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HUMAN ANTIRABIES GAMMA GLOBULIN*

THOMAS S. HOSTY, Ph.D.
Director of Laboratories, State Health Department, Montgomery, Ala., USA

R. E. KISSLING, D.V.M., M. SCHAEFFER, M.D.,**
GORDON A. WALLACE, D.V.M.
Virus and Rickettsia Section, Communicable Disease Center, Public Health Service, US Department of Health, Education, and Welfare, Montgomery, Ala., USA

E. H. DIBBLE, M.D.
Director, John Andrew Memorial Hospital, Tuskegee Institute, Tuskegee, Ala., USA

SYNOPSIS

To obviate the foreign protein reactions experienced with the use of hyperimmune serum in rabies-exposed individuals, an attempt was made to produce a rabies antiserum of human origin.

Five doses of an inactivated rabies virus duck-egg vaccine were administered to 34 volunteers at 4-day intervals (i.e., on days 0, 4, 8, 12 and 16). An additional dose of chick-embryo attenuated virus vaccine—Flury HEP (high egg passage)—was given on the 46th day, followed by a final booster dose of duck-egg vaccine on the 288th day. Twenty-four days later, i.e., on the 312th day after the first dose, the participants were bled and the serum pooled and converted to gamma globulin.

These volunteers, having no initial antibody, responded with variable titres, the pooled serum having a titre of 1:100 against 50 LD_{50} of rabies virus in neutralization tests and the gamma globulin prepared from this pool a titre of 1:300.

In five individuals inoculated with the antirabies gamma globulin, blood samples tested at intervals for residual antibody showed significant titres through 21 days.

While the passive antibody levels resulting from the administration of a more potent immune horse serum were much higher than those achieved by the weaker human antirabies gamma globulin used, the decrease in titre was more gradual with the human globulin. With more booster inoculations in a larger group of human volunteers, it is believed that a human rabies immune gamma globulin could be produced which would be equal in effect to immune horse serum. The advantages of a human source of antibody in rabies prophylaxis are discussed.

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** Chief, Virus and Rickettsia Section

In the State of Alabama, USA, for the last four years, the administration of an equine rabies hyperimmune serum simultaneously with vaccination has been advocated in certain cases of rabies exposure, particularly those with face bites. Previous experience had shown that a varying number of cases of serum sickness could be expected five to eight days after administration of the horse serum (Hosty & Hunter, 1953). Furthermore, since the serum dose was administered on a weight basis, reactions were more prone to occur in adults. These reactions did not preclude the continued judicious use of this serum where indicated, a procedure now recommended by the World Health Organization.

Owing to the significant number of individuals sensitive to horse serum, an attempt was made to produce a rabies hyperimmune serum of human origin. Arrangements were made with the Tuskegee Institute, School of Veterinary Medicine, Tuskegee, Ala., for volunteers who would submit to rabies vaccination and periodic bleeding for antibody determinations. Thirty-four volunteers were obtained for this study.

Methods

Immunization and blood-testing schedule

To minimize possible reactions to nerve tissue, a killed rabies vaccine of duck-egg origin (Eli Lilly and Company) was used for the primary course of immunization. On zero day blood specimens were collected from all volunteers and 1 ml of duck-egg vaccine was administered subcutaneously in the scapular region of each participant. This procedure was repeated every four days until a total of five injections of vaccine had been given. Thirty days after the fifth injection (i.e., on the 46th day) blood was again taken for antibody determinations and 0.2 ml of Flury HEP (high egg passage) chick-embryo vaccine (Lederle) was administered intracutaneously. Seventeen days after inoculation of Flury HEP vaccine blood samples were taken for serological study. On the 77th day, 500 ml of blood were drawn from each of the participants showing neutralization indices of not less than 80 and the pooled serum was converted to gamma globulin. Because of an accident during shipment almost all of this globulin was lost and it was necessary to wait until the beginning of the following school year in order to continue the experiment. On the 288th day a blood sample was taken from 26 of the same group of volunteers to determine antibody levels and 1 ml of duck-egg vaccine was again given. During the vaccination only a few reactions of a minor nature were encountered, such as erythema at the inoculation site and in one case a slight induration and malaise.

Twelve days later blood was taken and neutralization indices again determined on all available participants. On the 312th day (24 days after

the last booster dose) 500 ml of blood were drawn from each subject with significant antibody levels and the serum pooled and converted to gamma globulin. Five volunteers who showed no rabies-neutralizing antibody in a preliminary test were inoculated intramuscularly with the human rabies immune gamma globulin (approximately 8% solution) in the amount of 0.5 ml per kilogram of body-weight. Successive blood samples were taken at varying intervals from the 5th day up to and including the 90th day, the schedule and test results of which are shown in Table 3. The sera were tested by a modified procedure described later.

Gamma-globulin preparation

Initial separation of the plasma into five fractions and subfractionation of fractions II and III were performed by the methods of Cohn et al. (1956) and Oncley et al. (1949). Because the serum was fractionated to give a maximum yield of gamma globulin, more than the usual amount of lipoprotein was present in the reconstituted 8% solution of gamma globulin.

Neutralization techniques

Three neutralization techniques were used in this study, for the reasons explained below.

Method 1. Aliquots of undiluted serum were mixed with equal quantities of fixed rabies virus (CVS (standard challenge virus) 11) in increasing tenfold dilutions. A normal serum treated similarly served as a control. The mixtures were incubated overnight at 4°C before being inoculated intracerebrally into mice. The ratio of the titre of the virus in the test serum to the titre in the control serum is expressed as the neutralization index. Method 1 lacks the sensitivity of methods 2 and 3, but gives results that are more consistent from test to test. Since it was desirable to follow the antibody response as the immunization schedule progressed, method 1 was used for this phase of the study.

Method 2 was the " regular " neutralization test used by Atanasiu et al. (1957). In brief, increasing fivefold dilutions of serum were mixed with a single dose of virus calculated to contain 30 LD_{50} per mouse dose. The virus was titrated in the presence of normal serum to determine the actual dose used in each test. After 90 minutes' incubation at 37°C the mixtures were inoculated into mice. The results are expressed as that dilution of serum allowing 50% survival of the mice.

Method 3 was the " modified " neutralization test of Atanasiu et al. (1957). Nine parts of serum were mixed with one part of virus calculated to contain approximately 10 000 LD_{50} per mouse dose. After 90 minutes' incubation at 37°C, fivefold dilutions of the mixture were prepared and inoculated intracerebrally into mice. The results are expressed in logarithms

to the base 10 as the difference between the titre of the virus in the test serum and its titre in a normal control serum handled similarly.

Methods 2 and 3 were used so that the results of this study could be more closely compared with those of Atanasiu et al. (1957).

Results

The individual neutralization indices (method 1) obtained by periodic inoculations of rabies vaccine are shown in Table 1. It is of interest to note that on the 12th day 24 of the 26 sera had neutralization indices of 50 or better. The response on the 63rd day to a booster inoculation of Flury HEP vaccine administered 17 days before is shown. In retrospect there is no reason to feel that the killed duck-egg vaccine would not have produced at least as good results as the modified Flury HEP live virus vaccine. The pooled serum collected on the 77th day gave a neutralization index of 2000.

To compare our results with those of similar investigations of rabies immune serum of equine origin (Atanasiu et al., 1957), neutralization titres were determined by method 2 on this pooled serum and gamma globulin (77th day). Using 32 LD_{50} doses of rabies virus, the pooled serum had a titre of 1 : 22. The gamma globulin was reconstituted and tested in two different strengths; namely, 700 mg per 100 ml and 7 g per 100 ml, giving antibody titres of 1 : 2 and 1 : 28, respectively, as compared to a titre of 1 : 630 for a WHO standard (horse) serum.

Because this supply of gamma globulin was inadequate for human testing and also of inadequate strength, it was not further evaluated.

Serum obtained on the 312th day had a titre of 1 : 100 and 1 : 300 for the gamma globulin against 50 LD_{50} doses of virus by method 2. This gamma globulin was used for inoculation into human subjects. By contrast, the commercial antirabies serum used by Atanasiu et al. (1957) showed a protective titre of 1 : 5164 against 239 LD_{50} of fixed rabies virus.

Electrophoretic studies on ten selected sera were performed to determine the gamma-globulin concentration. Five sera showing low neutralization indices were compared to five with high neutralization indices. The results of this examination are shown in Table 2. In this limited study there was no correlation between the gamma-globulin levels and the antibody titres of the sera whose globulin content lay within the normal range. Complement-fixation tests run on the 300th-day sera likewise showed no correlation when compared to the neutralization indices (Table 1).

Table 3 shows the results of antibody determinations on the five individuals who received antirabies human immune gamma globulin. For comparison, the results of Atanasiu et al. (1957) with immune horse serum are included. There was considerable inconsistency in the neutra-

TABLE I. INDIVIDUAL NEUTRALIZATION INDICES OF VOLUNTEERS SUBMITTING TO RABIES VACCINATION

Day / Individual	0	4	8	12	16	46	63	77 *	288 **	300 ***	312 *
1	—	6.3	—	50	20	10	620	800	160	1 000 (4)	800
2	—	6.3	—	630	1 600	500	2 500	2 500	630	3 200 (16)	4 000
3	—	—	—	20	—	—	—		63	200 (8)	NT
4	50	200	32	160	50	25	620	1 000	1 000	500 (4)	500
5	—	—	—	—	50	50	2 500	1 600			
6	—	—	—	—	20	8	62		63	2 000 (16)	800
7	—	—	32	20	50	320	25 000	16 000			
8	—	—	—	—	—	16	250	NT			
9	—	—	—	50	63	32	62				
10	—	—	—	50	200	32	100	200	100	1 600 (4)	1 000
11	—	—	—	112	50	16	620	320	63	1 000 (4)	800
13	—	—	—	100	80	32	1 000	5 000	63	500 (8)	250
14	—	—	—	320	100	320	500	5 000	630	4 000 (16)	5 000
16	10	10	16	160	160	80	2 500	8 000			
17	—	—	—	2 000	5 000	200	160	200	63	2 000 (16)	800
18	—	—	32	100	32	25	250	32	63	1 000 (4)	NT
19	—	6.3	—	100	50	250	4 000	1 000	125	1 600 (8)	8 000
20	—	—	—	50	500	250	4 000	1 000			
21	—	—	—	500	2 000	1 600	2 500	10 000	3 200	2 000 (16)	25 000
22	—	—	16	200	50	16	25		63	160 (4)	NT
23	—	—	—	200	320	160	1 000	2 000	500	1 600 (8)	800
24	—	—	32	100	80	—	—	NT	63	20 (8)	
25	—	—	16	100	100	8	160	80			
26	—	12	16	100	500	100	1 600	NT	63	1 600 (16)	2 500
27	—	—	—	—	20	25	50	NT	63	500 (8)	250
28	—	—	—	—	16	—	25	NT	63	32 (32)	
30	—	—	32	116	160	32	500	1 600	160	2 000 (16)	500
31	—	—	16	200	1 000	80	500	320	63	1 600 (16)	4 000
33	—	—	—	116	50	8	250	100	63	500 (4)	80
35	—	—	63	80	NT	25	80	160	160	1 600 (8)	500
36	—	—	16	50	160	1 600	2 500	1 600	160	1 000 (8)	5 000
37	—	—	63	—	—	—	32				
38	32	—	320	—	—	NT	NT	NT	63	320	NT
39	—	—	32	—	80	8	1 000	8 000	63	320	NT
								Pooled serum: neutralization index, 2 000			Pooled serum: neutralization index, 5 000

Duck-egg vaccine was given on days 0, 4, 8, 12 and 16; Flury HEP vaccine was given on day 46.

* 500 ml of blood withdrawn

** Blood sample taken and duck-egg booster vaccine given

*** The figures in parentheses are the complement-fixation titres.

NT = not tested or not available on day of bleeding

TABLE 2. COMPARISON OF NEUTRALIZATION INDICES RESULTING FROM RABIES VACCINATION AND GAMMA-GLOBULIN LEVELS * OF TEN INDIVIDUAL HUMAN SERA

Gamma-globulin levels	Low to normal	Slightly elevated		Moderately elevated
Neutralization	320	20	5 000	32
indices	16 000	25	8 000	5 000
		25	10 000	

* Determined by electrophoretic studies

lization titres from one period to the next both in the individuals who received horse serum and in those who received human gamma globulin. The factors responsible for this variability have been discussed previously (Atanasiu et al., 1957).

Assuming that a neutralization index of 0.50 or more is significant, four of the five people treated with human gamma globulin had significant titres on the 15th day and all three of the individuals given horse serum had a significant antibody level at that time (Table 3). The same ratio persisted on the 21st day. On the 42nd day, two of the three inoculated with horse serum retained adequate titres, while only one of the five who received human globulin had adequate antibody levels. After this period significant levels of antibody were not observed.

Discussion

The results of these experiments provide sufficient evidence to encourage the belief that effective passive transfer of antibodies from human sources is feasible in antirabies prophylaxis. The disadvantages of animal serum are quite clear and the advantages of substituting human antiserum are too obvious to require further elucidation.

The dramatic incident in Iran (Habel & Koprowski, 1955), and the excellent results obtained there, provide the best evidence thus far of the efficacy of serum prophylaxis in humans following exposure to severe bites of rabid animals.

Our experience in Alabama has been that rabies in vaccinated humans, if infection develops, has occurred frequently on about the 15th to 20th day after exposure. In Table 3, four of the five human-serum treated volunteers had antibody levels of over 0.50 on the 15th day, as compared to all three of those receiving horse serum. However, while the horse serum shown in comparison had a much higher antibody content than the human gamma

TABLE 3. COMPARISON OF NEUTRALIZATION INDICES OBTAINED WITH ANTIRABIES HOMOLOGOUS GAMMA GLOBULIN (A) AND WITH HORSE IMMUNE SERUM (B)

Day / Individual	0	2	5	6	10	15	21	28	35	42	49	60	68	90
A. Human gamma globulin														
832	—	NT	1.08	NT	1.08	1.08	1.08	NT	0.69	NT	1.31	NT	0.0	0.52
833	—	NT	−0.22	NT	0.0	1.01	1.18	1.01	0.0	NT	−0.34	NT	−0.10	0.13
834	—	NT	1.40	NT	1.63	0.98	1.05	NT	0.48	NT	0.22	NT	0.22	0.22
874	—	NT	0.70	NT	0.44	0.82	0.58	0.12	0.35	NT	0.12	NT	0.0	0.26
875	—	NT	0.07	NT	0.67	0.48	0.22	0.67	−0.13	NT	−0.50	NT	−0.50	−0.36
B. Horse immune serum *														
S1	—	2.90	NT	3.60	2.10	2.20	2.10	1.85	NT	0.45	NT	0.30	NT	NT
S2	—	3.15	NT	3.85	3.40	1.70	2.20	2.00	NT	1.90	NT	0.70	NT	NT
S3	—	3.45	NT	2.70	3.40	2.80	1.75	1.55	NT	1.50	NT	0	NT	NT

* Results of Atanasiu et al. (1957)
NT = not tested

globulin, this disparity was not so apparent in the human recipients 15 days after administration. In Table 3 it will be noted that the horse serum titre dropped markedly after ten days, whereas that of the serum of human origin held generally through 21 days. This is consistent with the findings of others that homologous antibody in humans is far superior to horse antibody on a unit basis (Smolens et al., 1956).

Of the 26 volunteers tested in the second part of the experiment (Table 1), 6 (23%) had final neutralization indices of 4000 or more on the 312th day. This percentage of significant immunizers is consistent with the findings of others (Smolens et al., 1957) using different antigens.

From this preliminary study, which must be confirmed with larger numbers of individuals and more elaborate tests, the following encouraging conclusion may tentatively be drawn. Gamma globulin of human origin may serve to supply a treated individual with immediate antibody with which to inactivate virus introduced during exposure and possibly protect for a long enough period until active immunization is induced by the vaccine which is recommended to be given simultaneously. Moreover, it is anticipated that higher titres in the gamma globulin can be obtained if individuals are inoculated with more potent vaccines. If, subsequently, only those responding with high titres are selected for the serum pooling, the resulting gamma globulin titres would be still further increased.

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RÉSUMÉ

Les protéines étrangères du sérum antirabique préparé sur le cheval peuvent provoquer chez l'homme des réactions désagréables, que l'on cherche à éviter en mettant au point un sérum d'origine humaine.

Cinq doses de vaccin antirabique inactivé préparé sur œuf de canard ont été administrées à des volontaires, à intervalles de 4 jours, soit aux jours 0, 4, 8, 12 et 16. Une dose supplémentaire de vaccin atténué provenant d'embryon de poulet (Flury HEP) a été administré le 46e jour, suivie d'une dose de rappel du vaccin sur œuf de canard, le 288e jour. Le 312e jour après la première dose, du sang a été prélevé sur les sujets à l'étude, les sérums mélangés et la gamma globuline extraite.

Les sujets, qui n'avaient aucun anticorps antirabique au départ, présentaient des titres divers: 23% avaient des indices de neutralisation de 4000 et plus, le 312e jour. Le mélange de leurs sérums avait un titre de 1:100 contre 50 DL_{50} de virus rabique, dans les tests de neutralisation, et la gamma globuline obtenue à partir de ces sérums avait un titre de 1:300.

Cette globuline a été administrée à des sujets humains. On a prélevé à divers intervalles des échantillons de sang chez les 5 sujets ayant reçu la globuline pour déterminer la quantité d'anticorps résiduels. Un groupe de 3 témoins recevait du sérum préparé sur le cheval. Un indice de neutralisation de 0,50 était considéré comme significatif. Le 15e jour, 4 des 5 personnes ayant reçu la gamma globuline, et les 3 témoins, avaient des niveaux d'anticorps significatifs, qui ont persisté jusqu'au 21e jour. Le 42e jour, 2 des 3 témoins et 1 seul des 5 traités à la gamma globuline avaient encore un niveau d'anticorps convenable. A partir de ce moment, le niveau tomba en-dessous du titre significatif.

Le sérum de cheval avait à l'origine un titre en anticorps beaucoup plus élevé que la globuline humaine. Le titrage sur l'homme ne le laissait pas supposer. En effet, le titre du sérum de cheval baissa considérablement les 10 premiers jours, tandis que la globuline humaine gardait son titre jusqu'au 21e jour. Ces résultats confirment les observations selon lesquelles le sérum humain est de beaucoup préférable au sérum de cheval, sur la base de l'unité.

Les conclusions provisoires que l'on peut tirer de cette étude — qui doit être reprise sur des bases plus larges et au moyen d'autres tests — sont encourageantes. La gamma globuline d'origine humaine peut permettre de traiter immédiatement un individu exposé à l'infection, en lui fournissant des anticorps neutralisant le virus introduit par morsure, en attendant que ne s'installe l'immunité active résultant de l'injection de vaccin qui doit être faite en même temps. On peut espérer en outre qu'en administrant aux sujets producteurs d'anticorps des vaccins plus puissants que dans cette dernière étude, en n'utilisant que les sérums de sujets ayant fortement réagi, on obtiendrait des gamma globulines d'un titre protecteur encore plus élevé.

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