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Participation of Normal Human Immunoglobulins M, G, and A in Opsonophagocytosis and Intracellular Killing of *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* by Human Polymorphonuclear Leukocytes

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Restoration of the ability of hypogammaglobulinemic serum to support opsonophagocytosis and intracellular killing of *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* by human polymorphonuclear luekocytes was achieved by supplementation with normal human immunoglobulin M, but not with normal human immunoglobulin G. Polymorphonuclear leukocyte bactericidal activity in the presence of immunoglobulin A-deficient human serum was equivalent to that observed in the presence of normal human serum.

During the last decade, the clinical aspects of infections caused by members of the *Bacteroidaceae* have been studied extensively. *Bacteroides* has been shown to be the predominant genus of the intestinal anaerobic flora isolated in pure or mixed culture from clinical specimens (19), and *B. fragilis* has been shown to be the most commonly isolated species (6, 7, 13, 21). Despite the increased awareness of the importance of *Bacteroides* in infectious processes, minimal information is available regarding host defense mechanisms against these microorganisms.

In our previous studies, clinical isolates of *B. fragilis* and *B. thetaiotaomicron* were shown to be killed by human polymorphonuclear leukocytes (PMNs) in vitro in the presence of normal human serum, but not by either PMNs or serum alone (1). Subsequent studies demonstrated that the normal human serum factors which facilitated opsonophagocytosis and intracellular killing of the *Bacteroides* strains were immunoglobulin and components of the alternative complement pathway (2, 3). The purpose of the present investigation was to determine the participation of normal human immunoglobulin M (IgM), IgG, and IgA in opsonophagocytosis and killing of the *Bacteroides* strains by human PMNs.

IgG and IgM were purified from a pool of 25 normal adult sera by chromatography on Sephadex G-200 (10). Fractions containing IgM were chromatographed a second time over the G-200 column. IgG fractions were further chromatographed on diethylaminoethyl-cellulose (25). IgG and IgM fractions were concentrated by ultrafiltration and dialyzed against 0.01 M sodium phosphate-buffered saline, pH 7.0. By radial immunodiffusion (18), the IgM preparation contained 128 mg of IgM per 100 ml, 18 mg of IgA per 100 ml, and undetectable IgG. The IgG preparation contained 2,080 mg of IgG per 100 ml, and undetectable IgA and IgM. Neither immunoglobulin preparation contained detectable C1q, C4, C2, C3, C5, properdin, factor B, β1H, or C3b inactivator. Total protein in the IgM and IgG preparations, as determined by the method of Lowry et al. (17), was 462 mg/100 ml and 2,504 mg/100 ml, respectively. The immunoglobulin preparations were stored in small portions at −70°C and were centrifuged at 20,000 × *g* to remove aggregates before use.

Pooled normal human serum, hypogammaglobulinemic serum, and IgA-deficient human serum were divided into small portions which were frozen at −70°C. By radial immunodiffusion (18), hypogammaglobulinemic serum contained <100 mg of IgG per 100 ml and undetectable IgA and IgM. IgA-deficient human serum contained 1,250 mg of IgG per 100 ml, 105 mg of IgM per 100 ml, and undetectable IgA. Total hemolytic complement (15), C3 conversion by inulin and cobra venom factor (22), and immunochemical concentrations of C1q, C4, C2, C3, C5, properdin, factor B, β1H, and C3b inactivator (18) in the immunoglobulin-deficient sera and pooled normal human serum were found to be equivalent.

PMN bactericidal activity was measured under anaerobic conditions as previously described (1-3). PMNs were prepared by dextran sedimentation of heparinized whole blood from healthy adult volunteers followed by hypotonic lysis to

remove contaminating erythrocytes (16). The leukocytes were washed twice and resuspended to a final concentration of 1.0×10^7 PMNs/ml in deoxygenated Hanks balanced salt solution (Microbiological Associates Inc., Walkersville, Md.) containing 0.1% gelatin (HBG). *B. fragilis* 1365 and *B. thetaiotaomicron* 1343 were maintained in thioglycollate medium (BBL Microbiology Systems, Cockeysville, Md.) at −70°C. Before each experiment, a frozen culture was thawed and inoculated into a tube containing 10 ml of broth; the broth consisted of equal parts of Trypticase soy broth (BBL Microbiology Systems) and brain heart infusion broth (Difco Laboratories, Detroit, Mich.) supplemented with 0.05% thioglycollate (BBL Microbiology Systems). The tube was incubated at 37°C for 18 h in an anaerobic glove box (Coy Manufacturing Products, Inc., Ann Arbor, Mich.), which was monitored to contain 85% nitrogen, 10% hydrogen, 5% carbon dioxide, and not over 25 ppm of oxygen. The bacteria were washed twice and resuspended to a final concentration of 1.0×10^7 cells per ml in deoxygenated HBG. Combinations of 1.0×10^6 bacteria, 5.0×10^6 normal human PMNs, 10% of the test serum, 10% of the purified immunoglobulin preparation, and deoxygenated HBG were added to 12- by 75-mm plastic capped tubes (Falcon Plastics, Cockeysville, Md.) in a final volume of 1 ml. Deoxygenated HBG was substituted for the PMNs, serum, or immunoglobulin preparation in the controls. The tubes were rotated in the glove box for 3 h at 37°C, and 100-μl samples were removed at zero time and after 30, 90, and 180 min of incubation. The samples were diluted in deoxygenated distilled water to rupture the leukocytes, and serial 10-fold dilutions were plated by the pour plate method in agar; the agar consisted of equal parts of Trypticase soy agar and brain heart infusion agar (Difco Laboratories) supplemented with 0.5% yeast extract (BBL Microbiology Systems) and 0.05% thioglycollate. The plates were incubated anaerobically for 4 days, and the colonies were enumerated to determine the total number of surviving bacteria. In some experiments, the number of extracellular bacteria was determined by plating the supernatants of additional 100-μl samples after centrifugation at $200 \times g$.

To determine the participation of normal human IgM and IgG in opsonophagocytosis and intracellular killing of *B. fragilis* and *B. thetaiotaomicron*, PMN bactericidal activity was measured in the presence of hypogammaglobulinemic serum supplemented with increasing concentrations of purified normal human IgM or IgG. Dose-dependent restoration of the ability of hypogammaglobulinemic serum to support opsonophagocytosis and intracellular killing of both *Bacteroides* strains was achieved by supplementation with normal IgM (Fig. 1). PMN bactericidal activity was not demonstrated in the presence of a physiological concentration of normal IgM alone, indicating that IgM acted with additional normal serum factors to facilitate opsonophagocytosis and intracellular killing. Extracellular bacterial counts were found to be equivalent to total bacterial counts in reaction mixtures containing pooled normal human serum, hypogammaglobulinemic serum, or hypogammaglobulinemic serum supplemented with a physiological concentration of IgM, suggesting that restoration of PMN bactericidal activity was related to an effect of the serum factors on opsonophagocytosis rather than intracellular killing.

PMN bactericidal activity in the presence of

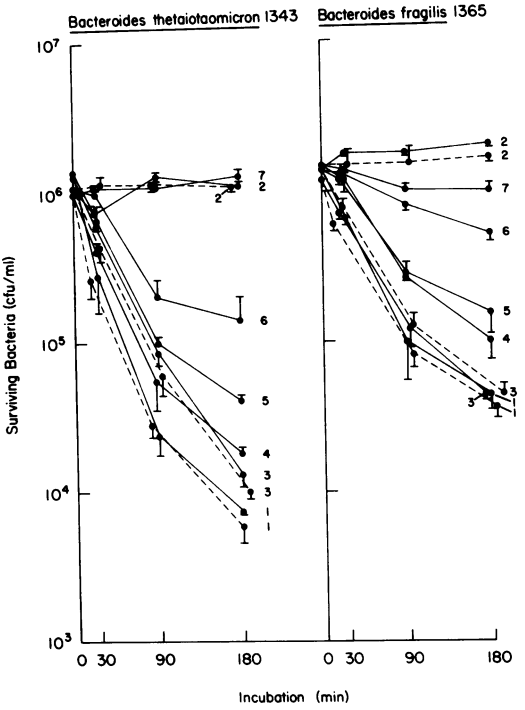

FIG. 1. *Participation of normal human IgM in opsonophagocytosis and killing of B. thetaiotaomicron 1343 and B. fragilis 1365 by human PMNs. Reaction mixtures consisted of bacteria, PMNs, diluent, and the following reactants: 1, pooled normal human serum serum; 2, hypogammaglobulinemic serum (HS); 3, HS and 128 μg of IgM; 4, HS and 64 μg of IgM; 5, HS and 32 μg of IgM; 6, HS and 16 μg of IgM; and 7, 128 μg of IgM. The solid lines represent total surviving bacteria, and the dotted lines represent surviving extracellular bacteria. The points represent mean values of duplicate determinations, and each vertical bar represents the standard error of the mean.*

normal IgG alone or hypogammaglobulinemic serum supplemented with normal IgG was equivalent to that observed in the presence of unsupplemented hypogammaglobulinemic serum (Fig. 2). The failure of normal IgG to restore opsonophagocytosis and intracellular killing was not related to aggregation of the immunoglobulin preparation as evidenced by the inability of normal IgG to consume complement in pooled normal human serum and thus reduce its opsonophagocytosis-promoting activity. Extracellular and total bacterial counts were found to be equivalent in reaction mixtures containing hypogammaglobulinemic serum supplemented with a physiological concentration of IgG, suggesting that IgG was unable to restore opsonophagocytosis of the bacteria rather than intracellular killing.

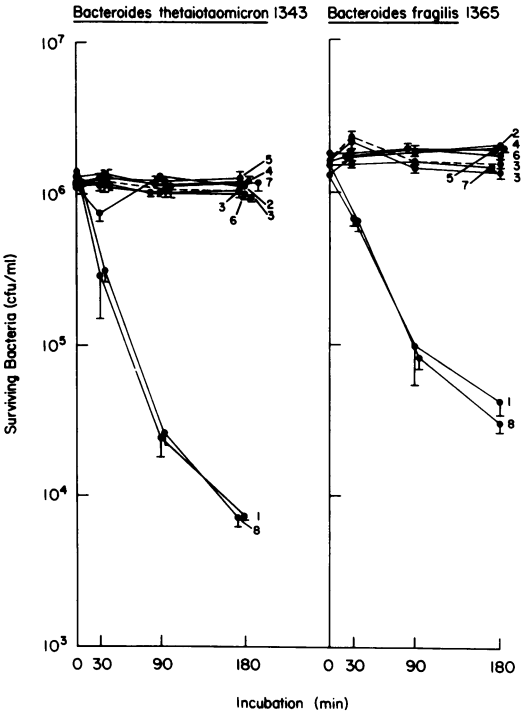

FIG. 2. *Participation of normal human IgG in opsonophagocytosis and killing of B. thetaiotaomicron 1343 and B. fragilis 1365 by human PMNs. Reaction mixtures consisted of bacteria, PMNs, diluent, and the following reactants: 1, pooled normal serum (PNHS); 2, hypogammaglobulinemic serum (HS); 3, HS and 2,080 μg of IgG; 4, HS and 1,040 μg of IgG; 5, HS and 520 μg of IgG; 6, HS and 260 μg of IgG; 7, 2,080 μg of IgG; and 8, PNHS and 2,080 μg of IgG. The solid lines represent total surviving bacteria, and the dotted lines represent surviving extracellular bacteria. The points represent mean values of duplicate determinations, and each vertical bar represents the standard error of the mean.*

The ability of IgA-deficient human serum to support opsonophagocytosis and intracellular killing of *B. fragilis* and *B. thetaiotaomicron* was determined next. PMN bactericidal activity in the presence of IgA-deficient serum was found to be equivalent to that observed in the presence of pooled normal serum (Fig. 3). These results suggested that serum IgA did not participate in opsonophagocytosis and intracellular killing of the *Bacteroides* strains.

Participation of IgM and components of the alternative complement pathway in opsonophagocytosis of bacteria by leukocytes has not been previously reported. IgG and alternative complement pathway activity in normal sera have been shown to facilitate opsonophagocytosis of *Pseudomonas aeruginosa* (4, 5), *Streptococcus pneumoniae* (26), and *Neisseria gonorrhoeae* (23). In addition, a requirement for IgG for alternative complement pathway-me-

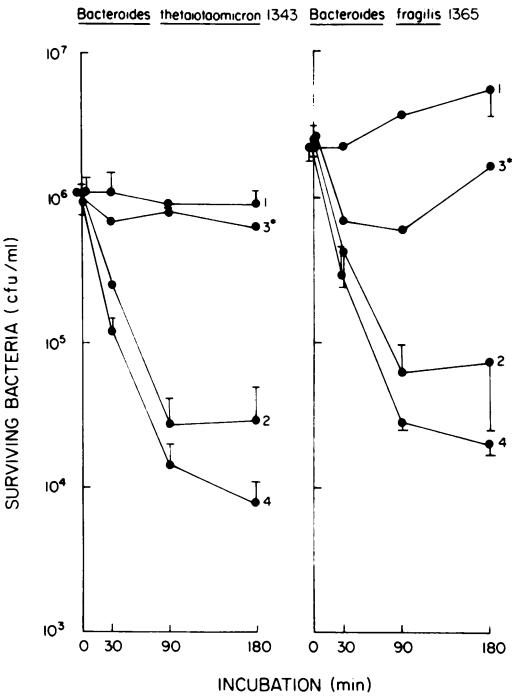

FIG. 3. *Participation of serum IgA in opsonophagocytosis and killing of B. thetaiotaomicron 1343 and B. fragilis 1365 by human PMNs. Reaction mixtures consisted of bacteria, diluent, and the following reactants: 1, PMNs; 2, pooled normal human serum and PMNs; 3, IgA-deficient human serum; and 4, IgA-deficient human serum and PMNs. Mean values of total surviving bacteria obtained from duplicate determinations are presented except for those designated by an asterisk which were obtained from a single determination. The vertical bars represent the standard error of the mean.*

diated lysis of measles virus-infected cells has been reported (8, 14, 24). Immunoglobulin and alternative complement pathway activity have also been shown to facilitate bacteriolysis of *Escherichia coli* (11) and *N. gonorrhoeae* (12) and cytotoxicity against *Trypanosoma rhodesiense* (9); however, the classes of immunoglobulin which participated in these events were not determined.

The only previous investigation regarding a comparison of the ability of various classes of immunoglobulin to facilitate an alternative complement pathway-mediated event has been reported by Polhill et al (20). Restoration of the ability of hypogammaglobulinemic sera to support optimal lysis of rabbit erythrocytes was accomplished by supplementation with normal human IgM, IgG, colostral IgA, an eluate from serum-sensitized rabbit erythrocytes, and the Fab'_2 fragment of IgG. The investigators concluded that "natural" antibodies of the IgM, IgG, and IgA classes exerted a rate-limiting effect on alternative complement pathway-mediated lysis of rabbit erythrocytes.

The reason why normal human IgM, and not IgG or IgA, acted synergistically with components of the alternative complement pathway to facilitate opsonophagocytosis of *B. fragilis* and *B. thetaiotaomicron* by human PMNs is uncertain. The most probable explanation for this observation is that antibody activity in normal human serum is required for the opsonization or ingestion process, and the antibodies belong to the IgM class.

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