Determination of Brucella Immunoglobulin G Agglutinating Antibody Titer with Dithiothreitol

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Received 4 February 1981/Accepted 23 March 1981

The routine brucella agglutination test measures both immunoglobulin M (IgM) and IgG brucella antibody titers; however, only an elevated IgG titer is significant for differentiating active from inactive disease in patients with symptoms lasting 3 or more weeks. The IgG antibody titer can be determined by treating the serum with 2-mercaptoethanol to inactivate the IgM brucella antibodies while leaving the IgG brucella antibodies intact. Dithiothreitol, which also inactivates IgM, was compared with 2-mercaptoethanol for the determination of IgG brucella agglutination titers. The dithiothreitol and 2-mercaptoethanol test results agreed within ±1 dilution step in 103 of 105 serum specimens tested, for a 98.1% rate of agreement. The results indicate that dithiothreitol can be used in place of 2-mercaptoethanol for determining IgG brucella agglutination titers. Dithiothreitol does not have the offensive odor or the irritant properties of 2-mercaptoethanol.

Brucellosis may present clinically as acute, as chronic after an acute attack, or as chronic and of insidious onset. The serological results may differ, depending on the clinical form and stage of the infection. Immunoglobulin M (IgM) brucella antibody predominates for the first week of the acute infection, after which the IgG antibody level starts to increase, reaches a peak after a few weeks, and predominantes over the IgM antibody level until adequate therapy eliminates the infection (3, 7). Adequate therapy reduces the IgG antibody level but not the IgM antibody level (7). If adequate therapy is not given, the infection may progress to a state of chronic brucellosis in which the serological activity is due mainly to IgG antibody (6). Individuals whose immune systems are repeatedly stimulated by contact with brucella organisms, such as veterinarians or abattoir workers, produce predominately IgG antibody and very little IgM antibody (4).

The laboratory diagnosis of brucellosis is made primarily by serological tests because the organism is isolated by cultural methods in no more than 20% of the cases (8). The routine brucella agglutination test is the most frequently used type of serological test for this purpose; however, it does not differentiate between active and inactive disease because it does not differentiate between IgG and IgM agglutinins.

The IgM titer can be eliminated by treating the serum specimen with 2-mercaptoethanol (2-ME), which breaks the disulfide bonds and depolymerizes the IgM (5). Depolymerized IgM does not contribute to agglutination; therefore, the titer is due primarily to 2-ME-resistant antibody (IgG). The 2-ME agglutination test is most useful for differentiating active from inactive brucellosis in persons having ill-defined complaints and sterile blood cultures. The presence of 2-ME-resistant (IgG) brucella agglutinins indicates active disease in these types of patients (7). In addition, the 2-ME agglutination test has been found to be superior to the routine agglutination test for determining the adequacy of antibiotic therapy (3). A fall in the 2-ME-resistant antibody titer indicates a satisfactory response to therapy.

A major objection to the use of the 2-ME test is that 2-ME irritates the eyes and mucous membranes. It also has a very strong and offensive odor. A reagent without these objectionable properties which could be used in place of 2-ME to determine IgG brucella agglutination titers would be an improvement. Dithiothreitol (DTT) depolymerizes IgM but not IgG and does not have the objectionable properties of 2-ME (9). The purpose of our investigation was to find out if DTT could be substituted for 2-ME in determining IgG brucella agglutination titers.

MATERIALS AND METHODS

Serum specimens. Various state health departments sent the human serum specimens used in the
study to our laboratory for the determination of brucella agglutination titer. Of the 105 specimens used in the study, 85 had titers of \( \geq 160 \) and 20 had titers of \(<160\) with the routine brucella microagglutination test. An unpaired serum specimen titer of \( \geq 160 \) is usually considered to be elevated (positive) and suggestive of a brucella infection (8).

Serological tests. The procedure for the routine brucella microagglutination test has been described previously (2). The same procedure was followed for the 2-ME and DTT tests except that the phosphate-buffered saline used to dilute the serum specimens contained 0.1 M 2-ME or 0.005 M DTT. The final concentrations of the sulfhydryl reagents after the addition of antigen to the tests were 0.05 M 2-ME and 0.0025 M DTT. The 2-ME was obtained from the Eastman Kodak Co., and the DTT was obtained from the Calbiochem-Behring Corp. The tests were done in conical (V)-bottom polystyrene microtitration plates. Reference sera of known high and low titers were included as controls in the routine test, the 2-ME test, and the DTT test. Phenol must not be included in the antigen diluent or in the serum diluent used in the 2-ME and DTT tests because it interferes with the action of 2-ME and DTT.

RESULTS AND DISCUSSION

A comparison of the DTT and 2-ME brucella agglutination titers is shown in Table 1. The same titer was obtained in both tests in 68 of the 105 serum specimens tested. The titer varied by only 1 dilution step in 35 of the remaining 37 specimens. Thus, the DTT and 2-ME agglutination test titers agreed within \( \pm 1 \) dilution step in 98.1% of the 105 specimens tested. This high rate of agreement indicates the DTT can be substituted for 2-ME in the microagglutination test for determining IgG brucella antibody titers. The advantages of using DTT in place of 2-ME are that unlike 2-ME, DTT does not have an offensive odor and does not irritate the eyes or mucous membranes.

<table>
<thead>
<tr>
<th>TABLE 1. Comparison of DTT and 2-ME brucella agglutination titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTT titer in relation to 2-ME brucella agglutination titer</td>
</tr>
<tr>
<td>Same</td>
</tr>
<tr>
<td>1 Dilution step lower</td>
</tr>
<tr>
<td>1 Dilution step higher</td>
</tr>
<tr>
<td>2 Dilution steps lower</td>
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</table>

There is no consensus in the literature as to what is considered to be a significant 2-ME-resistant (IgG) brucella agglutination titer or what is the lowest IgG titer considered to be indicative of an active chronic infection. Reddin et al. (7) found that 2-ME-resistant (IgG) brucella agglutinins appear to be a distinguishing feature in differentiating active from inactive disease in patients having ill-defined complaints, sterile blood cultures, and low titers of brucella agglutinins. However, they do not specify what constitutes a significant titer. According to Alton et al. (1), any positive titer in the 2-ME test should be regarded as indicating infection or should at least lead one to suspect infection. Buchanan and Faber (3) state that a negative (titer of \( \leq 80 \)) 2-ME test is strong evidence against a diagnosis of brucellosis.

In our laboratory, we consider a DTT- or 2-ME-resistant (IgG) brucella agglutination titer of \( \leq 20 \) on an unpaired serum specimen as indicating the absence of active infection and a titer of \( \geq 160 \) as suggestive of active infection. A titer of 40 or 80 is reported as inconclusive.

LITERATURE CITED