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Murine Monoclonal Antibodies Reactive with Human Heart and Group A Streptococcal Membrane Antigens

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Ten selected murine hybridoma cell lines that produce monoclonal antibodies against M type 5 *Streptococcus pyogenes* and human heart antigen were isolated. All of the monoclonal antibodies studied were determined to be the immunoglobulin M isotype. The antibodies were characterized on the basis of their reactions with Triton X-100-extracted whole human heart antigens, sodium dodecyl sulfate-extracted sarcolemmal antigens, and whole streptococci or their membranes. Enzyme-linked immunosorbent assays and Western immunoblotting techniques were used to compare the reactivity of the monoclonal antibodies. All 10 of the antibodies were first selected for their reactivity with Triton X-100-extracted heart antigens and whole group A, M type 5 streptococci. These antibodies were then divided into two categories: strong reactors or weak reactors with human sarcolemmal and streptococcal membranes. Among the strong reactors, two different types of monoclonal antibodies were observed on the basis of their immunobanding patterns with sarcolemmal and streptococcal membranes on Western blots. Monoclonal antibodies that were strong reactors with sarcolemmal and group A streptococcal membrane antigen were directed against a determinant on a family of proteins. The major reactants of sarcolemmal extracts were high-molecular-weight proteins near 200,000. Some monoclonal antibodies demonstrated more specificity for the heart than did others when reacted with separated Triton X-100-extracted tissue antigens from the heart, kidney, and skeletal muscle. One of the monoclonal antibodies that reacted with group A streptococci reacted with a Triton X-100-extracted heart antigen ca. 40,000 daltons in size. None of these monoclonal antibodies opsonized type 5 *Streptococcus pyogenes*, and in enzyme-linked immunosorbent assays most of the antibodies were found to react to a lesser degree with other groups of streptococci. Monoclonal antibody was used to probe normal and rheumatic sarcolemma for differences in reactivity. Although the rheumatic heart reacted more intensely, no major differences between the immunobanding patterns of normal and rheumatic hearts were observed.

The literature is replete with descriptions of cross-reactions between the group A streptococcus and heart tissue from a variety of animal and human sources. It is well established that immunization of rabbits with *Streptococcus pyogenes* induces the production of antibodies which bind to heart tissue (7, 11). Also, the sera of patients with acute rheumatic fever, uncomplicated streptococcal infections, and poststreptococcal glomerulonephritis have been shown to react with heart tissue (6, 13). Acute rheumatic fever sera have been shown to be four times more reactive than sera from other streptococcal diseases.

Most of the studies in the past have concentrated on cross-reactive streptococcal antigens rather than on antigens in the host that react with streptococcal antibody. Van de Rijn et al. (10) isolated four polypeptides from group A streptococcal membranes which absorbed heart-reactive antibodies from human sera, and Dale and Beachey (3) were able to stimulate heart-reactive antibodies in certain rabbits by immunization with type 5, pepsin-extracted M protein. Studies with streptococcal membranes have demonstrated that there are cross-reactive antigens in all group A streptococcal membranes (11). However, the work with type 5 M protein demonstrated a serotype-specific, cross-reactive antigen-antibody system. All of these studies have suggested that the cross-reactive host antigen is related to a sarcolemmal component of the heart. Other studies have associated cross-reactivity with immunodeterminants of the streptococcal group A carbohydrate and heart valves (5).

However, a major problem in the identification of the antigens related to both streptococci and the heart has been the complexity of conventional polyclonal antisera and the streptococcal antigens used for absorption of this antisera. The purpose of this study was to isolate hybridoma cell lines that secrete monoclonal antibodies against both group A streptococci and human heart antigens. Monoclonal antibodies represent a powerful probe for the related antigens of the streptococcus and human tissue.

In this study, 10 selected monoclonal antibodies were characterized, and their reactions with separated streptococcal and tissue antigens were studied. The monoclonal antibodies were classified on the basis of strong or weak reactivity with sarcolemmal and streptococcal membrane antigens. Some of the monoclonal antibodies demonstrated a strong specificity for specific extracted heart antigens, whereas other monoclonal antibodies reacted with similar epitopes extracted from heart, kidney, or skeletal muscle. In addition, their specificity for various groups of streptococci was determined. Monoclonal antibodies were also used to probe normal and rheumatic sarcolemma for potential differences. Although the rheumatic sarcolemma extract reacted more intensely, no major differences in immunobanding patterns were observed.

MATERIALS AND METHODS

Streptococci. The strain of *S. pyogenes* used in this study was M type 5 (Manfraedo) obtained from Edwin Beachey, Veterans Administration Hospital, Memphis, Tenn. Streptococcal groups B, C, P, and R, *Streptococcus mutans* (GS5), and *Streptococcus sanguis* were obtained from Joseph J. Ferretti, University of Oklahoma Health Sciences Center, Oklahoma City, Okla.

Streptococcal membranes. Membranes were purified from

whole streptococci by a procedure previously described (9). Twenty-five grams (wet weight) of streptococcal cells were suspended in 100 ml of phosphate buffer (pH 7). Subsequently, cells were broken with sterile glass beads in a Braun cell homogenizer. Two 90-s bursts were used to homogenize the cells at 0°C. Beads were separated from cells with a scintered glass filter. The homogenate was centrifuged twice at 12,000 × *g* for 20 min. The supernatant was then centrifuged at 100,000 × *g*, and the resulting pellet contained the streptococcal membranes. The pellet was suspended in 0.01 M phosphate buffer (pH 7.4) containing 0.04 M NaCl and 0.005 M $MgCl_2$. DNase and RNase (0.2 mg/ml each) were added, and the mixture was digested at 37°C for 2 h. The membranes were centrifuged at 100,000 × *g* and washed once with buffer. The membrane pellet was resuspended in distilled water and lyophilized.

Triton X-100-extracted human tissue antigens. Normal tissues were obtained from autopsy or transplant donors and frozen at −70°C. Postmortem time on tissues ranged from 1 to 24 h. The tissue was thawed and then homogenized in phosphate buffer (pH 7.0) containing 4% Triton X-100 (0.2 g [wet weight] per ml). Homogenization was performed by two 20-s bursts at 0°C with the Polytron homogenizer (Brinkmann Instruments Inc.). The homogenate was then centrifuged at 12,000 × *g* in a Sorvall RC-2B centrifuge, and the supernatant was frozen in 1- to 2-ml samples at −70°C. Tissues were routinely tested for bacterial contamination, and only those samples that were sterile were used.

Preparation of SDS-extracted human sarcolemmal antigen. Sarcolemmal membranes were prepared by a method previously described by Van de Rijn et al. (10). Briefly, 10 g of human heart tissue was homogenized in 0.05 M $CaCl_2$ by two 30-s bursts in a Polytron homogenizer. The homogenate was centrifuged, and the pellet was washed three times in saline. Autolysis of the membranes was performed in distilled water at 4°C for 18 to 24 h. After digestion with DNase and RNase, the membranes were centrifuged, washed three times, and lyophilized. Protease inhibitors (10^{-3} M) α-*N*-*p*-tosyl-L-lysine chloromethyl ketone and phenylmethylsulfonyl fluoride were included in solutions at each step. Membranes were extracted with 1% sodium dodecyl sulfate (SDS) at a concentration of 5 mg/ml and then heated to 100°C for 3 min. The mixture was centrifuged, and the supernatant was stored at 25°C.

Preparation of monoclonal antibodies and immunoassays. Hybridoma cell lines cloned in this study were produced, stored, and maintained by methods previously described (2, 4). The hybridomas were subcloned into soft agar containing Iscove modified Dulbecco medium (IMDM) plus 20% horse serum (Hyclone Laboratories). Additional enrichment of the plating medium was provided by adding twice the normal concentration of glutamine (2 mM), minimal essential amino acids (GIBCO Laboratories), vitamins (GIBCO Laboratories) as found in IMDM, and 5 × 10^{-2} M 2-mercaptoethanol. Bacto-Agar (0.3%; Difco Laboratories) was used for solidification of the plating medium. Plates were allowed to solidify for 2 h at room temperature and were then placed in a CO_2 incubator. Colonies appeared in the soft agar after ca. 2 weeks. Colonies were picked and placed into conditioned 96-well plates containing IMDM plus 20% horse serum. Culture fluids from picked clones were assayed for antibody activity in the enzyme-linked immunosorbent assay (ELISA) as previously described (2). In these ELISAs, Triton X-100-extracted heart and glutaraldehyde-fixed streptococci were bound to polyvinyl chloride micro-ELISA plates and used as the test antigen (2). Subclones found to be positive for antibody activity were recloned twice and then grown in IMDM plus 2.5% horse serum, using 75-cm^2 T flasks for large-scale antibody production. Ammonium sulfate precipitation (50% saturation) was used to concentrate antibody from culture fluid for testing the immunoglobulin class by agar gel immunodiffusion as previously described (2). Antibodies specific for mouse immunoglobulin G (IgG) (Cappel Laboratories), IgM (Cappel Laboratories), and IgA (Meloy Laboratories, Inc.) were reacted against concentrated antibodies. Radial immunodiffusion assays were performed with plates commercially prepared to measure mouse IgM (Meloy Laboratories Inc.), and a standard curve was plotted from reactions of a mouse IgM standard (2.22 mg/ml; Meloy Laboratories). Unknowns were calculated from this standard plot to determine approximate immunoglobulin concentrations in culture fluids. Culture fluids were continuously monitored for activity by ELISA, using heart and streptococcal antigens as previously described (2). The procedures used for SDS-polyacrylamide gel electrophoresis and Western immunoblotting are described elsewhere (2). Peroxidase- and alkaline phosphatase-conjugated antibodies were obtained as affinity-purified Fab′ fragments prepared against

TABLE 1. Characteristics of monoclonal antibodies reactive with the human heart

Hybridoma no.[a]	Reaction in ELISA[b] with:		Ig isotype	Reactions in Western immunoblot[c] with:		
	Heart[d]	Whole streptococci[e]		Heart[d]	Sarcolemma	Group A membranes
36.2.1	>2.0	1.306	IgM	+	+	+
36.2.2	>2.0	>2.0	IgM	+	+	+
49.8.2	1.507	1.260	IgM	+	Weak	Weak
49.8.9	1.662	1.422	IgM	+	Weak	Weak
54.2	>2.0	1.340	IgM	+	+	+
54.8	>2.0	1.121	IgM	+	+	+
54.13	>2.0	1.115	IgM	+	+	+
54.16	>2.0	1.065	IgM	+	+	+
63.3.9	1.648	0.827	IgM	+	Weak	Weak
65.10.7	1.562	0.692	IgM	+	Weak	Weak
Culture medium	0.052	0.089				

[a] Hybridomas of the 36, 49, and 54 series were from mice immunized with purified group A streptococcal membranes, and in the 63 and 65 series the immunogen was whole type 5, UV-killed group A streptococci.
[b] Absorbance at 405 nm.
[c] Immunoblots were developed with horseradish peroxidase-labeled anti-mouse immunoglobulins and 4-chloro-1-naphthol substrate.
[d] Human heart was extracted with 4% Triton X-100 and diluted 1:500 in coating buffer.
[e] Streptococci (group A, M type 5) were fixed with glutaraldehyde to ELISA plates.

mouse immunoglobulins. Opsonization tests were performed also as previously described (1).

RESULTS

Characterization of monoclonal antibodies. After hybridoma cell lines were subcloned in soft agar, the antibodies produced were reacted in ELISA and Western immunoblots with heart and streptococcal antigens. The data have been combined (Table 1) to illustrate the overall reactions of the monoclonal antibodies. The monoclonal antibodies were all reactive with Triton X-100 human extract and with whole M, type 5, group A streptococci. However, when the monoclonal antibodies were reacted in Western immunoblots with group A streptococcal membranes and SDS-extracted human sarcolemmal antigen, only 6 of the 10 antibodies gave a strongly positive reaction. The reaction of the monoclonal antibodies with human sarcolemma always coincided with their strong reactivity with group A streptococcal membranes. Likewise, those monoclonal antibodies which were weak to unreactive with sarcolemmal antigens were weak to unreactive with streptococcal membranes.

For the determination of immunoglobulin isotype, monoclonal antibodies in culture fluids were concentrated 50- to 100-fold by 50% ammonium sulfate saturation. All of the concentrated antibodies were unreactive with anti-IgG (gamma chain, Fc fragment specific) or anti-IgA but were strongly reactive with anti-IgM (mu chain specific). The reaction with anti-IgM was evident by production of a single precipitin band in Ouchterlony agar gel immunodiffusion. All of the monoclonal antibodies described herein were IgM.

Reaction of monoclonal antibodies with streptococcal antigens. None of the monoclonal antibodies were observed to be opsonic for type 5 *S. pyogenes*. However, the monoclonal antibodies were also evaluated for their reactions with other groups of streptococci in ELISA (Table 2). Monoclonal antibodies 36.2.1, 49.8.2, and 54.16 were used to demonstrate representative reactions of the different monoclonal antibody series. By comparison, the antibodies produced the strongest reaction with *S. pyogenes* (group A) and weaker reactions with groups B, C, P, and R and *S. mutans*. Most of the monoclonal antibodies were unreactive or the least reactive with *S. sanguis*. Other M types of group A streptococci, including M types 6, 24, and 56, were reactive (data not shown) with all of the monoclonal antibodies. All of these ELISAs were routinely performed at the same time with the same culture fluids. Of the 10 monoclonal antibodies that reacted with whole streptococcal cells, only 6 reacted strongly in Western immunoblots with separated streptococcal membrane antigens. These six comprised the monoclonal antibodies which reacted with sarcolemmal antigen (36 and 54 antibody series; Table 1). The 36 monoclonal antibody series produced different reaction patterns than did monoclonal antibodies of the 54 series when reacted with separated group A streptococcal membrane components in Western immunoblots (Fig. 1). The monoclonal antibody 36.2.2 reacted with a large family of membrane components from the group A, M type 5 membranes (Fig. 1A). The most reactive component was near 43,000 daltons. Monoclonal antibodies 49.8.2 and 63.3.9 were weakly reactive to negative with the membranes on the immunoblots (Fig. 1A). The reaction of the separated membrane components with IMDM plus 2.5% horse serum was negative. The reaction of monoclonal antibody 54.2 was limited primarily to a few bands (Fig. 1B), with the strongest reactions occurring with two high-molecular-weight components, the largest of which was near 94,000. A band of small molecular size (near 22,000) was also observed. The reactions of 36.2.2 and 54.2 with streptococcal antigens were distinctly different. Nonspecific binding of medium or serum to membrane components was not observed in controls. Occasionally a particular low-molecular-weight band near 30,000 was observed in controls but was always discounted.

TABLE 2. Reaction of monoclonal antibodies produced against heart and group A streptococci with various groups of streptococci

Microorganism	Reaction of:				
	Monoclonal antibody			Medium control	Buffer control
	36.2.1	49.8.2	54.16		
Streptococcus spp.					
Group A	1.527[a]	0.760	1.139	0.088	0.067
Group B	0.820	0.181	0.547	0.133	0.020
Group C	1.146	0.167	0.558	0.134	0.020
Group P	0.861	0.199	0.596	0.086	0.036
Group R	0.891	0.217	0.525	0.009	0.050
S. mutans	0.942	0.207	0.472	0.141	0.040
S. sanguis	0.706	0.039	0.380	0.005	0.007

[a] Average of duplicate ELISA tests; absorbancy at 405 nm.

Reaction of monoclonal antibodies with human sarcolemmal antigens. The reactions of monoclonal antibodies 36.2.2, 49.8.9, 54.8, 63.3.9, and 65.10.7 with SDS-extracted human sarcolemmal proteins from a rheumatic heart are shown in Fig. 2. Monoclonal antibodies 36.2.2 and 54.8 strongly reacted with a number of sarcolemmal proteins; however, the reactions demonstrated that they are different. Monoclonal antibody 49.8.9 was a weak reactant, with bands similar to those of the 54 series, whereas monoclonal antibodies 63.3.9 and 65.10.7 were weakly reactive, with bands similar to that of 36.2.2. The reaction of the entire 54 series of monoclonal antibodies and medium and normal mouse sera controls are shown in Fig. 3. All four monoclonal antibodies of the 54 series (54.2, 54.8, 54.13, and 54.16) reacted with the same protein bands in sarcolemmal extracts (Fig. 3). In addition, normal mouse sera were found to be totally unreactive with separated human sarcolemmal antigens, as was the medium control. More attention was focused on the 54 series of monoclonal antibodies because human sequellae sera that we have tested in preliminary studies have reacted with the same protein bands in sarcolemmal extracts as those seen in reactions with monoclonal antibodies of the 54 series (unpublished data).

Monoclonal antibody 54.8 was then used to probe both normal and rheumatic sarcolemma for differences in their immunobanding patterns (Fig. 4). The rheumatic heart was observed to have proteins which reacted more intensely with monoclonal antibody 54.8. Concentrations of both extracts were kept equivalent in the experiment, but the amount of the reactive proteins appeared to be in lower concentration in the normal sarcolemma as demonstrated in the amido black protein stain. Although the immunobanding patterns of the normal and rheumatic sarcolemma were quite similar (Fig. 4), the rheumatic sarcolemmal extract reacted more intensely. Except for the intensity, only minor differences were observed in their immunobanding patterns. No reaction was observed between sarcolemma and peroxidase-conjugated anti-mouse IgM (Fig. 4).

Further studies comparing four normal sarcolemmal preparations demonstrated similar banding patterns to that observed with normal sarcolemma in Fig. 4 (data not shown).

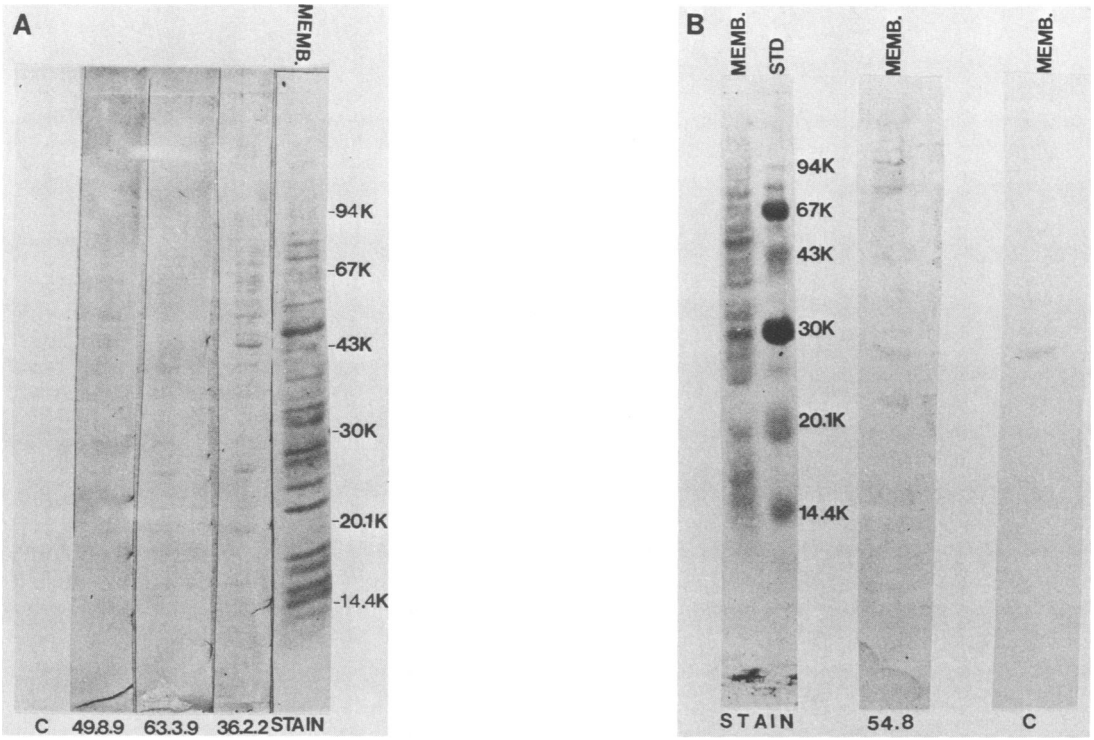

FIG. 1. Western immunoblots of electrophoretically separated *S. pyogenes*, M type 5 membranes (MEMB.) reacted with murine monoclonal antibodies against group A streptococci and human heart. STAIN, Amido black protein stain of electrophoresed and blotted membrane components; C, culture medium control (IMDM plus 2.5% horse serum). The immunoblot was developed by using horseradish peroxidase-conjugated anti-mouse immunoglobulins and 4-chloro-1-naphthol as substrate. Molecular weight standard proteins were electrophoresed simultaneously and marked (94K to 14.4K). (A) 36.2.2, 63.3.9, and 49.8.9 monoclonal antibodies reacted with separated membrane components; (B) 54.8 monoclonal antibody reacted with separated membrane components.

Reaction of monoclonal antibodies with Triton X-100-extracted heart, kidney, and skeletal muscle antigens. The specificity of monoclonal antibodies for extractable antigens in whole heart, kidney, and skeletal muscle was studied. Antigens were extracted from tissues with Triton X-100 and electrophoresed by SDS-polyacrylamide gel electrophoresis and electro-blotted onto nitrocellulose sheets. The banding pattern was observed by the amido black stain and by reaction with monoclonal antibodies. In the Triton X-100-extracted preparations the immunoreactive bands stained darkly with the amido black protein stain (Fig. 5). The reactivity of monoclonal antibody 36.2.2 is shown to be widely distributed among a number of proteins extracted from kidney, skeletal muscle, and human heart (Fig. 5A and B). It also was the antibody that most nearly resembled reactions of normal mouse sera (Fig. 5A) but with minor differences. However, monoclonal antibodies 49.8.2 and 49.8.9 reacted with a specific band in heart (molecular weight, ca. 40,000 [40K]) (Fig. 5B). Although the 49 series was strongly reactive with whole streptococci, it was weak to unreactive with membranes from group A streptococci and human sarcolemma (Fig. 3). Therefore, 36.2.2 and 49.8.2/49.8.9 appeared to be distinct from each other. The four monoclonal antibodies in the 54 series were weak to unreactive with extracts from kidney and skeletal muscle but reacted with three high-molecular-weight proteins in Triton X-100-extracts of human heart (data not shown). Therefore, it appeared that the 54 series was most specific for heart and primarily reacted with three high-molecular-weight proteins (100K to 200K) of Triton X-100-extracted heart. The 49 series was specific for a particular heart protein (extractable with Triton X-100) and others in kidney and skeletal muscle. The 36 series was the least specific, with a wide distribution of reactivity among proteins in all of the tissues studied, and resembled normal mouse sera reactions. The 63.3.9 and 65.10.7 antibodies were weakly reactive in the Western blot with Triton X-100-extractd antigens. The medium control was essentially negative (Fig. 5A). From this data, at least three distinctly different monoclonal antibody types were distinguished, one (36.2.1 and 36.2.2) representing antibodies reactive with membranes and perhaps found in normal animals, one (49.8.2 and 49.8.9) representing reactions associated with non-membrane-associated antigens, and one (54.2, 54.8, 54.13, and 54.16) representing reactions associated with the streptococcal and sarcolemmal membranes.

Comparison of the binding of monoclonal antibodies to heart and group A streptococci in ELISA. The concentrations of IgM concentrated from culture fluids were determined by radial immunodiffusion in agar plates containing anti-mouse IgM. The amount of IgM per milliliter was determined from a standard curve that was plotted from the reactions of known concentrations of mouse IgM. Various amounts of IgM from culture fluids were added to wells containing a constant amount of sarcolemmal antigen or group A streptococci. Although these experiments compare the relative binding of each of the monoclonal antibodies, the affinities or binding constants cannot be calculated from this limited data. Monoclonal antibodies 49.8.9, 63.3.9, 65.10.7, and 54.8 strongly reacted with group A streptococci, but 36.2.2 was weakly reactive (Fig. 6A). Reactivity of monoclonal antibody 36.2.2 with group A streptococci was compared with its reactivity with heart or sarcolemmal extracts (Fig. 6B and C). In this

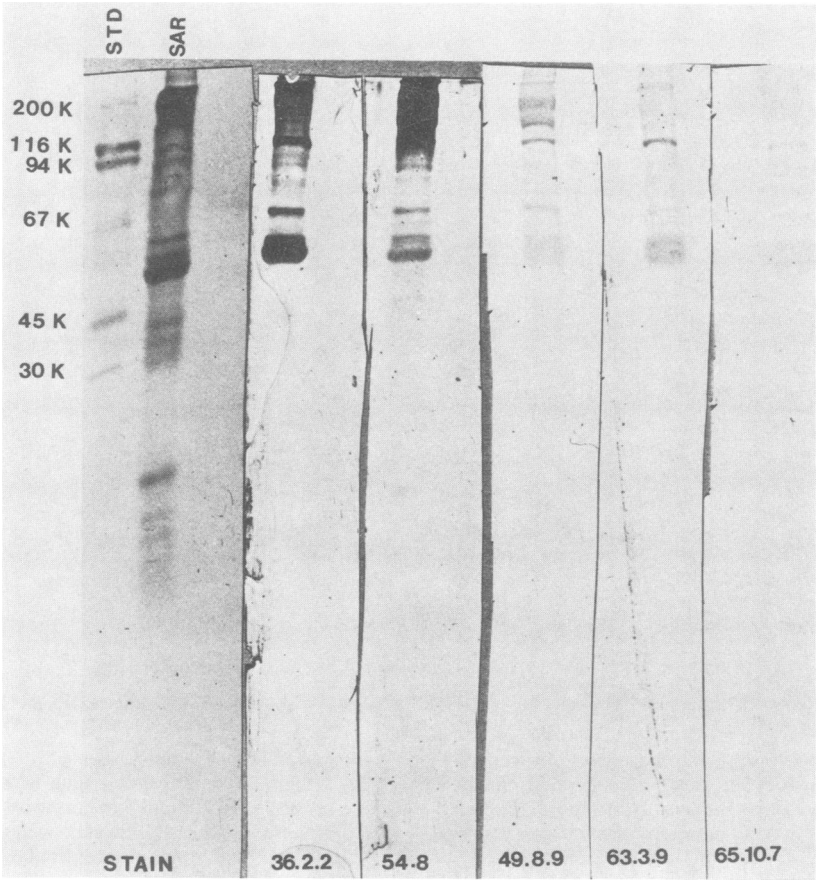

FIG. 2. Western immunoblots of electrophoretically separated sarcolemmal antigens from a rheumatic heart reacted with murine monoclonal antibodies against group A streptococci and human heart. STAIN, Amido black protein stain; STD, molecular weight standards marked 200K to 30K; SAR, SDS-extracted sarcolemma from rheumatic heart; 36.2.2, 54.8, 49.8.9, 63.3.9, and 65.10.7, monoclonal antibodies reacted with separated sarcolemma extract. Immunoblots were developed by using horseradish peroxidase-conjugated anti-mouse IgM (mu chain specific).

study monoclonal antibody 36.2.2 was more reactive with heart than with streptococci. Monoclonal antibody 49.8.9 reacted strongest with group A streptococci and heart-specific protein in Triton X-100-extracted heart preparations. Monoclonal antibody 49.8.9 surpassed all of the other antibodies in these binding assays. Monoclonal antibody 54.8 reacted equally well with streptococci and sarcolemma but was least reactive with the Triton X-100-extracted heart. Nonimmune mouse IgM was used as a control and did not react with heart or streptococcal antigens in ELISA. These data confirm the results of the Western immunoblots when monoclonal antibodies were reacted with heart and streptococcal antigens. In addition, the data indicate that monoclonal antibody 49.8.9 is the strongest binding antibody when compared with the other monoclonal antibodies which were evaluated.

DISCUSSION

Rheumatic heart disease has long been attributed to biological mimicry between the group A streptococcus and human heart tissue (8). Although these immunological similarities between the group A streptococcus and mammalian heart and other tissues have been described for many years, the antigens repsonsible for these cross-reactions are essentially still unknown. The production and study of monoclonal antibodies which react with both human heart and streptococci have allowed human tissue and streptococcal antigens to be probed for cross-reactivities. Studies in which monoclonal antibodies are used overcome some of the pitfalls inherent in the use of polyclonal sera. The initial findings reported here have described the characteristics of these antibodies which can be used to identify the cross-reactive epitopes of the group A streptococcus and human tissues. Of the stable murine hybridomas directed against both human heart and group A streptococcal antigens, all were IgM. This was an interesting finding since the mice used for fusion studies had been hyperimmunized before removal of their spleens. Considering the reactions of the monoclonal antibodies with a family of proteins, it may be speculated that these antibodies could be directed against carbohydrate epitopes on a family of glycoproteins. However, the reactants involved were demonstrated to be proteins, and the possibility of amino acid sequence or structural homology may also explain the multiple banding. The multiple banding was not likely due to degradation during the homogenization or isolation process because of the presence of protease inhibitors. This does not eliminate degradation during the postmorten period, and the multiple bands may represent the degradation of the large 200K protein before

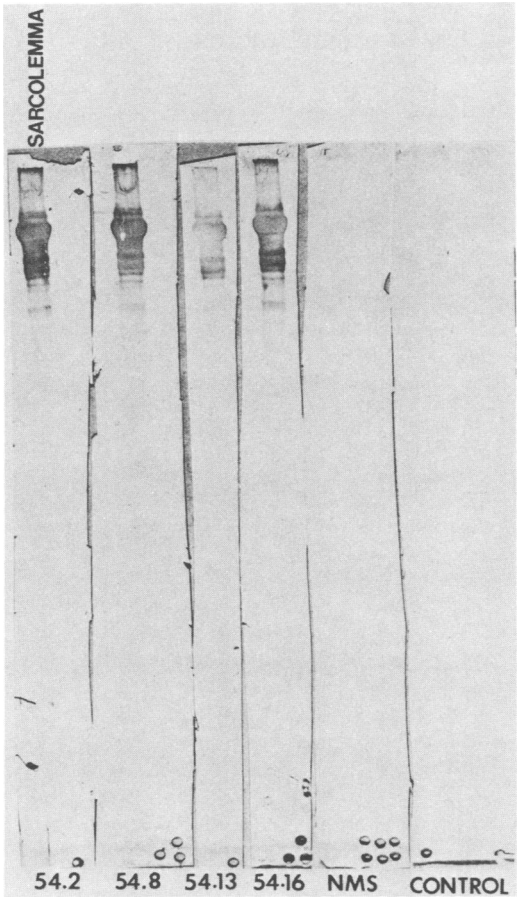

FIG. 3. Western immunoblots of electrophoretically separated sarcolemmal antigens from a normal heart reacted with murine monoclonal antibodies against group A streptococci and human heart. 54.2., 54.8, 54.13, and 54.16, Monoclonal antibodies reacted with separated normal sarcolemmal extract; NMS, normal mouse sera; Control, culture medium control (IMDM plus 2.5% horse serum).

extraction. Identification of the specific reactant(s) is under further investigation. Whether the reactants in the sarcolemmal extracts were actually structural membrane components or proteins simply associated with the membranes cannot be determined from this data.

The fact that the monoclonal antibodies were IgM eliminated the possibility of nonspecific binding by Fc receptor molecules present in any of the antigen preparations. Furthermore, serum and medium controls repeatedly demonstrated that nonspecific reactions did not occur.

When the monoclonal antibodies were compared in Western immunoblots, those of the 36 and 54 series reacted with extracted human sarcolemmal antigens (Fig. 3). These antibodies were also reactive with streptococcal membrane components. However, the 36 and 54 series of monoclonal antibodies were different, as demonstrated by their reactions against both of these antigens. The 54 series was most interesting since it strongly resembled reactions of human sera from streptococcal sequellae (unpublished data). Both of these cell lines (36 and 54 series) were from mice hyperimmunized with streptococcal membranes. These data support the earlier findings of Zabriskie and Freimer (11), who reported sarcolemmal immunofluorescent staining of heart tissues with rabbit antibodies against group A streptococcal membranes. Heart reactive antibody in human sera could also be absorbed with group A streptococcal membranes (10). Since none of these monoclonal antibodies demonstrated opsonic activity, they do not appear to be related to the serotype-specific antibody-antigen system most recently described by Dale and Beachey (3). They described type 5 M protein antibodies which were opsonic for M type 5 *S. pyogenes* and which reacted with a component of sarcolemmal extracts.

The results observed in ELISA, which was performed to compare relative binding of the monoclonal antibodies to heart and streptococcal antigens, suggests that the 36 series may be more strongly reactive with heart than with streptococci, although it is cross-reactive with whole streptococci or streptococcal membranes (Fig. 6). Immunoblots of antibodies 36.2.1. or 36.2.2 suggest that a clone is already present in the normal mouse, probably from previous exposure to these antigens (Fig. 5A). It would not be suprising to expect a clone of this nature. However, it is interesting that it is cross-reactive.

Monoclonal antibodies of the 49 series reacted primarily with a protein extracted from whole heart tissue by Triton X-100 (Fig. 5B). This protein had a molecular weight of ca.

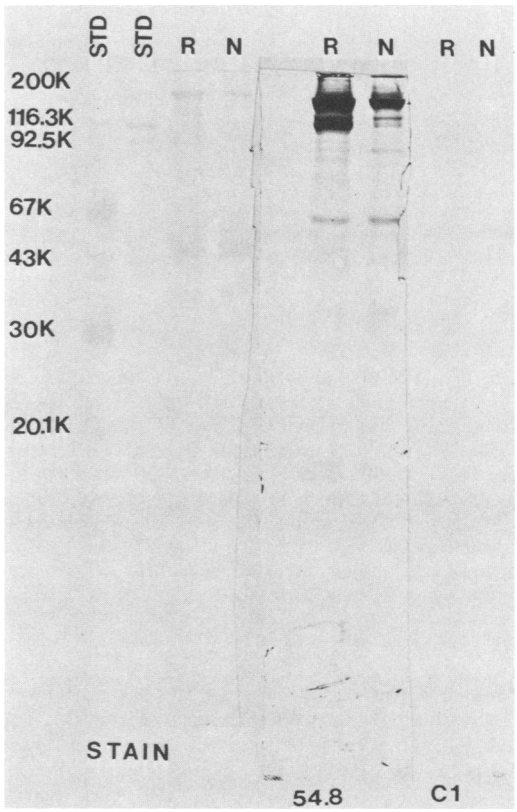

FIG. 4. Comparison of the reactions of normal and rheumatic sarcolemmal extracts with monoclonal antibody 54.8. STAIN, Amido black protein stain of molecular weight standards (STD) from 200K to 20.1K; R, rheumatic sarcolemma; N, normal sarcolemma; 54.8 monoclonal antibody reacted with separated sarcolemmal extracts; C1, culture medium (IMDM plus 2.5% horse serum) control. Immunoblots were developed by using horseradish peroxidase-conjugated anti-mouse IgM (mu chain specific) and 4-chloro-1-naphthol substrate.

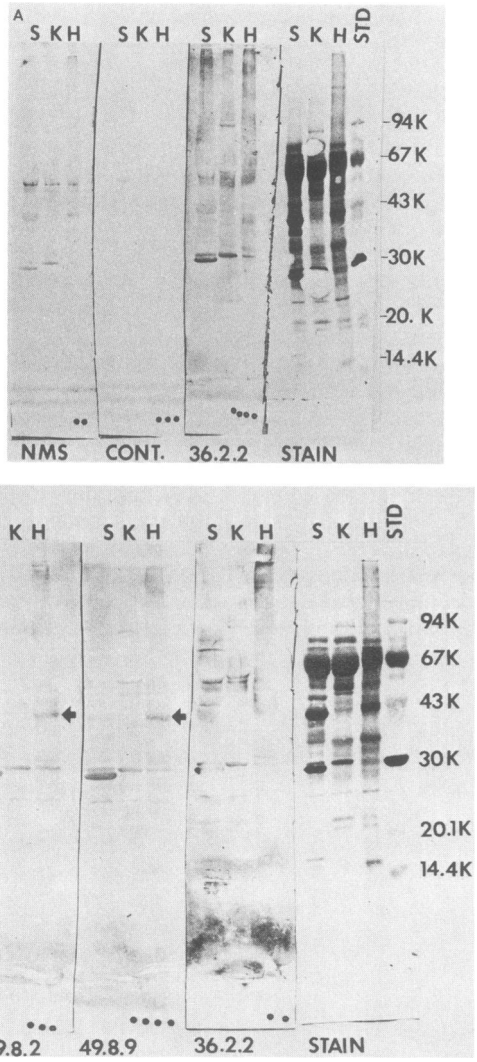

FIG. 5. Western immunoblots of Triton X-100 extracts of normal human heart, kidney, and skeletal muscle after reaction with murine monoclonal antibodies against group A streptococci and human heart. STAIN, Amido black protein stain of electrophoretically separated Triton X-100 extracts of human heart (H), kidney (K), and skeletal muscle (S). (A) NMS, Normal mouse sera reaction; CONT., culture medium (IMDM plus 2.5% horse serum) control; 36.2.2, monoclonal antibody. (B) 36.2.2, 49.8.2, and 49.8.2, Monoclonal antibodies. Stained molecular weight standards (STD) from 94K to 14.4K are indicated. Immunoblots were developed by using horseradish peroxidase-conjugated anti-mouse immunoglobulins and 4-chloro-1-naphthol as substrate.

40K, but its identity is not yet known. Recently, Cunningham and Russell demonstrated that reactions of immune rabbit anti-M, type 5 *S. pyogenes* sera reacted with heart proteins of molecular weight >94K, 70K, and 40K (2). The reactions of 49.8.2 and 49.8.9 antibodies resembled that of the antibodies in rabbit sera with a 40K protein in Triton X-100 extracts of human heart. Zabriskie and Friedman also have recently shown two proteins of molecular weights 43K and 38K in saline extracts of bovine heart to react with sera from acute rheumatic fever patients (12). Although antibodies of the 49 series reacted with antigen(s) in heart tissue extracted with Triton X-100, they did not react with sarcolemmal membrane extracts. Furthermore, the 49 series of

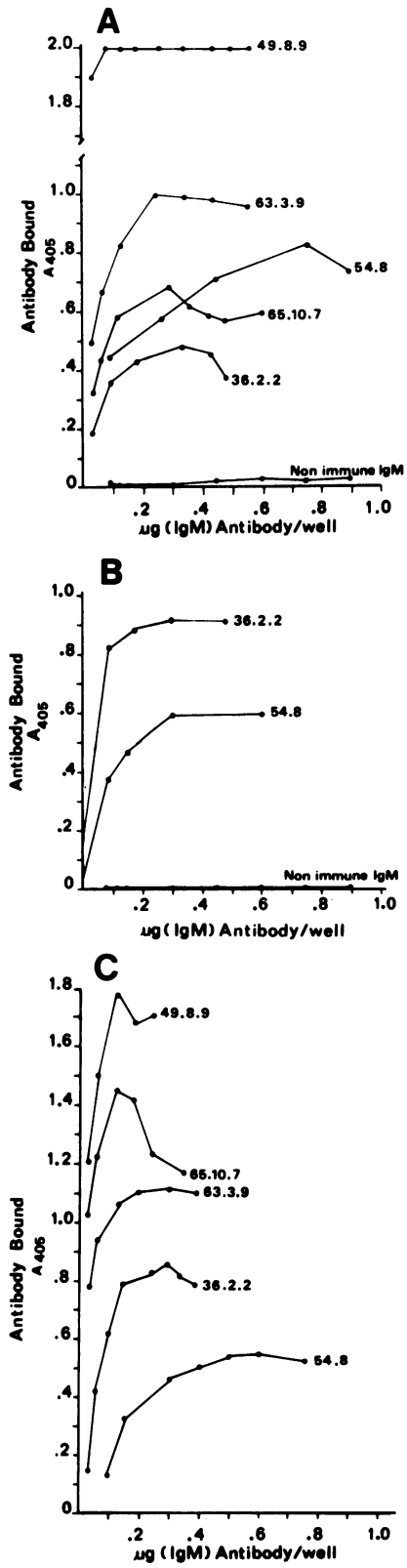

FIG. 6. Relative binding of murine monoclonal antibodies with human heart and streptococcal antigens in ELISA. The reaction of monoclonal antibodies with group A streptococci (A), sarcolemma extracts (B), and Triton X-100 heart extracts (C). Reactions were determined by using alkaline phosphatase-conjugated anti-mouse IgM (mu chain specific).

monoclonal antibodies was not reactive with streptococcal membrane components (Fig. 1). These results suggest that an epitope not associated with sarcolemmal or streptococcal membranes was associated with this cross-reactivity. The same was true for the 63.3.9 and 65.10.7 antibodies, although these antibodies appeared to have weaker binding capacities.

The reactions of all of the monoclonal antibodies with whole group A streptococci in ELISA suggested that these cross-reactive antigens were exposed somewhat on the outside of the streptococci. This idea could be supported by the fact that not all of the monoclonal antibodies reacted with separated streptococcal membrane components. The weaker reactions of the monoclonal antibodies with other Lancefield groups of streptococci indicated either that there were lower quantities of the epitope on the other groups streptococci or that the epitope was not identical but similar to that of the group A streptococci (Table 2). The reactions of the 36 series with steptococcal membranes demonstrated reactivity with many membrane components (Fig. 1). However, the 54 series reacted with a restricted number of components, including one high-molecular-weight band near 94K which stained the most intensely with antibody. Cell wall components were essentially unreactive with the 54 series of antibody (data not shown). The 49 antibody series and 63.3.9 and 65.10.7 antibodies failed to produce marked reactions with streptococcal membranes. This corresponded with their weaker reactivity with sarcolemmal membranes in Western immunoblots. Epitopes which were initially reactive with 63.3.9 and 65.10.7 in Triton X-100 extracts could be labile to SDS treatment. Our data actually suggest that this may be the case, but further studies will be needed to clarify this point.

When all of the data were compared, there were at least three different cross-reactive epitopes represented among this group of antibodies. The three types include: (i) clones of the 36 series which resemble normal mouse sera and may represent either clones stimulated by streptococci associated with normal flora or clones against the heart inherently present in the mouse, (ii) clones of the 49 series which represent antibodies reactive with a 40K protein in the heart, and (iii) clones of the 54 series which represent those antibodies reactive against high-molecular-weight proteins in Triton X-100 whole heart extracts, human sarcolemmal extracts, and group A streptococcal membranes.

The study of the specificity of these antibodies for antigens in other tissues besides heart indicated that there were extractable antigens in kidney and skeletal muscle which were strongly reactive with all of the monoclonal antibodies except those of the 54 series. The 54 series primarily reacted with high-molecular-weight proteins extractable with either Triton X-100 or SDS. These data suggest that the 54 antibody is directed against determinants associated primarily with heart tissue. The study focused more on the 54 series of antibody because its reaction with human sarcolemma resembled that of human sera from streptococcal sequellae (unpublished data). The comparison of four normal human sarcolemmal extracts indicated that no significant differences existed among the four antigen preparations after reaction with monoclonal antibody 54.8. The major reactant of human sarcolemma with antibodies of the 54 series or human sequellae sera was a 200K protein. Several lower-molecular-weight proteins usually reacted with these antibodies, indicating a common epitope shared among these proteins. When normal and rheumatic sarcolemma were probed with monoclonal antibody 54.8, no major differences in immunobanding patterns were observed. However, a difference in intensity and quantity of some of the protein bands was obvious. Minor differences seen in the immunobanding are most likely not signficant since other protein bands were present that were highly reactive. The significance of the greater intensity with rheumatic sarcolemmal extracts is not known. It is important to emphasize that the reaction was with a monoclonal antibody, and the results do indicate that reactive epitopes are present in both normal and rheumatic hearts. However, the more intense reaction seen with the rheumatic heart may reflect important epitope binding differences and will require further study. The role which any or all of these antibodies might play in immune disease processes has not been defined but is of great interest.

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