Prophylactic Immunization of Humans Against Rabies by Intradermal Inoculation of Human Diploid Cell Culture Vaccine

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The antirabies human diploid cell vaccine produced by l'Institute Mérieux, Lyon, France, was administered intradermally to 35 high-risk volunteers using 0.2-ml amounts and various immunization schedules. Three groups never before vaccinated against rabies developed virus-neutralizing antibodies, the titer of which was dose dependent. A single injection stimulated the formation of antibodies. Four inoculations induced the highest antibody levels and the longest persistence of antibody. The administration of a single intradermal booster inoculation was sufficient, even in the case of low-persisting antibody, to elicit a rapid increase of antibodies to high levels. A primary inoculation course of two injections induced a sufficient antibody level which, in case of exposure, could apparently be rapidly elevated by a 0.2-ml intradermal booster inoculation. Adverse side reactions were observed in 7 of 14 individuals after a 1- or 1.5-year intradermal booster inoculation. We therefore suggest that the intramuscular and subcutaneous routes continue to be used for primary vaccinations and that the highly effective intradermal route be restricted to booster inoculations. This is the first long term study of this vaccine and should be a guideline for the pre-exposure treatment of high-risk personnel.

Pre-exposure vaccination against rabies for high-risk personnel has been recommended for several years by the World Health Organization (WHO) (11). Vaccines prepared from mammalian nervous tissues are not suitable due to the high incidence of vaccine-derived complications after post-exposure treatment. Avian embryo vaccines have not found wide acceptance because the antibody response in humans is generally low. Potent pre-exposure rabies vaccines for man have now been developed using human diploid cell cultures (13), and have been successfully tested in animal experiments (8) and in human field trials (1, 3, 4, 14). The results obtained in these studies led us to use this type of vaccine in a pre-exposure vaccination study in our laboratory personnel using the intradermal route of injection and different schedules of vaccination.

The objectives of the study were to determine (i) the effectiveness of intradermal vaccinations using a minimal amount of vaccine, (ii) the most efficient inoculation schedule, (iii) the persistence of antibodies over a relatively long period of time, and (iv) the effect of intradermal booster inoculations.

MATERIALS AND METHODS

Vaccine. The antirabies human diploid cell vaccine (HDCV) was obtained from L'Institute

Mérieux, Lyon, France. The vaccine was a 10-fold concentrate of cell culture supernatant fluid concentrated by ultrafiltration. The Pitmann-Moore strain of rabies virus was grown in human diploid cells (WI-38) and was inactivated by beta-propiolactone before concentration. Vaccine lots no. SO172 and SO286 were used in the following studies. The lyophilized vaccine was reconstituted with sterile distilled water immediately before use. As compared to a reference vaccine (A 18, Behringwerke, Marburg, Germany, previously shown to be equal to the WHO international antirabies reference vaccine), the Mérieux HDCV showed an antigenic value of 0.6 in the antibody-binding test and an antigenic value of 1.2 in the NIH mouse test (7), using a challenge dose of 22 mean lethal doses/0.03 ml.

Schedule of vaccination. The vaccine was injected intradermally (i.d.) in each case in 0.2 ml amounts into the skin of the forearm. The volunteers were divided into four groups with various immunization schedules. Group 1 consisted of 10 subjects, all of whom had been previously immunized with various antirabies vaccines at time intervals dating back, in certain cases, to over 10 years. This group received one booster inoculation of HDCV, and serum samples were taken at the intervals indicated. The three other groups, which received various vaccine doses, had never been vaccinated against rabies. Group 2 consisted of nine individuals who received injections on days 1, 3, 8, and 28. Group 3 consisted of four individuals who were injected on days 1, 3, and 28, and group 4 of 12 individuals vaccinated on days 1 and 28. Booster

inoculations of a 0.2-ml vaccine were administered to group 3 after 1.5 years and to group 4 after 1 year; the same vaccine lot was used as for the primary immunization.

Serum antibody titration. The rise in virus neutralizing-antibody titers was followed at the times indicated in Fig. 1-4. Before testing, all serum samples were heat inactivated for 30 min at 56 C. The antibody response of each individual was determined by using a modification of the rapid fluorescent focus inhibition test (9). For this test, 0.3 ml of the serum dilution was mixed with 0.3 ml of a constant dilution of the challenge virus standard strain of rabies virus. After incubation for 90 min at 37 C, 0.2 ml of the serum-virus mixture was pipetted into the compartments of a Lab-Tek tissue culture chamber slide (Lab-Tek Products, Division of Miles Laboratories, Inc., Westmont, Ill.) and 0.2 ml of a 5×10^5 /ml concentration of BHK-21 C 13 cells (10) was added. Serum and virus dilutions were prepared in Eagle medium (5) containing 0.3% (wt/vol) bovine serum albumin. The BHK cells were diluted in Eagle medium containing 15% (wt/vol) calf serum.

The tissue culture chamber slides were incubated for 18 h in a controlled humidity, carbon dioxide incubator at 37 C. The medium was then removed and the cells were washed with phosphate-buffered saline, fixed in acetone at -20 C for 10 min, and stained for 20 min with antirabies conjugate (6). The slides were examined with a Zeiss fluorescence microscope. For each serum dilution, 20 low-power ($\times 80$) microscopic fields were counted per chamber and the reduction in the number of infected cells per field as compared to the control was recorded. Each tissue culture chamber slide contained a control consisting of the virus dilution plus an equal volume of Eagle medium containing 0.3% bovine serum albumin. That serum dilution in which the number of infected cells per microscopic field was reduced by 50% was considered to be the end point. For the neutralization assay, a challenge virus standard dilution was chosen which, under the conditions described, infected approximately 10 to 20 cells per microscopic field. In each experiment a reference serum was included which had been standardized against the WHO reference antiserum and against the U.S. standard reference antiserum. In this assay system it was found that a rapid fluorescent focus inhibition test titer of 1:200 was equal to 1 international unit (IU)/ml. As a control, the above mentioned reference sera were also tested by using the plaque reduction technique and the same results were obtained (Cox and Schneider, unpublished data).

Antibody binding test. For determining the antibody binding capacity (antigenicity) of the vaccines, the technique described by Arko et al. (2) was used with the following modifications: 0.3 ml of serial twofold dilutions of the vaccine were mixed with 0.3 ml of the standard immune serum in a dilution containing 25 to 100 antibody units (that is, 25 to 100 times the limiting concentration of the specific virus-neutralizing activity). After incubation of the vaccine serum mixture for 90 min at 37 C, 0.6 ml of the challenge virus standard dilution was added and incubation was continued for 90 min at 37 C, after

which 0.2 ml of each mixture was pipetted onto the Lab-Tek tissue culture chamber slides. After addition of 0.2 ml of the BHK cells (5×10^5 /ml), the slides were incubated for 18 h and handled as indicated above for serum antibody titrations. The end point was expressed as that vaccine dilution at which the infected cells were reduced by 50%.

RESULTS

The antibody profiles of the four vaccinated groups are shown in Fig. 1 through 5. The profiles represent the geometric means of the individual antibody titers. The individual titers are indicated by dots. In all figures the value of 0.6 IU/ml is indicated. This value is equivalent to an antibody level of 20 IU of passively administered antibody per kg of body weight (75 kg of body weight and approximately 2,500 ml of serum per average person) as given in postexposure rabies treatment.

Group 1. This group consisted of 10 individuals previously vaccinated against rabies. After a single intradermal booster inoculation, virus neutralizing antibody rapidly increased in these subjects, irrespective of the time period between the last injection and the booster inoculation. The increase of antibodies within 14 days after the booster inoculation and the antibody profile over a period of 2 years is shown in Fig. 1. The mean titers increased twofold after 2 days, 15-fold after 6 days, and 84-fold after 10 days. At this time the mean antibody titer was equivalent to 15.2 IU/ml. After 14 days no further increase in antibody titer was noted. After 1 year the mean titer was 1.7-fold that of day 0, and after 1.5 years the 0-day level was reached and remained constant for up to 2 years when this study was terminated.

Group 2. A group of nine individuals received a primary course of three injections (days 1, 3, and 8) and one booster on day 28 (Fig. 2). On day 28 postvaccination, the mean titer of virus neutralizing antibody was 2.93 IU/ml. With one booster inoculation this titer increased to about 11 IU/ml within 10 days. Compared to the 28-day values, the antibody level was reduced by half after 1 year, again by half after 1.5 years, and again by half after 2 years. Six of nine individuals still had titers of 0.6 IU/ml or above at 1.5 years, and three of nine after 2 years.

Group 3. A small group of four individuals received a primary course of two doses (days 1 and 3) and a booster inoculation on day 28. This group also received a booster inoculation 1.5 years postvaccination (Fig. 3). At 28 and 38 days postvaccination, the antibody profile was similar to that of group 2, however, the virus neutralizing antibody titers at the 1-year level were about threefold lower than those of group

FIG. 1. Antibody profile of group 1, consisting of 10 individuals previously vaccinated against rabies who received a booster inoculation of 0.2 ml i.d. of HDCV. The profile represents the geometric mean of the individual antibody titers. The individual titers are indicated by dots.

2. A pronounced decrease in titers was noted 6 months postvaccination. The administration of one booster injection after 1.5 years resulted in an increase over the 1.5 year titer level of 2.5-fold after 2 days, 15-fold after 6 days, and 52-fold after 10 days. The mean titer achieved 10 days postbooster inoculation was equal to that obtained 38 days after primary vaccination (16 IU/ml).

Group 4. A group of 12 individuals received a primary course of two injections (days 1 and 28). After 1 year, this group was also given a booster inoculation (Fig. 4). A single injection induced the formation of low titered antibodies (mean 0.52 IU/ml) which were raised more than fivefold by the second injection on day 28. At 1 year the mean titer was as low as 0.15 IU/ml, being approximately 10-fold lower than the corresponding value of group 2. A booster injection after 1 year resulted in an increase over the 1 year titer level of 6.3-fold after 6 days, and 47-fold after 10 days. It is interesting to note that the mean titer of 7.6 IU/ml achieved 10 days postbooster is approximately 2.7-fold higher than the maximum titer observed after the primary course of vaccination (day 38).

In Fig. 5 the antibody profiles of all four groups are compared.

Adverse reactions after vaccination. Of the 10 individuals of group 1, only one subject complained of a side reaction consisting of a swelling at the site of the inoculation which persisted for 3 days.

No adverse side reactions were reported among the individuals of the groups 2, 3, and 4 during the primary course of inoculation including the 28-day booster injection.

After the revaccination at 1 year of group 4, 5 of the 11 individuals reported a local reaction at the site of inoculation. The local symptoms, which were noted within 2 h after inoculation, consisted of slight-to-moderate swelling of the arm accompanied by redness, warmth, and increased sensitivity to touch. No fever was reported. The local symptoms disappeared within 3 to 4 days. A slight induration of the skin at the site of injection was notable for several weeks.

Identical local symptoms were reported in two of the three individuals of group 3 revaccinated 1.5 years after primary vaccination. The symptoms were more intense than those re-

FIG. 2. Antibody profile of group 2, consisting of nine individuals who received a primary course of three inoculations of 0.2 ml i.d., and a booster inoculation of 0.2 ml i.d. on day 28. Explanation of profile as in Fig. 1.

FIG. 3. Antibody profile of group 3, consisting of four individuals who received a primary course of two inoculations of 0.2 ml i.d., and a booster inoculation of 0.2 ml i.d. on day 28. A further booster inoculation of 0.2 ml i.d. was given 1.5 years postvaccination. Explanation of profile as in Fig. 1.

ported from group 4, but disappeared altogether within 3 to 4 days. However, 9 to 10 days after the booster inoculation these two individuals complained of symptoms resembling a hypersensitivity type of reaction. Initial itching all over the body was followed by the appearance of a generalized urticaria. Reddish skin eruptions varying from 0.5 to 5.0 cm in diameter appeared on various parts of the body. On day 10 intensive subcutaneous edema developed on the hips, hands, legs, and face which reached a maximum on day 11. The symptoms disappeared after application of calcium and antihistamines within the next 2 days. Due to the severe side reactions observed in the two individuals of group 3, the planned revaccination of group 2, 2 years postvaccination, was dispensed with.

DISCUSSION

This is the first study to show long-range antibody profiles of individuals 1, 1.5, and 2 years after vaccination with HDCV. In recent publications (1, 3, 4, 14) the early response to vaccinations after i.d. and intramuscular ad-

ministration of HDCV to humans was demonstrated. The results were in accord with ours on the following points: HDCV applied to humans elicited a good antibody response, multiple injections induced a high antibody level, a single booster inoculation given to a previously vaccinated subject resulted in the rapid formation of high-titered antibodies.

The i.d. inoculation required less vaccine than is recommended for intramuscular or subcutaneous administration; however, comparable titers were achieved. Although a single inoculation resulted in a 100% conversion rate, multiple injections were necessary for the persistence of high titering antibodies. The best results were obtained after a primary course of four injections given over 28 days (group 2). A reduction in the number of primary inoculations (groups 3 and 4) resulted in a lower antibody response (Fig. 5).

A single booster inoculation in subjects previously vaccinated with other types of antirabies vaccines resulted in a rapid increase of virus neutralizing antibodies, reaching a maximum after 14 days. The average titer was 15 IU/ml,

FIG. 4. Antibody profile of group 4, consisting of 12 individuals who received a primary course of one inoculation of 0.2 ml i.d., and a booster inoculation of 0.2 ml i.d. on day 28. A further booster inoculation of 0.2 ml i.d. was given 1 year postvaccination. Explanation of profile as in Fig. 1.

FIG. 5. A composite of the antibody profiles of groups 1 through 4. The effect of the booster inoculation given to groups 3 and 4 is indicated by the interrupted lines.

comparing well with the results obtained by Cabasso et al. (4) in a similar study. The same effect was observed when individuals of group 4 with the lowest antibody response observed in this study received a single booster inoculation 1 year later (Fig. 4). The antibody levels were threefold higher than the maximum levels previously observed in this group.

During the primary course of i.d. inoculations with HDCV no adverse side reactions were reported. However, after the 1- and 1.5-year booster inoculation, 7 of 14 individuals showed slight-to-moderate local reactions at the site of inoculation. Of the 10 individuals who received a HDCV booster after primary vaccination with other types of antirabies vaccines, only one reported a local reaction. This individual was known to be subject to allergic types of reactions.

In addition to local reactions, two individuals from group 3 experienced symptoms resembling either a delayed type of hypersensitivity reaction or a form of serum sickness after booster inoculation. It is not known whether the i.d. injections given during the primary course of vaccination may sensitize an individual against certain components of the vaccine so that a booster immunization at a later date elicits the appear-

ance of such reactions. In a similar vaccination study using HDCV administered intramuscularly, 10 individuals who received a booster inoculation 1 year after the primary course of injections experienced no adverse side reactions (T. J. Wiktor, personal communication). Until this problem has been fully resolved, multiple i.d. injections of rabies vaccine should be regarded as a potential hazard. We therefore recommend that the intramuscular and subcutaneous routes continue to be used for primary vaccinations, and that the i.d. administration of vaccine be restricted to booster inoculations where it has been shown to be extremely effective.

In conclusion it can be stated that regardless of the primary schedule of inoculations and the antibody titer present at the time of booster inoculation, a single 0.2-ml i.d. injection is sufficient to stimulate the development of high-titered virus neutralizing antibodies. In case of actual exposure to the virus, a single booster inoculation would seem to be sufficient for the postexposure treatment of man. Our findings strongly support a similar proposal recently recommended by the WHO Expert Committee on Rabies (12).

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