A Modified Absorption-Reduction Method to Detect Virus-Specific Hemagglutination Inhibiting and Neutralizing IgM Antibodies

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Abstract. IgG antibodies are preferentially absorbed by protein A-coupled sepharose beads while most of the IgM and IgA antibodies remain in solution. Even relatively low amounts of virus-specific IgM antibodies can then be detected unequivocally by a decrease of the antibody titer following the treatment with reducing agents. Ethandithiol proved superior to 2-mercaptoethanol in combination with neutralization assays.

Introduction

The determination in human sera of antiviral antibodies belonging to the IgM class is an established method to differentiate an acute and a remote virus infection. This is particularly important in the case of suspected rubella infections in early pregnancy.

In the last years a simplified technique for the detection of anti-rubella IgM antibodies by the absorption of IgG antibodies to staphylococcal protein A was described [1, 2, 3]. To avoid false positive results, the sensitivity to 2-mercaptoethanol of the antibodies remaining after absorption was assayed in two of these studies [2, 3]. False negative results were also reported, possibly due to some absorption of IgM to protein A or to other receptors present on the bacterial membrane [2].

In all these studies, the demonstration of rubella-specific antibodies was based on the hemagglutination inhibition assay.

The applicability of the protein A absorption technique in combination with neutralization tests has not yet been reported. We here describe a modified absorption-reduction technique for the detection of virus-specific IgM antibodies in human sera which can also be applied in combination with neutralization assays. To avoid non-specific absorption of IgM to staphylococcal surface structures other than protein A, purified protein A covalently coupled to sepharose beads was used. The shortcomings

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Abbreviations: ET = ethandithiol; HHI = hemagglutination inhibition test; 2-ME = 2-mercaptoethanol; MEM = Minimal essential medium; protein AS = protein A-sepharose CL-4 (Pharmacia)
in neutralization assays of 2-mercaptoethanol were overcome by using ethandithiol as a reducing agent.

Material and Methods

1. Preparation of Sera

a) for rubella hemagglutination inhibition test (HHI): The unspecific inhibitor of rubella virus hemagglutinin was removed by absorption with heparin and MnCl₂, the natural agglutinins were removed from sera by absorption with 50% chicken erythrocytes as described by Antoniadis [5]. By this pretreatment the sera were diluted 1:4.

b) for enterovirus neutralization test (NT): The sera were diluted 1:4 with Eagle’s MEM. The sera were heat-inactivated (56°C 30 min) after absorption of the sera by Protein AS, or after reduction of the IgM-antibodies by ethandithiol (ET).

2. Hemagglutination inhibition test was performed in microtiter plates according to the procedures described by the Center for Disease Control, Atlanta, USA [6].

3. The neutralization tests were performed in microtiter plates according to Phillips [4].

4. Protein A-sepharose Cl-4 was purchased from Pharmacia Chemicals. Appropriate amounts of the dried powder in Eppendorf centrifuge tubes were swollen overnight in 1 ml 0.1 M-phosphate buffer pH 7. Before absorption of sera for HHI, the gels were washed three times in this buffer. After the last centrifugation, the supernatant fluids were carefully removed. Before absorption of sera for NT, the gels were additionally washed twice with Eagle’s MEM.

5. Determination of total amounts of IgM and IgG: The total amounts of IgG and IgM were determined by immunodiffusion plates (Behringwerke) according to the technique described by Mancini [7]. For reference curves a standard human serum (Behringwerke) was used.

6. Procedures for the demonstration of IgM antibodies:

a) absorption: 0.4 ml of prepared sera (final dilution 1:4) were mixed with the protein-AS gels and incubated for 30 min in a 37°C waterbath. After sedimentation of the gels (Eppendorf centrifuge 3200, 2 min) the supernatants were harvested. One half of the supernatants was heat-inactivated (56°C 30 min) and directly used for HHI or NT.

b) Reduction: A mixture of 0.15 ml of absorbed serum and 0.05 ml of 2-mercaptoethanol (0.5 M) were incubated for 1 h at 37°C in a waterbath. Thereafter the serum was heat inactivated (56°C 30 min) and used for HHI.

For use in NT, a mixture of 0.1 ml of absorbed serum and 0.1 ml of ethandithiol (0.06 M) was kept for 3 h at room temperature. During the subsequent heat inactivation, the ethandithiol was evaporated. The final dilution of the absorbed and reduced serum corresponded to a dilution of 1:8.