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Influence of Granulocytes on Brain Edema, Intracranial Pressure, and Cerebrospinal Fluid Concentrations of Lactate and Protein in Experimental Meningitis

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Brain water content (brain edema), intracranial pressure, and cerebrospinal fluid (CSF) concentrations of lactate and protein increased significantly during 24 h of experimental meningitis due to *Streptococcus pneumoniae*, but changes were similar in normal and neutropenic rabbits. In sterile meningitis induced by *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), low and high doses of fMLP were equally effective in inducing CSF pleocytosis, whereas only high doses of fMLP caused brain edema. High doses of fMLP injected intracisternally during pneumococcal meningitis also increased brain water content. The fMLP did not significantly increase intracranial pressure or CSF concentrations of lactate or protein in sterile or pneumococcal meningitis, nor did it cause brain edema in neutropenic animals. Thus, granulocytes may contribute to brain edema during meningitis if adequately stimulated, but intracranial pressure and CSF protein and lactate concentrations appear independent of granulocytes. Stimulation does not appear to occur early in meningitis, when granulocytes were without effect on brain edema.

Morbidity and mortality from bacterial meningitis remain high [1–4]. Pneumococcal meningitis has, even in recent years, a death rate of almost 30% [3, 4]. This figure has not changed in the last 40 years, despite new antibiotics and an improved understanding of the principles of antibiotic therapy [5–7].

The functional and morphological substrates of brain damage induced by bacterial meningitis are only partially understood. Early pathological observations have indicated that brain edema and thrombosis of cerebral vessels may contribute to the loss of neuronal functions [8–10]. In addition, evidence exists that meningitis is often associated with increased intracranial pressure and that this factor may in turn impair cerebral blood flow and thus limit the supply of oxygen and nutrients to the brain [11–13]. Increased CSF outflow resistance [14], impaired cerebral circulation [15], increased intracranial pressure, and brain edema [16, 17] have been documented in animal models. These changes are likely to cause the brain to shift its energy production to anaerobic glycolysis and thus increase the production of lactate [18]. Increased lactate concentrations in CSF can be documented readily during meningitis [19, 20].

Some evidence exists that both mortality and the development of neurological sequelae may be related to inflammatory CNS alterations. In animal studies the time of death is associated with maximal inflammation in the subarachnoidal space [21], and one study suggested that neutropenic dogs with pneumococcal meningitis may survive longer than animals with a normal inflammatory reaction in the CSF [22]. Fishman et al. [23], Chan and Fishman [24], and Chan et al. [25] have demonstrated that products of leukocytes, such as polyunsaturated fatty acids and oxygen-free radicals, can induce brain edema, increased lactate production, and energy depletion in cortical brain slices of rats. However, the role of leukocytes in the mediation of brain edema during bacterial meningitis has not been examined. Whether other changes observed during

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meningitis, for example, increased intracranial pressure, are mediated by leukocytes is also not known.

Demonstrating harmful effects of leukocytes in the subarachnoidal space during meningitis could have therapeutic consequences. We therefore evaluated the influence of granulocytes on various pathophysiological parameters in an animal model of meningitis. Brain edema, increased intracranial pressure, and changes in CSF concentrations of lactate and protein were examined during bacterial meningitis in normal and in neutropenic rabbits. In addition, these studies were expanded using a model of sterile meningitis in which granulocytic pleocytosis in CSF was induced using a chemotactically active peptide.

Materials and Methods

Infecting organism. A type 3 encapsulated *Streptococcus pneumoniae* originally isolated from a clinical specimen [26] was grown on blood agar plates, resuspended in 0.9% NaCl, and stored at −70 C. For infecting animals, the thawed inoculum was either diluted directly to the desired concentration in 0.9% NaCl (first set of experiments) or was grown in Todd-Hewitt broth for 6 h, washed, and suspended in 0.9% NaCl (third set of experiments). The actual titer of the inoculum was determined by quantitative cultures on blood agar plates.

Model of experimental pneumococcal meningitis. The model of experimental meningitis in rabbits originally described by Dacey and Sande [27] was used. New Zealand white rabbits weighing 2–3 kg were anesthetized iv with 30 mg of pentobarbital/kg (Carter-Glogau Laboratory, Glendale, Ariz) for all experimental procedures. A helmet formed with dental acrylic was attached to the skull by four screws with the animal under general anesthesia, which allowed placement of the animals in stereotactic frames constructed to puncture the cisterna magna (provided by Dr. O. Zak, Ciba-Geigy, Basel, Switzerland). Three days after attachment of the helmet, the animals were again anesthetized with pentobarbital and placed in the stereotactic frames. The cisterna magna was punctured with a spinal needle (3.5 inches; 25 gauge; Becton, Dickinson and Co., Rutherford, NJ). After the pressure was recorded (see below), 0.5 mL of CSF was withdrawn, and 5–7 × 10^5 cfu of *S. pneumoniae* suspended in 0.5 mL of 0.9% NaCl was injected into the cisterna magna. Twenty-four hours later anesthesia was reinduced, the animals were placed in the stereotactic frames, and the cisterna was punctured for collection of CSF and measurement of the other experimental parameters. At the time of infection and after 24 h, blood was collected by cardiac puncture with a 25-gauge, 5/8-inch needle so that the white blood cell (WBC) and differential counts could be determined.

Neutropenic rabbits. Neutropenia was induced in some experimental groups of rabbits by injection of mechlorethamine HCl (nitrogen mustard; Merck Sharp & Dohme, West Point, Pa), 1.85 mg/kg iv, three days before infection or intracisternal injection of a chemotactic stimulus. Simultaneously with the injection of nitrogen mustard, the animals received 1.2 × 10^6 U of procaine penicillin im so that *Pasteurella multocida* pneumonia during neutropenia was prevented. At the time of induction of meningitis, three days later, peripheral WBC and differential counts were determined. Neutropenic animals were not visibly sick at this point.

Chemotactic peptide–induced sterile meningitis. Sterile meningitis was induced in normal and neutropenic rabbits by intracisternal injection of a solution of *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP; Sigma, St. Louis). Two doses of fMLP were examined: a low dose with 10^{-5} *M* fMLP and a high dose with 10^{-3} *M* fMLP. fMLP was diluted in 0.1% (vol/vol) dimethyl sulfoxide and PBS and was injected into the cisterna magna in a volume of 0.5 mL after removal of an equal volume of CSF. Animals received three injections of fMLP 5 h apart and underwent final examination 24 h after the experiment began. One injection of the fMLP carrier alone did not induce CSF pleocytosis of >50 WBCs/mm^3 in any of the five control animals. The carrier also did not affect the intracranial pressure of rabbits with pneumococcal meningitis. Before injections of fMLP and at the end of the experiment, WBC counts and all other experimental parameters were determined in CSF.

Stimulation of granulocytes in CSF of rabbits with pneumococcal meningitis. In some animals infected with *S. pneumoniae*, the cisterna magna was punctured 20 h after infection, 0.5 mL of CSF was removed and examined, and 0.5 mL of 10^{-3} *M* fMLP, prepared as described above, was injected intracisternally so that the WBCs present in the subarachnoidal space were stimulated. Control animals simultaneously received the same amount of 0.9% NaCl. The animals underwent final examination 4 h later as described above.

Experimental parameters. CSF from the cisterna magna was collected through the spinal needle. Bacterial titers in CSF were determined quantitatively by culturing 10-fold dilutions of CSF on blood agar plates incubated overnight at 35 C in room air with 5% CO_2. WBC counts in CSF and blood were determined in a Neubaur hemacytometer. Differential WBC counts were done using smears stained with Giemsa stain. For determination of lactate and protein concentrations, CSF was centrifuged for 30 s within minutes after collection. The supernatant was frozen immediately at −70 C. Samples were then analyzed using commercially available methods and a centrifugal analyzer in a routine chemical laboratory. Lactate content was determined using an enzymatic method that detects the generation of pyruvate in the presence of lactate dehydrogenase (Monotest Lakatat®; Boehringer, Mannheim, Federal Republic of Germany). Protein content was determined using a colorimetric method (total protein test; Bio-Rad, Glattbrugg, Switzerland).

Intracisternal pressure was determined through the spinal needle placed in the cisterna magna while the anesthetized rabbits were secured in a sitting position [16]. Pressure was recorded on a multichannel polygraph (Grass Instrument Co., Quincy, Mass; or Gilson Medical Electronics, Middleton, Wis) by connecting the needle to a water-filled mechanical pressure transducer (Gould Statham model P23ID; Gould, Oxnard, Calif). Each animal served as its own control, and results were expressed as change in pressure from the baseline (preinfection) value. Determination of pressure was considered accurate when mean pressure was stable during a 10-s period and when respiration-induced changes could be identified on the recording. This method revealed an intraassay reproducibility of <1 mm Hg.

At the end of the experiment, 24 h after infection or induction of sterile meningitis, animals were killed by an iv overdose of pentobarbital. The skull was opened beneath the helmet, and the brain was immediately removed and dissected on filter paper. One hemisphere was weighed and then dried to a stable dry weight in a vacuum oven at 105 C [16]; the other hemisphere was dissected into gray and subcortical white matter, and these fractions were also weighed and dried. Brain water content was then calculated and expressed as grams of water per 100 g of dry weight. Minimal technical modifications were introduced in an attempt to increase the reproducibility of the brain water determination. These modifications influenced the normal values of brain water content in uninfected controls. Thus in each phase of the experiments, separate groups of uninfected animals were included as controls. These animals were examined simultaneously with the corresponding experimental animals by using identical techniques.

Statistics. Results are expressed as mean ± SD values unless stated otherwise. Groups were compared by the Student's *t* test; paired values were examined by the paired *t* test.

Results

Normal vs. neutropenic rabbits. In a first set of experiments the role of granulocytes in the development of brain edema, increased intracranial pressure, and increased CSF concentrations of lactate and protein during meningitis was evaluated by comparing the effect of pneumococcal meningitis in normal and neutropenic rabbits. Fifty rabbits were divided into four groups: normal uninfected, normal infected, neutropenic uninfected, and neutropenic infected. On the day of the experiments, neutropenic rabbits had peripheral WBC counts of 550 ± 350/mm,^{3} compared with 6500 ± 2020 WBCs/mm^3 in normal rabbits ($P < .001$). In neutropenic rabbits, granulocytes consistently accounted for <10% of the peripheral WBCs, compared with 35.2% ± 14.4% in normal rabbits ($P < .001$). All other experimental parameters were not significantly different in neutropenic rabbits compared with healthy controls.

Table 1. WBC counts and bacterial titers in CSF of rabbits with experimental meningitis.

Experimental group (*n*)	WBC count	Bacterial titer
Infected		
Normal (14)	2200 (350–7050)*	5.7 ± 0.9
Neutropenic (12)	138 (13–500)†	5.9 ± 1.1
fMLP		
Low-dose (5)	1400 (340–4500)*	Sterile
High-dose (5)	700 (400–1000)*	Sterile
Normal infected, at 20 h after infection (29)	1900 (255–8000)*	6.0 ± 1.3
Infected (24 h)		
fMLP-stimulated (15)	3500 (595–9200)*	5.6 ± 1.0
Saline-injected (14)	3430 (720–9150)*	5.9 ± 1.5

NOTE. The WBC count (no. of cells/mm^3) is given as median value (range), and bacterial titer ($\log_{10}$ no. of cfu/mL) is given as mean ± SD.

* More than 90% of the WBCs were granulocytes.

† All WBCs were mononuclear cells.

Pneumococcal meningitis induced clinical disease (lethargy and fever) and progressive changes in all experimental parameters. Bacterial titers in CSF after 24 h of disease were identical in normal and neutropenic rabbits (table 1). WBC counts in CSF were, however, markedly different (table 1). In normal rabbits the median CSF WBC count was 2200/mm^3, and >90% of these cells were granulocytes. In contrast, neutropenic animals had a median of only 138 WBCs/mm^3 in CSF 24 h after infection, and all these cells were mononuclear cells ($P < .001$).

Brain water content increased as a result of the infection, but the increase was identical in normal and neutropenic rabbits (figure 1). The water content of hemispheres increased from 392 ± 8 g of water/100 g of dry weight in uninfected normal rabbits and 394 ± 9 g/100 g in uninfected neutropenic rabbits to 403 ± 10 and 403 ± 12 g/100 g, respectively ($P < .01$ for normal and neutropenic rabbits combined). The corresponding values for white matter water content were 240 ± 12 g/100 g in normal and 242 ± 19 g/100 g in neutropenic rabbits to 252 ± 12 and 251 ± 22 g/100 g, respectively ($P < .05$ for normal and neutropenic rabbits combined). There was also a slight but not statistically significant increase in gray matter water content during infection in both the normal and neutropenic animals.

Intracisternal pressure increased significantly ($P < .01$) during the 24-h infection in normal rabbits and slightly less in neutropenic rabbits (figure 2A; 4.6 ± 1.8 vs. 3.4 ± 1.8 mm Hg; difference not significant). CSF concentrations of lactate and protein increased markedly during meningitis, but there was no significant difference in changes between normal and neutropenic rabbits: for lactate, 1.3 ± 0.3 mmol/L in uninfected rabbits, 7.14 ± 3.7 mmol/L in normal infected animals ($P < .001$), and 5.25 ± 2.9 mmol/L in neutropenic infected rabbits (difference not significant); and for protein, 0.5 ± 0.2 g/L in uninfected controls, 2.0 ± 1.5 g/L in normal infected animals ($P < .01$), and 2.8 ± 1.4 g/L in neutropenic infected animals (difference not significant).

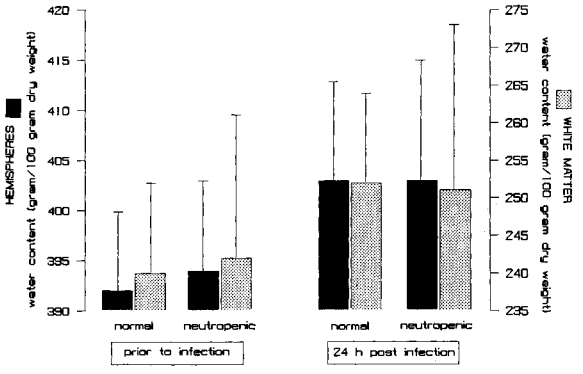

Figure 1. Brain water content in normal and neutropenic rabbits. Animals were examined before and 24 h after induction of pneumococcal meningitis. *Darker columns* represent brain water content of hemispheres; *lighter columns* represent brain water content of subcortical white matter. Data are mean ± SD (*bars*) values. The difference between uninfected and infected animals was significant ($P < .05$).

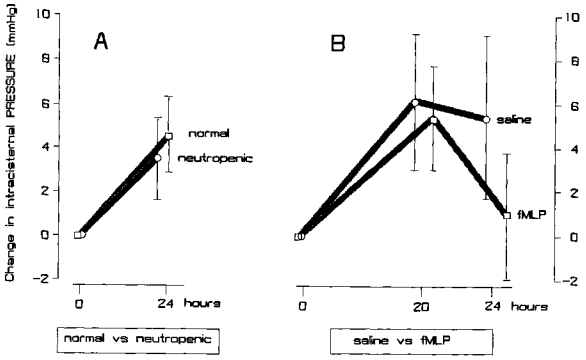

Figure 2. Changes in intracisternal pressure (mm Hg) in animals during pneumococcal meningitis. Data are mean ± SD (*bars*) values. *A*: Neutropenic animals had an increase in intracisternal pressure during infection, similar to that in the infected controls. *B*: In animals with pneumococcal meningitis injected with high-dose fMLP 20 h after induction of infection, pressure decreased significantly ($P < .01$) compared with control animals, which received saline at 20 h.

Chemotactic peptide-induced sterile meningitis. A second approach was designed for evaluating the role of granulocytes in the development of pathophysiological changes during meningitis. Sterile meningitis was induced by repeated (three times) intracisternal injection of fMLP into experimental groups of five or six rabbits. The chemotactic and neutrophil-stimulating properties of this oligopeptide have been well characterized in vitro [28–30]. Two doses of fMLP were examined: a low dose with 10^{-5} *M* fMLP per injection and a high dose with 10^{-3} *M* fMLP. If an ~100-fold dilution in CSF and tissue of animals is assumed, these doses were chosen so that in vivo concentrations in the range of those capable of inducing oxygen free radical generation and degranulation of granulocytes in vitro [29, 30] were achieved.

Injections with the lower dose of fMLP were as effective in inducing CSF pleocytosis as were injec-

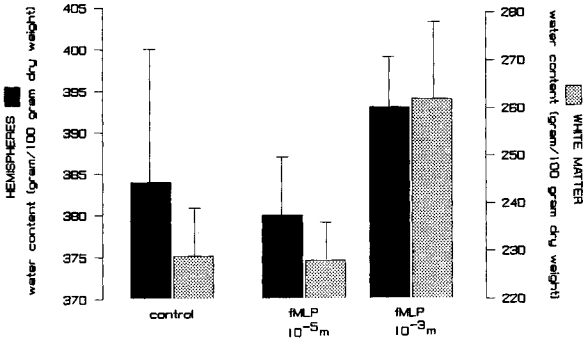

Figure 3. Brain water content in control animals and in rabbits receiving intracisternal injections of low or high doses of fMLP. *Darker columns* indicate water content of hemispheres; *lighter columns* indicate water content of subcortical white matter. Data are mean ± SD (*bars*) values. The differences between high-dose and low-dose fMLP were significant ($P < .02$).

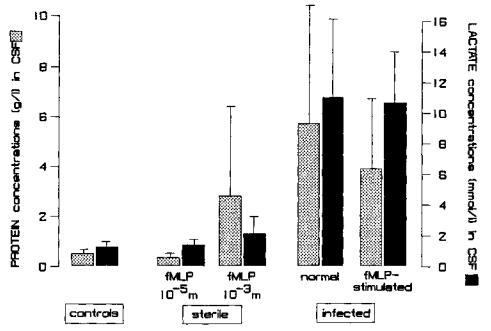

Figure 4. Protein (*lighter columns*) and lactate (*darker columns*) concentrations in CSF of rabbits with sterile and pneumococcal ("infected") meningitis. Data are mean ± SD (*bars*) values. Sterile meningitis was induced with three doses of fMLP. Infected animals were compared after intracisternal injection of saline or fMLP 20 h after infection, 4 h before they were killed. Infection induced a significant increase in lactate and protein concentrations ($P < .01$); all other differences were not significant.

tions of the higher dose (table 1). In all animals >90% of the cells in the CSF were granulocytes. The pleocytosis induced by low-dose fMLP was not associated with significant changes in any of the experimental parameters (figures 3 and 4). Brain water content of the hemispheres was 380 ± 7 g/100 g of dry weight compared with 384 ± 16 g/100 g in controls; white matter water content was 228 ± 8 g/100 g compared with 229 ± 10 g/100 g in controls (figure 3). CSF pressure did not change significantly during the 24-h course of sterile meningitis (0.1 ± 2.7 mm Hg). Similarly, CSF concentrations of lactate (1.6 ± 0.2 mmol/L) and protein (0.4 ± 0.14 g/L) were not different from control values (figure 4).

In contrast to the animals receiving low-dose fMLP, the CSF pleocytosis induced by high-dose fMLP had a significant effect on brain water content (figure 3): hemispheres, 393 ± 6 g/100 g of dry weight ($P < .02$ vs. low-dose fMLP; difference not significant vs. controls); and white matter, 262 ± 16 g/100 g ($P < .01$). This induction of brain edema was associated with an increase in CSF concentrations of protein and lactate (2.84 ± 3.60 g/L and 2.15 ± 1.12 mmol/L, respectively; figure 4). However, because not all animals in this group showed chemical alterations of CSF (the SD was large), these differences did not reach statistical significance. CSF pressure did not change significantly during the course of sterile meningitis induced by high-dose fMLP (0.8 ± 1.6 mm Hg).

The possibility that the observed development of brain edema was induced by fMLP itself was excluded by injection of high-dose fMLP into four neutropenic animals. Brain water content in these animals was not significantly different from that in control animals: hemispheres, 370 ± 5 vs. 375 ± 8 g/100 g of dry weight in controls; and white matter, 222 ± 14 vs. 220 ± 11 g/100 g. Thus these results show that only the CSF pleocytosis (predominantly granulocytes) induced with high-dose fMLP was associated with development of brain edema and a moderate increase in CSF concentrations of lactate and protein.

Stimulation of granulocytes with fMLP during pneumococcal meningitis. Results reported so far are compatible with the hypothesis that activated (high-dose fMLP), in contrast to inactive (low-dose fMLP), granulocytes can contribute to development of brain edema. The lack of a measurable effect of granulocytes on brain edema during the first 24 h of meningitis (first set of experiments) can be explained by the absence of a sufficient stimulation of granulocytes in the CSF during infection. This proposal is supported by the inability of granulocytes to reduce bacterial titers in CSF [31].

To test this hypothesis we examined the effect of stimulating granulocytes during pneumococcal meningitis. A group of 15 animals with pneumococcal meningitis was injected with high-dose fMLP intracisternally 4 h before they were killed (20 h after infection) in an attempt to stimulate the granulocytes then present in CSF. These animals were compared with 14 animals with pneumococcal meningitis

receiving 0.9% NaCl. In both groups, CSF WBCs increased to similar final counts between 20 and 24 h (table 1). Bacterial titers were also similar in the two groups (table 1).

Intracisternal injection of fMLP was associated with higher brain water content compared with values in infected controls (figure 5): hemispheres, 391 ± 12 vs. 381 ± 10 g/100 g of dry weight ($P <$ .03); and white matter, 244 ± 18 vs. 234 ± 13 g/100 g ($P <$ .1). Gray matter water content also increased slightly. As in the previous experiments, infected animals had higher brain water content than did uninfected controls (figure 5).

In parallel with the fMLP-induced increase in brain water, intracisternal pressure was reduced (figure 2B). Twenty hours after infection, when fMLP was injected, intracisternal pressure had increased by 5.4 ± 2.3 mm Hg ($P <$ .01). Four hours later the pressure had dropped to 0.8 ± 2.9 mm Hg ($P <$ .01), despite the concomitant increase in brain edema. In animals receiving saline intracisternally, the pressure remained stable between 20 and 24 h (final pressure, 4.7 ± 3.7 mm Hg; $P <$ .005 compared with final pressure in fMLP-treated animals). CSF concentrations of lactate and protein were not significantly affected by intracisternal injection of fMLP and the associated increase in brain edema (figure 4): lactate, 10.7 ± 3.3 vs. 11.0 ± 5.1 mmol/L; and protein, 3.9 ± 2.8 vs. 5.8 ± 5.7 g/L.

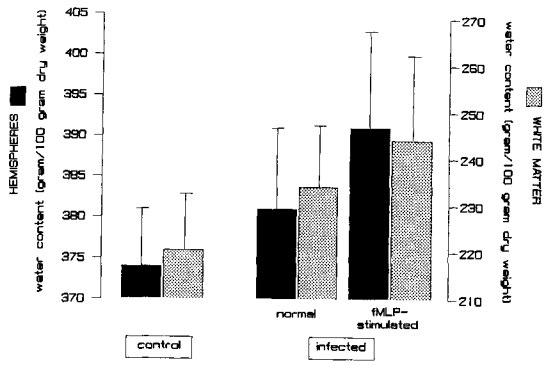

Figure 5. Brain water content in normal uninfected rabbits and in rabbits with pneumococcal meningitis receiving intracisternal saline or fMLP. Saline or high-dose fMLP was injected intracisternally 20 h after infection, 4 h before the animals were killed. *Darker columns* represent hemispheres; *lighter columns* represent subcortical white matter. Data are mean ± SD (*bars*) values. Injection of fMLP was associated with a significant ($P <$ .05) increase in brain edema.

Discussion

That various elements of the body's inflammatory response can be destructive for the host's own tissue has recently become clear. The most important cells involved in such inflammation-associated tissue damage are the neutrophils. Granulocytes have been implicated as playing a key role in the development of adult respiratory distress syndrome [32, 33], even though recent data suggest that this syndrome can also develop in neutropenic individuals [34]. In other situations granulocytic enzymes may develop their harmful activity in conjunction with microbial enzymes, a mechanism that has been documented in the bronchial system of children with cystic fibrosis colonized with *Pseudomonas aeruginosa* [35]. In arthritis the toxic products of granulocytes that accumulate in the inflamed joint also appear to contribute to development of chronic tissue damage [36, 37].

Some indirect evidence exists that granulocytes could also contribute to brain damage during meningitis. Petersdorf and Luttrell [22] found that neutropenic dogs with experimentally induced pneumococcal meningitis survived the infection an average of 62 h compared with 46 h for normal dogs with an unimpaired inflammatory response in CSF. The statistical significance of this difference has not been determined. McAllister et al. [21] observed an association between the maximal inflammation in CSF and the time of death in rabbits with experimental pneumococcal meningitis. Extensive work by Fishman et al. [23], Chan and Fishman [24], and Chan et al. [25] has implicated granulocytes as an important factor in development of brain edema. Both arachidonic acid and other free polyunsaturated fatty acids [38–40], which represent major constituents of the granulocytic cell wall and are found in high concentrations in pus, as well as oxygen-derived free radicals [25, 41] appear to be involved in the generation of brain edema in vitro and in vivo.

Despite these data the role of granulocytes in the pathogenesis and pathophysiology of bacterial meningitis has not been well defined. In one study Ernst et al. [31] showed that granulocytes in CSF of rabbits with pneumococcal meningitis were ineffective in reducing bacterial growth. This observation has been confirmed in the present study. In their study, changes in CSF concentrations of lactate, protein, and glucose were also examined. There was no obvious difference between normal and neutropenic

animals, but this conclusion was based on only three neutropenic rabbits examined [31]. A more recent study in rats indicated that leukocytes were not essential for development of increased blood-brain barrier permeability during experimental *Haemophilus influenzae* meningitis [42].

Here we measured pathophysiological changes that have previously been characterized in this model [16, 43]. Brain edema was examined because of its association with brain injury due to various causes [44]. In experimental meningitis the development of brain edema, albeit not massive, has been documented [16, 43, 45], and clinical evidence exists of severe brain edema in fatal cases of meningitis [8, 46]. According to Fishman et al. [23] and Fishman [44], brain edema during meningitis ("granulocytic edema") comprises all three types of brain edema, i.e., vasogenic edema, cytotoxic edema, and interstitial edema. As shown in this and previous studies, brain edema in experimental meningitis in rabbits develops primarily in the white matter, a localization that is typical for vasogenic edema [43]. Vasogenic edema is the expression of increased permeability of the blood-brain barrier typical for bacterial meningitis [42, 47]. Determination of CSF protein concentrations, which reflect this leakage into the interstitial space, have been included here.

Intracranial pressure was also monitored. Few studies have examined intracranial pressure during meningitis despite the general consensus that pressure increases during the disease [11–13, 17, 45]. Massively increased intracranial pressure may impair cerebral blood flow [48]. Increased brain volume due to swelling is thought to be one mechanism contributing to increased intracranial pressure, but our own experimental data indicate that this mechanism cannot be the only one, because infected animals treated with methylprednisolone had increased pressure despite the absence of brain edema [16].

CSF lactate concentrations were included as an experimental parameter to serve as an indicator of impaired glucose metabolism of the brain [18]. Glucose is the major source of energy of the CNS, and any alteration of this metabolism has potentially serious functional consequences [49, 50]. Elevated lactate concentrations in CSF, which appear to be only minimally influenced by direct lactate production of bacteria or leukocytes in CSF, have been associated with increased mortality from meningitis in humans [51] and in experimental pneumococcal meningitis [52].

The results of this study indicate that granulocytes are of minor relevance for the pathophysiological alterations examined. All parameters changed significantly when animals were infected, but the virtually complete absence of granulocytes in neutropenic rabbits did not have any significant effect on the magnitude of these changes. Obviously, factors other than granulocytes must be involved. Preliminary studies indicate that bacterial products may be important. Endotoxin released during treatment of *Escherichia coli* meningitis with a new cephalosporin was responsible for development of brain edema in one study [43]. Products of pneumococcal cell walls induce inflammation, increased intracranial pressure, and brain edema in the same animal model [53–55].

It was only when granulocytes in CSF were stimulated by high doses of fMLP that any effect attributable to granulocytes could be documented, i.e., increased brain edema. Moreover, the increase in brain edema was moderate and was not reflected by increased lactate production. Increased lactate concentrations in CSF would be expected if the additional edema was detrimental to the brain's glucose metabolism. Whether stimulation of granulocytes occurs during meningitis is unknown. The comparison between neutropenic and normal animals indicates that at least during the first 24 h of the disease there is no substantial stimulation, despite the pronounced inflammatory changes in CSF. The apparent lack of stimulation can be explained by the inefficient phagocytosis by granulocytes [31], which is reflected by the uniformly fatal course of the untreated disease [5]. The increase of brain edema after fMLP stimulation was associated with a decrease in intracisternal pressure. A similar reduction of intracranial pressure was observed in very sick animals with meningitis induced by high doses of pneumococcal cell walls [53]. These observations emphasize that increased brain volume is not necessarily the basis for the increased intracranial pressure.

In summary, these studies show that granulocytes are not important in the development of brain edema, increased intracranial pressure, or changes in CSF concentrations of lactate or protein during the first 24 h of experimental meningitis due to *S. pneumoniae*. This conclusion modifies the concept of Fishman et al. [23] and Fishman [44] and indicates that brain edema during meningitis may not be "granulocytic" edema. The mere presence of granulocytes in CSF appears to be insufficient to contribute to brain edema or other pathophysiological alterations dur-

ing bacterial meningitis. Rather, stimulation of the granulocytes is necessary. Further studies must clarify whether such stimulation plays a role in advanced stages of meningitis and whether the release of active products from the infecting bacteria after institution of therapy can stimulate granulocytes in the CSF [43, 56].

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