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Short Communication

Evidence for Intrathecal Synthesis of Alternative Pathway Complement Activation Proteins in Experimental Meningitis

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Complement has been shown to contribute to intrathecal inflammation in bacterial meningitis. However, the cellular source of complement in the infected central nervous system has not been determined. In this study, we analyzed protein and mRNA expression of two alternative pathway complement activation proteins, C3 and factor B, in the brains of mice with *Listeria monocytogenes* meningitis. Complement protein levels were found elevated in the cerebrospinal fluid of infected mice, compared with mock-infected animals. In the course of the disease, enhanced C3 and factor B mRNA expression was detected on pyramidal neurons and Purkinje cells within 6 hours, peaking at 12 hours and then gradually decreasing by 72 hours after infection. In addition, leukocytes infiltrating the subarachnoid space, within 12 to 24 hours, expressed mRNA for C3 and factor B. The cellular infiltration increased dramatically up to 72 hours. Intraperitoneal injection of tumor necrosis factor (TNF)-α up-regulated C3 and factor B mRNA expression on neurons in normal mice, suggesting that TNF-α may represent one cytokine regulating complement expression in this model of bacterial meningitis. However, additional mediators may be involved in regulation of intrathecal complement expression, as infected mice deficient of TNF/lymphotoxin-α genes did not demonstrate attenuated complement expression in the brain. ***(Am J Pathol 1997, 151:897–904)***

Despite the introduction of modern antibiotics,[1] the overall mortality rate in bacterial meningitis has hardly decreased over the last three decades, and the incidence of persistent neurological impairment remains high.[2] As the host inflammatory response within the intrathecal compartment appears, in large part, responsible for adverse outcome in bacterial meningitis,[3–5] it seems reasonable to suggest that further therapeutic improvements in the future will most likely arise from better understanding the basic molecular mechanisms of this disease, rather than from improvements in bactericidal therapy.[4,6]

Several studies suggest that activation of the complement cascade may contribute to intrathecal inflammation in bacterial meningitis, mainly by attracting blood-derived inflammatory cells into the subarachnoid space (SAS). The attraction of leukocytes across the blood-brain barrier (BBB) and the subsequent local release of pro-inflammatory mediators are associated with the development of brain edema, increased intracranial pressure, and loss of cerebrovascular autoregulation, altogether leading to irreversible neuronal damage and adverse outcome in bacterial meningitis.[7–11] Attraction of leukocytes into the SAS may involve chemokines produced by meningeal macrophages and cells of the plexus choroideus. However, as anti-chemokine antibodies only partially neutralize chemotactic activity in the cerebrospinal fluid (CSF) of *Listeria*-infected mice and patients with bacterial meningitis,[12,13] other chemotactic mediators must be involved as well. Two different models of experimental *Streptococcus pneumoniae* meningitis in rabbits have demonstrated the presence of complement-mediated chemotactic activity for neutrophils in the CSF.[14,15] Furthermore, the intracisternal application of the complement fragment C5a induced sterile meningitis in rabbits, characterized

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by a massive extravasation of neutrophils into the SAS and a disintegration of the BBB.[16,17] A recent study from our laboratory demonstrates up-regulation of the C5a receptor (C5aR, CD88) on neurons and infiltrating leukocytes in mice with experimental *L. monocytogenes* meningitis.[18] In humans, elevated CSF levels of complement components C3[19,20] and factor B[19] have been detected in bacterial but not in aseptic meningitis. Furthermore, intrathecal complement activation was demonstrated in patients with bacterial meningitis, based on the presence of increased complement-mediated opsonic activity in the CSF[20] and elevated C3d[20] and C5a[18] CSF levels.

Taken together, these studies emphasize the significance of complement as a potent mediator of intrathecal inflammation in bacterial meningitis. However, the cellular origin of complement proteins in bacterial meningitis has not been evaluated. Complement within the intrathecal compartment may be derived from 1) leakage of serum-derived complement proteins into the SAS across a dysfunctional BBB and 2) intrathecal complement biosynthesis by resident and/or infiltrating cells in response to bacterial antigens or to secondary host-derived mediators. Passive complement leakage across the injured BBB is very likely to occur *in vivo*, as alterations of the BBB integrity represent a hallmark of bacterial meningitis,[3,21] and physiological complement concentrations are several hundred-fold higher in serum than in CSF.[22,23] On the other hand, resident cells of the central nervous system (CNS) are capable of synthesizing an intact and functional complement system on stimulation by different inflammatory mediators (reviewed in Ref. 24), such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α, which are released into the CSF in bacterial meningitis.[25–27] It is therefore possible that intrathecal synthesis may contribute to elevated complement levels in the CNS in bacterial meningitis, in addition to leakage across a dysfunctional BBB.

In the present study, we investigated the expression of the complement components C3 and factor B in the brains of mice with experimental *L. monocytogenes* meningitis. The data demonstrate that both complement proteins are elevated in the CSF of infected mice and that intrathecal C3 and factor B synthesis occurs in the course of the disease. Furthermore, TNF-α, a key cytokine in the pathogenesis of bacterial meningitis,[3,25,26] was identified as a potential mediator of neuronal complement gene expression.

Materials and Methods

Outbred adult female ICR mice (Institut für Zuchthygiene, Tierspital Zürich, Switzerland) were inoculated intracerebrally with 620 colony-forming units (CFU) of *L. monocytogenes*, strain EGD (kindly provided by Dr. R. M. Zinkernagel, Institute of Experimental Immunology, University Hospital, Zürich, Switzerland), diluted in minimal essential medium (MEM) plus 1% heat-inactivated fetal calf serum (FCS), as previously described.[12,25,28] Mock infection was performed by intracerebral inoculation of MEM plus 1% FCS only. Infected mice and mock controls were ether anesthetized and perfused with Ringer solution (Braun Medical, Emmenbrücke, Switzerland) at the time points 6 hours (n = 2), 12 hours (n = 3), 24 hours (n = 3), 36 hours (n = 3), 48 hours (n = 2), and 72 hours (n = 2) after intracerebral injection. Additionally, three mice with inactivation of both the TNF and lymphotoxin-α genes[29] were infected as described above and sacrificed at time points 12, 24, and 48 hours. Furthermore, three normal wild-type animals were injected intraperitoneally with 1 μg of murine recombinant TNF-α (Boehringer Mannheim, Rotkreuz, Switzerland) and sacrificed after 24 hours. After perfusion, brains were quickly removed, immediately covered by Tissue-Tek OCT compound embedding medium (Miles, Elkhart, IN), frozen at −40°C in 2-methylbutane (Fluka Chemie, Buchs, Switzerland), and stored at −70°C until analyzed by immunohistochemistry and *in situ* hybridization.

Immunohistochemical analysis and *in situ* hybridization experiments were performed on 6-μm-thick horizontal cryosections. A modified version of the immunogold-silver technique described by Roth and colleagues[30] was used for staining of infiltrating myeloid cells (anti-mouse CD11b antibodies, Pharmingen, San Diego, CA; 1:100), activated macrophages and microglia (anti-mouse F4/80-antigen antibodies, Serotec, Washington, DC; 1:100), astrocytes (anti-GFAP antibodies, Accurate Chemical Co., Westbury, NY; 1:1000), and neurons (anti-synapsin I antibodies, Chemicon, Temecula, CA; 1:100), as previously described.[18] An isotype-matched primary control antibody (dilution, 1:100) was obtained from Southern Biotechnology, Birmingham, AL. *In situ* hybridization was performed using a nonradioactive method with digoxigenin-labeled riboprobes and detection by alkaline phosphatase, as previously described.[18] The factor B cDNA plasmid was generated by cloning a *Bam*HI-*Kpn*I fragment (145 bp, nucleotides 2161 to 2305, spanning exons 16 to 18) of the full-length factor B cDNA pmBf5[31] into Bluescript SK (Stratagene, La Jolla, CA). Nucleic acid sequencing confirmed orientation and fidelity of the template. The murine C3 cDNA plasmid was prepared as previously described.[32] *In vitro* transcription was performed using an RNA transcription kit (Promega, Madison, WI) and digoxigenin-labeled UTP (Boehringer Mannheim, Mannheim, Germany) for generation of murine C3 and factor B anti-sense and sense riboprobes.

Murine CSF samples were collected by puncture of the cisterna magna, as previously described.[12,25,28] Samples of 2 to 10 animals were pooled, centrifuged, and supernatants were stored at −20°C until assayed for C3 and factor B protein levels. Before analyzing the pooled CSF by Western blot analysis, a cocktail of proteinase inhibitors containing aprotinin (0.3 μmol/L), leupeptin (1 μmol/L), pepstatin (1 μmol/L; Boehringer Mannheim), and EDTA (1 mmol/L; Sigma) (all final concentrations) was added to each CSF sample to prevent endogenous cleavage of complement proteins. The CSF samples (diluted 1:3 to 1:12) and inoculation medium (MEM plus 1% FCS), as a control, were run out on a 12.5% sodium dodecyl sulfate polyacrylamide gel. After transferring the gel onto a nitrocellulose membrane (MSI, Westboro, MA), Western blot analysis was performed using an ECL Western blotting kit (Amersham, Little Chalfont, UK), accord-

ing to the manufacturer's instructions. A polyclonal anti-mouse C3 antibody (Organon Teknika Corp., West Chester, PA) and a monoclonal anti-human factor B antibody (Quidel, San Diego, CA), each diluted 1:1000, were used for detection of C3 and factor B in murine CSF.

Results

Elevated Complement Protein Levels in Infected CSF

Mice inoculated intracerebrally with *L. monocytogenes* developed symptoms within 8 to 12 hours and died within 4 days after infection, as described in previous studies.[25,28] Western blot analysis of pooled murine CSF samples demonstrated elevated protein levels of complement components C3 (Figure 1A) and factor B (Figure 1B) in the CSF of infected mice, compared with mock-infected animals, at 12 and 36 hours after intracerebral inoculation of *L. monocytogenes*. In Figure 1A, a protein band at ~110 kd, corresponding to the α-chain of murine C3,[33] was detected in mock- and *Listeria*-infected mice. Markedly elevated C3 levels were detected in the CSF of infected animals at both time points. The multiple smaller bands in the CSF of infected mice likely represent C3 cleavage fragments, suggesting complement activation. We were unable to detect factor B in the CSF of mock-infected mice (Figure 1B). However, in infected CSF, a protein band with an apparent molecular mass between 97 and 100 kd, corresponding to murine factor B,[34] was detected at 12 and 36 hours after infection. No factor B proteolytic fragments were detected in the CSF of infected mice, possibly due to the use of a cross-reactive antibody to human factor B, which may not recognize murine factor B activation fragments. No bands were detected when inoculation medium (MEM plus 1% FCS) was probed by Western blot analysis as a control (data not shown). It should be noted that the differences between mock and infected CSF samples is even more pronounced, as the mock samples were analyzed at a dilution of 1:3 (12 hours) and 1:4 (36 hours), whereas the infected CSF samples were diluted 1:12 (12 and 36 hours).

C3 and Factor B Synthesis by Neurons

In situ hybridization experiments demonstrated the induction of complement synthesis by CNS neurons in response to intracerebral infection with *L. monocytogenes* (Figure 1, C–K). Within 6 hours after infection, enhanced C3 and factor B mRNA was detected on pyramidal neurons of the isocortex, the dentate gyrus, and CA1 to CA3 regions of the hippocampus as well as on Purkinje cells in the cerebellum (data not shown). Maximal intensity of complement expression by neurons was seen 12 hours after inoculation of bacteria (Figure 1, D, F, G, J, and K). Figure 1D shows the expression of C3 mRNA in the cortical neuron layers II to IV of a *Listeria*-infected mouse sacrificed after 12 hours. In contrast, C3 mRNA was hardly detectable in the brains of mock-infected mice, sacrificed at the same time point (Figure 1C). Similarly, the strongest factor B mRNA expression by neurons was seen 12 hours after inoculation of *Listeria*, as shown for neurons in the cortical layer II (Figure 1F), Purkinje cells in the cerebellum (Figure 1J), and pyramidal neurons in the hippocampus (Figure 1K). Similar to C3 expression, only very low factor B transcript signals were detected in the brains of mock-infected mice (Figure 1E, cortex; Figure 1I, cerebellum). At later time points, the intensity of complement mRNA expression by neurons gradually decreased up to 72 hours after infection (see Figure 3F as a representative example and compare with Figure 1J). Immunohistochemical analysis of brain sections was performed to confirm neurons as the cellular source of C3 and factor B expression. Immunogold staining of brain sections using monoclonal anti-synapsin I antibodies demonstrated a similar cellular morphology and distribution pattern as the complement-expressing cells. A representative section is shown for factor B mRNA expression (Figure 1G) and immunogold staining for synapsin on a corresponding section (Figure 1H; same magnification as Figure 1G). Brain sections were also analyzed for glial fibrillary acidic protein and F4/80 antigen expression by immunohistochemistry. We observed a typical scattered astrocytic and microglial staining pattern, very distinct from the morphology of complement-expressing cells (data not shown). Furthermore, C3 and factor B mRNA was detected in typical neuronal cell layers, such as the stratum pyramidale in the hippocampus and the Purkinje cell layer in the cerebellum, as well as in the cortical layers II to IV but never in the stratum moleculare (layer I; see Figure 1). These data demonstrate that neurons represent the main source of complement expression within the brain parenchyma of infected mice.

TNF-Mediated Induction of Neuronal C3 and Factor B Gene Expression

Additional experiments were performed to evaluate a possible role of TNF-α, a pro-inflammatory cytokine released into the CSF of *L. monocytogenes*-infected mice,[25,28] in regulating complement synthesis by neurons. The expression of C3 and factor B mRNA by cortical neurons (not shown) and by Purkinje cells in *Listeria*-infected mice deficient in TNF and LT-α genes (TNF/LT-α $-/-$ mice)[29] was not significantly different from that of infected wild-type mice (Figure 2, A and B, respectively). No transcript signals for C3 and factor B were detected in the brains of mock-infected TNF/LT-α $-/-$ mice (data not shown). Western blot analysis of pooled CSF from infected TNF/LT-α $-/-$ mice revealed elevated C3 and factor B protein levels (data not shown), similar to the pattern seen in the CSF of *Listeria*-infected wild-type mice (Figure 1, A and B). Interestingly, intraperitoneal injection of murine recombinant TNF-α induced factor B (Figure 2D) and C3 (Figure 2E) mRNA expression on CNS neurons of wild-type mice within 24 hours, similar to the expression pattern seen in *Listeria*-infected mice. The low constitutive expression of factor B mRNA in the cortex of a normal wild-type mouse is shown in Figure 2C. Consti-

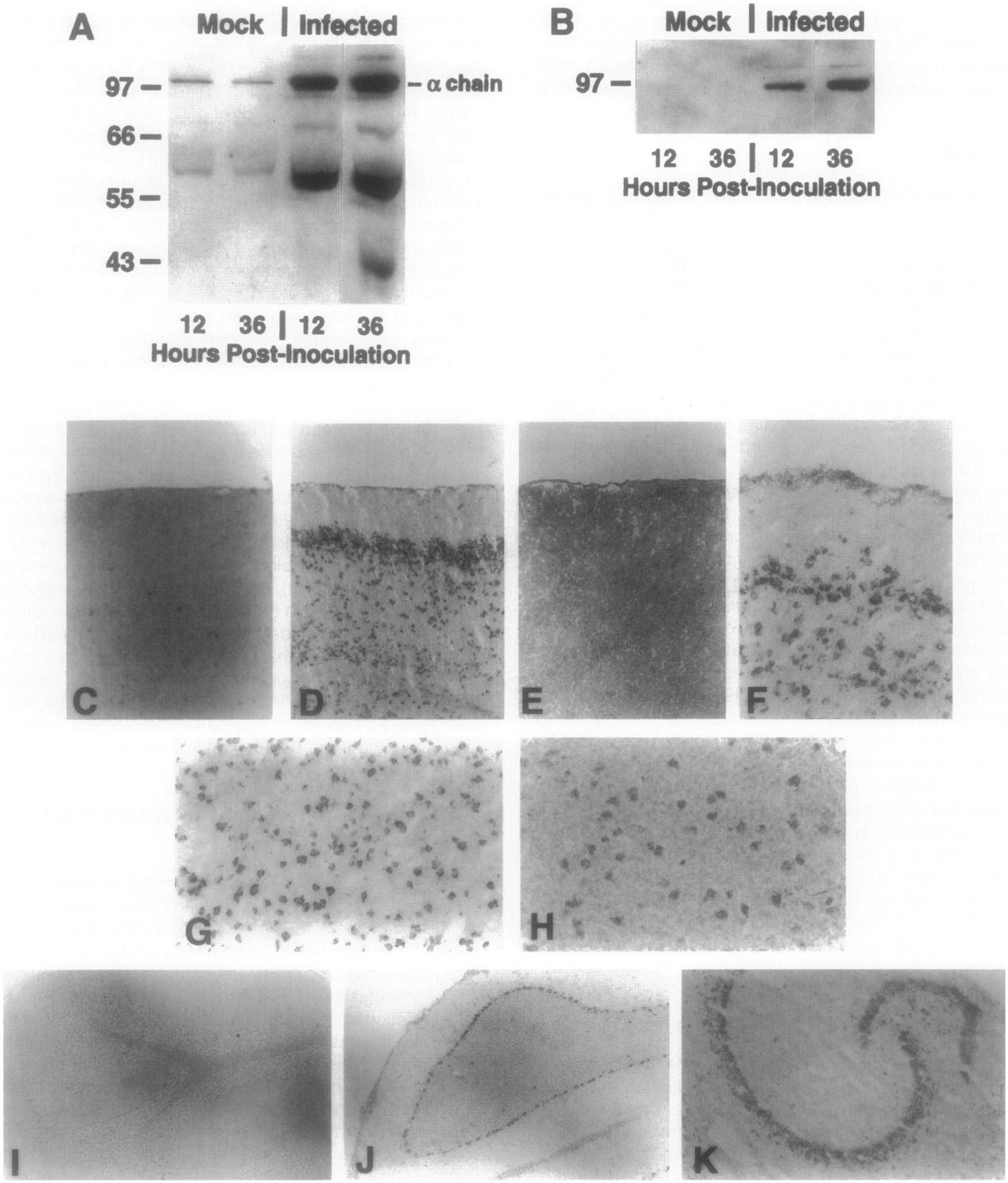

Figure 1. Elevated complement protein CSF levels and complement gene expression by neurons in mice with experimental *L. monocytogenes* meningoencephalitis. **A** and **B:** Western blot analysis of pooled CSF from mice inoculated intracerebrally with *L. monocytogenes* (infected) or medium alone (mock) and sacrificed after 12 or 36 hours. The CSF samples were run at a dilution of 1:3 (mock, 12 hours), 1:4 (mock, 36 hours), and 1:12 (infected, 12 and 36 hours). Complement protein levels were determined using a polyclonal anti-mouse C3 (**A**) and a monoclonal anti-human factor B (**B**) antibody. See text for details. **C** and **D:** *In situ* hybridization of 6-μm-thick brain sections from a mock-infected (**C**) and from a *Listeria*-infected (**D**) mouse sacrificed 12 hours after intracerebral inoculation, hybridized with C3 anti-sense riboprobes. Original magnification, ×20. **E** to **G** and **I** to **K:** *In situ* hybridization, using factor B anti-sense riboprobes, of brain sections from mock-infected (**E** and **I**) and *Listeria*-infected mice (**F**, **G**, **J**, and **K**), sacrificed after 12 hours. Original magnification, ×50 (**E** to **G**) and ×25 (**I** to **K**). **H:** Immunogold histochemistry of a section from the same brain as in **G**, using a monoclonal anti-synapsin I antibody. Original magnification, ×50. See text for explanations.

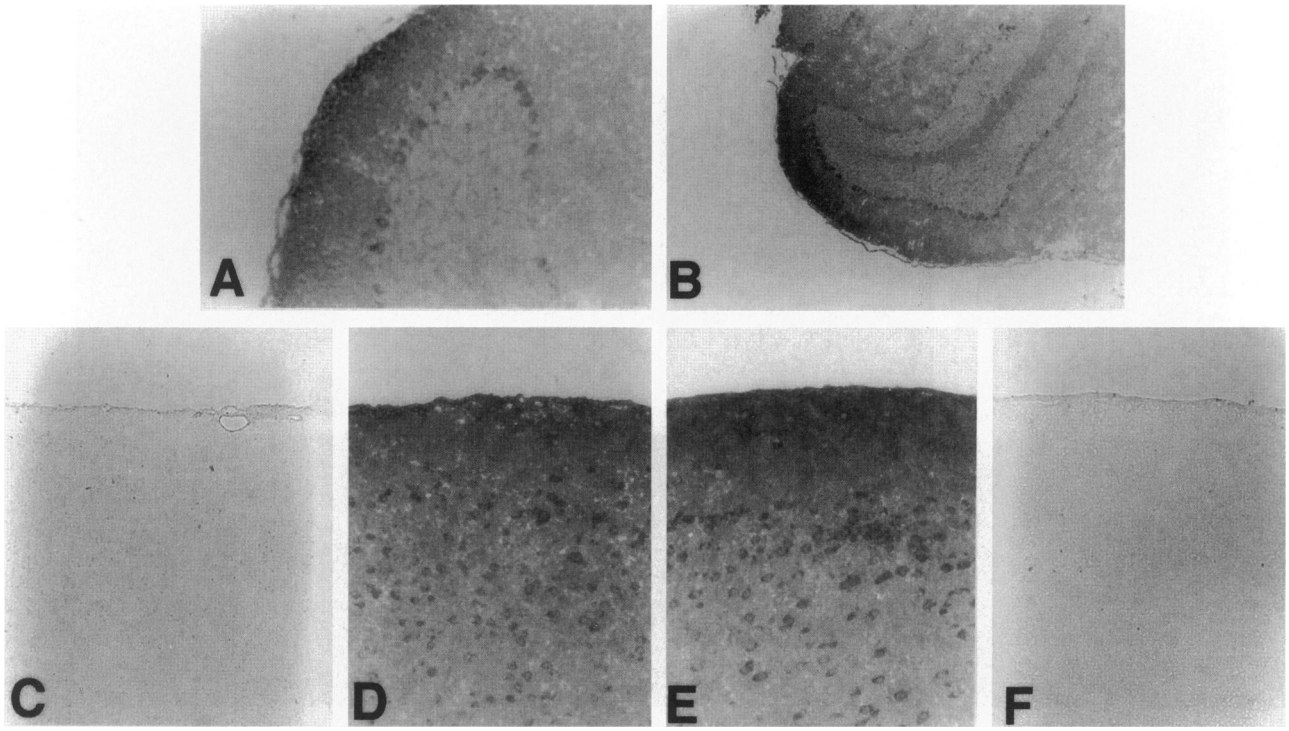

Figure 2. The role of TNF-α in the regulation of complement gene expression by neurons in mice with *L. monocytogenes* meningitis. **A** and **B**: *In situ* hybridization of 6-μm-thick brain sections from *Listeria*-infected TNF/LT-α −/− mice, sacrificed after 24 hours, using C3 (**A**) and factor B (**B**) anti-sense probes. Original magnification, ×50 (**A**) and ×20 (**B**). **C** and **D**: *In situ* hybridization of a brain section from a normal wild-type mouse (**C**) and from a wild-type mouse injected intraperitoneally with 1 μg of murine recombinant TNF-α and sacrificed after 24 hours (**D**), hybridized with factor B anti-sense riboprobes. **E** and **F**: Sections from the same brain as in **D**, hybridized with C3 anti-sense (**E**) and C3 sense riboprobes (**F**). Original magnification, ×50 (**C** to **F**).

tutive C3 expression in the brains of wild-type mice was similarly low (data not shown). No nonspecific signals were detected at any time point when brain sections were hybridized with C3 or factor B sense riboprobes. A representative example is shown in Figure 2F for C3.

Complement Expression by Infiltrating Leukocytes

Intracerebral inoculation of *L. monocytogenes* in mice induced a massive cellular infiltration in the meninges and choroid plexus of the ventricles within 12 to 24 hours, as previously shown.[12,18] The cellular infiltrates increased dramatically at 48 to 72 hours after infection (Figure 3). Staining for the complement receptor type 3 (CR3; CD11b/CD18) by immunohistochemistry demonstrates the myeloid origin of the infiltrating cells (Figure 3, A–C). No infiltrating cells were seen in the brains of mock-infected mice at any time point, as previously demonstrated.[18] Figure 3D shows immunohistochemical analysis for CR3 in the lateral ventricle of a mock-infected mouse, as a representative example. We have previously determined that the infiltrating cells in *Listeria*-infected mouse brains are mainly neutrophils during the first 12 to 48 hours after infection, whereas monocytes/macrophages represent the predominant infiltrating cell type at 72 hours after infection.[12] In the present study, the infiltrating leukocytes expressed C3 and factor B mRNA, as shown for factor B at 72 hours after inoculation of *Listeria* (Figure 3, E–J). The infiltrates in the meninges (Figure 3E) and in the lateral ventricle (Figure 3I) were strongly positive for factor B transcript expression (compare Figure 3E with the control shown in Figure 1E). At 72 hours, some scattered infiltrating CR3-positive cells were detected within the brain parenchyma in the proximity of the meningeal (Figure 3B, arrows) and ventricular infiltrates (Figure 3C, arrows), suggesting intraparenchymal diapedesis by blood-derived macrophages. By *in situ* hybridization, it was determined that these cells also express C3 (not shown) and factor B mRNA (Figure 3, F–I). Figure 3F illustrates the difference of the intensity of complement expression by neurons and infiltrating leukocytes at 72 hours; the factor B message on Purkinje cells (arrows) is clearly attenuated compared with 12 hours after infection (Figure 1J) and distinctly weaker than on the infiltrating cells at 72 hours (Figure 3, F and G; Figure 3G is a twofold magnification of 3F). Figure 3J, furthermore, shows infiltrating cells, expressing factor B mRNA, which may have migrated from a vessel into the cortical parenchyma.

Discussion

Ever since the first description of the presence of complement in the CSF of patients with bacterial meningitis more than 60 years ago,[35] numerous studies have been performed to investigate the significance of the complement system in the pathophysiology of bacterial meningitis (reviewed in Ref. 36). Although elevated comple-

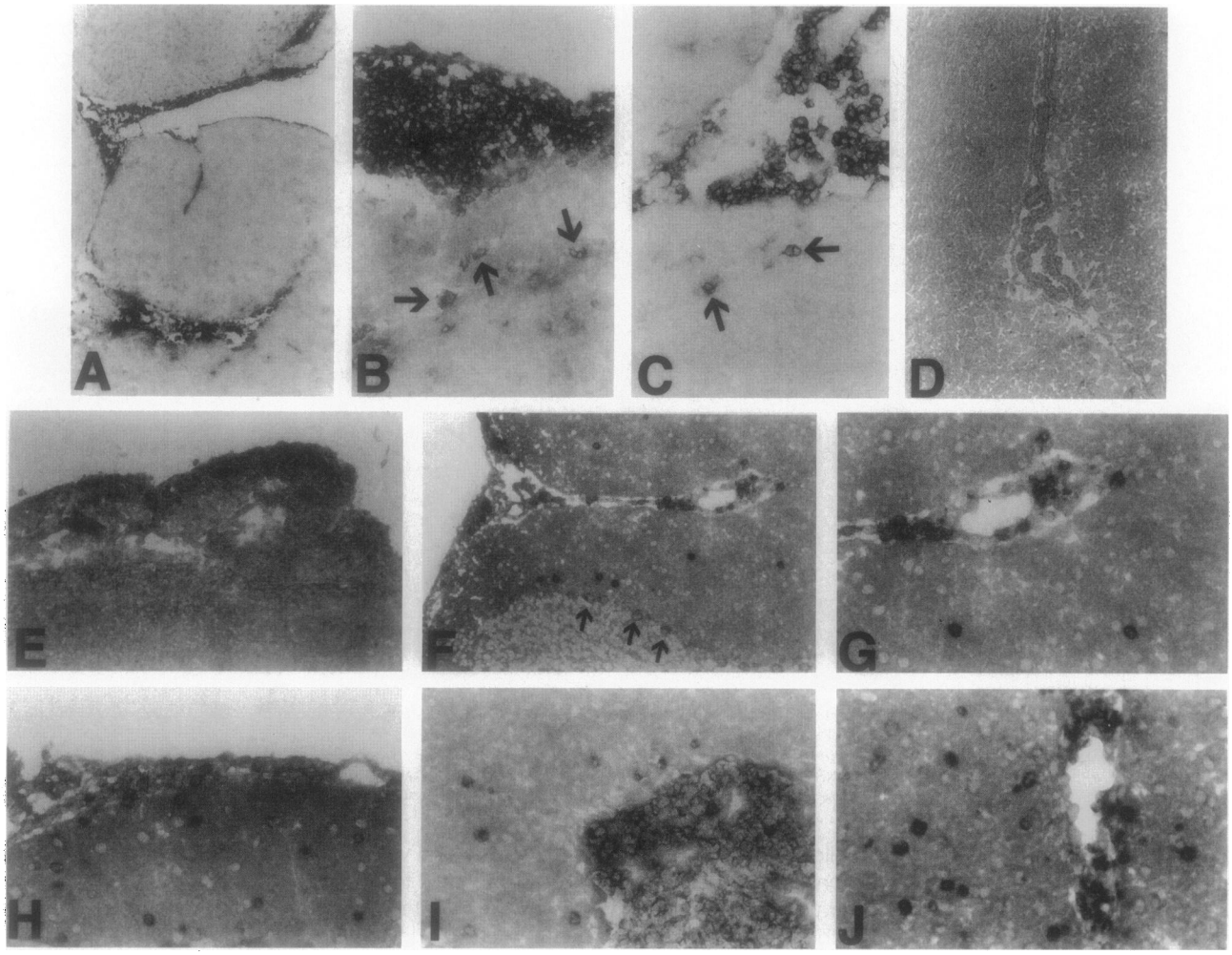

Figure 3. Complement gene expression by leukocytes infiltrating the meninges, ventricles, and the brain parenchyma in the course of experimental *L. monocytogenes* meningitis. **A** to **D:** Immunogold histochemistry of 6-μm-thick brain sections from mice sacrificed 72 hours after infection with *L. monocytogenes*, using a monoclonal anti-mouse CD11b antibody for detection of CR3-positive leukocytes. **A:** Infiltration with CR3-expressing cells in the meninges and lateral ventricle. Original magnification, ×10. **B** and **C:** CR3-positive leukocytes infiltrate the meninges (**B**) and the lateral ventricle (**C**); scattered infiltrating cells are seen within the brain parenchyma (**B** and **C, arrows**). Original magnification, ×100. **D:** Lateral ventricle of a mock-infected mouse. Original magnification, ×50. **E** to **J:** *In situ* hybridization of brain sections from infected mice sacrificed 72 hours after intracerebral inoculation with *L. monocytogenes*, probed with factor B anti-sense riboprobes. Infiltrating cells expressing factor B mRNA are seen in the meninges (**E**, **F**, and **H**), in the lateral ventricle (**I**), and within a vessel in the cortex (**J**). Furthermore, scattered cells infiltrating the brain parenchyma revealed strong factor B transcript signals (**F** to **J**). The **arrows** (**F**) indicate the Purkinje cell layer. See text for details. Original magnification, ×50 (**E** and **F**) and ×100 (**G** to **J**).

ment protein levels[19,20] and complement activation[18,20] have been demonstrated in the CSF of patients with bacterial meningitis, the source of complement within the SAS has not been determined. In the present study, we demonstrate intrathecal synthesis of the alternative pathway complement activation proteins C3 and factor B in experimental *L. monocytogenes* meningitis. Intracerebral inoculation of mice with *Listeria* induced C3 and factor B mRNA expression by pyramidal neurons in the cortex and hippocampus and by Purkinje cells in the cerebellum within 6 hours. Neurons were identified as the cellular source of complement, based on the morphological cellular pattern and confirmed by staining of adjacent sections for synapsin, a neuron-specific marker. Furthermore, C3 and factor B mRNA expression was detected within typical neuronal cell layers, such as the CA1 to CA3 regions of the hippocampus, the dentate gyrus, and the Purkinje cell layer in the cerebellum. Although neurons appear to be the primary source of C3 and factor B expression, it is likely that astrocytes and microglia also contribute to complement gene expression in this model. The production of complement components by glial cells has been well documented in several studies (reviewed in Ref. 24). Maximal complement expression by neurons was seen 12 hours after infection. Thereafter, the intensity of transcript signals on neurons decreased gradually until 72 hours, the last time point investigated. In addition, leukocytes infiltrating the meninges and choroid plexus of the ventricles within 12 to 24 hours were shown to express C3 and factor B mRNA for up to 72 hours after inoculation with *L. monocytogenes*.

To our knowledge, this is the first report on intrathecal complement synthesis in meningitis and the first demonstration of C3 and factor B biosynthesis by neurons. The expression of two classical pathway complement proteins, C1q and C4, has been previously demonstrated on

pyramidal neurons of the rat hippocampus in response to injection of neurotoxins.[37] In the present study, elevated C3 and factor B protein levels were detected in the CSF of infected mice, in accordance with data on human CSF.[19] The low C3 concentrations detected in the CSF of mock-infected animals may represent low constitutive C3 CSF levels, as previously shown for normal human CSF.[19,22,23] It should be noted that, although we detected increased C3 and factor B levels in the CSF of infected animals, we used pooled CSF samples for this study. Thus, the increases shown in Figure 1, A and B, do not show individual responses of the mice, which likely vary from animal to animal. As bacterial meningitis is strongly associated with a breakdown of the BBB,[3,21] it is reasonable to suggest that elevated complement CSF levels represent the sum of serum-derived complement leaking across a dysfunctional BBB and intrathecally produced complement proteins. As we did not assess the integrity of the BBB in this particular model of bacterial meningitis, the relative contribution of each complement source remains to be determined.

Little is known regarding the mediators that regulate complement expression by neurons. Experimental injection of neurotoxins, such as kainate and colchicine, induced neuronal C1q and C4 mRNA expression in rats.[37] The pro-inflammatory cytokine IL-6, which is released into the CSF in patients with bacterial meningitis,[27] has been shown to up-regulate C3 expression in the CNS, based on findings on transgenic mice with astrocyte-targeted overexpression of IL-6.[38] In these studies, however, increased C3 mRNA expression was detected on astrocytes, microglia, and ependymal cells but not on neurons, suggesting IL-6 does not induce C3 synthesis by neurons.[38] Another locally released cytokine in bacterial meningitis is TNF-α, a potent mediator of meningeal inflammation.[3,4,6] TNF-α CSF levels have been shown to be elevated in patients with bacterial meningitis[25–27] as well as in the murine model of *L. monocytogenes* meningitis used in the present study.[25,28] TNF-α was shown to up-regulate expression of the C5aR (CD88) on neurons in mice with *L. monocytogenes* meningitis.[18] Based on these findings, we sought to investigate whether this cytokine represents a crucial mediator of intrathecal C3 and factor B synthesis. Indeed, the intraperitoneal injection of murine recombinant TNF-α in mock-infected and normal mice induced C3 and factor B mRNA expression by neurons within 24 hours, similar to the expression seen in *Listeria*-infected animals. Peripherally administrated murine recombinant TNF-α has been previously shown to cross the BBB by a TNF-selective saturable mechanism and to be sequestered within the intrathecal compartment.[39] To further investigate the role of TNF-α in mediating complement expression in the CNS, we infected mice deficient of TNF and LT-α genes[29] with *L. monocytogenes*. The neuronal complement expression in infected TNF/LT-α $-/-$ mice was hardly attenuated, and very similar to the pattern of expression seen in infected wild-type animals. Furthermore, elevated C3 and factor B protein levels were also detected in the CSF of *Listeria*-infected TNF/LT-α $-/-$ mice. We therefore conclude that, although TNF-α is able to induce C3 and factor B expression by neurons *in vivo*, additional mediators must be involved in the regulation of intrathecal complement synthesis in the course of experimental *L. monocytogenes* meningitis.

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