure described by Gresser [5]. This serum, as well as control serum, was heated at 56°C for 30 min and absorbed on C3H mouse spleen and thymic cells sequentially at 37°C and 4°C for 30 min (1.2×10^8 cells per ml of serum). At a dilution of $10^{-5.6}$ the serum neutralized the antiviral effect of 9 units/ml of mouse L_{929} cell interferon by 50%. The protein concentration of the control serum was adjusted to that of the anti-interferon serum.

The experiments were done on 5-week-old, female, specific pathogen-free C3H/He mice (Charles River Japan, Inc.), housed in appropriate specific pathogen-free conditions. The mice were infected with virus (a mouse-adapted PR8 strain) by nebulization as described by Schulman and Kilbourne [15]. The conditions of this operation were strictly standardized. Groups of 10 mice were kept on a rotating (10 rpm) table in a circular steel cage, placed within a glass chamber. Aliquots of 10 ml of the virus suspension in phosphate-buffered saline (PBS) were nebulized in the chamber over a period of 30 min. The virus stock was titrated in LD_{50} and a standard dose of 0.5 LD_{50} was employed for the experiments.

Anti-interferon serum was administered by nasal instillation under anesthesia (50 mg/kg pentobarbital intraperitoneally). 20-μl doses of a 1 : 5 dilution of antiserum were given 1 and 24 h after the infection, and 40-μl doses were given 48 and 72 h after the infection. For survival experiments, the mice were observed for at least 15 days. For collection of blood or tissue samples, groups of 4 mice were killed at various time intervals. After blood was drawn by heart puncture, the pulmonary circulation was irrigated with 5 ml of saline. The lungs were homogenized in pool in a sterile, ice-chilled grinding vessel. The 20% homogenate in saline was clarified by centrifugation at $9\,000 \times g$ for 1 h. Virus titrations were done on MDCK cells grown in multiplates. According to the method described by Tobita [20], the cells were grown in Eagle's minimum essential medium (EMEM) supplemented with 10% foetal calf serum, 0.15% sodium bicarbonate and 0.0293% glutamine. The monolayers were thoroughly washed with PBS at 37°C and then inoculated with 0.1-ml aliquots of virus dilutions. After adsorption at 35°C in the CO_2 incubator, the cultures were re-fed with EMEM containing 0.2% sodium bicarbonate, 0.0005% trypsin, 0.22% sodium bicarbonate, 0.0293% glutamine, 0.2% glucose and a 4-fold excess of vitamins.

Interferon was titrated in a CPE inhibition assay with L_{929} cells using vesicular stomatitis virus as a challenge.

In the first type of experiment, groups of 10 influenza virus-infected mice were treated with anti-interferon serum, control serum or saline, or were left untreated. As shown in

Fig. 1, one mouse in the group treated with anti-interferon serum died 5 days post infection and the remainder of the group all died by the 7th day of infection (survival rate, 0/10). In the group treated with control serum, 6 mice died between the 6th and 8th day (survival rate, 4/10). In the untreated group 3 mice died (survival rate, 7/10). The

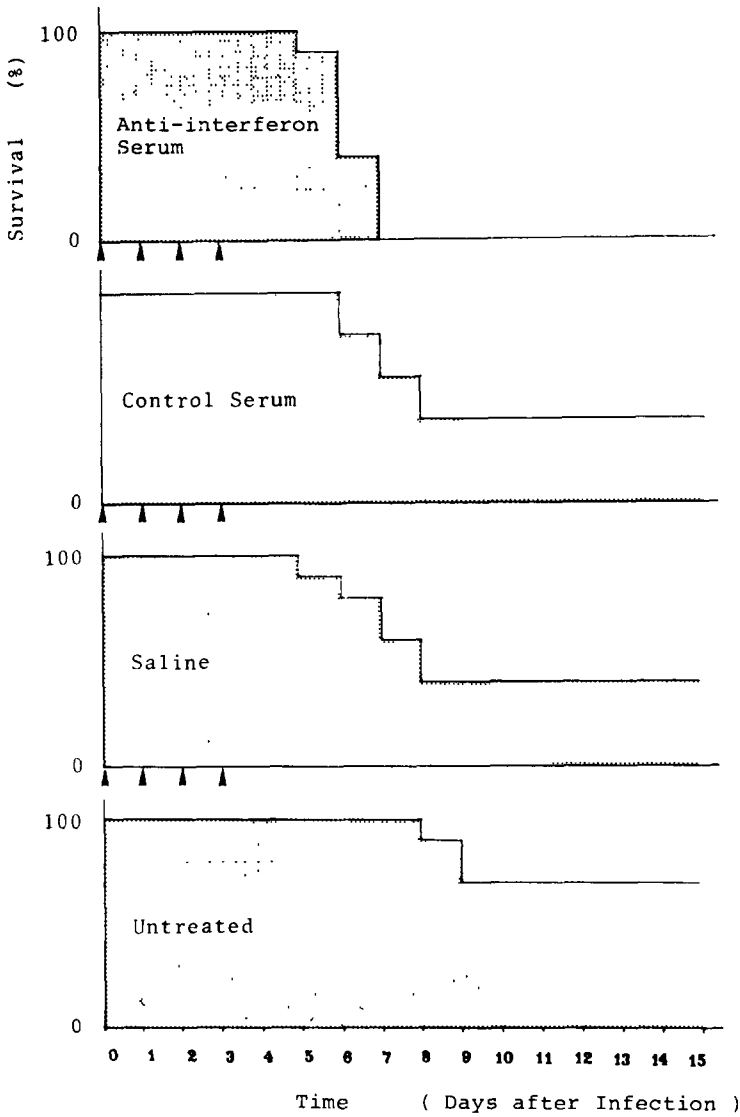

Fig. 1. Effect of anti-interferon serum on the course of influenza virus infection in mice. Mice were infected with influenza virus (0.5 LD_{50}) by nebulization and treated with intranasal doses of anti-interferon serum (arrows), control serum or saline; mice in the last group were infected but left untreated.

reason for the difference in the survival rate between the control serum or saline group and the group left untreated is not clear, though this difference was not statistically significant. Apparently mice treated with antiserum died earlier and had an increased mortality rate.

In order to reveal the mechanisms of impaired resistance against influenza virus infection in mice treated with anti-interferon serum, a study of the kinetics of virus multiplication in the lungs and the kinetics of IFN production in the lungs and the serum was undertaken. Three groups of 50 mice were infected with influenza virus. The first group was treated with anti-interferon serum after infection, as indicated before, the second group was treated with control serum and the last group was left untreated. Four mice in each group were sacrificed on the day indicated in Fig. 2, and lung and serum samples were analysed for virus and interferon content.

In the mice treated with control serum as well as in the untreated mice, the virus in the lungs increased gradually until the 3rd day ($10^{5.3}$ $TCID_{50}$ and $10^{5.2}$ $TCID_{50}$, respectively) and then rapidly decreased to become undetectable 12 days later (Fig. 2). However, in the mice treated with anti-interferon serum, the virus in the lungs showed a more rapid increase on the first 3 days, followed by a plateau phase until death of the animals.

In the mice treated with anti-interferon serum, interferon was undetectable in the lungs and sera until the 6th day of infection. However, in the mice treated with control serum and in untreated mice, interferon concentrations increased from day 2 and reached a peak on day 3 (9.3 log_2 and 9.2 log units in the lung, respectively and 5.7 log_2 and 5.5

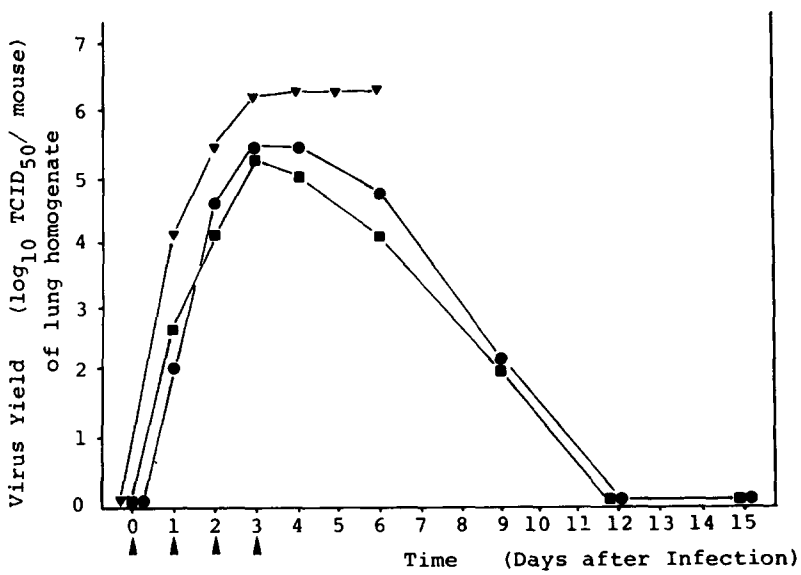

Fig. 2. Growth of influenza virus in the lungs of mice infected with influenza virus and treated with intranasal doses (arrows) of either anti-interferon serum (●) or control serum (▲). One group of mice was infected but left untreated (■). Each point is the value obtained for a pool of four mice.

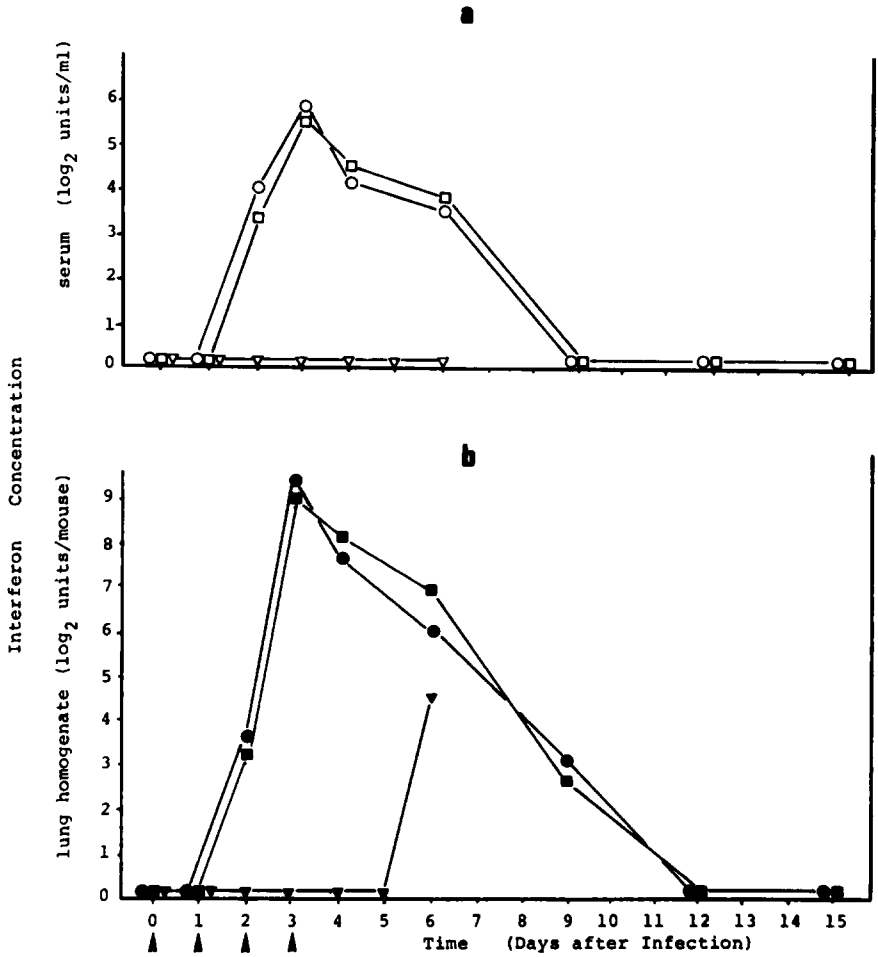

Fig. 3. Interferon in serum and lungs of mice, infected with influenza virus. Four mice in each group (○, ●. anti-interferon serum treated; △, ▲: control serum treated; □, ■: left untreated) were killed on the day indicated and interferon in lung homogenate and serum was assayed.

$\log_2$ units/ml in the serum, respectively) (Fig. 3a and b). These results suggest that treatment of the respiratory tract of influenza virus-infected mice with anti-interferon serum suppressed the formation of interferon in the lung and therefore enhanced the virus multiplication in the lung. Iwasaki et al. [7] also studied influenza virus infection in X-ray-irradiated mice that did not produce immunoglobulins or interferon. From these experiments the authors inferred that interferon played an important role in inhibiting virus propagation in the initial stages of infection, while antibodies played a more important role in later stages. By the use of intramuscular or intravenous administrations of anti-interferon antibody, Gresser et al. [5,6] could demonstrate the importance of endogenous interferon in various virus infections. In influenza virus infections, the anti-

sera had no effect. In our study topically administered anti-interferon antibody acted on influenza virus infection in much the same way as generalized anti-interferon serotherapy in systemic infections in Gresser's work. This indicates that endogenous interferon production is as important in influenza virus infections as in other virus infections.

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