Antibody and Immunoglobulin Response to Antirabies Vaccination in Man

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Serum neutralization antibody and immunoglobulin responses in 30 individuals were studied in paired serum samples which had been obtained before and 2 to 3 weeks after the administration of a recall dose of rabies tissue culture vaccine. The immune reaction consisted of a predominantly immunoglobulin G (IgG) antibody response. Also, a significant increase in neutralizing antibody titers was observed, but without a consistently correlated change of the IgG levels in the individual serum samples. The radial immunodiffusion test appears to contain the elements necessary for the development of a routine test to determine rabies antibody in single serum samples.

Antigenic stimulation caused by diseases of bacterial, viral, or even protozoan origin, or the immunization induced by appropriate vaccines, are both associated with increased immunoglobulin synthesis. In infectious diseases, the classes of immunoglobulin G and M (IgG and IgM, respectively) are known to predominate (5, 7, 8, 11, 14). After primary stimulation, the first serum antibody detected is IgM, which is gradually replaced in succeeding weeks by IgG. In contrast, the immediate response to secondary antigenic stimulation is predominantly production of IgG.

However, the sequence of immunoglobulin response can be altered by the nature and dosage of antigen (3, 4, 9), the use of adjuvant, and the route and timing of administration (16). The immunoglobulin response to antirabies vaccination has not been sufficiently investigated, although Rubin et al. (12) reported recently on responses in human sera to primary vaccination with duck embryo vaccine and found that the IgM antibody response was prolonged for at least 41 days.

Our serological investigations had three main objectives: (i) to establish the nature of immunoglobulin response to a recall dose of tissue culture rabies vaccine developed for preexposure immunization of persons in the high-risk category (6); (ii) to establish whether correlation exists between increases of immunoglobulin and those of neutralizing antibody titers in mice; and (iii) to assess the feasibility of developing a simple, rapid, inexpensive replacement test for the expensive and time-consuming neutralization test.

MATERIALS AND METHODS

Vaccine. The antirabies vaccine used in this study was one of tissue culture origin, produced in primary hamster kidney cells, inactivated with formaldehyde, and adjuvated with aluminum phosphate (1 mg/ml). A recall dose (1.0 ml/immunization) was given to a group of 30 students who 2 years previously received a primary preexposure rabies immunization course consisting of three doses of the above vaccine given 1 month apart.

Serum samples. Paired blood samples were collected from the vaccinees before and 2 to 3 weeks after the recall dose. The sera were separated, inactivated, and kept frozen until the performance of the tests.

Neutralization test. Serum neutralizing antibody titers were determined by intracerebral inoculation of mice with the mixture of serum dilutions and standard rabies virus strain (CVS) according to the method of the World Health Organization (2).

Determination of immunoglobulin levels. Radial immunodiffusion plates (Meloy Labs, Springfield, Va.) were used to quantify the level of immunoglobulins in the serum samples. For controls, different concentrations of reference human IgG or IgM preparations (Meloy Labs, Springfield, Va.) were employed. For the determination of IgG levels, 1:5 dilutions of the serum samples were applied to the plate, whereas for the determination of IgM levels undiluted samples were used.

Nonspecific antigens. Since the vaccine used may have contained traces of bovine serum and hamster kidney cell antigen, whereas nonspecific antigens from other sources would have been negligible, the 30 postrecall serum samples were examined by an Ouchterlony test for the possible presence of antibody against the above mentioned antigens.

Controls. To demonstrate the specificity of the test, two groups of control sera were included: (i) eight sera from persons whose blood samples were taken 2 to 3 weeks after recall rabies vaccination, and five
samples from persons never vaccinated before. Portions of these samples were absorbed with suspensions of rabies-infected mouse brain (RMB), with Western equine encephalitis-infected mouse brain (WMB), or finally with normal mouse brain (NMB). IgG and IgM levels were determined as described above, and the differences among the three serum portions were evaluated. (ii) The effect of adjuvant alone on the immunoglobulin levels was examined in six paired samples taken from persons who regularly received recall vaccination. These samples were taken prior to and 2 weeks after an intramuscular injection of 1 ml of T.C. medium containing 1 mg of aluminum phosphate.

Statistical comparisons were based on a standard t-test for paired data (13).

RESULTS

Prerecall neutralizing antibody titers ranged from <1/5 to 1/56; after the administration of the recall dose they had increased to the range of 1/11 to 1/279. The enhancing effect of the recall dose was evident and found to be highly significant ($P < .001$), with a mean increase in reciprocal titer of $50.6 \pm 11.1$ (standard error) (Fig. 1).

Regarding the total serum IgG levels, the prerecall values ranged from 350 to 1,700 mg/100 ml; after the recall dose, they had increased to the range of 625 to 1,880 mg/100 ml (Fig. 2). The mean increase was $207 (\pm 41)$ mg/100 ml or $23.9 (\pm 4.8)\%$. This increase was found to be highly significant ($P < .001$).

The prerecall dose total serum IgM levels ranged from 30 to 240 mg/100 ml; after the administration of the recall dose, they were found in the range of 28 to 280 mg/100 ml (Fig. 3).

Although a mean increase of $5.0 (\pm 5.2)$ mg/100 ml and a mean percent increase of $4.4 (\pm 4.6)\%$ could be calculated, this did not prove to be statistically significant. In fact in 8 out of the 30 postrecall dose samples the IgM levels had decreased.

The same 30 samples proved to be negative in the Ouchterlony test, indicating that the vaccine did not induce the production of detectable antiovine or anti-hamster precipitating antibody.

The specificity of the radial immunodiffusion
The effect of absorption with infected or control mouse brain on the levels of IgG and IgM in serum samples of vaccinated people

<table>
<thead>
<tr>
<th>Absorbing agents</th>
<th>Mean difference ± standard error</th>
<th>Probability</th>
<th>Absorbing agents</th>
<th>Mean difference ± standard error</th>
<th>Probability</th>
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<tbody>
<tr>
<td></td>
<td>IgG</td>
<td></td>
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<td></td>
<td>mg/100 ml</td>
<td>%</td>
<td>Probability</td>
<td>mg/100 ml</td>
<td>%</td>
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<td>NMB abs minus RMB abs</td>
<td>165 ± 27.0</td>
<td>14.6 ± 2.4</td>
<td>&lt;0.001</td>
<td>11 ± 6.5</td>
<td>5.8 ± 3.4</td>
</tr>
<tr>
<td>WMB abs minus RMB abs</td>
<td>151 ± 23.0</td>
<td>13.5 ± 2.0</td>
<td>&lt;0.005</td>
<td>18.3 ± 7.2</td>
<td>9.6 ± 3.7</td>
</tr>
</tbody>
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Abbreviations: NMB, normal mouse brain; RMB, rabies-infected mouse brain; WMB, western equine encephalitis-infected mouse brain; abs, absorbed; NS = not significant.

test was evaluated in the control groups. After absorption with RMB, as shown in Table 1, the eight vaccinated control sera had significantly less IgG than when absorbed with WMB (P < .005) or NMB (P < .001), and there was no difference between WMB- and NMB-absorbed samples. In the same group, RMB-absorbed sera had significantly less IgM than when absorbed with WMB (P < .05). A difference in IgM level that was consistent with expectation, but not significant, was found between RMB- and NMB-absorbed samples; again there was no difference between WMB- and NMB-absorbed sera.

In the unvaccinated group, the levels of immunoglobulins were not differentially affected by absorption with any of the above brain suspensions.

In the pre- and postinjection serum samples of the six primed persons treated with adjuvant alone, the radial immunodiffusion test did not reveal any difference between immunoglobulin levels. This was considered as a strong indication that administration of adjuvant without antigen does not effect changes in IgG or IgM concentration.

DISCUSSION

To determine the immune response after antirabies vaccination, antibody in human sera is titrated mainly in the intracerebral inoculation of serum-virus mixtures into mice. By this technique alone, however, the nature of protection cannot be demonstrated in its entirety because of the heterogeneity of the antibody involved (10) and because the result does not indicate the quantitative involvement of the different classes of immunoglobulins.

By using radial immunodiffusion technique, after recall immunization, the mean increase of IgG levels in the sera of 30 persons has been determined as 23.9% and found to be highly significant. This increase is far above the average individual variation of IgG levels (1), and it obviously represents a response to the administration of the recall dose. In contrast, the IgM levels were not found to be significantly altered.

The nature of the antigen and the use of an adjuvant affect not only the degree of increase in immune response, but may also modify the sequential appearance of the different classes of immunoglobulins (10, 16). Since the administration of adjuvant alone to primed persons did not affect the levels of immunoglobulins, it is reasonable to assume that the adjuvanted vaccine used in this study, beside being highly immunogenic, also provided the conditions suitable for the induction of a predominantly IgG antibody response.

The validity of any serological study depends on proof of its specificity, which in this case is rendered in part by the negative outcome of the Ouchterlony test for nonspecific antibody and by the lack of any effect of the adjuvant when given alone.

The fact that there is a significant decrease in the immunoglobulins of postrecall sera after absorption with specific rabies-antigen (RMB) and the absence of such an effect when a nonspecific antigen (WMB) is used strongly suggest the specific nature of the immunodiffusion test used in this study.

In the individual postrecall serum samples, the correlation coefficient for the percent change in IgG levels versus neutralizing antibody titers was 0.02, which is not significant. It is therefore likely that changes in neutralizing antibody titer are not consistently paralleled by changes in levels of IgG. Such a phenomenon could be explained by the fact that it is not only the level of IgG, but also the avidity of the molecules that determines the value of the neutralization titer (10, 15).

Based on our study, it appears that the degree of response to a recall dose can be assessed by using this radial immunodiffusion technique, provided paired serum samples are available. Additional study is needed to develop further the described method so as to allow the determina-
tion of the degree of protection against rabies in a single serum sample. This seems to be possible by the application of a comparative assay in which an unabsorbed part of the serum is tested against another portion, absorbed with viral antigen.

LITERATURE CITED


