Streptococcus penumoniae meningitis

Clinical and experimental studies

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1. INTRODUCTION

Before the introduction of antibiotics (sulphonamides in the 1930's and penicillin 1940's), meningitis due to *Streptococcus pneumoniae* ended without exception in the death of the patients (Netter 1887; Southard and Keene 1906). Several desperate therapeutic attempts such as drainage of cerebrospinal fluid and treatment with optochin, bile salt, or pneumococcal antiserum were performed on experimental basis during the pre-antibiotic period, but without clinical success (Kolmer 1929). Although treatment with antibiotics made *S. pneumoniae* meningitis a curable disease (Appelbaum and Nelson 1945; Finland et al 1938), the morbidity and mortality from the disease have not changed significantly over decades (Figure 1) and remain unacceptably high, despite continuous improvements in intensive care technology and the introduction of new more potent antibiotics (Swartz 2004).

The exact mechanism leading to the devastating outcome of *S. pneumoniae* meningitis is not fully elucidated but may include a direct harmful effect of the pathogen itself and a host immune reaction against the invading pathogen that continues to evolve after the bacteria are killed from antibiotic therapy. Therefore, therapeutic intervention should not only be directed against the invading pathogen, but also against the harmful effects of the host immune response. This has led to an increasing interest in studying the pathogenesis and pathophysiology of bacterial meningitis and to a search for new adjunctive therapeutic strategies to improve the outcome of the disease (for a review: (Koedel et al 2002a; Meli et al 2002; Nau and Bruck 2002)).

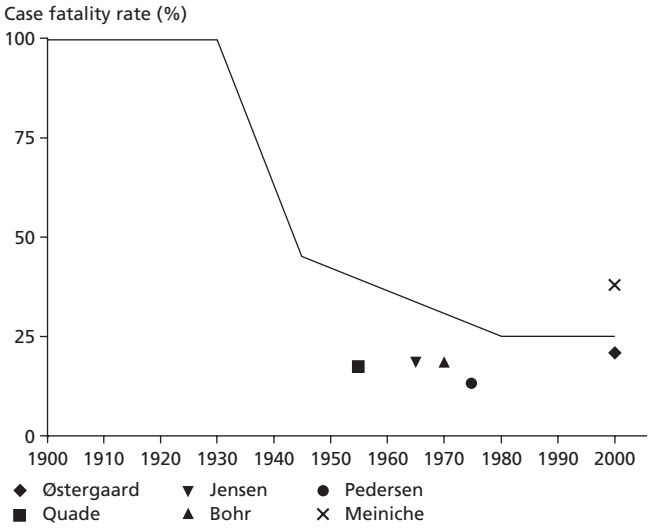

Figure 1. Mortality of pneumococcal meningitis over decades. The figure is reproduced according to (Swartz 2004) and includes case fatality rates from Danish studies.

Although therapeutic intervention – from the clinician's perspective – predominantly should target pathological events occurring late during the course of meningitis, studies of all aspects of the disease may not alone contribute to an increasing knowledge of pathogenesis and pathophysiology of bacterial meningitis, but also to a better outcome of the disease. Indeed, further progress may rather come from prevention of pneumococcal meningitis as has been the case with *Haemophilus influenzae* meningitis and from early identification of risk factors and predisposing conditions than from improvements in the treatment regimens (Swartz 2004). Strikingly, most therapeutic interventions used for more than a half century in the treatment of patients with bacterial meningitis (e.g. agents, dose and duration of antibiotic therapy (Prasad et al 2004), fluid restriction (Møller et al 2001a; Oates-Whitehead et al 2005), osmotic therapy (Nau 2000), hyperventilation (Ashwal et al 1994)) are not based on randomised clinical trials and are still controversial (Tunkel et al 2004), and also recent results of the efficacy of adjunctive therapy with dexamethasone are conflicting (de Gans and van de Beek 2002; Molyneux et al 2002). Therefore, better documentation and new treatment options are still warranted.

The clinical meningitis studies generated until now can primarily be grouped as descriptive and intervention studies. The descriptive studies are most frequent in numbers and include: 1) characterisation of the bacterial aetiology, 2) clinical characteristics including epidemiological data and outcome data, 3) non-invasive measurements (e.g. MR, Laser-Doppler flowmetry), 4) CSF and blood analysis for the evaluation of antibiotic pharmacokinetics, characterisation of pathophysiological mediators and their diagnostic- and prognostic use, and 5) autopsy studies (histopathology and immunohistochemistry). The intervention studies include the treatment efficacy of 1) antibiotics, 2) adjunctive therapy with corticosteroids, and 3) other kind of therapy (e.g. hyperventilation, fluid restriction, osmotic therapy) and are few in numbers, because it demands considerable resources during several years to perform multicentre randomised clinical trials. In addition, there are obvious limitations in clinical meningitis studies due to lack of possibilities for invasive sampling procedures during the disease. Therefore, the use of animal models is essential for a better understanding of the pathogenesis and pathophysiology of pneumococcal meningitis.

Aim of own studies. In continuation of the work presented in the Ph.D. thesis "The inflammatory response in bacterial meningitis. An experimental meningitis model" (Østergaard 2000), which was obtained in the rabbit meningitis model, we wanted to further address various aspects of the pathogenesis and pathophysiology of bacterial meningitis with special focus on pneumococcal meningitis. When appropriate, we wanted to test our research goals and hypothesis using clinical and experimental studies. We wanted to study the following issues:

1. Evaluation of clinical features and prognostic factors (VIII, IX, X, XI).
2. CSF evaluation for the identification of new diagnostic and prognostic tools (I, VI, VII).
3. Pharmacokinetic and pharmacodynamic study of potential new antibiotics in the treatment of meningitis (II).
4. A further characterisation of the mechanism behind the meningeal inflammatory response (III, IV, V, VIII, XI).
5. A characterisation of histopathological alterations (VIII).

2. EPIDEMIOLOGY

In Denmark, pneumococcal meningitis accounts for approximately 100 cases per year (incidence of ~2/100,000 cases per year) (Østergaard et al 2005), which is 10-1000 times lower than the incidence of

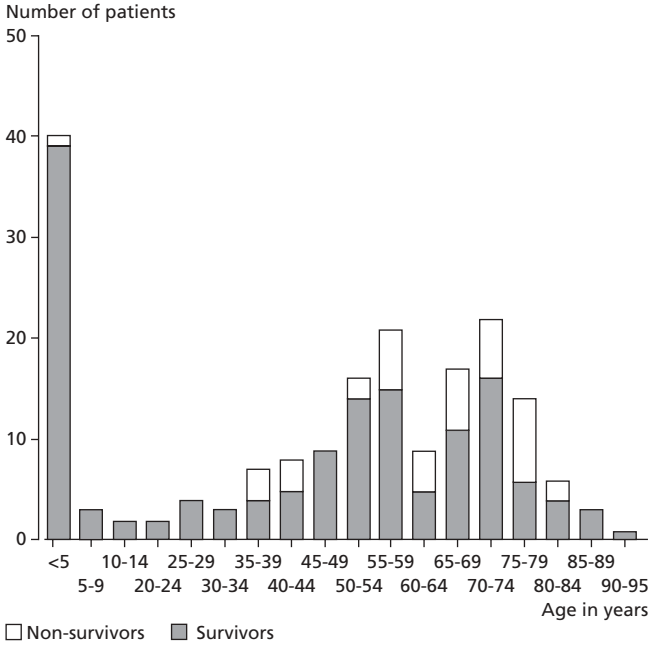

Figure 2. Age distribution and mortality of pneumococcal meningitis in Denmark in 1999-2000.

other invasive pneumococcal diseases (e.g. bacteraemia: ~20/100.000 cases per year (Konradsen and Kaltoft 2002), pneumonia: ~300/100.000 cases per year (Austrian 1981b)). The incidence of pneumococcal meningitis seems not to have varied significantly over decades in Denmark (Kaltoft et al 2000; Konradsen and Kaltoft 2002; Lund 1970; Nielsen and Henrichsen 1993; Østergaard et al 2005; Pedersen and Henrichsen 1983) or to differ significantly between industrilised countries (Eriksson et al 2000; Lexau et al 2005; Rendi-Wagner et al 2004; Weisfelt et al 2006), whereas exact epidemiological data from developing countries still are lacking (Gordon et al 2000).

The incidence of pneumococcal meningitis is highest during the winter season and varies according to age groups with more cases in children under two years of age (~25% of total cases) and in adults with increasing age (Figure 2, (Kaltoft et al 2000; Østergaard et al 2005)). The use of paediatric conjugate vaccine or polysaccharide vaccines to adults may change the epidemiology of meningitis, because the use of pneumococcal vaccines has shown a beneficial effect by reducing the risk of predisposing conditions to pneumococcal meningitis such as bacteraemia (Cutts et al 2005; Jackson et al 2003; Lexau et al 2005), and otitis media (Eskola et al 2001; Prymula et al 2006) and nasopharyngeal carriage (Dagan et al 2002). Indeed, a reduced transmission to non-vaccinated groups (herd immunity) seems to be an important issue for preventing invasive pneumococcal disease (Kyaw et al 2006; McIntosh et al 2005). However, serotype replacement that has been observed after the introduction of pneumococcal conjugate vaccine (Lexau et al 2005) is a cause of concern and should be monitored carefully in future studies.

In Denmark less than 10% of CSF isolates have reduced susceptibility to penicillin (Østergaard et al 2005), whereas high prevalence of penicillin – and cephalosporin resistance has emerged in countries like Australia, Spain, South Africa and USA (Whitney et al 2000). Importantly, the use of pneumococcal vaccines also had an effect by reducing the risk of invasive disease caused by resistant strains (Kyaw et al 2006).

3. CLINICAL PRESENTATION

Predisposing condition for developing pneumococcal meningitis is besides leak of the blood/brain barrier, which is found in ~11% of cases (Østergaard et al 2005) (e.g. oto – and rhinorrhoae after basilar skull fractures (Ratilal et al 2006), cochlear implants (Reefhuis et al 2003)), an increased susceptibility of the host for invasive pneumococcal disease. This includes an impaired immune response against pneumococcal infection (e.g. asplenia, various immune deficiencies, use of immunosuppressing therapy (Fraser et al 1973)), but also recurrent otitis media and day care attendance have been found to dispose for invasive pneumococcal disease (Takala et al 1995). We found that approximately 1 of every 3 cases with pneumococcal meningitis are secondary to an otogenic focus, whereas ~20% are due to a lung focus, ~8% to a sinusitic focus and in ~40% no primary infection focus can be found (Østergaard et al 2005), results which have persistently been observed over decades (Bohr et al 1985; Geiseler et al 1980). The presence of an accompanying focus depended on age groups with a lung focus more frequently observed among adult cases than among children (26% vs. 7%, respectively), whereas a higher proportion of children had no primary infection focus (64% vs. 35% in adult cases). This could reflect the higher prevalence of nasopharyngeal carriage in children than in adults (Leino et al 2001). However, an otogenic focus was as frequent in adults as in children, despite that the incidence of otitis media most likely is significantly higher among children than among adults. Our results also suggested that inadequate antibiotic therapy of otitis media could be a risk factor for developing pneumococcal meningitis (Østergaard et al 2006a).

The classical signs of meningitis (fever, back rigidity, decreased consciousness, and convulsion) are also characteristic clinical findings in pneumococcal meningitis and was found in 96%, 55%, 94%, and 12% of cases, respectively (Østergaard et al 2005), which is in accordance with recent studies in children (Casado-Flores et al 2005) and adults (Weisfelt et al 2006). Other clinical findings on admission include headache (~70% of adults), paresis (~10%), cranial nerve palsies (~10%), papilloedema (~5%), and tense fontanel (~50-70% of young children) (Casado-Flores et al 2005; Weisfelt et al 2006). Since back rigidity was less frequently observed than fever and a decreased consciousness, which are clinical features found in patients with other diseases than meningitis (e.g. sepsis), it might result in differential diagnostic difficulties. Indeed, not all lumbar punctures were performed on admission, and we found that a diagnostic CT-scan was performed in ~10% of cases before lumbar puncture, which delayed the initiation of antibiotic therapy (Østergaard et al 2005). Strikingly, ~57% of Dutch adult cases had a CT-scan before lumbar puncture (Weisfelt et al 2006), which could reflect difference in treatment protocols between countries. Indeed, the majority of Danish adult cases were admitted to internal medicine departments in contrast to The Netherlands, where adult meningitis patients were admitted to neurological departments.

4. CSF EVALUATION

Diagnostic use. Routine examination of the CSF for bacteria, WBC including differential counts, and concentrations of glucose and protein is the primary investigation to diagnose meningitis. Pneumococci can be visualized in Gram staining in ~90% and can be grown from the CSF in 98% of documented cases with pneumococcal meningitis, and in addition a positive blood culture is observed in ~67% of cases (Gray and Fedorko 1992; Østergaard et al 2005). However, in ~40% of a patient cohort with purulent meningitis admitted to a Danish hospital, the causative pathogen was not detected (Østergaard et al 2002b; Østergaard et al 2004a). Improvements in molecular techniques have emerged in the microbiological laboratory during the last two decades, but until now polymerase chain reaction (PCR) techniques have not improved the sensitivity in the detection of CSF bacterial pathogens (Corless et al 2001), in contrast to the significant improvements that have been obtained in the determination of viral pathogens (Koskiniemi et al 2001). Collection of the CSF in enriched culture media for transport to the microbiological laboratory has been shown to augment the determination of the bacterial pathogen (Lessing and Bowler 1996), and in cases receiving antibiotics before the CSF tap, PCR techniques may have a beneficial role (Cherian et al 1998).

In pneumococcal meningitis, we found that WBC counts varied

between 1-2 cells/μL to up to 50.000 cells/μL (Østergaard et al 2005), whereas the elevation in CSF protein levels and the decrease in CSF glucose levels often were more pronounced in pneumococcal meningitis than in other forms of community-acquired bacterial meningitis including *Neisseria meningitidis* meningitis (Østergaard et al 2002b; Østergaard et al 2004a), however, the results of CSF biochemical analysis are not always conclusive to distinguish bacterial from viral meningitis. Therefore, clinical studies of other CSF candidates (see Table 1 for a selected number of studies) may be useful for differential diagnostic purposes.

We studied IL-8 in meningitis, because it has a key role in neutrophil chemotaxis and recruitment in vitro and in vivo (Harada et al 1994; Smith et al 1991). CSF IL-8 levels were highly elevated en meningitis and were to some degree useful in distinguishing between bacterial and viral meningitis with a sensitivity, specificity and the positive predictive value of ~81%, ~92%, and ~96%, respectively (Østergaard et al 1996). CSF levels of TNFα and IL-1β (Lopez-Cortes et al 1993; Ramilo et al 1990a) and serum levels of CRP (Roine et al 1992) and PCT (Schwarz et al 2000) may also help to distinguish between bacterial and viral meningitis.

Prognostic value. A high number of bacteria in the CSF or a high CSF concentration of bacterial antigens (Feldman 1977; Mertsola et al 1991) as well as a high CSF concentration of the pneumococcal degradation product, lipoteichoic acid have been correlated with a poor outcome of meningitis (Schneider et al 1999). In pneumococcal meningitis, we found a significant association between poor outcome and alterations in routine CSF analysis (e.g. low number of CSF WBC, low CSF/blood glucose ratio, high CSF protein levels) (Østergaard et al 2005), which is in accordance with some studies (Kastenbauer and Pfister 2003; Weisfelt et al 2006), whereas others have not been able to show such an association (Bohr et al 1985).

Also, we studied YKL-40, a chitinase-related protein, in meningitis, because it had a prognostic value in other diseases such as pneumococcal bacteraemia and cancer (Cintin et al 1999; Kronborg et al 2002). We found that CSF YKL-40 levels were elevated in meningitis – in particular in patients with pneumococcal meningitis – and were related to the severity of the infection (Østergaard et al 2002b). We also studied suPAR, the soluble form of the urokinase-type plasminogen activator receptor, which is involved in proteolysis of the basement membrane and leukocyte migration (Blasi and Carmeliet 2002), because it had a prognostic value in pneumococcal bacteraemia and cancer (Stephens et al 1999; Wittenhagen et al 2004). We found that CSF levels of suPAR were elevated in bacterial meningitis and were correlated to a poor outcome (Østergaard et al 2004a).

In conclusion, clinical meningitis studies of various CSF parameters have contributed to a better understanding of the meningeal inflammatory process. However, several limitations in clinical studies of CSF parameters exist: 1) Patients may have individual immune response, 2) most studies include few and poorly characterised patients, and 3) variation in sampling time occurs during the course of meningitis. Thus, despite that CSF mediators to some degree were useful for diagnostic and prognostic purposes, no study has until now found a single marker that alone can discriminate between bacterial and viral meningitis or that alone predicts the outcome of bacterial meningitis. However, protein array analysis of CSF from patients with meningitis may be an interesting screening method for new diagnostic and prognostic CSF markers (Kastenbauer et al 2005).

5. PROGNOSIS AND OUTCOME

The bacterial pathogen itself is found to be an important determinant for the clinical outcome of meningitis with *S. pneumoniae* resulting in the highest mortality and morbidity among pathogens causing community-acquired meningitis in developed countries (e.g. *S. pneumoniae*: ~25%, *Neisseria meningitidis*: ~5-10% (van de Beek et al 2004)). Among cases with pneumococcal meningitis, we also showed that serotype-related differences in mortality existed (Figure 3, (Østergaard et al 2004b)), whereas the outcome did not rely on the susceptibility of the pneumococcal isolate (Fiore et al 2000; Kellner et al 2002; Østergaard et al 2005). Future testing of clinical pneumococcal CSF isolates for other virulence factors in relation to outcome may determine important targets for new protein based pneumococcal vaccines.

Table 1. Selected studies of CSF inflammatory mediators.

CSF analysis	Authors	Comments
Expression of TNFα and transforming growth factor (TGF)β-1	Ossege et al 1994	In situ hybridisation
Expression of TNFα, interferon (IFN)γ, IL-1, TGFβ, endothelin-1	Rieckmann et al 1995	Semi-quantitative RT-PCR
TNFα	Lopez-Cortes et al 1993	Differential value
Caspase-1	Koedel et al 2002b	
IL-1β,	Mustafa et al 1989	Prognostic value
sTNFαR	Ichiyama et al 1996	Differential and prognostic value
TNFα and TGFβ-1	Ichiyama et al 1997	Prognostic value
IFNγ	Ohga et al 1994	
IL-6	Chavanet et al 1992	
IL-8	Østergaard et al 1996	Differential value
GROα, monocyte chemotactic protein-1, macrophage inflammatory protein (MIP)-1α, and RANTES	Sprenger et al 1996	
IL-10	Frei et al 1993	Differential value
IL-12, IFNγ	Kornelisse et al 1997	
IL-2, sIL-2R	Larsen and Bjerager 1990	
IL-18 and IFNγ	Fassbender et al 1999	
Macrophage migration inhibitory factor	Østergaard et al 2002a	
sCD14	Nockher et al 1999	
sL-selectin and sELAM	Fassbender et al 1997	
Intercellular adhesion molecule (ICAM)-1	Lewczuk et al 1998	
CRP	Stearman and Southgate 1994	
C3	Whittle and Greenwood 1977	
Matrix metalloproteinases (MMP)	Leppert et al 2000	Prognostic value
Nitric oxide	Kornelisse et al 1996	
S-fragtalkine	Kastenbauer et al 2003	
YKL-40	Østergaard et al 2002b	Prognostic value
SuPAR	Østergaard et al 2004a	Prognostic value
Follistin	Michel et al 2000	
α-melanocyte-stimulating hormone	Ichiyama et al 2000	Prognostic value
Glutamate	Spranger et al 1996	Prognostic value
Nitrotyrosine	Kastenbauer et al 2002a	Prognostic value

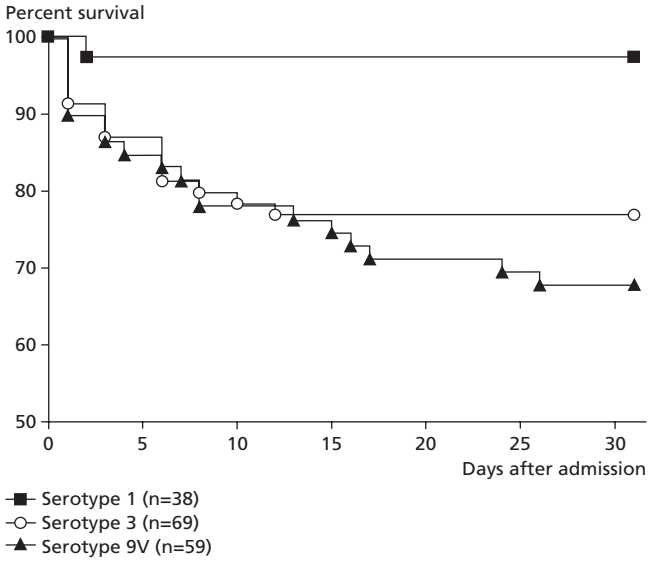

Figure 3. Kaplan-Meier survival curve of pneumococcal meningitis according to serotypes. Significant difference between groups (Log rank test: P=0.0047). Patients infected with serotype 3 and 9V had significantly higher mortality rate than patients infected with serotype 1 (Log rank test P=0.0065 and P=0.0006, respectively).

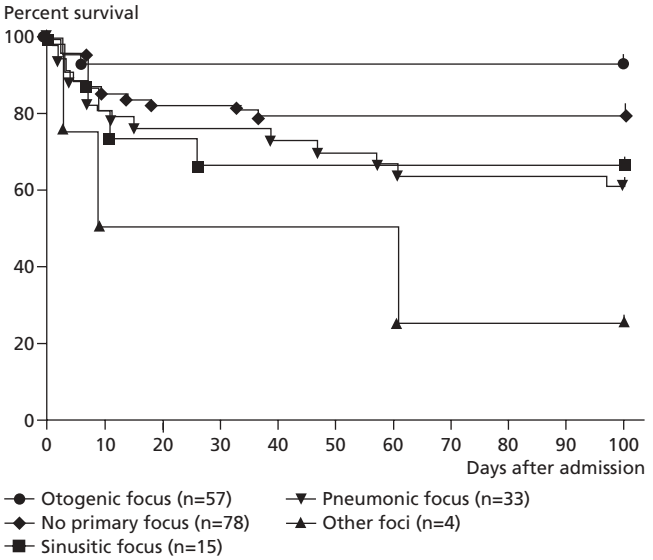

Figure 4. Kaplan-Meier survival curve of pneumococcal meningitis according to the focus of the infection. Significant difference between groups (Log rank test: P=0.0005). Otogenic focus vs. pneumonic focus, sinusitic focus, other foci, and no primary infection focus: P=0.0002, 0.008, <0.0001, and 0.03, respectively. Other foci vs. no primary infection focus: P=0.01

Two out of every three deaths from pneumococcal meningitis occurred within the first week of hospitalisation, but death related to the disease was observed up to 3 months after admission (Østergaard et al 2004b; Østergaard et al 2005; Weisfelt et al 2006). Therefore, our results may suggest a longer study period when studying pneumococcal meningitis than a mortality at 14 days as the endpoint, as previously suggested for studying community-acquired bacterial meningitis (McMillan et al 2001). The causes of death from pneumococcal meningitis were due to both neurological complications (e.g. increases intracranial pressure, brain oedema, cerebral incarceration in ~50%) and to systemic complications (e.g. septic shock, stress haemorrhagic ulcers, multiorgan failure in ~50%), and neurological sequelae such as hearing loss, mental retardation, limp paralysis occurred in up to half of survivors, (Bohr et al 1985; Kastenbauer and Pfister 2003; Østergaard et al 2005; Weisfelt et al 2006).

Several studies have tried to identify risk factors associated with fatal outcome and the development of sequelae from pneumococcal meningitis, but because most studies have been relatively small in size and included different or selected study populations (e.g. adults vs. children or patients admitted to intensive care units (Auburtin et al 2002), respectively), results have differed between studies. However, the case fatality rate of pneumococcal meningitis has consistently been shown to be twice as high in developing countries (Baird et al 1976; Goetghebuer et al 2000; Gordon et al 2000) as in industrialised countries (Østergaard et al 2005), to be higher in adults (Kastenbauer and Pfister 2003; Weisfelt et al 2006) than in children (Casado-Flores et al 2005; Fiore et al 2000; Kornelisse et al 1995; Laxer and Marks 1977), and to be associated with advanced age among adult cases (Bohr et al 1985; Kastenbauer and Pfister 2003; Østergaard et al 2005; Weisfelt et al 2006) (Figure 2). Less consistent findings include an increased risk of fatal outcome in cases, who had an accompanying underlying disease, who had an altered mental status on admission, or who developed complications during hospitalisation (e.g. seizures, need for assisted ventilation), respectively (Bohr et al 1985; Henneberger et al 1983; Kornelisse et al 1995; Østergaard et al 2005; Weisfelt et al 2006; Weiss et al 1967). Moreover, presence of bacteraemia or pneumonia has been associated with increased mortality (Bohr et al 1985; Kastenbauer and Pfister 2003; Laxer and Marks 1977), whereas we showed that presence of an otogenic focus conversely was associated with a lower case fatality rate (Figure 4, (Østergaard et al 2005)). Furthermore, meningitis patients receiving antibiotics prior to admission were found to have a lower mortality than patients, who did not receive antibiotics (Bonsu and Harper 2001; The Research Committee of the BSSI 1995), and a delay in diagnosis and start of antibiotic therapy (e.g. due to CT-scan before diagnostic lumbar puncture) during hospitalisation was associated with worsened outcome (Aronin et al 1998; Østergaard et al 2005), emphasising the importance of prompt initiation of antibiotic therapy.

6. PATHOLOGY

Autopsy studies performed in the pre-antibiotic era (Southard and Keene 1906) and from patients receiving antibiotic therapy (Cairns and Russell 1946; Quade and Kristensen 1962) have shown that pneumococcal meningitis is characterised by inflammation within the subarachnoidal space and along the cerebral vasculature predominantly of neutrophil origin and various degrees of histopathological alterations within the brain parenchyma. The inflammatory reaction within the subarahnoidal space appears as purulent exudates on the surface of the brain, and the inflammatory reaction around the vessels appears as artheritis and phlebitis with thrombosis occasionally observed within the vascular lumen. In addition, abscess formation may be found around inflamed vessels affecting the surrounding brain parenchyma. Within the brain parenchyma, brain oedema and signs of cerebral incarceration due to increased intracranial pressure may be found as well as focal cortical necrosis and ischemic lesions. In hippocampus neural apoptosis is observed in the dentate gyrus (Nau et al 1999a). Only a limited number of brain autopsies is available, which represents a selected material from only fatal cases, and which may be of poor technical quality due to the degenerative processes taking place after death, until the brain is preserved. Therefore, animal models are essential for studying the pathogenesis and pathophysiology of bacterial meningitis and in elucidating optimal antibiotic and adjunctive therapies.

7. ANIMAL MODELS

Optimally, the animal model should resemble the natural course of human pneumococcal meningitis (e.g. route of infection, histopathological alteration, systemic complications), but the experimental design may depend on the scientific issue studied and on the end-point monitored. In all experimental animal models, CNS bacterial invasion or inoculation of pneumococci directly into the CNS causes an accumulation of leukocytes within the subarachnoidal

space, increased blood/brain barrier permeability, development of brain oedema and increased intracranial pressure. In contrast, the causes of death and degree of brain damage vary according to the model (O'Reilly et al 2005).

The mouse meningitis model has been used to study the pathogenesis of pneumococcal meningitis, because it has been possible to induce meningitis after intranasal inoculation either with or without the use of hyaluronidase as facilitating agent (Orihuela et al 2003; Zwijnenburg et al 2001). Also, two alternative routes of pneumococcal inoculation (intracisternal (Echchannaoui et al 2002; Koedel et al 2003) and intracerebral (Gerber et al 2001)) have been used. An increasing number of studies using gene-modulated knockout mice have recently been performed and have been very useful in the study of host immune reactions (Paul et al 2003a). Cortical brain damage (Klein et al 2006) and neural apoptosis in hippocampus (Mitchell et al 2004) is observed in this model.

The rabbit meningitis model has been used extensively in studying CSF dynamics (CSF penetration of antibiotic, CSF bacterial growth – or kill rate, CSF components of the meningeal inflammatory response) during the course of pneumococcal meningitis, because sequential tapping is possible (Dacey and Sande 1974). Another advantage of this model is that the immune response phylogenetically is closer to man than for other rodents (Graur et al 1996), and IL-8 is present in rabbits (Harada et al 1993) contrary to in mice and rats. We adjusted this model, so that it was possible to study the meningeal inflammatory response in the early phase of pneumococcal meningitis before and during the start of CSF pleocytosis to identify important regulatory mediators (Østergaard 2000). However, the rabbit model has limitations in studying mortality and brain damage, because a rapid secondary bacteraemia develops, and death primarily occurs due to systemic complications (e.g. septic shock, ARDS) (Stewart 1927). In addition, no brain damage has been found in the cortex (Figure 5), whereas neural apoptosis in hippocampus has been detected (Braun et al 1999; Zysk et al 1996). On the other hand, the rabbit model has been useful in the study of hearing loss due to meningitis (Bhatt et al 1993).

The rat meningitis model. The infant rat meningitis model has consistently shown histopathological alterations close to human pneumococcal meningitis with both cortical and hippocampal involvement (Leib et al 2000; Leib et al 2003). Less consistent results have been obtained in the adult rat model, where we and others have established a model with histological alteration that include significant cortical involvement (e.g. focal cerebral abscess formation and cortical necrosis, (Figure 5)) (Brandt et al 2004; Tauber et al 1992). Others find less significant brain damage and have monitored intracranial pressure, brain oedema and blood/brain barrier permeability as endpoint parameters (Koedel et al 1995). Discrepancies could be due to difference in virulence of the pneumococci used, since this has been crucial for the development of brain damage in our model (Brandt et al 2004). Indeed, distinct differences in degree and pattern of brain damage was observed with the use of different serotypes in the adult rat model (serotype 1: vasculitis and cortical

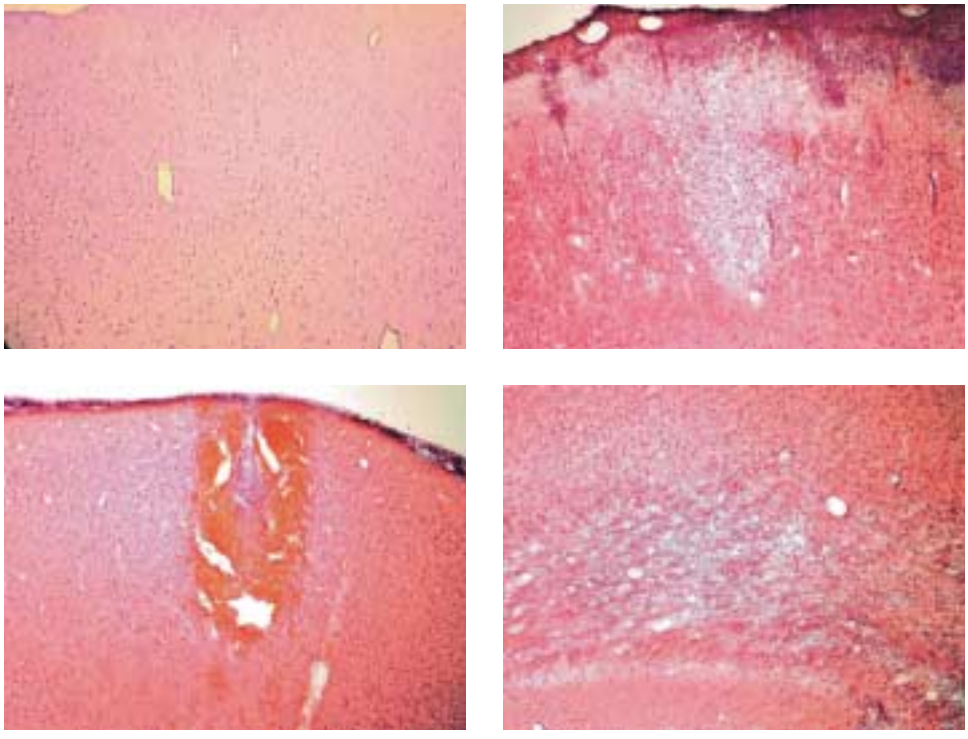

Figure 5. Brain damage in rabbits and rats due to *S. pneumoniae* meningitis. No brain damage was observed in rabbits (upper left), whereas rats had various degree and pattern of cortical involvement, i.e. ischaemia/necrosis (upper right), abscess formation, vasculitis and haemorrhagia (lower feft) as well as subcortical abscess formation (lower right).

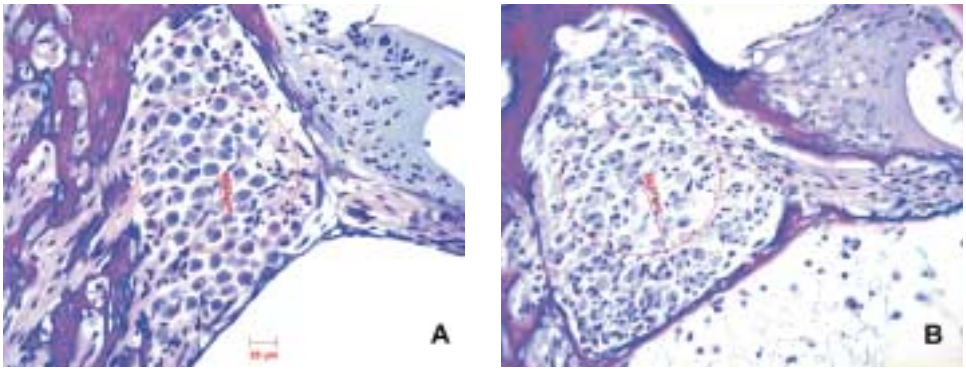

Figure 6. Cochlea damage due to experimental *S. pneumoniae* meningitis in rats. Section of the spiral ganglion from the basal turn of the cochlea. Comparison between a control animal (A) and a G-CSF pre-treated animal (B), which displays a nearly complete loss of spiral ganglion neurons. PAS-alcian blue staining (Brandt et al 2006a).

haemorrhagia, serotype 3: cortical necrosis and abscess formation, and serotype 9V: subcortical (callosal) abscess formation (Østergaard et al 2004b)). Significant hippocampal involvement has not been observed in the adult rat model, which could indicate that adult neurons are less susceptible to neuroexcitatory stress than neonatal neurons. The adult rat model has also been valuable in the study of hearing loss (Brandt et al 2006a; Klein et al 2003) (Figure 6).

8. PATHOGENESIS

Sternberg and Pasteur discovered the *S. pneumoniae* simultaneously in 1881 (for a review: (Austrian 1981a)). Important pathogenic structures of the pneumococcus are the polysaccharide capsule (variation in its antigenic structure allows to distinguish in more than 90 different serotypes according to the Danish nomenclature) and the cell wall. A thorough morphological decription of the various structural components of the pneumococcus has previously been presented in a Danish Doctoral thesis (Sørensen 1995). One important characteristic of the pneumococcus is its ability to adapt to the environment and up- and down regulate several surface structures that help the pneumococcus to survive and even take advantage of host reactions (Gillespie and Balakrishnan 2000). Such a variation between different phases can be visualised phenotypically by its morphological colony appearance on agar plates, and transparent and opaque colonies were found to express large amount of cell wall (e.g. choline binding proteins) and capsule (polysaccharides), respectively (Cundell et al 1995b).

No clinical studies have until now demonstrated the exact route and mechanism for developing pneumococcal meningitis, but theoretically it may either be through direct invasion from a primary infection focus close to the meninges (e.g. ear, sinus, dura disruption) or through haematogenous spread from a distant infection focus (e.g. lung, nasopharynx colonisation). To further address this issue we studied the presence of bacteraemia according to the focus of the infection and found that bacteraemia was observed in ~67% of cases with pneumococcal meningitis with no significant difference between cases with a close or a distant primary infection focus (Østergaard et al 2005), indicating that pneumococcal meningitis predominantly may be introduced haematogenously. However, secondary bacteraemia is found to occur within ~4-8 hours after intracisternal inoculation of animals with pneumococci (Østergaard 2000). Therefore, studies with quantitative cultures of CSF and blood (La Scolea and Dryja 1984) are still needed in cases with pneumococcal meningitis due to different foci to yield additional information about pneumococcal CNS invasion.

It is well-known that the pneumococcal capsule is an important virulence factor, since all invasive pneumococcal isolates are encapsulated to survive the innate immune response within the systemic compartment (Austrian 1981b). Moreover, some serotypes has – from unidentified reasons – more frequently been isolated from patients with meningitis (e.g. 12F, 6B) than from patients with bacteraemia (e.g. 1 and 14 (Hausdorff et al 2000; Konradsen and Kaltoft 2002)). Sparse information about other pneumococcal virulence factors has yet been generated from clinical isolates, among these, strains isolated from patients with pneumococcal infection (including meningitis) were more frequently producing hyaluronidase than carrier strains (Kostyukova et al 1995). However, significant new knowledge about pneumococcal virulence factors has been obtained experimentally. Signature-tagged mutagenesis (Polissi et al 1998) and micro-array analysis (Orihuela et al 2004b) have provided useful screening methods for potential pneumococcal virulence factors that may lead to the design of genetically modulated pneumococcal mutants for further testing in in vitro models and in animal models. Moreover, advances in bioluminescence imaging has provided a useful non-invasive method for studying the progression of pneumococcal meningitis in vivo (Kadurugamuwa et al 2005) and in a mice model it was found that serotype-related differences in the ability to cause meningitis exist after intranasal inoculation of various serotypes (Orihuela et al 2003). Furthermore, a recent study has thoroughly described the role of various virulence factors in the integrated processes taking place from nasopharyngeal colonization eventually leading to CNS invasion (Orihuela et al 2004a):

Colonization and epithelial transmigration. Pneumococci produce IgA proteases able to degrade mucosal IgA, which may protect them from the mucosal immune system (Poulsen et al 1998). Pneumococci produce neuraminidases that enhance epithelial adherence (Tong et al 2000). Pneumococci upregulate cholin-binding proteins (e.g. CbpA) that bind to epithelial polymeric immunoglobulin receptor, which is important for the adherence and the migration of pneumococci through epithelial cells (Cundell et al 1995a; Zhang et al 2000). In experimental meningitis, neuraminidase, pyruvate oxidase and CbpA all contributed to nasopharyngal colonisation, whereas pneumolysin, pyruvate oxidase, and autolysin contributed to further systemic invasion (Orihuela et al 2004a). Also, hyaluronidase facilitated systemic invasion after intranasal inoculation of pneumococci, probably by degrading the basement membrane (Zwijnenburg et al 2001).

Survival in the systemic circulation. For survival in the bloodstream, pneumococci upregulate the expression of capsular polysaccharide (Kim and Weiser 1998) and release pneumolysin, resulting in an attenuation of the innate immune response (Gillespie and Balakrishnan 2000). In experimental meningitis, pneumolysin and autolysin contributed to an increased degree of bacteraemia (Orihuela et al 2004a).

CNS invasion. Upregulation of CbpA facilitates binding to the endothelial platelet activating factor receptor and subsequent transmigration of pneumococci through the endothelial cell into the CNS as shown in vitro (Ring et al 1998) and in experimental meningitis (Orihuela et al 2004a). Once entered the CSF, an exponential growth of pneumococci may occur as shown in experimental meningitis models. However, the CSF is a poor growth media for pneumococci, as shown in Figure 7.

Virulence factors and outcome. A direct causal role of the pneumococcal toxins (e.g. pneumolysin and H2O2, pneumococcal adherence and virulence factor A) for mortality and for the development of hippocampal neural apoptosis has been documented in experimental meningitis using genetically modulated mutants of pneumococci, whereas neuraminidase- and hyaluronidase deficient strains did not influence the outcome (Braun et al 2002; Pracht et al 2005; Wellmer et al 2002). As shown in human pneumococcal meningitis, an association between CSF concentrations of lipoteichoic/teichoic acid and a poor outcome has also been documented experimentally (Gerber et al 2003; Nau et al 1999b).

9. PATHOPHYSIOLOGY

The immune reaction in pneumococcal meningitis against the invading pathogen includes both local – and systemic host inflammatory reactions and involves both innate and adaptive immune responses. An important characteristic of the immune response within the CNS that clearly differentiates it from the systemic immune response is an impaired opsonic – and phagocytic activity (Simberkoff et al 1980) and the incapability of controlling the infection within the CNS compartment, which uniformly leads to the death of the patients. Particularly, the adaptive immune response is impaired within the CNS compartment and active immunization with heat-killed pneumococci intracisternally as well as intravenously did not induce a significant intrathecal antibody production able to attenuate CSF bacterial growth contrary to the significant systemic antibody production after immunization capable of controling the systemic infection (Østergaard et al 2006b; Stewart 1927). Also, the innate immune response is impaired in the CNS compartment (e.g. impaired complement activity (Crosson, Jr. et al 1976; Zwahlen et al 1982)). Therefore, intravenously and/or intrathecally administered serotype-specific antibodies experimentally

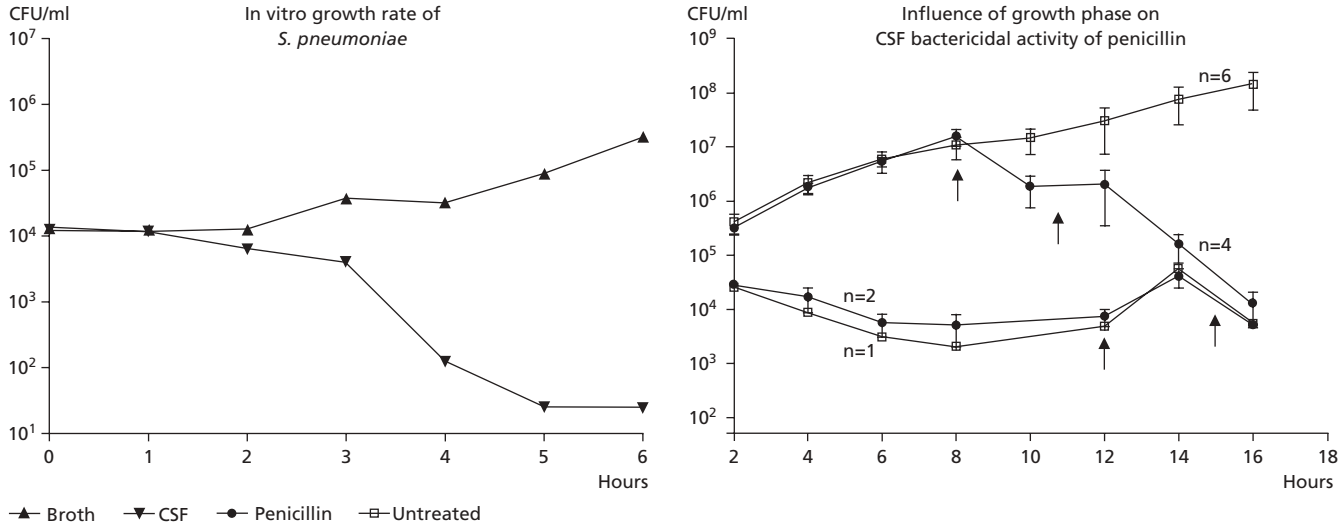

Figure 7. CSF bacterial growth and bactericidal activity of penicillin. A. Time-kill studies of *S. pneumoniae* in broth and in human CSF. B. Bacterial concentrations in CSF from rabbits infected with *S. pneumoniae.* Arrows indicate dosing of penicillin (0.16 MIE/kg as 1. dose and 0.08 MIE/kg as 2. dose).

used in the pre-antibiotic era did not lead to the survival of patients with pneumococcal meningitis (Kolmer 1929), which was in contrast to the better outcome of systemic pneumococcal infection with serum therapy (Avery et al 1917). But besides impaired infection control by the CNS host response, which can be compensated with antibiotic therapy, the host response may cause harmful effects, since injections of heat-killed pneumococci into cisterna magna induced pathophysiological CNS alterations resulting in the death of rabbits (Tuomanen et al 1989).

A complex cascade of events takes place with the activation of the innate immune system that includes recruitment of leukocytes from the systemic compartment and release within the CNS of inflammatory mediators (e.g. cytokines, chemokines, reactive oxygen radicals, excitatory amino acids and proteolytic enzymes). Experimental studies have investigated modulation of the various steps in the inflammatory cascade of pneumococcal meningitis to describe a functional role. These studies include pharmacological intervention and recently a number of studies using knock out mice have been performed.

Activation. Intracisternal inoculation with live or heat-killed pneumococci or various pneumococcal components are capable of inducing a meningeal inflammatory response in animals that mimic the meningeal alterations observed in patients with pneumococcal meningitis (Tuomanen et al 1985a). Various experimental procedures influenced the extent and the pattern of meningeal inflammatory response (e.g. CSF pleocytosis, cytokine kinetics) during experimental pneumococcal meningitis (e.g. inoculum size, the use of heat-killed or living bacteria, serotypes, antibiotic therapy) (Østergaard 2000). For example, inoculation of high inoculum sizes of living serotype 3 (~10^8 CFU = ~10^7 CFU/mL CSF) induced a proinflammatory cytokine response with TNFα and IL-1β preceding the pleocytosis, which was not observed during CSF bacterial growth after inoculation with a low inoculum size (Figure 8). Also, intracisternal injection of pneumococcal cell wall material (> 0.2 μg) and serotype 3 capsule (>200μg) as well as LPS from Gram-negative bacteria induced a proinflammatory cytokine response preceding the pleocytosis ((Tuomanen et al 1985b), Østergaard, C., unpublished data). Moreover, inoculation of heat-killed as compared to living pneumococci resulted in an enhanced proinflammatory cytokine response, however with a significantly attenuated pleocytosis (Figure 8). Various components of the pneumococcal cell wall (teichoic acid and peptidoglycan) have been identified as main inducers of the meningeal inflammatory response during experimental meningitis (Tuomanen et al 1985a).

The molecular explanation for the recognition of pneumococci and the downstream activation of the innate immune response has been studied extensively in vitro and recently in experimental meningitis using knockout mice. LPS binding protein (LBP), an acute phase protein that binds to pneumococcal peptidoglycans causing an activation of the innate immune system through Toll-like-receptor (TLR)-2, played a biological role in pneumococcal meningitis, since LBP was upregulated during pneumococcal meningitis, and LBP-deficient mice had an attenuated pleocytosis that could be restored after intrathecal injection of recombinant LBP as compared to wild type mice (Weber et al 2003). TLR's were upregulated in the CNS during experimental pneumococcal meningitis (TLR-2, 4 and 9 mRNA) (Bottcher et al 2003b), however TLR-2 deficient mice showed no difference in pleocytosis compared to wild type mice (Echchannaoui et al 2002; Koedel et al 2003) suggesting that other TLR's than TLR-2 are involved in activation of the innate immune response in pneumococcal meningitis. Indeed, intracisternal inoculation of bacterial DNA induced pleocytosis primarily of monocytic origin (Deng et al 2001) and most likely through TLR-9 (Hemmi et al 2000). The intracellular transmission of the activation signal from membrane-bound TLR to the nuclear transcriptional factor is mediated through myeloid differentiation factor 88 (MyD88), and in pneumococcal meningitis, MyD88-deficient mice had an attenuated pleocytosis and cytokine response (Koedel et al 2004). The intracellular transcription factor-κB (NFκB) is the final step in the activation by the invading pathogen resulting in the subsequent production of inflammatory mediators and upregulation of adhesion molecules promoting leukocyte recruitment into the CNS, and NFκB was upregulated in experimental pneumococcal meningitis, and inhibition of NFκB attenuated both pleocytosis and CSF cytokine response (Koedel et al 2000).

Adhesion and migration. With activation of NFκB, selectin adhesion molecules are upregulated on the luminal surface of the endothelial cells, which perform a weak and incomplete binding to selectins on the leukocyte resulting in leukocyte rolling along the cerebral vasculature. In experimental pneumococcal meningitis, therapy with the selectin-blocker fucoidin inhibited leukocyte rolling along endothelial cells (Granert et al 1994) and attenuated the pleocytosis (Angstwurm et al 1995; Granert et al 1998; Granert et al 1999; Østergaard et al 2000b). Also, P- and E-selectin deficient mice had decreased pleocytosis after cykine-induced meningitis (Tang et al 1996), and therapy with peptides derived from pertusis toxin, causing a competitive inhibition of the binding to selectins, reduced pleocytosis in experimental pneumococcal meningitis (Rozdzinski et al 1993; Sandros et al 1994).

The next step in the adhesion process is the strong binding between integrins on the leukocytes and immunoglobulins on the endothelial cells resulting in firm attachment. Therapy with mono-

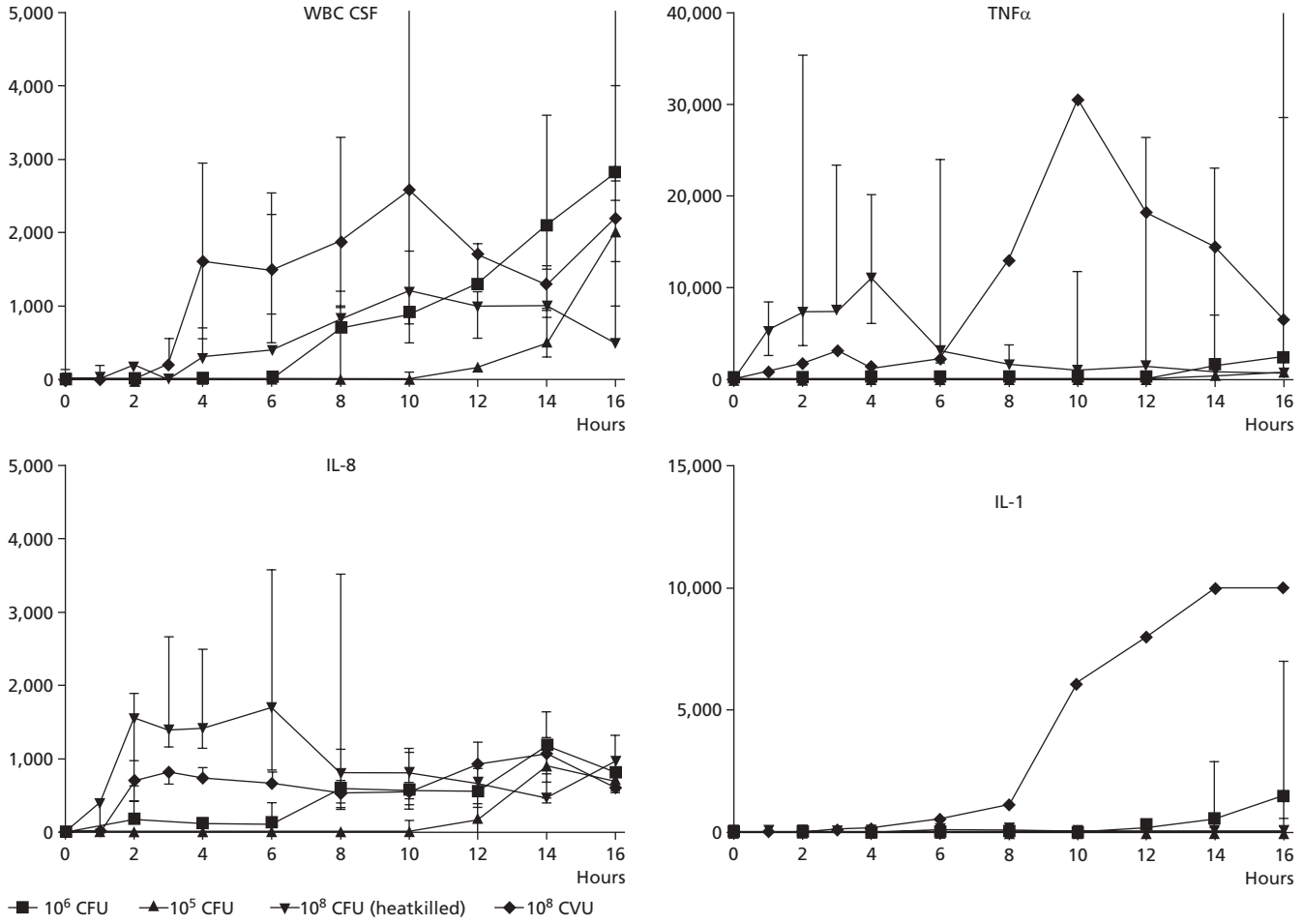

Figure 8. Inoculum size and meningeal inflammatory response during experimental pneumococcal meningitis. CSF kinetics in rabbits infected with *S. pneumoniae* (10^5 CFU (n=7), 10^6 CFU (n=7), 10^8 CFU (n=5), 10^8 CFU heat-killed (n=10)).

clonal antibodies to CD18 (b2 integrin) (Tuomanen et al 1989; Zysk et al 1996) or therapy with peptides derived from filamentous hemagglutinin of Bordatella pertusis, causing an competitive inhibition of binding to integrins, attenuated pleocytosis in experimental pneumococcal meningitis (Rozdzinski et al 1995a; Rozdzinski et al 1995b). Also, therapy with monoclonal antibodies to the endothelial immunoglobulin, CD54 in experimental pneumococcal meningitis (Weber et al 1995) or to junctional adhesion molecule in cytokine-induced meningitis attenuated the pleocytosis (DelMaschio et al 1999).

The transmigration of leukocytes through the endothelial cells into the CNS compartment is induced by local chemotactic gradients (e.g. chemokines as discussed below) and by a release of proteolytic enzymes from the leukocyte (e.g. gelatinase, elastase, uPAR) breaking down the basement membrane on the apical side of the endothelial cell. Intracerebral injection of MMP resulted in blood/brain barrier disruption (Lukes et al 1999), and therapy with MMP inhibitors reduced blood/brain barrier disruption, but not pleocytosis in experimental meningitis (Paul et al 1998), whereas MMP-deficient mice had no significant difference in pleocytosis and blood/brain barrier permeability as compared to wild type mice during pneumococcal meningitis (Bottcher et al 2003a). Also, intracisternal injection of elastase increased blood/brain barrier permeability, but only marginally pleocytosis (Temesvári et al 1995). In contrast, uPAR deficient mice had an attenuated pleocytosis, but no alterations in blood/brain barrier permeability in experimental pneumococcal meningitis, suggesting a chemotactic role of uPAR (Paul et al 2005).

Cytokines and chemokines. Cytokines and chemokines are produced by local cells (e.g. endothelial cells, astrocytes, microglial cells) and by blood-derived leukocytes, and they play a key role in endothelial activation and leukocyte recruitment and function (Tauber and Moser 1999). A functional role of cytokines and chemokines as well as the cellular origin of cytokine production has been investigated in experimental pneumococcal meningitis (Zwijnenburg et al 2006). However, conflicting results have been obtained, which may be due to species differences and/or to differences in methods used.

In the rabbit model, expression of TNFα and IL-1β was primarily observed in mononuclear cells within the cellular infiltrate (Bitsch et al 1997), whereas preliminary results showed that IL-8 was predominantly detected along the cerebral vasculature (C. Østergaard and D. Hougaard, unpublished data). Pleocytosis has been induced with intracisternal injection of rabbit TNFα and IL-1β (Ramilo et al 1990b), human MIP-1 and 2 (Saukkonen et al 1990), whereas rabbit or human IL-8 did not induce pleocytosis (Dumont et al 2000; Østergaard et al 2000a). Also, therapy with monoclonal antibodies against TNFα (i.c.), IL-1β (i.c.) , IL-8 (i.v., but not i.c.) (Dumont et al 2000; Østergaard et al 2000a; Saukkonen et al 1990) or the inhibition of IL-1β by caspase inhibition (Braun et al 1999) attenuated CSF pleocytosis in experimental pneumococcal meningitis. In contrast, therapy with IL-10 did not attenuate pleocytosis but did reduce CSF TNFα levels in experimental meningitis (Paris et al 1997).

In the rat model, various cytokines are upregulated during pneumococcal meningitis (Diab et al 1997). Pleocytosis was induced by injection of human TNFα and IL-1β intracisternally (Quagliarello et al 1991) and intraspinally, but not intracerebrally (Schnell et al 1999), and in experimental pneumococcal meningitis pleocytosis was attenuated by IL-10 (i.v. but not i.c.) (Koedel et al 1996) and by caspase inhibition (Koedel et al 2002b).

In the mouse model, intracerebral injection of TNFα, IL-1, IL-8, PAF (Andersson et al 1992) and intracisternal injection of MIP-2

and KC (Zwijnenburg et al 2003a) resulted in pleocytosis. However, mice deficient of TNFα or its receptors (Gerber et al 2004; Wellmer et al 2001), mice deficient of IL-1R type 1(Zwijnenburg et al 2003c), mice deficient of IL-10 (Zwijnenburg et al 2003d) and mice deficient of IL-18 (Zwijnenburg et al 2003b), respectively, had no attenuation in CSF pleocytosis in experimental pneumococcal meningitis. In contrast Caspase-1 deficient mice had attenuated pleocytosis (Koedel et al 2002b), whereas IL-6 deficient mice had enhanced CSF pleocytosis (Paul et al 2003b).

Influence of the systemic infection/inflammation on the meningeal inflammatory response. The CSF pleocytosis is influenced by events taking place within the systemic compartment: There was significant correlation between blood WBC and CSF WBC in patient with pneumococcal meningitis (Østergaard et al 2006b), and in experimental pneumococcal meningitis an attenuated CSF pleocytosis was observed with the induction of leukopenia (Ernst et al 1983) or after depletion of mononuclear cells in the systemic compartment (Zysk et al 1997b), but not after depletion of CNS macrophages (Trostdorf et al 1999). Moreover, an earlier onset of bacteraemia caused an attenuated pleocytosis most likely due to a decrease in number of peripheral leukocytes (Østergaard et al 2006b), and also G-CSF pretreatment resulted in an attenuated pleocytosis most likely due to decreased chemotactic ability of leukocytes (Østergaard et al 1999).

In meningitis patients, high TNFα and IL-1β levels were detected in the CSF with low or not detectable corresponding levels in the blood, whereas no pleocytosis was observed during bacteraemia with significantly lower CSF cytokine levels as compared to blood levels (Waage et al 1989). In further support that cytokines are released locally in the CSF during meningitis, a net efflux of cytokines from the brain to the blood was observed in patients with pneumococcal meningitis (Møller et al 2005) contrary to no observed efflux of cytokines after intravenously injection of LPS (Møller et al 2002). In experimental pneumococcal meningitis, attenuation of the CSF pleocytosis, however, resulted in decreased CSF levels of cytokines predominantly produced by blood-derived cells (e.g. TNFα (Bitsch et al 1997; O'Reilly et al 2007), IL-1 (Østergaard et al 2000b; Zysk et al 1997b), whereas cytokines produced by local cells (e.g. IL-8) responsible for the chemotactic signal were augmented (Østergaard et al 2000b; Østergaard et al 2006b).

Leukocyte activation. With the development of pleocytosis several pathophysiological events occur that include both direct cytotoxic alterations and vascular alterations. The exact causal and temporal role of these events are not completely elucidated, but when entering the CNS, the leukocyte is able to release several activation products in the defence against invading microorganisms (Borregaard 1996). These include reactive oxygen species and nitric oxide, which were markedly elevated during experimental pneumococcal meningitis (Koedel and Pfister 1999). However, such products are not only toxic for the microorganisms but also for the host cells, and besides the effects on brain tissue, nitric oxide and reactive oxygen radicals also affect endothelial cells and cause changes in the tonus of the cerebral vasculature (vasodilatation and vasoconstriction, respectively), as will be described below.

Brain oedema and increased intracranial pressure. With the disruption of the blood/brain barrier that normally forms a tight barrier between the systemic compartment and the CNS, an increased permeability (elevated CSF protein levels) may lead to brain oedema (Quagliarello et al 1986). Blocking of leukocytes entry into the CSF reduced CSF protein levels (Østergaard et al 2000b; Tuomanen et al 1989), whereas a decrease in peripheral WBC was not associated with decreased blood/brain barrier permeability in experimental pneumococcal meningitis (Ernst et al 1983; Østergaard et al 2006b; Tauber et al 1988). Also, an increased outflow resistance most likely caused by the meningeal inflammation may participate in the development of brain oedema (Scheld et al 1980). Recent MR-findings showed that during experimental pneumococcal meningitis, vasogenic (extacellular) oedema is the earliest event observed followed by a shift to cytotoxic (intracellular) oedema (Brandt et al 2006b). Because the rigidity of the cranial cavity limits the expansion of the brain volume, brain oedema may result in an increased intracerebral pressure that may lead to fatal brain herniation and/or a global decrease in cerebral perfusion/blood flow. Osmotic therapy (glycerol, manitol, diuretics) may be tried to reduce ICP, however, the documentation of treatment efficacy is still lacking (van de et al 2006). Also, continuous measurements of ICP after placement of a ventricular shunt with active intervention against elevated ICP levels may be a promising future treatment option (Grande et al 2002; Lindvall et al 2004) in bacterial meningitis.

Cerebral blood flow. After an increase in cerebral blood flow initially (Pfister et al 1990b), further progress in the disease resulted in a global decrease in cerebral blood flow as observed in experimental pneumococcal meningitis (Tauber et al 1991) and in meningitis patients (Paulson et al 1974). A loss of cerebral autoregulation was found in patients with bacterial meningitis (Møller et al 2001b) and in experimental pneumococcal meningitis (Tureen et al 1990). Consequently, the induction of systemic hypotension was recently shown to result in a decrease in global cerebral blood flow during experimental pneumococcal meningitis, whereas an augmentation of the mean arterial blood pressure by norepinephrine increased cerebral blood flow (Pedersen et al 2007). Interestingly, hyperventilation partially restored normal autoregulation in experimental pneumococcal meningitis (Pedersen et al 2007) and in meningitis patients (Møller et al 2000b). Also, hydration status influenced global cerebral perfusion in experimental pneumococcal meningitis (Tureen et al 1992).

Focal cerebral perfusion abnormalities were found in patients with bacterial meningitis (Förderreuther et al 1992; Møller et al 2000a), and the focal nature of brain damage (e.g. wedge-shape necrosis/ischemia around occluded vessels) found in pneumococcal meningitis (Brandt et al 2004) suggest that several mediators locally released during the inflammatory process may influence local cerebral perfusion. Indeed, inhibition of mediators causing vasoconstriction such as superoxide radicals (Auer et al 2000; Koedel and Pfister 1997) and endothelins (Koedel et al 1998; Pfister et al 2000) increased cerebral blood flow and attenuated development of brain damage in experimental pneumococcal meningitis. Inhibition of mediators causing vasodilatation such as nitric oxide (NO) and endothelial NO synthase (NOS) decreased blood flow and increased ischemic brain damage (Koedel et al 1995; Koedel et al 2001), whereas deficiency of inducible NOS was beneficial in experimental pneumococcal meningitis (Winkler et al 2001).

Neurotoxicity. As documented in stroke models, ischemiacally induced elevation in CNS concentrations of neuroexitatory amino acids has been detected (Guerra-Romero et al 1993) and has been shown to contribute to development of brain damage in experimental meningitis (Leib et al 1996b).

10. THERAPY

Antibiotics. The most important step in the treatment of bacterial meningitis is the prompt initiation of antibiotic therapy. Randomised clinical meningitis trials evaluating different antibiotic treatment regimes have been performed (Saez-Llorens et al 2002; Schaad et al 1990), but a Cochrane review showed no difference in clinical outcome between the use of broad-spectrum and narrow-spectrum antibiotics (Prasad et al 2004). Therefore, the choice of antibiotic therapy for treatment of bacterial meningitis depends primarily on local susceptibility patterns for meningeal pathogens, the age of the patient, and on considerations of CSF pharmacokinetic and pharmacodynamic properties of antibiotics. Such pk/pd data have predominantly been obtained from experimental studies using the rabbit meningitis model. Empiric therapy with a third generation cephalosporin in combination with penicillin will cover most meningeal pathogens in Denmark (Meyer et al 2004), whereas in countries with high penicillin – and cephalosporin resistance, recommended

therapy includes the addition of vancomycin and/or rifampicin (Tunkel et al 2004), but also therapy with newer fluoroquinolones (e.g. moxifloxacin) should be considered (Saez-Llorens et al 2002).

CSF penetration. The blood/brain barrier forms a tight membrane that limits free passage of antibiotics into the CNS. The penetration of antibiotics across the blood/brain barrier into the CNS was found to be facilitated by high lipid solubility (lipophilic drugs: fluoroquinolones, rifampicin, metronidazole, hydrophilic drugs: b-lactams) and by low molecular weight (i.e. high: vancomycin, low: fluoroquinolones) of the drug (Nau et al 1994), whereas the role of protein binding has not been fully elucidated. Inhibition of an active efflux pump by probenecid increased the CSF penetration of penicillin (Dacey and Sande 1974). However, the most important factor for the CSF penetration is the meningeal inflammation that may increase the CSF penetration 10-fold (Figure 9) (O'Reilly et al 2005; Østergaard et al 1998; Østergaard et al 2003). The CSF penetration of various antibiotics obtained in the rabbit meningitis model is shown in Figure 10.

CSF bacterial killing. CSF bactericidal activity of antibiotics is compromised because of poor bacterial growth rate in CSF (Figure 7). Therefore, higher antibiotic concentrations are required to obtain maximal bacterial killing within the CSF than in the systemic compartment, also for drugs normally showing minimal concentration-dependent killing (e.g. β-lactams: ~10-100 × the MBC vs. ~4 × the MBC, respectively (Tauber et al 1984)). It is possible to sterilise the CSF within ~10-12 hours after start of antibiotic therapy for most antibiotics in the therapy of pneumococcal meningitis (Kanegaye et al 2001), however, regrowth may occur with premature withdrawal of antibiotic therapy (Østergaard et al 1998). In addition, clinical treatment failures have been observed with vancomycin, which has been explained by a lower CSF penetration of the drug with the resolvement of meningitis and the restoration of the blood/brain barrier integrity (Viladrich et al 1991).

Pd properties. Several aspects influence, why pd properties in bacterial meningitis differ from "normal" pk/pd: 1) The fluctuation in antibiotic concentration is less pronounced in the CSF than in the blood due to a ~3-4 times longer elimination half life in CSF than in serum, resulting in almost steady state pk/pd. 2) Complete sterilization of the infection is needed, because the immune system within the CNS cannot help clearing the bacterial infection, as discussed in detail above.

For concentration-independent antibiotics, $T_{>MBC}$ was the pd parameter correlating best with CSF bacterial killing during ceftriaxone therapy (Lutsar et al 1997). However, treatment with ceftriaxone and other β-lactams still caused additional CSF bacterial killing at CSF peak concentrations 10-100 × MBC (Tauber et al 1984). In contrast to this, therapy with vancomycin caused no additional killing at CSF concentrations 4 × MBC (Ahmed et al 1999).

For concentration-dependent antibiotics (e.g. fluoroquinolones) additional killing occurred at concentrations at least 40 × MBC. AUC/MBC was the pd parameter correlating best with CSF bacterial killing during fluoroquinolone therapy, but regrowth occurred when CSF concentrations fell below the MBC (Lutsar et al 1998; McCoig et al 1999; Østergaard et al 1998).

Combination therapy. Several studies have investigated various combinations of antibiotics. In general, combination of bacteriostatic and bactericidal antibiotics resulted in antagonism (Østergaard et al 2003), whereas combination of bactericidal antibiotics caused synergism in some studies, but indifference in others (Cottagnoud and Tauber 2004). The clinical importance of not combining bacteriostatic and bactericidal antibiotics was documented half a century ago, where therapy with penicillin in combination with aureomycin resulted in a higher mortality than therapy with penicillin alone (Lepper and Dowling 1951). However, recent experimental results showed that such antagonism could be compensated with the use of higher β-lactam doses (Meli et al 2006).

CSF bacterial killing and mortality. Antibiotic induced CSF bacterial killing may cause an increased meningeal inflammatory response (Friedland et al 1995; Tuomanen et al 1987), and the injection of heat-killed bacteria into cisterna magna of rabbits not only induced

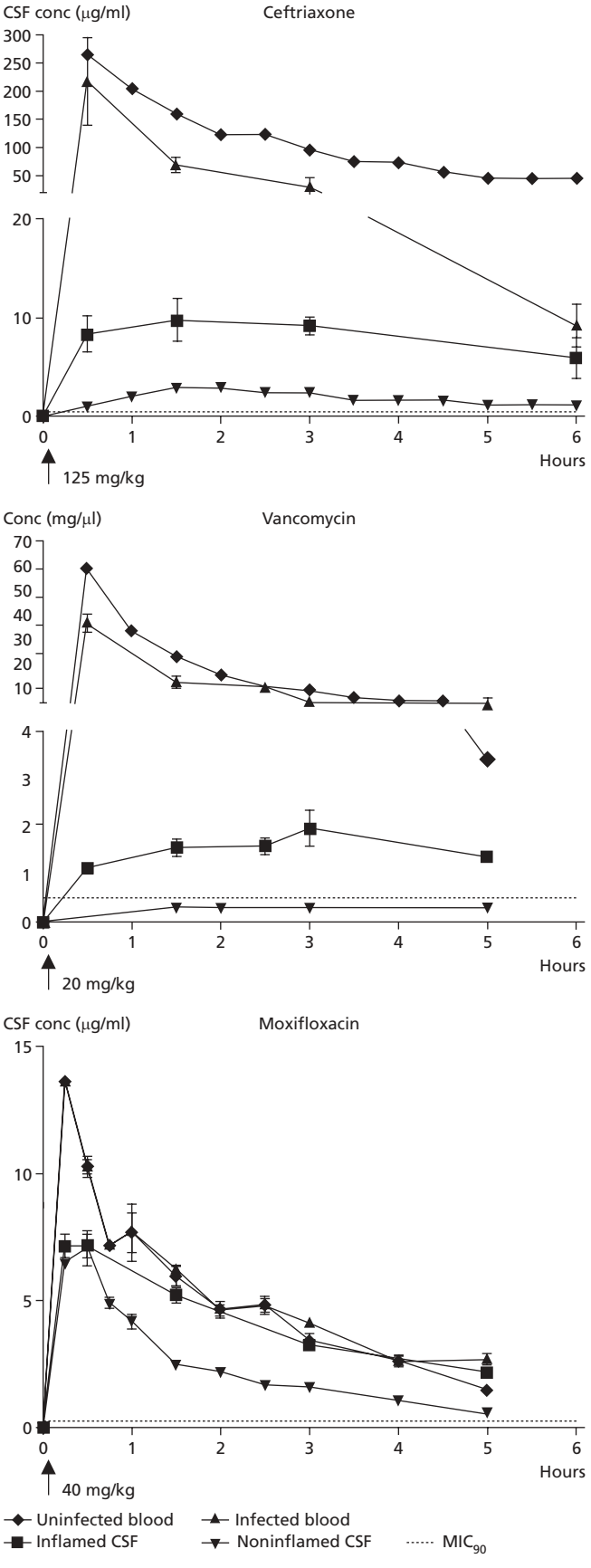

Figure 9. CSF antibiotic penetration through inflamed and non-inflamed meninges in rabbits. The CSF penetation (AUC_{CSF}/AUC_{blood}) of ceftriaxone, vancomycin, and moxifloxacin was 15%, 13%, and 81%, respectively for infected CSF, and 1.9%, 1.1%, and 47%, respectively for uninfected CSF.

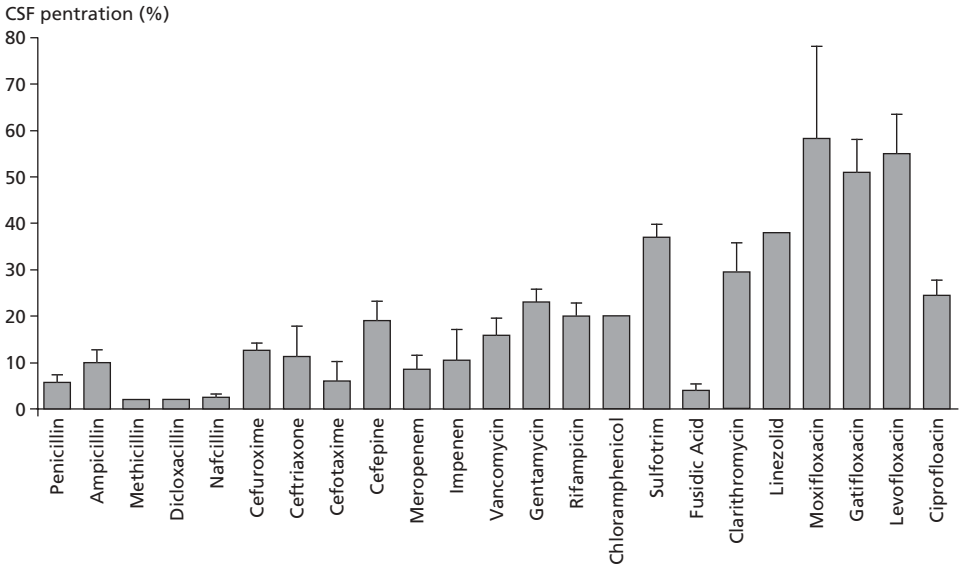

Figure 10. CSF penetration of various antibiotics obtained in the rabbit meningitis model.

a meningeal inflammatory response as discussed above, but also led to the death of the animals (Tuomanen et al 1989). However, the pattern and extent of the meningeal inflammatory response after the initiation of antibiotic therapy are not fully elucidated, and may depend on several parameters such as the time-point for the start of antibiotic therapy (Pfister et al 1994) and the ability of the pathogen to lyse with antibiotic therapy (Tuomanen et al 1988) as well as the antibiotic used. Indeed, some antibiotics induced a high release of bacterial cell wall fragments (e.g. ceftriaxone) during CSF bacterial killing, whereas others that influence the protein synthesis (e.g. rifampicin, clindamycin) did not, but on the other hand, the antibiotic induced release of lipoteichoic acid and teichoic acid within the CSF was lower with antibiotic therapy than with no treatment (Bottcher et al 2004; Spreer et al 2003; Stuertz et al 1999). Treatment with rifampicin or clindamycin in comparison with ceftriaxone reduced brain damage and/or mortality in experimental pneumococcal meningitis (Bottcher et al 2000; Bottcher et al 2004; Nau et al 1999b), whereas treatment with moxifloxacin resulted in a similar mortality as with ceftriaxone therapy (Djukic et al 2005). Therefore, future studies should investigate, whether mortality or degree of brain damage are better parameters for antibiotic efficacy than CSF bactericidal effect for the study of pk/pd in bacterial meningitis.

Adjunctive therapy with corticosteroids. A Cochrane review including 18 randomised clinical trials evaluating corticosteroids in bacterial meningitis of 1853 patients has concluded that dexamethasone has a beneficial effect on mortality in adults and on hearing loss in children (van de Beek et al 2003). Recently, a prospective, randomised European multicentre study on adults showed a beneficial effect of dexamethasone in particular on mortality in pneumococcal meningitis (de Gans and van de Beek 2002), whereas a Malawian study on children did not (Molyneux et al 2002). Including these two trials in the analysis of the efficacy of adjunctive therapy with corticosteroids (C. Østergaard, unpublished data), there was a significant beneficial effect on survival from bacterial meningitis with the use of corticosteroids (86% (1194/1386) vs. 83% (1138/1365), OR: 1.24 (95% C.I.: 1.01-1.53), *P*=0.043). The beneficial effect of dexamethasone on survival was dependent on the bacterial pathogen (*S. pneumoniae*: n=757, OR: 1.48 (1.07-2.07), *P*=0.017; *N. meningitidis*: n=570, OR: 1.33 (0.61-2.90), *P*=0.455; *H. influenzae:* n=810, OR: 1.33 (0.82-2.14), *P*=0.248; other pathogens: n= 100, OR: 0.79 (0.34-1.83), *P*=0.582; unknown pathogen: n=205, OR: 0.75 (0.33-1.69), *P*=0.484), on age groups (adults: n=674 ,OR: 1.92 (1.29-2.86), *P*=0.001; children: n= 2077, OR: 1.04 (0.81-1.33), *P*=0.776), and on socioeconomic status (industrialised countries: n=1427, OR: 1.56 (1.01-2.41), *P*=0.045; developing countries: n= 1235, 1.24 (0.96-1.61), *P*=0.098). Among pneumococcal cases, a beneficial effect was observed only among adult patients (industrialised countries: n=195, OR: 2.26 (1.20-4.29), *P*=0.012; developing countries: n=91, OR: 3.14 (1.31-7.51), *P*=0.01), whereas no significant effect was observed among children (industrialised countries: n=110, OR: 0.71 (0.04-11.7), *P*=0.814; developing countries: n=361, OR: 1.22 (0.78-1.89), *P*=0.389). Moreover, a beneficial effect of dexamethasone may be associated with the risk of dying from the infection (Figure 11), as previously documented for the effect of anti-inflammatory therapy in sepsis studies (Eichacker et al 2002). In addition, most of the beneficial effect of dexamethasone has been related to an effect on systemic complications rather than on neurological complications (van de Beek and de Gans 2004). Therefore, further meningitis studies should evaluate whether lower doses of corticosteroids as used for treatment of septic patients would be the optimal dosing regimens, as has been documented in sepsis (Annane et al 2002).

Anti-inflammatory therapy and outcome. When studying outcome of pneumococcal meningitis using animal models, several factors have to be taken into consideration. 1) The most obvious outcome parameters would be mortality or neurological sequelae (e.g. motor performance, learning deficits, hearing loss). Also, degree of brain damage has been useful due to a direct association with mortality or sequelae (Brandt et al 2004; Leib et al 2003), and because damaged brain tissue will not recover, whereas other end-point parameters such as bacterial concentrations, meningeal inflammation, intracra-

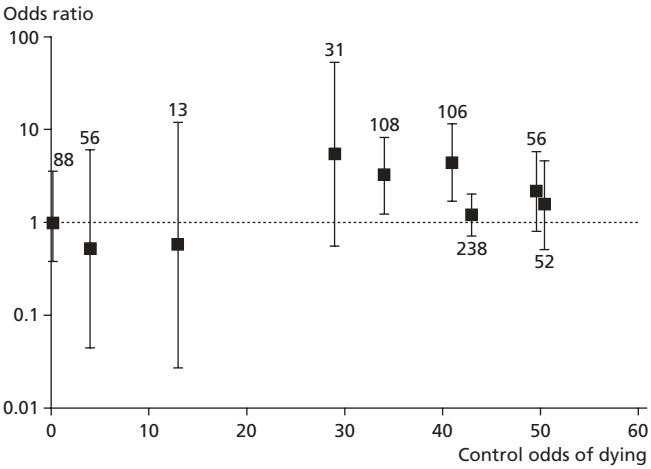

Figure 11. Adjunctive therapy with corticosteroids and mortality rate. The studies shown on the figure include studies from a Cochrane review (van de Beek et al 2003) and studies by (de Gans and van de Beek 2002) and (Molyneux et al 2002).

nial pressure, brain oedema and blood/brain barrier permeability are not always related to mortality and will resolve with the recovery from meningitis (Koedel et al 2004). 2) The animal model should optimally have a similar mortality rate as in human pneumococcal meningitis (~25-30%), because the mortality rate itself has been shown to be of significant importance for the efficacy of anti-inflammatory therapy in experimental sepsis models (Eichacker et al 2002). A beneficial effect of anti-inflammatory treatment in experimental sepsis was only observed with high mortality rates, when high inoculum sizes were used, in contrast to a harmful effect observed in clinical trials with a lower mortality rate. A likely explanation could be that high inoculum sizes induce an overwhelming inflammatory burst (e.g. high CSF cytokine levels), as shown in pneumococcal meningitis as discussed above, which may be an "artificial" situation, where anti-inflammatory treatment in particular may have a beneficial effect. 3) Adult and neonatal animal models may provide conflicting results due to difference in susceptibility to the infection (e.g. neonatal neurons become more likely apoptotic than adult neurons during oxidative stress). Thus, the choice of animal model and endpoint parameters may have significant impact on the results obtained experimentally.

In our laboratory, we have established an adult rat model using inoculum sizes of $\sim 1 \times 10^6$ CFU live pneumococci for studying outcome (Brandt et al 2004), because we believe that this model closely resembles human pneumococcal meningitis. Adjunctive therapy should be initiated during fully developed meningitis and at the time of initiation of antibiotic therapy, when studying the efficacy of adjunctive therapy (e.g. anti-inflammatory treatment). Conversely, experimental designs with initiation of adjunctive therapy at time of infection or with the use of gene-modified animals may be useful for the characterisation of important mediators in the pathophysiology of meningitis, but does not reflect treatment efficacy in a "realistic" situation that may lead to further clinical testing. In conclusion, experimental studies with pharmacological intervention or the use of gene-modified animals in pneumococcal meningitis have shown that anti-inflammatory therapy has detrimental effects in pneumococcal meningitis (Table 2): 1) A reduction in pleocytosis or intracerebral complication by anti-inflammatory therapy may be associated with increased systemic complication due to an impaired host response that may result in a poorer outcome (Brandt et al 2005; Koedel et al 2004). 2) The efficacy of adjunctive therapy may depend on disease stage or bacterial pathogens (Leib et al 1996a), i.e. an inhibition of the vasodilator NO was beneficial in some studies (Koedel et al 1995; Koedel et al 2001), but harmful in other studies (Leib et al 1998; Winkler et al 2001). 3) Hippocampal injury may have another pathogenesis than other IC complications, since therapy with dexamethasone aggravates neural apoptosis in the hippocampus (Leib et al 2003), but reduces other intracranial complications (Koedel et al 1994). Thus, testing of various anti-inflammatory interventions should optimally be addressed in various animal models before further clinical trials.

11. CONCLUSION

In the clinical studies, we found that the most common predisposing focus was otitis media in both adults and children (~30% of cases), and our results suggest that inadequate antibiotic therapy of otitis media may be associated with the development of pneumococcal meningitis. Of the classic signs of meningitis, fever and decreased consciousness were found in almost all patients with pneumococcal meningitis, whereas back rigidity was found only in half the cases, suggesting that meningitis also should be considered in unconscious febrile patients without back rigidity. We found that the case fatality rate of pneumococcal meningitis in Denmark has not improved significantly over half a century, and the death of the patient was due to both neurological and systemical complications. Risk factors for a poor clinical outcome of pneumococcal meningitis were advanced age, the predisposing focus of the infection, the infecting serotype, having a CT-scan prior to lumbar puncture, development of adjacent complications (e.g. convulsion, respiratory insufficiency) and various CSF alterations (low WBC, high protein levels, low CSF/blood glucose ratio), respectively. CSF analysis showed that IL-8 to some degree was useful as diagnostic marker in patients suspected of having meningitis on admission, and YKL-40 and sUPAR were useful as prognostic markers. However, no single CSF parameter alone distinguishes bacterial from viral meningitis or predicts the clinical outcome.

In the experimental studies, we showed that several factors influenced the CSF pleocytosis: 1) the infecting pathogen, 2) blocking of adhesion molecules (the selectin-blocker, fucoidin), 3) inhibition of chemotactic signal (IL-8), 4) inhibition of chemotactic ability of blood leukocytes (G-CSF-pretreatment) and 5) reducing the number of peripheral leukocytes (induced by early onset bacteraemia). Moreover, we documented an accelerated CSF IL-8 production with attenuation of leukocyte entry into the CSF suggesting a local release of IL-8 in the CSF during pneumococcal meningitis. Furthermore, serotype-related differences in histopathological alterations were found, indicating that the pathophysiology may vary according to the serotype. Finally, we showed that moxifloxacin had an excellent CSF penetration through inflamed and uninflamed meninges and excellent bacterial killing suggesting it to be a promising candidate for treatment of bacterial meningitis.

12. PERSPECTIVE

Because the case fatality rate has not decreased significantly over decades, further studies on pneumococcal meningitis are still warranted. The following issues should be addressed in future studies:

A better understanding of risk factors for developing meningitis and for the outcome. The analysis of genetic polymorphism from meningitis cases could identify groups of patients with a high risk for developing meningitis or with a high case fatality rate, and the analysis of pneumococcal isolates from meningitis patients could help identifying other virulence factors than the serotype of importance for both developing meningitis and mortality. Such studies could help the designing of new vaccines and the identification of patients groups with a specific need for vaccination programs. Also, experimental studies using gene-modified animals and genetically modulated pneumococcal mutants could be beneficial in addressing the interaction between various pneumococcal virulence factors and host immune reactions. Finally, international studies on pneumococcal meningitis due to different predisposing infections (e.g. otitis media) could help identifying optimal antibiotic treatment regimes for such infections, which in turn may reduce the risk of developing meningitis.

Improvements in therapy of systemic complications in pneumococcal meningitis. Because approximately half the deaths from pneumococcal meningitis are due to systemic complications, experimental studies addressing this issue are warranted. In particular, studies investigating the effect of passive immunisation with serotype-specific antibodies could result in promising adjunctive treatment options in future clinical trials, but also experimental studies with "sepsis" doses of corticosteroids or with activated protein C would be interesting study issues.

Improvements in the control of cerebral autoregulation in pneumococcal meningitis. Loss of autoregulation and the development of brain oedema are characteristic features of pneumococcal meningitis, however little is known about this issue. Future experimental studies investigating the effect of various therapeutic interventions (e.g. hyperventilation) in restoring the cerebral autoregulation and its impact on preventing brain oedema, increased intracranial pressure, and brain damage are still needed and could lead to improved treatment options. Other potential adjunctive therapies could include erythropoietin and ventricular drainage/continous monitoring of intracranial pressure and pharmacological intervention to reduce the intracranial pressure.

Table 2. Selected outcome studies of immune modulation in experimental pneumococcal meningitis.

Intervention	Animals	Outcome
Activation		
TLR-2 deficiency	Mice	↑mortality (Echchannaoui et al 2002), ↑ICP, ↑BBB permeability (Koedel et al 2003).
MyD88 deficiency	Mice	↑mortality, ↓ICP, ↓ BBB permeability (Koedel et al 2004)
NfκB1 deficiency	Mice	↑mortality (Kastenbauer et al 2004)
NFκB inhibition (pre-treatment)	Rats	↓ICP, ↓BBB permeability (Koedel et al 2000)
Cytokines		
TNFα-deficiency	Mice	↑mortality, ↑HNA, ↑LD (Gerber et al 2004), →mortality, →HNA (Wellmer et al 2001)
Caspase-1 deficiency	Mice	↓ICP, ↓BBB permeability (Koedel et al 2002b)
Caspase-3 inhibition (pre-treatment)	Rats	↓HNA (Gianinazzi et al 2003)
Caspase inhibition (pre-treatment)	Rabbits	↓HNA
(treatment)		→HNA (Braun et al 1999)
IL-1 receptor deficiency	Mice	↑mortality (Zwijnenburg et al 2003c)
IL-6 deficiency	Mice	↓ICP, ↓BBB permeability (Paul et al 2003b)
IL-6 inhibition (pre-treatment and treatment)	Rats	↓ICP (Paul et al 2003b)
IL-10 deficiency	Mice	→mortality (Zwijnenburg et al 2003d)
IL-10 (pre-treatment and treatment)	Rats	↓ICP, ↓BBB permeability (Koedel et al 1996)
IL-18 deficiency	Mice	↓mortality (Zwijnenburg et al 2003b)
TGFβ2 pre-treatment	Rats	↓ICP and ↓BBB permeability (Pfister et al 1992a)
CD95-deficiency	Mice	→mortality and →ICP, →BBB permeability (Paul et al 2004)
Adhesion molecules		
Selectin antagonist (pre-treatment)	Rats	↑mortality, →cortical injury
(treatment)		→mortality, →cortical injury (Brandt et al 2005)
CD11/18 blockage (pre-treatment)	Rabbits	↓mortality and ↓brain oedema (Tuomanen et al 1989), ↓HNA (Braun et al 1999)
ICAM-deficiency	Mice	→HNA (Gerber et al 2001)
Leukocyte activation		
MMP inhibition (pre-treatment)	Rats	↓cortical necrosis (Leib et al 2000)
MMP and TNFα converting enzyme inhibition	Rats	↓mortality, ↓cortical injury, ↓HNA, and ↓LD (Leib et al 2001; Meli et al 2004).
(treatment and pre-treatment)		
MMP-9 deficiency	Mice	→mortality (Bottcher et al 2003a)
Antioxidants		
NAC pre-treatment	Rats	↓mortality, ↓cortical injury (Auer et al 2000), ↓HL (Klein et al 2003)
MnTBAP treatment	Rats	↓ICP (Kastenbauer et al 2002b), ↓HL (Klein et al 2003)
Urate (pre-treatment)	Rats	↓ICP, ↓BBB permeability (Kastenbauer et al 1999)
(treatment)		→ICP (Kastenbauer et al 2002b)
PBN pre-treatment	Rats	↑HNA and ↑LD (Loeffler et al 2001)
Superoxide dismutase pre-treatment and treatment	Rats	↓ICP and ↓brain oedema (Koedel et al 1995; Pfister et al 1990a; Pfister et al 1992c).
DFO pre-treatment	Rats	↓ICP and ↓brain oedema (Pfister et al 1992c), ↓cortical injury (Auer et al 2000)
Lipid peroxidation inhibition (pre-treatment)	Rats	↓ICP, ↓brain oedema (Lorenzl et al 1995), ↓cortical injury (Auer et al 2000)
(treatment)		↓ICP, ↓brain oedema (Lorenzl et al 1995)
Catalase pre-treatment	Rats	↓ICP and ↓brain oedema (Pfister et al 1992b)
NOS inhibition (pre-treatment)	Rats	↓ICP, ↓brain oedema
(treatment)		↑mortality (Haberl et al 1994), ↓ICP and ↓brain oedema (Koedel et al 1995)
eNOS deficiency	Mice	↑ICP, ↑BBB permeability, ↑mortality (Koedel et al 2001)
iNOS deficiency	Mice	↓BBB permeability (Winkler et al 2001)
$P47^{phox}$ deficiency	Mice	↑BBB permeability (Schaper et al 2003)
$Gp91^{phox}$ deficiency	Mice	→BBB permeability (Schaper et al 2003)
Poly(ADP-ribose) polymerase-1 deficiency	Mice	↓BBB permeability and ↓ICP (Koedel et al 2002c)
Corticosteroids		
Methylprednisolone (pre-treatment, treatment)	Rats	↓ICP, ↓brain oedema (Koedel et al 1994)
Methylprednisolone (treatment)	Rabbits	↓brain oedema (Tauber et al 1985)
Dexamethasone (treatment)	Rats	↑HNA and ↑LD (Leib et al 2003)
Dexamethasone (treatment)	Rabbits	↑HNA (Zysk et al 1996), ↓HL (Bhatt et al 1995), ↓brain oedema, ↓ICP (Tauber et al 1985)
Neuroexitation		
Glutamine synthetase inhibition (treatment)	Rabbits	↑HNA (Tumani et al 2000)
Glutamate receptor antagonist (pre-treatment)	Rats	→HNA (Kolarova et al 2003)
Autoregulation		
Endothelin-B-receptor deficiency	Rats	↑HNA (Ehrenreich et al 2000)
Endothelin antagonist (pre-treatment)	Rats	↓cortical injury (Pfister et al 2000) and ↓ICP, ↓brain oedema (Koedel et al 1998)
Bradykinin receptor antagonist (treatment)	Rats	↓ICP and ↓brain oedema (Lorenzl et al 1996)
Other types of immune modulation		
UPAR- and tPA-deficiency	Mice	→ICP, →BBB permeability (Paul et al 2005).
M-CSF deficiency	Mice	→HNA (Gerber et al 2001)
G-CSF (pre-treatment)	Rats	↓mortality and ↓cortical injury (Brandt et al 2004), ↑HL (Brandt et al 2006a)
(treatment)		→mortality and →cortical injury (Brandt et al 2004), →HL (Brandt et al 2006a)
α-melatonin stimulating hormone (treatment)	Rats	→ICP, →BBB permeability (Kastenbauer et al 2001)
Melatonin pre-treatment	Rabbits	↓HNA (Gerber et al 2005)
Pentoxifylline treatment	Rabbits	→HNA (Zysk et al 1997a)
Indomethacin (pre-treatment)	Rabbits	↓brain oedema (Tureen et al 1991)
Indomethacin (pre-treatment)	Rats	↓brain oedema (Pfister et al 1990a)
Bcl-2 deficiency	Mice	↑mortality, →HNA (Wellmer et al 2004)

A better understanding of the pathophysiological events leading to hearing loss in pneumococcal meningitis. The relative significance of the meningeal pathogen and host inflammatory reactions for the development of hearing loss in pneumococcal meningitis are not fully elucidated. Serotype-related differences or other pneumococcal virulence factors may be of importance, and anti-inflammatory treatment (e.g. blocking of leukocyte recruitment) could be a beneficial treatment option.

Introduction of new antibiotics with a favourable pharmacodynamic profile (e.g. causing less inflammation) in the treatment of pneumococcal meningitis. Both experimental and clinical trials are needed to investigate the treatment effect of new antibiotics. In particular, antibiotics inhibiting bacterial protein synthesis should be tested (e.g. fluoroquinolones, clindamycin, and rifampicin).

Use of non-invasive techniques in experimental pneumococcal meningitis. Non-invasive techniques (e.g. bioluminescence) may be important tools in studying the pathogenesis and pathophysiology of pneumococcal meningitis. Also, improvements in MRI-techniques make it possible to monitor disease progress (e.g. blood/brain barrier alterations, cerebral oedema, cerebral blood flow, brain damage) over time in each individual animal. Moreover, marking pneumococci with iron, plasmids etc. – possible to visualise by non-invasive techniques – could give information of the in vivo distribution of pneumococci during the course of meningitis. Furthermore, MRI can detect fluoromolecules and thereby visualise in vivo distribution of fluoroquinolones and interaction with bacteria.

International cooperation between meningitis groups. International cooperation between meningitis groups will be beneficial for future testing of new treatments regimes in pneumococcal meningitis, because the effect of anti-inflammatory therapy may vary between experimental meningitis models.

13. SUMMARY

This thesis is based on the work performed during a 10-year period at Statens Serum Institut and Department of Infectious Diseases, Hvidovre Hospital. The investigations were performed as both clinical and experimental studies.

Based on national registration of pneumococcal meningitis cases, we showed in clinical studies that the incidence of pneumococcal meningitis was ~2/100.000 cases per year, and the most common predisposing infection focus was otitis media in ~30% of cases, results comparable with previous Danish studies. Our results suggest that inadequate antibiotic therapy of otitis media may be associated with the development of pneumococcal meningitis. Fever and decreased consciousness were found in almost all patients with pneumococcal meningitis, whereas the classical meningitis sign back rigidity was found in 1 out of every 2 cases, suggesting that meningitis also should be considered in unconscious febrile patients without back rigidity. We found that the case fatality rate of pneumococcal meningitis in Denmark has not improved significantly over half a century and remains as high as ~21% (adults: ~30% and children: ~5%) and was due to both neurological and systemical complications. Risk factors for a poor clinical outcome of pneumococcal meningitis were advanced age, the predisposing focus of the infection, the infecting serotype, having a CT-scan prior to lumbar puncture, development of adjacent complications (e.g. convulsion, respiratory insufficiency) and various CSF alterations (low WBC, high protein levels, low CSF/blood glucose ratio).

CSF samples obtained from patients admitted to Department of Infectious Diseases, Copenhagen University Hospital Hvidovre suspected for meningitis were analysed for the content of various inflammatory markers and correlated to clinical data to describe, whether such analyses could have diagnostic and prognostic value. Our results showed that the chemotactic factor IL-8 was highly elevated in patients with meningitis and that IL-8 to some degree was useful as diagnostic marker. YKL-40 and sUPAR, inflammatory prognostic markers in other infections and cancer diseases, were elevated in CSF from meningitis patients and were to some degree useful as prognostic markers. However no single CSF parameter alone distinguishes bacterial from viral meningitis or predicts the clinical outcome.

In experimental studies using a rabbit meningitis model, we studied various mechanism leading to the influx of leukocytes into the CSF and found that several factors influenced the CSF pleocytosis: 1) the infecting pneumococcal serotype, 2) blocking of adhesion molecules (the selectin-blocker fucoidin), 3) inhibition of chemotactic signal (IL-8), 4) inhibition of chemotactic ability of blood leukocytes (G-CSF-pretreatment), and 5) decrease in number of peripheral leukocytes due to early onset bacteraemia. We documented an accelerated CSF IL-8 production associated with an attenuated pleocytosis indicating local release of IL-8 into the CSF. Also, we used the rabbit meningitis model for pharmacokinetic and pharmacodynamic studies of antibiotics. We showed that moxifloxacin had an excellent CSF penetration through inflamed and uninflamed meninges and caused an excellent bacterial killing suggesting it to be a promising candidate for treatment of bacterial meningitis.

Because the rabbit model is not suitable for investigating outcome (mortality, sequelae, brain damage), we established a rat meningitis model and found that the degree and pattern of brain damage was dependent on the infecting pneumococcal serotype. This model is excellent to investigate the efficacy of various anti-inflammatory treatments and other therapeutic regimes.

Future progress may rather come from prevention (e.g. vaccination) and early identification and treatment of disposing factors. Optimal future therapeutic treatment strategies should not only involve therapy against intracranial complications but also against systemic complications.

ABBREVIATIONS

LD:	Learning deficit
CSF:	Cerebrospinal fluid
CNS:	Central nervous system
CT:	Computer tomography
MR:	Magnetic resonance
WBC:	White blood cell
IL:	Interleukin
TNF:	Tumour necrosis factor
LPS:	Lipopolysaccharide
BBB:	Blood brain barrier
OR:	Odds ratio
MIC:	Minimal inhibitory concentration
MBC:	Minimal bactericidal concentration
$T_{>MIC}$:	Duration of the concentration curve above MIC
AUC:	Area under the concentration curve
CFU:	Colony forming units
HNA:	Hippocampal neural apoptosis
HL:	Hearing loss
BBB:	Blood/brain barrier
ICP:	Intracranial pressure

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