Serodiagnosis of Infectious Mononucleosis with a Bovine Erythrocyte Glycoprotein

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A glycoprotein from bovine erythrocyte membrane was evaluated in two immunoassays as a reagent for the serodiagnosis of infectious mononucleosis (IM). We previously reported that a partially purified preparation of this glycoprotein, when attached to latex beads, agglutinated in the presence of IM heterophile antibody. In the present study, we used a highly purified form of the glycoprotein both as an agglutinating reagent, covalently bound to latex, and in a solid-phase sandwich-type radioimmunoassay (RIA) for IM antibody detection in a larger population of patients. We tested serum samples from college students with symptoms suggestive of IM with the latex reagent (143 samples) and with the RIA (245 samples). Correlation of these two tests, both with each other and with the classical differentially absorbed, agglutination tests for Paul-Bunnell antibody in IM sera, using fresh sheep or horse cells, was excellent (>97% agreement). The new tests also corresponded in most cases with a rapid, unabsorbed preserved horse erythrocyte slide test. However, in this study of 245 samples, both apparent false-positives (5 samples) and apparent false-negatives (3 samples) were observed with this slide test. In conclusion, we found that the bovine glycoprotein as a reagent can facilitate the diagnosis of IM, giving results comparable to those with erythrocyte agglutination tests on differentially absorbed sera. The advantages are ease and speed of performance (latex test), potential for automation (RIA test), stability and uniformity of the glycoprotein reagent (latex and RIA tests), and most importantly, the ability to use unabsorbed sera (latex and RIA tests).

Bailey and Raffel (1) described, in 1935, that sera from patients with infectious mononucleosis (IM) hemolyzed and, to a lesser extent, agglutinated bovine erythrocytes. They also noted that the cross-reactivity of these heterophile antibodies in IM sera was greater for bovine erythrocytes than for sheep erythrocytes. In 1950, Gleeson-White et al. (11) reported that bovine erythrocytes are resistant to agglutination by IM antibody, unless they first are treated with protease. Subsequently, Coombs et al. (4) showed that these erythrocytes can be made to agglutinate by building up an antiglobulin-globulin lattice. In 1971, Fletcher and Woolfolk reported the isolation of crude IM antigen from bovine erythrocytes (9). A partially purified form was used to prepare a latex reagent for the detection of IM antibody (17). In a small group of 99 samples, this reagent discriminated between sera from normal controls and sera from patients with IM. The bovine erythrocyte antigen was further purified and characterized (6). Its properties include the following: amino acid content, 73%; carbohydrate content, 27%; sub-

unit molecular weight, 34,000; and aggregate molecular weight, 107,000. In the present study, the usefulness of the highly purified antigen for the immunoassay of IM heterophile antibody, by both a latex agglutination test and by a radioimmunoassay (RIA), was evaluated and compared with some standard assays.

MATERIALS AND METHODS

Preparation of antigen. Antigen was prepared by hot, aqueous ethanol extraction of hemoglobin-free bovine erythrocyte stroma, as previously described by Fletcher and Woolfolk (9). This crude antigen extract was further purified by phosphocellulose chromatography, ethanol precipitation, sequential extractions with ethanol-ether and chloroform-methanol, and DEAE chromatography (in 1% Emulphogen as described by Dejter-Juszynski et al. for the removal of complex glycolipids [5]). The antigen was recovered as a single peak. The properties of the highly purified material, a sialoglycoprotein, were recently reported (6). Radiolabeled glycoprotein was prepared by the Iodogen (Pierce Chemical Co., Rockford, Ill.) method of Fraker and Speck (10). Labeled glycoprotein was separated from ¹²⁵I by passage over a Sephadex G-25 column previously equilibrated with 0.5% bovine se-

rum albumin. The specific activity of the labeled glycoprotein was 10^6 cpm/ μ g.

Collection of sera. A total of 245 coded samples were obtained from the University of Miami Student Health Service. All samples were from patients from whom blood was drawn for an IM heterophile antibody test. All sera were inactivated at 56°C for 30 min and stored at -20°C .

RIA. Sandwich-type, solid-phase RIAs were carried out in polystyrene microtiter plates (U-bottom; Dynatech Laboratories, Inc., Alexandria, Va.). The assay, using purified horse erythrocyte glycoprotein (Ho GP) prepared as described by Caldwell et al. (3), was done as previously described by Fletcher et al. (8). In brief, wells were coated sequentially, with extensive appropriate washing between each step, with glycoprotein, gelatin, test serum, and ^{125}I -labeled Ho GP. The plates were air dried, the wells were cut out, and radioactivity was determined. The assay in which purified bovine erythrocyte glycoprotein (Bo GP) was used was done in the same way. Preliminary tests were carried out to determine the optimum concentrations of glycoproteins for coating the wells. Decreasing the coating from 1 to 0.1 μ g per well increased the sensitivity of

the test. A plateau of sensitivity was observed when the coating was decreased from 0.1 μ g to 10 ng per well. Subsequent tests were done, using 10 ng of Bo GP per well. The effect of the concentration of ^{125}I -labeled glycoprotein was evaluated. Optimum binding of 30% by 1:100 dilution of a standard IM serum pool was seen at 1 ng of ^{125}I -labeled Bo GP per well. Figure 1 shows the dilution binding curve of the standard pool of IM serum as compared with a standard pool of normal serum. Both serum pools were tested with wells coated either with Bo GP or with buffer alone. More than 200 normal sera were tested, and the total counts bound were less than 2% of the total counts in both the wells coated with buffer and those coated with Bo GP. The specific binding of IM serum was calculated by subtracting the counts bound in wells coated with the buffer control from the counts bound in wells coated with Bo GP.

Latex test. Purified Bo GP was coupled to carboxyl-modified uniform-diameter latex particles (average diameter, 0.455 μ m; Dow Diagnostics, Indianapolis, Ind.) with a water-soluble carbodiimide [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride], and the latex agglutination test was carried out on glass slides as previously described (17).

Other heterophile antibody tests. The standard tests used for the detection of IM heterophile antibody included the following. (i) The sheep erythrocyte test used was that originally described by Wöllner (19). In this test, a sample of the serum of the patient is absorbed with an equal volume of washed, papain-treated sheep erythrocytes. This step removes from the serum the non-IM heterophile agglutinins, such as the Forssman glycolipid, which react with sheep erythrocyte antigens resistant to papain digestion. The absorbed serum is then titrated against native sheep erythrocytes. Any agglutination seen at this point is considered to be due to IM heterophile antibody (18). (ii) Two horse erythrocyte tests were used, a preserved horse erythrocyte slide test (Monotest; Wampole Laboratories, Cranbury, N.J.) and a fresh horse erythrocyte differential tube agglutination test in which unabsorbed serum, guinea pig (Difco Laboratories, Detroit, Mich.)-absorbed serum, and bovine erythrocyte-absorbed serum were separately titrated against horse erythrocytes (16).

RESULTS

The new RIA procedure in which ^{125}I -labeled Bo GP is used was compared with the previously described RIA done with ^{125}I -labeled Ho GP (8) by testing a total of 147 serum samples with both procedures (Fig. 2). All sera binding less than 2% of the ^{125}I -labeled tracer were considered negative, and those which bound 2% or more of the ^{125}I -labeled Bo GP were marked positive. For this group of sera, collected from patients for whom IM was part of the differential diagnosis, 129 samples were negative and 18 samples were positive in both RIAs. Thus, for this series, the agreement between these RIAs was 100%. Of these sera, 57 were tested also by the fresh horse erythrocyte differential test. Agreement between this sensitive agglutination test and the

FIG. 1. Antibody dilution curve showing the binding of labeled Bo GP by: IM sera on Bo GP-coated wells (●), IM sera on phosphate-buffered saline-coated wells (■), normal sera on Bo GP-coated wells (○), and normal sera on phosphate-buffered saline-coated wells (□).

FIG. 2. Comparison of the Bo GP and Ho GP RIAs for the detection of IM antibody. The specific binding of the ^{125}I -labeled glycoprotein by the test sera is shown.

Bo BP RIA was 98% (Fig. 3). Only one sample was negative in the horse cell test but positive in the RIA. Another group of 97 samples of sera drawn for heterophile antibody testing were

FIG. 3. Comparison of the Bo GP RIA with the fresh horse erythrocyte differential hemagglutination test which was done on absorbed sera.

FIG. 4. Comparison of the Bo GP RIA with the Wöllner test for the detection of IM antibody.

tested by the Bo GP RIA and by the sheep cell test of Wöllner (Fig. 4). Again, agreement was very high, 97%. A total of 33 samples were negative and 61 samples were positive in both tests. One sample was weakly positive in the sheep cell test but negative in the RIA, and two samples were positive (2.6 and 6% binding) in the RIA but negative (titer less than 1/2) in the Wöllner test. A total of 245 samples were tested by a rapid spot test with preserved horse erythrocytes (Monotest) and by the Bo GP RIA. In this series (Fig. 5), 162 samples were negative and 74 were positive in both tests. Four samples were negative in the Monotest and positive in the Bo GP RIA. These samples were also evaluated by the Wöllner test; three were positive and one was negative. There were five samples which were positive in the Monotest and negative in the Bo GP RIA. Because these five sera were also negative in the fresh horse erythrocyte differential test and in the Ho GP RIA, they probably represent false-positives in the Monotest. Overall agreement, however, between this preserved horse erythrocyte test and the Bo GP RIA was good, 96%. We also tested 143 of these sera with the Bo GP latex test and found that this type of rapid slide test corresponded to the preserved horse erythrocyte slide test 97% of the time (Fig. 6). Not surprisingly, the Bo GP latex test agreed very well with the Bo GP RIA in all but 4 of 153 samples.

DISCUSSION

We have successfully isolated sialoglycoproteins from the erythrocytes of four mammalian

FIG. 5. Comparison of the Bo GP RIA with the preserved horse erythrocyte test (Monotest) for the detection of IM antibody.

species (13); these sialoglycoproteins are reactive with the Paul-Bunnell heterophile antibodies in the sera of patients with IM. These glycoproteins are potentially useful as diagnostic reagents for the detection of Paul-Bunnell antibodies. All can be covalently coupled to latex beads which agglutinate when IM serum is added (15). Because they are, in each case, glycoproteins, they do not react with some other heterophile antibodies in IM sera, such as the Forssman antibodies, which are directed toward determinants associated with complex glycolipids (9, 14). We evaluated the Ho GP, both in a latex agglutination test and in a solid-phase RIA, and found that it is a potentially useful reagent for IM antibody detection (7, 8). However, the Bo GP, which we recently obtained in purified form, has the greatest degree of cross-reactivity with IM antibodies. It is 4 times more reactive than the Ho GP and 25 times more reactive than

the sheep erythrocyte antigen (15). Also, the Bo GP has determinants that are reactive with at least two distinct antibodies present in IM sera (6). One of these sites is dependent on the presence of sialic acid and is found in sheep, goat, and horse glycoproteins. The other is probably not a carbohydrate in nature and is found only in the Bo GP. In a previous study of

FIG. 6. Comparison of the Bo-GP latex test with the preserved horse erythrocyte test (Monotest) for the detection of IM antibody.

99 serum samples, a partially purified form of the Bo GP was evaluated for its utility as a reagent to detect IM antibody in a latex agglutination test (17). In the present study, we used highly purified glycoprotein, covalently attached to latex and tested this material by using it in testing an additional 143 samples of human sera. All of these sera were drawn from college students with symptoms suggesting IM as part of the differential diagnosis. We compared the results obtained with the new latex reagent with those given by a rapid slide test based on preserved horse erythrocytes. In these two studies, agreement between the Bo GP test and the Monotest was 94.5%. Eleven samples were negative in the Monotest but positive in the Bo GP latex test, and three were positive in the Monotest but negative in the latex test. In the cases of disagreement between these two rapid tests, titration tests with absorbed sera, using fresh horse or sheep erythrocytes, confirmed, with two exceptions, the results with the Bo GP latex test.

In the present study, we used the purified glycoprotein to devise a sensitive RIA for IM antibodies. The Bo GP was coated to polystyrene as the solid phase, and ^{125}I -labeled Bo GP was used as the tracer. This test also corresponded well with the two fresh erythrocyte (horse and sheep) differential tests and with the preserved horse erythrocyte slide test.

In conclusion, the Bo GP latex test has the best features of the bovine hemolysin test, sensitivity and specificity (12), and the best features of the preserved horse erythrocyte slide test, ease and speed of performance (2). The Bo GP RIA takes 3 days to perform and requires facilities for handling and counting radioactive materials. The actual processing time per sample is short, however, and the test could be automated. In large centers at which many samples are processed it might be the preferred procedure. The Bo GP could also be easily adapted to an enzyme-linked immunoassay procedure.

ACKNOWLEDGMENTS

We are grateful to Eugene Flipse of the University Health Service for access to patient materials.

This project was supported by Public Health Service grant AM 16763 from the National Institutes of Health.

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