Differential Immune Response to Attenuated Rubella Virus Vaccine

JIBAN D. GUPTA, VINCENT J. PETERSON, AND ALAN M. MURPHY

Children's Medical Research Foundation, Royal Alexandra Hospital for Children, Camperdown, N.S.W. 2050, Australia, and Institute of Clinical Pathology and Medical Research, Lidcombe, N.S.W. 2141, Australia

Received for publication 6 September 1971

Serum samples collected from 18 volunteers 1, 2, and 3 months after vaccination with Cendehill strain of attenuated rubella virus vaccine were fractionated on a Sephadex G-200 column to test for immunoglobulin M (IgM) antibody. Seventeen of the 18 had demonstrable IgM antibody in serum collected 1 month postvaccination whereas only 5 and 3 had IgM antibody 2 and 3 months, respectively, post-inoculation. The geometric mean titers of hemagglutination-inhibiting antibody in the 19S fraction were 38, 7, and 6 at 1, 2, and 3 months after vaccination, whereas the corresponding 7S titers were 29, 53, and 57, respectively. Four other sero-negative volunteers vaccinated with Cendevax were tested serially for total antibody only in whole blood obtained by finger pricks. Three of them showed appearance of antibody between 14 and 17 days and one between 17 and 21 days. It appears that vaccine-induced immune response is similar to natural infection especially with regard to the time of appearance of antibody and the relative proportions of IgM and IgG antibody thus produced.

Vaccine-induced antibody response differs from natural infection in that a much lower antibody titer is obtained on vaccination (3, 11). Also, much weaker complement-fixing antibody and fluorescent antibody, detected only in particular cell lines, are produced on vaccination (2, 12). Although the time sequence of appearance and disappearance of immunoglobulin M (IgM) antibody on natural infection has been studied (1, 8), no such study has been made on antibody produced on vaccination. Our previous work (8) on the separation of IgM and immunoglobulin G (IgG) antibody by fractionation of convalescent and immune sera on a Sephadex G-200 column, coupled with rubella hemagglutination inhibition (HI) test in the fractions thus obtained, prompted us to use the same techniques to detect IgM antibody in sera collected 1, 2, and 3 months after vaccination. Any differences observed as compared to natural infection could thus provide a marker for vaccine-induced antibody.

MATERIALS AND METHODS

Vaccines. Forty-five sero-negative volunteers were vaccinated for a clinical trial (J. M. Forrest et al., Med. J. Aust., 2:470, 1971) of the attenuated rubella virus vaccine, Cendevax (Smith Kline & French). Sera were collected from each person, all of whom had sero-converted, 1, 2, and 3 months after vaccination. Out of these 45, 18 sets of sera were used for Sephadex G-200 fractionation for detection of IgM antibody, the only criterion of selection being the availability of enough of serum for fractionation of the Sephadex column.

Fractionation of serum. A 2-ml amount of each serum sample was fractionated on a Sephadex G-200 column (2.5 by 40 cm), and 3-ml fractions were collected. Because we used formalinized sheep red blood cells (formalinized-SRBC) in N-2-hydroxy ethylpiperazine-N’2’-ethane-sulfonic acid (HEPES) buffer (5, 6) for the rubella HI test, we used the same buffer but without albumin as eluant for fractionation. The tubes under the ascending and descending parts of the 19S peak were pooled to obtain fractions 1 and 2, respectively, and the corresponding tubes of the 7S peak were designated fractions 3 and 4. Fractions 1 and 2 were diluted, if necessary, to 12 ml and fractions 3 and 4 to 16 ml with the same HEPES buffer, so that they were initially diluted 1:6 and 1:8, respectively, with respect to the original volume of serum.

Rubella antibody determination. HI titer in each fraction was determined by using formalinized sheep erythrocytes (5, 6) in the HI test instead of avian erythrocytes. A 0.3-ml amount of each fraction was treated with 0.1 ml of heparin-manganese chloride mixture, prepared by adding equal volumes of heparin (750 units/ml) and 0.2 M manganese chloride. After 0.5 hr in the cold with intermittent shaking, 0.1 ml of 25% formalinized-SRBC in HEPES, and an addi-
tional 0.1 ml of HEPES buffer in the case of fractions 3 and 4 only, were added. The mixture was then treated as described previously (5, 6) for rubella antibody titration. The initial dilution of fractions 1 and 2 was thus 1:10, and that of 3 and 4 was 1:16 with respect to the original volume of serum fractionated.

Antibody determination in whole blood obtained 10, 14, 17, 21, 28, and 60 days after vaccination was done as described by Gupta and Peterson (7). A 0.1-ml amount of capillary blood obtained by finger prick was added to 0.2 ml of heparin-manganese chloride, kept in the cold for 0.5 hr, and shaken intermittently, followed by addition of 0.2 ml of 12.5% formalinized-SRBC in HEPES. This mixture was then treated in the same way as the fractions, but antibody was now titrated at an initial dilution of 1:5 with respect to the whole blood.

Antibody titer in all cases was expressed as the reciprocal of that dilution which completely inhibited agglutination by rubella hemagglutination antigen.

Mercaptoethanol treatment. A 0.1-ml amount of serum or 0.3 ml of fractions were incubated at room temperature for 5 hr with one-tenth volume of 2 mM mercaptoethanol. An equivalent amount of buffer was added to control fractions. These mixtures were then tested for HI antibody as described before, ignoring the dilution due to the addition of mercaptoethanol.

RESULTS

Serial HI antibody titration in whole blood in four vaccinated persons is shown in Fig. 1. Three of the four showed detectable antibody between 14 and 17 days and one between 19 and 21 days. In three of them, highest antibody levels were attained by 28 days, and there was no increase in antibody titers in samples collected 2 months after vaccination.

On fractionation of serum on a Sephadex G-200 column using HEPES buffer as eluant, three well-defined peaks corresponding to 19S and 7S globulins and albumin are obtained; the separation characteristics of sera are not affected by using HEPES buffer as eluant instead of the commonly used tris(hydroxymethyl)aminomethane-sodium chloride for such fractionation. In Fig. 2, the manner in which tubes under the first two peaks were pooled to obtain four fractions from each serum is shown.

Figure 3 represents the distribution of HI antibody, in fraction 1 only, from each of the eighteen sets of serum samples collected 1, 2, and 3 months after vaccination. Antibody was detected in this fraction from 17, 5, and 3 samples of serum collected 1, 2, and 3 months postvaccination, respectively. Two of the three persons who had the highest level of antibody in fraction

![Fig. 1. Hemagglutination-inhibiting (HI) titer of whole blood collected serially from four vaccinees.](http://iai.asm.org/)

![Fig. 2. Sephadex G-200 fractionation of a vaccinee's serum with HEPES buffer used as an eluant. Four fractions were obtained from each serum as indicated.](http://iai.asm.org/)

![Fig. 3. Hemagglutination-inhibiting (HI) titer of fraction 1 obtained from all the sera collected 1, 2, and 3 months after vaccination.](http://iai.asm.org/)
1 (80) as well as in the serum itself (320) in 1-month samples continued to have high antibody in fraction 1 even at 2 and 3 months. One of them was tested 6 months after vaccination and still had comparable HI antibody in fraction 1. That the antibody in fraction 1 is IgM was confirmed by complete abolition of antibody activity in this fraction on treatment with mercaptoethanol. The effect of mercaptoethanol treatment on the reduction of antibody titers of the four fractions obtained from each of the 18 sera collected 1 month postvaccination is shown in Table 1. Only antibody in fractions 1 and 2 was reduced, whereas antibody in fractions 3 and 4 was unaffected. On mercaptoethanol treatment of whole sera, at least fourfold reduction in titer was obtained in only 3 out of 18 sera tested. This was indicative of the lack of sensitivity of such treatment for demonstration of the presence of IgM antibody in whole serum.

Table 2 shows the distribution of HI titers in all serum samples collected 1, 2, and 3 months postvaccination, in their four fractions, and also in 19 and 75S fractions. The antibody titer of the 19S fraction was obtained by the addition of the mean geometric titers of fractions 1 and 2. Antibody titer of the 7S fraction was obtained similarly from fractions 3 and 4. One month after vaccination, 19S antibody was about equal to 7S antibody, and it then decreased to low levels at 2 and 3 months. On the other hand, 7S antibody increased to almost twice the 1-month level at 2 months, and only slightly thereafter.

**Table 1. Reduction of hemagglutination-inhibiting (HI) antibody titer of 18 sera collected 1 month after vaccination, and of their fractions (Fr) obtained by Sephadex G-200 gel filtration, on treatment with mercaptoethanol**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Reduction of antibody titer (no. positive/no. tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Completeb</td>
</tr>
<tr>
<td>Serum</td>
<td>0/18</td>
</tr>
<tr>
<td>Fr1</td>
<td>17/17</td>
</tr>
<tr>
<td>Fr2</td>
<td>15/17</td>
</tr>
<tr>
<td>Fr3</td>
<td>0/17</td>
</tr>
<tr>
<td>Fr4</td>
<td>0/17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>Partialc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>3/18</td>
</tr>
<tr>
<td>Fr1</td>
<td>0/17</td>
</tr>
<tr>
<td>Fr2</td>
<td>2/17</td>
</tr>
<tr>
<td>Fr3</td>
<td>0/17</td>
</tr>
<tr>
<td>Fr4</td>
<td>0/17</td>
</tr>
</tbody>
</table>

* One serum sample with HI titer of 20 did not have any measurable antibody in any of its four fractions, probably due to an even distribution in all fractions.

* Reduced to a titer of <10 in the case of fractions 1 and 2 and <16 in the case of fractions 3 and 4.

* Fourfold or higher reduction, but not to a negative titer.

**Table 2. Hemagglutination-inhibiting (HI) antibody titer in sera collected 1, 2, and 3 months after vaccination and in their fractions (Fr) obtained by Sephadex G-200 gel filtration**

<table>
<thead>
<tr>
<th>Months after vaccination</th>
<th>Geometric meana HI titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Fr1</td>
</tr>
<tr>
<td>1</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>79</td>
</tr>
</tbody>
</table>

a In calculating geometric mean titer, the negative titers were taken as zero.

**DISCUSSION**

The appearance of antibody between 14 and 21 days after vaccination is similar to that in natural infection. In natural infection, there is an asymptomatic incubation period of about 2 weeks after which rubelliform rash and clinical symptoms appear; antibody is detected a few days thereafter. Initial trials of different vaccines had also shown a similar time course of appearance of antibody. Our observations thus also point to the suitability of rubella antibody determination in whole blood for testing the efficiency of vaccine in eliciting antibody response. It should be emphasized, however, that the whole blood titer is generally half of the corresponding serum antibody titer (7) as would be expected from the approximately 1:1 ratio of cells to fluid in whole blood.

Although various studies have been done on the overall antibody response on vaccination, not much work has been reported on the type of immunoglobulin antibody thus produced. By using immunofluorescence techniques in rubella virus-infected BHK21 cells, Brown et al. (2) demonstrated IgM antibody in 9 out of 13 sera collected 6 weeks after vaccination. We reported earlier the suitability of Sephadex G-200 fractionation of serum coupled with rubella HI test for the detection of rubella-specific IgM antibody (8). The same technique has been utilized here to demonstrate the presence of IgM antibody in the sera of vaccinees. Out of 18 sera collected 1 month after vaccination, 17 showed the presence of antibody in fraction 1 which was completely reduced on mercaptoethanol treatment. Although antibody in fraction 1 does not reflect the absolute amount of IgM antibody present in the serum, it gives a semiquantitative estimate of this type of antibody with respect to the total antibody. From the titers obtained with fractions 1 and 2 and from the result of mercaptoethanol treatment, it appears that IgM antibody, when present in vaccinees' sera, is about equally distributed in frac-
rubella appears to be readily explained, in IgG immune sera. In most cases, it was partially reduced (unpublished data). Similarly, even though in naturally immune serum there was no antibody in fraction 1, there was almost always some antibody in fraction 2. Both of these observations can be explained on the basis of much higher antibody titers of the convalescent and immune sera as compared to vaccinees' sera resulting in some spillover of IgG antibody in fraction 2. But it is also conceivable that fraction 2, in the case of natural infection, may contain specific immunoglobulin A (IgA) antibody which cannot be differentiated by the technique we used. It has recently been reported (4, 10) that the incidence of reinfection in vaccinated persons is much higher than in persons with naturally acquired antibody; this may be explained as due to local immunity provided by IgA antibody in natural infection. Our results only indirectly point to the possibility of IgA antibody being absent in the sera of vaccinees and being present in naturally immune sera.

In spite of this suggestive difference, the IgM response on vaccination is very similar to that of natural rubella infection. In natural infection, IgM antibody can generally be demonstrated in sera collected within 4 weeks after the appearance of rubella rash (1, 8); similarly, on vaccination also, IgM antibody in most cases disappears between 1 and 2 months with a concomitant increase in IgG antibody. The continued IgM response at an appreciable level in two subjects cannot be readily explained, but such prolonged response on natural infection has also been reported (9). Overall, the immune response on vaccination appears to be qualitatively similar to natural rubella infection, especially with reference to the variation of relative proportion of IgM and IgG antibody with time after vaccination.

ACKNOWLEDGMENTS

This investigation was supported by a grant from the National Health and Medical Research Council of Australia. We thank ATN channel 7, Sydney, for providing a fellowship for one of us (J. D. G.).

We are indebted to Jill Forrest and Margaret Menzer for the vaccinees' sera.

LITERATURE CITED