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Mapping T-Cell Epitopes in Group A Streptococcal Type 5 M Protein

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Group A streptococcal cell surface M proteins elicit highly protective, serotype-specific opsonic antibodies and many serotypes also elicit host cross-reactive antibodies, which may contribute to the pathogenesis of poststreptococcal autoimmune disease. To date, studies aimed at designing safe (non-host-cross-reactive, defined-epitope) M vaccines have focused almost exclusively on antibody epitopes. Here we identify T-cell epitopes recognized by T cells from BALB/c, C57BL/6, and CBA/Ca mice immunized with purified, recombinant serotype 5 M protein (rM5). The responses of rM5-specific, major histocompatibility complex class II-restricted, T-cell clones to synthetic peptides representing most of the M5 sequence identified at least 13 distinct T-cell recognition sites, including sites recognized by more than one major histocompatibility complex haplotype of mice. Although none of these sites appeared to be strongly immunodominant, an N-terminal peptide, sM5[1-35], was recognized by lymph node T cells of rM5-immunized mice and by a larger proportion of rM5-specific T-cell clones than any other individual peptide. The fine specificity of these clones was mapped with subpeptides to a single site at or overlapping the sequence ELENHDL at residues 21 to 27, which is in close proximity to previously mapped protective antibody epitopes. Other T-cell recognition sites are distributed throughout the M protein and include several in the highly conserved C-terminal region of the molecule. One of these C-terminal sites, located within residues 300 to 319, was recognized by a significant proportion of T-cell clones from two strains of mice. Helper T-cell epitopes located in the C-terminal region of M5 are likely to be widely conserved between different M serotypes and could be particularly useful in designing multivalent, defined-epitope M vaccines.

The cell surface M proteins of group A streptococci (*Streptococcus pyogenes*) are important both as virulence factors and protective antigens (for reviews, see references 8, 15, and 18). More than 80 distinct serotypes of M protein have been identified to date, and, with rare exceptions, only one serotype is expressed by each strain. DNA sequencing and hybridization studies have shown that the C-terminal halves of M proteins are highly conserved between serotypes but that the N-terminal halves are highly variable. M proteins form fibrillar, α-helical coiled-coil dimers on the streptococcal cell surface (23), with the conserved C-terminal ends closely associated with the streptococcal cell membrane and the variable N-terminal ends protruding outwards from the cell (4). These fibrils confer the cell with resistance to phagocytosis (8, 18). In addition, many serotypes of M protein elicit human host tissue cross-reactive antibodies, and it has been suggested that such antibodies may contribute to the pathogenesis of the poststreptococcal autoimmune sequelae, acute rheumatic fever and acute glomerulonephritis (8, 15). In humans, systemic immunity to group A streptococcal infections is due predominantly to type-specific opsonic antibodies, directed against the highly variable N-terminal regions of M proteins (8, 15). To facilitate the design of safe (non-host-cross-reactive), defined-epitope M vaccines that could elicit effective systemic protection, extensive efforts have been made over the past 10 years to identify and characterize M protein antibody epitopes (reviewed in reference 15). To date, however, the locations of M protein helper T-cell epitopes have not been defined.

Studies of experimental protein antigens such as chicken ovalbumin, hen egg lysozyme, pigeon cytochrome *c*, and sperm whale myoglobin have indicated that helper T-cell recognition is focused on a small number of antigenic sites, with T cells restricted to different major histocompatibility complex (MHC) haplotypes recognizing largely different sites (reviewed in references 5 and 19). These immunodominant sites represent clusters of epitopes, each recognized by independent T-cell clones differing in their fine recognition specificity. The major conclusions from these earlier studies have been confirmed by a comprehensive investigation of helper T-cell epitopes in hen egg lysozyme, defining major and minor determinant regions by the pin synthesis system (10), as well as by reports on a limited number of bacterial, viral, and parasite antigens, including *Mycobacterium leprae* 18-kDa protein (14), *Mycobacterium tuberculosis* 65-kDa protein (17), staphylococcal nuclease (7) influenza virus hemagglutinin and nucleoprotein (1, 11), human immunodeficiency virus gp160 (13), bacteriophage lambda *c*I repressor (24), *Chlamydia trachomatis* major outer membrane protein (25), the major surface glycoprotein of *Leishmania major* (27), and malarial circumsporozoite protein (12). Systematic mapping of T-cell epitopes in a variety of MHC haplotypes of mice has shown that these antigens possess multiple immunodominant T-cell recognition sites, some of which are antigenic for more than one mouse MHC haplotype, suggesting that while most epitopes are MHC haplotype restricted, some may be universal in respect of MHC-restricted T-cell recognition.

Here we report that streptococcal type 5 M protein possesses at least 13 distinct T-cell recognition sites, that some of these sites are antigenic for more than one MHC

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haplotype in mice, and that T-cell recognition sites are located in both the highly variable N-terminal and the highly conserved C-terminal halves of the molecule, the last suggesting that some helper T-cell epitopes may be widely conserved between serotypes.

MATERIALS AND METHODS

Mice. BALB/c mice ($H\text{-}2^d$) were obtained from the Comparative Biology Centre, University of Newcastle upon Tyne, Newcastle upon Tyne, United Kingdom. CBA/Ca ($H\text{-}2^k$) and C57BL/6 ($H\text{-}2^b$) mice were purchased from Bantin and Kingman, Hull, United Kingdom.

Recombinant M5 protein (rM5) and pepsin-cleaved M5 protein (pepM5). The cloning and expression of the M5 determinant from the type 5 group A streptococcal strain Manfredo and the sequence of the cloned gene have been described previously (16, 22). rM5 was purified to >95% homogeneity, as judged by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, from *Escherichia coli* LE392 (20) expressing the cloned M5 determinant (16). Nine, 1-liter cultures in L broth (21) supplemented with 100 μg of ampicillin per ml were grown at 37°C and 200 rpm in an orbital incubator to an A_{600} of between 0.8 and 1.2. The cells were harvested by centrifugation at 10,000 × *g* for 5 min, and all subsequent steps were carried out at 4°C. Cells were washed and resuspended to 450 ml in buffer A (25 mM Tris-HCl [pH 8.0], 50 mM NaCl), containing 1 mM EDTA and 5% (vol/vol) glycerol. Lysozyme was added to a concentration of 60 μg/ml, and phenylmethylsulfonyl fluoride (from 50 mM stock solution in isopropanol) was added to a concentration of 1 mM. After 30 min, 45 ml of 250 mM EDTA (pH 8.0) was added and incubation was continued for a further 15 min to achieve maximum release of periplasmic proteins. Some degree of cell lysis also occurred under these conditions. Unlysed spheroplasts and cell debris were removed by centrifugation at 16,000 × *g* for 20 min, and the supernatant was passed through a column (40 by 2.5 cm) of DEAE-Sephacel (Pharmacia LKB, Uppsala, Sweden), preequilibrated with buffer A. The column was washed with buffer A, and fractions (12 ml) with an A_{280} of >0.2 were pooled. Ammonium sulfate was added slowly to give 80% saturation, and precipitated protein was collected by centrifugation at 16,000 × *g* for 45 min and resuspended in 75 ml of buffer B (4.0 mM Na_2HPO_4, 0.75 mM KH_2PO_4 [pH 7.4]) containing 2 M NaCl. This mixture was loaded directly (without dialysis) onto a column (30 by 2.5 cm) of Octyl-Sepharose CL-4B (Pharmacia) preequilibrated with buffer B containing 2 M NaCl. The column was washed with the same buffer. When the A_{280} of the eluate began to fall, the washing buffer was changed to buffer B containing 250 mM NaCl and 10-ml fractions were collected. Fractions containing rM5 were identified by SDS-polyacrylamide gel electrophoresis and immunoblotting with anti-pepM5 sera as described previously (16). Desired fractions were pooled, dialyzed against buffer C (50 mM sodium phosphate buffer [pH 8.0]) containing 250 mM NaCl and loaded onto a Phenyl-Sepharose CL-4B (Pharmacia) column (20 by 25 cm), preequilibrated with the dialysis buffer. The column was washed with 150 ml of the same buffer and then eluted with a decreasing NaCl gradient (250 to 25 mM) in buffer C. Fractions (5 ml) containing rM5 were identified as described above, and desired fractions were pooled, concentrated by dialysis against phosphate-buffered saline (PBS) (0.137 M NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4 [pH 7.2]) plus 20% (wt/vol) polyethylene glycol 8000.

The pepM5 antigen was generously provided by M. Kotb and the late Edwin Beachey (University of Tennessee, Memphis). pepM5 corresponds to the N-terminal half of the M5 protein released from *S. pyogenes* Manfredo by limited pepsin cleavage and purified as described previously (2). Purified rM5 and pepM5 were dialyzed against PBS, filter sterilized, and stored in aliquots at −80°C.

Synthetic peptides. Peptides numbered sM5[1-10], sM5[1-20], sM5[1-35], sM5[28-54], sM5[55-70], sM5[61-90], sM5[70-102], sM5[103-132], sM5[133-162], sM5[175-208], sM5[329-359], sM5[359-388], sM5[389-425], and sM5[426-450] were generously provided by the late Edwin Beachey. Other peptides were synthesized on an Applied Biosystems automated peptide synthesizer (model 431A; Applied Biosystems, Warrington, United Kingdom) by using Fmoc chemistry and purified by high-performance liquid chromatography on a C_8 reverse-phase column, according to Applied Biosystem's instructions, after which samples were checked by hydrolysis and amino acid composition analysis. Purified peptides were dialyzed against PBS, filter sterilized, and stored in aliquots at −80°C.

Production of rM5-specific T-cell clones. Mice were footpad immunized with 500 nM (25 μg) rM5 protein emulsified in Freund's complete adjuvant (FCA). Seven days later, rM5-specific T cells were cloned directly by limiting dilution from popliteal lymph node cells (PLN) into 60-well Terasaki plates (Nunc; Gibco Ltd., Paisley, Scotland) by using RPMI 1640 (Gibco Ltd.) supplemented with 3 mM glutamine, 10% fetal bovine serum (Sigma Chemical Co., Poole, Dorset, United Kingdom), and 5×10^{-5} M 2-mercaptoethanol (RF10). PLN at 10^3, 3×10^3, and 10^4 per well were cultured with 5×10^4 irradiated syngeneic spleen cells and 12.5 μg of rM5 per ml, and positive wells were scored at day 7 and day 14 by using an inverted microscope. Plates with more than 10% positive wells were discarded, and the remainder were considered to be clonal. Positive wells were restimulated with rM5 and irradiated spleen cells every 2 to 3 weeks through 96- and 24-well plates (Costar; NBL Ltd., Cramlington, Northumberland, United Kingdom) as the clones expanded. After each restimulation, T-cell blasts were expanded in RF10 containing 10% supernatant from concanavalin A-stimulated rat spleen cells for 4 days and maintained in 2% rat spleen cells thereafter.

T-cell proliferation assay. Mice were footpad immunized with 25 μg of rM5 in Freund's complete adjuvant. Seven days later, PLN were assayed in round-bottom multiwell plates (Costar; NBL Ltd.) by using 2×10^5 cells per well for 72 h at 37°C in a humidified CO_2 incubator. T-cell clones were assayed in flat-bottom multiwell plates (Costar) by using 2×10^4 cells per well with 7.5×10^5 irradiated syngeneic spleen cells for 48 h. Peptides were used at 1,000, 200, and 40 nM, and both rM5 and pepM5 were used at 250 nM. Triplicate wells were pulse-labelled with tritiated thymidine (TRA 310; specific activity, 2 Ci/mmol; Amersham International plc, Amersham, Buckinghamshire, United Kingdom) for the final 4 h of culture, harvested onto glass fiber, and prepared for scintillation spectroscopy. PLN responses were scored as positive if the number of disintegrations per minute in the presence of antigen was 2.5-fold or greater than the number of disintegrations per minute of PLN alone.

RESULTS

PLN responses of BALB/c, C57BL/6, and CBA/Ca mice. In preliminary experiments, PLN and spleen cells from unim-

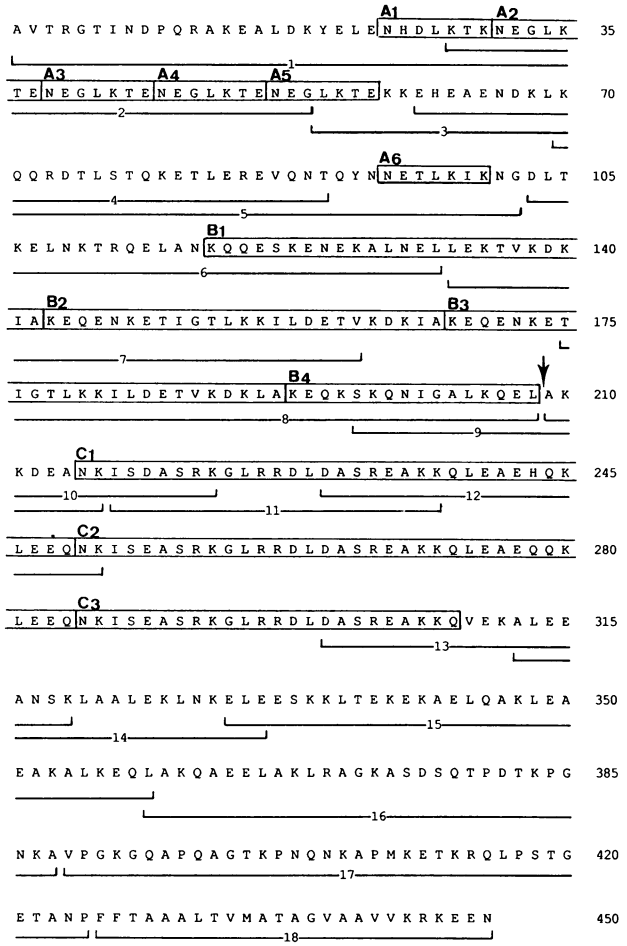

FIG. 1. Synthetic peptides of M5 protein. The numbers to the right of the sequence indicate amino acid positions. The sequence of the mature M5 protein (excluding the 42-residue N-terminal signal peptide) is shown, and therefore residue 1 corresponds to residue 43 of the previously described sequence (22). The boxes highlight the three types of tandemly repeated sequences, previously designated the A, B, and C repeats (22). The sequences corresponding to the synthetic peptides used in this study are identified by underlining and are numbered 1 to 18. The pepsin cleavage site defining the pepM5 N-terminal fragment is shown by an arrow.

munized mice and mice immunized with saline and adjuvant, as well as a number of ovalbumin-specific T-cell clones, were assayed against doses ranging from 20 nM to 2 μM of intact rM5 purified from *E. coli* and the N-terminal pepM5 fragment purified from *S. pyogenes*. No responses above background were observed, indicating that neither rM5 nor pepM5 polyclonally activated murine T cells (data not shown). PLN from rM5-immunized BALB/c, CBA/Ca, and C57BL/6 mice were tested for their responsiveness to rM5, pepM5, and 18 synthetic peptides corresponding to most of the M5 sequence (Fig. 1). Significant responses were observed at doses of rM5 above 20 nM, peaking at 1 μM, and antibody inhibition experiments confirmed that these responses are MHC class II restricted (data not shown). pepM5 recalled a large proportion of the rM5-specific response in all three strains of mice, indicating that there are T-cell epitopes located in the N-terminal half of the molecule (Fig. 2). None of the synthetic peptides, however, recalled

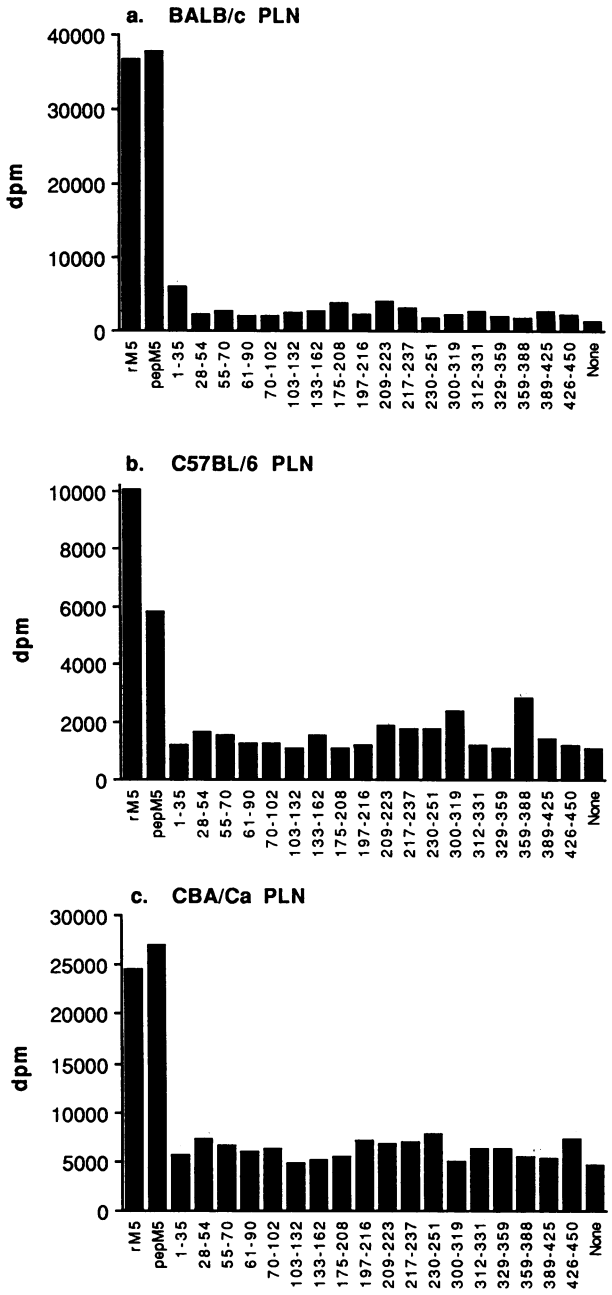

FIG. 2. Proliferation responses of PLN cells to rM5, pepM5, and synthetic peptides. Four mice from each of three strains were immunized with 500 nM of rM5 in complete adjuvant, and proliferation responses of PLN were measured 7 days later (see Materials and Methods for assay conditions). The responses of representative BALB/c (a), C57BL/6 (b), and CBA/Ca (c) mice are shown. Results are expressed as mean disintegrations per minute (dpm) at 250 nM rM5 and pepM5 and as the highest count of disintegrations per minute from a dose titration of 1,000, 200, or 40 nM for each of 18 peptides representing the majority of the M5 sequence. None of the responses to peptides were significant (see Materials and Methods for criteria), except for peptide 359 to 388 for the C56BL/6 PLN and peptides 1 to 35, 175 to 208, and 209 to 233 for BALB/c PLN.

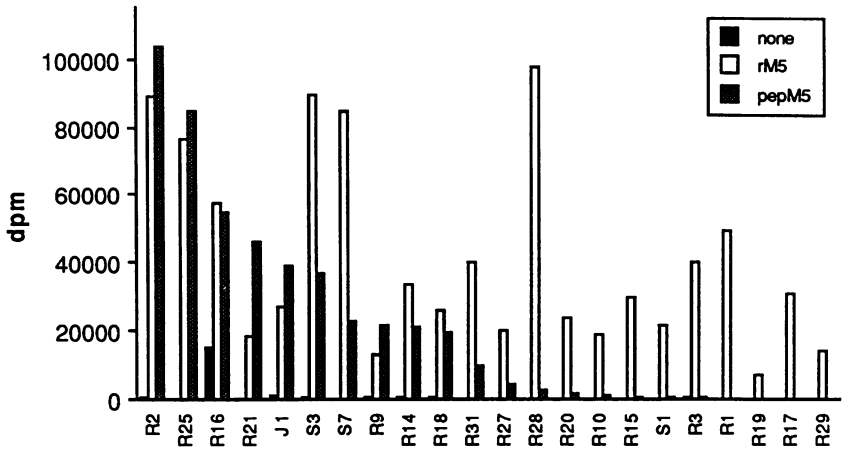

FIG. 3. Proliferation responses of BALB/c T-cell clones. The proliferation responses of 22 BALB/c rM5-specific T-cell clones (see Materials and Methods for assay conditions) are shown. Results are expressed as mean disintegrations per minute (dpm) in the presence or absence of 250 nM rM5 or pepM5. Clones are ordered left to right by the magnitude of response to pepM5.

more than a small proportion of the PLN response, although some peptides appear to be more antigenic than the majority. Although several peptides induced small but significant responses in PLN (Fig. 2), only those observed in three of four rM5-immunized BALB/c mice to peptide sM5[1-35] and in two of four rM5-immunized C57BL/6 mice to peptide sM5[359-388] could be considered to be in any way consistent. These results suggest that none of the peptides used correspond to strongly immunodominant T-cell recognition sites.

Response of BALB/c T-cell clones to rM5 and pepM5. rM5-specific T-cell clones were generated from four BALB/c mice by direct cloning in a limiting dilution system from PLN, 7 days after footpad immunization with rM5 in Freund's complete adjuvant. All clones tested were $CD4^+$ by immunofluorescence. The responses of 22 clones to rM5 and pepM5 are described in Fig. 3. Twelve clones clearly recognized pepM5, indicating that these clones were specific for epitopes in the N-terminal half of M5. pepM5 induced responses of equal magnitude to rM5 in many clones (e.g., R2), but, in a number of clones (e.g., S3), pepM5 induced clearly detectable responses that were considerably lower than those induced by rM5. The remaining clones responded to pepM5 poorly or not at all, suggesting that these clones recognized antigenic sites in the C-terminal half of M5 (see below).

Specificity of BALB/c T-cell clones for synthetic peptides. The proliferative responses of rM5-specific BALB/c T-cell clones to the synthetic peptides corresponding to most of the M5 sequence (Fig. 1) were examined. The results are summarized in Table 1, and representative responses of eight clones are described in Fig. 4. In nearly all cases, a single peptide recalled the majority of the response to rM5, suggesting that the T-cell clones recognized epitopes within the peptide sequence with high affinity. In a few cases, additional peptides also recalled a proportion of the response (e.g., peptide 329 to 359 for clone R17). The specificities of 29 of 55 clones were mapped with these peptides. However, two clones (R18 and S1) each recognized two nonoverlapping N-terminal peptides and could correspond to a mixture of two clones with distinct specificities. One clone (R28) recognized peptides sM5[133-162] and sM5[175-208] which are not contiguous but share a region of sequence from the B repeats (Fig. 1), suggesting that this clone was specific for the common sequences. All other clones recognized individual peptides. Antigenic sites were located in eight of the peptides, six corresponding to sequences in the N-terminal half of M5 and two corresponding to sequences in the conserved C-terminal half. The majority of T-cell clones which recognized peptides corresponding to N-terminal M5 sequences also responded to pepM5 (Fig. 3). However, three clones (S1, R17, and R28) recognized N-terminal M5 peptides but not pepM5, suggesting that there are subtle differences between pepM5 and the corresponding N-terminal

TABLE 1. Numbers of rM5-specific T-cell clones that recognize individual synthetic peptides

Peptide	M5 sequence	No. of T-cell clones[a]		
		BALB/c	C57BL/6	CBA/Ca
1	1–35	14	1	0
2	28–54	2	0	1
3	55–70	1	0	1
4	61–90	0	0	0
5	70–102	0	0	0
6	103–132	2	0	0
7	133–162	2	0	0
8	175–208	2	0	1
9	197–216	0	0	0
10	209–223	0	0	1
11	217–237	0	1	0
12	230–251	0	0	0
13	300–319	6	0	5
14	312–331	0	1	0
15	329–359	3	0	0
16	359–388	0	3	0
17	389–425	0	1	0
18[b]	426–450	0	0	0

[a] T-cell clones whose response to a peptide was approximately of the same magnitude as the response to rM5 were considered to be positive (see Fig. 4 for examples).

[b] Immunoblotting experiments with antisynthetic peptide antibodies have suggested that the C-terminal hydrophobic tail, which may correspond to a membrane anchor (1), is proteolytically detached from the bulk of the rM5 protein purified from *E. coli* periplasm (unpublished observations). Thus, rM5-specific T-cell clones are unlikely to recognize peptide 18.

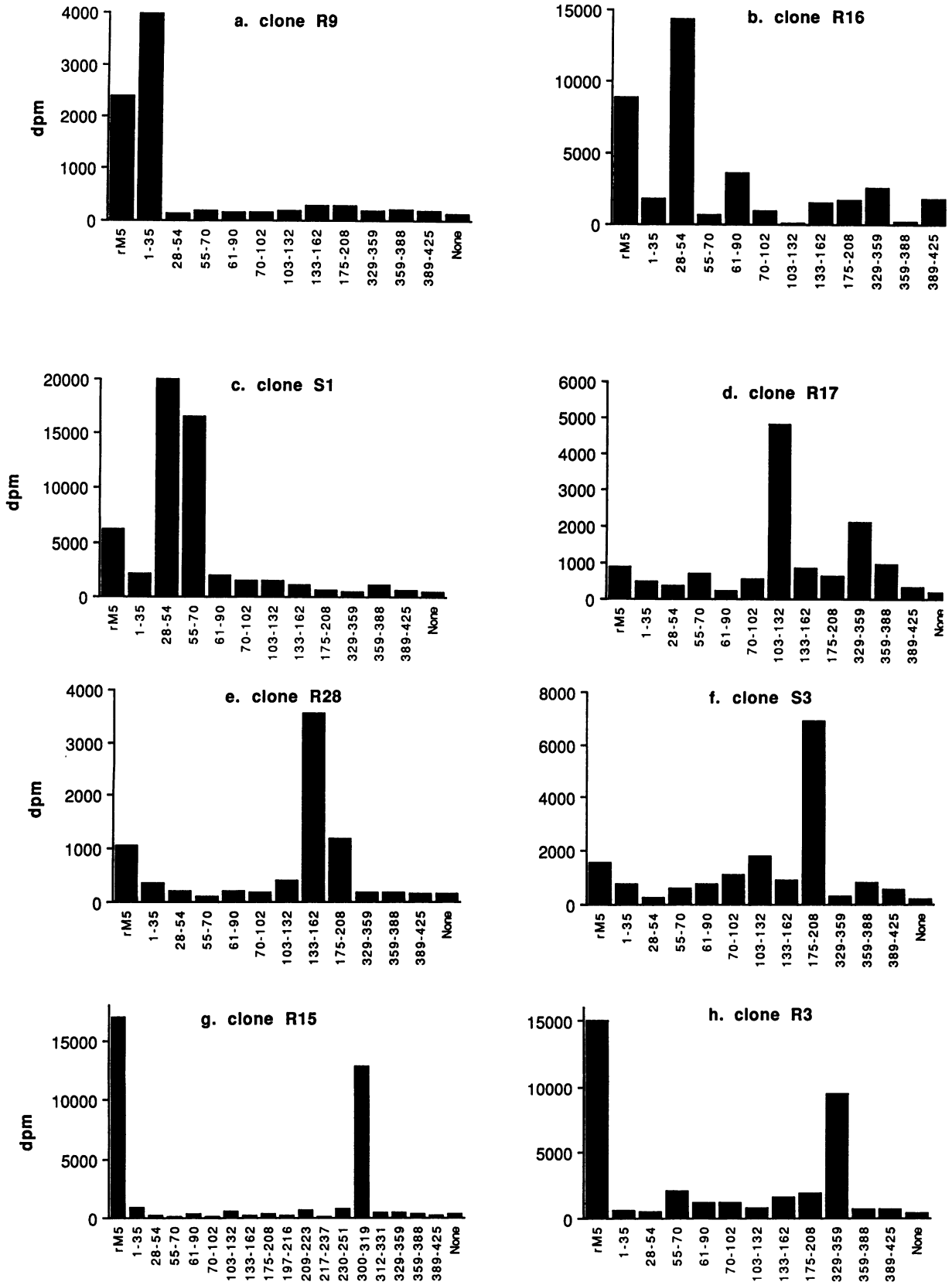

FIG. 4. Proliferation responses of BALB/c T-cell clones to synthetic peptides. The results of proliferation assays from representative BALB/c rM5-specific T-cell clones, namely, R9 (a), R16 (b), S1 (c), R17 (d), R28 (e), S3 (f), R15 (g), and R3 (h), recognizing each of the eight T-cell peptides, are shown as mean disintegrations per minute (dpm) for 250 nM rM5 or the number of disintegrations per minute from the dose (1,000, 200, or 40 nM) of each peptide giving the maximum response.

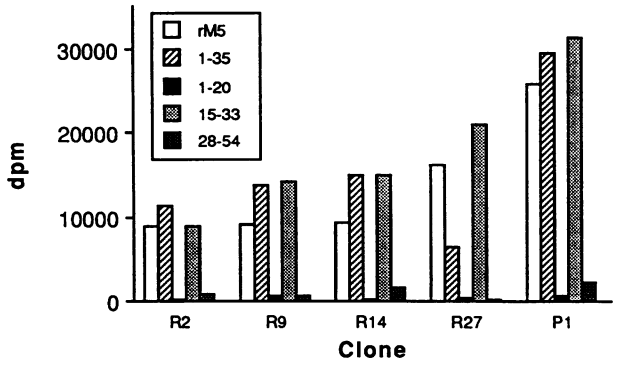

FIG. 5. Fine specificity of T-cell clones specific for M5(1-35). The results of proliferation assays of four BALB/c and one C57BL/6 (P1) rM5-specific clones to peptides sM5[1-35], sM5[1-20], sM5[15-33], and sM5[28-54] are shown as mean disintegrations per minute (dpm) for 250 nM rM5 or the number of disintegrations per minute from the dose (1,000, 200, or 40 nM) of each peptide giving the maximum response.

region in the intact rM5. While most T-cell clones recognize N-terminal peptides, the recognition of a significant proportion of T-cell clones mapped to two different peptides corresponding to C-terminal sequences.

Specificity of C57BL/6 and CBA/Ca clones. The specificities of a limited number of rM5-specific T-cell clones from C57BL/6 (9 clones) and CBA/Ca (17 clones) mice were also mapped (Table 1). Clones from C57BL/6 mice recognized T-cell epitopes on five distinct peptides. These include sM5[1-35], which was also recognized by several BALB/c rM5-specific clones, as well as four peptides which were not recognized by any of the BALB/c clones tested. Three C57BL/6 T-cell clones recognized peptide sM5[359-388], which induced a weak proliferative response in polyclonal C57BL/6 PLN cells. The specificities of eight CBA/Ca T-cell clones were mapped to five separate peptides corresponding to sequences in either N-terminal or C-terminal regions of M5, four of which were also recognized by one or more BALB/c clones. The peptide sM5[300-319] was therefore recognized by a significant number of T-cell clones from both CBA and BALB/c mice.

Fine specificity of T-cell clones recognizing sM5[1-35]. Fifteen T-cell clones, whose specificities are described in Table 1, recognized peptide sM5[1-35]. All 15 clones were shown to be specific for sM5[15-33]. Mapping of the fine specificity of five of these clones (four BALB/c and one C57BL/6) is shown in Fig. 5. Each clone responded clearly to sM5[15-33] but not to sM5[1-20] or sM5[28-54], indicating that all five clones recognize an epitope in the region of M5 residues 21 to 27. We have subsequently investigated 11 of the BALB/c sM5[15-33]-specific clones, which were all shown to be *I-E*^{d} restricted by anti-MHC class II antibody blocking (data not shown).

DISCUSSION

Previous studies of T-cell responses to M proteins have been limited. It has been reported that both pepM5 and pepM24 elicit in vitro proliferation of lymphocytes from rabbits immunized with a poly-L-lysine-conjugated synthetic peptide corresponding to M24 residues 109 to 120 (4). This response, however, could not be recalled by the sM24[109-120] peptide nor by a larger peptide, sM24[92-126]. A similar observation has been reported for rabbits immunized with a keyhole limpet hemocyanin-conjugated trivalent hybrid peptide, corresponding to M5 residues 1 to 10, linked in tandem to residues 1 to 11 of M6 and residues 1 to 12 of M24. Lymphocytes from the immunized rabbits responded to pepM5, pepM6, and pepM24, but not to the relevant synthetic peptides (3). These earlier studies did not provide clear indications of the numbers or locations of T-cell epitopes in M proteins.

The studies reported here have defined at least 13 distinct MHC class II-restricted T-cell recognition sites in the type 5 M protein. None of these sites appear to be particularly immunodominant, with the possible exception of the site within residues 1 to 35 for BALB/c and the site within residues 359 to 388 for C57BL/6 mice, which were recognized by a higher proportion of clones than any other region of the M5 molecule and which stimulated a small but significant proliferation response in PLN from rM5-immunized mice. Eight recognition sites have been defined by rM5-specific T-cell clones from BALB/c mice, and a further five sites have been defined by C57BL/6 and CBA T-cell clones. The specificity of around 60% of rM5-specific T-cell clones from three strains of mice studied in detail were mapped to these recognition sites. The remainder of the clones were tested and did not recognize any of these peptides described in Fig. 1, suggesting that there may be additional T-cell epitopes overlapping the junctions of certain peptides, for example the junctions of peptides 15 and 16, 16 and 17, and 17 and 18 in the C-terminal end of the molecule. Thus, M5 appears to contain a larger number of distinct T-cell recognition sites than has been reported to date for other protein antigens.

Surprisingly, a number of clones which recognized peptides corresponding to several distinct sequences in the N-terminal half of M5 did not respond to the N-terminal pepM5 fragment. Although to date we have observed no differences in the reactivities of pepM5 and rM5 with fibrinogen or anti-pepM5 monoclonal and polyclonal antibodies, it is possible that there are subtle conformational differences between pepM5 and the corresponding region of rM5 that affect antigen processing and the effective presentation of certain pepM5 sequences to rM5-specific mouse T-cell clones. Such conformational differences would be consistent with other observations on the mitogenic properties of pepM5 and rM5 for human T cells. Although pepM5 behaves as a conventional antigen in mice and other laboratory animals (6), it has recently been reported to act as a superantigen for human T cells (26). We have found that neither the intact rM5 nor pepsin-cleaved rM5 elicits the proliferation of nonimmune human T cells (unpublished observations). If conformation influences antigen processing during conventional antigen presentation, our results would imply that T-cell responses to recombinant antigens, purified antigens, or both could differ from responses during natural infections, where the antigen is encountered in the context of more complex structures. It seems likely that the immunization (infection) route and expression of other products by the pathogen could also modulate responses during natural infections. To explore these potential differences, we are now examining T-cell responses to M5 during streptococcal infections in mice.

Like other serotypes of M proteins that have been sequenced, a large proportion (just over 50%) of the M5 protein consists of tandemly repeated sequences. M5 con-

tains three distinct types of repeat sequences, termed A, B, and C (Fig. 1). Although one or more T-cell recognition sites have been defined in each of the three types of repeats (Table 1), it is interesting to note that none of these sites are particularly immunodominant. Studies of antibody responses to M proteins in rabbits and humans have suggested that antibody epitopes in the B repeats are immunodominant and that the production of protective (i.e., opsonic) antibodies to the more proximal A repeats and unrepeated N-terminal sequences is delayed (9). Although there appear to be several distinct T-cell recognition sites in the B repeats, the most frequently recognized site identified to date is located in unique, unrepeated sequences. The fine specificity of all clones which recognized peptide sM5 [1-35] were mapped and found to recognize a single site, at or overlapping the sequence ELENHDL (residues 21 to 27). This site was recognized by clones from two different MHC haplotypes of mice and is located close to sequences where a number of protective antibody epitopes have been mapped (8, 15). This may be analogous to recent reports that several antigenic sites in influenza virus hemagglutinin contain coextensive or overlapping T- and B-cell epitopes (1). At present, our understanding of how antibody production might be influenced by structural relationships between individual T- and B-cell epitopes is too limited to suggest that there may be any significance in the relative locations of this T-cell recognition site and protective antibody epitopes. The M5 protein could prove to be an interesting model for studying such relationships. It is an important protective antigen in which the location of several opsonic antibody epitopes have been defined as well as epitopes that evoke human heart cross-reactive antibodies that might contribute to the pathogenesis of an autoimmune disease, namely acute rheumatic fever. Furthermore, M protein vaccines safe to use in humans would need to be based on a defined-epitope immunogen to avoid potentially harmful host cross-reactivity. It will be interesting to determine whether T-cell recognition sites in the widely conserved C-terminal M sequences could provide effective help for protective, opsonic-antibody epitopes which are restricted to serotype-specific N-terminal sequences. If so, well-defined (non-host-cross-reactive) C-terminal sequences encompassing several T-cell recognition sites, including sites recognized by more than one MHC haplotype, could form the basis for an effective carrier for multiple, type-specific B-cell epitopes that would evoke protection against a variety of epidemiologically important serotypes.

ACKNOWLEDGMENTS

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