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Pathogenesis of Bacterial Meningitis: Contributions by Experimental Models in Rabbits

Summary: Rabbit models of bacterial meningitis have contributed substantially to our understanding of the disease, although the technical characteristics of these models only allow the study of specific aspects of the disease. Bacterial multiplication in the subarachnoidal space is not substantially influenced by host defense mechanisms, mainly because of the lack of sufficient amounts of specific antibodies and functional complement in infected CSF. The multiplying bacteria induce profound changes in the blood-brain barrier, an influx of serum proteins into the CSF and the invasion of polymorphonuclear leukocytes at the site of the infection. The presence of polymorphonuclear leukocytes in CSF not only appears to be of limited value in combating the infection, but also seems to produce deleterious effects on the central nervous system. Components of the leukocytes, such as unsaturated fatty acids, arachidonic metabolites and free oxygen radicals, may contribute to the profound hydrodynamic, structural and metabolic changes that are currently under study in experimental models of the disease. A better understanding of the pathophysiology of bacterial meningitis may allow us to design more effective therapeutic strategies and improve the outcome of this disease.

Zusammenfassung: *Pathogenese der bakteriellen Meningitis: Experimentelle Studien mit Kaninchenmodellen.* Experimentelle Untersuchungen der bakteriellen Meningitis unter Verwendung von Kaninchenmodellen haben wesentlich zu unserem Verständnis dieser Erkrankung beigetragen, obwohl sich Kaninchenmodelle aus technischen Gründen nur zum Studium ganz spezifischer Aspekte eignen. Die Vermehrung von Bakterien im Subarachnoidalraum bleibt von der körpereigenen Immunabwehr weitgehend unbeeinflußt, in erster Linie, weil im infizierten Liquor ungenügende Mengen von spezifischen Antikörpern und funktionsfähigem Complement vorhanden sind. Die sich vermehrenden Bakterien zerstören die Integrität der Blut-Hirnschranke und führen zum Einstrom von Serumeiweiß und zur Invasion polymorphkerniger Leukozyten an den Ort der Entzündung. Die Gegenwart von Leukozyten im Subarachnoidalraum ist unzureichend, um mit der Infektion fertig zu werden; vielmehr scheinen Leukozyten schädigende Wirkungen auf das Zentralnervensystem zu haben. Verschiedene Stoffwechselprodukte der Leukozyten, so zum Beispiel ungesättigte Fettsäuren, Arachidonsäuremetaboliten und freie Sauerstoffradikale, kommen als Mediatoren für die tiefgreifenden hydrodynamischen, strukturellen und metabolischen Veränderungen in Frage, die derzeit in experimentellen Modellen untersucht werden. Dank vertieftem Verständnis der pathophysiologischen Zusammenhänge der bakteriellen Meningitis wird es möglicherweise in Zukunft gelingen, therapeutische Strategien zu entwickeln, welche die Prognose dieser Krankheit weiter verbessern.

Introduction

Experimental studies performed with rabbit models of bacterial meningitis have contributed substantially to our understanding of many aspects of the pathophysiology, clinical and laboratory manifestations, and treatment of bacterial meningitis. The use of an animal model has been particularly important for the study of meningitis, since the devastating nature of this disease greatly limits studies in humans. The purpose of this review is to summarize some of the important work performed in this area, focusing primarily on information derived from the model designed by *Dacey* and *Sande* in 1974 (1). The characteristics of this rabbit model of experimental meningitis favor the study of certain aspects of the disease, whereas it precludes th study of other areas. However, where appropriate, closely related aspects derived from *in vitro* work or from studies with other animal models will be included. The mechanisms of infection in all experimental rabbit models of meningitis do not mimic the normal physiological process found in man. In humans, bacteria gain access to the CNS by hematogenic spread in the majority of cases. In the rabbit model, animals are either infected by direct injection of the organism into the *cisterna magna* (1) or by i.v. injection of a relatively large inoculum minutes after the integrity of the meninges has been altered by puncture, or following the intracisternal injection of mucin (2). The intracisternal mode of infection is most commonly used, since it results in infection in virtually 100%

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of the animals, whereas i.v. challenge after spinal tap results in infection in only half of the animals (2). Injecting a sterile mucin suspension into the CSF prior to the production of the bacteremia increases the infection rate to 84% (2), probably as a result of the more generalized disturbance of the meningeal integrity. The size of the inoculum which produces a consistent, progressive and ultimately fatal infection after intracisternal injection depends on the infecting organism and the immune status of the host animal. For *Streptococcus pneumoniae* and *Escherichia coli,* 10^5 colony forming units (cfu) produce meningitis in all animals, although as few as 10 cfu produce disease when the bacteria are in the logarithmic growth phase at the time of injection (*Decazes, J. M., Sande, M. A.*, unpublished observations). In contrast, 10^9 cfu of *Haemophilus influenzae* must be injected in order to produce a fatal infection. This high inoculum is necessary for this organism, because bactericidal antibodies against *H. influenzae* type b make rabbits relatively resistant to this pathogen (3); the organism is cleared from the CSF without producing a fatal disease if lower inocula (10^8 cfu or less) are used.

Bacterial Growth in CSF

Since meningitis must be induced "artifically" in rabbits, models using this animal species are not suited for studying the mechanism by which bacteria gain access to the subarachnoidal space in naturally occurring disease. Moreover, these models have not been used extensively to define the virulence factors of pathogenic organisms. In contrast, the rabbit model originally described by *Dacey* and *Sande* (1) allows frequent (almost continuous) sampling of relatively large amounts of CSF. In this model, the animals are secured under anesthesia in a stereotactic frame, thus allowing a spinal needle to be placed in the *cisterna magna.* The needle may be left *in situ* for the duration of the experiment (up to 48 hours). Thus, this model is well suited for examining the characteristics of bacterial growth in CSF *in vivo. In vitro,* most bacteria grow less well in CSF than in broth, and the reduction in the growth rate seems to vary between bacterial species (4). For instance, *Staphylococcus epidermidis,* which is a rare meningeal pathogen found almost exclusively in patients with CNS shunts (5), does not grow in CSF *in vitro. Staphylococcus aureus,* another rare cause of meningitis, does not grow well in CSF *in vitro* either, and the infection in rabbits is self-curing (6). In our laboratory we found that *Streptococcus pneumoniae* had an average generation or doubling time of 21 minutes in broth (7). The doubling time increased to 37 minutes in *ex vivo* CSF (7) and to 67 minutes in CSF in infected rabbits (8). Even though part of the observed difference between the animals and the test tube may be due to a loss of bacteria into the circulation *in vivo* (9), it appears that bacteria do grow more slowly in CSF than in broth under optimal conditions. This may be of clinical importance for the efficacy of certain antibiotics (beta-lactams) which exert their maximal bactericidal activity during rapid growth of the infecting organism (10).

The explanation for this reduced bacterial growth rate in CSF is not clear and several reasons are possible. CSF does lack some essential nutrients, such as iron (11), which are present in sufficient amounts in broth. Moreover, rabbits with pneumococcal meningitis generate high body temperatures of 41°C which may slow the cell growth of the heat-sensitive pneumococci (12, 13). In our laboratory we found that rabbits whose hyperthermic reaction was suppressed by using a long-lasting anesthetic (urethane) developed higher bacterial titers in CSF than control rabbits who were unanesthetized and developed high degrees of fever during the infection (author's unpublished data).

Host Defense in CSF

In contrast to the CSF itself, specific host defense mechanisms do not substantially affect multiplication of meningeal pathogens in the subarachnoidal space. The interactions between microorganism and host defenses have been studied in detail in pneumococcal meningitis in rabbits. Bacterial growth rates and maximal bacterial counts were similar in normal and neutropenic rabbits with less than 100 WBC/mm^3 in their CSF (8). However, bacterial titers in blood were higher in neutropenic rabbits. Thus, while polymorphonuclear leukocytes seem to play a role in clearing pneumococci from the systemic circulation, they are unable to effectively phagocytize and kill the organisms in the CSF *in vivo.* The explanation for this function deficiency of the WBC in CSF has been clarified recently in part. The presence of sufficient amounts of type-specific anticapsular antibodies and complement are required for maximal phagocytosis of the encapsulated pneumococci (14, 15). Concentrations of both immunoglobulins and complement are low in normal and infected CSF (16–20). While the spleen provides the major clearance mechanism for poorly opsonized or particularly virulent pneumococci in the systemic circulation (21), no such back-up system exists in the subarachnoidal space. Recent studies by *Scheld* and colleagues on rabbits have confirmed the essential role of antibodies and complement for the effective phagocytosis and killing of pneumococci in CSF (22). When serum of immunized rabbits was injected repeatedly into the *cisterna magna* of infected rabbits, the animals were able to rapidly clear the organisms from the CSF. This effect was not observed after the injection of non-immune serum or after the immune serum had been pre-absorbed with the infecting strain. We also found that pneumococci grew more slowly and reached lower final titers in the CSF of animals previously immunized with live organisms (23). Both studies confirm the critical role of type-specific antibodies in CSF. Complement levels are also very low in normal CSF, and although the concentration increases during meningitis, the complement-mediated opsonic activity of infected CSF remains undetectable or far below values observed in se-

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rum in humans and rabbits with bacterial meningitis (24, 25). One mechanism responsible for the low complement activity in infected CSF may be the destruction of functional complement by bacterial or leukocytic proteases *in vivo*. A similar observation has been made in pleural empyema fluid, where leukocyte neutral proteases destroy complement at the site of the infection (26). Recent studies in rabbits suggest that a similar process may be involved in the CSF (22). When a non-specific protease inhibitor (phenyl methyl sulfonylfluoride; PMSF) was repeatedly injected into the CSF of rabbits with pneumococcal meningitis, bacterial titers rapidly declined compared to controls, and the CSF was sterilized. PMSF itself did not affect the growth of pneumococci *in vitro,* nor did it influence the degree of pleocytosis in CSF. In summary, low concentrations or the absence of specific antibodies and complement-mediated opsonic activity in infected CSF seem to be major factors responsible for the inability of polymorphonuclear leukocytes to eliminate the infecting organism from the CSF. Although no such studies are available, it is reasonable to assume that similar defects in host defenses – like those shown for pneumococci – are also present for other encapsulated meningeal pathogens.

Alterations of the Blood-brain Barrier during Meningitis

The presence of viable bacteria in the subarachnoidal space has profound effects on the central nervous system. One of the earliest changes involves the integrity of the blood-brain barrier (BBB). This functional barrier restricts the exchange of macromolecules between the intravascular and the extravascular space of the CNS in the normal host. Thus, it is responsible for the low protein concentration in normal CSF and for the limited delivery of most drugs, including antibiotics, to the brain and the CSF (27). Anatomically, the BBB consists in part of neurocapillaries that have tight junctions between the endothelial cells, rare pinocytotic vesicles and many mitochondria in the endothelial cells (28). In contrast, capillaries in other parts of the body are fenestrated and show many pinocytotic vesicles, but few mitochondria. As a consequence of the BBB disruption during meningitis, serum protein extravasates into the CSF and the penetration of drugs is enhanced five to ten-fold (29). In rabbits, an increase in protein is observed 10 to 15 hours after intracisternal infection with *S. pneumoniae* (8). Although the mechanism by which the microorganisms affect the BBB have not been identified specifically, the morphologic changes of the BBB during bacterial meningitis were characterized recently (30). After being isolated, metabolically active brain capillaries were exposed to a culture of *E. coli in vitro*; an opening of the tight junctions and a marked increase in endothelial pinocytotic vesicles could be demonstrated by transmission electron microscopy. The same morphological changes were also noted in brain capillaries of rats sacrificed 17 hours after intracisternal infection with the same organisms. It is likely that these changes, which in fact represent a loss of the characteristics of intact brain capillaries, represent the morphological basis for the decreased integrity of the BBB during meningitis. It should be noted that the changes *in vitro* were mediated by the organisms alone without the presence of WBC's or other mediators of inflammation.

The Inflammatory Response in Bacterial Meningitis

In rabbits, WBC appear in the CSF when the protein concentration of the CSF begins to rise. As the disease progresses, the CSF pleocytosis becomes more pronounced and animals with fully developed disease have CSF leukocyte counts similar to those found in man. In one study in our laboratory, the median WBC count 24 hours after infection was 4,000 WBC/mm^3 with a range of 800 to 27,000 mm^3 (31). Approximately 95% of the WBC in CSF are polymorphonuclear leucocytes, which are characteristically eosinophilic in rabbits.

The chemotactic stimuli in infected CSF, which cause the attraction of WBC, have been identified. *Nolan* and colleagues (32) first examined the chemotactic activity in CSF of rabbits with pneumococcal meningitis. CSF of uninfected animals showed no chemotactic activity, whereas CSF from infected rabbits exhibited progressively increasing activity over 72 hours. These authors identified two chemotactic components in CSF. One appeared to be produced by the infecting organism itself, since it could be partially blocked by specific pneumococcal antiserum. The other product appeared to be a protein with characteristics very similiar to the potent chemotactic complement component C5a. Recent studies in our laboratory established the essential role of C5a as a major chemotactic factor in the CSF of rabbits with pneumococcal meningitis (33).

McAllister et al. (34) used a rabbit model of pneumococcal meningitis to quantitate the inflammatory response in the subarachnoidal space by computer assisted histological techniques. Maximal inflammatory mass was present on the ventral surface of the brain in the region of the posterior cerebrum, the proximal brain stem and the cerebellopontine portion. The inflammation increased progressively and reached a maximum intensity after about 72 hours. The inflammatory mass, determined by microscopy, correlated closely with the concentration of LDH in CSF, suggesting a possible role for the determination of the LDH concentration in estimating the degree of inflammation in the subarachnoidal space. In a subsequent study, the same group of investigators found that methylprednisolone reduced the inflammatory mass in the course of the disease (35). The effect of the steroids was dose-dependent and appeared to be associated with a reduced adhesiveness of the circulating granulocytes to capillary endothelium. Impaired adhesiveness reduces the ability of the WBC to leave the vascular bed and gain access to the site of the infection. The reduction in inflammation resulting from the steroid therapy was paralleled by a reduced LDH concentration in the CSF. On the other hand, the total WBC count in the CSF was not reduced

by the steroid treatment. This indicates that WBC counts in the CSF may not reflect the degree of inflammation in meningitis accurately, at least when the natural course of the disease is altered by anti-inflammatory drugs. The slow response of CSF WBC's was confirmed in our studies in which we found no decrease in the WBC count in the CSF 24 hours after beginning antibiotic treatment which rapidly sterilized the CSF (31).

The pathophysiological consequences of the presence of WBC's in the subarachnoidal space are conflicting. As previously discussed, the influence of polymorphonuclear leukocytes on bacterial multiplication is negligible in the absence of opsonizing antibodies (8, 23). Nevertheless some clinical and experimental data suggest that a very low WBC count in CSF in the presence of high bacterial titers is a bad prognostic sign, perhaps indicating some protective role of the WBC's (36–39). The reasons for this discrepancy have not been studied. The majority of experimental studies which have examined the role of the inflammation in the CSF not only failed to show a protective role, but actually demonstrated that leukocytes had a harmful effect on brain tissue. In the study by *McAllister* et al. (34), the time of the death of rabbits with pneumococcal meningitis correlated with the time of maximal inflammation. Those animals which survived longer than average had less inflammation at the time of death. In an early study of pneumococcal meningitis in dogs, neutropenic animals (with less meningeal inflammation) survived for an average of 62 hours, whereas normal dogs survived for an average of only 46 hours (40). We found that neutropenic rabbits with WBC counts below 200/mm^3 in CSF developed less brain edema during pneumococcal infection than normal animals. Moreover, a sterile inflammation (induced by the intracisternal injection of formyl-meth-leu-phen) produced brain edema of a similar degree to that in pneumococcal meningitis. The degree of edema in sterile meningitis closely correlated with the WBC count in CSF (41).

Fishman and his colleagues (42, 43) have demonstrated one possible mechanism by which WBC's can damage the brain. They showed that free unsaturated fatty acids, which originate from the cell membranes of granulocytes and brain cells and are present in high concentrations in pus, induce brain edemas. *In vitro* studies with rat brain slices demonstrated profound changes in the energy metabolism of brain tissue with a shift to anaerobic glycolysis induced by arachidonic, linoleic or linolenic acid. The metabolic changes were associated with cellular swelling. Similar swelling could also be induced when unsaturated fatty acids were injected into the brains of rats. Free oxygen radicals, generated by polymorphonuclear leucocytes during phagocytosis (44), also appear to be involved in the detrimental effects of leukocytes on brain tissue (45). Further studies are needed to clarify the relative importance of these factors in bacterial meningitis.

CSF Lactate and Glucose during Meningitis

Increased lactate and decreased glucose concentrations are closely related changes in bacterial meningitis which can be readily documented in humans and experimental animals (8, 46, 47). The mechanisms responsible for these changes are not fully understood. Early studies in dogs suggest that glucose transport is impaired across the BBB by means of facilitated diffusion (48, 49). It has been suggested that the presence of WBC's is necessary to induce hypoglycorrhachia (40), but this finding has not been confirmed, since neutropenic rabbits develop the same changes in glucose and lactate concentrations in CSF as do normal rabbits (8). The *in vitro* studies mentioned above show that unsaturated fatty acids induce increased glucose utilization and increased lactate production in brain tissue under controlled, constant oxygen tension (42, 43). These studies suggest that stimuli other than decreased oxygen supply can induce a shift of brain metabolism to anaerobic glycolysis. In addition, decreased cerebral blood flow as a consequence of increased intracranial pressure or thrombosis is likely to be a major stimulus for anaerobic brain metabolism. Despite the fact that many aspects of brain metabolism during meningitis need to be clarified, it is reasonable to assume that lactate production in the brain as a consequence of anaerobic glycolysis is the major source of the increased lactate concentrations in CSF during bacterial meningitis (50).

Changes in CSF Hydrodynamics

CSF hydrodynamics and intracranial pressure are profoundly affected by bacterial meningitis. We documented an early and consistent increase in intracranial pressure in rabbits with experimental pneumococcal meningitis (31). Therapy which sterilized CSF rapidly normalized the pressure. Treatment with high-dose dexamethasone but not with methylprednisolone also significantly reduced the CSF pressure (51). We previously examined parameters of CSF hydrodynamics in the same rabbit model with *E. coli* and pneumococcal meningitis (52). Artificial CSF was infused at different rates into the subarachnoidal space of normal and infected rabbits and the outflow resistance of the CSF (across the arachnoidal villi-saggital sinus system) was calculated by determining the intracranial pressure at steady state flow rates. Animals infected with either organism showed a marked increase in outflow resistance compared to controls. After sterilization of the CSF, these changes persisted for more than two weeks, whereas steroids markedly reduced the resistance. These results suggest that the inflammation profoundly affects the function of the arachnoid villi, the main clearance system for CSF from the subarachnoidal space into the saggital venous sinus (53). The mechanism by which steroids affect these changes are not clear. The findings suggest, however, that increased CSF outflow resistance is not the major mechanism for the increased intracranial pressure in pneumococcal meningitis. Although the outflow resistance was normalized by prednisolone in one study, the

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intracranial pressure remained virtually unchanged after administration of the same drug in the other study. However, the observed changes in the CSF hydrodynamics may well provide a basis for the relatively frequent development of non-absorptive hydrocephalus observed after meningitis (54).

Systemic Effects of Meningitis

The effects of meningitis on respiration and circulation have been examined systematically using a rabbit model of experimental meningitis (55). An increase in CSF lactate concentrations and a decrease in pH were followed by spontaneous hyperventilation of all the animals, a possible mechanism to maintain the acid-base homeostasis. At the same time, cardiac output was almost doubled, while peripheral and pulmonary vascular resistance decreased slightly. Ventilation, vascular resistance and blood pressure declined in the last hour before death, which was caused by respiratory arrest in the animals examined until death. Although it is not certain that the increased lactate concentrations in CSF are in fact the cause for the observed alterations, these studies suggest that respiratory disturbances do play an important role in the clinical picture of pneumococcal meningitis.

Conclusions

Our understanding of the pathophysiological processes that occur during bacterial meningitis is at best incomplete. A great amount of the data available at present has been gained by experimental studies in rabbits. Such work has been particularly fruitful in studying the treatment of the disease with antibiotics (56), a topic not covered in this review. Although the development of rational therapeutical strategies has helped, these optimal therapies have not substantially improved the morbidity and mortality of meningitis in the last thirty years (57). We must, therefore, further improve our understanding of the mechanisms by which the bacterial infection in the subarachnoidal space damages the host. The influence of the infecting organism, the inflammatory response and systemic, metabolic and physiological changes in the development of increased intracranial pressure, impaired CSF hydrodynamics, brain dysfunction and brain edema must be further studied. Only a detailed understanding of the different components of the inflammatory response will allow us to develop additional therapeutical strategies, which will eventually lead to a further improvement of the prognosis of bacterial meningitis.

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