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Immunology

Cytotoxic and viral neutralizing antibodies crossreact with streptococcal M protein, enteroviruses, and human cardiac myosin

(molecular mimicry/autoimmunity)

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ABSTRACT **The development of autoimmunity in certain instances is related to infectious agents. In this report, cytotoxic monoclonal antibodies (mAbs) that recognize epitopes on both enteroviruses and the bacterium *Streptococcus pyogenes* are described. Murine anti-streptococcal mAbs that were crossreactive with streptococcal M protein, human cardiac myosin, and other α-helical coiled-coil molecules were found to neutralize coxsackieviruses B3 and B4 or poliovirus type 1. The viral-neutralizing anti-streptococcal mAbs were also cytotoxic for heart and fibroblast cell lines and reacted with viral capsid proteins on a Western immunoblot. Alignment of amino acid sequences shared between streptococcal M protein, coxsackievirus B3 capsid protein VP1, and myosin revealed 40% identity in a 14- to 15-amino acid overlap. Synthetic peptides containing these sequences blocked mAb reactivity with streptococcal M protein. The data show that antibodies against α-helical structures of bacterial and viral antigens can lead to cytotoxic reactions and may be one mechanism to explain the origin of autoimmune heart disease.**

Viruses as well as other microbial agents have been associated with autoimmune diseases (1–6). In rheumatic carditis (7–9) and myocarditis (10–16), substantial evidence has connected a bacterial or viral infection directly with autoimmunity. In many autoimmune diseases, the connection with infectious agents is not clear; however, molecular mimicry may play a role in deregulating the immune system in susceptible hosts (1–6, 9–16).

In rheumatic carditis, antibodies to heart tissue were shown to react with the group A streptococcus, the etiologic agent of acute pharyngitis and acute rheumatic fever (8). Heart-reactive antibodies were also found in sera of patients with myocarditis, a disease that often results from a coxsackievirus (CV) group B infection (10–14), and in sera of mice with CVB3-induced myocarditis (15, 16). Antibodies to heart tissue in both diseases recognized myosin as a major autoantigen (9, 17–20). Studies of murine and human monoclonal antibodies (mAbs) directed against the group A streptococcus and myosin revealed two distinct groups of heart-reactive antibodies (9, 21, 22). One group recognized α-helical coiled-coil molecules such as myosin, keratin, and tropomyosin and included antibodies recognizing actin, whereas the other group recognized DNA and myosin (21, 22). A recent report (23), unrelated to the streptococcal studies, identified a mAb that crossreacted with the capsid protein VP1 of CV serotype B4 and with myosin. Although there are many studies, the role of immunological crossreactivity in disease has not been established.

The streptococcal M protein has been conclusively shown to crossreact immunologically with myosin (9, 21, 22, 24–26) and tropomyosin (22), and myosin-specific antibodies affinity-purified from sera of patients with acute rheumatic fever have been shown to crossreact with M protein (9, 26). In fact, a pentameric amino acid sequence (Gln-Lys-Ser-Lys-Gln) in M protein was involved in the crossreaction of anti-myosin antibodies from acute rheumatic fever patients with synthetic peptides representing the M protein molecule (26). Murine and human mAbs that were crossreactive with streptococcal M protein and myosin were also found to recognize synthetic peptides that contained the same pentameric sequence (26). The α-helical coiled-coil structure of the streptococcal M protein (27–29) provides a basis for immunological crossreactivity with myosin (9, 21, 22, 24–26). Little is known about the conformation of the epitopes of infectious agents that are associated with induction of autoantibodies. The hypothesis that different types of infectious agents such as viruses and bacteria share similar or common structures has not been established. Groups or families of autoantigenic structures may be important in generating autoimmune responses in genetically predisposed individuals with deleterious consequences. Although it is clear that autoantibodies to myosin, actin, and many other host proteins can be present in individuals without serious consequences (2, 30, 31), antibodies crossreactive with host cell surface epitopes may be cytotoxic and cause tissue damage (32).

Because viruses and group A streptococci have been implicated in autoimmune diseases of the heart, we tested the hypothesis that viruses, such as group B CVs, might share epitopes with the group A streptococcus. Streptococcal M protein and viral capsid proteins are surface targets for antibody and complement-mediated clearance (33–35). To test this hypothesis, a panel of murine IgM mAbs reactive with group A streptococcal M protein and heart tissue antigen was used in this study. These antibodies have been extensively characterized (17, 18, 21, 22, 26, 32) and shown to be broadly crossreactive with α-helical coiled-coil protein structures including myosin (9, 17, 18), tropomyosin (22), keratin (21), vimentin (36), and laminin (32). We now present evidence that this crossreactivity between α-helical structures gives these anti-streptococcal mAbs dual specificity for both bacterial and viral pathogens. We show that these mAbs recognize specific viral capsid proteins and neutralize virus that is intimately associated with the production of autoimmune heart disease in mice (10, 11) and humans (11–14).

MATERIALS AND METHODS

Preparation of Virus. Viruses were prepared as described (37) and were purified by ultracentrifugation (38). Purified

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Abbreviations: mAb, monoclonal antibody; HCMB, human cardiac myosin β chain; CV, coxsackievirus.

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virus particles were dissolved in 1% SDS and viral polypeptides were separated by SDS/PAGE (21). Separated proteins were blotted onto nitrocellulose and incubated with antibody culture fluids as described (21).

mAbs. Murine mAbs were produced, maintained, and stored as described (18, 21). Antigen specificities of IgM mAbs 36.2.2, 49.8.9, 54.2.8, 101.4.1, 24.1.2, and 654.1.1 have been described (17, 18, 21, 22, 26, 32, 36). Briefly, mAb 36.2.2 has been shown to react with group A streptococcal M proteins serotypes 1, 5, and 6 and with the following host proteins: myosin, tropomyosin, actin, keratin, and laminin. mAb 49.8.9 has been shown to react with streptococcal M5 and M6 proteins and with the host protein vimentin (36). mAb 54.2.8 reacts with streptococcal M5 and M6 proteins and with the host proteins myosin and tropomyosin as well as with DNA (21, 22). mAbs 101.4.1, 654.1.1, and 24.1.2 have also been shown to react with various streptococcal M proteins and myosin (22).

ELISA. Virus antigen was applied to microtiter plates at 50 ng/ml. M6 protein was applied to microtiter plates at 10 μg/ml. The ELISA was performed as described (17, 18). Results were calculated from triplicate measurements.

Plaque-Reduction Assay. Neutralization of CVB3 or CVB4 variants and poliovirus type 1 by anti-streptococcal mAbs 36.2.2, 49.8.9, and 54.2.8 was tested in plaque-reduction assays. A known number of virus plaque-forming units were mixed 1:1 (vol/vol) with mAb or culture medium control. Antibodies or controls were partially purified by 50% ammonium sulfate saturation and precipitation. IgM concentrations of purified mAb preparations were tested by ELISA (9). The mAb/virus mixture was incubated 45 min at 37°C, 0.1 ml was plated in duplicate on HeLa cell monolayer cultures, and a standard plaque assay was performed (37). The average number of plaques in the virus controls without antibody ranged between 80 and 352 for the CVB3 variants, 79 and 146 for CVB4, 40 and 86 for poliovirus type 1, and 30 and 117 for $CVB3_m$.

Competitive Inhibition ELISA. Competitive inhibition of mAb binding to streptococcal M6 protein was tested using synthetic peptides. Percent inhibition was determined after incubating the mAb with peptide (500 μg/ml), 1:1 molar ratio, as described (18, 26), and the mixture was then incubated with the M6 protein in the ELISA. Results were calculated from triplicate measurements. Peptides contained the following sequences: M6 peptide was Lys-Leu-Thr-Glu-Lys-Glu-Lys-Ala-Glu-Leu-Gln-Ala-Lys-Leu-Glu-Ala-Glu, beginning at residue 326 of the mature streptococcal M protein (39); CVB3 peptide was Tyr-Ala-Glu-Trp-Val-Leu-Thr-Pro-Arg-Gln-Ala-Ala-Gln-Leu-Arg-Arg-Lys-Leu-Glu-Phe-Phe, beginning at residue 87 of the CV VP1 (40); human cardiac myosin, β-chain (HCMB) peptide was Glu-Ala-Glu-Ala-Ser-Leu-Glu-His-Glu-Glu-Gly-Lys-Ile-Leu-Arg-Ala-Gln-Leu-Glu-Phe-Asn, beginning at residue 1544 of the myosin heavy chain (55).

Cell Lines and Cytotoxicity Assay. Cell lines were obtained from the American Type Culture Collection as a primary rat heart cell line (ATCC CRL-1446), a primary rat fibroblast cell line (ATCC CRL-1213), or a primary rat liver cell line (ATCC CRL-1439). Cells were cultured overnight in sterile 96-well microtiter plates at 1×10^4 cells per well at 37°C and 5% CO_2/95% air. The cells were labeled with $Na_2{}^{51}CrO_4$ (DuPont) at 5 μCi per well (1 Ci = 37 GBq) for 2 h at 37°C. Culture medium was removed and the attached cells were washed three times with culture medium containing 20% (vol/vol) fetal bovine serum and incubated for 1 h prior to addition of antibody. Antibody was added to the cells at 50 μl per well for 1 h at 37°C. An equal volume of guinea pig complement (Whittaker Bioproducts) was added and the mixture was incubated for 1 h at 37°C. Supernatant fluids were harvested by a Skatron harvester system and ^{51}Cr release was measured in a LKB γ-counter (LKB-1282 Compugamma). Minimum lysis was calculated from cells treated with culture medium alone and 100% lysis was calculated from cells treated with 1 M HCl for maximum ^{51}Cr release. Percent lysis was determined by calculating [(test sample release − minimum release)/(maximum release − minimum release)] × 100.

RESULTS

Reaction of Virus with Anti-Streptococcal mAbs. In the ELISA (Table 1), anti-streptococcal IgM mAbs 36.2.2, 49.8.9, and 54.2.8 were incubated with purified $CVB3_m$ particles, a variant highly myocarditic for mice (38). The mAbs that recognize (17, 18, 21, 26) shared epitopes on streptococcal M protein and cardiac myosin also recognized epitopes on the partially denatured CV particles. A mouse anti-$CVB3_m$ serum reacted strongly with the virus particles in the ELISA (Table 1). Mouse IgM, a medium control, and two other IgM anti-streptococcal mAbs (101.4.1 and 24.1.2) did not react with the virus in this assay. Electrophoretically separated capsid proteins from purified $CVB3_m$ and from poliovirus type 1 were incubated with anti-streptococcal mAbs 36.2.2, 49.8.9, or 54.2.8 on Western blots (Fig. 1). All three anti-streptococcal mAb probes uniquely recognized viral proteins of $CVB3_m$ but did not react with proteins of poliovirus type 1. mAb 36.2.2 reacted most strongly with VP3 of $CVB3_m$, whereas mAb 54.2.8 reacted exclusively with VP1 of $CVB3_m$. mAb 49.8.9 recognized an epitope in VP1, VP2, and VP3 of $CVB3_m$. The reaction of mAb 49.8.9 with more than one capsid protein may be surprising; however, recent data show (41) that a mAb against enterovirus 70 neutralized the virus and reacted with two viral capsid proteins. Nevertheless, our data clearly document epitope sharing in group A streptococcal M protein, cardiac myosin, and the $CVB3_m$ capsid proteins.

Neutralization of Virus by Anti-Streptococcal mAbs. After establishing that anti-streptococcal mAbs reacted with $CVB3_m$ capsid proteins, the mAbs were tested for virus neutralization in a plaque-reduction assay (37). CVs (one CVB4 variant and eight CVB3 variants) and poliovirus type 1 were incubated with partially purified mAbs 36.2.2, 49.8.9, and 54.2.8 (Table 2). By using 50% reduction in plaque number as the endpoint, mAbs 36.2.2 and 54.2.8 neutralized poliovirus type 1, whereas CVB4 and the other CVB3 variants were not consistently or significantly (≥50%) neutralized. Conversely, mAb 49.8.9 neutralized seven of eight CVB3 variants and a CVB4 variant but did not consistently or significantly neutralize poliovirus type 1. Cross neutralization of viral serotypes was recently shown to occur when an anti-poliovirus type 2 mAb neutralized poliovirus type 1 (42). The anti-streptococcal mAbs possessed various neutralizing capabilities with significant virus neutralization concentrations ranging from 86 μg of IgM per ml for mAb 49.8.9 to ≤1 μg of IgM per ml for mAbs 36.2.2 and 54.2.8. The neutralizing capacity of mAb 49.8.9 was not as great as observed with mAbs 36.2.2 and 54.2.8, which were tested at

Table 1. Binding of anti-streptococcal mAbs to $CVB3_m$ in the ELISA

Antibody	A_{405}
mAb 36.2.2	>1.999
mAb 49.8.9	1.805
mAb 54.2.8	1.999
mAb 101.4.1	0.191
mAb 24.1.2	0.070
Anti-CVB3 serum	>1.999
Medium control	0.004
Mouse IgM (20 μg/ml)	0.080

IgM concentrations were ≤20 μg/ml.

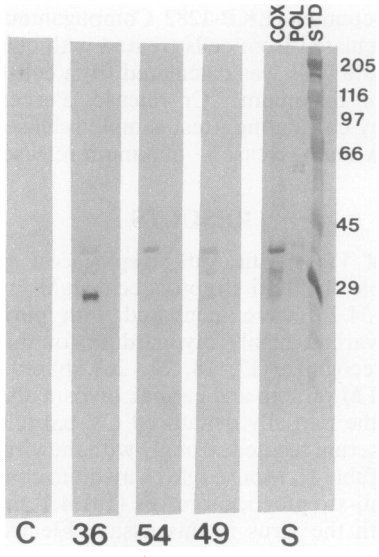

FIG. 1. Reaction of mAbs 36.2.2 (lane 36), 49.8.9 (lane 49), and 54.2.8 (lane 54) with SDS-extracted proteins of purified particles of $CVB3_m$ (lane COX) or poliovirus type 1 (lane POL) in a Western blot. The stained viral polypeptides (lanes S) have molecular sizes consistent with reported values (ref. 37; H.-P. Vosberg, personal communication): CVB3 [VP1 (34.5 kDa), VP2 (31 kDa), and VP3 (26.5 kDa)] and poliovirus type 1 [VP1 (37 kDa), VP2 (36 kDa), and VP3 (32.5 kDa)]. Positions of molecular mass standard proteins are indicated (lane STD) in kDa. The control strip (lane C) shows the lack of reaction of the antibody-peroxidase conjugate and Iscove's modified Dulbecco's culture medium containing 10% horse serum with either $CVB3_m$ or poliovirus type 1 capsid proteins. Other IgM mAbs 101.4.1 (100 μg/ml) and 654.1.1 (5 μg/ml) did not react with any proteins from either virus (data not shown). Three Western blot experiments demonstrated similar results with all mAbs tested.

concentrations ranging from 10–20 μg/ml to <250 ng/ml. Two other purified IgM mAbs 24.1.1 and 654.1.1, at concentrations ranging from 5 to 100 μg of IgM per ml, did not neutralize any of the viruses tested (data not shown). To eliminate the possibility that serum in the culture media might be responsible for the observed neutralization, partially purified sham medium controls containing 10 or 15% horse or fetal bovine serum in Iscove's modified Dulbecco's medium were tested and no virus neutralization was found. At all concentrations tested, the mAbs were not cytotoxic for 1×10^4 HeLa cells after incubation at 37°C for 24 h in the absence of complement. Incubation of HeLa cells with the mAbs prior to addition of virus did not protect the HeLa cells against virus-induced cytopathic effects. Therefore, unique and reproducible differences were found in plaque-reduction assays with mAbs 36.2.2, 49.8.9, and 54.2.8.

Table 2. Neutralization of CVs and poliovirus by anti-streptococcal mAbs in a plaque reduction assay

	% plaque reduction		
Virus variant	mAb 49.8.9	mAb 36.2.2	mAb 54.2.8
PV1	12–46	68–83	48–85
$CVB4_{TM}$	58–85	0–4	0–7
$CVB3_m$	53–81	34–39	25–39
$CVB3_{AS}$	0	0	34
$CVB3_{DO}$	84	0	0
$CVB3_{GA}$	87	0	0
$CVB3_{GB}$	56	0	0
$CVB3_{OL}$	72	0	0
$CVB3_{RE}$	90	9	0
$CVB3_{ZU}$	84	0	0

Plaque reduction of ≥50% was considered significant and was reproducible. Values ≥50% are underlined. The range of plaque reduction values obtained in four assays for poliovirus type 1 (PV-1), $CVB4_{TM}$, and $CVB3_m$ is shown; the other CVB3 variants were assayed only once. The highest dilution (lowest concentration) of mAb that significantly neutralized virus was as follows: mAb 49.8.9, 86 μg of IgM per ml against $CVB4_{TM}$ and CVB3 variants; mAb 36.2.2, 0.3–0.6 μg of IgM per ml against PV1; mAb 54.2.8, 1–9 μg of IgM per ml against PV1.

The likely explanation why mAbs 36.2.2 and 54.2.8 neutralized poliovirus but did not react with poliovirus proteins in the Western blot (Fig. 1) may be that highly conformational epitopes are involved in neutralization (43). These same two mAbs (36.2.2 and 54.2.8) recognized nonneutralizing epitopes in $CVB3_m$ proteins as detected by immunoblot assay. mAb 49.8.9 neutralized several CVB3 variants in addition to $CVB3_m$ and gave positive reactions with $CVB3_m$ proteins in the Western immunoblot assay, suggesting the possible recognition of a linear epitope. Linear neutralizing epitopes have been reported for all three poliovirus serotypes (43–45). mAbs 36.2.2 and 54.2.8 apparently recognize epitopes on poliovirus type 1 capsid proteins in a neutralization assay and epitopes on $CVB3_m$ capsid proteins in an immunoblot assay.

Cytotoxicity of Anti-Streptococcal mAbs. mAbs 36.2.2, 49.8.9, and 54.2.8 were tested for participation in complement-mediated cytotoxicity on three cell lines in culture. mAb 36.2.2 was cytotoxic for rat heart and fibroblast cell lines at mAb concentrations of 4 μg of IgM per ml (Fig. 2). mAb 36.2.2 produced ≈60% lysis of these two cell lines, as assessed in a ^{51}Cr release assay, whereas mAbs 49.8.9 and 54.2.8 were minimally cytotoxic to the cell lines at the same concentration. At concentrations at least 10 times that of mAb 36.2.2, mAbs 49.8.9 and 54.2.8 were also cytotoxic in the presence of guinea pig complement (data not shown). mAb 36.2.2 participated in complement-mediated lysis of heart cells and fibroblasts in a dose-dependent manner, because antibody at as little as 0.6 μg/ml participated in 20% lysis. None of the mAbs tested were cytotoxic to a hepatocyte cell line. The IgM isotype controls, anti-myosin and anti-streptococcal mAbs 101.4.1 or 654.1.1, did not cause release of ^{51}Cr. Both guinea pig and rat complement were effective in tests with mAb 36.2.2, and no lysis was observed with complement or mAbs alone. The data suggest that mAb 36.2.2 is reactive with epitopes on the surface of heart cells and fibroblasts and can participate in lysis in the presence of complement. In addition, mAb 36.2.2 was much more cytotoxic than mAbs 49.8.9 or 54.2.8.

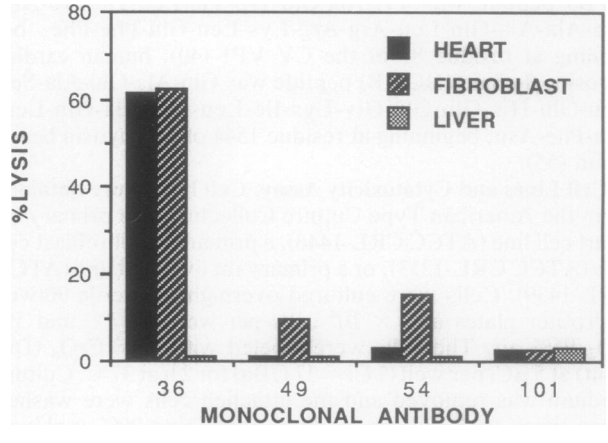

FIG. 2. Complement-mediated cytotoxicity of anti-streptococcal mAbs 36.2.2 (bars 36), 49.8.9 (bars 49), 54.2.8 (bars 54), and 101.4.1 (bars 101) on rat heart, fibroblast, and liver cell lines. mAbs, partially purified by 50% ammonium sulfate saturation, were incubated with 4 μg of IgM per ml with cell lines and then with guinea pig complement.

Sequence Homology Shared Between Streptococcal M6 Protein, Human Cardiac Myosin, and CV VP1 Capsid Protein. When the amino acid sequence of streptococcal M6 protein (M6) (39) was compared with HCMB (55) and CVB3 capsid protein VP1 (40), ≈40% identity was observed in a 14- to 15-amino acid overlap between these three proteins (Fig. 3). No significant homology was observed between the streptococcal M6 protein and the poliovirus type 1 capsid proteins, although 29% identity was seen between the poliovirus type 1 capsid protein VP1 and human cardiac myosin in a 28-amino acid overlap (data not shown).

Competitive Inhibition of mAb Binding to Streptococcal M6 Protein by Synthetic Peptides. Peptides containing the M6, HCMB, and CVB3 overlapping amino acid sequence homologies (Fig. 3) were synthesized to determine if they could inhibit the reaction of cytotoxic mAbs 36.2.2, 49.8.9, and 54.2.8 with streptococcal M6 protein (Table 3). The reaction of mAb 36.2.2 with M6 protein was inhibited strongly by the HCMB peptide, partially inhibited by the CVB3 peptide, and weakly inhibited by the M6 peptide. mAb 54.2.8 was inhibited strongly by HCMB peptide, weakly inhibited by M6 peptide, and not inhibited by the CVB peptide. mAb 49.8.9 was not inhibited by any of the peptides. The HCMB peptide strongly inhibited the binding of mAbs 36.2.2 and 54.2.8 to M6 protein, with peptide at as little as 7–15 μg/ml required for inhibition. The other peptides were unable to inhibit binding to the M protein at these lower concentrations.

Table 3. Competitive inhibition of mAb binding to streptococcal M6 protein by synthetic peptides

mAb	% inhibition		
	CVB3 VP1	HCMB	M6
36.2.2	32	78	19
54.2.8	0	63	14
49.8.9	0	8	0

Peptides were tested at a final concentration of 500 μg/ml and mAbs were at ≈10 μg/ml.

DISCUSSION

The data clearly show that anti-streptococcal mAbs that crossreact with myosin or other α-helical molecules and the streptococcal M protein were capable of neutralizing viruses. One mAb, 36.2.2, was highly cytotoxic for heart and fibroblast cell lines and recognized $CVB3_m$ protein in the Western blot. mAb 36.2.2 is a unique antibody in that it recognizes a broad range of α-helical molecules including myosin, tropomyosin, keratin, and laminin (21, 22, 32). It is possible that recognition of a particular helical structure by mAb 36.2.2 contributes to the cytotoxic property. Whatever the reason for the crossreactivity, the data represent unique information that may lead to a better understanding of immunological crossreactions not only between viruses and bacteria but also between the host and infectious agents.

The reactivity of these anti-streptococcal antibodies with CV proteins is of particular interest since members of the CV group B have been implicated in the pathogenesis of autoimmune myocarditis. The immunological similarity between myosin and proteins of these infectious agents could be important in the overall symptomatology seen in both rheumatic carditis and chronic autoimmune myocarditis, which are distinctly different diseases.

Studies over the past several years have shown that streptococcal M proteins share structural (27–29) and immunological (17, 18, 21, 22, 24–26) similarities with myosin and other α-helical coiled-coil proteins such as tropomyosin, keratin, and vimentin (18, 21, 22, 36, 46, 47). The amino acid sequences shared among streptococcal M6 protein, VP1 of CVB3, and human cardiac myosin have several unique features in addition to their α-helical structure. The shared amino acid sequence in streptococcal M protein is located within the cell wall in the carboxyl-terminal C- and D-repeat regions of the molecule (39). In addition, several types of M proteins including M5, M6, and M24 contain the identical M6 peptide sequence that shares homology with the CV and myosin peptides. This region in M protein was recently shown to share 31% identity with human cardiac tropomyosin (22). The homology between these four proteins within α-helical segments of these molecules enhances the probability that these sites might share conformational determinants. Furthermore, an epitope common to acute rheumatic fever-associated streptococcal strains has been proposed to exist in the carboxyl-terminal C-repeat region of the M protein (48), adjacent to the region of homology found here.

The shared sequences in human cardiac myosin (amino acids 1552–1566) were found in the α-helical rod region in the light meromyosin tail fragment (55). Reactions of mAbs 36.2.2 and 54.2.8 with human cardiac myosin have been mapped to the light meromyosin and S2 fragments, respectively (18). When the shared sequence within VP1 of CVB3 (amino acids 84–112) was compared with the known structure of VP1 of poliovirus type 1, it appeared to be within an α-helical segment in the capsid protein (49, 50). Identification of the human cardiac myosin sequence involved in crossreactions with M protein revealed that the myosin sequence (Fig. 3) was homologous but not identical to sequences reported for crossreactive epitopes of M proteins (26).

Because of their flexibility, only a small proportion of the peptides used in a competition assay will assume the conformation of the native molecule, thus a large excess of free peptide is required to compete for antibody directed against native structures (26). Inhibition of the mAbs by the synthetic peptides suggests that the predicted sites that share amino acid sequence homology and α-helical structure may be important in the binding sites of the crossreactive mAbs. In fact, the myosin peptide clearly defined a crossreactive epitope in competitive inhibition assays. In direct ELISAs to measure mAb reactivity directly with the peptides bound to a surface, mAb 49.8.9 reacted strongly with the CV and myosin peptides (0.8–1.0 A_{405} unit), whereas mAb 36.2.2 and 54.2.8 strongly recognized the M6 peptide and moderately recognized the myosin and CV peptides on microtiter plates (data not shown). These results suggest that the native conformation in the whole molecule is recognized over the smaller peptide sequences that, in the absence of the whole molecule, are recognized by all three mAbs.

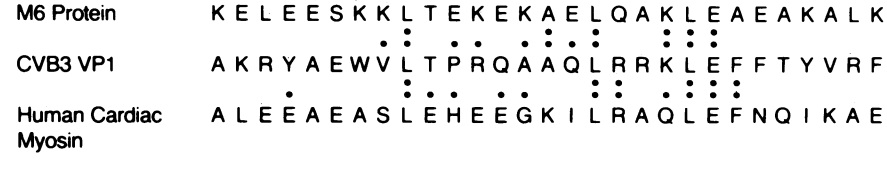

FIG. 3. Overlapping sequence homology between VP1 of CVB3 (40) beginning at residue 84, streptococcal M6 protein (39) beginning at residue 326 of the mature protein, and human cardiac myosin (55) beginning at residue 1552. Sequence comparisons were performed using the James M. Pustell DNA/Protein Sequencing Program from International Biotechnologies. Colons represent identities and periods represent conserved substitutions.

The host may take advantage of the shared α-helical coiled-coil structures of many bacterial and viral proteins (51) and respond to a common conformational determinant. Therefore, immunologic crossreactivity between man and microbe may be a primordial immune response used by the host to protect itself against a variety of pathogenic agents and may explain multispecificity often reported for autoantibodies (18, 21, 22, 52–54). Autoantibodies specific for intracellular cytoskeletal proteins may remain innocuous unless targeted for shared epitopes on the host cell surface. In individuals predisposed to hyperresponsiveness against infectious agents, production of crossreactive immune responses may lead to the development of autoimmunity and damage to host tissues in diseases such as acute rheumatic fever, myocarditis, and perhaps other autoimmune diseases.

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