A NEW APPROACH TO THE DIAGNOSIS OF MENINGOCOCCAL INFECTION: LATEX-ERYTHROCYTIC AGGLUTINATION

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The rapid diagnosis of meningococcal infection (MI) and of suppurative bacterial meningitis (SBM) still remains an urgent problem in practical health care. To detect nonviable cells and specific antigens, test systems of enzyme immunoassay are used in laboratory practice, accompanied on an ever-increasing scale by the use of kits for use in the latex agglutination (LA) test, enabling the etiologic agent of MI and SBM to be identified within a few minutes actually at the patient's bedside. As pathological material in this case, cerebrospinal fluid, blood serum, and urine are traditionally used. The conduct of analysis in such cases may be rendered more difficult by the presence of certain proteins, more especially of rheumatoid factor [4], in the biological fluids, where they may cause the development of a false positive reaction.

Adsorption of polysaccharide (PS) and lipopolysaccharide (LPS) antigens on the stroma of erythrocytes also is a well-known fact and it lies at the basis of the preparation of erythrocytic diagnostic sera. The presence of this process, in principle, may cause a high proportion of antigens to be in the bound state, so that the search for them in the patient's serum proves unsuccessful. In this paper we suggest a method of performing the LA test with erythrocytes, which may be of fundamental importance for the diagnosis of MI.

EXPERIMENTAL METHOD

To prepare diagnostic latex preparations (LP) we used polystyrene carboxylated latex, obtained by emulsifier-free polymerization of styrene with the addition of methacrylic acid. Details of the latex were given in [1]. The latex was sensitized with immunoglobulins, isolated from meningococcal serum of serogroup A (Leningrad Research Institute of Vaccines and Sera) by adsorption under the conditions described in [2].

In the experiment whose aim was to determine optimal concentrations of components for the LA test, we used formalinized sheep's erythrocytes, sensitized with meningococcal PS of serogroup A, and diagnostic LP.

Model experiments were carried out with freshly isolated human and mouse erythrocytes; the variable parameters were the concentration of the erythrocyte suspension and the sensitizing dose of PS of meningococci of serogroup A. Human erythrocytes were obtained from blood taken from the finger of blood donors belonging to different ABO blood groups. They were washed 3 times to remove serum proteins with 0.15 M NaCl solution, using centrifugation to precipitate them. Erythrocytes were sensitized at 37°C for 30 min, and equal volumes of suspensions of erythrocytes (in 0.15 M NaCl) with concentrations of 0.5, 0.25, and 0.12%, and of a solution of meningococcal PS, of serogroup A, in concentrations of 50, 5, 0.5, and 0.05 μg/ml. After incubation the erythrocytes were washed 3 times with 0.15 M NaCl solution, followed by sedimentation by centrifugation. The mouse erythrocytes were sensitized in the same way.

In the mouse model, noninbred mice (weighing 16-20 g) were given an intravenous injection of meningococcal PS of serogroup A in a dose of 50 μg and in a volume of 0.15 ml. Blood was taken from the orbital sinus of the eye 1.5, 3, 6, 12, 24, and 48 h after injection of PS. There were 40 mice in the group. The control group consisted of 10 mice, receiving an intravenous injection of 0.15 M NaCl solution in a volume of 0.15 ml. The agglutination test was carried out with a 0.2% suspension of mouse erythrocytes and with diagnostic LP in order to detect meningococci of serogroup A [2].

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TABLE 1. Dependence of Intensity of LA on Concentration of Components of Reaction for Determining Meningococcal Polysaccharide of Serogroup A, Adsorbed on Formalinized Sheep's Erythrocytes, with the Aid of Diagnostic LP (reaction temperature 21-24°C, incubation time after mixing 3-5 min)

Concentration of suspension of diagnostic LP, %	Concentration of suspension of formalinized sensitized sheep's erythrocytes (%)					Concentration of suspension of formalinized sheep's erythrocytes (specificity control, %)			
	0,5	0,25	0,12	0,06	0,03	0,5	0,25	0,12	
1	4+	2+	—	—	—	—	—	—	—
0,5	4+	4+	3+	2+	—	—	—	—	—
0,25	1+	2+	3+	4+	3+	—	—	—	—
0,12	2+	—	—	—	—	—	—	—	—
0,06	1+	—	—	—	—	—	—	—	—

Legend. Protein concentration in 1% suspension of LP was 15 μg/ml. Sensitizing dose of meningococcal polysaccharide of serogroup A was 50 μg/ml.

TABLE 2. LET with Human Erythrocytes, Sensitized by Various Doses of Meningococcus of Serogroup A

Blood group	Erythrocyte concentration	Specificity	Sensitizing dose of PS-A, μg/ml			
			50	5,0	0,5	0,05
I(O)	0,5	—	4+	4+	2+	—
	0,25	—	4+	4+	1+	—
	0,12	—	4+	1+	—	—
II(A)	0,5	—	4+	4+	2+	—
	0,25	—	4+	4+	1+	—
	0,12	—	4+	1+	—	—
III(B)	0,5	—	4+	3+	1+	—
	0,25	—	4+	3+	±	—
	0,12	—	4+	2+	+	—
IV(AB)	0,5	—	4+	4+	2+	—
	0,25	—	4+	4+	±	—
	0,12	—	4+	1+	—	—

TABLE 3. LET with Mouse Erythrocytes Sensitized by Different Doses of Meningococcal PS of Serogroup A

Concentration of mouse erythrocytes	Specificity control	Sensitizing dose of PS-A, μg/ml			
		50	5,0	0,5	0,05
1,4	—	4+	4+	4+	±
0,28	—	4+	4+	1+	±
0,14	—	4+	1+	—	—

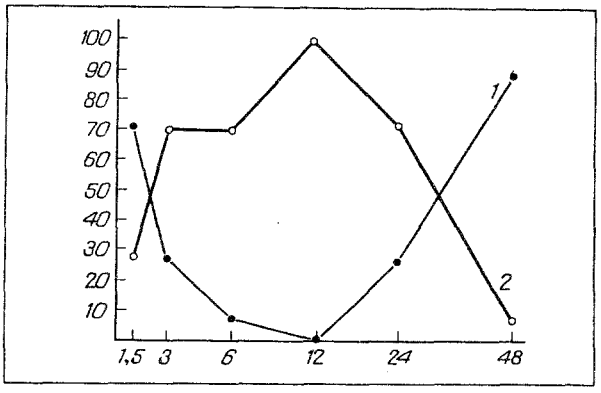

Fig. 1. Results of the "mouse model" of LET. Abscissa, time after intravenous injection of meningococcal PS antigen; ordinate, percentages. 1) Number of negative tests (according to times of observation), 2) number of positive tests.

The intensity of the agglutination reaction of erythrocytes and latex particles was estimated visually and expressed on a traditional four + system.

EXPERIMENTAL RESULTS

On the basis of the results given in Table 1, obtained on formalinized sheep's erythrocytes, the optimal concentration of LP suspension from the point of view of maximal agglutination effect of 0.25% (i.e., containing 0.25 g of polymer per 100 g of preparation), at which the suspension exhibited maximal sensitivity, can be estimated.

The intensity of agglutination in the latex-erythrocytic test (LET) depended directly on the sensitizing dose of PS and the concentration of the erythrocytic suspension. Analysis of the results in Table 2 shows that for a 0.5% suspension of human erythrocytes LET was positive with a sensitizing dose of antigen of between 0.5 and 50 μg/ml. Lowering the concentration of the erythrocyte suspension fourfold reduced the sensitivity of the test by 2 orders of magnitude (to 50 μg/ml). A similar picture also was observed in model experiments with mouse erythrocytes (Table 3).

It is important to note that in all cases the specificity control of the LET (i.e., the reaction of LA with unsensitized erythrocytes) was negative.

Adsorption of meningococcal PS of serogroup A on erythrocytes in vivo was observed in mice (mouse model). The time course of LET for this case is shown in Fig. 1. As early as 1.5 h after injection of the antigen, a positive LET was recorded in 27.3% of cases, and in 9% of cases the intensity of the reaction was 3+. After 12 h a positive LET was found with all animals. It is interesting to note that even 48 h after injection of the meningococcal PS the reaction of LA with erythrocytes was positive in 9.1% of cases. In the control group of animals, all the tests gave a negative result.

Thus the possibility of agglutination of LP with erythrocytes was confirmed in principle in experiments on mice. The mouse model also made it possible to estimate the length of time during which the LET gives useful results. The time course of adsorption of meningococcal PS antigen on the stroma of the animal's erythrocytes was such that the intensity of the agglutination reaction was maximal 12 h after the time of injection of the antigen, and the agglutination test remained positive in the majority of animals (72.7%) for 24 h.

When discussing the experimental data described above, it could be postulated that for the first few days of the disease, in generalized forms of MI, positive results of the LA with erythrocytes of a sick individual would be expected. We studied seven samples of erythrocytes obtained from patients with generalized forms of MI, and with a bacteriologically confirmed diagnosis (meningococci of serogroup A were isolated from all of them). A 0.5% suspension was prepared from the solid residue of erythrocytes from blood taken from the finger, and this was used subsequently as pathological material for LA. In four samples the LET was positive, and with three samples the result was negative. The reason was evidently the times at which the erythrocytes were taken. For instance, all positive reactions were observed with erythrocytes obtained from patients during the first 2 days of the disease, and negative reactions after the 3rd day of the disease.

Because of the small number of clinical observations, the question of the duration of circulation of erythrocytes with meningococcal PS adsorbed on their stroma in the blood stream of patients with MI remains unsolved. After tests of LET on a sufficient number of specimens of pathological material, it will be possible to reach a final decision about its diagnostic value, with a view to recommending the test for practical application.

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