INFECTION AND IMMUNITY, Nov. 1982, p. 573–579
0019-9567/82/110573-07$02.00/0

Vol. 38, No. 2

Type-Specific Antibodies to Structurally Defined Fragments of Streptococcal M Proteins in Patients with Acute Rheumatic Fever

ALAN L. BISNO,[1]* XIMENA BERRIOS,[2] FRANCISCO QUESNEY,[2] DANIEL M. MONROE, JR.,[1] JAMES B. DALE,[1,3] AND EDWIN H. BEACHEY[1,3]

Division of Infectious Diseases, Department of Medicine, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163[1]; Veterans Administration Medical Center, Memphis, Tennessee 38104[3]; and Sotero del Rio and Josefina de Martinez Hospitals and Catholic University, Santiago, Chile[2]

Received 28 May 1982/Accepted 26 July 1982

Group A streptococci of M protein type 5 have been epidemiologically related to acute rheumatic fever in a number of reported outbreaks. Preliminary bacteriological evidence suggests that M5 may be an important "rheumatogenic" type in Santiago, Chile. To assess further the relationship of this streptococcal serotype to rheumatic fever in Chile, sera of 34 patients with rheumatic fever and an equal number of age-, sex-, and race-matched controls were assayed for antibodies to types 5, 6, and 24 in an enzyme-linked immunosorbent assay with purified pepsin extracts of the respective M proteins as solid-phase antigens. Sera of 11 rheumatic fever patients (32%) were positive (titer > 1:800) for type 5 antibodies, but only 1 (3%) of the matched controls was positive ($P < 0.01$). Neither the patients nor the controls had antibodies to type 24. Although 38% of the patient sera contained antibodies to type 6, 29% of the control sera also had such antibodies ($P > 0.20$). The enzyme-linked immunosorbent assay served as an accurate predictor of which sera contained type 5 opsonic antibodies as measured by the opsonophagocytic test. Although antigenic cross-reactivity exists between M protein type 5 and type 6 group A streptococci, this phenomenon is unlikely to have accounted for the preferential occurrence of type 5 antibodies in rheumatic fever sera. The enzyme-linked immunosorbent assay and opsonic antibody results suggest that M5 is an important rheumatogenic type in Chile.

Acute rheumatic fever is a delayed, nonsuppurative sequela of upper respiratory infection due to group A streptococci. A growing body of evidence now suggests that individual strains of streptococci may vary in their ability to elicit rheumatic fever (10–12, 19, 23, 24, 28, 30). Indeed, representatives of only a limited number of the more than 70 serotypes of M protein have been epidemiologically associated with rheumatic fever (9).

Attempts at worldwide control of rheumatic fever have focused upon proper diagnosis and therapy of streptococcal pharyngitis (primary prevention) and upon continuous antimicrobial prophylaxis of individuals with a history of the disease (secondary prevention) (13). Such programs have met with only limited success in developing nations, wherein the problem remains most severe. An alternative approach would be the development of a group A streptococcal vaccine(s). Such an approach is attractive because the principal virulence factor of these organisms is known to be M protein, and type-specific antibodies against this cell wall antigen confer protective immunity. Several groups of investigators have reported upon purification techniques for M protein (7, 17) and upon preliminary trials of immunization in humans (8, 18, 22).

The formulation of effective polyvalent vaccines would require the knowledge of those M serotypes in any geographic area which are most prevalent and most highly rheumatogenic. Unfortunately, throat cultures of patients with rheumatic fever often fail to clarify this issue because of the long latent period between the initial streptococcal infection and the onset of acute rheumatic fever (often 3 to 4 weeks) and the frequency with which antibiotics have been administered before attempts have been made to isolate streptococci.

The present study was conducted in Santiago, Chile, among a population group in which rheumatic fever is still common. Sera of patients with rheumatic fever and of controls were assayed for type-specific antibodies to three serotypes of

group A streptococci by an enzyme-linked immunosorbent assay (ELISA) utilizing highly purified fractions of M protein (2). The presence of type-specific opsonic antibodies in ELISA-positive sera was confirmed by opsonophagocytic tests. The results indicated (i) a high prevalence of antibodies to type 5 (a strongly "rheumatogenic" type [9]) in sera of Chilean patients with acute rheumatic fever, (ii) an excellent correlation between the presence of type-specific M5 antibodies in human sera as assayed by ELISA and opsonophagocytic tests, and (iii) the presence of immunological cross-reactivity between two of the M types tested, M5 and M6.

MATERIALS AND METHODS

Patients with rheumatic fever admitted to Sotero del Rio Hospital or Josefina de Martinez Pediatric Hospital, Santiago, Chile, between March 1978 and December 1979 who agreed to participate were enrolled in the study. All patients fulfilled the modified Jones criteria. For each patient, a control subject of the same race, sex, and socioeconomic status and of approximately the same age was recruited. Control subjects were selected either from individuals hospitalized at the same time as the index case (but without streptococcal infections or rheumatic fever) or from persons residing in the same geographic area as the index case (usually within the same city block or apartment building).

Throat swabs obtained from each patient and control were inoculated onto 5% sheep blood agar plates and incubated aerobically overnight at 37°C. Beta-hemolytic streptococci were isolated in pure culture, serogrouped and serotyped by the capillary tube precipitin method (29) with antisera produced by the Bacteriologic Institute, Santiago, and the Centers for Disease Control, Atlanta, Ga. Serum specimens were obtained from rheumatic fever patients and controls upon entry into the study and again 1 month later.

ELISA. M proteins of types 5, 6, and 24 were extracted by limited peptic digestion and purified by ammonium sulfate precipitation, ion-exchange chromatography, and isoelectric focusing as previously described (1, 8). The resultant material was obtained in pure form as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, quantitative amino acid analysis, and Edman degradation (5, 7) and was designated pep M. Rabbit antisera raised against these M proteins contained type-specific, antiopsonic, and immunoprecipitating antibodies (7).

ELISA for M antibodies was performed as previously described (8) in polystyrene tubes (12 by 75 mm) (Falcon Labware, Div. of Becton-Dickinson & Co., Inc., Oxnard, Calif.) with 5 μg of purified M protein per ml to coat each tube. Antibodies in patient serum were detected by using alkaline phosphatase-conjugated rabbit anti-human immunoglobulin G (Microbiological Associates, Walkersville, Md.) with *p*-nitrophenylphosphate (Sigma Chemical Co., St. Louis, Mo.) as substrate. The titer of the test serum was recorded as the highest serum dilution which, when compared with control tubes, gave an absorbance of >0.1 at 400 nm on a Beckman 26 spectrophotometer.

Opsonophagocytic tests. The assay mixtures consisted of 0.4 ml of fresh human blood supplemented with 10 U of heparin per ml and 0.05 ml of a standard suspension of streptococci preopsonized (1 volume of cells to 1 volume of serum for 15 min at 37°C and 15 min at 0°C) with test serum. The ratio of streptococcal colony-forming units to polymorphonuclear leukocytes was approximately 10:1. Penicillinase (50,000 U) (BBL Microbiology Systems, Cockeysville, Md.) was added to each tube before the addition of streptococci. The percentage of neutrophilic leukocytes with associated streptococci (percent phagocytosis) was estimated by microscopic counts of stained smears obtained after 15, 30, and 45 min of rotation at 37°C. A test was considered positive if the percent phagocytosis in the presence of test serum exceeded 25 and was at least threefold greater than that in a control tube containing normal rabbit serum. In practice, most control tubes demonstrated a percent phagocytosis of approximately 10% at 30 min, whereas tubes containing positive sera yielded values in the range of 35 to 70%. A statistical analysis of replicate specimens indicated that such differences were unlikely to be due to chance ($P < 0.01$).

Absorption studies. Three volumes of serum were absorbed with one volume of whole heat-killed streptococcal cells at 37°C for 30 min. After centrifugation, sera were passed through a 0.45-μm filter before use in ELISA or opsonophagocytic tests. In certain experiments, the ability of pepsin-extracted M protein to competitively inhibit ELISA reactions was tested. Ten micrograms of pep M antigen was added to each of the 1-ml tubes of diluted sera just before these sera were transferred to the tubes containing fixed antigen. The assay was then performed in the usual manner.

RESULTS

ELISA titers in rheumatic fever patients and matched controls. We chose the ELISA method of assaying type-specific serum antibody to M protein because of its applicability to screening large numbers of specimens and because in studies of M protein immunization in humans (8), type-specific antibodies detected by ELISA correlated closely with those detected by opsonophagocytic tests. We studied antibodies to M types 5, 6, and 24 for the following reasons: (i) all three types have been epidemiologically associated with rheumatic fever in various parts of the world (9); (ii) highly purified antigens suitable for use in ELISA were available in our laboratories; and (iii) in preliminary bacteriological studies, M5 streptococci have been recovered from the throats of five Chilean rheumatic fever patients (two were recovered before the initiation of these studies, one was recovered during the study reported here, and two were recovered subsequently). Types 6 and 24 have knowledge, been recovered from Chilean rheumatic fever patients.

Sera from 34 patients and 34 matched controls were assayed. In most cases, an acute-phase serum sample and at least one sample collected 1

TABLE 1. Anti-M antibodies (determined by ELISA) in 34 acute rheumatic fever patients and 34 control subjects in Santiago, Chile, 1978–1979

Subjects	No. (%) of patients positive (serum titer > 1:800) for antibody to:		
	M5	M6	M24
Acute rheumatic fever	11 (32)[a]	13 (38)[b]	0
Control	1 (3)	10 (29)	0

[a] Acute rheumatic fever versus controls, $P < 0.01$.
[b] Acute rheumatic fever versus controls, not statistically significant.

TABLE 2. Comparison of ELISA and opsonophagocytic (OP) test results[a]

Antigen	ELISA titer (reciprocal)	No. of sera tested	No. (%) of sera positive by OP test (vs homologous type)
Pep M5	1,600–≥51,200	15	14 (93)
	200–800	14	4 (29)
	<200	17	0 (0)
Pep M6	1,600–≥51,200	13	10 (77)
	200–800	5	2 (40)
	<200	2	0 (0)
Pep M24	1,600–≥51,200	0	0 (0)
	200–800	2	0 (0)
	<200	8	0 (0)

[a] The data in this table include all available sera on which both ELISA and OP tests were performed.

month later were available for all rheumatic fever patients, and similarly timed sera were obtained from control subjects. Based upon comparisons with opsonophagocytic tests (see below), ELISA titers higher than 1:800 were considered positive for type-specific antibody. A total of 32% of the patients with rheumatic fever had evidence of type-specific antibody to pep M5 as measured by ELISA, compared with only 3% of the controls (Table 1; $P < 0.01$). There was no statistically significant difference in the prevalence of positive ELISA tests for M6 antibodies between patients and matched controls. None of the sera were positive for M24 antibodies. Eight rheumatic fever patients and one control had type-specific antibodies to both type 5 and type 6.

Comparison of ELISA titers and opsonophagocytic results. Opsonization tests for type 5 antibodies were performed on 46 sera (including paired sera from the same patients in several instances). A total of 93% of sera with ELISA titers of >1:800 contained type 5 opsonic antibodies, whereas none of 17 sera with ELISA titers of <1:200 contained such antibodies (Table 2). Approximately one-third of sera with intermediate ELISA titers (1:200 to 1:800) contained opsonic antibodies. The data for M6 were less clear-cut. A total of 77% of sera with ELISA titers higher than 1:800 contained detectable type-specific antibodies by the opsonic test, whereas two of five sera with intermediate titers gave positive opsonic tests. No sera were encountered with ELISA titers against pep M24 of >1:800 or with positive opsonic antibody tests for this serotype.

The results of the opsonic antibody assays were clear-cut and type specific in sera in which there were marked dissociations between the ELISA titers of anti-M5 and anti-M6. For example, serum S-36, which had ELISA titers of 1:1,600 against pep M5 antigen and <1:200 against pep M6 antigen, was markedly opsonic for type M5 (Fig. 1A) but not for type M6 (Fig. 1B) streptococci.

Cross-reactivity between M5 and M6. Recent studies by Fischetti (16) have demonstrated significant antigenic similarities between types 5 and 6 as well as between other serotypes of M protein. We (14) have also recently shown immunological cross-reactivity between M protein fragments isolated from strains of M serotypes 5 and 6. Such immunological cross-reactivity was apparent in studies of naturally acquired anti-M antibodies in human sera collected from our Chilean subjects.

In general, type 5 and type 6 cells each absorbed ELISA antibodies to both M5 and M6, but there were exceptions (Table 3). Similar results were obtained by using pep M5 or pep M6 antigens as competitive inhibitors in ELISA tests. In most instances, opsonic antibodies were removed by a single absorption with cells of the homologous M type; absorption of all of the opsonins by the heterologous type was variable (Table 3).

In further assessing these cross-reactions, we compared ELISA titers to each of the two serotypes in individual sera. A total of 124 sera were available for analysis from the 68 rheumatic fever and control subjects considered in this study. A total of 43 of the 124 sera were positive by ELISA (i.e., titer > 1:800) for either pep M5 or pep M6, but only 11 (25%) of these 43 sera were positive for both. Moreover, there was no correlation between the ELISA titers against pep M5 and those against pep M6 in individual sera (by the method of least squares, $r = 0.109$ and $P > 0.20$).

Fourfold rises or falls in M5 or M6 ELISA titers were observed in 36% of the rheumatic fever patients for whom paired sera were available and in 8% of the control subjects. There

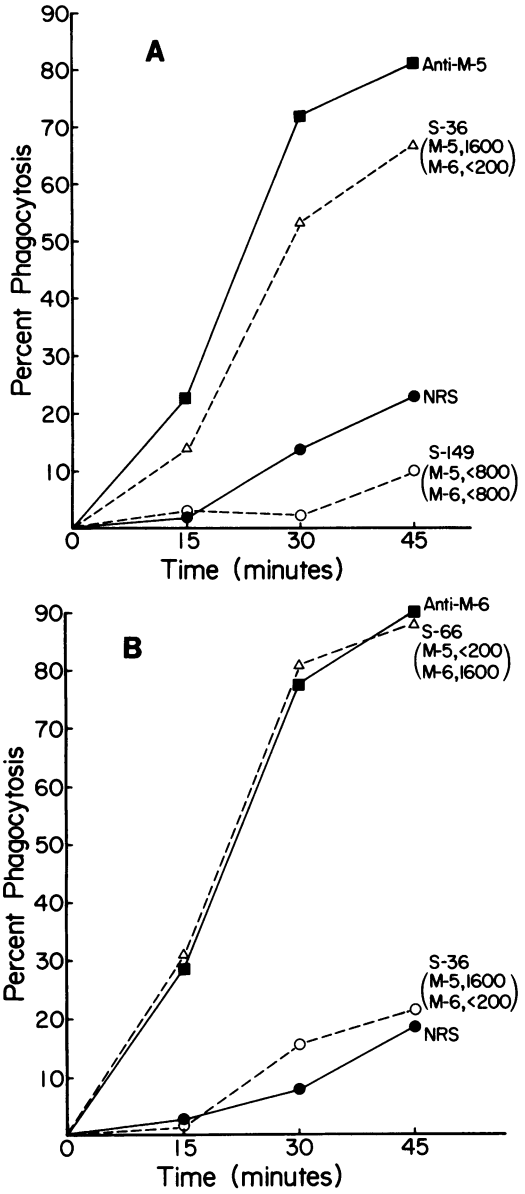

FIG. 1. Representative opsonophagocytic test results. The test organisms were an M type 5 group A streptococcus (A) and an M type 6 organism (B). Reciprocal ELISA titers against pep M5 and pep M6 antigens are indicated for each of the human sera. Sera with titers of >1:800 are markedly opsonic for the homologous but not for the heterologous streptococcal type. NRS, Normal rabbit serum; anti-M5 and anti-M6, hyperimmune rabbit antisera; S-36, S-149, and S-66, sera from Chilean study patients.

was no parallelism between temporal rises or falls in M5 and M6 antibody titers. Representative data from five patients are presented in Table 4.

DISCUSSION

The association of a particular serotype with rheumatic fever is most readily established during those epidemics of well-documented streptococcal upper respiratory infections which clearly give rise to rheumatic sequelae. A review of such outbreaks recorded in the English literature (9) reveals that: (i) a limited number of serotypes are involved; (ii) certain serotypes, particularly type 5, are strongly rheumatogenic; and (iii) certain extremely prevalent serotypes, such as type 12, have seldom if ever been documented to produce rheumatic fever outbreaks. Although the classic prospective studies at Warren Air Force Base, Wyo., in 1949–51 (25) demonstrated a constant rheumatic fever attack rate regardless of infecting serotype, in those studies only three types (5, 14, and 24) gave rise to enough infections to allow the calculation of an attack rate. All three types have been documented as rheumatogenic in other settings as well.

The task of relating specific streptococcal serotypes to rheumatic fever cases is much more difficult when the disease occurs sporadically in open populations. Nevertheless, our studies in Memphis, Tenn., have suggested the presence of rheumatogenic and nonrheumatogenic strains in such a population (9–11). The investigations of Potter et al. (23) in Trinidad have revealed that streptococcal infections antecedent to acute rheumatic fever and to poststreptococcal acute glomerulonephritis occurring in the same population at the same time are due to different serotypes.

Studies of endemic rheumatic fever, such as those conducted in Memphis, Trinidad, and Chile, are hampered by the relatively low isolation rate of group A streptococci from patients who contract the disease, even when the antecedent streptococcal infection is well documented by antibody studies. The reasons for this are unclear but probably relate at least in part to the relatively long interval between the upper respiratory infection and the onset of rheumatic manifestations, the still-further delay in diagnosing rheumatic fever and obtaining a throat culture, and the frequency with which antibiotics are administered before the initial culture. An alternative strategy, that of assaying type-specific antibodies to potentially rheumatogenic serotypes in the blood of rheumatic patients, presents certain technical difficulties. The standard assay, the bactericidal test of Lancefield, is cumbersome, does not readily lend itself to the processing of large numbers of sera, and is more difficult to interpret when human sera (which may contain only modest amounts of antibody) are used than when hyperimmune rabbit sera are tested.

TABLE 3. Absorption of anti-M5 and anti-M6 ELISA and opsonic antibodies by homologous and heterologous streptococcal cells

Serum code	Serum absorption	ELISA titer (reciprocal)		OP test[a]	
		M5	M6	M5	M6
B635	None	6,400	6,400	+	+
	M5 cells	200	800	−	+
	M6 cells	800	400	−	−
B173	None	<200	12,800	−	+
	M5 cells	<200	12,800	ND	+
	M6 cells	<200	1,600	ND	−

[a] OP, Opsonophagocytic; +, positive; −, negative; ND, not done.

In this study we used ELISA to detect anti-M protein antibodies (26) because it is highly sensitive, exhibits less variability than tests of phagocytic killing, and lends itself to the study of large numbers of samples. The utility of ELISA in measuring type-specific antibody is dependent upon the availability of highly purified M antigens (2, 15). ELISA results correlated well with our assay for opsonic antibodies. No sera with ELISA titers of <1:200 against pep M5, pep M6, or pep M24 were opsonic against the homologous streptococcal organisms (Table 2). In contrast, 93% of sera with anti-M5 titers of >1:800 and 77% of sera with similarly elevated anti-M6 titers had detectable opsonic antibodies. Because ELISA is more sensitive than opsonic assays (8, 14, 15, 26), it is not surprising that a number of sera with low or intermediate ELISA titers had negative opsonophagocytic tests.

Using the ELISA in conjunction with opsonophagocytic assays, we detected an immunological response to type 5 streptococcal M protein in approximately one-third of rheumatic fever patients admitted to Sotero del Rio and Josefina de Martinez hospitals during the study. Whether or not these M5 immune responses were due entirely to type 5 streptococcal infection is difficult to establish unequivocally from our studies because of the high degree of immunological cross-reactivity which has been observed between type 5 and type 6 streptococci and their M proteins (14–16). Indeed, in some sera (Table 3), the ELISA antibody titers against M5 and M6 could both be absorbed with either type 5 or type 6 streptococci, suggesting that some of the antibody in such sera was directed against common determinants, perhaps as a result of structural homologies (3, 20, 21, 27) shared among certain M protein serotypes.

Several lines of evidence, however, make it most unlikely that the M5 antibodies found in sera of rheumatic fever patients were the result of type 6 infections. First, type 5 ELISA antibodies were rarely detected in control sera, despite the high prevalence of M6 antibodies in the same sera (Table 1). Second, there was no correlation in individual sera between their titers of anti-M5 and anti-M6 antibodies as measured by ELISA. Indeed, anti-M5 and anti-M6 titers rose and fell independently over time. Finally, type 5 streptococci have been recovered on five occasions over the past few years from the throats of patients with acute rheumatic fever, whereas none of the patients have been positive for type 6.

If, as we believe, the type 5 immune responses we detected are type specific, how might such a phenomenon be explained, given the known cross-reactivity between M protein types 5 and 6? One might hypothesize the presence of a dominant, type-specific immunodeterminant preferentially exposed on intact type 5 streptococci during these naturally occurring infections. Minor cross-reactive determinants may, on the other hand, be fully exposed in purified M protein extracted from the cell wall. Indeed, this concept is supported by recent evidence that each M protein may contain several type-specif-

TABLE 4. M5 and M6 ELISA antibody titers in paired sera

Patient	Serum phase	Titer (reciprocal)	
		M5	M6
ARF[a] 18	Acute	200	<200
	Conv[b]	1,600	<200
ARF 14	Acute	25,600	<200
	Conv	3,200	200
Control 33	Acute	200	<200
	Conv	<200	1,600
ARF 42	Acute	<200	1,600
	Conv	<200	<200
ARF 52	Acute	1,600	6,400
	Conv	6,400	800

[a] ARF, Acute rheumatic fever.
[b] Conv, Convalescent.

ic determinants among many subpeptides of the isolated M molecule (4, 6, 14). If such purified peptide fractions of M protein can elicit broadly protective immunity, then the task of developing a multivalent vaccine against rheumatogenic serotypes may be greatly facilitated.

The results presented here suggest that, as in the United States and England (9), type 5 is a major rheumatogenic serotype in Chile. Further studies of streptococci that elicit rheumatic fever and of the protective immune response to them and their purified M proteins are required both to identify prevalent rheumatogenic serotypes in a given geographic area and to define cross-reactions that may aid in the development of broadly reactive M protein vaccines.

ACKNOWLEDGMENTS

This work was supported by a grant-in-aid from the American Heart Association (no. 80-981), by Public Health Service research grant AI-10085 from the National Institutes of Health, and by institutional research funds from the U.S. Veterans Administration. E.H.B. is the recipient of a Medical Investigatorship Award from the U.S. Veterans Administration. J.B.D. is the recipient of Research Traineeship Award GR-AM07317 from the National Institutes of Health.

The secretarial assistance of Pamela Swann is gratefully acknowledged.

LITERATURE CITED

1. **Beachey, E. H., G. L. Campbell, and I. Ofek.** 1974. Peptic digestion of streptococcal M protein. II. Extraction of M antigen from group A streptococci with pepsin. Infect. Immun. **9**:891–896.
2. **Beachey, E. H., E. Y. Chiang, J. M. Seyer, A. H. Kang, J. M. Chiang, and G. H. Stollerman.** 1977. Separation of the type specific M protein from toxic cross-reactive antigens of group A streptococci. Trans. Assoc. Am. Physicians **90**:390–400.
3. **Beachey, E. H., and J. M. Seyer.** 1982. Primary structure and immunochemistry of group A streptococcal M proteins. Sem. Infect. Dis. **4**:401–410.
4. **Beachey, E. H., J. M. Seyer, J. B. Dale, W. A. Simpson, and A. H. Kang.** 1981. Type-specific protective immunity evoked by synthetic peptide of *Streptococcus pyogenes* M protein. Nature (London) **292**:457–459.
5. **Beachey, E. H., J. M. Seyer, and A. H. Kang.** 1978. Repeating covalent structure of streptococcal M protein. Proc. Natl. Acad. Sci. U.S.A. **75**:3163–3167.
6. **Beachey, E. H., J. M. Seyer, and A. H. Kang.** 1980. Primary structure of protective antigens of type 24 streptococcal M protein. J. Biol. Chem. **255**:6284–6289.
7. **Beachey, E. H., G. H. Stollerman, E. Y. Chiang, T. M. Chiang, J. M. Seyer, and A. H. Kang.** 1977. Purification and properties of M protein extracted from group A streptococci with pepsin: covalent structure of the amino terminal region of type 24M antigen. J. Exp. Med. **145**:1469–1483.
8. **Beachey, E. H., G. H. Stollerman, R. H. Johnson, I. Ofek, and A. L. Bisno.** 1979. Human immune response to immunization with a structurally defined polypeptide fragment of streptococcal M protein. J. Exp. Med. **150**:862–877.
9. **Bisno, A. L.** 1980. The concept of rheumatogenic and nonrheumatogenic group A streptococci, p. 789–803. *In* S. E. Read and Z. B. Zabriskie (ed.), Streptococcal diseases and the immune response. Academic Press, Inc., New York.
10. **Bisno, A. L., I. A. Pearce, and G. H. Stollerman.** 1977. Streptococcal infections that fail to cause recurrences of rheumatic fever. J. Infect. Dis. **136**:278–285.
11. **Bisno, A. L., I. A. Pearce, H. P. Wall, M. D. Moody, and G. H. Stollerman.** 1970. Contrasting epidemiology of acute rheumatic fever and acute glomerulonephritis: nature of the antecedent streptococcal infection. N. Engl. J. Med. **283**:561–565.
12. **Bloomfield, A. L., and L. A. Rantz.** 1943. An outbreak of streptococcal sore throat in an army camp. J. Am. Med. Assoc. **121**:315–319.
13. **Committee on Rheumatic Fever and Bacterial Endocarditis, American Heart Association.** 1977. Prevention of rheumatic fever. Circ. Suppl. **55**:S1–S4.
14. **Dale, J. B., I. Ofek, and E. H. Beachey.** 1980. Heterogeneity of type-specific and cross-reactive antigenic determinants within a single M protein of group A streptococci. J. Exp. Med. **151**:1026–1038.
15. **Dale, J. B., I. Ofek, and E. H. Beachey.** 1980. Enzyme-linked immunosorbent assay of the human immune response to a streptococcal M protein vaccine, p. 862–864. *In* J. D. Nelson and C. Grassi (ed.), Current chemotherapy and infectious disease, vol. 2. American Society for Microbiology, Washington, D.C.
16. **Fischetti, V. A.** 1977. Streptococcal M protein extracted by nonionic detergent. II. Analysis of the antibody response to the multiple antigenic determinants of the M-protein molecule. J. Exp. Med. **146**:1108–1123.
17. **Fischetti, V. A., E. C. Gotschlich, G. Siviglia, and J. B. Zabriskie.** 1976. Streptococcal M protein extracted by non-ionic detergent. I. Properties of the antiphagocytic and type-specific molecules. J. Exp. Med. **144**:32–53.
18. **Fox, E. N., R. H. Waldman, M. K. Wittner, A. A. Mauceri, and A. Dorfman.** 1973. Protective study with a group A streptococcal M protein vaccine. J. Clin. Invest. **52**:1885–1892.
19. **Kuttner, A. G., and E. Krumweide.** 1941. Observations of the effect of streptococcal upper respiratory infections on rheumatic children: a three-year study. J. Clin. Invest. **20**:273–287.
20. **Manjula, B. N., and V. A. Fischetti.** 1980. Studies on group A streptococcal M proteins: purification of type 5 M protein and comparison of its amino terminal sequence with two immunologically unrelated M protein molecules. J. Immunol. **124**:261–267.
21. **Manjula, B. N., and V. A. Fischetti.** 1980. Tropomyosin-like seven residue periodicity in three immunologically distinct streptococcal M proteins and its implications for the antiphagocytic property of the molecule. J. Exp. Med. **151**:695–708.
22. **Polly, S. M., R. H. Waldman, P. High, M. K. Wittner, A. Dorfman, and E. N. Fox.** 1975. Protective studies with a group A streptococcal M protein vaccine. II. Challenge of volunteers after local immunization in the upper respiratory tract. J. Infect. Dis. **131**:217–224.
23. **Potter, E. V., M. Svartman, I. Mohammed, R. Cox, T. Poon-King, and D. P. Earle.** 1978. Tropical acute rheumatic fever and associated streptococcal infections compared with acute glomerulonephritis. J. Pediatr. **92**:325–333.
24. **Potter, E. V., M. Svartman, T. Poon-King, and D. P. Earle.** 1977. The families of patients with acute rheumatic fever or glomerulonephritis in Trinidad. Am. J. Epidemiol. **106**:130–138.
25. **Rammelkamp, C. H., F. W. Denny, and L. W. Wannamaker.** 1952. Studies on the epidemiology of rheumatic fever in the armed services, p. 72–89. *In* L. Thomas (ed.), Rheumatic fever. University of Minnesota Press, Minneapolis.
26. **Russel, H., R. R. Facklam, and L. R. Edwards.** 1976. Enzyme-linked immunosorbent assay for streptococcal M protein antibodies. J. Clin. Microbiol. **3**:501–505.
27. **Seyer, J. M., A. H. Kang, and E. H. Beachey.** 1980. Primary structural similarities between types 5 and 24 M

proteins of *Streptococcus pyogenes*. Biochem. Biophys. Res. Commun. **92**:546–553.
28. **Stollerman, G. H.** 1975. The relative rheumatogenicity of strains of group A streptococci. Mod. Concepts Cardiovasc. Dis. **64**:35–40.
29. **Swift, H. F., A. T. Wilson, and R. C. Lancefield.** 1943. Typing group A hemolytic streptococci by M precipitin reactions in capillary pipettes. J. Exp. Med. **78**:127–133.
30. **Widdowson, J. P., W. R. Maxted, C. M. Notley, and A. M. Pinney.** 1974. The antibody responses in man to infections with different serotypes of group A streptococci. J. Med. Microbiol. **7**:483–496.