

EFFICIENCY TESTING OF PASSIVE IMMUNIZATION AGAINST VENEZUELAN EQUINE ENCEPHALOMYELITIS IN MICE

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Summary. — Heterogeneous gamma-globulin from immune rabbits, given in a dose of 0.08 g per kg body-weight, was very efficient in protecting mice from death following respiratory or subcutaneous infection with Venezuelan equine encephalomyelitis (VEE) virus. Effective protection was obtained when gamma-globulin was administered before or within 24 hours after infection (p.i.). Serological examination of surviving animals suggested that in a part of the mice the infection developed in the presence of gamma-globulin, which resulted in active immunization. In unprotected animals, the infectious and lethal doses were equal and there was no survival of immune animals.

The effects of prophylactic therapy by passive immunization have been described for experimental infections caused by several arboviruses (Sabin, 1936; Šimková, 1959; Hanzal, 1959; Berge *et al.*, 1961; Daneš *et al.*, 1962; Hsieh *et al.*, 1963; etc.).

In the present work we were interested to see whether gamma-globulin administration would prove effective in the case of an infection with a very short incubation period and rapid penetration of virus through the blood-brain barrier, like the respiratory infection of mice with VEE virus (Hrušková *et al.*, 1969b).

The VEE virus strain used in the form of mouse brain suspension was the same as previously employed (Hrušková *et al.*, 1969a,b). Mice of strain H, weighing 16 g, were used.

Gamma-globulin from the plasma of hyperimmune rabbits was prepared by Dr. F. Škvařil, Research Institute of Immunology, Prague. It was administered to the mice in amounts of 0.08 g/kg body-weight, given in 0.5 ml volumes subcutaneously. The neutralization index of the solution employed was $10^{4.7}$.

The inhalation infection was carried out as described by Hrušková *et al.* (1969a). The doses inhaled were calculated from aerosol concentration and expressed in mouse inhalation LD_{50} values. In subcutaneous inoculation, the inoculum volume was 0.1 ml.

Fifteen groups of 20 mice each were used. Five groups each were infected by inhalation with 1, 10 and 63 LD_{50} of VEE virus, respectively. With each inoculum, 4 groups were given gamma-globulin at intervals indicated in Table 1 and one group remained untreated as control.

The results summarized in Table 1 showed that the amount of gamma-globulin employed offered effective protection to the mice infected with any of the 3 doses of virus used in the form of aerosol. The results also indicate that the protective effect was not lowered even when gamma-globulin was administered as late as 24 hours p.i.

Table 1. Protective effect of anti-VEE gamma-globulin in mice infected by inhalation of VEE virus

| Gamma globulin given | Dose of VEE virus inhaled | | |
|----------------------|---------------------------|---------------------|---------------------|
| | 1 LD ₅₀ | 10 LD ₅₀ | 63 LD ₅₀ |
| Before inf. | 20/20 | 19/20 | 16/20 |
| 6 hr p.i. | 20/20 | 18/20 | 18/20 |
| 12 hr p.i. | 18/20 | 19/20 | 17/20 |
| 24 hr p.i. | 20/20 | 15/20 | 16/20 |
| Untreated controls | 10/20 | 1/20 | 1/20 |

Numerator: number of mice surviving 3 weeks p.i.; denominator: number of mice per group. Death of mice occurred mainly 5 and 6 days p.i., irrespective of the kind of treatment.

The experiment on subcutaneous challenge was simplified. VEE virus was administered in doses of 1, 10 and 100 guinea pig intracerebral LD₅₀ to groups of mice, parts of which either were given gamma-globulin (0.08 g/kg body-weight) before infection or 24 hours p.i., or remained untreated. The results are presented in Table 2.

Table 2. Protective effect of anti-VEE gamma-globulin in mice subcutaneously infected with VEE virus

| Gamma globulin given | Dose of VEE virus inoculated | | |
|----------------------|------------------------------|---------------------|----------------------|
| | 1 LD ₅₀ | 10 LD ₅₀ | 100 LD ₅₀ |
| Before inf. | 10/10 | 10/10 | 10/10 |
| 24 hr p.i. | 8/10 | 8/10 | 10/10 |
| Untreated controls | 15/20 | 2/20 | 1/20 |

Numerator and denominator as in Table 1.

To obtain information as to whether the amount of gamma-globulin administered protected the mice only from a lethal infection or whether it prevented the development of infection and the immune response, 5 weeks p.i. we examined in neutralization tests in chick embryo cells the sera from a part of mice which survived. Sera from 3 animals of a given group were pooled; 56 serum pools were examined. Sera from animals which received no gamma-globulin contained no detectable amounts of antibody (starting dilution of 1 : 5). Sixteen of 45 serum samples from animals infected by inhalation and given gamma-globulin at any of the intervals mentioned contained neutralizing antibody in titres from 1 : 5 to 1 : 80. The occurrence of antibody was unaffected either by the virus dosis or the time of gamma-globulin administration.

In a control group of 63 mice given gamma-globulin we found that their serum contained neutralizing antibody in titres of 1 : 40, 1 : 20—1 : 40 and 1 : 5 on the 1st, 7th and 14th day after the injection of gamma-globulin,

respectively. No antibody was found in 11 serum pools tested 5 weeks after the injection of gamma-globulin.

Our results suggest that passive immunization might prove effective even in protecting non-vaccinated laboratory personnel from accidental infection, including the penetration of virus into the respiratory tract.

The high protective effect of gamma-globulin in case of respiratory infection of mice with VEE virus seems to offer indirect evidence that the virus penetrates from the nasal mucosa into target organs, in the case of mice to the central nervous system, by the blood or lymphatic routes rather than via the fila olfactoria. As yet there is no direct evidence which of these ways prevails.

The results of serological examination indicate that, under the influence of passive immunization, active immunization after VEE virus infection may occur in some individuals, like in the case of subgroup B arbovirus infections.

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