Relationship and Significance of Specific Immunoglobulin M Antibody Response in Clinical and Subclinical Melioidosis

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Thirty-six hospitalized patients with circulating antibody to Pseudomonas pseudomallei were classified as having either clinical or subclinical melioidosis after full clinical examinations and supplementary radiological and culture investigations. Qualitative estimations of immunoglobulin M (IgM) antibody response were determined by complement fixation, indirect hemagglutination, and immunofluorescence, and the estimations were correlated with the clinical findings in each patient. An attempt was made to appraise the importance of IgM antibody, as determined by each test, in establishing the diagnosis of clinical melioidosis. Results obtained from serological tests were of no value in differentiating between active and latent infections. However, the IgM-immunofluorescent test appeared to be relevant to the diagnosis of clinical melioidosis, since the presence and absence of IgM-immunofluorescent antibody bore a close relation to clinical and subclinical disease, respectively. Surveillance studies indicated that the IgM-immunofluorescent test may also be of value in monitoring the activity and treatment of the infection, since the results of the test were generally negative 3 to 6 months after administration of chemotherapy appropriate for melioidosis.

The serological diagnosis of clinical melioidosis is presently established by demonstrating rising antibody titers to Pseudomonas pseudomallei with modifications of the complement fixation (CF) test of Nigg and Johnston (9) and the indirect hemagglutination (IHA) test of Ileri (4). However, in a significant number of instances, a stable high titer is present by the time the patient is first seen by a physician. Since antibody titers to P. pseudomallei may remain elevated for several years after the initial infection, despite apparent clinical cure, and can occur in the normal population in endemic areas (1), one cannot conclude from a stable positive titer obtained by present methods that a clinical illness is due to melioidosis.

Previous studies have indicated that there is no correlation between initial titer and prognosis, although persistence of high serological titers or rising titers may preindicate relapse of infection (3). On occasion, patients have remained seronegative or became seronegative during illness (11), whereas other patients will show a negative reaction with one serological technique and show a positive reaction with another (1). It has also been suggested that serological tests are of limited value as an aid to the diagnosis of melioidosis (6). However, since diagnosis of this disease relies entirely on either isolation of P. pseudomallei or on serological techniques, it is essential that the antibody status of the patient be clearly defined, since cultures for P. pseudomallei from clinical specimens are frequently negative and melioidosis mimics many other disease processes.

The differentiation of various infectious diseases into active and past infections has been elucidated by the presence or absence in serum of specific immunoglobulin M (IgM) antibody, because IgM is usually formed early in response to infection, and IgG is formed later. A simple, rapid method, applicable to small diagnostic laboratories, for estimating IgM fraction is mild treatment of the serum with a sulfhydryl compound which splits the IgM fraction into 6 to 7 subunits. In serum treated in this manner, IgM antibodies are inactivated, whereas the serological behavior of IgG is unaffected (8). Preliminary studies have also suggested that detection by immunofluorescence of IgM antibody specific to P. pseudomallei may be a useful procedure in the diagnosis of clinical melioidosis (1a).

Since no previous investigation has been carried out to correlate, by serological and immunofluorescence methods, clinical findings and the presence of IgM antibody specific to P. pseudomallei, this study was undertaken to ascertain whether the use of such techniques is of value in...
differentiating between active and latent infections with *P. pseudomallei.*

**MATERIALS AND METHODS**

**Subjects.** In a period of just over 3 years, 36 hospitalized patients who were found after routine testing at the Australian Department of Health, Pathology Laboratory, Townsville, to have positive antibody titers for melioidosis, as determined by IHA, or were known, from positive cultures, to have active infections with *P. pseudomallei* were carefully examined at the Townsville General Hospital by Senior Medical Officers. These consultations included a detailed history of the past and present symptoms of each patient as well as a complete physical examination, culturing of clinical specimens for *P. pseudomallei,* and radiological assessment, which consisted of straight X-ray examination of the chest. The physicians then recorded their findings and, basing their judgments on clinical grounds, indicated whether the patients were suffering from clinical or subclinical melioidosis.

**Sera.** I collected the initial test sera from patients with known positive cultures before appropriate chemotherapy for melioidosis was begun. To follow the progression of antibody production, I obtained subsequent sera from patients with clinically active melioidosis at regular intervals of approximately 3 months. The small volumes of sera available from some patients limited the number of tests which could be performed. Whenever possible, all sera from the same patient were run in parallel during testing. Prolonged storage of sera at −20°C did not appear to affect specific IgM or total antibody titers.

Sera, selected at random from 84 hospitalized patients at the Townsville General Hospital, were also tested for antibody to *P. pseudomallei.*

**Reductive cleavage of IgM.** Dithiothreitol (Sigma Chemical Co., St. Louis, Mo.), used in procedures essentially the same as those previously described (10), was employed to inactivate the IgM fraction.

**Demonstration of antibodies to *P. pseudomallei.** Sera were tested for antibodies specific to *P. pseudomallei* by CF, IHA, and indirect fluorescent-antibody (IFA) methods. The CF test was carried out by using a microtitration adaptation of the methods of Laws (7), and the IHA test was performed by using a microtitration adaptation of the technique of Jones and Hambie (6). The IFA test for IgM and total immunoglobulins, using fluorescein-labeled anti-human immunoglobulin prepared in sheep (MF01; Wellcome Research Laboratories, Beckenham, England) and fluorescein-labeled anti-human IgM prepared in sheep (MF04; Wellcome) is described elsewhere (1a). Appropriate controls were included in each series of tests.

**RESULTS**

Of the 36 patients examined, 6 felt entirely well and free from symptoms of melioidosis. Another seven patients showed mild symptoms but were considered by the examining physicians not to be suffering from active infection and were classified as having subclinical melioidosis. Of the remaining 23 patients, 5 were classified as having acute, 10 as having subacute, and 8 as having chronic forms of melioidosis. The symptoms most frequently encountered were anorexia, weight loss, cough, fever, night sweats, tachypnea, and hepatomegaly. *P. pseudomallei* was recovered from the cultures of 16 of these patients. Abnormal radiographic pictures were seen in 11 of 36 patients, but the X-ray films from only 7 patients showed pulmonary involvement consistent with melioidosis. Patients diagnosed as having clinical melioidosis were treated with appropriate chemotherapy for periods ranging from 1 to 12 months. The clinical findings for six of these patients have been described more fully elsewhere (2).

As determined from the initial sera collected from the 36 patients, the serological reactions were positive in all tests on untreated sera except for tests on the sera from two patients in which the CF and IFA tests gave positive reactions but the IHA test failed to detect specific antibody. Sera from these patients were collected while they were suffering from bacteremia caused by *P. pseudomallei.*

An antibody reduction ratio (ARR) for each test was determined by dividing the reciprocal of the antibody titer of serum treated with dithiothreitol by the reciprocal of the antibody titer obtained from the sample of untreated serum. Hence, the detection of IgG would only be expressed as an ARR equivalent to 1; an ARR of 0 would reflect the presence of IgM alone, and an ARR of 0.5 would indicate a twofold difference in antibody titer between dithiothreitol-treated serum and untreated serum. The ARR calculated for serum samples by CF and IHA were compared with the results of the IgM-IFA test (Table 1).

After reductive cleavage, a significant decrease in antibody titer (ARR, ≤0.25) was noted by CF in 31 sera, suggesting that IgM was the predominant class of antibody detected by this method. Except in sera from two patients, the IHA test was found to measure IgM antibody almost exclusively in that all specific antibody was inactivated by dithiothreitol, resulting in titers of 1:5 or less. Although CF and IHA detected significant levels of specific IgG antibody, there appeared to be no correlation among initial antibody titers determined by these tests, ARR, and disease status in the 36 patients under study (Table 2).

The IgM-IFA test was positive (titer, ≥1:10) for untreated sera from 25 of the 36 patients but gave negative reactions in all 36 sera treated...
TABLE 1. Comparison of findings of specific IgM, as determined by CF, IHA, and IFA, in sera from 36 patients with melioidosis

<table>
<thead>
<tr>
<th>IgM-IFA test*</th>
<th>CF test</th>
<th>IHA test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Negative (11)</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Low positive (2)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>High positive (23)</td>
<td>7</td>
<td>11</td>
</tr>
</tbody>
</table>

* Negative, Titer of <1:10; low positive, titer of 1:10 to 1:20; high positive, titer of ≥1:40. Numbers in parentheses indicate numbers of reactions.

b ARR was determined by dividing the reciprocal of the antibody titer of serum treated with dithiothreitol by the reciprocal of the antibody titer obtained from the sample of untreated serum.

TABLE 2. Relationship between IgM antibody to P. pseudomallei, as measured by CF, IHA, and IFA, and disease status in 36 patients with melioidosis

<table>
<thead>
<tr>
<th>Disease status*</th>
<th>Significant antibody reduction*</th>
<th>IgM-IFA test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CF test</td>
<td>IHA test</td>
</tr>
<tr>
<td>Subclinical melioidosis (13)</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Clinical melioidosis (23)</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Clinical symptoms only (7)</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Positive cultures (16)</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

* Numbers of patients in each classification are given in parentheses.

* Significant antibody reduction, ARR ≤0.25 or a fourfold or greater decrease in titer after treatment of serum with dithiothreitol.

* No reactions.

with dithiothreitol. The sera from all 23 patients with clinically active melioidosis gave positive reactions in the IgM-IFA test. Sera from the 16 patients with positive cultures showed high specific IgM-IFA titers ranging from 1:40 to 1:640, whereas the lowest IgM-IFA titer obtained from sera from the remaining 7 patients with clinical melioidosis was 1:40. Of the 13 patients with subclinical melioidosis, the sera from only 2 patients gave positive IgM-IFA test results, and the titers were found to be 1:20 or less (Table 2).

Specific antibody, detected by IHA over various time periods after diagnosis and treatment of the 23 cases of clinical melioidosis, persisted in all patients for the first 6 months, and only three patients after that period were found to be devoid of IHA antibody after 2 years of surveillance. In contrast, sera from 9 patients were negative (titer, <1:10) for IgM-IFA antibody within 3 months, and 16 patients showed negative reactions within 6 months. In the sera from five patients, IgM-IFA antibody persisted for 1 year, whereas sera from two patients remained reactive at low titers for 2 years. Despite high dosage, long-term chemotherapy, relapse of infection, proven by positive cultures, occurred in three patients, all of whom had persistent low titers (1:10 to 1:20) of IgM-IFA antibody. Recrudescence of disease was reflected by significant changes in titer (1:80 to 1:320) in the IgM-IFA test.

Of the sera collected from 84 hospitalized patients, all were negative by the IHA test for P. pseudomallei and were subsequently tested with the IgM-IFA test to determine if a false-positive test may have resulted from various unrelated illnesses. All 84 sera were negative in this test.

**DISCUSSION**

It was thought that indirect demonstration of specific IgM by CF or IHA would help to differentiate between active and latent infections with P. pseudomallei. However, the present study has shown that information obtained from serological results after treatment of sera with dithiothreitol was of no value and bore no better relation to clinical findings than results from serological tests on untreated sera. Consequently, it can be concluded that CF or IHA titer does not reflect the stage of infection nor is a measure of activity.

Preliminary studies (1a) suggested that detection of specific IgM antibodies by immunofluorescence may be helpful in assessing active infection with P. pseudomallei. Results from this investigation have confirmed a close relationship...
between clinical disease and the presence of specific IgM-IFA antibody. Of the 13 patients with subclinical melioidosis, only the sera from 2 were positive at low titers in the IgM-IFA test. In contrast, the sera from all 23 patients with active infections gave positive results at high titers for IgM-IFA antibody, and no false-positive reactions in this test were seen in the sera from 84 patients who had various unrelated diseases and who were IHA antibody negative.

Persistence of IgM-IFA antibody at low titer after treatment was demonstrable in a small number of cases, some patients showing positive IgM-IFA reactions for longer than 2 years. The reason for the continuance of both IHA and IgM-IFA antibodies is uncertain but is probably the persistence of viable organisms in tissue despite appropriate chemotherapy and apparent clinical cure. In most cases, however, IgM-IFA titer fell to a low dilution or disappeared within several months after administration of suitable chemotherapy, indicating that the IgM-IFA test may be valuable in monitoring disease progression after treatment.

The findings from the present study suggest that the absence of IgM-IFA antibody in the presence of a positive serological reaction for specific antibody would most probably preclude the diagnosis of clinical melioidosis, since the sera from all 23 patients with active infection were positive in IgM-IFA tests before treatment. The presence of IgM-IFA antibody at low titer may not be diagnostically indicative of active infection, since IgM-IFA antibody persisted for a considerable period in a small number of patients without clinical symptoms. The presence of IgM-IFA antibody at high titer, together with relevant clinical symptoms, appeared to be compatible with a diagnosis of clinical melioidosis since, at a titer of 1:40 or greater, the IgM-IFA test correlated well with positive cultures and clinical disease.

However, additional assessment of a larger number of cases of melioidosis is necessary before these findings can be accepted as definitive diagnostic criteria for determining infection. The IgM-IFA test has been performed routinely for more than 1 year at the Australian Department of Health, Pathology Laboratory, which is the reference center for serological studies of melioidosis in Northern Australia, and the test has been particularly beneficial in establishing a diagnosis of clinical melioidosis when the organism cannot be recovered from clinical material.

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LITERATURE CITED