PHAGOCYTOSIS IN SUBACUTE BACTERIAL ENDOCARDITIS

Localization of the Primary Opsonic Site to Fc Fragment*

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Previous studies have indicated that immune γG-opsonins constitute the main molecular class of antibody involved in phagocytosis and killing of infecting organisms in subacute bacterial endocarditis of man (1–3). High titers of γG-opsonins were documented among patients infected with a wide variety of Gram-positive bacterial species, including *Streptococcus viridans*, *Microaerophilic streptococcus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Diplococcus pneumoniae* (1). Moreover, antiopsonic effect was consistently noted when isolated human 19S anti-γ-globulin factors or rabbit antisera specific for γG were added to phagocytic systems (2). In vitro competition between heat-labile serum factors and human anti-γ-globulin factors could be readily demonstrated. Since anti-γ-globulin factors as well as the complement component sequence show primary reactivity for structures present on the Fc portion of γG, it seemed important to explore the role of the Fc region of γ-globulin in phagocytosis. An attempt was therefore made to examine the specific contribution of Fc structures in conferring specific opsonic activity to 7S γ-globulin molecules. The effects of enzymatic digestion, mild reduction with mercaptoethanol, or treatment of γG-opsonin with the oxidizing agent sodium metaperiodate were studied. It appeared that several procedures capable of degrading or altering sites on the Fc portion of 7S γ-globulin abolished opsonic activity.

Materials and Methods

Opsonins.—In most instances, patients with active bacterial endocarditis furnished the specific immune opsonins. Because only certain strains of bacteria showed a high degree of reproducibility and consistency in the phagocytosis system, all of the experiments utilized

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bacterial strains and isolated γG-opsonin from eight patients infected with organisms growing well in pour plates. These organisms and characteristics of the human antibacterial antibodies are given in Table I.

γ-Globulin possessing strong opsonic properties was isolated from immune serum by DEAE-cellulose chromatography using pH 8.1, 0.02 M phosphate buffers as eluting agent (4, 5). In some instances, 7S fractions from 10–40% sucrose gradient ultracentrifugal separations of 0.3-ml serum samples were used as source of opsonin. Sera were inactivated at 56°C for 30 min before separation on sucrose gradients.

Bacteria.—Seven of the eight bacterial strains studied in phagocytosis experiments were derived from blood cultures of patients with bacterial endocarditis and were studied with

TABLE I

Infecting Bacterial Species and Characterization of Antibacterial Antibodies in Eight Patients with Bacterial Endocarditis

Name of patient	Type of organism	Agglutinating antibacterial antibody		Complement-fixing antibacterial antibody		Opsonic activity*	
		7S‡	19S‡	7S‡	19S‡	7S‡	19S‡
Shr	*Staph. aureus*	16	0	64	0	+	0
Bec	*Staph. aureus*	128	4	16	0	+	0
Fos	*Staph. aureus*	64	16	4	0	+	0
Har	*Staph. epidermidis*	16	8	16	4	+	0
Flu	*Staph. aureus*	128	8	32	4	+	0
Red	*Str. viridans*	32	8	AC§	AC§	+	0
Fre‖	*S. marcescens*	32	8	16	4	+	0
Ell	*Staph. epidermidis*	32	4	8	0	+	0

* Opsonic activity was defined as positive if greater than 90% of bacteria incubated were phagocytized and killed at 120 min incubation.

‡ 7S and 19S fractions of sera separated by preparative ultracentrifugation in a 10–40% sucrose gradient at 35,000 rpm for 16 hr.

§ Whole serum and separated fractions anticomplementary in CF tests.

‖ This patient had chronic septicemia from an infected arteriovenous cannula.

their own matching immune opsonin. In one instance, a *Serratia marcescens* was studied to represent an example of at least one chronic human Gram-negative septic condition. This patient had an infected arteriovenous cannula being used for chronic hemodialysis.

Phagocytic System.—The quantitative phagocytosis system of Maaløe (6) as modified by Hirsch and Strauss (7) was used. Details of the procedure have been previously outlined (1–3). The phagocytic cells used in all instances were polymorphonuclear leukocytes from normal healthy donors. No mandatory requirement for C'1 complement component was apparent in any of the phagocytic systems used in these studies. However, the participation of trace amounts of complement adherent to the washed polymorphonuclear leukocytes used cannot be excluded and has been previously examined (1, 2).

Procedures Specifically Designed to Alter γG-Opsonins.—Enzymatic digestion of γG-opsonins by pepsin was carried out at pH 4.1 using 7S γG-opsonin obtained from individual sera by DEAE-cellulose chromatography and equilibrated by dialysis with pH 4.1, 0.1 M acetate buffer. An enzyme to protein ratio of 1:100 was used. Digestion with pepsin was carried out

at 37°C for ½, 4, 8, and 16 hr, and terminated by dialysis against several changes of pH 7.4, 0.1 M phosphate buffer. Monitoring of pepsin digestion employed titration of persistent Gm(a) and/or Gm(b) antigenic activity after various periods of digestion. Since both Gm(a) and Gm(b) sites are located on Fc fragment (8–10), progressive digestion of the latter by pepsin could be most sensitively monitored using residual Gm activity as a marker.

In addition to enzymatic digestion, mercaptoethanol reduction of isolated 7S γG-opsonins was accomplished using 0.01 or 0.1 M mercaptoethanol for 1 hr at 4°C. Blocking with a molar excess of iodoacetamide was subsequently immediately done and the preparations dialyzed for 12 hr against three changes of Hank's balanced salt solution. Control observations included incubation of human polymorphonuclear leukocytes with mercaptoethanol-reduced γ-globulin preparations followed by assay of leukocyte phagocytic and bactericidal capacity with untreated opsonin. Mild reduction of opsonins was studied in conjunction with complement-fixing antibody to specific infecting bacteria since previous studies by several groups (11–12) had shown loss of complement-fixing activity after disulfide bond reduction of 7S γ-globulins.

Periodate alteration of γG-opsonins followed the procedure outlined by Andersen and coworkers (13) using 0.01 M periodate for periods of 1 hr and 5 hr in the dark followed by sodium arsenite. All periodate-treated opsonins were dialyzed for 24 hr against balanced Hank's solution before use. The physical and immunochemical effects of periodate treatment were monitored by amino acid analysis, analytic ultracentrifugation, and optical rotatory dispersion. In addition, testing for stability of Gm(a), Gm(b), and Gm(f) (14, 15) was used as a measure of the effect of sodium metaperiodate treatment of human γG-globulins.

Comparative Opsonic Properties of Human 11S Colostrum.—As a contrast to the situation with 7S γG where both complement interaction and antiopsonic effect of rheumatoid factors seemed to involve structures of Fc(2), the opsonic properties of normal human 11S colostrum were studied. This was done in an attempt to dissect out the contributions of complement-fixing sites on immune opsonins from those not necessarily involving complement. Thus, 11S colostrum would provide a source of antibacterial antibody but presumably possessing distinct structural differences from those on the Fc portion of γG. Such differences or others intrinsic to γA-antibodies have been presumed to be responsible for lack of γA-complement fixation (16–18).

Colostrum from 10 puerperal women was pooled and the 11S γA separated as described by Tomasi et al. (19). Final preparative sucrose gradient ultracentrifugation or Sephadex G-200 gel filtration resulted in an initially homogeneous 11S preparation. Subsequent analytical ultracentrifugal analysis revealed formation of 20–40% higher polymeric forms (15–18S). Degree of preparative purification of colostrum is shown in Fig. 1. Starting and final 11S preparations are shown on immunoelectrophoresis against anti–whole human serum. Of interest in this regard was the presence of β_1C-globulin complement component in the whole but not in the pure preparation.

Agglutinating antibody to bacterial antigens was assayed in pooled normal human colostrum by agglutination of Rh-negative human erythrocytes coated with clear supernatant from an overnight bacterial culture. Bacteria were killed by heating for 1 hr at 70°C and separated from culture supernatant by ultracentrifugation for 30 min at 20,000 rpm. In certain instances bacterial polysaccharide antigens were used to coat erythrocytes. Representative titers of 11S colostral agglutinating antibody to various bacterial antigens are shown in Table II. In addition, no complement fixation was demonstrated between bacterial antigens tested and the 11S γA-colostral preparation.

RESULTS

11S-Colostral γA.—No opsonic activity was demonstrable in purified human colostral 11S γA when tested at a concentration range between 2.0 mg/cc and

0.02 mg/cc with seven different organisms including both Gram-positive and Gram-negative species (Table II).

Enzymatic Digestion.—Pepsin digestion of 7S γG-opsonins revealed distinct loss of opsonic capacity as Fc antigenic structures were lost. Complete digestion of opsonin to 5S $F(ab')^2$ fragment resulted in disappearance of opsonic proper-

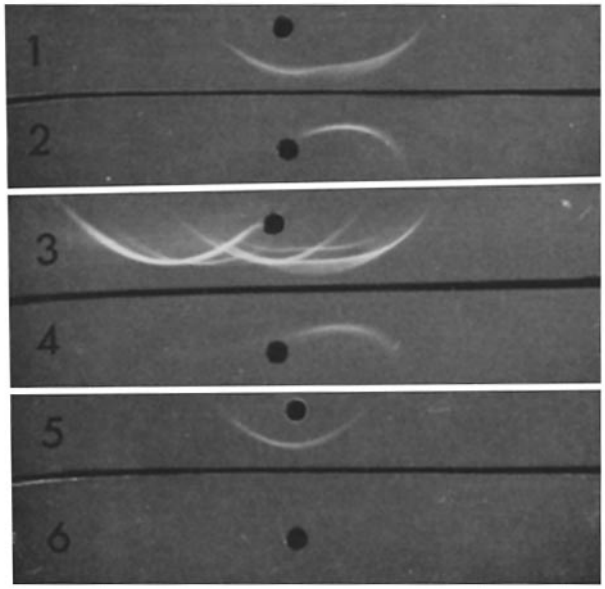

FIG. 1 *a*. Shows whole colostrum, 1, and isolated 11S-colostral γA, 2, on immunoelectrophoresis against anti-γA-antiserum (trough). The limited mobility of 11S-colostral γA is apparent. Anode is to the left, cathode to the right.

FIG. 1 *b*. Shows whole colostrum, 3, and 11S-colostral γA, 4, developed with antiserum to whole human serum. Similar patterns were obtained with several rabbit antisera to whole human colostrum. Only one line was noted with the isolated 11S γA-preparation, 4.

FIG. 1 *c*. Again shows whole colostrum above, 5, and 11S-colostral γA below, 6, developed with antiserum to β_{1C}-globulin component of human complement. The whole colostrum, 5, shows easily detectable β_{1C}-globulin whereas this is absent in the purified 11S γA-preparation, 6.

ties (Fig. 2). Moreover, in timed digestion experiments proceeding over ½, 4, 8, and 12 hr, opsonic capacities were reduced in a general parallel fashion to loss of Gm(a) or Gm(b) antigens. In this regard, loss of Gm(b) was most closely related to loss of opsonic capacity. These results are shown in Fig. 3. Opsonin of high titer was also available from a patient with chronic *Serratia marcescens* septicemia. In this instance as well, progressive pepsin digestion as monitored by loss of Gm(a) and Gm(b) γG-antigens was accompanied by stepwise reduction in opsonic power (Fig. 3). Control experiments were done using normal

polymorphonuclear leukocytes incubated with pepsin-digested γG-preparations, and subsequently assayed for phagocytic capacity using immune untreated γG-opsonin and bacteria. No decrease was noted in leukocyte phagocytic or bactericidal capacity after such treatment. Pepsin-digested γ-globulin controls showed no killing of bacteria.

Despite loss of opsonic activity after pepsin digestion, 5S pepsin fragments of antibacterial antibody could be shown to combine directly with bacteria

TABLE II

Agglutinating Antibody Titers in 11S γA from Pooled Normal Colostrum Against a Panel of Bacterial Antigens as Measured by Agglutination of Human Rh-Negative Cells Coated with Bacterial Culture Supernates or Isolated Bacterial Antigens

Source of bacterial antigen	Log_2 dilution* of agglutination titer in 11S colostral γA	Log_2 dilution mean and range of agglutination in 10 normal control sera	
		Mean	Range
E. coli (126)	8	8.0	3–12
E. coli (026)	0	2.3	1–5
E. coli (0111)	2	3.4	2–6
E. coli (K-12)‡	2‡	2.9	2–5
S. marcescens‡	3‡	4.8	3–7
Salmonella minnesota	0	3.8	2–5
Salmonella typhimurium	0	2.3	1–5
Pseudomonas aeruginosa	0	2.2	1–3
Klebsiella‡	5‡	2.0	1–4
Str. viridans	1	2.2	1–3
Staph. aureus 1‡	0‡	6.8	3–10
Staph. aureus 2‡	3‡	5.8	3–9
Staph. aureus 3‡	2‡	5.0	2–8
Pneumococcus XIV	2	2.0	0–4
Pneumococcus VI	2	4.8	2–7
Paracolon‡	0‡	4.1	3–5

* Starting dilution was 1:4.

‡ These organisms tested with 11S-colostral preparation for opsonic activity.

using bacterial agglutination reactions, washing, and subsequent Coombs' tests with rabbit anti-5S pepsin fragment antisera previously absorbed with bacteria. These results can be seen in Table III.

Since important sites for complement fixation appear to reside on Fc structures (20, 21), it seemed of interest to study the effect of adding labile serum factors—presumably complement—to specific pepsin-digested opsonin. Fresh normal human serum was titered to a dilution so as not to show any direct opsonic effect in the test system. When this dilution of fresh normal serum was added to 5S pepsin fragment of specific opsonin, no potentiation was noted.

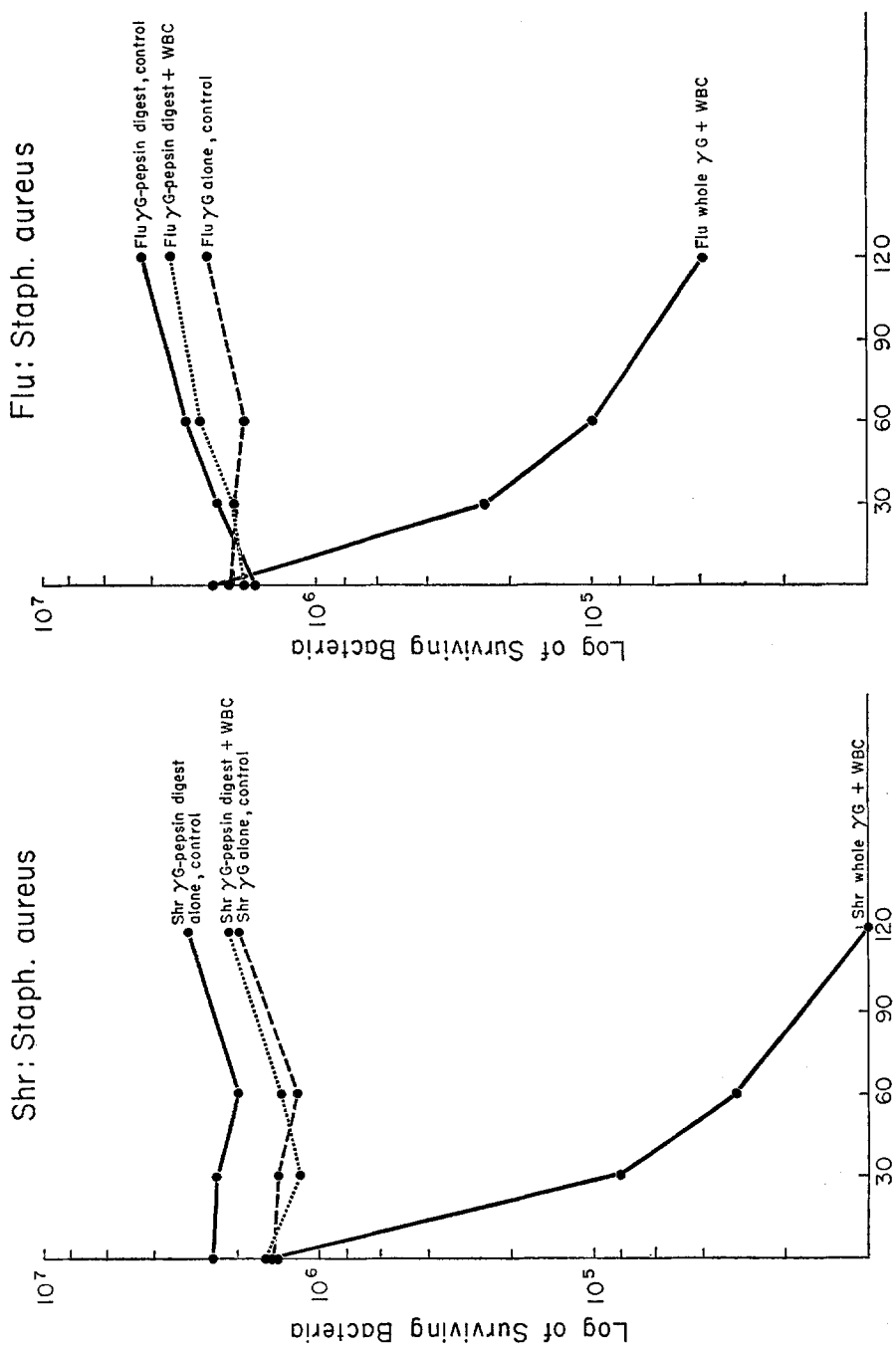

FIG. 2. Phagocytic curves of two γG-opsonins before and after pepsin digestion of γG-opsonin. On the left is shown the opsonic curve for γG-opsonin Shr (Shr whole γG + white blood cells [WBC]) and the curve using pepsin-digested Shr opsonin (Shr γG-pepsin digest + WBC). The marked loss of opsonic activity is apparent after pepsin digestion. Control curves for whole γG-opsonin pepsin digest alone show no lytic or bactericidal activity. On the right, marked loss of opsonic activity is apparent after pepsin digestion of γG-opsonin Flu. Designation of curves is the same as that for the experiment shown to the left. In both experiments, whole γG and pepsin-digests were adjusted to 1.5 mg/cc protein concentration. However, in other experiments when a range of concentrations of both pepsin-digested and whole γG were studied, no opsonic effect of pepsin digests could be shown when as much as 80 times the concentration of pepsin-digested γ-globulin opsonin (4.0 mg/cc) was used in comparison with distinct opsonic activity of whole γG at 0.05 mg/cc.

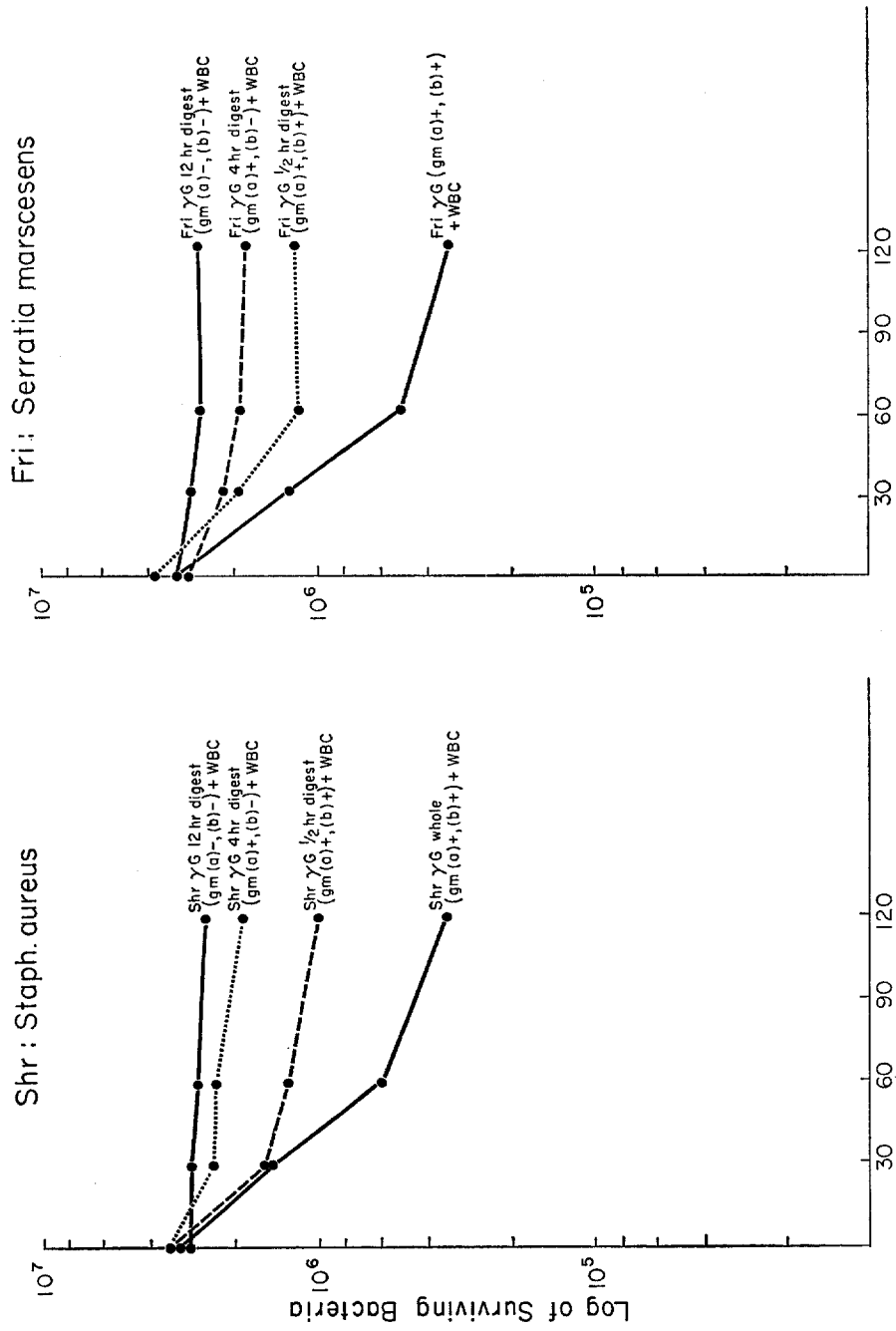

FIG. 3. Results of timed pepsin digestion experiments with two γG-opsonins—Shr γG (*Staph. aureus*) and Fri γG (*S. marcesens*). Opsonins were digested with pepsin at pH 4.1 for ½ hr., 4 hr, and 12 hr and pepsin activity halted by adjusting pH to 7.4 and equilibrating with balanced Hanks' solution. In the experiment shown on the left, γG-opsonin Shr Gm a(+), b(+) can be seen progressively to lose opsonic activity after ½, 4, and 12 hr of pepsin digestion. The same result is noted with the Gm a(+), b(+) γG-opsonin for the Gram-negative *Serratia marcescens* organism studied in the experiment shown on the right. In both instances, Gm (a) antigenicity is not completely lost until 12 hr of pepsin digestion, whereas Gm (b) activity is lost after 4 hr of pepsin digestion. This discrepancy may be related to differences in absolute relative numbers of γG-molecules bearing these determinants but was used as a serologic guide to monitor pepsin digestion. All γG-preparations used in these experiments were adjusted to 1.0 mg/ml protein concentration.

Loss of opsonic activity previously demonstrated when 7S opsonin interacts with 19S rheumatoid factors or 7S rabbit anti-γG-antibody (2), as well as abolition of opsonic property following pepsin digestion of γG, strongly implicated Fc structures as being intimately involved with the property which makes an antibody an opsonin. Because of the recent demonstration that cytophilic 7S γ-globulins apparently bind to macrophages through specific Fc receptor sites (22, 23), a series of experiments were done to determine whether similar specific opsonic sites existed on human polymorphonuclear leukocytes.

Attempts were made to inhibit specific γG-opsonin using 3.5S fragments obtained after papain digestion of Cohn Fraction II, and separation of Fab and

TABLE III

Comparative Bacterial Agglutinating, Complement-fixing, Opsonic Activity, and Gm Typing of Shr γG after Pepsin Digestion or Mild Reduction with Mercaptoethanol

Preparation tested	Bacterial agglutinating titer	Complement-fixing titer	Opsonic activity*	Results of Gm(a) and (b) testing
Shr whole serum	64	32	+	Gm(a)+ (b)+
Shr-isolated γG‡	64	16	+	Gm(a)+ (b)+
Shr 5S pepsin-digested γG‡	64	0	0	Gm(a)− (b)−
Shr γG reduced with 0.01 M mercaptoethanol‡	32	4	0	Gm(a)+ (b)−
Shr γG reduced with 0.1 M mercaptoethanol‡	32	0	0	Gm(a)+ (b)−

* Opsonic activity was defined as positive if greater than 90% of bacteria incubated were phagocytized and killed at 120 min of incubation.

‡ All isolated γG-preparations adjusted to 3 mg/ml before testing in bacterial agglutination and complement fixation with bacteria; Gm testing started with γG-preparation at 1 mg/ml.

Fc fragments by starch block electrophoresis. In a second set of experiments, specific Fab and Fc fragments of individual immune γG-opsonins were studied. Polymorphonuclear leukocytes were incubated with Fc or Fab fractions before addition to the phagocytic test system containing bacteria and whole 7S opsonin. These experiments were designed to block specific opsonic Fc receptor sites, if present, on the polymorphonuclear leukocytes. In neither type of experiment (Fig. 4) was inhibition or a distinct antiopsonic effect of Fc fragment demonstrable. Thus, pepsin digestion of opsonin Fc destroyed its property of facilitating phagocytosis, however, isolated opsonin Fc alone could not block nor inhibit the opsonic phagocytic mechanism for polymorphonuclear leukocytes.

Mercaptoethanol Treatment.—When stepwise reduction of isolated γG-opsonin was accomplished using 0.01 M and separately 0.1 M mercaptoethanol, progressive loss of opsonic capacity was noted (Fig. 5). Control observations indicated

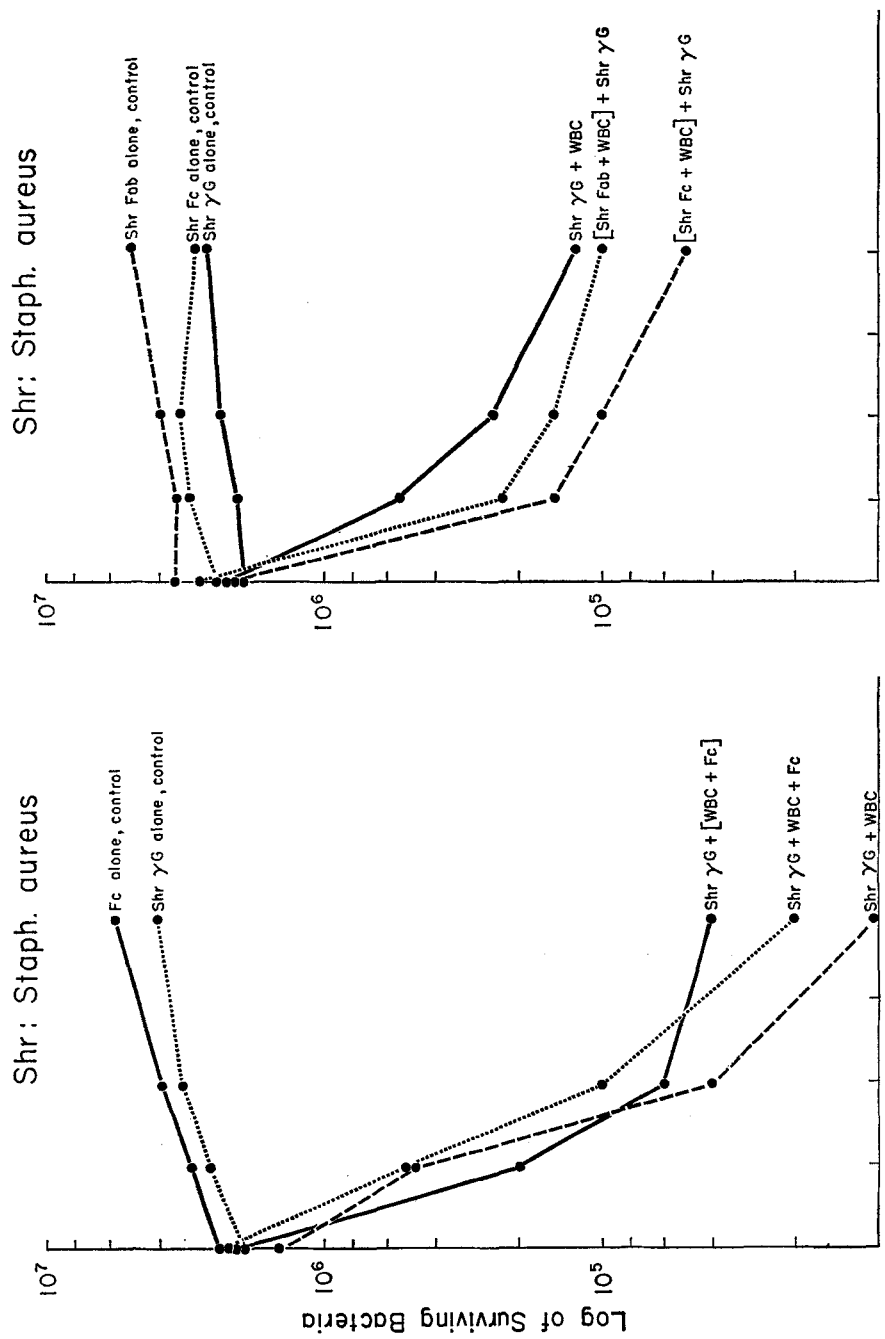

FIG. 4. These experiments were undertaken to attempt to block possible opsonic receptor sites on polymorphonuclear leukocytes by using preparations of isolated Fc fragment. On the left are charted results of opsonic curves using Fc fragment prepared from Cohn Fr II. In the curve labeled Shr γG + [WBC + Fc], leukocytes were preincubated with Fc before being added to the opsonic system using whole Shr γG-opsonin. In the curve labeled Shr γG + WBC + Fc no preincubation with leukocytes was performed. In the curves shown on the right, Fc and Fab prepared from the specific Shr γG-opsonin were preincubated with leukocytes before addition to the phagocytic system. In all instances, Fc and Fab were used at concentrations between 0.3-0.5 mg/cc. It can be seen that no inhibition of opsonic activity could be attributed to Fc from Cohn Fr II or from specific opsonin Shr. Control curves indicated that Fc, Fab, and Shr γG alone did not affect bacterial counts.

that incubation with mercaptoethanol-reduced γ-globulin preparations produced no major deterrent effect on phagocytic or bactericidal function of

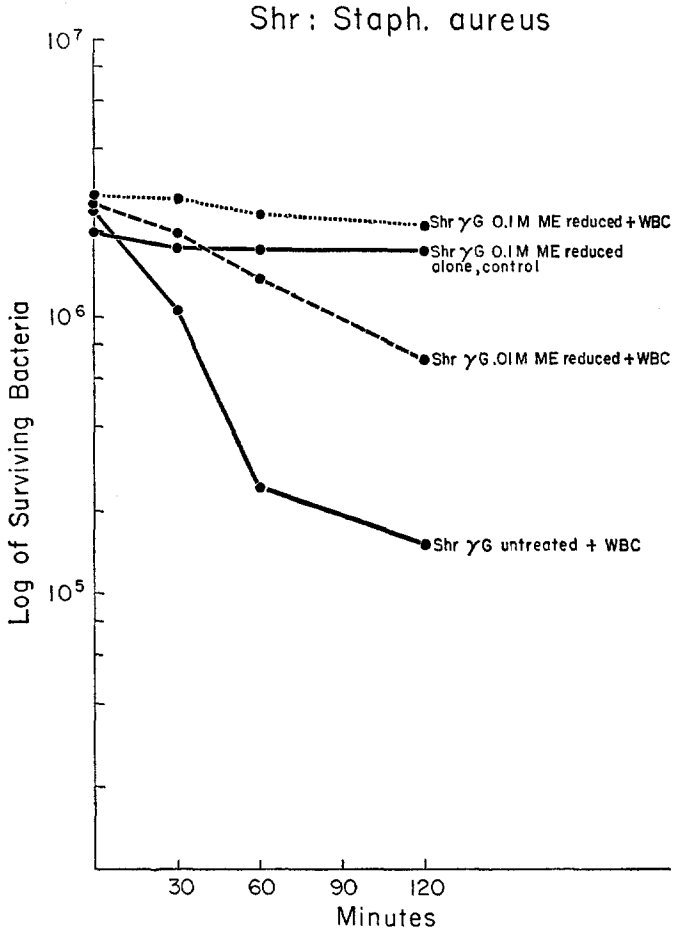

FIG. 5. These opsonic curves indicate partial abolition of Shr γG-opsonic capacity after mild reduction with 0.01 M mercaptoethanol (Shr γG 0.01 M ME reduced + WBC) and complete loss of opsonic activity after reduction with 0.1 M mercaptoethanol (Shr γG 0.1 M ME reduced + WBC). Preservation of Gm(a) antigenicity but loss of Gm(b) activity as well as complement-fixing ability were noted after these reductive procedures (Table III). In the opsonic curves shown in Fig. 5 the Shr-reduced γG was used at 1.0 mg/cc and the mercaptoethanol-treated γG at 1.5 mg/cc. Opsonic activity could be demonstrated in untreated Shr γG at 0.05 mg/cc but none in the 0.1 M-reduced materials in concentrations up to 3.0 mg/cc.

polymorphonuclear leukocytes. Alkylation alone without prior reduction of γG-opsonin did not alter its opsonic properties. Immunoelectrophoresis against rabbit antisera specific for Fc fragment showed no gross alteration after this

treatment; testing for residual Gm antigens revealed preservation of Gm(a) but loss of Gm(b) after these mild reduction procedures. In addition, a direct parallel was noted between loss of opsonic activity after mercaptoethanol reduction and ability to fix complement with the various infecting bacteria. No

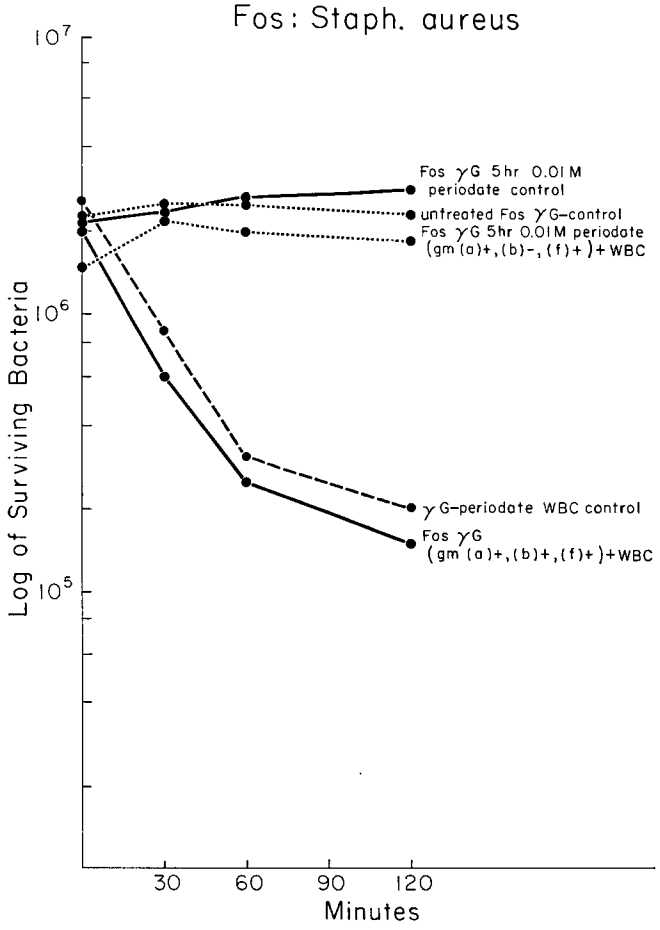

FIG. 6. These phagocytic curves indicate abrupt loss of opsonic activity after treatment of γG–Fos opsonin for 5 hr with 0.01 M metaperiodate. Coincident with loss of opsonic activity, the periodate-treated γG-preparation lost antigenicity for Gm(b) systems, though Gm(a) and Gm(f) were preserved. The curve labeled γG-periodate WBC control indicates that when periodate-treated γG was preincubated with WBC, the mixture washed, and the WBC subsequently used with untreated opsonin, no intrinsic adverse effect of periodate-treated γG on polymorphonuclear leukocytes was detected. In these experiments untreated Fos γG was used at 1.0 mg/cc and opsonic activity could be demonstrated in Fos γG through dilutions containing 0.1 mg/cc; the periodate-treated Fos γG shown in the figure was 1.5 mg/cc. No opsonic activity was present in periodate-treated Fos γG at concentrations up to 3.0 mg/cc.

marked reduction in antibacterial agglutinating antibody was noted. Representative findings are summarized in Table III.

Periodate Treatment of Opsonin.—The experiments with sodium metaperiodate were designed with the intent to oxidize carbohydrate moieties most of

TABLE IV

Comparative Effects of Inhibition of Gm Systems after Treatment of Isolated γG-Opsonins with Sodium Metaperiodate 0.01 M

Opsonin preparation tested	Log_2 dilution of inhibition*		
	Gm(a) system	Gm(b) system	Gm(f) system
Har γG, untreated	9*	8	10
Har γG, 0.01 M periodate for 1 hr	8	0	8
Har γG, 0.01 M periodate for 5 hr	8	0	8
Fos γG, untreated	7	6	9
Fos γG, 0.01 M periodate for 5 hr	6	0	8

* All γG-preparations tested in doubling dilutions; first dilution of log_2 1 was 1 mg/ml in all instances.

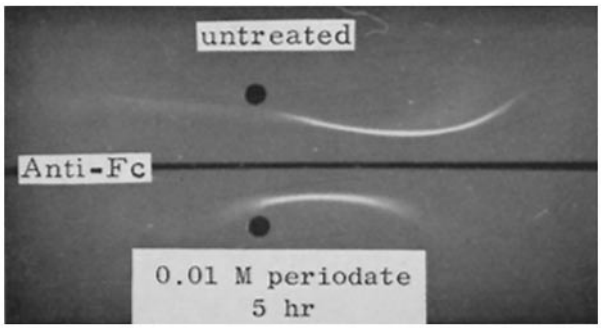

FIG. 7. Immunoelectrophoresis showing γG-opsonin Fos before and after treatment with 0.01 M sodium metaperiodate for 5 hr in the dark. Central trough contains rabbit antiserum specific for determinants on human Fc. Anode is to left, cathode to right. A distinct increase is apparent in electrophoretic mobility after periodate treatment.

which appear to reside on the Fc portion of γ-globulin (24, 25). It was recognized that oxidation of particularly susceptible amino acids might also be produced or that this reagent might produce other structural changes in the protein not related to any preferential oxidation of carbohydrate units.

Treatment of 10 mg of γG-opsonin (2.0 mg/cc) with 0.01 M sodium metaperiodate for 5 hr in the dark completely abolished opsonic activity (Fig. 6). Also even short treatment (0.01 M periodate for 1 hr) destroyed Gm(b) antigenic activity (Table IV). In addition to the effects of opsonic activity and Gm(b)

antigens, a shift in electrophoretic mobility was noted after treatment of γG with the periodate reagent (Fig. 7). Amino acid analysis of two γ-globulin preparations before and after 5 hr of 0.01 M metaperiodate indicated minor but significant losses of several amino acids including serine, glutamic acid, phenylalanine, and histidine. Examination in the analytic ultracentrifuge showed a slight increase in sedimentation coefficient from 6.8 to 7.2 $s_{20,w\infty}$ after periodate treatment. Optical rotatory dispersion measurements in the Cary 60 spectropolarimeter indicated slight but consistent decreases in negative specific rotation particularly below $[\alpha]_{365m\mu}$ (Table V).

TABLE V

Recorded Changes in Optical Rotatory Dispersion $[\alpha]_D$ of 7S γ-globulin before and after Treatment with 0.01 M Sodium Metaperiodate in the Dark at 4°C

Sample studied*	[α]589 mμ	[α]436 mμ	[α]384 mμ	[α]365 mμ	[α]284 mμ	[α]245 mμ	[α]234 mμ
Untreated γ-globulin in phosphate buffer pH 7.4 0.1 M	−42.0	−96.0	−123.0	−142.0	−384.0	−800.0	−1220.0
γ-globulin after 1 hr periodate treatment	−40.0	−85.0	−123.0	−130.0	−334.0	−700.0	−1000.0
γ-globulin after 5 hr periodate treatment	−42.0	−85.0	−115.0	−122.0	−334.0	−700.0	−1130.0

* All samples studied at same protein concentration (60 mg/100 ml in 0.1 M phosphate buffer, pH 7.4).

DISCUSSION

The results presented here add further weight to the concept that the opsonic property of immune γG resides on the Fc portion of 7S γ-globulin. Furthermore, a close relationship between the opsonic γG-site and that involved in complement fixation was apparent. As supporting evidence, the following observations can be cited.

1. γG-opsonic activity is inhibited in phagocytic systems to which human 19S anti-γ-globulin factors or heterologous rabbit anti-γ-globulin antisera are added (2). Both these reagents show primary specificity for antigenic determinants present on Fc fragment (26, 27).

2. In vitro competition for bacteria preincubated with opsonin can be demonstrated between heat-labile serum components and isolated human anti-γ-globulin factors (1, 2). Since both rheumatoid factors and complement components can be shown to compete for reactive sites on antibodies opsonizing bacteria, it would appear that their attachment sites on immune complexes may be very similar or closely adjacent.

3. Pepsin digestion of γG-immune opsonin abolishes opsonic activity, though residual 5S pepsin fragments can be shown to combine with respective bacteria. Furthermore, progressive pepsin digestion of opsonin Fc shows gradual loss of residual Gm(b) activity. This could be shown to parallel quantitative loss of opsonic property.

4. 11S colostral γA-antibacterial antibody obtained from pooled normal colostrum shows no in vitro opsonic property. In addition, this molecular class of antibacterial antibody also does not fix complement and presumably possesses H chain structures distinct from Fc of γG (17).

5. Periodate treatment of whole γG-opsonin effectively obliterated its opsonic properties. Such treatment destroyed the Gm(b) Fc antigen though Gm(a) and Gm(f) remained intact. The majority of carbohydrate of γG being located on Fc fragment (24, 25), this further supports the importance of integral Fc structure to opsonic property, although some alteration of amino acid composition, optical rotatory dispersion, and sedimentation coefficient was also induced by this treatment.

6. Mild reduction of whole γG–immune opsonin by mercaptoethanol abolished opsonic properties in parallel with ability of the reduced antibody to fix complement with bacteria. In this instance, Gm(a) antigenicity, resident on Fc fragment, remained intact after reduction as did agglutinating antibody for bacteria. Of interest was the partial loss of Gm(b) after this treatment. Thus, ability to fix complement was destroyed by the reducing conditions applied, but interaction with anti-γ-globulin factors, as measured in Gm(a) reactivity, was not affected.

These lines of evidence support the idea that phagocytizing cells interact with an opsonic site available after bacteria have combined with specific antibody. It was for this reason that attempts were made to inhibit phagocytosis of γG-opsonized bacteria by preincubating polymorphonuclear leukocytes with isolated Fc derived from the specific immune opsonin being tested. The recent report by Lo Buglio and coworkers (23) indicates that specific attachment sites for Fc of γG are present on macrophages participating in phagocytosis. It was considered that a similar phenomenon is involved with phagocytizing polymorphonuclear leukocytes. The absence of specific Fc inhibition of phagocytic mechanisms in our system still does not rule out this possibility. It might well be that the logistics of interaction require far more dense packing of Fc sites in conjunction with polymorphonuclears than we were able to muster in the experiments undertaken. Still another possibility is that the Fc-opsonic site requires conformational stabilization only conferred upon it by the stability of the whole γG-molecule. It may also be that receptor sites on polymorphonuclear leukocytes and macrophages are qualitatively different. Other methods capable of inducing only minor or subtle conformational changes particularly in the Fc region are needed before this question can be finally answered.

There is considerable additional evidence that the Fc portion of antibody interacts with macrophages in phagocytic systems. In line with experiments reported here, pepsin digestion of γG was noted by Berken and Benacerraf (22) to destroy the ability of cytophilic antibody to interact with macrophage receptors. Recently, Rabinovitch (28) has shown that pepsin digestion of 7S rabbit γ-globulin opsonin markedly diminished phagocytosis in a system employing macrophages and glutaraldehyde-treated red cells. These findings lend further support to the notion that the opsonic site resides on Fc.

The experiments relating to attempts to reconstitute opsonic activity by adding fresh heat-labile serum factors to pepsin-digested opsonin are worthy of some comment. Previous studies have shown (29, 30) that certain levels of complement fixation are demonstrable on the 5S pepsin fragment of specific antibody. However, in our system it appeared that opsonic and complement-fixing activity were destroyed by pepsin digestion procedures employed. An interesting parallel observation was the lack of opsonic property in 11S colostral γA which contained antibacterial antibodies but did not fix complement.

Finally, the results of periodate treatment of specific opsonin indicate that opsonic activity was lost in parallel with some alteration on Fc as manifested by loss of Gm(b) activity. The studies by Andersen et al. (13) have indicated considerable loss of γG-carbohydrate after such treatment of rabbit γ-globulin, as well as some alterations in amino acid composition and sedimentation behavior, are induced by such periodate treatment. Our observations confirm this and indicate that slight changes in optical rotatary dispersion as well as selective loss of Gm(b) antigenicity occurred after periodate treatment of γ-globulin opsonins. Of interest in this regard was the marked fall in PCA reactivity noted by Andersen et al. after periodate treatment of rabbit antibodies (13). The primary functions now ascribed to structures residing on Fc have in several instances been related to tissue binding or cytophilic antibody function. This was clearly described by Berken and Benacerraf (22). Similar functions of binding to tissue in PCA reactions (31) or mechanisms of active placental transfer of γG-antibodies (32, 33) have been shown to reside on Fc fragment. It is of interest that many of these physiological functions are similar in that they seem to involve reaction of Fc structures with fixed or circulating tissue receptors, the exact nature of which is as yet not known. Current studies can now be directed at mechanisms of binding of cytophilic, opsonic, PCA, or placentally transferred γG-antibody to tissue receptor sites.

SUMMARY

The opsonic properties of immune γG-globulins isolated from patients with chronic septicemic conditions, principally subacute bacterial endocarditis were studied. Opsonic capacity as well as complement-fixing properties of γ-globulins appeared to be closely associated with integrity of Fc structures. Progressive pepsin digestion of immune γG-globulins, as monitored by successive loss of

Gm(a) and Gm(b) antigens, abolished opsonic activity. Colostral γA, containing agglutinating antibacterial antibodies but no demonstrable complement-fixing activity, was devoid of opsonic capacity. Reduction of γ-globulin opsonins with 0.01 or 0.1 M mercaptoethanol progressively abolished opsonic activity in parallel with loss of ability of treated γ-globulins to fix complement with bacteria. Treatment of γ-globulin opsonins with 0.01 M sodium metaperiodate also produced complete loss of opsonic capacity in parallel with loss of Gm(b) Fc antigens. These findings, together with antiopsonic effects demonstrable with anti-γ-globulin factors showing primary reactivity with Fc structures, indicate that the opsonic property of immune γ-globulins requires the participation of structures integral to the Fc region of γ-globulin.

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