Effects of Homologous or Heterologous Antiserum on Neutralizing-Antibody Response to Rabies Vaccine*

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Heterologous antirabies serum is commonly used in the treatment of persons exposed to rabies. However, the high incidence of serum sickness which accompanies its use has prompted work to develop a homologous human product. As human antirabies serum is expensive and difficult to obtain in large quantities, a series of experiments was done on guinea-pigs to test the effects of homologous and heterologous antirabies serum.

Similar amounts of homologous and heterologous antiserum administered to guinea-pigs produced similar circulating neutralization titres one day later. The homologous antibody titres, however, decreased more slowly than the heterologous antibody titres.

When homologous antiserum was given, followed by duck-embryo rabies vaccine, an apparent response to the vaccine was suppressed or delayed longer than when heterologous antiserum and vaccine were administered. However, when homologous antiserum was given with suckling-mouse-brain vaccine, of a much higher potency, the response to vaccine was apparent in the presence of a passive titre of 1 : 120.

If a similar relationship is seen in man with the use of a homologous antirabies product, it will be essential to use high potency vaccines or alter the established vaccination schedules in order to overcome the inherent interference problems.

The high incidence of serum sickness in persons treated with equine antirabies serum has prompted efforts to produce and test an antirabies serum or globulin of human origin (Hosty et al., 1959; Anderson & Sgouris, 1965; Winkler, Schmidt & Sikes, personal communication, 1968). Since human rabies immune plasma is expensive and difficult to obtain in quantity, homologous antiserum was tested in various animal species to evaluate homologous passive treatment against rabies. Veeraraghavan et al. (1958) demonstrated that homologous antiserum may be superior to heterologous antiserum when used to treat challenged guinea-pigs. Anderson & Sgouris (1965) had similar results using monkeys. Winkler et al. (personal communication, 1968) obtained similar results in guinea-pigs and dogs, and also observed that antiserum interfered with vaccine when they were given together. Atanasiku et al. (1961, 1967) had previously observed interference between heterologous antiserum and vaccine in humans. The experiments reported here compared the potency, persistence and interference of homologous and heterologous antirabies serum with 2 vaccines in guinea-pigs.

MATERIALS AND METHODS

Animals

Hartley-strain guinea-pigs weighing approximately 400 g were used in the experiments. Swiss white mice (ICR strain) were used for the serum neutralization tests.

Serum

Rabies immune serum was obtained from donkeys and guinea-pigs that had been immunized with both beta-propiolactone-inactivated and virulent fixed rabies virus of suckling-mouse brain origin.
purified by ECTEOLA\(^1\) chromatography, or fixed rabies virus propagated in hamster-kidney tissue-culture. The potencies of the sera were determined by comparing neutralization titres with a standard reference serum from the US National Institutes of Health (NIH). Immune serum was diluted with non-immune serum from the same species to obtain the desired antibody levels. Immune serum was administered intramuscularly in 1.0-ml doses in the left rear leg.

**Vaccines**

Duck-embryo rabies vaccine (lot OMW45A)\(^2\) and suckling-mouse-brain vaccine (lot R-150)\(^3\) were used. The duck-embryo vaccine (DEV) had an antigenic value of 0.4, and the suckling-mouse-brain vaccine (SMBV) had an antigenic value of 2.6 in the NIH potency test (Seligmann, 1966). The SMBV had an infectivity titre of \(2 \times 10^9\) mouse intracerebral LD\(_{50}\) per g of tissue before inactivation. Vaccines were given subcutaneously in 0.5 ml-amounts of the concentrations prepared by the manufacturers for human use. DEV was prepared as a 10% wet weight, tissue suspension, while SMBV was prepared as a 1% wet weight, tissue suspension.

**Challenge virus**

The challenge virus was the NYC-GA strain of street-rabies virus stored as a 10% suspension of infected canine salivary gland. It had an infectivity titre of \(1.5 \times 10^7\) LD\(_{50}\) per g of tissue. The challenge was 0.2 ml of an appropriate dilution given intramuscularly in the right rear leg. After each challenge, the virus suspension was titrated by intracerebral inoculation of mice.

**Serum neutralization tests**

Neutralizing antibody was measured by incubating 5-fold serial dilutions of serum with 20–160 LD\(_{50}\) of standard challenge virus (CVS-27) for 90 min at 37°C, and injecting 0.03 ml of each mixture intracerebrally into five 3-week-old mice. All sera were inactivated at 56°C for 30 min before testing. The Reed & Muench calculation (1938) was used to determine the 50% end-points.

**Post-challenge protection test**

Ninety-eight guinea-pigs were challenged with 6700 LD\(_{50}\) of rabies virus intramuscularly in the right rear leg and were treated 24 hours later with serial dilutions of donkey or guinea-pig antiserum in the left rear leg. These guinea-pigs were held for 45 days after challenge.

**First serum–vaccine interference test**

Inoculations of 1.6, 6.3, 25, or 100 IU of donkey or guinea-pig antiserum alone or in combination with 14 daily injections of DEV plus boosters on days 24 and 34 were given to 11 groups of 15 guinea-pigs each. Serum samples were obtained from all treated guinea-pigs 1, 3, 15, 22, 31, 47 and 78 days after the serum injections. Pools of serum consisting of equal volumes of serum obtained at the same bleeding from each guinea-pig within a group were tested for neutralizing antibody. Individual serum neutralization tests were done on all guinea-pig sera 78 days after treatment began and from earlier bleedings of selected groups. All guinea-pigs were challenged intramuscularly with

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\(1\) Ecteola-epichlorohydrin triethanolamine cellulose, Carl Schleicher and Schuell Company, Keene, New Hampshire, USA. (Trade names are provided for identification only and their mention does not imply endorsement by the Public Health Service or the United States Department of Health, Education, and Welfare.)

\(2\) Rabies vaccine (duck-embryo) dried killed virus, Eli Lilly and Company, Ind'ianapolis, Indiana, USA.

\(3\) Rabies vaccine (suckling-mouse-brain), killed virus, Instituto Bacteriologico de Chile, Santiago, Chile.

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**Table 1**

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Mortality (deaths/total)</th>
<th>50% Effective dose (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Amount (IU)</td>
<td></td>
</tr>
<tr>
<td>Homologous</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5/11</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>11/11</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>10/11</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>10/11</td>
</tr>
<tr>
<td>Heterologous</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6/11</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>4/10</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10/11</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>11/11</td>
</tr>
<tr>
<td>None (control)</td>
<td></td>
<td>10/10</td>
</tr>
</tbody>
</table>

\(a\) Antiserum was given in 1.0-ml amounts intramuscularly in right rear leg.

\(b\) 6700 LD\(_{50}\) NYC-GA rabies virus was given intramuscularly in left rear leg.
EFFECTS OF HOMOLOGOUS AND HETEROLOGOUS ANTIRABIES SERUM

26,000 LD_{50} rabies virus 79 days after the first treatment day.

Second serum–vaccine interference test

Seven groups of 12 guinea-pigs each were inoculated with 100 IU of guinea-pig antiserum alone or in combination with 13 daily injections (the 10th injection was inadvertently omitted) of DEV or SMBV plus boosters on days 24 and 34. In 2 groups, the serum was given 14 and 7 days before beginning the vaccine injections. Serum samples were obtained from all treated guinea-pigs 1, 3, 15, 31, 50, and 80 days after the first vaccine injection.

RESULTS

Post-challenge protection test

Results of the post-challenge protection test are shown in Table 1. The 10-fold difference in the 50% effective dose of each serum, as calculated by the method of Reed & Muench (1938), is consistent with results reported by other workers (Winkler et al., personal communication, 1968). All but 2 of the rabies deaths occurred within 12 to 31 days after challenge; the 2 exceptions occurred 42 and 43 days after challenge.

First serum–vaccine interference test

The serological responses of guinea-pigs in the first serum–vaccine interference test are shown in Fig. 1–4.

Antibody in guinea-pigs treated with DEV alone first appeared after 6 days and reached peak titres after 24 days, when the first booster injection was given (Fig. 1). With homologous antiserum, 100 IU produced a passive neutralizing titre similar to that produced by 100 IU of heterologous antiserum;

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**FIG. 1**

COMPARISON OF MEAN a RABIES SERUM NEUTRALIZING TITRES b OF GUINEA-PIGS c

- Legend:
  - 16 DOSES DUCK-EMBRYO VACCINE
  - 100 UNITS HOMOLOGOUS ANTISERUM
  - 100 UNITS HETEROLOGOUS ANTISERUM
  - 100 UNITS HOMOLOGOUS ANTISERUM PLUS 16 DOSES VACCINE

- Arrow indicates 0.5 ml injection of duck-embryo vaccine.

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a Titre of serum pools made by mixing equal volumes of serum from each guinea-pig in a group.
b Reciprocal of 50% end-point serum dilutions tested against 10 to 100 LD_{50}.
c Arrow indicates 0.5 ml injection of duck-embryo vaccine.
however, homologous passive antibody levels decreased by 50% every 7 days, and heterologous passive antibody levels decreased by 50% every 1.5 days. Of the guinea-pigs that received 100 IU of homologous antiserum alone 2 had low levels of antibody (1:7 and 1:9) 78 days later.

The apparent active response to DEV was delayed by 100 IU of homologous antiserum until after the booster injection on the 24th day (Fig. 1). No detectable response to the vaccine occurred in 4 guinea-pigs in this group and only 1 responded with a titre greater than 1:50. With heterologous antiserum 100 IU delayed a detectable response to DEV until after the 10th daily injection of vaccine. However, the response that followed was nearly as high as the response to this vaccine given without serum.

Detectable response to DEV was delayed by 25 IU of homologous antiserum until after the 15th day, and the response was slightly less than without serum (Fig. 2); 25 IU of heterologous antiserum given with DEV reduced the 10th-day titre to one-quarter of the normal response, but by the 15th day the difference was slight.

Inoculation with 6.3 IU of either homologous or heterologous antiserum, together with DEV, resulted in low initial passive titres. Rapidly increasing active titres followed and these were only slightly less than the normal response to this vaccine by the 6th day (Fig. 3).

Inoculation with 1.6 IU of either homologous or heterologous antiserum did not result in detectable passive titres or interference with the response to DEV (Fig. 4).

**FIG. 2**
COMPARISON OF MEAN a RABIES SERUM NEUTRALIZING TITRES b OF GUINEA-PIGS c

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*a* Titre of serum pools made by mixing equal volumes of serum from each guinea-pig in a group.

*b* Reciprocal of 50% end-point serum dilutions tested against 10 to 100 LD₅₀.

*c* Arrow indicates 0.5 ml injection of duck-embryo vaccine.
The results of challenge of guinea-pigs in the first serum-vaccine interference test are given in Table 2. With few exceptions, the presence of detectable serum neutralizing antibodies was effective in protecting guinea-pigs against challenge. Neutralization titres were determined for individual guinea-pigs at the last bleeding prior to challenge so that resistance to challenge could be related to presence of antibody. Of the guinea-pigs that received homologous antiserum but no vaccine, 2 had low residual passive titres (1:7 and 1:9) 78 days later, but both died as a result of challenge. Of 13 guinea-pigs treated with DEV and 100 IU of homologous antiserum, 8 had titres less than 1:5 one day before challenge, and all 8 died as a result of challenge. Of 25 guinea-pigs treated with DEV and 25 IU of homologous antiserum, 2 had titres less than 1:5 one day before challenge, and these 2 also died. All guinea-pigs that received less than 25 IU of homologous antiserum and DEV had neutralizing antibody one day before challenge, and all survived. Of 14 guinea-pigs treated with 100 IU of heterologous antiserum without vaccine, 13 died after challenge. One guinea-pig treated with 100 IU of heterologous antiserum and DEV died of rabies, although it had a 1:230 antibody titre one day before challenge. All other guinea-pigs treated with heterologous antiserum and duck-embryo vaccine survived. Of 15 untreated control guinea-pigs, 14 died.

**Second serum-vaccine interference test**

The serological responses of guinea-pigs in the second serum-vaccine interference test are shown in Fig. 5–7.
### TABLE 2

**RESULTS OF CHALLENGE a OF FIRST SERUM-VACCINE INTERFERENCE TEST**

<table>
<thead>
<tr>
<th>Homologous antiserum (IU)</th>
<th>Heterologous antiserum (IU)</th>
<th>Vaccine (No. of doses)</th>
<th>No. of survivors/ total in group</th>
<th>No. with SN titre $&gt;1:5^b$ / total in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>—</td>
<td>—</td>
<td>1/15 c</td>
<td>2 d/15</td>
</tr>
<tr>
<td>100</td>
<td>—</td>
<td>16</td>
<td>5/13</td>
<td>5/13</td>
</tr>
<tr>
<td>25</td>
<td>—</td>
<td>16</td>
<td>13/15</td>
<td>13/15</td>
</tr>
<tr>
<td>6.3</td>
<td>—</td>
<td>16</td>
<td>13/13</td>
<td>13/13</td>
</tr>
<tr>
<td>1.6</td>
<td>—</td>
<td>16</td>
<td>11/11</td>
<td>11/11</td>
</tr>
<tr>
<td>—</td>
<td>100</td>
<td>—</td>
<td>1/14</td>
<td>0/14</td>
</tr>
<tr>
<td>—</td>
<td>100</td>
<td>16</td>
<td>12/13 e</td>
<td>13/13</td>
</tr>
<tr>
<td>—</td>
<td>25</td>
<td>16</td>
<td>13/13</td>
<td>13/13</td>
</tr>
<tr>
<td>—</td>
<td>6.3</td>
<td>16</td>
<td>13/13</td>
<td>13/13</td>
</tr>
<tr>
<td>—</td>
<td>1.6</td>
<td>16</td>
<td>14/14</td>
<td>14/14</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>16</td>
<td>13/13</td>
<td>13/13</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1/15 (controls)</td>
<td></td>
</tr>
</tbody>
</table>

a Challenge was 26 000 LD$_{50}$ NYC-GA strain of rabies virus injected intramuscularly in right rear leg 79 days after treatment with various combinations of vaccine and antisera began.

b Serum neutralization titres one day before challenge.

c Surviving guinea-pig had SN titre $<1:5$.

d Two guinea-pigs had residual passive SN titres of 1:7 and 1:9.

e Guinea-pig that died had SN titre of 1:230.

### TABLE 3

**RESULTS OF CHALLENGE a OF SECOND SERUM-VACCINE INTERFERENCE TEST**

<table>
<thead>
<tr>
<th>Treatment-group</th>
<th>Vaccine Type</th>
<th>No. of doses</th>
<th>No. of survivors/ total in group</th>
<th>No. with SN titre $&gt;1:5^b$ / total in group</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 (14 days before vaccine)</td>
<td>DEV c</td>
<td>15</td>
<td>8/9</td>
<td>9/9</td>
</tr>
<tr>
<td>100 (7 days before vaccine)</td>
<td>DEV</td>
<td>15</td>
<td>3/11</td>
<td>4/11</td>
</tr>
<tr>
<td>100 (same day as vaccine)</td>
<td>DEV</td>
<td>15</td>
<td>8/10</td>
<td>4/10</td>
</tr>
<tr>
<td>100 (same day as vaccine)</td>
<td>SMBV d</td>
<td>15</td>
<td>11/11</td>
<td>11/11</td>
</tr>
<tr>
<td>100</td>
<td>—</td>
<td>—</td>
<td>3/10</td>
<td>1/10</td>
</tr>
<tr>
<td>—</td>
<td>DEV</td>
<td>15</td>
<td>10/10</td>
<td>10/10</td>
</tr>
<tr>
<td>—</td>
<td>SMBV</td>
<td>15</td>
<td>9/9</td>
<td>9/9</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2/14 (controls)</td>
<td>0/14</td>
</tr>
</tbody>
</table>

a Challenge was 3200 LD$_{50}$ NYC-GA strain of rabies virus injected intramuscularly in right rear leg.

b Serum neutralization titres one day before challenge.

c Duck-embryo vaccine.

d Suckling-mouse-brain vaccine.
Higher antibody titres were produced by SMBV than by DEV (Fig. 5). With the guinea-pig antiserum used in this experiment 100 IU produced higher initial passive titres than the antiserum used in the first experiment; however, the slopes of the passive titre curves were the same in both experiments.

Inoculation with 100 IU of homologous antiseraum together with DEV prevented a detectable response to the vaccine (Fig. 6). Although the response to SMBV was decreased by the antiserum, sufficient antibody was produced for a response to this vaccine to be detectable in the presence of a high (1:120) passive antibody titre.

Inoculation with 100 IU of homologous antiseraum 7 days before the first vaccine injection prevented a detectable response to DEV (Fig. 7). However, when 100 IU of homologous antiserum were given 14 days before the first vaccine injection, a detectable response to the vaccine was apparent after the passive antibody titre decreased to 1:30.

The results of challenge of the guinea-pigs in the second serum-vaccine interference test are in Table 3. A neutralization titre greater than 1:5 one day before challenge was effective in protecting guinea-pigs against challenge. In contrast to the results of the first experiment, however, several guinea-pigs in the second experiment, with titres of less than 1:5, also survived challenge. This difference may be due to the lesser challenge (3200 LD_{50}) in the second experiment than in the first (26 000 LD_{50}).
FIG. 5
COMPARISON OF MEAN a RABIES SERUM NEUTRALIZING TITRES b OF GUINEA-PIGS c

DISCUSSION

The results of the first serum–vaccine interference test indicate that a passive neutralizing titre greater than about 1:20 will interfere with the response to DEV in guinea-pigs. As illustrated in Fig. 1, 2, 3, and 7, detectable active antibody did not appear in the circulation until passive titres decreased to about 1:20. However, in the second serum–vaccine experiment when SMBV, with a higher potency, was given with homologous antiserum, a response to this vaccine became apparent in the presence of passive titres of about 1:120. Although approximately 10 times as much DEV as SMBV (0.5 ml of 10% suspension DEV versus 0.5 ml 1.0% suspension SMBV) was given on a tissue concentration basis, by comparing infectivity titres of the vaccines before inactivation (5 x 10^5 to 5 x 10^6 LD_{50}/g tissue and 2 x 10^9 LD_{50}/g tissue, respectively) it is evident that more viral antigen was given with SMBV than with DEV.

Guinea-pigs treated with 25 IU of antiserum (approx. 60 IU/kg) and vaccine received approximately the amount of antiserum (40 IU/kg) recommended for the treatment of severely exposed persons (WHO Expert Committee on Rabies, 1966). This amount of homologous passive antibody persisted long enough in guinea-pigs to cause greater interference with a response to DEV than a similar amount of heterologous antiserum. A clinical study of antirabies globulin of human origin, which would determine the persistence of passive homologous
antibody and also the relationship between passive titre and interference with vaccine in humans, would be necessary before sound recommendations for the use of human immune globulin could be made.

The experimental results reported here indicate that improved, more potent vaccines would reduce the problem of interference. Methods of growth and concentration procedures are currently available to produce rabies vaccines with higher potency values.

Limited evidence is also given that varied treatment schedules may be used to offset the increased interference seen with homologous antiserum.

CONCLUSIONS

(1) When given after challenge with rabies virus, homologous passive antibody is more effective than heterologous passive antibody in protecting guinea-pigs.

(2) Similar amounts of homologous and heterologous passive antibody administered to guinea-pigs result in similar circulating neutralization titres one day later.

(3) Homologous passive rabies antibody persists longer than heterologous passive antibody.

(4) The longer persistence of homologous antiserum delays the apparent response to rabies vaccine longer than heterologous antiserum.

(5) A more antigenic vaccine will elicit a detectable active response in the presence of a higher passive antibody titre than a less antigenic vaccine.

FIG. 6
COMPARISON OF MEAN a RABIES SERUM NEUTRALIZING TITRES b OF GUINEA-PIGS c

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a Titre of serum pools made by mixing equal volumes of serum from each guinea-pig in a group.
b Reciprocal of 50% end-point serum dilutions tested against 20 to 160 LD₅₀.
c Arrow indicates 0.5 ml injection of vaccine.
FIG. 7
COMPARISON OF MEAN 
RABIES SERUM NEUTRALIZING TITRES 
OF GUINEA-PIGS

LEGEND:
100 UNITS HOMOLOGOUS ANTISERUM FOLLOWED IN 14 DAYS BY 15 DAILY DOSES OF DEV
100 UNITS HOMOLOGOUS ANTISERUM FOLLOWED IN 7 DAYS BY 15 DAILY DOSES OF DEV
100 UNITS HOMOLOGOUS ANTISERUM GIVEN WITH FIRST OF 15 DAILY DOSES OF DEV

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On administer couramment du sérum antirabique hétérologue au cours du traitement des personnes exposées à l'infection rabique. Etant donné la fréquence des cas de maladie du sérum résultant de l'application de cette méthode, on s'est efforcé d'autre part de mettre au point un sérum ou des gammaglobulines d'origine humaine. Les essais sur l'animal décrits dans le présent article ont été entrepris pour préciser certains aspects de l'utilisation de l'un ou l'autre type de préparation.

On a recherché chez le cobaye les effets de deux préparations de sérum antirabique, un sérum homologue et un sérum hétérologue, afin de comparer leur pouvoir immunisant, la durée de la protection et l'ampleur des phénomènes d'interférence en cas d'emploi concomitant de vaccin antirabique.

Des titres similaires d'anticorps neutralisants ont été décelés après un jour chez des cobayes traités par des doses équivalentes (100 UI) de sérum homologue ou de sérum hétérologue, mais dans le premier cas, les titres sériques ont décru beaucoup moins rapidement que dans le second. Après injection de sérum homologue, suivie de l'administration de sérum antirabique préparé sur embryon de canard, la réponse immunitaire postvaccinale a été nulle, ou davantage retardée que lorsque la vaccination a été pratiquée après injection de sérum hétérologue. On n'a décelé aucune réaction au vaccin aussi longtemps que les titres sériques se sont maintenus à un niveau supérieur à 1 : 20. Après administration de sérum homologue et d'un vaccin antirabique beaucoup plus actif préparé sur tissu cérébral de souriceau à la mamelle, une réponse immunitaire a été enregistrée alors que les titres passifs d'anticorps étaient encore de 1 : 120.

Au cas où des observations du même ordre seraient faites chez l'homme lors de l'administration de préparations homologues, il serait indispensable d'utiliser des vaccins antirabiques très actifs ou de modifier les schémas de traitement actuels pour contrebalancer les phénomènes d'interférence.
REFERENCES


