Preparation and Characterization of Monoclonal Antibodies to Group-Specific Antigenic Determinant of Group A Streptococcus Polysaccharide

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Group A streptococcus polysaccharide contains at least two different group-specific determinants including N-acetyl-β-D-glucosamine (more important) and rhamnose. Monoclonal antibodies to group-specific determinants of group A streptococcus polysaccharide do not react with tissue antigens. Cross reactions with tissues were detected in tests with monoclonal antibodies to rhamnose-enriched epitopes of group A streptococcus polysaccharide.

Key Words: *group A streptococcus; microorganism cell wall polysaccharide; monoclonal antibodies; autoimmunity*

Group A streptococcus polysaccharide (A-PS) is a polymer of L-rhamnose and N-acetyl-β-D-glucosamine (βGlcNac). A-PS was believed to contain several rhamnose residues and one group-specific determinant (βGlcNac) [9,11]. However hybridization methods changed our notions on the structure of group-specific A-PS determinant. Group-specific monoclonal antibodies (MAb) were obtained, directed to the A-PS determinant including βGlcNac and rhamnose [13]. Group-specific MAb were revealed, differing by their characteristics and presumably reacting with different sites of A-PS molecule [8]. Therefore, the structure and number of group-specific determinants of A-PS are still not clear. A-PS inhibits functional activity of T suppressors due to a determinant in which βGlcNac plays the key role [1,4]. Some immunoregulatory disorders characteristic of streptococcal diseases can be due to A-PS determinants similar to epidermal thymic antigens. Group-specific determinant of A-PS is believed to play an important role in this phenomenon [6,12]. It has not been proven yet whether group-specific A-PS determinant is cross-reactive.

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We obtained MAb to A-PS group-specific determinant and studied their properties.

MATERIALS AND METHODS

Heat-inactivated pepsin-treated [11] streptococcal strains J-17A4, No. 6/49 (group A) and No. 43/59 (group L) from Prague collection and A variant No. 32/18, a gift of Dr. McCarty, were used as sources of polysaccharides. A-PS was isolated by formamide extraction [9]. In addition, hydrochloric acid streptococcus extracts of 19 various groups were used [2].

The specificity of MAb to certain A-PS determinant was studied by competitive inhibition of MAb-A-PS enzyme immunoassay [3]. The inhibitors were synthetic L-rhamnose preparations simulating different A-PS epitopes, kindly given by L. V. Bakinovskii and N. E. Nifantiev, Institute of Organic Chemistry, Russian Academy of Sciences: 1) dirhamnoside with 1-2 bond, αL-Rha(1-2)-αL-Rha-O$(CH_2)_6NH_3$ — R1-2R; 2) dirhamnoside with 1-3 bond, αL-Rha(1-3)-αL-Rha-O$(CH_2)_6NH_3$ — R1-3R; 3) trirhamnoside with 1-2 and 1-3 bonds, αL-Rha(1-3)-αL-Rha(1-2)-αL-Rha-O$(CH_2)_6NH_3$ — R1-3R1-2R; 4) polyrhamnoside with 1-2 and 1-3 bonds, [-αL-Rha(1-2)-αL-Rha(1-3)]-

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αL-Rha-O$(CH_2)_6NH_2$] — PR; 5) βGlcNac connected by 1-3 bond to αL-rhamnose, βGlcNac(1-3)-αL-Rha-OCH_3 — βGlcNac1-3R; 6) βGlcNac connected by 1-3 bond to rhamnose disaccharide αL-Rha(1-2)-αL-Rha-OCH_3 — βGlcNac1-3R1-2R.

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β-D-GlcNac-

Besides, paranitrophenyl-N-acetyl-β-D-glucosamine (pβGlcNac) and N-acetyl-D-glucosamine (GlcNac) (Chemicals) were used.

For obtaining MAb, female BALB/c mice weighing 17-20 g were intraperitoneally immunized with heat-inactivated pepsin-treated group A streptococcus (3×10^9 bacterial bodies 3 days running every week during 3 weeks). Challenge dose (3×10^9 bacterial cells) was injected intravenously on day 25 after the last injection to mice with high anti-A-PS antibody titer. After 2 days splenocytes were fused with murine plasmacytoma cells [5]. Antibodies to A-PS in the sera, supernatants, and ascitic fluids containing MAb were detected by ELISA [7], or agar gel immunodiffusion [2]. The reaction of MAb with epidermal antigens was studied by indirect immunofluorescence on cryostat sections of labial skin of BALB/C mice.

RESULTS

Nineteen stable monoclones were obtained. Supernatants of 10 clones contained MAb reacting in EIA with groups A and L streptococci and A variant or group A and A variant (group 1). MAb in supernatants of the rest 9 clones were group-specific and reacted only with group A streptococcus (group 2). In this group, pβGlcNac inhibited interaction of MAb with group A streptococcal culture by more than 70%. A-PS intensely inhibited (by 70%) the reaction of MAb of 96G10 and 24C6 clones. For other clones, MAb reactions were inhibited by no more than 30%.

For more detailed characterization of group-specific antibodies, ascites were induced by clones 96G10, 48B6, and 24C6. The reaction of MAb isolated from ascitic fluid with A-PS (100 μg/ml) was assayed by ELISA in multiwell plates with a high adsorption activity. Complete inhibition of MAb with A-PS indicates its specificity.

Immunodiffusion showed that MAb 96G10 and 24C6 formed a precipitation line with A-PS (7-250 μg/ml), while MAb 48B6 exhibited no precipitation. Investigation of MAb 96G10 and 24C6 by this method with hydrochloric acid extracts of 19 streptococcus groups showed that they reacted only with group A streptococcus. MAb 96G10 and 24C6 are IgG3 and MAb 48B6 is IgM.

Competitive inhibition of interaction between MAb and A-PS showed that A-PS more intensely inhibited the reaction of MAb 96G10 than of MAb 48B6. The same regularity was observed when synthetic preparations containing GlcNac were added to MAb (monosaccharides pβGlcNac, GlcNac, and trisaccharide βGlcNac1-2R, Table 1). Disaccharide βGlcNac1-3R did not inhibit MAb 48B6 and weakly inhibited MAb 96G10, which virtually did not differ from the control with maltose (5.3×2.0%). Preparations containing rhamnose alone: dirhamnosides R1-2R, R1-3R, trirhamnoside R1-3R1-2R, and polyrhamnoside PR did not inhibit the reaction of MAb 96G10 (2.3-6.8% inhibition). Dirhamnosides did not suppress the reaction of MAb 48B6, while tri- and polyrhamnoside inhibited it by 19.9±4.4 and 19.8±3.5%, respectively, i. e. significantly higher than maltose ($p<0.01$).

Supernatants of 19 clones and MAb 96G10, 48B6, and 24C6 isolated from ascitic fluid were tested on skin sections of BALB/c mice by indirect immunofluorescence. MAb reacted with epidermal antigens only in 4 cases. The cytoplasm of basal cells fluoresced with antigens of clone 1, perinuclear cytoplasm in differentiated layers adjacent to the basal layer fluoresced with clone 2, and the cytoplasm of differentiated epidermal layers fluoresced with clones 3 and 4. All clones, whose antibodies reacted with epidermal antigens belong to group 1. Reactions with epidermal cells were stopped after supernatant adsorption with pepsin-treated culture of group A streptococcus or by adding A-PS.

Hence, the characteristics of MAb directed to various group- and non-group-specific determinants of A-PS have been studied for the first time. Three MAb to group-specific A-PS determinant were examined: MAb 24C6 and 96G10 are precipitating and 48B6 non-precipitating. MAb pβGlcNac completely inhibited the reaction between MAb and A-PS, which in-

TABLE 1. Inhibition of Immunoenzyme Reaction of MAb by A-PS and Synthetic Analogs of Its Epitopes

Inhibitor	Inhibition of MAb reaction, %	
	96G10	48B6
A-PS	75.5±7.5	31.0±4.6
pβGlcNac	88.2±3.0	61.2±7.0
GlcNac	48.0±7.5	24.5±4.8
βGlcNac1-3R	13.3±5.0	0*
βGlcNac1-3R1-2R	36.7±5.8	24.0*
R1-R2	4.9±3.2	5.3±2.7
R1-3R	6.8±3.6	3.3±0.5
R1-3R1-2R	5.1±3.0	19.9±4.4
PR	2.3±1.6	19.8±3.5
Maltose	5.3±2.0	

Note. *One measurement.

dicates that their specificity is due to βGlcNac. The absence of reactions of MAb 24C6 and 96G10 with hydrochloric acid extracts of streptococci other than group A (including group L streptococcus, whose polysaccharide contains βGlcNac) in the immunodiffusion test [10] indicates that MAb are directed towards group-specific determinant of A-PS. The same is true for non-precipitating 48B6 antibodies reacting with group A streptococcus, but not with group L and A variant.

Data on competitive inhibition of MAb 96G10 and 48B6 indicate that the corresponding epitopes include both βGlcNac and rhamnose. A-PS and preparations containing GlcNac inhibit both MAb, the highest inhibitory effect being observed with pβGlcNac. Its inhibitory effect surpasses that of GlcNac. This may be due to the fact that GlcNac is a mixture of 2 isomers, α and β. The inhibition of MAb 96G10 by βGlcNac-containing preparations is more pronounced than that of MAb 48B6. This may be due to different affinity of the examined MAb. Precipitating antibodies to A-PS are high-affinity, while non-precipitating are characterized by low affinity [8]. Presumably, the precipitating and non-precipitating antibodies are directed to different sites of polysaccharide chain, which is confirmed by our results. None of the agents containing rhamnose alone suppressed the reaction of MAb 96G10. On the other hand, tri- and polyrhamnoside inhibited MAb 48B6, though not so intensely as GlcNac. Apparently, MAb 48B6 are directed towards A-PS determinant containing βGlcNac and rhamnose. The rhamnose site of this determinant apparently consists of at least 3 rhamnose units, because dirhamnosides do not inhibit MAb reaction with A-PS.

Presumably rhamnose is present in the determinant to which MAb 96G10 are directed, because βGlcNac1-3R1-2R notably inhibited the reactions of these MAb with A-PS. MAb 96G10 may be similar to precipitating antibodies to A-PS which are directed towards repeated βGlcNac moieties along the polysaccharide chain [8]. MAb 48B5 seem to be directed to a smaller determinant.

Therefore, MAb directed to different group-specific A-PS determinants do not react with epidermal antigens. Neither non-precipitating (48B6) nor precipitating MAb (96G10 and 24C6) react with epidermal antigens. The absence of reaction does not depend on the class of antibodies, because neither MAb 96G10 and 24C6 (IgG) nor MAb 48B6 (IgM) reacted with epidermal antigens. Interestingly, cross-reactions with epidermal antigens were detected in testing MAb reacting with A-PS and with polysaccharides of streptococci of group L and A variant. Rhamnose is a common component of these polysaccharides [9-11]. βGlcNac is a component of A-PS and group L streptococcus polysaccharide, but not of A variant polysaccharide. This suggests that MAb reacting with tissue antigens are directed to the polysaccharide epitopes in which rhamnose plays the dominant role.

The results of these and previous [5] studies indicate that A-PS contains at least 4 cross-reacting determinants. The dominant role of rhamnose in this epitopes suggests the presence of cross-reacting determinants common for skin epithelial and thymic antigens in group A streptococci and in other microorganisms, whose polysaccharide contains rhamnose. Therefore, immunoregulatory disorders in infectious diseases of different etiology are probably mediated via common mechanisms determined by carbohydrate components of bacterial cell wall.

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