Antibody Response to *Chlamydia* Agents: Lack of Immunoglobulin M Antibodies During the Secondary Immune Response

ABDALLAH M. ISA

*Department of Microbiology and Division of Immunobiology, Meharry Medical College, Nashville, Tennessee 37208*

Received for publication 29 November 1972

In previous studies we reported that the antibody response elicited in man (5) and monkey (4) after experimental infection and inoculation with *Chlamydia* agents consisted primarily of immunoglobulin G (IgG) (7S) antibodies. More recently, however, we were able to demonstrate, by immunofluorescence with fluorescein-tagged goat anti-human immunoglobulin (IgG) antisera, the production of high levels of IgM (19S) antibodies after injection of the monkey with a single massive dose of *Chlamydia* agent particles (3).

In the present investigation we present evidence suggesting that the lack of IgM anti-chlamydial antibodies previously reported may have been related to the previous exposure of the host to cross-reacting antigens.

**MATERIALS AND METHODS**

**Animals.** Young (1- to 2-year-old) male and female African green (*Cercopithecus aethiops*) and rhesus monkeys (*Macaca mulatta*) (Primate Imports Co., Port Washington, N.Y.) were employed throughout these experiments.

**Antigens.** The following trachoma and inclusion conjunctivitis (TRIC) strains of *Chlamydia* agents were used for immunization and for slide antigen preparation: (i) TRIC/USA-Cal-15/ON, commonly known as IC Cal 8, a genital strain causing inclusion conjunctivitis and containing 1.2 X 10⁸ particles per gram of yolk sac material; and (ii) TRIC/USA-Aris/Cal-4/OT, commonly known as Ap-2, an ocular strain causing trachoma and containing 6 X 10⁸ particles per gram of yolk sac material.

**Immunization procedure.** Monkeys were anesthetized by the injection of sodium pentobarbital (Abbott Lab., North Chicago, Ill.) prior to immunization and bleeding. Each animal received a primary injection of 0.5 ml of 50% yolk sac material containing 1.5 X 10⁸ to 3 X 10⁸ particles of either IC Cal 8 or Ap-2 intramuscularly. Secondary immunizations consisting of 0.5 ml of 50% yolk sac material containing IC Cal 8 or Ap-2 particles were given 6 weeks after the primary immunization. Animals that received IC Cal 8 for primary immunization received Ap-2 in the secondary immunization and vice versa.

**Antisera.** Monkeys were bled at 3-day intervals for the first 2 weeks after immunization and at variable intervals thereafter. Sera were separated and stored at -20 C until used.

**Sephadex G-200 gel filtration.** Separation of immunoglobulin fractions by gel filtration was described earlier (5).

**Immunofluorescence.** The antibody levels in whole monkey sera and on separated immunoglobulin fractions were estimated by an indirect fluorescent-antibody method that employed fluorescein-tagged antisera specific to human IgM and IgG immunoglobulins (Hyland Lab., Costa Mesa, Calif.). Slide antigens for immunofluorescence were prepared by the method of Hanna and Bernkopf (2) and consisted of fluorocarbon-purified particles from IC Cal 8 and Ap-2 strains of *Chlamydia* agents. These antigens were fixed to microscope slides in absolute methanol at -20 C for 1 h.

**RESULTS**

To determine the specificity of the IgM anti-chlamydial antibody response in the monkey, each serum sample was tested against slide antigens prepared from homologous and heterologous chlamydial agents. Although higher titers were observed with homologous antigens, the anti-
bodies produced reacted to high levels with heterologous antigens.

In an effort to determine the nature of the antibody response of the monkey after secondary immunization with a related *Chlamydia* agent, African green monkeys were first injected with IC Cal 8 and 6 weeks later were challenged with Ap-2. As seen in Table 1, the animals produced antibodies of the IgM and IgG classes after primary immunization. These IgM and IgG antibodies were reactive with the homologous (IC Cal 8) and heterologous (Ap-2) antigens. When later challenged with a heterologous (Ap-2) antigen, no significant levels of IgM antibodies were detected, but the animals reacted by the production of increased amounts of IgG antibodies. When the order of antigen administration was reversed and Ap-2 was the primary antigen and IC Cal 8 was the secondary antigen, high levels of IgM antibodies were produced after primary immunization with Ap-2. When challenged 6 weeks later with IC Cal 8, monkey M-5 failed to produce antibodies of the IgG class, but instead produced increased levels of IgG antibodies (Table 2).

**Table 1. Distribution of antibodies reactive by indirect immunofluorescence in whole serum of African green monkeys at various times after intramuscular inoculation of IC Cal 8 and Ap-2 strains of *Chlamydia* agents**

<table>
<thead>
<tr>
<th>Weeks after inoculation</th>
<th>No. of animals</th>
<th>Immunizing antigen</th>
<th>Titers&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IC Cal 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Ap-2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgM&lt;sup&gt;e&lt;/sup&gt;</td>
<td>IgG&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>0&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>64</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>64</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>64</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>512</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>512</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>32</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Primary inoculation consisted of 0.5 ml of 50% yolk sac material (1.5 × 10<sup>6</sup> particles) of IC Cal 8 and was challenged with the same volume of Ap-2 (1.5 × 10<sup>6</sup> particles) 6 weeks later.

<sup>b</sup> Reciprocal serum dilution.

<sup>c</sup> Fluorescein-labeled goat anti-human IgM antiserum 1/5.

<sup>d</sup> Serum negative when tested undiluted.

**DISCUSSION**

The results reported here describe the serum antibody response of rhesus and African green monkeys after primary and secondary inoculation with *Chlamydia* agent particles.

The serum antibody response after primary immunization with antigens from genital *Chlamydia* strain IC Cal 8 resulted in the production of both IgM (19S) and IgG (7S) antibodies. The IgM antibodies produced were not specific to the homologous strain (IC Cal 8) but rather were broadly specific and reacted to high levels with an ocular *Chlamydia* strain (Ap-2). This may suggest that antibodies were elicited in response to group antigens shared by the various strains of *Chlamydia* agents. Upon challenge of the same animals with antigens from ocular *Chlamydia* strain Ap-2, the animals reacted by the production of increased amounts of IgG (7S) antibodies with no significant production of IgM (19S) antibodies (Table 1). When the order of antigen administration was reversed and the primary immunogen was Ap-2 and the secondary immunogen was IC Cal 8, a similar response was
elicited; high levels of IgM antibodies were produced after primary immunization and only IgG antibodies were produced after secondary immunization (Table 2). The nature of the antibody response and the speed with which they were produced after secondary immunization with heterologous antigens are characteristic of an anamnestic response and suggest that immunological memory has been established by the primary immunogen.

TRIC strains of Chlamydia agents cause localized infections of mucous membranes, particularly the conjunctiva of man. The role of circulating antibody in resistance to localized Chlamydia agent infections is not clearly understood, but it is known that circulating antibodies do not effectively modify the disease pattern (1). High levels of circulating IgG anti-chlamydial antibodies failed to protect guinea pigs against challenge with live particles of a guinea pig inclusion conjunctivitis strain of chlamydial agent (7).

To explain the presence of IgM antibodies in the present studies and lack of such antibodies in the previous ones (4, 5), at least two possibilities must be considered. First, the lack of demonstrable levels of IgM antibodies in the human studies (5) may have been related to the small antigenic mass used to inoculate the volunteers; however, massive doses of a virulent strain of lymphogranuloma venereum Chlamydia agent strain 434 failed to elicit an IgM antibody response in the monkey (4). Secondly, previous exposure of the host to the same or an antigenically related organism and then reexposure causes the host to react by producing IgG antibodies only. This may explain the lack of IgM antibodies (4, 5), although the nature, source, and time of primary exposure of these hosts to the antigens could not be ascertained. The accelerated response and the production of increased levels of IgG antibodies observed in the present studies after stimulation of primed monkeys with antigenically related chlamydial antigens indicates that immunological memory was established by the primary immunogen. The detection of IgM antibodies in monkey whole sera after primary immunization and its absence in purified IgM fractions obtained after secondary immunization rules out the possibility that IgG antibodies interfered with the detection of IgM antibodies (6).

The findings presented here complement our earlier studies (4, 5) and strongly suggest that the lack of IgM antibodies in these studies may be related to the previous exposure of the hosts, and may be related in nature to cross-reacting antigens, and that perhaps these hosts reacted in a secondary immune response after exposure to the antigens(s) in the laboratory. This is clearly indicated by the findings reported in the present studies, since all animals produced IgM antibodies after primary immunization, only antibodies of the IgG class after secondary immunization, and only antibodies of the IgG class after restimulation with cross-reacting antigens.

ACKNOWLEDGMENTS

This research was supported by grant G-444-C2 from Fight for Sight, Inc., New York, and Public Health Service grant 5 S01 RR05422-11 from the National Institutes of Health.

LITERATURE CITED


