PRODUCTION OF INTERFERON AND OF VIRUS-NEUTRALIZING ANTIBODIES IN ADULT MICE INFECTED WITH ENTEROVIRUSES IN THE NEONATAL PERIOD

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Newborn albino mice were infected with three strains of enteroviruses and reinfected 6 weeks later with homologous or heterologous strains. Strains with relatively low pathogenicity for newborn mice were shown to lead to the formation of specific immunologic memory. If virus of high pathogenicity was used, prolonged production of specific antiviral antibodies and absence of a marked secondary immune response were observed in the surviving animals. Ability to produce interferon was independent of the pathogenicity of the strains for newborn animals and of the presence of antibodies against them.

KEY WORDS: enteroviruses; interferon; neutralizing antibodies; immunologic memory.

The problem of formation of immunoreactivity in the early postnatal period is one of the most important in modern immunology [3, 4]. Accordingly the question of immunologic reactivity of newborn animals to viruses is of considerable interest. It is important to establish whether a specific immunologic memory for virus antigens arises in infected newborn animals and to study the effectiveness of interferon production in response to virus inducers in adult individuals infected in the neonatal period. In the investigation described below these problems were studied in experiments on mice infected with enteroviruses immediately after birth.

EXPERIMENTAL METHOD

Viruses. Strains of enteroviruses isolated in Estonia from patients and healthy subjects were used: ECHO 11 strain No. 484, Coxsackie B5 strain No. 48, and Coxsackie B5 clone L_1, isolated by the plaque method on a primary trypsinized culture of human embryonic skin and muscle tissue. All three viruses were subcultured twice in cells of the transplantable MK (monkey kidney) line.

Animals. Altogether 175 newborn noninbred albino mice were used. Before infection, half the animals of each litter were suckled by a different mother.

Scheme of Experiment. The viruses were injected intraperitoneally in 0.05 ml culture fluid on the 1st or 2nd day after birth of the mice. The same volume of fluid, taken from an uninfected MK culture, was injected into the control animals. Reproduction of the viruses in the mice was demonstrated by their isolation from the brain, heart, and spleen on HEp-2 cells and by the results of the second passage in newborn mice. Six weeks after infection the surviving animals were divided into three groups. The mice of group 1 received the same (homologous) virus, those of group 2 received a different (heterologous) virus, and those of group 3 the culture fluid taken from uninfected MK cells. Blood was taken after 18-20 h from half of the animals of each group for estimation of serum interferon. The remaining animals were killed 2 weeks later for determination of antiviral antibodies. The suitability of the choice of these times was confirmed by data of Reyes and Lerner [5].

Interferon was titrated on a culture of transplantable mouse cells of the L line by the method of delay of the cytopathic effect induced by 100 TCD_{50} of vesicular stomatitis virus. Antibodies were titrated in the neutralization test against 100 TCD_{50} of the corresponding virus on a culture of transplantable HEp-2 or RH cells. Type-specific Coxsackie B5 and ECHO 11 sera were used as the controls.

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TABLE 1. Interferon Titers (in $\log_2$) in Mice 18-20 h after Reinoculation with Homologous or Heterologous Strains of Enteroviruses

Material inoculated	Titer of interferon induced homologous strain	Material inoculated		Titer of interferon induced by heterologous strain
		first injection	second injection	
Strain No. 48	4,3±0,3	Strain No. 484	Strain No. 48	4,7±0,2
		Clone L_1	Strain No. 48	4,7±0,3
		C. f.	Strain No. 48	4,0±0
Clone	3,5±0,7	Strain No. 48	Clone L_1	4,5±0,7
Strain No. 484	5,5±1,2			

Legend. 1. Geometric mean of interferon titers calculated on the basis of titration of samples from 3-8 mice. 2. Interferon titers in adult animals 18-20 h after infection with strains Nos. 48 and 484 were 5.0 ± 0.5. 3. Here and in Table 2, C.f. indicates culture fluid taken from continuous culture of MK cells.

TABLE 2. Titers of Antibodies (in $\log_2$) of Mice 2 weeks after Reinoculation with Homologous or Heterologous Strains of Enteroviruses

Material inoculated		Titers of antibodies to:		
first injection	second injection	strain No. 484	clone L_1	strain No. 48
Strain No. 484	Strain No. 48	<2		4,7±0,3
Strain No. 484	Strain No. 484	7,0±0		
C. f.	Strain No. 484	2,5±0,2		
Clone L_1	Strain No. 48		<2	5,0±1,0
Clone L_1	Clone L_1		6,8±0,7	
Strain No. 48	C. f.			3,0±0,2
Strain No. 48	Clone L_1			4,0±0,8
Strain No. 48	Strain No. 48			5,2±0,3
C. f.	Strain No. 48			7,7±0,3
C. f.	C. f.	<2	<2	<2

Legend. Geometric mean of antibody titers calculated on the basis of titration of samples from 3-8 mice.

EXPERIMENTAL RESULTS

Of 33 newborn mice infected with strain No. 484 of ECHO 11 virus in a dose of $5 \cdot 10^5$ TCD_{50}, five died, mostly during the first 4 days. Virus was isolated from the brain of the dying and surviving animals in most cases before the 4th day in a titer not exceeding 10^{-1} TCD_{50}.

Of the 31 animals infected with clone L_1 of Coxsackie B5 virus in a dose of $5 \cdot 10^{3.5}$ TCD_{50} eight died during the first 4-6 days. The virus was isolated in only one case in a titer of 10^{-3} TCD_{50}.

Strain No. 48 of Coxsackie B5 virus proved to be highly pathogenic for the newborn albino mice: on infection with 500 TCD_{50}, 20 of the 25 animals died during the first week, whereas after infection with 50 TCD_{50}, 27 of 54 mice died within 2 weeks. The virus was isolated from the brain between the 1st and 8th days in a titer of 10^{-1}-10^{-2} TCD_{50}. Characteristically the disease followed a sluggish course with paralyses of the hind limbs, and growth of some of the surviving mice was delayed.

Of the 16 control newborn mice receiving an intraperitoneal injection of the culture fluid taken from uninfected MK cells, only one mouse died.

It can be concluded from the data on isolation of the viruses from the organs of the dying and outwardly healthy animals as a result of the second passage in 16 newborn mice that all the enteroviruses studied multiplied in the animals, even when no outward signs of infection were present. The presence of infection without clear clinical manifestations in newborn albino mice infected with ECHO 1 and ECHO 12 viruses has also been observed by Koroleva et al. [2]. Of the enteroviruses used in the present experiments, strain No. 48 reproduced most intensively in newborn mice.

As Table 1 shows, interferon production in the mice was independent of whether homologous or heterologous virus was used for reinoculation: the difference between the interferon titers was not statistically significant.

According to the data of Table 2, in adult mice inoculated in the perinatal period with strain No. 484 and clone L_1, no antibodies against these viruses were detected. Reinfection of the adult mice with strain No. 484

caused a sharp increase in the antibody titer. The titer was significantly higher than in control animals receiving a single injection of that virus at the age of 6 weeks. Reinfection with clone L_1 also was accompanied by a marked increase in antibody synthesis against this virus. It can accordingly be concluded that infection of newborn mice with enteroviruses of the above-mentioned strains is accompanied by the formation of specific immunological memory.

A different situation was observed after infection of the newborn mice with strain No. 48. Eight weeks later antibodies could be detected in the surviving mice against the virus, but the titer was lower than in mice of the control group, receiving a single injection of this virus at the age of 6 weeks. A second injection of strain No. 48 into mice infected with this virus in the perinatal period caused no increase in synthesis of specific antiviral antibodies. The absence of a secondary response in this case may be due to the fact that at the time of reinfection the mice were still continuing to produce antibodies against strain No. 48.

The following conclusion can be drawn from these results. If the virus has relatively low pathogenicity for newborn animals (such as strain No. 484 and clone L_1, for example) it leads to the formation of specific immunologic memory, whereas the productive phase of the primary immune response is evidently of short duration. In the case of high pathogenicity of the virus for newborn animals (strain No. 48), with the result that it propagates intensively, the surviving animals respond to injection of this virus by prolonged production of the specific antibodies. The immune response to reinfection with the virus is weaker in this case than to a single injection of the virus into adult animals.

Ability to produce interferon in adult mice, it must be noted, is independent of the pathogenicity of the corresponding strain for newborn animals and also of the presence of specific immunologic memory for virus antigens or antibodies against them in the mice. The fact that interferon production is independent of the biosynthesis of antibodies against the virus used as inducer has also been demonstrated in experiments with influenza virus [1].

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