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Experimental Pneumococcal Meningitis: Role of Leukocytes in Pathogenesis

JOEL D. ERNST,[1] JEAN MARIE DECAZES,[2] AND MERLE A. SANDE[1]*

Medical Service, San Francisco General Hospital Medical Center, and Department of Medicine, University of California, San Francisco, California 94110,[1] and l'Hopital Claude Bernard, Paris, France[2]

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Two groups of rabbits with experimental meningitis induced by direct intracisternal inoculation of *Streptococcus pneumoniae* cells were studied. One group was rendered profoundly leukopenic by nitrogen mustard, and the other had normal leukocyte counts. The two groups had comparable bacterial growth rates (mean generation time, 60 versus 67 min) and ultimate bacterial populations in the cerebrospinal fluid (CSF) (mean $\log_{10}$ CFU, 9.1 versus 8.7); therefore leukocytes did not effectively slow or limit the growth of penumococci in the CSF in vivo. Increased CSF protein, decreased CSF glucose, and increased CSF lactate levels were similar in both groups, suggesting that leukocytes are not essential for these changes to occur. Quantitative blood cultures revealed identical levels of pneumococcal bacteremia until 13 to 14 h after the initiation of infection, when the leukopenic rabbits showed a larger number of pneumococci in the blood, ultimately exceeding the number reached in nonleukopenic rabbits by 100-fold. Leukocytes therefore limit the extent of pneumococcal bacteremia after infection of the CSF despite their lack of effect on the course or the CSF manifestations of experimental meningitis.

Purulent meningitis due to *Streptococcus pneumoniae* remains an important cause of morbidity and mortality. In the United States, approximately 6,000 cases occur annually, and the fatality rate of 20 to 30% has remained unchanged over the past three decades despite therapy with active antibiotics and aggressive supportive care. In addition to its high mortality rate, many survivors are left with permanent neurological deficits. It seems to be clear that if the morbidity and mortality of this disease are to be further reduced, new therapeutic strategies are needed. The role of the inflammatory response, especially that of polymorphonuclear leukocytes in the control of or recovery from pneumococcal meningitis, has never been clearly defined. It does seem likely that the products of inflammation in the cerebrospinal fluid (CSF) have a harmful effect on the cranial nerves, brain, and CSF hydrodynamics (8). Modulation of the inflammatory response could prove to be an important therapeutic intervention; however, better understanding of host-bacteria relationships and the pathogenesis of the infection is necessary as an initial step.

The rabbit model of experimental pneumococcal meningitis has proved useful for studying several aspects of this infection, including the effect of antibiotics (7, 9) the nature of the inflammatory response (4, 5), and the effect of the infection on CSF hydrodynamics (8).

The purpose of these studies was to examine the effect of leukocytes on the kinetics of bacterial growth in vivo in the CSF and in the bloodstream, as well as on the chemical changes in the CSF during the course of infection.

MATERIALS AND METHODS

Sixteen New Zealand white rabbits weighing 3 to 4 kg were prepared and infected by intracisternal inoculation of stationary-phase pneumococci as previously described (2). *S. pneumoniae* type III (clinical isolate, San Francisco General Hospital, Clinical Microbiology Laboratory) was grown on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with 5% defibrinated sheep blood (TSA-blood agar plates) at 37°C in 10% CO_2 after being isolated from the CSF of a previously infected rabbit. Colonies were washed from the surface of the plates with sterile normal saline, and the bacterial suspension was divided into portions and stored at −70°C. All the rabbits in these studies were infected with organisms from simultaneously prepared samples.

For studies of infection in leukopenic rabbits, 10 animals were given nitrogen mustard (Mustargen; kindly supplied by Merck Sharp & Dohme, West Point, Pa.), 3 mg/kg intravenously after light sedation with pentobarbital 72 h before the initiation of infection. In addition, rabbits treated with nitrogen mustard were given procaine penicillin G, 1.2×10^6 U intra-

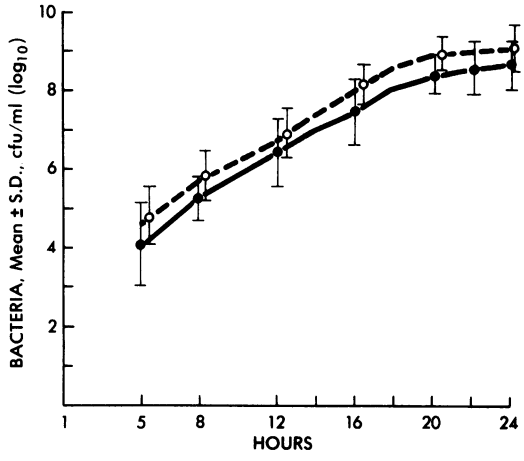

FIG. 1. Bacterial growth curves in CSF of normal and leukopenic rabbits. Bacterial generation time (time required for the population to double) was (mean ± standard deviation) 67 ± 5.3 min in normal rabbits and 59.5 ± 8.3 min in leukopenic rabbits ($P > 0.5$). Maximum bacterial populations in CSF were 8.7 ± 0.5 $\log_{10}$ CFU in normal rabbits ($n = 5$) and 9.1 ± 0.6 $\log_{10}$ CFU in leukopenic rabbits ($n = 8$) ($P > 0.20$).

muscularly at the time of administration of nitrogen mustard, to prevent overwhelming infection with *Pasteurella multocida*. (This dose of penicillin did not affect the course of pneumococcal infection.)

On the day of study, rabbits were anesthetized by slow intravenous injection of urethane (ethyl carbamate; Sigma Chemical Co., St. Louis, Mo.) at 1.75 g/kg. After placement of femoral arterial catheters, the animals were placed in stereotaxic frames and infected by intracisternal inoculation of 3.9 to 4.5 $\log_{10}$ CFU of lag-phase *S. pneumoniae* organisms in 0.4 ml of normal saline after the withdrawal of 0.4 ml of CSF. The inoculum was quantitated by serial dilution in normal saline and incubation on TSA-blood agar plates in 10% CO_2 at 37°C.

Erythrocyte (RBC) counts in blood and CSF were performed in a hemacytometer after serial dilution in normal saline. Leukocyte (WBC) counts were done in a hemacytometer after serial dilution of the samples in normal saline and lysis of the RBCs with glacial acetic acid.

Quantitative blood cultures were obtained hourly via the indwelling femoral arterial catheter, serially diluted in saline, plated on TSA-blood agar plates, and incubated at 37°C in 10% CO_2. CSF samples were taken by the intracisternal needle before the injection of pneumococci and at 5, 8, 12, 14, 16, 18, 20, and 24 h after inoculation. The CSF samples were divided, and RBCs and WBCs were counted immediately. Another portion of the CSF was quantitatively cultured by serial 10-fold dilution in normal saline, plated on TSA-blood agar, and incubated for 18 h at 37°C in 10% CO_2. The remaining CSF was centrifuged, and the supernatant was frozen at −70°C until chemical analysis was performed.

Chemical analysis of the CSF was performed by the Clinical Chemistry Department of the San Francisco General Hospital Clinical Laboratory. Serum glucose levels were determined on samples taken late in the infection to confirm that the rabbits had not become hypoglycemic. Glucose in CSF was assayed by a glucose oxidase method with a Beckman Astra 8 Analyzer. CSF lactate was measured by the rapid lactate method (Calbiochem-Behring, San Diego, Calif.). CSF protein concentrations were determined by trichloracetic acid precipitation, followed by determination of turbidity at 360 nm in a Gilford 400 spectrophotometer and comparison with a standard curve. All determinations for each rabbit were performed simultaneously to minimize interassay variation. Due to the limited volume of CSF obtainable from each rabbit, all studies could not be done on all rabbits; therefore, the number of tests included in the results differs from one study to another.

Bacterial generation time during the rapid growth phase was calculated from a regression line determined after plotting the bacterial population in CFU per milliliter of CSF versus time by using a Hewlett-Packard 33C calculator.

Statistical comparisons of the data were done with Student's *t* test.

RESULTS

Nitrogen mustard rendered the rabbits profoundly leukopenic. At the time of initiation of infection, peripheral blood leukocyte counts ranged from 0 to 900 per mm^3. When WBCs were counted in peripheral blood samples taken 8 h later, the rabbits that initially had detectable circulating leukocytes were more leukopenic, with peripheral blood WBC counts of 0 to 30 per mm^3. Rabbits that did not receive nitrogen mustard did not become leukopenic during the course of infection.

Bacterial growth in CSF. The bacterial growth characteristics were identical for the normal and leukopenic animals (Fig. 1). Active bacterial multiplication in the CSF began before the 5 h

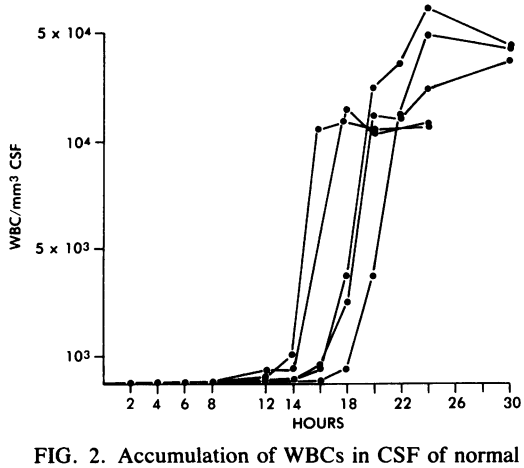

FIG. 2. Accumulation of WBCs in CSF of normal rabbits with *S. pneumoniae* meningitis. Each line represents data for one rabbit.

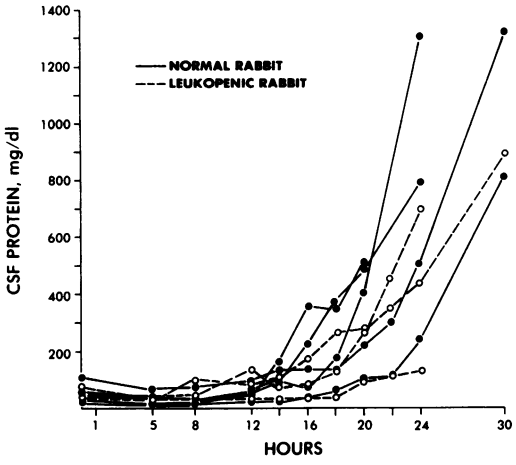

FIG. 3. CSF protein concentrations during *S. pneumoniae* meningitis. Each line represents data for one normal (●——●) or leukopenic (○- - -○) rabbit.

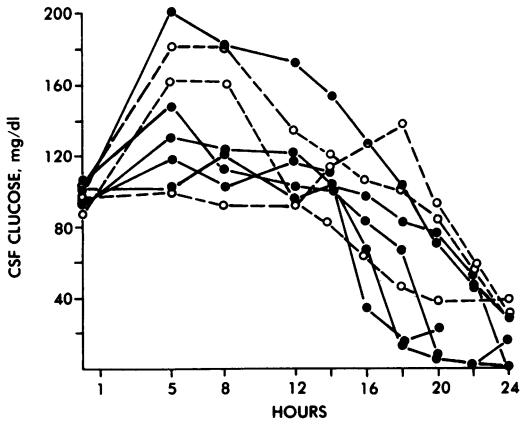

FIG. 4. CSF glucose concentrations during *S. pneumoniae* meningitis. Each line represents data for one normal (●——●) or leukopenic (○- - -○) rabbit.

postinfection sampling time. The linear slope of bacterial proliferation was nearly constant until approximately 18 h, when multiplication slowed and reached a plateau. Peak or maximum bacterial populations were reached in the CSF 24 h after inoculation and were not significantly different between the normal and leukopenic rabbits. Few rabbits survived beyond this time, and in those that did the bacterial population changed very little.

CSF leukocyte dynamics. Leukocytes began to appear in the CSF of normal rabbits approximately 12 to 16 h after inoculation and rapidly increased to a plateau 4 to 6 h later (Fig. 2). Maximum WBC counts in the CSF reached approximately 10^4 cells per mm^3 in all of the normal rabbits. Leukopenic rabbits rarely had WBCs in the CSF. Only one leukopenic rabbit had more than 100 WBC per mm^3 of CSF, and that was found for one determination only.

Time course of CSF chemical changes. CSF protein concentrations began to increase approximately 12 h after inoculation and increased progressively until the rabbits died (Fig. 3). The protein content of the CSF varied considerably between rabbits, but the time course and magnitude of increase were similar in both groups. CSF glucose concentration consistently decreased 14 to 18 after infection in both groups (Fig. 4). The low point of CSF glucose concentration was similar in both groups, although a tendency for lower concentrations to occur in the rabbits with normal WBCs was observed. An unexplained early increase in CSF glucose concentration was observed in all but two of the rabbits (one in each group). This increase was out of proportion to the increases in the level of serum glucose measured simultaneously and was not due to introduction of glucose with the bacterial inoculum, since assay of the inoculum revealed no detectable glucose.

CSF lactate concentrations progressively increased in both groups of rabbits (Fig. 5). The increase began approximately 14 h after the initiation of infection (at the same time that CSF glucose concentrations began to decrease), and the time course and magnitude of increase were essentially identical in rabbits with and without WBCs in their CSF.

Bacteremia. Quantitative cultures of blood revealed the development of pneumococcal bacteremia in all rabbits 2 to 7 h after infection. The quantity of pneumococci in the blood of infected animals was nearly identical in both the leukope-

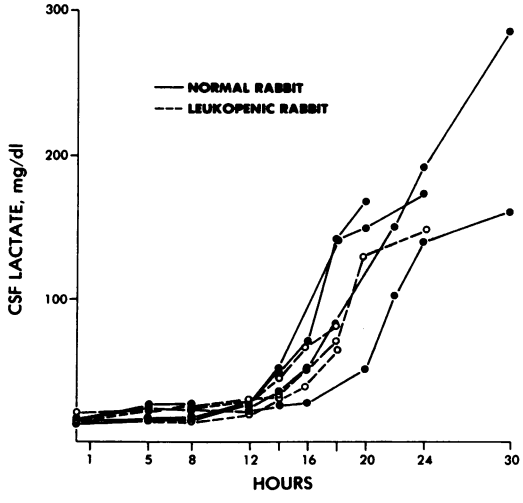

FIG. 5. CSF lactate concentrations during *S. pneumoniae* meningitis. Each line represents data for one normal (●——●) or leukopenic (○- - -○) rabbit.

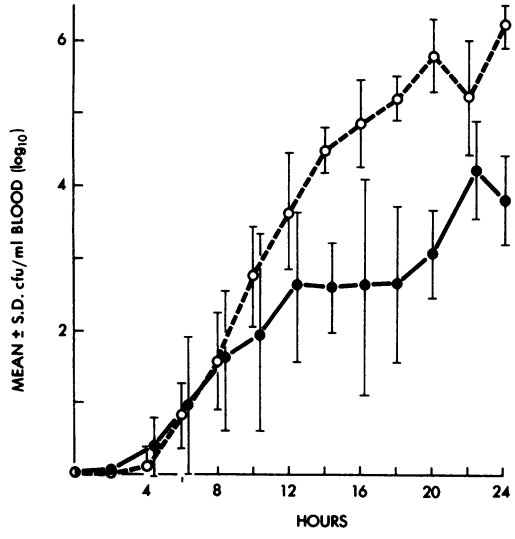

FIG. 6. Quantitative pneumococcal bacteremia in five normal (●——●) and eight leukopenic (O- - - -O) rabbits with *S. pneumoniae* meningitis. Bars represent standard deviations from the mean.

nic and normal rabbits until 13 to 14 h postinfection (Fig. 6). After that time, bacteremia in the leukopenic rabbits became quantitatively greater, and at 24 h was more than 100-fold higher in leukopenic than in the normal rabbits. (At 24 h the mean was 6.2 $\log_{10}$ CFU/ml of blood in leukopenic rabbits and 3.8 $\log_{10}$ CFU/ml of blood in normal rabbits [$P < 0.001$].)

DISCUSSION

The results of these studies indicate that host-bacteria relationships and chemical changes in the CSF in experimental pneumococcal meningitis can be studied so that the course of infection and the effects of various manipulations of the host can be defined. The growth of pneumococci in CSF in vivo mimics that observed in broth in vitro: after a stationary phase, the bacteria enter a rapid growth phase, and when the bacterial population reaches a certain level bacterial multiplication slows and may cease. The plateau seen in the bacterial population in vivo was not a consequence of equilibrium between active division and phagocytosis and killing by leukocytes, since a similar growth curve and ultimate bacterial populations were observed in rabbits with and without leukocytes in the CSF.

Bacterial growth in the CSF precedes chemical changes in the CSF by several hours. The clinical observation that patients may have meningitis (as indicated by bacteria isolated from the CSF) without abnormalities in cell counts, glucose, protein, or lactate may be due to the lag before these changes develop. The individual rabbits in each group were remarkably similar in CSF bacterial growth patterns, WBCs, glucose, and lactate concentration; however, the protein concentration varied up to 10-fold between individual animals. The reasons for this variation are not clear.

The time course and extent of increase in CSF protein were similar in the leukopenic and normal rabbits. This observation suggests that WBCs are not essential for the increase in meningeal permeability that allows plasma proteins to enter the CSF. Microbial factors or host mediators of inflammation other than those contained in leukocytes could account for the increased permeability. Recent work in vitro supplied evidence supporting the role of bacterial factors. Scheld and Long (Clin. Res. **30**:378A, 1982) showed evidence of endothelial cell damage, an opening of the tight junctions between the endothelial cells, and increased pinocytotic activity of the isolated brain capillaries (blood brain barrier) when these tissues were incubated with either *S. pneumoniae* or *Escherichia coli*.

Neither CSF protein nor lactate concentrations achieved a plateau, but increased progressively with time until the animals died. Leukocytes were not essential for the increase in lactate or decrease in CSF glucose. All of the rabbits studied developed hypoglycorrhachia relative to the concentration of glucose in the CSF early in the infection. This change occurred without concomitant hypoglycemia, and was accompanied in each rabbit by a marked increase in CSF lactate, indicating a shift of glucose metabolism to anaerobic glycolysis. That such changes in CSF glucose occur in the absence of WBCs is contrary to the results reported by Petersdorf and Harter in dogs (6). In those studies, only 1 of 21 dogs made leukopenic by total body irradiation and given pneumococcal meningitis developed a decrease in CSF glucose. This discrepancy may indicate a fundamental difference in meningeal glucose transport or leukocyte metabolism between rabbits and dogs, or it may be due to the greater bacterial populations achieved in the CSF in current experiments (10^8 to 10^9 CFU/ml) compared with those achieved in the earlier study (10^6 CFU/ml).

Although there was no difference in bacterial growth or chemical changes in the CSF in leukopenic compared with normal rabbits, a marked difference in bacteremia occurred late in the infection. Bacteremia was quantitatively similar in both groups of rabbits until 13 to 14 h postinfection. As the infection progressed, the number of bacteria in the bloodstream increased slowly in nonleukopenic rabbits, but continued to increase rapidly in the leukopenic rabbits and had reached much higher levels by the time of death. Preliminary studies in our laboratory failed to

reveal a difference in the rate of clearance of organisms from the bloodstream of normal and leukopenic rabbits after intravenous injection of type III *S. pneumoniae* organisms. Further studies are necessary to define the mechanism of this phenomenon.

The evidence from these studies indicates that the presence of WBCs in the CSF does not affect the rate of bacterial growth or the ultimate size of the bacterial population, but this failure of WBCs to contain the infection remains unexplained. In vitro studies indicate that pneumococci must be opsonized before phagocytosis by polymorphonuclear leukocytes, and in vivo studies have shown that opsonization by either complement or immunoglobulin G (IgG) or IgM enhances bloodstream clearance of pneumococci (1, 3). There are conflicting reports of opsonic activity in CSF obtained from human patients with meningitis. Simberkoff et al. (10) studied CSF opsonic activity and found that CSF from patients with meningitis had minimal opsonic activity in vitro against several bacterial species, including a type 9 pneumococcus. These investigators found small but measurable amounts of IgG, IgM, and complement components in CSF from patients with meningitis. Zwahlen et al. (12) examined CSF from patients with bacterial meningitis and found complement-mediated opsonic activity against a test strain of *Staphylococcus aureus* in 15 of 27 patients, and the presence of opsonic activity correlated with a favorable outcome. Another possible explanation for the lack of effectiveness of polymorphonuclear leukocytes in the CSF is that, as previously suggested (11), they are unable to phagocytose and kill encapsulated pneumococci (even if opsonized) within the open spaces of infected CSF (lack of so-called surface phagocytosis).

These studies showed that bacterial growth and chemical changes in the CSF of rabbits inoculated intracisternally with type III pneumococci are not appreciably affected by the presence of leukocytes. Bacterial factors or (other) host-derived mediators of inflammation may account for the chemical changes observed in CSF during meningitis. These studies did not evaluate central nervous system damage during experimental meningitis, and the role of leukocytes in contributing to such damage requires further study. Leukocytes are unable to effectively contain bacterial growth in the CSF, but more studies will be necessary to define the defect prohibiting phagocytosis and killing of pneumococci in the CSF. The intriguing finding of quantitatively greater bacteremia in leukopenic rabbits implies that the presence of leukocytes is of some value in limiting bacteremia during the infection. This finding suggests that leukocytes are able to contain or impede the dissemination of pneumococci outside the CSF.

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