Horse Erythrocyte Glycoprotein-Latex Reagent That Reacts with Infectious Mononucleosis Heterophile Antibody

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Received 2 October 1981/Accepted 13 April 1982

A sialoglycoprotein from horse erythrocytes was isolated in essentially homogeneous form and found to contain the neuraminidase-sensitive determinant of the horse erythrocyte for Paul-Bunnell heterophile antibodies of infectious mononucleosis. This reactivity was retained after covalent coupling of the antigen to latex particles. The latex reagent has greater stability (>3 years) than either fresh or preserved horse erythrocytes. It can be used in a direct slide test; no absorption of the serum is necessary. The new test compared favorably with some standard tests for infectious mononucleosis antibody which use horse erythrocytes. Agreement of the latex test with the absorbed fresh horse cell test was 100%, and agreement with a stabilized horse erythrocyte spot test was 94%. The latex test also agreed 100% with a radioimmunoassay for the detection of heterophile antibody with ^{125}I -labeled horse erythrocyte glycoprotein. The new latex test was compared with a bovine erythrocyte glycoprotein-latex test, and a correlation of 94% was observed. In addition, it was compared with the Wöllner test (enzyme-treated sheep cell-absorbed sheep cell agglutination test) with which it agreed in 92% of samples tested.

In 1936, Beer reported that horse erythrocytes reacted with the heterophile antibodies of infectious mononucleosis (IM) at higher titers than with sheep erythrocytes (2). Thirty years later, Hoff and Bauer demonstrated that horse cells preserved in Formalin were still sensitive indicators of IM antibody (15). Lee et al. found, however, that whereas horse cell agglutination titers alone did not always differentiate between IM and non-IM sera, after absorption with guinea pig kidney and bovine erythrocytes the residual horse agglutinins did sharply differentiate (17). Lee et al. (18) reported that horse blood preserved in 3.8% sodium citrate remained usable for 3 months and was three to four times more sensitive than sheep erythrocytes and 16 times more sensitive than formalinized horse cells. They devised a differential slide test which, when compared with the Paul-Bunnell, Davidsohn-modified differential test using sheep erythrocytes by Basson and Sharp (1) on sera from 372 patients with suspected IM, correlated very well except for five apparent false-positive tests. In these five cases, the preserved horse cell suspension was over 3 months old. False-negative spot tests are reported to occur in leukemia (20), lymphoma (25), and pancreatic carcinoma (22). Horowitz et al. studied five patients who continued to react positively in a spot test for 4 to 6 years but who were repeatedly negative in the fresh horse erythrocyte differ-

FIG. 1. Electropherogram of ^{125}I -labeled horse erythrocyte glycoprotein in 0.1% sodium dodecyl sulfate-7.5% polyacrylamide gel buffered with 0.1 M phosphate (pH 7.0). The sample was preincubated in 0.01 M phosphate (pH 7.0) containing 1% sodium dodecyl sulfate and 0.1% mercaptoethanol at 37°C for 30 min. After electrophoresis, the gel was sliced into 35 segments, and the radioactivity of each slice was determined. The arrow shows the migration of marker dye.

FIG. 2. Comparison of two slide tests for the detection of IM antibody: a stabilized horse erythrocyte spot test (Monotest) and the horse (HO) GP-latex test.

ential test (16). A recent editorial in the *British Medical Journal* also pointed out the tendency of horse cell slide tests to give false-positive results (4). Golubjatnikov et al. reported a false-positive rate of 6 to 13% with two types of the spot test (13). In contrast, Feorino et al., studying cases of IM early after the onset of disease, reported false-negative results in 9 to 11% of patients tested (6).

In our laboratory, we have isolated an antigen from horse erythrocytes which reacts with IM antibody but not with Forssman or serum sickness antibody. This IM activity is associated with a sialoglycoprotein and is completely abolished by treatment with neuraminidase (11). A partially purified form of the glycoprotein was used to develop a sensitive and specific sandwich-type radioimmunoassay (RIA) for the detection of small quantities of IM heterophile antibody or antigen (9). Previously, we successfully prepared a latex reagent for the detection of IM antibody with bovine erythrocyte glycoprotein (19). The aim of the present study was to prepare a latex reagent from horse erythrocyte glycoprotein and to compare it with two standard horse cell-based tests and

FIG. 3. Comparison of the absorbed horse cell agglutination test with the horse (HO) GP-latex test for detection of IM antibody.

with the two other tests developed in our laboratory as well as with a sheep cell differential test.

MATERIALS AND METHODS

Horse erythrocyte glycoprotein. The extraction of glycoprotein from hemoglobin-free horse erythrocyte membranes with hot aqueous 75% ethanol has been previously described (11). The 75% ethanol extract was then further purified by cation exchange chromatography and delipidation (10). As a final step, the glycoprotein was dissolved in 1% Emulphogene (GAF Corp. Chemical Products, New York, N.Y.) and chromatographed on DEAE-cellulose as described by Dejter-Juszynski et al. for the removal of complex glycolipids (3). The properties of this highly purified erythrocyte membrane antigen have been reported (2a) and are summarized as follows. Composition: polypeptide, 44.9 g/100 g; carbohydrate, 55.1 g/100 g; residues: *N*-glycolylneuraminic acid, 26 mol/30,000 g; galactose, 19 mol/30,000 g; mannose, 1 mol/30,000 g; *N*-acetylgalactosamine, 19 mol/30,000 g; and *N*-ace-

FIG. 4. Comparison of sensitivity of two horse (Ho) glycoprotein tests for the detection of IM antibody: the RIA and the latex test.

tylglucosamine, 1 mol/30,000 g. The apparent molecular weight of the subunits is 30,000, and the apparent molecular weight of the aggregate is 383,000. The activity with IM antibody (the amount that inhibits the agglutination of sheep erythrocytes by four agglutinating doses of IM serum) is 0.2 μ g of native glycoprotein per ml and >5,000.0 μ g of neuraminidase-treated glycoprotein per ml.

Preparation of GP-latex reagent. Purified horse erythrocyte glycoprotein (GP) was coupled to carboxyl-modified, uniform latex particles (0.455 μ m average diameter, Dow Diagnostics) with a water-soluble carbodiimide [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride] by the procedure previously described (19).

Collection of sera. A total of 245 coded sera were collected from the University of Miami Student Health Service. All 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.

Heterophile antibody tests. The tests used for the detection of IM heterophile antibody included the following. (i) Two horse erythrocyte tests were used, a preserved horse cell slide test (18) (Monotest; Wampole Laboratories, Cranbury, N.J.) and a fresh horse cell differential tube agglutination test in which unabsorbed serum, guinea pig kidney (Difco Laboratories)-absorbed serum, and bovine erythrocyte (Difco Laboratories)-absorbed serum were separately titrated against horse cells (2). (ii) The sheep erythrocyte test used was developed by Wöllner (26). In this test, a sample of patient serum is absorbed with an equal volume of washed, papain-treated sheep erythrocytes.

This step removes from the serum the non-IM heterophile agglutinins that react with sheep erythrocyte antigens resistant to papain digestion, such as the Forssman glycolipid. 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 (24). (iii) Two tests were done with horse erythrocyte glycoprotein. The latex agglutination test was carried out on glass slides as previously described (19). Sera were diluted 1:4 initially, and agglutination at this dilution was considered positive. In some cases the sera were titrated in doubling dilutions. The horse glycoprotein RIA was done as a sandwich-type assay in microtiter plates as previously described (9). Glycoprotein was labeled with ¹²⁵I by the Iodogen (Pierce Chemical Co., Rockford, Ill.) method (12). The ¹²⁵I-labeled glycoprotein behaved as the native glycoprotein on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (11), as shown in Fig. 1. Also, its reactivity with IM antibody was found to be intact by agglutination inhibition tests. The slide test for the detection of IM antibody with bovine erythrocyte GP-latex reagent was done as previously described (19).

RESULTS

Detection of IM heterophile antibody in serum with GP-latex reagent. Preliminary tests were carried out to determine the optimum concentration of horse glycoprotein for attachment to the latex particles. There was little difference in serum titers with glycoprotein at concentrations of 10 to 150 μ g/ml. However, increasing the

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WÖLLNER TEST

FIG. 5. Comparison of tests for the detection of IM antibody: a sheep erythrocyte agglutination test on papain-digested sheep erythrocyte-absorbed sera (Wöllner test) and the horse (HO) GP-latex test.

concentration above that range decreased the titer of a standard IM serum, and decreasing the glycoprotein below 10 $\mu\text{g}/\text{ml}$ increased sensitivity but also had a prozone effect. It was also noted that the concentration of the carbodiimide could be varied between 0.5 and 20 mg/ml without affecting the titers of IM serum with the reagent. Results reported in this study were obtained with horse GP-latex reagent prepared with 10 to 30 μg of glycoprotein per ml. The reagent was used in a direct slide test with the coded sera. A total of 245 samples were tested with the preserved horse cell slide test and with the GP-latex slide test (Fig. 2). Overall agreement was 94%: 5 samples were negative in the Monotest and positive in the GP-latex test, and 10 were positive in the Monotest and negative in

the GP-latex test. All of these samples were also tested with either an absorbed fresh erythrocyte agglutination test (the Wöllner test or the horse cell differential test) or the horse glycoprotein RIA, or both. Figure 3 shows that in the 54 sera tested by both the GP-latex test and the horse cell differential test, agreement was 100%. Similarly, 100% agreement was seen between the results with the latex test and the RIA, which are both based on purified horse erythrocyte glycoprotein (Fig. 4). There was also good correlation between latex test titers and percent specific binding for these two tests. Figure 5 shows the results of the GP-latex test and the Wöllner test on 94 samples. Six of these were positive in the modified sheep cell-absorbed test and negative in the latex test, and two were negative in the Wöllner test and positive in the latex test. Table 1 shows the serological findings for those 15 patients for whom the results of the two slide tests (latex and Monotest) disagreed. In 11 of these, the results with an absorbed fresh erythrocyte agglutination test (either Wöllner or horse cell differential test) agreed with the latex test, and in 4 cases there was agreement with the horse erythrocyte spot test. A total of 244 sera were tested with both the new horse GP-latex test and the previously reported bovine GP-latex test. Overall agreement was high (94%). In 98 of the sera, titers were compared (Fig. 6). Of these, 35 were negative in both tests, and 15 were positive and of equal titer. In the remaining samples, 73% had higher titers in the bovine GP-latex test, whereas 27% gave a higher titer in the horse GP-latex test.

DISCUSSION

The data of Evans et al. (5) indicate that over 96% of clinical cases of IM are positive by the fresh horse erythrocyte agglutination test on serum absorbed with guinea pig kidney. This test also detects IM antibody for over 1 year in many cases and in almost one-half of the asymptomatic cases of Epstein-Barr virus infection, the etiological agent for IM (14). Evans et al. also point out that the only test for Epstein-Barr virus antibody with diagnostic utility is that devised by Schmitz and Scherer (23) for the demonstration of immunoglobulin M antibodies to viral capsid antigen. They note, however, that this test is less sensitive than the horse erythrocyte agglutination test, less specific than the bovine hemolysin test, and technically very difficult to perform.

For nearly 50 years, the demonstration of the heterophile antibody first noted by Paul and Bunnell (21) has remained the method most useful in the laboratory diagnosis of IM. The antibody is detected by a fortuitous cross-reac-

TABLE 1. Serological findings for patients for whom disagreement between GP-latex test and horse erythrocyte spot test occurred

Patient no.	Horse GP-latex test	Horse erythrocyte spot test ^a	Wöllner test ^b	Fresh horse cell differential test ^c	Horse GP RIA ^d
18	+	-	-	ND ^e	ND
36	+	-	+	ND	ND
44	-	+	+	ND	ND
62	+	-	+	ND	ND
64	+	-	+	ND	ND
81	-	+	-	ND	ND
84	+	-	+	ND	ND
98	-	+	+	ND	ND
99	-	+	+	ND	ND
110	-	+	ND	-	-
128	-	+	ND	-	-
129	-	+	ND	-	-
134	-	+	ND	-	-
148	-	+	ND	-	-
149	-	+	ND	-	-

^a Monotest (Wampole).
^b Sera absorbed with papain-treated sheep erythrocytes and tested with untreated sheep erythrocytes.
^c Sera absorbed with guinea pig kidney and bovine erythrocytes and tested with horse erythrocytes.
^d Solid-phase RIA with ¹²⁵I-labeled horse erythrocyte glycoprotein.
^e ND, Not determined.

tion with antigens present on certain animal erythrocytes. Until recently, all methods were based upon either hemagglutination or hemolysis and required that the serum be suitably

absorbed (or diluted) to remove other heterophile antibodies commonly present in human serum which are unrelated to IM. Methodology developed in our laboratory has

FIG. 6. Comparison of IM antibody titers of sera with the bovine (Bo) GP-latex reagent and the horse (Ho) GP-latex reagent.

made possible for the first time the development of tests without erythrocytes or absorption of serum. We have isolated and purified glycoprotein antigens from goat, sheep, bovine, and horse erythrocytes, all of which are reactive with IM heterophile antibody (7, 8, 10). All of these can be used to make reagents for the detection of IM antibody. A specific and sensitive RIA was developed with horse glycoprotein (9), and later a latex test was developed based on the bovine glycoprotein (19). Both of these procedures compared favorably with standard tests. In the present study, a homogeneous sialoglycoprotein from the horse erythrocyte membrane was covalently coupled to latex beads for use as a slide test. This reagent was compared with some other methods for IM antibody detection. It gave results exactly comparable to those of the fresh horse cell-absorbed test and the horse glycoprotein RIA and in substantial agreement with a preserved horse cell spot test and an absorbed sheep cell agglutination test. We also compared the new test with our previously reported bovine GP-latex test (19). In this case also agreement was high as to classifying a given sample as positive or negative. However, titers for individual sera tended to be higher in the bovine-based test. We have reported that glycoprotein extracted from bovine erythrocytes, when compared with the glycoproteins from horse, sheep, or goat erythrocytes, is more reactive with the Paul-Bunnell type in mononucleosis serum and in fact reacts with additional antibody in IM serum for which these latter glycoproteins lack receptors (2a, 10; N. G. Klimas, K. E. Caldwell, P. L. Whitney, and M. A. Fletcher, *Dev. Comp. Immunol.*, in press; M. A. Fletcher, K. E. Caldwell, and Z. A. Latif, *Vox Sang.*, in press). Thus, a latex test based on the bovine glycoprotein may be preferred over the new horse GP-latex test. However, our preliminary results suggest that, because of its ability and ease of standardization, the horse GP-latex reagent would be preferred over the other horse erythrocyte-based tests available.

ACKNOWLEDGMENTS

This project was supported by Public Health Service grant AM16763 from the National Institutes of Health.

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

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