Inhibitory Effect of Passive Antibody on Active Immunity Induced Against Rabies by Vaccination

T. J. WIKTOR, 1 R. A. LERNER 2 & H. KOPROWSKI 3

The administration of rabies-immune serum in addition to regular vaccination has been recommended as routine treatment in man after severe exposure to rabies virus. The inhibitory effect of passive antibody on active immunity induced against rabies by vaccination was investigated in rabbits. It was demonstrated that a dose of rabies-immune serum could completely block the neutralizing antibody response engendered by a very potent rabies vaccine. The inhibitory effect could be partially overcome if the number of vaccine doses was increased, if vaccination was started 15 days after serum administration, or if immune serum was given 12–24 hours after vaccination. Even under these circumstances, the antibody level was lower than that observed after administration of vaccine alone. Both 19S and 7S antibody exerted similar effects.

Field trials conducted in Iran in 1954 indicated that rabies-immune serum, when combined with 21 doses of Fermi-type (phenolized brain tissue) vaccine, markedly improved the survival rate of subjects severely bitten by rabid wolves, in comparison with the results of treatment with vaccine alone (Baltazard & Bahmanyar, 1955). Since then, immune serum has been recommended as routine treatment in man after severe exposure to rabies virus (WHO Expert Committee on Rabies, 1957). Experimental justification for including immune serum in post-exposure treatment has been obtained from laboratory studies on the protection of mice, hamsters, and guinea-pigs (Habel, 1945; Koprowski & Black, 1954; Koprowski, Van der Scheer & Black, 1950), by treatment with serum alone or in combination with vaccine.

Observations made during the past few years have indicated, however, that heterologous immune serum may inhibit the antibody response induced by active immunization in man and that an increase in the dosage of vaccine, combined with booster doses of vaccines administered at long-term intervals after the initial treatment, may overcome this effect (Atanasiu et al., 1957).

These findings were supported by the results of experimental studies in guinea-pigs, which showed that the administration of homologous rabies-immune serum inhibited active immunization following vaccination much more effectively than did heterologous serum (Archer & Dierks, 1968).

Recent evidence indicates that the number of failures of human post-exposure treatment after therapy with both immune serum and vaccine, followed by booster doses of vaccine, is increasing (Anderson, Daly & Kidd, 1966; Dehner, 1970). In view of this increase, and of the availability on the one hand of a potent preparation of homologous immune serum for the treatment of man, and, on the other hand, of a potent experimental vaccine that necessitates only one vaccinating injection (Wiktor et al., 1969; Sikes et al., 1971), we decided to evaluate, on a more quantitative basis, the effect in experimental animals of passive immunity on active immunity induced by vaccination.

MATERIALS AND METHODS

Animals

Young adult New Zealand rabbits (2–3 kg body weight) of both sexes were used in all experiments, as well as for the preparation of immune serum.
Virus

Tissue-culture-adapted PM, CVS, ERA, or HEP strains of rabies virus (Wiktor, Fernandes & Koprowski, 1964) were purified by cloning, using a plaque technique employing agarose-suspended BHK-S13 cells (Sedwick & Wiktor, 1967). The cloned virus stocks were propagated in monolayer cultures of BHK-S13 cells in a medium containing 0.1% bovine serum albumin according to methods described previously (Sokol et al., 1968). A roller-type culture was used for virus propagation.

Immune serum

The immunizing antigens were PM, CVS, ERA, and HEP rabies virus prepared by the concentration of infected tissue-culture fluid by ultrafiltration (Strohmaier, 1967) followed by 2 cycles of high-speed centrifugation at 12 500 rev/min in a No. 30 rotor, Spinco model L centrifuge. The final sediment was resuspended in NT buffer (0.13 M NaCl and 0.05 M 2-amino-2-[hydroxymethyl]-1,3-propanediol ["Tris"]) hydrochloride, pH 7.8) containing 0.1% human serum albumin.

Rabbits immunized with HEP virus were inoculated intravenously with $3 \times 10^8$ plaque-forming units (PFU) of infectious virus once a week for 4 weeks and bled 7 days after the last inoculation. For the preparation of immune serum against the PM, CVS, and ERA strains, animals were inoculated intramuscularly once a week for 2 weeks with $4 \times 10^8$ PFU of virus inactivated by ultraviolet irradiation (Wiktor et al., 1969) and emulsified in equal parts of incomplete Freund's adjuvant. The animals then received 2 intravenous inoculations of $1 \times 10^8$ PFU of live virus, and were bled 7 days after the last inoculation.

Serum neutralization test

The level of serum neutralizing antibody in immunized animals was determined in vitro by a plaque assay technique (Sedwick & Wiktor, 1967). Two-fold dilutions of inactivated (heated at 56°C for 60 min) serum were tested by incubation with a virus preparation (PM strain) diluted to contain 500 PFU/ml. The neutralizing antibody titre was expressed as the reciprocal of the highest dilution of immune serum causing a 50% reduction in plaque numbers.

Virulent challenge

Ninety days after the initial vaccination, rabbits, together with a number of unvaccinated control animals, were challenged by intramuscular inoculation with $10^{5-7}$ mouse LD$_{50}$ of a DR street rabies virus of vampire bat origin.$^1$

---

$^1$ Obtained through the courtesy of Dr Boris Szefres, Director, Centro Panamericano de Zoonosis, Buenos Aires, Argentina.
RESULTS

Combined treatment of rabbits with immune serum and vaccine

In the experiments summarized in Fig. 1, one group of rabbits received 1 dose of vaccine, one group received 1 dose of immune serum (100 IU/kg), and one group received simultaneously 1 dose of vaccine and 1 dose of immune serum. The results indicate that whereas the passively acquired immunity could be detected on the first day after administration of serum, antibodies engendered by 1 dose of vaccine could be demonstrated only on the third day after injection. From then on, however, the antibody levels in the group receiving 1 injection of vaccine rose rapidly, reaching a peak on the 7th day after vaccination, with an antibody titre of 4600. This antibody level was maintained for 26 days and had declined only slightly by the 50th day of observation. In marked contrast, the antibody level observed following administration of immune serum alone remained stationary for 7 days, then declined rapidly, reaching undetectable levels on the 50th day after administration.

When a combination of vaccine and serum was administered, the antibody level paralleled that observed after the administration of serum alone for 10 days, and then declined even more rapidly. On the 50th day after immunization, with the serum and vaccine combined, however, a titre of 30 was still detectable.

In the next set of experiments, attempts were made to overcome the inhibitory effect of passive immunity on the induction of antibodies by vaccine through the use of a reduced dose of 50 IU/kg of immune serum, corresponding to a dose recommended for human post-exposure treatment, and also through administration of a larger number of vaccine doses than in the first experiment.

The results (Fig. 2) indicate that although, in all instances, passive immunity lowered the antibody level induced by vaccination, the daily administration of 5 doses of vaccine following the administra-

---

**Fig. 1.** Dynamics of virus-neutralizing antibody formation in rabbits inoculated with 1 dose of rabies vaccine alone, 100 IU of homologous rabies antibody alone, and a combination of both.

**Fig. 2.** Dynamics of virus-neutralizing antibody formation in rabbits inoculated with 3 or 5 doses of rabies vaccine alone and in combination with 50 IU of homologous rabies antibody given as a single dose together with the first dose of vaccine.
Fig. 3. Dynamics of virus-neutralizing antibody formation in rabbits inoculated with 1 dose of rabies vaccine alone, and in combination with 20, 4, and 0.8 IU of homologous rabies antibody.

Fig. 4. Dynamics of virus-neutralizing antibody formation in rabbits inoculated with 1 dose of rabies vaccine alone, and in combination with 5, 1, and 0.2 IU of homologous rabies 19S antibody.

tion of serum seemed to overcome, to a certain extent, the inhibitory effect of passive immunity. Conversely, the administration of 3 doses of vaccine, by comparison with 1 dose, seemed to make little difference in overcoming the inhibitory effect of passive immunity.

In the next set of experiments, we tried to demonstrate on a more quantitative basis the inhibitory effect of immune serum. Three groups of rabbits received 3 different doses of immune serum—namely, 20, 4, and 0.8 IU/kg, respectively—simultaneously with 1 dose of vaccine, as used in the previous experiments.

The results (Fig. 3) indicate that the amount of serum administered determined the level of passive immunity observed on the first day after administration; the smallest dose of serum (0.8 IU/kg) had the smallest inhibitory effect on the development of antibodies following vaccination, whereas the largest dose (20 IU/kg) had the greatest effect. It should be emphasized that the dosage of 20 IU/kg is less than that recommended at present for post-exposure treatment of man.

**Effect of 19S antibody in the combined immune serum-vaccine treatment**

To explore the possibility that 19S rabies antibody administered to rabbits may stimulate rather than inhibit antibodies induced by active immunization (Henry & Jerne, 1968), we immunized rabbits with rabies vaccine and obtained serum 5 days after immunization. Euglobulin was precipitated from the serum twice by the method already described. After the neutralizing antibody content had been determined by the plaque reduction method, euglobulin was given to groups of rabbits in three dosages—namely, 5, 1, and 0.2 IU/kg—together with vaccine. The results (Fig. 4) indicate that no antibody level could be detected on the first day following the administration of the 0.2 IU/kg dose. The antibody
level determined on the same day following administration of 1 and 5 IU/kg corresponds to that observed after administration of the 7S antibody (Fig. 3). There was no clear indication, however, that passive immunity engendered by 19S antibody is less inhibitory to active immunization induced by vaccine than that engendered by 7S antibody.

Following the administration of either 5 IU/kg or 1 IU/kg of 19S antibody and vaccine, the antibody level seemed to be the same as that after administration of 4 IU/kg or 0.8 IU/kg, respectively, of 7S antibody and vaccine (Fig. 3).

Effect of delayed administration of vaccine on the combined immune serum—vaccine treatment

Because the half-life of passively acquired antibody (see Fig. 1) is approximately 14 days, immune serum (7S) was administered to rabbits at the rate of 50 IU/kg and delayed injections of vaccine were begun 8 and 15 days later; 3 doses of vaccine were administered in each case. The results (Fig. 5) indicate that whereas initiation of vaccine treatment on the 8th day after the injection of serum did not overcome the inhibitory effect of passive immunity, initiation of vaccine treatment on the 15th day seemed to induce much higher antibody levels; these levels, however, were still lower than those observed after administration of 3 doses of vaccine alone.

Effect of delayed administration of immune serum on the combined immune serum—vaccine treatment

In another series of experiments, groups of rabbits were vaccinated 3, 12, or 24 hours before the administration of immune serum. Control groups received vaccine or serum alone. The results (Fig. 6) indicate that in all instances the inoculation of immune serum lowered the antibody level induced by vaccination. When, however, immune serum was administered 12 or 24 hours after vaccination, the interference was partially overcome.
DISCUSSION

It is well known that the presence of passively acquired antibodies may interfere with the actual formation of antibodies against an antigen (Uhr & Baumann, 1961). It was somewhat surprising, however, to find that the rabies-immune serum so effectively blocked the neutralizing antibody response engendered by an otherwise very potent rabies vaccine. The inhibitory effect could be partially overcome if the number of vaccine doses was increased from 1 to 5, when vaccination was started on the 15th day after administration of immune serum, or when immune serum was given 12–24 hours after vaccine inoculation. Even under these circumstances, however, the antibody level was lower than that observed after the administration of vaccine alone.

Blocking of the antibody response by immune serum made the animals susceptible to challenge with street virus, even though rabbits in one group received a smaller dose—namely, 50 IU/kg—of immune serum followed by 3 injections of the vaccine (see accompanying table). Animals that received vaccine alone always withstood challenge with virulent virus.

Although all the experiments reported here were performed on rabbits, the blocking action of immune serum has also been observed in other species of animal and against a different type of vaccine. Winkler et al. (1969) showed that the treatment of dogs with 83 IU/kg of rabies-immune serum of horse origin effectively blocked the protective effect of 14 injections of duck-embryo vaccine. Only when the dose of immune serum was decreased to an undetectable level in the serum of the inoculated animals was the blocking effect eliminated. This dosage of serum (Fig. 4) would be ineffective per se in preventing the spread of rabies virus if administered after exposure to virulent virus.

The blocking effect of passive immunity could not be related to a particular fraction of immune serum, since both 19S and 7S antibody exerted a similar effect.

The rationale of post-exposure treatment was based on the fact that rabies is probably the only infection in which the time of actual exposure to the virus can be determined exactly and in which the spread of the virus from the site of infection to the central nervous system occurs relatively slowly. Within this incubation time, the virus may become bound to an antibody engendered by either active or passive immunization. The latter treatment would be particularly important in cases of severe exposure, in which arrest of the spread of the virus would have to take place almost immediately. Since in virus-antibody complexes the virus may not be completely inactivated (Wiktor & Koprowski, unpublished data), it was thought that an assurance of constant surveillance by antibody produced as a response to active immunization had to be secured by administering vaccine following treatment with immune serum.

If, however, administration of immune serum in doses that convey a certain level of passive immunity to the animal results in blocking, or decreasing, the antibody response engendered by vaccine, the pattern of post-exposure treatment with immune serum and vaccine may have to be more carefully scrutinized and a scheme of treatment devised to provide an exposed person or animal with an adequate level of rabies antibodies. An attempt to face this problem directly was made by the WHO Expert Committee on Rabies (1957), which recommended booster doses of vaccine after the completion of the standard course of treatment, if the patient had also received immune serum. Since the vaccine currently used in northern-hemisphere countries has a lower immunogenicity than the vaccine used previously, combined treatment with serum and vaccine may have to be followed with great care in order to determine how many injections of the vaccine should be given, and at what dosage level, before a patient can be assured of adequate immunological surveillance over a long period.

Once the new and potent rabies vaccine becomes available, it is not inconceivable that the use of the immune serum may become obsolete.

Protection of rabbits treated with vaccine, immune serum, or a combination of both. Vaccine, concentrated rabies vaccine of tissue culture origin, antigenic value = 30; immune serum, homologous rabies-immune serum, 400 IU/ml; challenge, DR street rabies virus, 10⁶.⁷ mouse LD₅₀, administered by intramuscular inoculation, 90 days after vaccination.

<table>
<thead>
<tr>
<th>Treatment (doses)</th>
<th>Serum (doses)</th>
<th>Mortality ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>0/4</td>
</tr>
<tr>
<td>1</td>
<td>100 IU</td>
<td>4/4</td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>0/4</td>
</tr>
<tr>
<td>3</td>
<td>50 IU</td>
<td>3/4</td>
</tr>
<tr>
<td>—</td>
<td>100 IU</td>
<td>4/4</td>
</tr>
<tr>
<td>—</td>
<td>—</td>
<td>4/4</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENT

The authors gratefully acknowledge the excellent technical assistance of Mrs Doris Grella.

RÉSUMÉ

EFFET INHIBITEUR DES ANTICORPS PASSIFS SUR L'IMMUNITÉ ANTIRABIQUE ACTIVE INDUITE PAR LA VACCINATION

Des essais pratiques menés en Iran en 1954 ont montré qu'en associant l'administration de sérum antirabique à l'injection de 21 doses d'un vaccin type Fermi on augmentait notablement les chances de survie des sujets mordus par des loups enragés. Depuis lors, l'emploi d'immunserum a été recommandé chez l'homme comme traitement de routine après une exposition grave. Cependant, on a signalé récemment un nombre croissant d'écussons du traitement mixte sérum-vaccin suivi de doses de rappel de sérum chez les sujets exposés à l'infection. Le fait a incité les auteurs à étudier chez le lapin l'effet suppressif des anticorps passifs homologues sur la réponse immunitaire active suscitée par la vaccination.

On a utilisé comme vaccin la souche PM de virus rabique propagée sur culture tissulaire, concentrée par ultrafiltration, partiellement purifiée par centrifugation à vitesse élevée et inactivée par la β-propiolactone. La valeur antigénique de cette préparation était 30-45 fois supérieure à celle du vaccin de référence NIH.

Les séums antirabiques ont été préparés chez des lapins immunisés par plusieurs inoculations de virus rabique de culture tissulaire, vivant ou inactivé. Quant aux anticorps 19S, ils ont été obtenus par inoculation à des lapins d'une dose unique de sérum antirabique inactivé 5 jours avant la saignée, la fraction euglobulinique du sérum étant isolée par précipitation à pH 5,5.

La détermination des titres d'anticorps a été faite par l'épreuve de neutralisation sur plaque.

Au cours d'une série d'expériences, il a été démontré que la réponse immunitaire à la vaccination est complètement abolie chez les animaux traités par administration simultanée d'une dose d'immunserum et d'une dose d'un vaccin de culture tissulaire à forte activité immunogène. L'effet suppressif n'est que partiellement surmonté par l'injection quotidienne de vaccin pendant les 5 jours suivant l'injection du sérum. L'injection de 3 doses de vaccin se révèle pratiquement impuissante à contrecarrer l'action inhibitrice de l'immunité passive. L'effet inhibiteur est fonction de la dose de sérum et la dose de 40 UI/kg actuellement recommandée pour le traitement de l'homme exposé à l'infection rabique a un pouvoir d'inhibition considérable. Les fractions globuliniques 19S et 7S ont une action analogue.

Si le traitement vaccinal est instauré 8 jours après l'administration de sérum, il ne réussit pas à vaincre l'inhibition due à l'immunité passive; s'il est commencé après 15 jours, il suscite une réponse immunitaire active beaucoup plus forte, mais qui reste cependant inférieure à celle qu'entraîne le traitement vaccinal seul. Le blocage de la réponse immunitaire active peut être partiellement empêché si le sérum est administré 12 à 24 heures après la vaccination.

REFERENCES

Atanasiu, P. et al. (1957) Bull. Wld Hlth Org., 17, 911
Atanasiu, P. et al. (1966) Laboratory techniques in rabies, Geneva, World Health Organization