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NOTES

Unique and Common Protective Epitopes Among Different Serotypes of Group A Streptococcal M Proteins Defined with Hybridoma Antibodies

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A set of four monoclonal antibodies was produced against a highly purified pepsin extract of type 5 streptococcal M protein. Three of the four antibodies cross-reacted with purified M proteins from heterologous serotypes and opsonized the respective heterologous organisms. Our studies suggest that monoclonal antibodies may be useful in identifying subpeptides of various M proteins containing common, protective epitopes that are capable of evoking antibodies that would protect against several different potentially "rheumatogenic" serotypes.

The surface M protein of group A streptococci evokes a humoral immune response that protects the host against subsequent infection with the same M serotypes (16). Although M protein antibodies are for the most part serotype specific, Hirst and Lancefield (13) as early as 1939 demonstrated that certain epitopes are shared among different serotypes. More recently, it has been shown that some cross-reactive M antibodies were directed against common, protective epitopes and, therefore, were able to opsonize the heterologous organisms (6, 7, 10, 11). Thus, it appears that the M protein of one serotype contains a heterogenous population of antigenic determinants, some that are unique to the particular serotype and others that give rise to cross-reactive antibodies that opsonize organisms of a different serotype. In addition, recent studies in our laboratory have identified a third set of M protein epitopes that evoke opsonic antibodies that cross-react with human heart tissue (6).

The ability to identify precisely the different regions of the M protein molecules containing type-specific and cross-reactive antigens, in addition to those which may be immunologically identical to host tissues, may shed light on the mechanisms of protective and tissue cross-reactive immunity evoked by group A streptococci. In the present study we used a set of four monoclonal antibodies directed against a polypeptide fragment of type 5 M protein to define cross-reactive and type-specific antigenic determinants of types 5, 6, 19, and 24 M proteins. We present data to show that certain antigenic regions of the M protein molecules are conserved and that antibodies directed against a single common epitope are sufficient to opsonize homologous and heterologous serotypes of group A streptococci.

M protein (pep M) was purified to homogeneity from limited peptic digests of types 5, 6, 18, 19, and 24 group A streptococci (5, 6, 15). Mouse monoclonal antibodies against pep M5 were produced by methods previously described (12). Antibody-secreting hybridomas were detected by an enzyme-linked immunosorbent assay (ELISA) as described below. Specific M protein monoclonal antibodies were purified from ascites fluids (12) by affinity chromatography with a column of pep M5 covalently linked to Sepharose 4B (Pharmacia Fine Chemicals, Upsala, Sweden) (6). The antibodies adsorbed to the column were eluted with 0.2 M glycine–0.2m NaCl, pH 2.8, dialyzed extensively against 0.02 M phosphate–0.15 M NaCl, pH 7.4, and then concentrated to the original volume by membrane filtration (YM30 membrane; Amicon Corp., Lexington, Mass.). The samples were sterilized by filtration and stored at 4°C.

Culture supernatants, ascites fluids, and affinity-purified samples were tested for the presence of type-specific and cross-reactive M antibodies by ELISA (6, 7) with pep M5, pep M6, pep M18, pep M19, and pep M24 (5 μg/ml) adsorbed to polystyrene cuvettes as the solid-phase antigen. In some instances ELISAs were performed with whole streptococci as particle-phase antigens (12) to demonstrate the binding of antibodies to the surface M protein in its native state.

Type-specific and cross-reactive opsonic antibodies were detected by in vitro opsonophagocytic assays as described elsewhere (5). The test mixture consisted of 0.4 ml of fresh human blood supplemented with 10 U of heparin per ml, 0.05 ml of a standard suspension of streptococci, and 0.1 ml of test antibody. The percentage of neutrophils with associated streptococci (percent phagocytosis) was estimated by microscopic counts of stained smears prepared from the assay mixture after rotating it at 37°C for 45 min. Indirect bactericidal tests were performed with the same test mixtures, except that fewer CFU of streptococci were added (5). Indirect immunofluorescence tests for heart cross-reactive

TABLE 1. ELISA titers of pep M5 monoclonal antibodies against homologous and heterologous pep M antigens

Monoclonal antibody[a]	ELISA titer against:				
	pep M5	pep M6	pep M19	pep M24	pep M18
IB7	102,400	102,400	102,400	<200	<200
IE11	25,600	3,200	<200	<200	<200
IIID11	102,400	<200	<200	12,800	<200
IB11	102,400	<200	<200	<200	<200
CD3[b]	<200	<200	<200	<200	<200

[a] Assays were performed with ascites fluids from BALB/c mice (see the text).

[b] An unrelated IgG1 hybridoma antibody raised against purified type 1 fimbriae of *E. coli* (1).

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TABLE 2. Opsonization of types 5, 6, 19, and 24 streptococci by pep M5 monoclonal antibodies

Monoclonal antibody[a]	Percentage of PMN[b] with associated streptococci				
	Type 5	Type 6	Type 19	Type 24	Type 18
IB7	98	76[c]	28	16	0
IE11	98	20	4	2	2
IIID11	36	0	8	18	0
IB11	58	0	0	4	0

[a] Opsonophagocytic assays were performed with affinity-purified antibodies (see the text) that were concentrated to an ELISA titer equivalent to that of the original ascites fluids.

[b] PMN, Polymorphonuclear leukocytes.

[c] Underlined values represent significant levels of opsonization of heterologous organisms.

antibodies were performed with purified sarcolemmal membranes as described elsewhere (6).

All of the monoclonal antibodies raised against pep M5 reacted in high dilution with the immunizing antigen, as determined by ELISA (Table 1). Double immunodiffusion assays revealed that each of the antibodies was of the immunoglobulin G1 (IgG1) subclass. One of the hybridoma antibodies (IB7) cross-reacted with pep M6 and pep M19, with a titer of 102,400 against each heterologous M protein (Table 1). The IE11 clone produced antibody that cross-reacted with pep M6 but not pep M19 or pep M24, and IIID11 cross-reacted with pep M24 but not pep M6 or pep M19. None of the antibodies cross-reacted with pep M18. Thus, three of the four monoclonal antibodies were directed against epitopes that were shared by heterologous serotypes of M protein. An unrelated IgG1 hybridoma antibody, CD3, raised, against type 1 fimbriae of *Escherichia coli* (1) did not react with any of the pep M proteins tested.

To determine whether the antibodies were binding to protective M protein determinants that were exposed on the surface of the organisms, in vitro opsonophagocytic assays were performed with each hybridoma antibody, using homologous or heterologous serotypes of group A streptococci (Table 2). All of the antibodies opsonized type 5 organisms, indicating that each was directed against a protective epitope that was exposed on the native type 5 M protein. In addition, the antibodies that cross-reacted with heterologous pep M proteins also opsonized the respective heterologous serotypes of group A streptococci. The most highly cross-reactive antibody, IB7, opsonized type 6, type 19, and type 24 organisms in addition to the homologous type 5 M serotype.

The finding that the IB7 antibody opsonized type 24 streptococci, yet did not react with pep M24 in the ELISA, suggested that it was directed against a cross-reactive epitope on the native M protein that was not present on the pepsin-derived polypeptide fragment of M24. To test this hypothesis we performed a particle-phase ELISA with type 24 streptococci as the test antigen. The titer of the IB7 antibody against the intact organism was 800, whereas the titer of IE11, which did not opsonize type 24 streptococci, was <100. These results confirm the presence of a cross-reactive, protective epitope of type 24 M protein that is not represented on the pep M24 peptide fragment.

The ability of the IB7 hybridoma antibody to opsonize homologous and heterologous serotypes of group A streptococci was confirmed by indirect bactericidal assays (Table 3). Although the test organisms were not completely eradicated in the presence of the antibody, there was a more significant reduction of CFU than in control mixtures, confirming that the antibody was directed against a presumably protective M protein determinant on all four serotypes.

Because previous studies in our laboratory have demonstrated the presence of antigenic determinants on type 5 M protein that are cross-reactive with sarcolemmal membranes of human heart (6), we tested each of the hybridoma antibodies for heart tissue reactivity. None of the antibodies cross-reacted with purified sarcolemmal membranes, as determined by indirect immunofluorescence tests.

Recently, there has been considerable interest in defining the minimum peptide structures of various group A streptococcal M proteins necessary to elicit protective antibody responses. The concept that immunity against only a portion of the M protein molecule is sufficient to protect against the intact organism is supported by recent studies in our laboratory (2, 3, 5a, 8). Several chemically synthesized peptides representing only limited regions of type 24 (2, 3, 5a) and type 5 M proteins (8) evoked protective immune responses in rabbits. Furthermore, monoclonal antibodies raised against a polypeptide fragment of type 24 M protein opsonized type 24 organisms (12). In each case the immune responses were type-specific and afforded no protection against heterologous serotypes.

The present study provides the most definitive evidence that antibodies directed against a single epitope on one M protein molecule are sufficient to opsonize heterologous serotypes sharing the same epitope. The level of opsonization obtained with monoclonal antibodies was generally lower than that observed with polyclonal antisera (6, 7). Perhaps the lower levels of opsonization were the result of inefficient complement fixation by the mouse IgG1 subclass (9). In addition, the data obtained by ELISA suggest that some of the hybridoma antibodies bind to cross-reactive epitopes either with lower affinity or in lesser amounts, which may result in relatively sparse concentrations of antibody on the surface of the organisms. Nonetheless, with the recent advances in defining the covalent structures of several M proteins (2, 4, 8, 14, 15), hybridoma antibodies should be useful as probes to identify small subpeptides that contain common protective, as opposed to tissue cross-reactive, epitopes. Such peptides, either natural or chemically synthesized, could then be used to evoke cross-protective, polyclonal immune responses against many different serotypes of group A streptococci.

TABLE 3. Indirect bactericidal activity of monoclonal antibody IB7 against types 5, 6, 19, and 24 *Streptococcus pyogenes*

Antibody[a]	No. of colonies after 3 h of growth in test mixtures (inoculum)				
	Type 5 (20)	Type 6 (26)	Type 19 (35)	Type 24 (30)	Type 18 (32)
Control	>2,000	>2,000	>2,000	>2,000	>2,000
IB7	222	134	249	544	>2,000

[a] Assays were performed with affinity-purified antibodies (see text) that were concentrated to an ELISA titer equivalent to that of the original ascites fluid. The control was 0.02 M phosphate–0.15 M NaCl, pH 7.4.

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