A Mouse Model for Post-Exposure Rabies Prophylaxis: 
The Comparative Efficacy of Two Vaccines and of Antiserum Administration

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SUMMARY

Mice challenged with rabies virus were vaccinated 24 h later with one of two types of rabies vaccine. Vaccine prepared from a BHK cell substrate resulted in the production of serum interferon and neutralizing antibody, whereas vaccine prepared from a human diploid cell substrate gave rise to neutralizing antibody but no interferon. Only the first vaccine was effective in reducing mortality.

Various combinations of mouse hyperimmune antiserum, purified IgG, complement and RNase were administered to other groups of mice challenged with rabies, but these had no significant effect on their survival.

INTRODUCTION

Our previous study on the pathogenesis and treatment of rabies in mice (Baer & Cleary, 1972) was especially designed to mimic the human disease and its prophylaxis: the incubation periods after infection with a bobcat strain of rabies virus varied from 17 to 120 days, the group mortality was approx. 50% and treatment was initiated 24 h after challenge. The only regimens that significantly reduced the number of deaths were the administration of (1) antiserum and daily doses of vaccine (the schedule recommended for man by the World Health Organization, 1973), or (2) a single dose of a highly potent vaccine produced from baby hamster kidney (BHK) cells (Wiktor et al. 1969). In the first case, the protective effect could be correlated with the neutralizing antibodies present from the first day of treatment, while in the second, the crucial factors appeared to be the early presence of interferon followed by the rapid rise of neutralizing antibody. We also noted that the hyperimmune serum administered after inoculation of the challenge virus did not reduce the mortality but merely prolonged the incubation period. The present studies have examined the minimum potency of the BHK vaccine needed to protect mice after exposure and the efficacy of a potent commercial human diploid vaccine. We have also examined the in vivo effectiveness of a series of possible treatments which have been shown to be virucidal in vitro, including (a) administering a combination of antiserum, complement and RNase, shown to be virolytic against AKR leukaemia virus in tissue culture (Oroszlan & Gilden, 1970), and (b) administering IgG and then anti-IgG, a combination which is a much more potent reducer of vaccinia plaques (in tissue culture) than IgG alone (Weeke-Lüttmann, 1970).
METHODS

Viruses. A $10^{-2}$ dilution of the bobcat virus previously described (Baer & Cleary, 1972) was used. The second virus came from the salivary glands of a rabid grey fox. Both virus isolates were supplied by Mrs Dora F. Woodall, Arizona State Department of Health.

Mice. Groups of 50 4-week-old female white Swiss ICR strain mice were used to evaluate treatments. Weaning mice were used for intracerebral virus titration.

Vaccines. BHK (baby hamster kidney) vaccine was prepared by growing the ERA strain of rabies vaccine virus (Abelseth, 1964) on BHK cells (MacPherson & Stoker, 1962); the harvest titre was $10^{6.5}$ mouse intracerebral LD$_{50}$ (MICLD$_{50}$)/0.03 ml. but the virus was concentrated to a titre of $10^{8.5}$ MICLD$_{50}$/0.03 ml by the method described by Mikhailovsky, Tsiang & Atanasiu (1971) and Obijeski et al. (1974). After inactivation with a 1:4000 dilution of $\beta$-propiolactone, the vaccine had an antigenic value (AV) of 100 when tested against the National Institutes of Health (NIH) reference vaccine, batch 178.

HDCS (human diploid cell) vaccine was prepared on human diploid cells (lot S0201) with the PM strain of virus and had an antigenic value of 5 when tested against NIH reference vaccine, batch 178.

Ribonuclease (beef pancreas RNase) (Worthington Biochemical Corporation, Freehold, N.J.) was used at a concentration of 10 $\mu$g/ml.

Complement (C'). Guinea pig whole complement (Cordis Laboratories, Miami, Fla.) had, when reconstituted, a potency of 260 complement haemolytic (CH)50 units/ml.

Hyperimmune serum. Rabies hyperimmune serum was obtained from 5 to 6-month-old mice injected intraperitoneally with commercial ERA vaccine on eight occasions. It contained 35 international units/ml when tested by the rapid fluorescent focus inhibition test (RFFIT; Smith, Yager & Baer, 1973) against NIH reference serum lot 4 (10/7/71).

Purified mouse rabies immunoglobulin (IgG). The globulin was partially purified from the mouse serum described above. IgM was removed from the serum by overnight rate zonal centrifugation in a Beckman P-15 titanium rotor on a 10 to 35% sucrose gradient (Fischer & Kanning, 1968). Approximately 50 fractions were collected and analysed by direct radial immunodiffusion (Mancini, Carbono & Hermens, 1963) with monospecific antisera for mouse IgM, IgG1, IgG2 and IgA. Pooled fractions were concentrated back to the original volume by membrane ultrafiltration; the concentrate contained 67% of the original concentration of IgG1 and IgG2, and 80% of the IgA.

Anti-IgG. An antiserum to mouse IgG, prepared in goats, was purchased from Melpar Laboratories (Springfield, Va.).

Mouse challenge. Eleven groups of mice, 10 treated and one control, were inoculated in the left hind footpad with 0.03 ml of bobcat virus containing approx. 10 peripheral LD$_{50}$. Three additional groups, two treated and one control, were inoculated in the left hind footpad with a $10^{-23}$ dilution of fox salivary gland virus.

Mouse treatment schedules. These schedules are summarized in Table 1. Unless otherwise stated, a single treatment of each type was given 24 h after virus inoculation.

Vaccines. A 0.1 ml dose of vaccine was injected into the right hind leg muscles. (a) BHK vaccine: the same ERA-BHK vaccine was used in different dilutions in groups 1, 2, 3, 12 and 13. Group 1: undiluted vaccine (AV 100). Groups 2 and 12: 1:4 diluted vaccine (AV 25). Groups 3 and 13: 1:20 diluted vaccine (AV 5). (b) HDCS vaccine; Group 4 mice received 0.1 ml of this vaccine.

Other treatments. With these, 0.03 ml was injected into the left footpad and 0.07 ml into
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the left leg muscles. The amounts shown for each treatment are the total in the 0.1 ml injected into each mouse. Group 5: RNase (1 μg); Group 6: hyperimmune serum (3.5 units); Group 7: complement (4.3 CH₅₀ units), together with hyperimmune serum (3.5 units); Group 8: complement (4.3 CH₅₀ units), hyperimmune serum (3.5 units) and RNase (1 μg); Group 9: purified rabies immune globulin (4 international units); Group 10: purified rabies immune globulin (4 units) 24 h after virus inoculation. In addition, 0.1 ml of undiluted antiserum to mouse IgG was injected into the left leg after a further 24 h (0.03 ml into the footpad and 0.07 ml into the leg muscles).

Interferon assays. In the vaccine-dosed groups, 1 to 4, six mice were bled from the orbital sinus (Stone, 1954) 24 h after vaccination. Interferon concentrations were determined by a plaque reduction technique, with mouse L cells and vesicular stomatitis virus (Janis & Habel, 1972). Approximately 1.5 of the endpoint dilution units determined by this assay correspond to 1 mouse interferon reference reagent unit (based on standard G-002-904-511).

Neutralization titres. Virus neutralizing titres were determined by the RFFIT (Smith et al. 1973). Six mice from groups 1 to 4 were bled on days 4, 8, 16, 36 and 100, and average serum titres were calculated.

Mouse observation. Mice were observed daily for the first 2 months, and then three times a week for 1 year duration of the study. Signs of rabies were recorded, and the incubation period is considered as the number of days between inoculation of virus and the appearance of signs of rabies. The brains of all dead mice were examined for rabies antigen by the fluorescent antibody technique (Goldwasser & Kissling, 1958). Only those mice found to be positive are included in the tabulation.

RESULTS

The mortality in the control mice, as in the previous study, was almost 50%, but none of the incubation periods exceeded 30 days (Baer & Cleary, 1972). The mortality was significantly reduced (P < 0.01) in all the five groups given one dose of BHK vaccine, even if the antigenic value was as low as 5 (Table 1). A maximum of four mice (8%) died in any of these groups, which contrasts sharply with the 44% mortality in the animals given HDCS vaccine, and the 49 and 40% mortality in the two groups of untreated controls. Deaths in the groups given hyperimmune mouse serum (either alone, or in combination with complement or RNase) or purified mouse immune globulin (without or with anti-IgG) were also reduced to about 20% in each instance, but RNase alone had no effect on the mortality.

The neutralization titres in serum from the vaccinated mice in groups 1 to 4 did not correlate with the protective effects of the vaccines administered. Mice in all four groups had appreciable antibody at the various times tested (Fig. 1), and indeed at 16 and 36 days, titres in those given the ineffective human diploid vaccine (AV 5) were higher than in those given BHK vaccine diluted to the same AV, which were protected. The relative efficacy of the BHK vaccine and HDCS vaccine were, however, related to the amounts of interferon resulting in the serum: concentrations of 800, 400 and 100 dilution endpoint units/ml were found in mice given, respectively, undiluted and 1/4 and 1/20 diluted BHK vaccine, but no interferon was detected in the serum of mice given HDCS vaccine.

DISCUSSION

This study, the second in a series investigating the basis of effective post-exposure rabies prophylaxis in mice, again emphasizes the importance of interferon induction by rabies
Table 1. Group mortality and incubation periods in mice infected with a rabies virus and treated in various ways

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>Mortality</th>
<th>Incubation periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>(A) Bobcat virus challenge</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Vaccines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>ERA-BHK (AV100)</td>
<td>1/48†</td>
<td>2:1**</td>
</tr>
<tr>
<td>2</td>
<td>ERA-BHK (AV 25)</td>
<td>1/47</td>
<td>2:1**</td>
</tr>
<tr>
<td>3</td>
<td>ERA-BHK (AV 5)</td>
<td>3/48</td>
<td>6:3**</td>
</tr>
<tr>
<td>4</td>
<td>PM-HDCS (AV 5)</td>
<td>20/48</td>
<td>43:8</td>
</tr>
<tr>
<td>(ii) Other treatments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>RNase</td>
<td>29/48</td>
<td>60:5</td>
</tr>
<tr>
<td>6</td>
<td>Mouse antirabies</td>
<td>12/49</td>
<td>24:5***</td>
</tr>
<tr>
<td>serum</td>
<td></td>
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<td>7</td>
<td>C’+serum</td>
<td>8/39</td>
<td>20:4***</td>
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<tr>
<td>8</td>
<td>C’+serum+RNase</td>
<td>10/49</td>
<td>20:4***</td>
</tr>
<tr>
<td>9</td>
<td>Mouse rabies immune</td>
<td>10/48</td>
<td>20:8***</td>
</tr>
<tr>
<td>globulin (IgG)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>IgG + anti-IgG</td>
<td>10/46</td>
<td>21:7***</td>
</tr>
<tr>
<td>11</td>
<td>Controls (bobcat virus)</td>
<td>24/49</td>
<td>49</td>
</tr>
<tr>
<td>(B) Fox virus challenge</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Vaccines</td>
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<td></td>
</tr>
<tr>
<td>12</td>
<td>ERA-BHK (AV 25)</td>
<td>1/50</td>
<td>2**</td>
</tr>
<tr>
<td>13</td>
<td>ERA-BHK (AV 5)</td>
<td>4/49</td>
<td>8:2**</td>
</tr>
<tr>
<td>14</td>
<td>Controls (fox virus)</td>
<td>19/47</td>
<td>40:4</td>
</tr>
</tbody>
</table>

† Reduction in the denominator indicates that deaths were due to causes other than rabies.

** P < 0.01.

*** P < 0.05.

Thus, a BHK vaccine with an antigenic value as low as 5, which nevertheless gave rise to appreciable amounts of serum interferon, gave virtually complete protection against a severe challenge. In contrast, a vaccine prepared from human diploid cells which had the same antigenic value but did not give rise to any detectable interferon, gave no protection. However, it should be noted that the antigenic potencies of these vaccines were determined according to the standard convention by pre-exposure testing, while the true evaluation of
the life-saving effect of vaccine is its use after exposure. The efficacy of vaccines prepared on BHK cells, when given after infection, has been demonstrated in rhesus monkeys (Sikes et al. 1971b) and mice (Baer & Cleary, 1972), and contrasts to the failure of human diploid vaccine in these same species (K. Sikes et al. unpublished data). Further, studies in rabbits, mice and hamsters have shown that interferon, whether induced by specific inducers such as poly(rI).poly(rC) (Fenje & Postic, 1970, 1971; Janis & Habel, 1972; Janis & Harmon, 1974; Harmon & Janis, 1975), or by rabies vaccines (Baer & Cleary, 1972; Wiktor et al. 1972), or even by vaccines against other viruses (Wiktor et al. 1972), is an important factor in preventing rabies, at least in those species of animals tested.

Although actively induced neutralizing antibody to rabies can protect if induced before infection in various species of animals (Dean, Evans & Thompson, 1964; Abelseth, 1966; Sikes et al. 1971a, b), it apparently has no immediate beneficial role if induced after exposure. 'Treatment failures' after post-exposure vaccine administration have been noted in numerous animal species (Markowski & Legezynski, 1929; Koprowski, Van der Scheer & Black, 1950; Ercegovac, 1956) and in man (McKendrick, 1940; Proca & Bobes, 1940; Greenwood, 1945/46; Otten, 1947; Clark, Wiktor & Koprowski, 1975). Antiserum administration apparently serves to keep the virus from advancing from the initially invaded muscle tissue (Murphy et al. 1973; Murphy & Bauer, 1974) and, when given without vaccine, prolongs the incubation period (Baer & Cleary, 1972). Although there was a reduction in mortality in the present study after administration of the antiserum alone, it was less marked than that

Fig. 1. Titres of neutralizing antibody in the serum of mice treated with rabies vaccines. Average titres are shown for 6 mice tested on each day shown. ERA-BHK vaccine: ○○, undiluted vaccine (AV 100); ■■, 1:4 vaccine (AV 25); ●●, 1:20 vaccine (AV 5). PM-HDCS vaccine: ▼▼, undiluted (AV 5).
achieved by other treatments; in our previous study (Baer & Cleary, 1972) no reduction was noted whatsoever. The combination of antibody given initially in passive form as immune serum, and antibody formed subsequently in response to vaccine remains the most effective prophylactic combination in rabies (WHO, 1973; Clark et al. 1975) at the present time. However, there is definite interference between the passive antibody and subsequent active antibody formation (Winkler, Schmidt & Sikes, 1969; Wiktor, Lerner & Koprowski, 1971), and numerous daily injections of vaccine are required to elicit an ‘adequate’ antibody response (Atanasiu et al. 1957).

In the present study none of the treatments involving serum, not even the combination of antiserum, complement and RNase, which was found so effective in vitro against a C-type RNA virus by Oroszlan & Gilden (1970), reduced mortality to the extent that one dose of BHK vaccine did. Both antiserum and IgG prolonged the incubation periods, but purified IgG was no more effective in reducing mortality than the unfractionated mouse antiserum.

Cells of the BHK line of baby hamster kidney are transformed (MacPherson & Stoker, 1962) and therefore potentially malignant, and thus they may not be acceptable as the source for a human vaccine, even if inactivated as in the present study. However, a combination of interferon and subsequent active antibody comparable to that found here could be produced by administering exogenous interferon, or inducing interferon, before giving one of the more potent human vaccines, such as those prepared from human diploid cells (Wiktor, Plotkin & Grella, 1973; Cabasso et al. 1974) or suckling mouse brains (Fuenzalida, 1972).

Since these newly developed vaccines commonly have an antigenic value of 3 to 30 times that of the currently used duck embryo vaccine (Dean & Sherman, 1962), a shorter regimen of only two to five doses should be enough to induce a comparable level of neutralizing antibody. The efficacy of such a combination of interferon and vaccine has recently been shown in mice by Harmon & Janis (1975): in post-exposure studies, the administration of poly(rI).poly(rC) together with one dose of human diploid vaccine was more effective than poly(rI).poly(rC) alone, while vaccine alone was without effect. We recently have shown (G. M. Baer et al. unpublished data) that administration of one dose of a human diploid vaccine together with a dose of either nuclease-resistant poly(rI).poly(rC) complex (Levy et al. 1974) or exogenous interferon completely protected mice against rabies.

We have also shown (G. M. Baer et al. unpublished data) that combinations of interferon or an interferon inducer and vaccine are effective in subhuman primates as well. Thus data presented here and elsewhere (Harmon & Janis, 1975; G. M. Baer & D. Baughcum, unpublished data) suggest that a combination of interferon and a potent rabies vaccine produces post-exposure protection which is as good as, or better than, that produced by the currently recommended immune serum and vaccine. Since side effects are likely to be less, the former combination may prove to be the most practical and simple post-exposure regimen for rabies in man.

Purification of the IgG was performed and verified by Dr Charles Reimer, Bureau of Laboratories, Center for Disease Control, Atlanta, Ga. The human diploid vaccine was supplied by Dr R. Lang, Institut Merieux, Lyon, France. The interferon assays were performed by Dr Burton Janis, Salt Lake City.
REFERENCES


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