Immunogenicity of a Subviral Herpes simplex Type 1 Preparation: Reduction of Recurrent Disease in Mice

Brief Report

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Summary

Immunization of mice with a relatively small dose of subviral herpes simplex type 1 vaccine used prior to homotypic virus infection reduced the frequency of recurrent disease. However, the same vaccine dose administered after virus infection did not result in significant reduction of recurrences.

In preceding papers we reported on the preparation of an experimental subviral herpes simplex (HSV) type 1 vaccine and the development of a chromium release inhibition test (CRIT) for determining the antigenic potency of such preparation. We also showed that this vaccine is capable of conferring immunity on mice against intraperitoneal challenge with homotypic virus and intradermal challenge with homotypic or heterotypic virus. The vaccine also markedly reduced the establishment of latent infection of sensory ganglia in rabbits. In general, there was excellent agreement between the immunogenicity of the vaccine in vivo and the results of CRIT, indicating that this test really measures the content of protective antigens.

In the present series of experiments we proceeded two steps further. We investigated whether immunization prior to infection reduces recurrent disease, and whether the recurrences can be prevented by intervening with the vaccine after latent infection has been established.

White mice (strain H) 4—5 weeks old weighing 9—11 g and HSV type 1, strain KAD, were used in the present experiments. The virus (3 × 10⁵ PFU unless stated otherwise) was inoculated on damaged skin of the right pinna as described by ŠLICHTOVÁ et al. Mice which developed erythema without necrosis and then recovered were selected for recurrence induction. At four weeks after infection these mice were mechanically irritated with celophane tape; the tape was applied and removed six times. The final stripping was followed by nine
superficial punctures with a metal needle. The efficiency of the procedure as observed in four repeated experiments is shown in Table 1. It can be seen that recurrent disease was induced in 26—33 per cent of the animals; this figure is close to that reported by Hill (1).

In subsequent experiments groups of mice were immunized with 40 CRIT units of the vaccine (1 CRIT unit corresponds to the least amount of antigen causing 50 per cent inhibition of 51Cr release (3)) in complete Freund adjuvant. Control mice were injected with the adjuvant alone. Three weeks after the injection all mice were infected with KAD virus and four weeks later the induction of recurrence was attempted in mice which had fully recovered from the disease (i.e. were without evident necrosis of pinna). The results are shown in Table 2.

In the first experiment the mice were challenged with $3 \times 10^2$ PFU. It can be seen that a markedly lesser number of immunized than of control mice developed disease and died. Since the symptoms in the immunized mice were generally less severe than in controls, a higher proportion of immunized diseased mice was available for recurrence induction than in the case of control animals. However the clinical findings in both the immunized and control animals ultimately used for recurrence induction were comparable. In terms of frequency the induction was about twice as effective in control than in immunized mice.

In the second experiment, four different virus doses, $3 \times 10^2$, $3 \times 10^3$, $3 \times 10^4$, and $3 \times 10^5$ PFU, were used for the infection of immunized mice, while control mice were infected with $3 \times 10^2$ PFU. Under these conditions the disease was as frequent in immunized as in control animals; but it was much milder in the former. This was reflected by a low death rate among the immunized animals and also by the fact that more of these were available for the recurrence induction test. Again, recurrences were about twice as frequent among controls as among previously immunized mice. These differences were statistically significant ($p < 0.01$).

In two subsequent experiments we followed the effects of vaccine administration after latent infection with HSV had been established. Mice were infected with $3 \times 10^2$ PFU of virus. Animals that developed erythema and then fully recovered were divided into two groups. Four weeks after the infection one group received 40 CRIT units of the vaccine in complete Freund adjuvant, while the other animals were inoculated with adjuvant only. Three weeks later the induction of recurrent disease was attempted. The results are summarized in Table 3. Although in both experiments the immunized animals developed recurrent disease somewhat less frequently than non-immunized ones, the differences were not statistically significant.

The present results indicate that, in mice, immunization with the subviral herpes simplex vaccine used prior to virus infection is capable of reducing the frequency of recurrent disease. This effect was achieved with the relatively low vaccine dose of 40 CRIT units; as reported elsewhere (4) one HSV-infected LEP 1200 ml bottle culture routinely yields 1000 to 2000 CRIT units. This corroborates the previous demonstration that the administration of this type of vaccine limits the rate of infection of sensory ganglia in rabbits (5). On the other hand, no significant reduction in induced recurrences was seen in the present experiments when the same dose of vaccine had been administrated after virus infection. It is