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THE EFFECT OF INTERFERON ON VIREMIA IN ARBOVIRUS INFECTION

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SUMMARY

This paper presents the results of a study of viremia in mice experimentally infected with Venezuelan equine encephalomyelitis virus. It was shown that higher sensitivity to infection by this virus in suckling mice than in adult mice is reflected in higher viremia. It was established that the highest levels of viremia coincide with maximum interferon content in the blood. In absolute terms interferon production in suckling mice was lower than in adult mice, but virus titers in the blood of the suckling mice was considerably higher than in adult mice. The work discusses theories on the influence of interferon on neurovirus infection in the viremia phase.

Viremia is inherent to the majority of viral infections. Moreover, the intensity and duration of viremia varies with different viral infections. Viremia arises as a result of escape of the virus from cells and its entry into the blood stream. It has been established that viremia is the principal pathogenic symptom for arboviruses transmitted by the bite of arthropods.

The intrusion of the virus into the macroorganism stimulates a specific immunological reaction, as well as a complex of nonspecific reactions. Interferon production is one of these nonspecific reactions of the organism to viral infection [10].

Newborn mice exhibit a higher sensitivity to infection by various arboviruses than do adult mice. For this reason the question arises whether this higher sensitivity of newborn mice is not associated with their lower capacity to produce interferon. The purpose of our investigation was to study the characteristics of the development of viremia in adult and suckling mice experimentally infected with the virus of Venezuelan equine encephalomyelitis (VEE), to compute the interferon in the blood serum of the infected animals and to study the effect of interferon on viremia.

MATERIALS AND METHODS

Viruses. VEE virus passed several times through mouse brain was used to produce experimental infection in mice. Throughout the experiment passages were carried out on suckling mice, infecting them with virus cultures of 10^{-5}. The virus titer was computed on a culture of chick fibroblasts by the plaque method. Newcastle disease virus (NDV) was used to obtain control samples of interferon. Vesicular stomatitis virus (VSV), Indiana strain, served as the indicator virus in interferon titration. All viruses were obtained from the virus museum of the D. I. Ivanovskiy Virology Institute of the USSR Academy of Medical Sciences.

Infection of animals and taking of blood. Nonvarietal white mice weighing 18-20 grams and 2 or 3-day-old sucklings of nonvarietal white mice were used in the experiments. The adult animals were inoculated intracerebrally with 0.04 ml of virus suspension, and the sucklings with 0.02 ml.

Blood was taken from the adult mice by puncturing the retroorbital venous vessels, as described by Khan Shi-tsze and V. V. Pogodina [5]. In the suckling mice blood was taken from dissected vessels with a Pasteur pipet through an incision in the anterior thoracic wall.

Tissue cultures and their infection. In the viremia experiments the virus was titrated on trypsinized cells of chick embryos. Trypsinization of the 9 or 10-day-old chick embryos was carried out by the usual method. The cells were grown in 50-ml distilling flasks, into each of which 10 ml of cell suspension was introduced in a concentration of $1.5 \cdot 10^6$ cells per ml. The growth medium consisted of 0.5% lactalbumin hydrolizate in Hanks solution containing 10% normal bovine serum, 100 units of penicillin and 100 mg of streptomycin per 1 ml of medium. The flasks were incubated at 37°. In 48 hours a solid cellular monolayer was formed. Before infection the growth medium was poured off, the cells were washed once in Hanks solution, and the remaining nutrient medium was removed with a pipet. After infection (0.2 ml of infectious material was introduced into each flask) and incubation for ½ hour at 37° with subsequent removal of the unabsorbed virus, the cells were covered with an agar layer as suggested by L. G. Karpovic [2], with the exception that normal bovine serum was used to culture the group-B arboviruses instead of the

newborn-calf serum which he recommended. The plaques were counted after 48 hours.

An interwoven culture of L-cells was used for interferon titration. The L-cells were peeled from the glass through the use of 0.25% trypsin solution heated to 37°. After the trypsin had been in contact with the cells for five minutes and removed, medium in which the cells were resuspended was introduced into the flasks. The resultant cell suspension was dispersed into test tubes at the rate of 200,000 cells per 1 ml and incubated at 37°. Formation of a monolayer of the necessary quality took 24 hours.

Production and titration of interferon. The source of the experimental interferon was the blood serum of mice infected with VEE virus, taken at various times after infection. Control samples of interferon were produced in mice weighing 18-20 g which were inoculated intravenously with 1 ml of allantoid culture of NDV containing 10^7-10^8 CPE_{50}. These mice were exsanguinated after four hours, since it has been established that in that time interferon is regularly found in the blood in a sufficiently high titer [3]. The blood sera were treated with a 0.1 M solution of HCl to pH 2.0 in order to free them from virus. After maintaining the serum at 4° for 48 hours, it was alkalized with sodium bicarbonate solution to pH 7.2-7.4. A total absence of viral activity was found in the samples.

The material containing interferon was titrated in test-tube cultures of L-cells. The experiment was accompanied by a control

(serum of normal mice not containing interferon, and mouse serum obtained 4 hours after infection with NDV and containing interferon in a titer of 1:1,024). After the experiment had begun, the culture liquid was poured off and 1 ml of double dilution of interferon in medium 199 was added to each test tube. The interferon was then poured off and 100 CPE_{50} of vesicular stomatitis virus in 1 ml of medium 199 were added. This dose of virus caused complete destruction of the cellular monolayer in 48 hours. The interferon titer was taken as the maximum dilution of serum which suppressed the cytopathogenic effect of the vesicular stomatitis virus in half of the infected test tubes.

RESULTS

In the experiments on viremia in mice infected intracerebrally with a dose of virus containing $4 \cdot 10^4$ PFP/ml, the accumulation of virus in the blood was determined at various intervals -- from 2 minutes to 120 hours. At each interval a group of ten animals was examined. Table 1 shows that viremia of $7.9 \cdot 10^3$ PFP/ml is found two minutes after the suckling white mice are inoculated intracerebrally. During the first three hours after infection the concentration of virus in the blood of the suckling mice decreases very slowly and reaches $5 \cdot 10^2$ PFP/ml after three hours. Viremia then increases again, so that two distinct viremia peaks are found. The first wave of viral concentration in the blood corresponds in time with the 4th to 6th hour after infection of the suckling mice, and the second begins 18 hours after the animals are infected. Around 30-48 hours viremia in the suckling mice reaches its maximum, i.e. $2.5 \cdot 10^9$-$3 \cdot 10^{11}$ PFP/ml respectively. Around this period

Table 1

Viremia in White Mice in Experimental Venezuelan Encephalomyelitis

Time after infection	Viremia in PFP/ml -- sucklings	adult mice	Time after infection	Viremia in PFP/ml -- sucklings	adult mice
2 min.	$7.9 \cdot 10^3$	n.p.	5 hrs.	$4.2 \cdot 10^5$	50
5 "	$7.2 \cdot 10^3$	0	6 hrs.	$3 \cdot 10^6$	$1 \cdot 10^2$
10 "	$2.9 \cdot 10^3$	0	18 "	$4 \cdot 10^6$	$2.5 \cdot 10^5$
15 "	$2.1 \cdot 10^3$	0	24 "	$8 \cdot 10^7$	$1.75 \cdot 10^7$
30 "	$2 \cdot 10^3$	0	30 "	$2.5 \cdot 10^9$	n.p.
1 hr.	$1.6 \cdot 10^3$	0	48 "	$3 \cdot 10^{11}$	$1.43 \cdot 10^7$
2 hrs.	$1.3 \cdot 10^3$	0	72 "	n.p.	$1.52 \cdot 10^6$
3 hrs.	$5 \cdot 10^2$	0	96 "	n.p.	$3.7 \cdot 10^5$
4 hrs.	$2.6 \cdot 10^4$	0	120 "	n.p.	$4 \cdot 10^3$

Legend (here and in table 2): n.p. -- experiment not performed; 0 -- virus not detected.

the symptom complex of experimental encephalomyelitis is observed in the animals and they begin to die. In adult mice, as table 1 shows, no virus is detected in the blood during the first four hours. The virus begins to appear in the blood stream five hours after infection, and in six hours it is circulating in the blood in a low titer -- $1 \cdot 10^2$ PFP/ml. In 18 hours viremia reaches $2.5 \cdot 10^5$ PFP/ml and then the concentration of virus in the blood gradually increases, attaining its maximum in 24 hours -- $1.75 \cdot 10^7$ PFP/ml. This intensity of viremia lasts for 48 hours after infection. The virus titer in the blood then gradually decreases and in 72 hours reaches $1.52 \cdot 10^6$ PFP/ml, and in

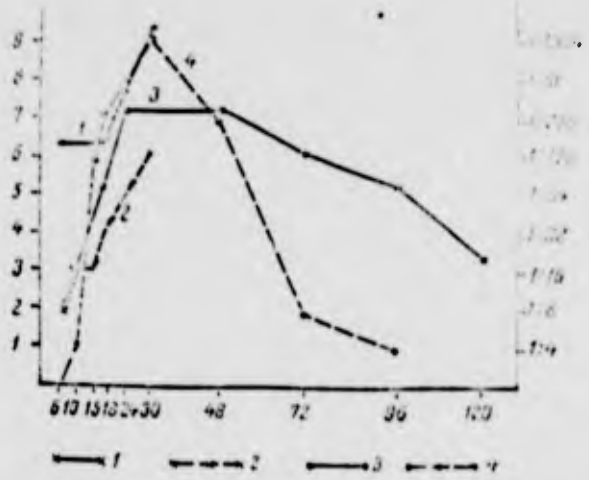
120 hours $4 \cdot 10^3$ PFP/ml. The first symptoms of infection appear in 48 hours: the mice are somewhat listless and their fur dishevelled. After 72 hours the symptoms are aggravated: listlessness increases and paresis and paralysis appear. The animals begin to die, exhibiting the clinical picture of encephalitis. The maximum life span of the adult mice was 99-120 hours from the moment of infection.

Interferon (table 2) appears in the blood serum of suckling mice 6 hours after infection and gradually increases in titer. Interferon titer in the blood serum of sucklings reaches its maximum, 1:128, 30 hours after infection. In the bodies of adult mice interferon production began 20 hours after infection and gradually increased. Thirty hours

Table 2

Interferon Production in Mice

Time of blood removal after infection	Interferon production in -- sucklings	adults
6 hrs.	1:8	0
10 "	1:16	1:4
15 "	1:16	1:128
18 "	1:32	1:256
24 "	1:64	1:512
30 "	1:128	1:1024
48 "	n.p.	1:256
72 "	n.p.	1:8
96 "	n.p.	1:4
120 "	n.p.	0



Ratio between virus titer and interferon production in the blood serum of mice. 1 -- viremia in suckling mice; 2 -- interferon titers in suckling mice; 3 -- viremia in adult mice; 4 -- interferon titers in adult mice. Abscissa -- time after infection in hours; left ordinate -- infectivity in PFP/ml; right ordinate -- interferon titer.

after infection interferon titer in the blood serum of adult mice reached 1:1024 and then began to decrease. Only residual amounts of interferon were found in the adult mice 96 hours after infection, and after 120 hours interferon could no longer be detected in the blood serum. The ratio between the interferon and virus titers detected in the blood serum of the infected animals is shown in the figure.

DISCUSSION

The degree of susceptibility of an animal to viral infection depends, of course, on the age of the host. Young animals are regularly more susceptible than adult animals to arboviruses. Opinions differ regarding the mechanisms which cause animals to have different age susceptibility

to viral infections. Since its discovery, interferon has been identified as one of the factors causing resistance [9]. Our experiments, conducted on animals of varying ages, revealed a marked difference in interferon production by animals of different age groups. In determining the dynamics of interferon content in the blood serum of infected animals, we observed a correlation with virus propagation. As the figure shows, virus content in the blood of infected sucklings reached a maximum in 30 hours, and in adults in 24-48 hours, i.e. at approximately the same period of time after infection, but viremia in these animals was of different intensities. Higher viremia is developed in the sucklings. Moreover, maximum interferon titers in suckling and adult mice are also detected at the same time (see figure), i.e. thirty hours after infection, but interferon titers in the adult animals were eight times higher than in the sucklings.

It should be noted that viremia does not affect the clinical picture of the infection. The clinical symptoms of illness are absent when the level of virus in the animals' blood is high. At the same time quite high interferon titers are detected in the blood serum of these animals. In the case of suckling mice (see figure) we see that interferon does not confine the viremia, which increases. The further course of virus and interferon in the bodies of the sucklings could not be traced, since the animals died with the high viremia and the considerable titers of interferon detectable in the blood serum.

Another picture was observed in the adult mice. The first symptoms of illness began to appear when the virus titers in the blood were still

not high, but the interferon content had already begun to decrease, i.e. 48 hours after infection (see figure). The illness manifests a clear clinical picture in 72 hours, when viremia is already displaying a clear tendency to decrease, and interferon is detectable in low titers. The violent development of infection and early death of the suckling mice may apparently be associated with less intensive production of interferon in the bodies of these animals than in adult mice. Moreover, the low sensitivity of suckling mice to the antiviral action of interferon has been established [1].

With regard to the adult mice, it may be assumed that interferon suppresses to some extent virus reproduction by sensitive cells and for this reason the outflow of virus into the blood is lower.

The cause of the fatal outcome of infection in adult mice may include loss of ability by the organism to manufacture interferon in that period of infection when antibodies have not yet appeared or when they are circulating in low titers. These data agree with the work of Baron et al. [6, 7, 8] and V. D. Solovyev et al. [4].

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