The bactericidal capacity of mononuclear and polymorphonuclear phagocytes obtained from normal newborn infants and from healthy adults was evaluated in vitro, using two group B beta-hemolytic streptococci (GBBHS) serotypes (GBBHS-IIa-SS-615/28 and GBBHS-III-SS-620/50) and uniform opsonic conditions. No intertype differences in bacteriolysis of these two serotypes were observed among leukocytes from newborns or adults. As a group, only polymorphonuclear phagocytes from newborns disclosed a significantly lower mean bactericidal capacity than their adult cellular counterpart, and only with respect to GBBHS-III-SS-620/50. On the other hand, 4 of 16 polymorphonuclear samples from newborns tested revealed significantly low bactericidal capacities against both GBBHS serotypes, and an additional sample revealed a bactericidal capacity against GBBHS-III-SS-620/50 alone. Interstrain variates in the intrinsic bactericidal capacity of polymorphonuclear phagocytes from newborns against GBBHS-III may exist, as suggested by a single observation made by using four clinical isolates of GBBHS-III. Such deviant phagocytic capacities of polymorphonuclear phagocytes from newborns may constitute an additional selective risk factor in the genesis of GBBHS sepsis of the newborn.

Group B beta-hemolytic streptococci (GBBHS) have become a leading cause of serious infection in the neonatal period (11). In most cases, the newborn acquires the microorganism from the maternal genital tract, and as many as 70% of the offspring of mothers colonized with GBBHS harbor GBBHS at birth (2). However, only about 1% of known colonized infants will develop serious GBBHS disease (12). This low colonization-to-disease ratio suggests that a combination of bacterial virulence factors and altered neonatal defense mechanisms have to converge to culminate in a clinical case of GBBHS disease. The inculcation of maternal GBBHS colonization (19) and other features of the microorganism (10, 16, 21) have been proposed as bacterial virulence determinants. On the part of the host, the most important resistance factor appears to be the presence of adequate titers of transplacentally acquired anti-GBBHS opsonic antibody in the newborn (3). Prospective studies to identify the precise attack rate for GBBHS disease among infants lacking such antibody have not been performed, and it is possible that other host factors, such as the intrinsic phagocytic capability of leukocytes from newborns, may also influence the pathogenesis of these infections (5).

The unusual virulence of the GBBHS serotype GBBHS-III in human newborns contrasts with its harmlessness when injected into 21-day-old mice, in which sepsis can be easily induced by using other GBBHS serotypes (14). This striking resistance to GBBHS-III is due to an intrinsic ability of the murine phagocytic system to clear this serotype even in the absence of specific antibody or complement (13, 25). It is not known whether such variations according to serotype in the phagocytosis of GBBHS exist in the human newborn. Phagocytosis of newborn leukocytes, although generally regarded as normal (15), may be so under less-than-optimal in vitro conditions (17). This can add to the physiological immunodeficiency of the newborn; this, however, does not readily explain the low colonization-to-disease ratios observed in the case of GBBHS, since it affects all neonates. However, individual variations in phagocytosis could exist and could constitute a risk factor for GBBHS disease. Therefore, we evaluated the intrinsic phagocytic capacity of mononuclear phagocytes (MP) and polymorphonuclear phag-
ocytes (PMN) of normal newborn infants against two representative GBBHS serotypes (Ia and III) in search of intertype variations in phagocytic capacity. In three instances, phagocytes from newborns were also tested against four clinical isolates of GBBHS-III for possible interstrain variations in phagocytosis.

MATERIALS AND METHODS

Preparation of bacteria. Prototype strains GBBHS-Ia-SS-615 and GBBHS-III-SS-620 were originally obtained from the Centers for Disease Control (Atlanta, Ga.) through the courtesy of Hazel Wilkinson. These strains were passaged in mice 28 and 50 times, respectively, by the method of Lancefield (14) and were designated GBBHS-Ia-SS-615/28 and GBBHS-III-SS-620/50, respectively (kindly provided by S. P. Gotoff, Chicago, Ill.). GBBHS-III strains HGM-1, HGM-2, HGM-3, and HGM-4 are clinical isolates from four newborns with meningitis (generously supplied by E. Calderón, Hospital General de México, Mexico City). Samples of overnight cultures of these bacteria on tryptic soy–5% defibrinated sheep blood agar (SBA) were inoculated into 100 ml of modified Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) and grown overnight for 12 h. This modified medium was prepared by increasing eightfold the concentrations of Na<sub>2</sub>HPO<sub>4</sub> and dextrose for adequate buffering and capsular polysaccharide production, respectively (3, 4). A 3-ml sample of these cultures was placed in 27 ml of modified Todd-Hewitt broth and cultured for an additional 5 h at 37°C to midlog phase and an optical density of 0.70 at 550 nm (Spectronic 20 spectrophotometer, Bausch & Lomb, Rochester, N.Y.), which corresponds to 1 x 10<sup>8</sup> to 5 x 10<sup>8</sup> bacteria per ml as confirmed by spread plating on SBA plates incubated for 24 h at 37°C. Samples in (2 ml) 80% glycerol of these midlog-phase bacteria were placed in screw-cap glass tubes, frozen in an acetone-dry ice bath, and stored at −70°C until used. When used, samples were rapidly thawed in a 56°C water bath and immediately placed on ice. The bacterial suspensions were washed twice in phosphate-buffered saline (PBS) (pH 7.4) to remove the glycerol and were resuspended in PBS to a concentration of 1 x 10<sup>9</sup> to 5 x 10<sup>9</sup>. Viability and concentration of the microorganisms in colony-forming units per ml were determined in each experiment by spread plating on SBA plates. Variations in bacterial concentration and viability throughout the experiment were negligible.

Collection and preparation of leukocytes. Amounts (30 to 50 ml) of heparinized venous blood were drawn from the placentas of 19 normal, full-term deliveries within 5 min of birth and from 10 healthy, fasting adult donors. The 19 newborns included in this study remained healthy at discharge and at 1 month of age. Half of each sample was processed for MP preparation by the gradient centrifugation technique of Böyum (7). Samples of 5 ml were layered over a 3-ml mixture of 24 parts of 9% Ficoll (Pharmacia Fine Chemicals, Uppsala, Sweden) and 16 parts of 33% Hypaque (Winthrop Laboratories, New York, N.Y.) in polystyrene tubes (17 by 100 mm). After centrifugation at 400 x g for 40 min at 20°C, the interface was removed, washed twice in Hank's balanced salt solution (pH 7.4) with 0.1% gelatin (Hanks; Difco), and suspended in Hanks-G–50% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.) at a concentration of 1 x 10<sup>5</sup> to 2 x 10<sup>6</sup> mononuclear cells per ml; 30 to 40% of the cells were MP and the rest lymphocytes, as ascertained microscopically. The other half of the blood sample was used for PMN isolation by a modification of the method of Quie (20). Blood was mixed in equal parts with Dextran (molecular weight 173,000; Sigma Chemical Co., St. Louis, Mo.) in 5% glucose–PBS. Samples (7 ml) of this mixture were allowed to sediment in Falcon tubes (16 by 125 mm) for 45 min at 37°C. The supernatant cell-rich plasma was washed twice at 400 x g for 10 min at 20°C with Hanks-G, and the cell suspension was placed on the same Ficoll–Hypaque gradient described above. After centrifugation at 400 x g for 40 min at 20°C, the cell button was removed and washed twice with Hanks-G at 400 x g for 10 min at 20°C, and the cells were resuspended in Hanks-G with 50% fetal calf serum at a concentration of 0.5 x 10<sup>9</sup> to 1.0 x 10<sup>9</sup> leukocytes per ml, PMN comprising more than 95% of them. The viabilities of MP and PMN were 95 to 98% as determined by trypan blue dye exclusion.

Bactericidal assay. The bactericidal capacities of MP and PMN were simultaneously assayed in duplicate by a modification of the methods of Alexander et al. (1) and Steigbigel et al. (24), in which interference with bacterial growth by phagocytic cells is measured. A mixture containing 0.2 ml of the bacterial suspension, 0.1 ml of 50% single-batch fresh human AB Rh+ serum (from a pool of eight healthy adult donors, stored in 1-ml portions at −70°C until used) in Hanks-G, and 0.2 ml of phagocytic cells was placed in polyethylene tubes (12 by 75 mm). The bacteria-to-phagocyte ratio ranged from 5:1 to 10:1 as confirmed by spread plating performed for each experiment. Control tubes without phagocytes or without bacteria were run simultaneously to ascertain bacterial growth and detect extraneous bacterial contamination. Both leukocyte types from each individual were incubated with GBS-Ia-SS-615/28 and GBS-III-620/50 in each experiment. In three sets of leukocytes from newborns, both cell types were tested simultaneously against GBBHS-III-620/50 and the four clinical GBBHS-III isolates. All tubes were rotated at 10 rpm end-over-end at 37°C for 120 min in a multipurpose tube rotor (Scientific Industries, Springfield, Ill.). At 120 min a 0.1-ml sample was removed from each tube and placed in 4.9 ml of sterile distilled water. Tubes were shaken for 45 s at high speed to lyse leukocytes and disperse bacteria. Tenfold dilutions were made in PBS, and 0.1-ml samples were poured plated in triplicate onto SBA plates. The colony-forming units formed after 24 h of incubation at 37°C were counted and averaged, and the results were expressed by the following bactericidal index (BI):

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BI = \frac{\text{colony-forming units in suspensions of cells and bacteria}}{\text{colony-forming units in suspensions of bacteria alone in the same period (120 min)}}
\]
This index compares the number of viable bacteria cultured in the presence of phagocytic cells with the number of viable bacteria cultured alone, and thus measures both the intracellular and extracellular bactericidal activity of leukocytes (24).

Statistical analysis of results. Nonparametric statistics were determined by using the Mann-Whitney U test for analysis by ranks and the Moses tests for extreme reactions (23).

RESULTS

Individual and mean BIs of the eight experimental groups are shown in Fig. 1. By rank analysis, the BIs of MP or PMN from newborns against GBBHS types Ia-SS-615/28 and III-SS-620/50 were comparable. Likewise, no differences were found when BIs of MP or PMN from adults against the two GBBHS serotypes were compared. There were no differences in BIs against either GBBHS type when MP from newborns were compared with MP from adults. On the other hand, when PMN from newborns were compared with PMN from adults, a small difference (P < 0.05) was found in their bactericidal capacities against GBBHS-III-SS-620/50, but not against GBBHS-Ia-SS-615/28. A comparison of the BIs of MP with those of PMN from individuals within the same age group and against the same bacterial serotype revealed that only MP from adults had lower BIs than did PMN from adults (P < 0.025), and then only against GBBHS-Ia-SS-615/28.

Considerable scatter of BI values was found, especially in the group of PMN from newborns, in which 4 of the 16 samples were found to have BIs at or below an arbitrary level of 40% against both GBBHS serotypes, and an additional sample of PMN from a newborn showed bactericidal activity only against GBBHS-III-SS-620/50. The Moses test showed these extreme reactions to be significantly deviant at a P < 0.05 level. Such low BIs were not observed among phagocytes from adults, and the BIs below 40% found among MP from newborns were not significant.

Two of the three sets of leukocytes from newborns that were tested simultaneously against the GBBHS-III prototype and the four clinical GBBHS-III isolates disclosed no differences in their BIs, and these were comparable to those shown in Fig. 1. In contrast, the third set of leukocytes from newborns, while revealing normal BIs by their MP, disclosed low BIs by their PMN against the prototype GBBHS-III-SS-620/50 and against one of the clinical strains (GBBHS-III-HGM-1) but disclosed normal BIs against the remaining three clinical isolates (Table 1).

DISCUSSION

A human equivalent to the striking intertype difference in intrinsic phagocytosis of GBBHS observed in rodents (13) was not found in our survey. When comparing the bactericidal performance of phagocytes from newborns with

![Fig. 1. Individual and mean BIs of MP and PMN from newborns and adults against GBBHS. $\bar{x}$, Mean; S.E.M., standard error of the mean.](http://iai.asm.org/)
that of cells from adults, we found that only PMN from newborns had, as a group, lower mean BIs against GBS-III-SS-620/50 than those of their counterparts from adults. This is an observation of questionable clinical significance because of the magnitude of the difference (8). This overall normal intrinsic phagocytic ability of newborn leukocytes against GBBHS types Ia and III is, then, in keeping with reports of normal neonatal intrinsic phagocytosis at a variety of microorganisms, provided adequate opsonic conditions and bacteria-to-cell ratios are employed (15). Shigeoka et al. found normal neonatal PMN phagocytosis against GBBHS-III by using radiolabeled bacterial uptake and chemiluminescence production assays, but 3 of the 16 infants included in their study disclosed a significant diminution in chemiluminescence production (22). A relatively large number (25%) of PMN samples from healthy newborns in our study also revealed BIs at or below 40% against the two GBBHS serotypes tested. Since opsonic conditions and bacteria-to-phagocyte ratios were kept constant throughout the experiment, this observation suggests that there are significant individual variations in the intrinsic phagocytic ability of PMN of apparently healthy newborns, and this in turn could constitute an additional selective risk factor in GBBHS disease in newborns (5).

The question of interstrain variation in intrinsic GBS bactericidal capacity of phagocytes from newborns was partially explored in our study. PMN of one of three healthy newborns revealed BIs at or below 40% against two of the five GBBHS-III strains used in the assay. This observation needs further exploration, but it suggests that interstrain GBBHS-III variations in bacterial killing by PMN from newborns may exist. Interstrain differences in opsonic requirements cannot explain this finding, since BIs in the other two infants were normal, including those for the two strains in question, and the BIs by MP against all GBBHS-III strains tested were uniformly normal in all three infants. Moreover, variations in opsonic requirements by different strains within a single GBBHS serotype have been found by some authors (21) but have been disclaimed by others (9). Should such variations exist, they could represent an additional factor in determining the phagocytic rate against a particular invading strain.

Isolated low BIs are not infrequently recorded in bactericidal studies (18, 24), but their biological significance has not been adequately explored. Attachment, ingestion, and killing may be equally affected in such abnormal cases. Our results do not allow a distinction between these steps, but it has been suggested that the attachment capacity of phagocytes constitutes the principal limiting factor of phagocytosis, and that ingestion and killing proceed at a constant rate proportional to the number of bacteria attached (6). With borderline opsonic conditions, or when an overload of microorganisms invades the newborn, such phagocytic defects may be critical in the genesis of neonatal GBBHS sepsis.

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LITERATURE CITED


