Specificity of Monoclonal Antibodies Obtained by Immunization of Mice with Trypsin-Treated Group A Streptococcus Culture

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Hybridomas producing monoclonal antibodies intensively reacting with group A streptococcus antigens in enzyme immunoassay were obtained as a result of immunizing mice with pepsin-treated cultures of group A streptococcus. All antibodies were referred to class M immunoglobulins. The reactions of monoclonal antibodies were completely inhibited by the pepsin-treated culture of group A streptococcus. The degree of inhibition with A-polysaccharide was lower, being 17.5 to 50.0 in different monoclonal antibodies. All the monoclonal antibodies obtained cross-reacted with antigens of murine and human epithelial tissues of the thymus and skin.

Key Words: *streptococcus; A-polysaccharide; monoclonal antibodies; cross-reacting antigens*

Antibodies to streptococcal group A polysaccharide (A-PS) are known to be produced for immunization of mice with a pepsin-treated culture of group A streptococcus [9]. A-PS represents an L-rhamnose homopolymer with alternating α-1-2 and α-1-3 bonds, to which N-acetylglucosamine is connected by the β-1-3 bond [8]. Recently A-PS was shown to contain several antigenic determinants including both N-acetylglucosamine and rhamnose [4]. β-N-acetylglucosamine is a component of the specific determinant and is responsible for the group appurtenance of streptococcus [8]. It is also a component of the determinant common to A- and L-polysaccharides [1,10]. The rhamnose component of A-PS is identical to polysaccharide of the A variant of streptococcus, which is an L-rhamnose homopolymer [8].

Antibodies to A-PS have been detected in various streptococcal infections and in complications after streptococcal infections, such as rheumatic fever and glomerulonephritis. The spectrum of antibodies to different determinants of A-PS differs in patients with different patterns of the rheumatic process [2,3].

At present A-PS is known as an inductor of autoantibodies reacting with antigens of various mammalian tissues: epithelial (skin, thymus, intestine), muscular, and connective [5,12]. Using a panel of murine monoclonal antibodies (MAb) to A-PS it was shown that a population of antibodies directed to N-acetylglucosamine reacts with antigenic determinants of the tissue proteins actin, vimentin, myosin, and keratin [12].

All this prompted further studies of antibodies to A-PS and led to the creation of a panel of antibodies of different specificity followed by analysis of their functional activity using different models, including cell cultures of different origin. In this research immunization of mice with pepsin-treated group A streptococcus permitted us to obtain several hybridomas; the specificities of MAb

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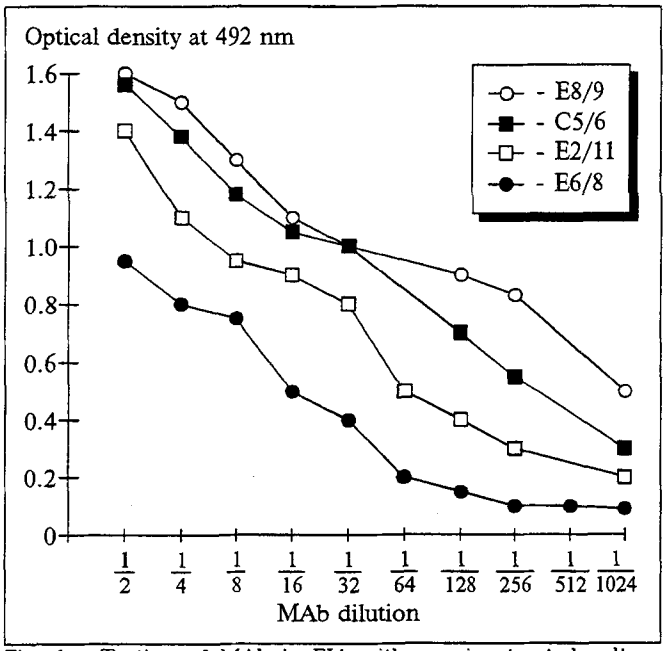

Fig. 1. Testing of MAb in EIA with pepsin-treated culture of group A streptococcus.

produced by them were estimated and cross-reactions with mammalian tissues studied.

MATERIALS AND METHODS

Cultures of groups A and L (Prague collection) and A-variant streptococcus (a gift of Dr. McCarthy, USA) were used. BALB/c mice weighing 17 to 20 g were immunized with pepsin-treated group A streptococci [9]. The culture was injected intraperitoneally in a dose of 2×10^9 bacterial cells per animal 3 times a week for 4 weeks. The resolving dose of 3×10^9 bacterial cells was likewise injected intraperitoneally 3 days before hybridization. Murine plasmacytoma Sp-2/0 cells were used to obtain hybridomas. For fusion, polyethyleneglycol 4000 (Merck) was used. Hybridization was carried out routinely [11]. Cells were grown in RPMI-1640 medium (Gibco) with 10-20% fetal calf serum (manufactured by the N. F. Gamaleya Research Institute of Epidemiology and Microbiology). The limiting dilutions method was used for cloning. The hybrid clones were screened and MAb in supernatants assessed by enzyme immunoassay (EIA) with antibodies to murine immunoglobulins conjugated with peroxidase (manufactured by the N. F. Gamaleya Research Institute). Pepsin-treated streptococcal cultures, groups A, A-variant, and L, in concentrations of 2.5×10^8 bacterial cells, were layered onto plates. The reaction was estimated with a Titerteck Multiskan photometer at wavelength 492 nm. MAb appurtenance to a certain class of murine immunoglobulins was determined in EIA with sera to individual murine IgG and IgM subclasses (the sera were kindly supplied by Dr. E. V. Sidorova, Research Institute of Viral Preparations, Russian Academy of Medical Sciences).

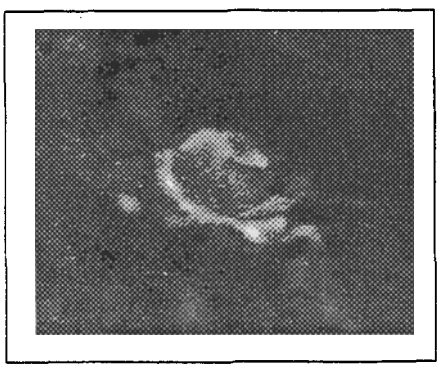

Fig. 2. Testing of MAb C5/6 on slices of human thymus. Reaction with epitheliocytes of Hassall's bodies. Obj. 40, homal 3.

For reaction inhibition in EIA we used pepsin-treated cultures of groups A, A-variant, and L streptococcus; A-PS obtained by formamide extraction from bacterial cells [7]; N-acetylglucosamine (BDH, UK), and L-rhamnose synthetic disaccharides with α-1-2 and α-1-3 bonds (P-1-2 and P-1-3) [4]. For experiments with streptococcus cultures, equal volumes of MAb and bacterial cells in a concentration of 10^{11}/ml were mixed. Inhibition with carbohydrates was performed as described previously [4]: an equal volume of carbohydrate solution in a concentration of 2 mg/ml was added to MAb diluted 1/8 to 1/16. Maltose was used in these experiments for control. The mixture was incubated for 2 h at 37°C and 18 h at 4°C. The percent share of reaction inhibition was estimated by the formula

$$\frac{\text{MAb optical density without inhibitor - MAb optical density with inhibitor}}{\text{MAb optical density without inhibitor}}\times100 \text{ [6].}$$

MAb cross-reactions with tissues were studied in indirect immunofluorescence with antibodies to murine immunoglobulins labeled with fluorescein isothiocyanate (manufactured by the N. F. Gamaleya Research Institute). The antibodies were tested on cryostat slices of murine skin and murine and human thymus and myocardium as described previously [5]. The reaction was assessed using a LYuMAM I-1 fluorescent microscope with objective 40.

RESULTS

Four stable monoclones were obtained. MAb produced by them were referred to IgM. All MAb intensively reacted with pepsin-treated group A streptococcus culture up to dilutions of 1/512 to 1/1024 in EIA (Fig. 1). Testing of antibodies on cultures of groups A, A-variant, and L streptococ-

cus also showed positive reactions, though less intensive than with group A streptococcus: 0.15-0.4 in dilution 1/32.

For characterization of determinants to which the resultant MAb were directed, experiments were carried out on inhibition of antibodies with pepsin-treated cultures of groups A, A-variant, and L streptococci, with A-PS, N-acetylglucosamine, and dirhamnosides. After this MAb were tested on plates sensitized with pepsin-treated group A streptococcus. Group A streptococcus was found to inhibit the reaction of virtually all MAb by 100%. A-variant streptococcus culture inhibited the reaction by 97-85%. Group L culture inhibited the MAb reaction to a far lesser degree, the most pronounced inhibition (31%) being observed with E6/8 MAb (Table 1).

The addition of A-PS to MAb C5/6 resulted in 27.3% reaction inhibition. The degree of inhibition was lower with N-acetylglucosamine (11.7%) and rhamnosides P-1-2 and P-1-3 (11.3 and 6.5%, respectively). A more pronounced inhibition (43.9%) with A-PS was observed with MAb E2/11. N-acetylglucosamine inhibited the reaction by 29.2%, dirhamnosides by 34.3 and 25.5%, respectively. MAb E6/8 were the most intensively inhibited by A-PS: 50%. The addition of N-acetylglucosamine resulted in 44.7% reaction inhibition, whereas rhamnosides inhibited it by only 26-27%. In contrast to other MAb, E8/9 were inhibited with A-PS to a far less extent, by only 17.5%, and were not inhibited with N-acetylglucosamine at all. Rhamnosides inhibited the reaction by 16 and 24%. It is noteworthy that a twofold increase of the carbohydrate dose did not boost the inhibition.

Hence, the MAb we obtained were completely inhibited by pepsin-treated group A streptococcus culture and were inhibited to a much lesser extent by A-PS and its component carbohydrates. All MAb cross-reacted with tissue antigens. Testing of MAb E2/11, E8/9, and E6/8 on murine skin slices showed that the reaction was localized in the basal layer of the epithelium and basement membrane. MAb C5/6 reacted with all layers of skin epithelial tissue. There was no reaction on murine thymus slices. On human thymus slices MAb C5/6 and E2/11 reacted with epitheliocytes of Hassall's bodies (Fig. 2). No reactions were observed on human myocardium, but there were weakly expressed reactions on murine myocardial slices, localized in the endothelium of small vessels.

The antibodies we obtained were similar in specificity to MAb A6/1D, which were slightly inhibited by A-PS and the carbohydrates composing it [4]. On the other hand, MAb A3/2 were 92% inhibited with N-acetylglucosamine and MAb D4/1 60% inhibited with rhamnosides. Hence, it is possible to obtain antibodies to A-PS of different specificity by immunizating animals with a pepsin-treated culture of group A streptococcus.

The low affinity of MAb may be responsible for poor inhibition by carbohydrates. IgM antibodies are known to possess a lower affinity in comparison with IgG. On the other hand, since the MAb reaction is completely inhibited by pepsin-treated group A streptococcus, it is possible that the specificity of the resultant antibodies depends on the determinant including, besides A-PS, some additional structures.

All the resultant MAb cross-reacted with antigens of murine epithelial tissue, and two of them cross-reacted with human thymus epithelial tissue. Cross-reactions with various mammalian tissues is one of the most important properties of antibodies to A-PS. Until recently, cross-reactions were considered to be caused by the presence of the relevant monosaccharides in the tissues. However, analysis of cross-reactions of different MAb to A-PS revealed that: 1) MAb react with proteins whose molecules have a spiral structure, namely, keratin, vimentin, myosin, and recombinant streptococcal M5 and M6 proteins; 2) none of the resultant

TABLE 1. Inhibition of MAb Reactions by Streptococcus Cultures and Carbohydrates in EIA

Preparations used for inhibition	Inhibition of MAb reaction, %			
	C5/6	E2/11	E6/8	E8/9
Streptococcus cultures				
A	100	100	98	95
A—variant	97	93	78	85
L	0	10.5	31.8	16.2
Carbohydrates				
A—PS	27.3	43.9	50.0	17.5
N—acetylglucosamine	11.7	29.2	44.7	0
P—1—2	11.3	34.3	26.1	24.3
P—1—3	6.5	25.5	27.8	16.5
Maltose	0	10.1	6.3	8.7

MAb to A-PS reacts with bovine serum albumin, polynucleotides aggregated with immunoglobulin, etc. The structure with which MAb do react represents a peptide [12]. Polyclonal and monoclonal antibodies to N-acetylglucosamine obtained from rheumatic patients have been found to react with the same peptide. It has been hypothesized that N-acetylglucosamine and the peptide have a similar conformational structure [12]. Hence, MAb to A-PS cross-reactinig with certain mammalian proteins represent a new group of multireactive antibodies that are of diagnostic and prognostic importance in human diseases of streptococcal etiology.

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