Streptococcal M Protein Antibodies After Infection

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Immunity to infection due to the group A hemolytic *Streptococcus* is considered to be specific for each serological type and depends on the production of antibodies to M protein. The time-honored method for detecting and measuring type-specific M protein antibodies has been the bacteriostatic test, which relies on the phenomenon that streptococci are usually killed after phagocytosis (S. Rothbard, J. Exptl. Med. 82: 93, 1945). Since the hemagglutination technique shows promise as a method for detecting and measuring type-specific M antibodies, it is important that it be evaluated critically and compared with the accepted bacteriostatic test.

Comparative studies of this type are extremely difficult to accomplish nowadays because of the widespread use of antibiotics for the treatment of upper respiratory infections of diverse etiology including those due to the group A hemolytic *Streptococcus*. Production of type-specific M antibodies is markedly inhibited by treatment of patients with penicillin. (G. Daikos and L. Weinstein, Proc. Soc. Exptl. Biol. Med. 78:160, 1951). We were fortunate in obtaining acute and convalescent sera (made available through the kindness of F. W. Denny, Department of Pediatrics, University of North Carolina, School of Medicine, Chapel Hill) from young adult male patients with streptococcal infections of the upper respiratory tract due to group A, types 5 or 6; they had not received antibiotics, but bacteriostatic tests and antistreptolysin O titrations had been performed on their sera. (F. W. Denny and L. Thomas, J. Clin. Invest. 32:1085, 1953).

The hemagglutination technique (R. W. Quinn and P. N. Lowry, J. Infect. Diseases 113:33, 1963) consists essentially of mixing appropriate dilutions of the patient's serum with tannic acid-treated chicken erythrocytes which have been sensitized with type-specific M protein of group A streptococci. The presence of type-specific M antibody results in antigen-antibody combination which causes the erythrocytes to agglutinate.

The essential point is well demonstrated in this and other studies (M. C. Holmes, Australian J. Exptl. Biol. Med. Sci. 36:395, 1958; R. W. Quinn and P. N. Lowry, J. Infect. Diseases 113:33, 1963; K. L. Vosti and L. A. Rantz, J. Immunol. 92:183, 1964; E. N. Fox, J. Immunol. 93:826, 1964; T. Nishimura, N. Kumagai, and T. Kobayashi, Japan. Heart J. 6:4, 1965): the hemagglutination test does detect and measure M protein antibodies, and the results are quite similar to those obtained by the bacteriostatic test (Table 1). Hemagglutinin titers for type 5 were consistently higher than those for type 6. This may reflect a difference in patient response to the type-specific M antigen or in antigenicity of the infecting *Streptococcus* species. It should be pointed out that the sera had been stored in the frozen state for about 12 years after the bacteriostatic tests had been completed and before the hemagglutination tests were done. Possibly, this long storage period resulted in lowering the hemagglutinin titers. Tests on fresh sera in our laboratory have consistently shown the hemagglutination test to be very sensitive, detecting M antibodies earlier than the bacteriostatic test and reaching very high titers in some patients.

The finding that hemagglutinin and bacteriostatic titers were still increasing at a time when antistreptolysin O titers were decreasing is consistent with the well-known observation that the appearance of detectable levels of M antibody after streptococcal infections is usually slower than the appearance of antibodies to extracellular products (F. W. Denny, W. D. Perry, and L. W. Wannamaker, J. Clin. Invest. 36:1092, 1957; S. Rothbard et al., Arch. Internal Med. 82:229, 1948; G. Daikos and L. Weinstein, Proc. Soc. Exptl. Biol. Med. 78:160, 1951; R. C. Lancefield, J. Exptl. Med. 110:271, 1959).

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TABLE 1. *Comparison of results of bacteriostatic, hemagglutination, and antistreptolysin O (ASO) tests on sera from patients with group A streptococcal infections*

Patient		Streptococcal group and type	Serum date	Bacteriostatic titers ^a	Hemagglutinin titers ^b	ASO titers in units
No.	Name					
1	Ma.	A 5	3-28-51	10 ⁻⁶	0	50
			5- 9-51	10 ⁻⁶	0	317
			8- 1-51	10 ⁻²	1:1,600	159
2	Bl.	A 5	3-21-51	10 ⁻⁶	0	50
			5- 3-51	10 ⁻⁵	0	250
			7-16-51	10 ⁻⁴	1:200	125
3	Cu.	A 5	3-28-51	10 ⁻⁶	0	50
			4-25-51	10 ⁻⁵	0	159
			8- 1-51	10 ⁻³	1:400	125
4	Da.	A 5	3-15-51	10 ⁻⁶	0	100
			4-12-51	10 ⁻³	1:25	400
			7-16-51	10 ⁻²	1:800	250
5	Er.	A 5	2-10-51	10 ⁻⁵	0	317
			4-10-51	10 ⁻⁴	1:100	1,250
			6-19-51	10 ⁻³	1:400	1,000
6	Sm.	A 5	3-24-51	10 ⁻⁶	0	62.5
			5- 4-51	10 ⁻⁵	0	200
			8- 2-51	10 ⁻²	1:1,600	200
7	Ch.	A 5	3-17-51	10 ⁻⁶	1:25	50
			4-19-51	10 ⁻⁵	1:25	83.3
			7-16-51	10 ⁻³	1:200	83.3
8	Co.	A 6	3-27-51	10 ⁻⁶	0	<50
			3-30-51	10 ⁻³	1:25	<50
			5-21-51	10 ⁻¹	+	62.5
			6-14-51	10 ⁻⁴	1:3,200	125
9	Pe.	A 6	3-28-51	10 ⁻⁶	1:2	83.3
			4-25-51	10 ⁻²	1:2	159
			8- 1-51	10 ⁻²	1:8	159
10	Har.	A 6	3-27-51	10 ⁻⁶	0	125
			4-16-51	10 ⁻⁶	1:2	159
			6-18-51	10 ⁻³	1:2	159
11	Sham.	A 6	2- 6-51	10 ⁻⁶	0	200
			5-29-51	10 ⁻²	1:32	625
12	Hay.	A 6	4-14-51	10 ⁻⁵	1:4	200
			8- 2-51	10 ⁻²	1:8	100

^a The interpretation of the bacteriostatic test is as follows. When it is indicated that the streptococcal culture was diluted to 10⁻⁶, this number of bacteria grew in undiluted serum. F. W. Denny considered an inhibition of two logs to be significant. For example, a reduction of dilutions from 10⁻⁶ to 10⁻⁴, or from 10⁻⁵ to 10⁻³, or anything greater, would be a significant increase in antibodies since the same amount of serum would now inhibit the growth of a much larger number of bacteria.

^b Unless otherwise indicated, all sera were diluted 1:25 prior to being tested. When hemagglutinins were not detected at this dilution, the serum was then used undiluted, followed by doubling dilutions of 1:2, 1:4, etc. Numbers under hemagglutinin titers represent the maximal dilution of patient's serum which would cause hemagglutination.

^c Titer not available.

TABLE 1.—(Continued)

Patient		Streptococcal group and type	Serum date	Bacteriostatic titers ^a	Hemagglutinin titers ^b	ASO titers in units
No.	Name					
13	Shar.	A 6	3-15-51	10 ⁻⁶	0	100
			4- 5-51	10 ⁻³	0	200
			6-21-51	10 ⁻³	1:4	125
14	Ne.	A 6	3-24-51	10 ⁻⁶	0	83.3
			5-19-51	10 ⁻⁵	0	200
			8- 1-51	10 ⁻³	0	159
15	Wa.	A 6	1-31-51	10 ⁻⁶	0	100
			2-23-51	10 ⁻⁴	0	400
			5-16-51	10 ⁻²	1:25	250
16	Mcl.	A 6	3-30-51	10 ⁻⁶	0	100
			4-24-51	10 ⁻³	0	500
			6-19-51	10 ⁻²	1:25	400
17	Ba.	A 5	4- 3-51	10 ⁻⁵	1:25	317