A Model in Mice for the Study of the Early Death Phenomenon after Vaccination and Challenge with Rabies Virus

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SUMMARY

The reactions of mice were studied for a period of 10 days after their vaccination with an inactivated rabies vaccine. The kinetics of their resistance to an intracerebral challenge and neutralizing antibody activity of their serum were determined daily. Protection began on the fourth day after vaccination and was approximately correlated with virus-neutralizing antibody titres from the sixth to the tenth day. However, in vaccinated mice still unprotected, death following the intracerebral challenge occurred earlier than in unvaccinated control mice. This 'early death phenomenon' is proposed as a model for immuno-pathological study of reaction to rabies vaccines.

In an investigation of the early immune response to rabies vaccination and challenge, previous observations by Bijlenga & Joubert (1977), Sikes et al. (1971) and Wiktor et al. (1976) that vaccinated and infected animals may succumb much earlier than unvaccinated control animals have been confirmed: a practical model for the study of this 'early death phenomenon' is investigated here.

Six- to 8-week-old specific pathogen-free Swiss outbred female OF1 mice (IFFA CREDO St Germain/L'Arbresle, France) were used in this study. A French national reference preparation (FNRP) of rabies vaccine was used. This preparation was made from suckling mouse brain infected with CVS virus and inactivated by β-propiolactone (1/4000). A comparison of results of the NIH potency test (Atanasiu, 1974) on the FNRP and the WHO Second International reference rabies vaccine preparation (used as standard) showed the FNRP to have an antigenic value of 1. A placebo preparation was used which had the same composition as the FNRP but was made from suckling mouse brain that had not been infected with rabies virus. Animals received a single i.p. inoculation of 0.5 ml vaccine or placebo. Mice were infected by i.c. inoculation of 30 LD50/0.03 ml standard rabies challenge virus (CVS) of mouse brain origin (Blancou et al. 1979). Animals were kept under observation for 28 days after challenge. Levels of virus-neutralizing antibody (VNA) were determined by the mouse inoculation technique (Atanasiu, 1974) in separate groups of animals. They are expressed in international units (iu) by reference to the VNA levels of the standard WHO rabies antibody preparation titrated at the same time.

After inoculation with the FNRP or placebo preparation, the appearance of VNA and resistance to rabies challenge were evaluated in groups of ten mice daily for 10 days. All unvaccinated mice died after 7 to 10 days. No VNA or resistance was noted in animals challenged on day 0, 1, 2 or 3 after vaccination (Fig. 1). A low level of VNA (0.25 iu) was detected on day 4 after vaccination and coincided with the survival of 50% of vaccinated mice challenged at this time. Antibody level and resistance to challenge increased gradually during the next 4 days, reaching 65 iu and 90 to 100% protection on day 8 after vaccination. No VNA was detected in mice given the placebo inoculation and none of these control animals resisted the CVS challenge (data not shown). However, it was noted that some FNRP-vaccinated mice challenged on day 2 or 3 after vaccination died earlier than animals which had received the placebo inoculation and been challenged similarly.
To confirm this observation, groups of 50 mice were given a CVS challenge 3 days after inoculation with either the FNRP or the placebo preparation. As in the previous experiment, none of the animals in either of these two groups survived challenge and all had died after 11 days. However, deaths in the vaccinated group again occurred much earlier than in the group that had received the placebo preparation. Daily cumulative percentages of mortalities in each experimental group are represented in Fig. 2. On day 6 after challenge, 42% of the mice in the FNRP group were already dead, whereas all had survived in the placebo group; on day 7, 80% of the FNRP-vaccinated mice were dead and 98% of the placebo group had survived. Mortality was still significantly higher in FNRP than in the control group on days 8 and 9 after challenge, but by days 10 and 11, the number of animals that had died in the two groups was similar.

When Pasteur type rabies vaccine, which contains live virus, was used, it was suggested that anti-rabies treatment could sometimes bring about death from rabies after a shorter incubation period than in untreated persons; the proposed explanation for this was that the live, fixed virus inhibited defence mechanisms and accelerated the passage of street virus (Proca & Bobes, 1940). This ‘early death phenomenon’ was reported in experiments involving the use of inactivated vaccine in monkeys (Sikes et al. 1971; Wiktor et al. 1976) and mice (Baer & Cleary, 1972; Bijlenga & Joubert, 1977) and may have occurred in some cases after the post-exposure treatment of humans with inactivated rabies vaccine prepared from suckling mouse brain (Bijlenga & Joubert, 1977). These observations implicate immunological mechanisms stimulated by dead antigen rather than by live virus present in the vaccine (Kaplan et al. 1975; Wiktor et al. 1977).
Until now, further investigation of this problem has been hampered by the lack of a convenient animal model. However, the model described here provides a useful and economical tool for the investigation of this interesting phenomenon.

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