Enhancing Effect of Type Specific Antistreptococcal Antibodies on Emergence of Streptococci Rich in M-Protein.* (31736)

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M-protein, a constituent of the cell walls of group A streptococci, in some unknown way impedes the phagocytic destruction of group A streptococci and in so doing contributes to their virulence. Group A streptococci can be classified into distinct serologic types on the basis of differences in M-protein. These proteins determine the production of protective, homologous antibodies against each type(1). Streptococci rich in M-protein proliferate rapidly in the presence of human polymorphonuclear neutrophils during rotation with freshly drawn lightly heparinized human blood without antibodies to M-protein of homologous type(2). In contrast, in the presence of these antibodies group A streptococci are readily destroyed by human polymorphonuclear neutrophils during rotation with human blood(3,4). Antobodies to M-protein are considered to be the basis of type specific immunity to infections with group A streptococci(5).

The purpose of this communication is to report the observation that the M-protein content of group A streptococci which survived rotation with mixtures of human blood and diluted homologous type specific antistreptococcal rabbit sera was greater, as much as 8-fold, than the M-protein content of streptococci which survived rotation with either human blood alone or mixtures of human blood and diluted heterologous type specific rabbit antistreptococcal sera.

Materials and methods. Group A streptococci: Group A streptococci of the following serologic types were used: type 1 (strains Mary, T1, and K43), type 3, type 12 (strain B225), type 19 (strain J17D), and type 49.

Antisera to streptococci: Antisera to group A streptococci of the above serologic types were obtained from New Zealand red or chinchilla rabbits which had received either repeated intravenous injections of heat-killed streptococci or repeated intracutaneous inoculations of living streptococci. The presence of type specific antistreptococcal antibodies was determined according to the indirect bactericidal test described by Maxted(4). Prior to use in experiments described below, sera containing type specific antistreptococcal antibodies were diluted in 4-fold steps to 1:256.

Rotation of "parent" streptococci with human blood: The details of this procedure have been fully described(6). In brief, streptococci of "parent" culture, i.e., streptococci which had never been rotated with human blood, were inoculated into 10 × 75 mm pyrex tubes containing 2.4 ml of freshly drawn, heparinized human blood without homologous type specific antibodies. The concentration of heparin in blood was 10 units/ml. The tubes containing the mixture of streptococci and blood were sealed with siliconized rubber stoppers and rotated end-over-end at 10 revolutions per minute at 37°C for 3 hours. After one period of 3 hours' rotation, 0.1 ml of the mixture was transferred to 2.4 ml of freshly drawn heparinized human blood and rotation was carried out as described above for a second period of 3 hours. At the end of this period, samples were streaked on 5% rabbit blood agar. Streptococci arising from microorganisms which survived rotation with human blood for 2 periods of 3 hours each will be referred to as "selected."

Rotation of "selected" streptococci with human blood alone or mixtures of human blood and either homologous type specific antistreptococcal rabbit sera, heterologous type specific antistreptococcal rabbit sera, or normal rabbit sera: Approximately 10^3 to 10^4 selected streptococci were inoculated into 10 × 75 mm pyrex tubes containing 0.1 ml samples of dilutions of either homologous or heterologous type specific antistreptococcal

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rabbit sera or of normal rabbit sera. These mixtures were briefly agitated. Similar inocula were placed in empty tubes. A sample (2.4 ml) of freshly drawn, lightly heparinized human blood was added to each tube. The tubes were stoppered and rotated for 3 hours as described above. At the end of this period samples were streaked on 5% rabbit blood agar.

Preparation of extracts of streptococci and semi-quantitative determination of M-protein content: Blood agar plates were streaked with parent streptococci, selected streptococci, or selected streptococci which survived rotation with human blood alone or with either mixtures of human blood and dilutions of homologous or heterologous type specific antistreptococcal rabbit sera, or with mixtures of human blood and normal rabbit serum. From each of these plates many colonies were inoculated into 40 ml samples of Todd-Hewitt broth. Morphologically these colonies were representative of the majority of colonies on the plate from which they were sampled. After 18 hours incubation at 37°C the turbidity of all cultures was measured in a Coleman Jr. spectrophotometer. Only cultures of approximately the same density were centrifuged. Acid extracts of the bacterial sediments were made according to the method of Lancefield(7), brought to equal volume and serially diluted in physiological saline of pH 7.4, containing phosphate buffer.

Four ml of 1% agar gel (Ionagar No. 2) in phosphate buffer, pH 7.4, was placed on a 3″ × 1″ glass slide. A rosette-like pattern, consisting off a central well surrounded at a distance of 7 mm by nine equally spaced peripheral wells, was cut in the layer of agar. Equal volumes of serial 2-fold dilutions of acid extract of a given culture were placed in 8 of the peripheral wells. The central well was filled with homologous, type specific antistreptococcal rabbit serum. Undiluted acid extract of selected streptococci of an heterologous type was placed in the remaining peripheral well as control. The reagents in the wells were permitted to diffuse for several days at 4°C. The agar gel slides were then washed with saline followed by distilled water, dried, and stained with naphthalene black. The greatest dilution of streptococcal acid extract forming a line of precipitate with homologous type specific antibodies was taken at the titre of M-protein in the extract.

Results. Streptococci rich in M-protein and resistant to phagocytic destruction emerged from populations of streptococci poor in M-protein and susceptible to phagocytic destruction during rotation with human blood without homologous type specific antistreptococcal antibodies. Cultures of these selected streptococci contained as much as 8 times more M-protein than parent cultures (Table I). In experiments here reported and in previous experiments(6) further rotation of populations of these selected streptococci with human blood alone did not result in emergence of streptococci any richer in M-protein (Table I). In contrast, when populations of the selected streptococci were rotated with mixtures of human blood and dilutions of homologous type specific antistreptococcal rabbit sera, streptococci emerged which were significantly richer in M-protein. Populations of these streptococci and their progeny contained as much as 8 times more M-protein than the populations of selected streptococci (Table I). Often, the enhancing effect of an homologous type specific antiserum on the emergence of streptococci especially rich in M-protein increased in stepwise fashion as the antiserum was diluted several-fold. Dilution of the antiserum beyond the zone of optimum enhancing effect often resulted in stepwise reduction or loss of enhancing effect. In control experiments, when populations of the selected streptococci were rotated with mixtures of human blood and dilutions of heterologous type specific antistreptococcal rabbit sera or mixtures of human blood and normal rabbit sera, the M-protein content of surviving streptococci was no greater than that of the selected streptococci (Table II).

Discussion. When populations of selected streptococci, *i.e.*, streptococci which survived rotation with human blood, were rotated with mixtures of human blood and certain dilutions of homologous type specific antistreptococcal rabbit sera, streptococci emerged which were significantly richer in M-protein than popu-

TABLE I. M-Protein Content* of Parent and Selected Group A Streptococci, of Selected Streptococci Which Survived Further Rotation with Human Blood, and of Selected Streptococci Which Survived Rotation with Human Blood and Added *Homologous* Type Specific Antistreptococcal Rabbit Sera.

Streptococci	M-protein content of: Parent streptococci	Selected streptococci	Selected streptococci which survived further rotation with human blood	Dilution of homologous, type specific antistreptococcal rabbit sera added to human blood	M-protein content of selected streptococci which survived rotation with human blood and homologous type specific antistreptococcal rabbit sera
Type 1 (Mary)	No precipitate	1:4	1:4	1:1	1:4
				1:4	1:16
				1:16	1:16
				1:64	1:8
				1:256	1:4
Type 1 (T1)	No precipitate	1:8	1:8	1:1	1:32
Type 1 (K43)	1:8	1:8	1:8	1:1	1:8
				1:4	1:8
				1:16	1:8
				1:64	1:8
				1:256	1:32
Type 3	1:1	1:8	1:8	1:1	1:8
				1:4	1:32
				1:16	1:16
				1:64	1:16
				1:256	1:16
Type 12 (B225)	No precipitate	1:4	1:4	1:1	1:4
				1:4	1:8
				1:16	1:32
				1:64	1:16
				1:256	1:4
Type 19 (J17D)	1:1	1:4	1:4	1:1	1:4
				1:4	1:8
				1:16	1:32
				1:64	1:16
				1:256	1:4

* M-protein content is measured as the greatest dilution of M-protein extract precipitating with type specific antiserum.

lations of the microorganisms which survived rotation with human blood alone. The enhancing effect of type specific antiserum on the emergence of streptococci especially rich in M-protein of homologous type was observed in some instances to increase and then decrease in stepwise fashion as the antiserum was diluted. This observation suggests the following: In the presence of a large quantity of antibody to homologous M-protein, all of the streptococci including those especially rich in M-protein are sufficiently opsonized and emergence of the latter fails to occur; in the presence of a lesser quantity of the antibody only streptococci moderately rich in M-protein are sufficiently opsonized, permitting streptococci especially rich in M-protein to emerge; and in the presence of still less of the antibody even streptococci moderately rich in M-protein are insufficiently opsonized and, therefore, streptococci especially rich in M-protein fail to emerge selectively and do not predominate. The emergence of streptococci especially rich in M-protein of a given type is only enhanced by homologous type specific antisera and not by type specific antisera to other serotypes of group A streptococci.

From these observations of an *in vitro* system, several inferences can be made concerning infections of man with group A streptococci. Selection of streptococci containing at least enough M-protein to resist phagocytic destruction may occur in the local inflammatory response following colonization of the human host with a population of group

TABLE II. M-Protein Content* of Parent and Selected Group A Streptococci and of Selected Streptococci Which Survived Rotation with Human Blood and Added *Heterologous* Type Specific Antistreptococcal Rabbit Sera or Added Normal Rabbit Sera.

Streptococci	M-protein content of		Dilution of heterologous type specific antistreptococcal rabbit sera added to human blood	M-protein content of selected streptococci which survived rotation with human blood and	
	Parent streptococci	Selected streptococci		Heterologous type specific antistreptococcal rabbit sera	Normal undiluted rabbit serum
Type 1 (Mary)	No precipitate	1:4	1:1	1:4	1:4
			1:4	"	
			1:16	"	
			1:64	"	
			1:256	"	
Type 3	No precipitate	1:8	1:1	1:8	1:8
			1:4	"	
			1:16	"	
			1:64	"	
			1:256	"	
Type 12 (B225)	No precipitate	1:4	1:1	1:4	1:4
			1:4	"	
			1:16	"	
			1:64	"	
			1:256	"	
Type 19 (J17D)	1:1	1:4	1:1	1:4	1:4
			1:4	"	
			1:16	"	
			1:64	"	
			1:256	"	

* M-protein content is measured as the greatest dilution of M-protein extract precipitating with type specific antiserum.

A streptococci. These selected streptococci then stimulate the host to produce type specific antibodies to streptococcal M-protein. When these antibodies rise to a certain titre they may enhance the emergence of microorganisms especially rich in M-protein from the population of infecting streptococci. Thus, type specific antibodies may be responsible for a second phase of selection of virulent streptococci. The consequences of this second phase of selection may be several. Streptococci surviving it may prolong the infection until the titre of type specific antibodies is high enough to eliminate even these streptococci. The emergence of streptococci especially rich in M-protein shortly after the host has begun to form antistreptococcal antibodies may result in a secondary immunologic stimulus to the host. This secondary stimulus may contribute to the persistent nature of the immune response to streptococcal M-protein(5). In some hosts it may also contribute to the development of non-suppurative sequelae of streptococcal infection. Finally, streptococci which have survived in one human host in the presence of type specific antibodies and which are especially rich in M-protein may have acquired increased capacity to infect other human hosts.

Summary. Streptococci rich in M-protein emerged by natural selection from populations of group A streptococci poor in M-protein during rotation with human blood without homologous type specific antistreptococcal antibodies. When populations of these selected streptococci were then rotated with mixtures of human blood and diluted homologous type specific antistreptococcal rabbit sera, the M-protein content of surviving streptococci was greater, as much as 8-fold, than that of streptococci which survived rotation with human blood alone. In control experiments, when populations of the selected streptococci were rotated with mixtures of human blood and diluted heterologous type

specific antistreptococcal rabbit sera, the M-protein content of surviving streptococci was no greater than that of streptococci which survived rotation with human blood alone.

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