Serological Response to Cholera Revaccination in a Semi-closed Community in Calcutta*

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A. H. ABOU-GAREEB,2 & D. L. SHRIVASTAVA4

Serological response to cholera revaccination has been studied in a semi-closed community consisting of individuals mostly in the 2–20-years age-group. The subjects had been inoculated against cholera every year at the beginning of the local epidemic season. Pre- and post-vaccination sera were obtained from 29 subjects inoculated with cholera vaccine (test group) and 28 from subjects inoculated with TAB vaccine (control group). These sera were tested for vibriocidal and agglutination titres. The geometrical means of the vibriocidal and agglutination titres of the post-vaccination sera in the test group rose by 490% and 463% respectively. This booster effect was observed mostly in individuals in the 2–14-years age-group, who had low titres (vibriocidal <320 and agglutination <40) in their prevaccination samples. Revaccination, therefore, appears to be useful as a booster for individuals having low titres.

As an adjunct to the studies of cholera carriers and the serological investigations early in 1967, the serological response to revaccination against cholera was studied in a semi-closed community consisting of about 100 persons, most of whom were between 2 and 20 years of age. The movements of these persons were restricted and they were not permitted to take any food or drink outside the community. Some of the children were allowed to go out once a year, however, to meet their relations, who were usually of low socio-economic status. The inmates had regular medical examinations and they were inoculated against cholera every year at the beginning of the local cholera season; the interval between the previous inoculation and the present study was, therefore, about a year. Their personal hygiene was regularly supervised by the authorities and was satisfactory.

None of the single stool samples obtained showed evidence of excretion of vibrios. Pre- and post-vaccination samples of blood were obtained from as many of the subjects as possible, and this paper presents data concerning the titres of agglutinating and vibriocidal antibodies in these sera.

MATERIALS AND METHODS

Immunization

Alternate members of the community were given cholera vaccine (test group) and TAB vaccine (control group). Both the vaccines had been manufactured by the West Bengal Government Vaccine Laboratory, and were the ones normally used for mass immunization in the State. The cholera vaccine was the conventional agar-grown, phenol-killed and phenol-preserved vaccine and contained 4×10⁸ organisms per ml of each of the Inaba and Ogawa serotypes. The dosage schedule was the same as that followed in the cholera vaccine trial in 1964 (Das Gupta et al., 1967).

Sera

A sample of blood was taken from each individual just before administration of the vaccine. Another sample was collected 11–13 days after vaccination.

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when the titres were expected to be at their peak levels. The sera were separated, filtered through Millipore membrane filters (0.45 μ) and preserved in sealed ampoules at −20°C. No preservative was used. Only 57 pairs of sera were available for serological studies, 29 in the test group and 28 in the control group. The test group comprised 20 sera from individuals aged 2–14 years and 9 from individuals aged 15 years or over. The corresponding figures for the control group were 24 and 4, respectively.

Antigens

Two strains of *Vibrio cholerae*, Ogawa 465 and Inaba 22463, were used as antigens. Both of these were from the stock strains preserved in stab cultures at this Centre.

Complement

Lyophilized guinea-pig complement obtained from Markham Laboratories (Chicago, Ill., USA) was employed.

Vibriocidal test

A microtechnique (Benenson et al., 1968a, 1968b) was used as already described in an earlier publication (Deb et al., 1969).

Agglutination test

The usual tube dilution method was used as well as the microtechnique (Benenson et al., 1968a, 1968b) for determining agglutination titres. The results were practically the same by both the methods.

The microtechnique was carried out with the following slight modification. Sera were not inactivated. Micropipettes were used to dispense 0.025 ml of normal saline into each cup of the Microtiter plates (U-shaped). The serum was diluted 1 : 5 in normal saline and 0.025 ml of this was added to the first cup. Twofold serial dilutions were made with help of 0.025-ml microdilutors and 0.025 ml of *V. cholerae* suspension in saline was added to each cup. The cultures had been grown for 18 h on heart-infusion agar slants, and were subsequently adjusted to an opacity reading of 30 on the scale of the Klett Summersorn colorimeter using green filter No. 54. The final dilution of serum in the first cup was thus 1 : 20. The plates were sealed with sealing tape and incubated at 41°C in the water-bath for 1 hour. Readings were taken after keeping the plates overnight at 4°C. Both in the vibriocidal and the agglutination titrations, a rise or fall of less than 4-fold was not considered to be significant.

RESULTS

The numbers of sera with high and low titres, both before and after vaccination, are shown in Table 1. The prevaccination vibriocidal and agglutination titres ranged from <20 to 5120 and from <20 to 640, respectively. In both the pre- and post-vaccination samples of sera in the control group, there were equal numbers in the low (<320) and high (>640) vibriocidal titre ranges (14). In the test group, however, the corresponding figures in the prevaccination samples were 17 (58.6%) and 12 (41.4%) and in the post-vaccination samples 7 (24.1%) and 22 (75.9%), respectively, in the low and high titre ranges. The geometrical means of the pre- and post-vaccination reciprocal titres in the test group were 119.4 and 704.7, respectively, as compared with 193.6 and 167.1, respectively, in the control group. Prevaccination agglutination titres in the control group were equally distributed in the low titre (<40) and high titre (>80) ranges. This distribution did not change after vaccination. In the test group, however, pre- and post-vaccination figures in the low titre range were 11 (37.9%) and 4 (13.8%), respectively. The corresponding figures in the high titre range were, respectively, 18 (62.1%) and 25 (86.2%). The geometric means of pre- and post-vaccination reciprocal titres were 56.4 and 317.7, respectively, in the test group and 25.9 and 30.3, respectively, in the control group.

An analysis of the geometric means of the vibriocidal and agglutination titres (reciprocals) by age-groups (2–14 years and >15 years) is shown in Table 2. In the younger age-group, after inoculation with cholera vaccine, the geometric mean of the post-vaccination vibriocidal titres rose by 646% compared with the rise of 251% in the older age-group. The corresponding percentage rises in geometric means of agglutination titres were 727 and 139 in the two age-groups. It will further be noted that in the younger age-group the geometric means of prevaccination titres were somewhat lower than those in the older age-group.

Table 3 shows the percentage distribution of the sera, of both the test and control groups, that showed vibriocidal response in the post-vaccination samples against both Ogawa and Inaba antigens. It will be seen that in the test group a 4-fold or greater rise of antibody levels against both Ogawa and Inaba

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SEROLOGICAL RESPONSE TO CHOLERA REVACCINATION

TABLE 1
NUMBERS OF SERA WITH HIGH AND LOW TITRESa BEFORE AND AFTER VACCINATION

<table>
<thead>
<tr>
<th>Titre</th>
<th>Control group</th>
<th>Test group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-vaccination</td>
<td>Post-vaccination</td>
</tr>
<tr>
<td>Vibriocidal titre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥320</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>≤640</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Geometrical mean titres</td>
<td>193.6</td>
<td>167.1</td>
</tr>
</tbody>
</table>

| Agglutination titre |          | | | |
|≥40                  | 14         | 14          | 11            | 4             |
|≤80                  | 14         | 14          | 18            | 25            |
| Geometrical mean titres | 25.9 | 30.3 | 56.4 | 317.7 |

a The titres, shown as reciprocals, were determined against both Ogawa and Inaba antigens, the higher of the two titres (usually against the former) being shown in the table.

antigens was observed in 41% (12 out of 29) and 38% (11 out of 29) of the sera, respectively. Of these, 9 sera (31%) were from individuals in the 2-14-year age-group. One serum in the control group showed a 4-fold or greater rise against Ogawa and 2 showed similar rises against Inaba: these rises may have been due to an infection with cholera just before the first bleeding or immediately after it, that remained undetected by the single stool sampling procedure.

On the other hand, a 4-fold or greater fall in titre was observed in 14% and 11% of sera of the control group against the Ogawa and Inaba antigens, respectively, compared with none in the test group. Of the sera in the control group, 82% had no significant change in titre as against 59% and 62% in the test group against Ogawa and Inaba, respectively. It is also evident that there was hardly any difference in the figures between the two antigen groups. This may be due to the presence of both the antigens in the cholera vaccine.

The percentage distribution of the agglutination responses of the post-vaccination serum samples, of the test and control groups, against both antigens,

TABLE 2
GEOMETRIC MEANS OF VIBRIOCIDAL AND AGGLUTINATION TITRES (RECIPROCALS) BY AGE-GROUPa

<table>
<thead>
<tr>
<th>Age-group (years)</th>
<th>Test group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vibriocidal titre</td>
<td>Agglutination titre</td>
</tr>
<tr>
<td></td>
<td>Pre-vaccination</td>
<td>Post-vaccination</td>
</tr>
<tr>
<td>2-14</td>
<td>106.9 (20)</td>
<td>798.0 (20)</td>
</tr>
<tr>
<td>&gt;15</td>
<td>152.4 (9)</td>
<td>535.8 (9)</td>
</tr>
</tbody>
</table>

a Figures in parentheses indicate the numbers of individuals whose sera were tested.
is shown in Table 4. In the test group, there was a 4-fold or greater rise in agglutination titres in 59% (17 out of 29) of sera against the Ogawa and the Inaba antigens, compared with 11% in the control group. The 17 sera in the test group that showed a 4-fold or greater rise in titre included 14 in the 2–14 years age-group and 3 in the 15-years and over age-group. In the control group 4% of the sera showed a 4-fold or greater fall in titre as compared with 3% in the test group. The titres did not change in 85% of control and 38% of the test-group sera.

Post-vaccination responses of individuals having vibriocidal and agglutination titres in the low and high ranges are shown in Table 5. Since the results against both Ogawa and Inaba were similar, only those against the former are given in this table. It will be seen that 53% of the sera with the low initial vibriocidal titre had a 4-fold or greater rise in titre and 47% showed no change, perhaps due to repeated vaccinations. The corresponding figures for sera having high initial titres were 25% and 75%, respectively. As regards agglutinating antibody, 82% of the sera with a low initial titre exhibited a 4-fold or greater rise as compared with only 44% of the sera in the high initial titre group. In the low initial titre group 18% and in the high titre group 50% did not show any change.

**DISCUSSION**

Pollitzer & Burrows (1959) remarked that “The few observations made in regard to changes in the bactericidal titres as a result of revaccination against cholera gave strikingly divergent results.” Karwatzki (1906) noted a marked increase in the bactericidal power after revaccination. On the other hand, Ahuja & Singh (1948) stated that injection of a booster dose did not increase the bactericidal power of the serum to any marked extent. Goodner & Smith (1965) observed that “the booster effect was obtained chiefly in persons having little persistent antibody.” Recently Mosley et al. (1969) carried out random serological surveys of children aged between 3 months and 14 years and showed that a single dose of vaccine acts as booster.

Although the data presented in the present communication are not sufficient to draw any definite conclusions, some indications of the effect of revaccination against cholera on the circulating vibriocidal
and agglutinating antibodies are, however, available. Since complete records were maintained for each individual in the community in which this investigation was carried out, the information with regard to immunization against cholera in previous years was reliable. This was further confirmed by the initial serological titres (Table 1) which may be assumed to be residual titres. The geometrical means of the initial vibriocidal titres were 193.6 and 119.4 in the control and test groups, much higher than the figure of 17 reported by Mosley et al. (1969).

The rise in the geometrical mean reported by Mosley et al. (1969) was 337% after inoculation with a commercial vaccine. The rise reported here was mostly in the 2-14-years age-group (Table 2). An analysis of the vibriocidal titres showed that in the test group, 12 sera (41%) exhibited a 4-fold or greater rise against Ogawa and 11 (38%) sera showed a similar rise against Inaba, 9 of these being in the 2-14-years age-group: the corresponding figures for the control group were 1 (4%) and 2 (7%). In the control group, 4 sera (14%) showed a 4-fold or greater fall in titre against Ogawa and 3 (11%) showed a similar fall against Inaba as against none in the test group. Similar results will be observed in the agglutination titres, the geometrical means of which increased from 56.4 to 317.7 (463%). Incidentally there does not appear to be any appreciable anamnestic response due to TAB vaccine as far as titres against V. cholerae are concerned.

It will further be noted (Table 5) that in the test group 53% (9) and 82% (8) of the sera in the low titre group showed a 4-fold or greater rise in the vibriocidal and agglutination titres respectively, whereas only 25% (3) and 44% (8) in the high titre group showed such rises. This indicates that the main booster effect of revaccination against cholera is usually in those individuals whose vibriocidal and agglutination titres are in the lower ranges. On the other hand, most of the people who had higher titres did not appear to have had a significant rise in their post-vaccination titres. Therefore, if circulating antibodies may be taken as an index of immunity against cholera (Mosley et al., 1969), revaccination would appear to be useful for low titre as well as high titre individuals, as a booster for the former, particularly in the lower age-group. This, however, may not protect individuals against developing asymptomatic infection with cholera, i.e., the carrier state (Sinha et al., 1967; Joint ICMR-GWB-WHO Cholera Study Group, Calcutta, India, 1970).

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RÉSUMÉ

RÉPONSE SÉROLOGIQUE À LA REVACCINATION CONTRE LE CHOLÉRA DANS UNE COMMUNAUTÉ SEMI-FERMÉE DE CALCUTTA

Les réactions sérologiques provoquées par une revaccination anticholérique ont été étudiées dans une petite communauté indienne groupant environ 100 personnes âgées pour la plupart de 2 à 20 ans. Ses membres faisaient l'objet d'une surveillance médicale régulière et étaient vaccinés chaque année contre le choléra avant le début de la période propice aux épidémies de cette affection. Pour la présente étude, on a administré à la moitié d'entre eux une injection de vaccin anticholérique (sérotypes Ogawa et Inaba) tandis que l'autre moitié (groupe témoin) recevait du vaccin TAB. Vingt-neuf paires de sérums pré- et postvaccinaux ont été prélèvées dans le premier groupe et vingt-huit dans le groupe témoin pour la recherche des anticorps vibriocides et des agglutinines.

On décelait dans 50% des sérums prévaccinaux des titres significatifs d'anticorps résiduels dirigés contre les antigènes Ogawa et Inaba. Chez les sujets revaccinés contre le choléra, les moyennes géométriques des titres d'anticorps vibriocides et agglutinants ont présenté une hausse de 490% et 463% respectivement, alors que dans le groupe témoin la réponse sérologique a été quasiment nulle. L'effet de rappel, marqué par une élévation de quatre fois ou davantage
des titres, a été particulièrement net chez les sujets âgés de 2 à 14 ans chez lesquels les anticorps vibriocides et les agglutinines n'étaient présents qu'à des titres faibles (≤ 1 : 320 et ≤ 1 : 40 respectivement) avant la vaccination.

La revaccination anticholérique apparaît comme une mesure utile, agissant comme une dose de rappel chez les sujets porteurs d'anticorps à des titres faibles et assurant à ceux qui sont déjà relativement immunisés une protection renforcée.

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