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RABIES ANTISERUM INTERFERENCE WITH ANTIGENICITY OF VACCINE IN MICE

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SYNOPSIS

Experimental work with mice designed to explore the interference phenomenon noted in human rabies prophylaxis is described. It was demonstrated quantitatively that when passive antibody from antiserum administration is present, active antibody response is reduced and the production of immunity to virus challenge is affected. The work appeared to confirm that the practice of giving "booster" doses of vaccine after the 10th day of treatment may overcome this interference.

Studies in unexposed human beings receiving various schedules of antirabies vaccine with and without a dose of rabies antiserum have indicated that the presence of passively administered antibody early in the course of vaccine treatment may suppress the subsequent active antibody response of the individual to the antigenic stimulus of the vaccine.[1] Since no direct evidence can be obtained in man as to whether this suppression of antibody response reflects a decrease in actual immunity to infection, the experiments described here were designed to test this interference phenomenon in mice, where both antibody response and immunity to virus challenge could be evaluated simultaneously.

The procedure followed involved the immunization of duplicate groups of mice with varying schedules of vaccine, one group also receiving antiserum. Mice were bled on the 14th day of treatment and challenged intracerebrally (IC) on the 15th day. Pooled serum samples were quantitated for neutralizing antibody levels.

Materials and Methods

Vaccine

A commercially prepared phenolized inactivated virus vaccine [2] was used. The potency of this vaccine was determined by the Habel test to be 630 LD_{50}.

[1] Atanasiu, P. et al. (1956) *Bull. Wld Hlth Org.* **14**, 593

[2] Prepared and kindly supplied by Sharp and Dohme, and used one year after preparation; original potency 8000 LD_{50}

Antiserum

A commercially prepared concentrated antirabies horse serum [1] was employed. Its potency was 1 : 3125 when tested against 32 LD_{50} of virus.

Challenge virus

The standard US National Institutes of Health challenge fixed virus (CVS) was used from a frozen working pool of virus. Challenge virus was always given by the IC route to the mice.

Immunization

The vaccine was diluted to 1% brain emulsion, and 0.2 ml was given intraperitoneally (IP) at each dose. The antiserum was used undiluted, 0.1 ml being administered subcutaneously at each dose. Sixty mice were used for each treatment group.

Serum neutralization test

Serial fivefold dilutions of pooled mouse sera inactivated at 56°C for 30 minutes were mixed with equal volumes of CVS virus diluted to contain 32 LD_{50} in 0.03 ml of the mixture. After incubation at 37°C for one and a half hours each of the mixtures was inoculated IC into 5 mice.

All calculations of 50% end-points were done by the Reed and Muensch method.

Results

Table I presents the results of one experiment in which all treatment groups except the serum control group received the same vaccine schedule

TABLE I. INTERFERENCE OF PASSIVELY ADMINISTERED ANTIBODY WITH ANTIGENICITY OF RABIES VACCINE IN MICE

Treatment schedule (days)	LD_{50} protection (log)	50% neutralization (serum dilution)
Vaccine: 1, 2, 3, 10; serum: 1	< 0.9	17
Vaccine: 1, 2, 3, 10; serum: 1, 5	1.5	< 5
Vaccine: 1, 2, 3, 10; serum: 1, 5, 10	1.5	17
Vaccine: 1, 2, 3, 10	2.3	9
Serum: 1, 5, 10	1.8	46

60 mice per group; vaccine dose IP of 0.2 ml of 1% phenolized vaccine; serum dose 0.1 ml subcutaneously. Neutralization test by serum dilution against 100 LD_{50} of virus. Mice bled on day 14 and challenged on day 15 with CVS fixed virus IC.

[1] Prepared and kindly supplied by Lederle Laboratories

of doses on days 1, 2, 3 and 10, while the serum dosage varied from one to three doses at 5-day intervals. It is readily apparent that the antibody levels in all groups are low, and those in the groups receiving antiserum are probably due to a persistence of the passively administered antibody. However, it is obvious that the level of immunity to actual challenge was highest in those mice receiving vaccine alone and lowest in the group receiving vaccine and a single dose of serum. This suggests definite interference by the antiserum with the antigenicity of the vaccine. It also suggests that the intermediate levels of immunity in the two groups receiving more than one dose of serum may have been due to the persistence of the passive antibody rather than to the antigenicity of the vaccine.

Table II investigates the effect of the vaccine schedule on the interference caused by a single dose of antiserum. Groups of mice received vaccine doses on the days indicated and duplicate groups were given one dose of antiserum on day 1. In the groups receiving vaccine alone, we again see a confirmation of results previously reported [1] that best antibody responses and immunity to challenge are produced by a vaccine schedule involving several primary doses followed by at least one "booster" dose after the 10th day. However, with every vaccine schedule the early administration of a single dose of antiserum almost completely suppressed the antibody response and definitely reduced the degree of immunity to

TABLE II. EFFECT OF A SINGLE DOSE OF ANTISERUM ON ANTIGENICITY OF VACCINE

Group	Day of treatment		IC challenge LD_{50} protection (log)	Serum neutralization *
	vaccine	serum		
A	1, 2, 3, 10	—	2.4	115
B	1, 2, 3, 12	—	2.3	76
C	1, 2, 3, 10, 11, 12	—	2.9	76
D	1, 5, 10	—	1.9	25
E	1, 2, 3	—	1.7	19
F	1, 2, 3, 10	1	1.5	3
G	1, 2, 3, 12	1	1.5	< 0
H	1, 2, 3, 10, 11, 12	1	1.9	19
J	1, 5, 10	1	< 1.5	2
K	—	1	< 1.5	6

60 mice per group; vaccine dose IP of 0.2 ml of 1% brain emulsion; serum dose 0.1 ml subcutaneously. Mice were bled on day 15 and challenged on day 15 with CVS fixed virus IC.

* Reciprocal of serum dilution which protected 50% of mice when mixed with 32 LD_{50} of virus

[1] Habel, K. (1956) *Bull. Wld Hlth Org.* **14**, 613

virus challenge. The best response by both methods of testing was in the serum group which received the greatest number of " booster " doses of vaccine after the 10th day.

Discussion

The use of rabies antiserum in conjunction with a course of vaccine has now become accepted as the most effective method of post-exposure rabies prophylaxis in man. However, in experiments in non-exposed human volunteers it has been shown [1] that if passive antibody from antiserum administration is present in high concentration over too long a portion of the active immunization period, the antigenicity of the vaccine, as measured by neutralizing antibody response, is reduced. This interference by antiserum can apparently be overcome by giving serum in a single early dose and using at least 14 daily doses of vaccine; this is now the recommended procedure for the combined treatment. The experiments reported here show that this interference effect is a true phenomenon which can be quantitatively demonstrated in experimental animals, and that the interference with active antibody response to the vaccine is also paralleled by an interference with the production of immunity to virus challenge. This work also tends to confirm the fact that " booster " doses of vaccine given after the 10th day of treatment are active in overcoming such interference.

RÉSUMÉ

L'emploi de sérum antirabique combiné à la vaccination représente, de l'avis général, le moyen le plus efficace de protéger contre la rage les sujets mordus. Des études effectuées sur des sujets humains non mordus ont révélé une action antagoniste du sérum sur le vaccin, c'est-à-dire que l'antigénicité du vaccin — mesurée par la production d'anticorps neutralisants — est diminuée, si, tandis qu'elle se développe, des anticorps passifs en forte concentration se trouvent dans le sang durant une assez longue période. Cet inconvénient n'est cependant pas à redouter si l'on administre une dose de sérum au début d'une série de 14 vaccinations quotidiennes. Aussi cette dernière posologie est-elle actuellement recommandée.

L'antagonisme sérum-vaccin peut être évalué quantitativement sur la souris. L'expérience sur l'animal a montré d'autre part que des doses de rappel de vaccin, administrées après le 10e jour de traitement, suppriment l'action inhibitrice du vaccin par le sérum.

[1] Atanasiu, P. et al. (1956) *Bull. Wld Hlth Org.* **14**, 593