REDUCTION OF INFLAMMATION, TISSUE DAMAGE, AND MORTALITY IN BACTERIAL MENINGITIS IN RABBITS TREATED WITH MONOCLONAL ANTIBODIES AGAINST ADHESION-PROMOTING RECEPTORS OF LEUKOCYTES

By ELAINE I. TUOMANEN,* KIRSI SAUKKONEN,* STEPHEN SANDE,* CHRISTINE CIOFFE,‡ and SAMUEL D. WRIGHT§

*From the Laborotories of *Microbiology, ‡Animal Research Center, and §Cellular Physiology and Immunology, The Rockefeller University, New York, New York 10021*

Approximately 20,000 cases of bacterial meningitis occur in children per year in the United States (1). Depending on the etiology, up to 30% of these children die and half experience severe sequelae (2). Paradoxically, morbidity and mortality have not changed over 30 years despite the introduction of and continued improvement in bactericidal antibiotics (1, 3) and it is now recognized that breakthroughs in limitation of tissue damage in meningitis will not come from greater antibiotic-induced killing (4). Recently, evidence has accumulated that adjuncts to antibiotic therapy that reduce inflammation can achieve reduced mortality (5–9). Since a large inflammatory mass is associated with a poor outcome of disease (10), it is possible that some of the benefits of these agents derive from their ability to decrease accumulation of leukocytes in cerebrospinal fluid (CSF).[1]

The contribution of leukocytes to neuronal and endothelial injury during meningitis is unresolved. Studies using cyclophosphamide to induce neutropenia, have provided evidence both against (11, 12) and for (11, 13–15) leukocyte-mediated damage, but side effects of cyclophosphamide limit their reliability. We have re-examined the role of leukocytes by using mAbs against the CD18 family of adhesion-promoting receptors on leukocytes (reviewed in reference 16) to block their movement. The CD18 complex is composed of three members, CD11a/CD18 (LFA-1), CD11b/CD18 (CR3 or Mac1), and CD11c/CD18 (p150,95) (17, 18). Expression of functional CD18 molecules appears essential for migration of inflammatory cells since patients with a genetic defect in CD18 (LAD, reference 18) fail to mobilize leukocytes to sites of inflammation, and infusion of anti-CD18 mAbs into animals blocks emigration of leukocytes into peripheral sites in response to noninfectious stimuli (19, 20). In vitro

This work was supported in part by grants from the U.S. Public Health Service (AI-22003, AI-24775, AI-23459, AI-16794, RR-07044) and by Biomedical Research Support Grant SO7 RR-07065. E. Tuomanen is a Career Investigator of the American Lung Association and an Established Investigator of the I. T. Hirschl Trust. S. D. Wright is an Established Investigator of the American Heart Association. Address correspondence to E. Tuomanen, The Rockefeller University, 1230 York Avenue, Box 152, New York, NY 10021.

[1] *Abbreviations used in this paper:* CSF, cerebrospinal fluid; HSA, human serum albumin; i.c., intracisternally.

studies show that CD18 molecules are necessary for the initial binding of leukocytes to endothelium (21, 22).

Here we show that intravenous infusion of antibodies against CD18 into rabbits inhibits the recruitment of leukocytes across the blood brain barrier in response to acute inflammatory stimuli of bacterial origin. Antibody-induced limitation of leukocyte emigration into the CSF is associated with decreased blood brain barrier injury and cerebral edema and prevention of mortality.

Materials and Methods

Bacterial Strains and Products. *Streptococcus pneumoniae* strain SIII, type III, and an unencapsulated strain R6 were grown in semisynthetic medium (23). *Haemophilus influenzae* strain Eagan, Type B, or *Neisseria meningitidis*, type b, were grown in brain heart infusion broth supplemented with 5% Fildes enrichment media (BBL Microbiology Systems, Cockeysville, MD) (23). Pneumococcal cell wall (preparation B, reference 24) or LPS (Re; List Biological, Campbell, CA) were suspended in pyrogen-free saline by sonication before injection into the rabbits.

mAb IB4 (IgG2A) directed against CD18 and mAb OKM1 (IgG2B) directed against CD11b were as described (17). mAb 60.3 (IgG2A) against CD18 was a generous gift of Dr. John Harlan (University of Washington, Seattle, WA) (22). Antibodies were administered either as ascites fluids or as purified Ig with equivalent results.

Induction of Meningitis. 2-kg female New Zealand white rabbits (Hare Marland, Nutley, NJ) were anesthetized with a combination of Ketamine (Parke-Davis, Morris Plains, NJ)/Xylazine (Miles Laboratories, Shawnee, KS) at a dose of 35 and 2.5 mg/kg, respectively, by the intramuscular route and a dental acrylic helmet was affixed to the calvarium (23). 24 h later the rabbits were reanesthetized with subcutaneous ethyl carbamate (Urethane, 1.75 g/kg; Aldrich Chemical Co., Milwaukee, WI) followed by pentobarbital (Nembutal, 15 mg/kg; Abbott Laboratories, Abbott Park, IL) intravenously and then placed on stereotaxic frames. A Quincke spinal needle (24 gauge by 3.5 in; Becton Dickinson & Co., Parsippany, NJ) was introduced into the cisterna magna with a geared electrode introducer. After withdrawal of 300 μl of CSF, the inoculum (200 μl) was introduced followed by repeated sampling of CSF (200 μl) at 2-h intervals.

Study Design. Animals were studied in groups of eight; half of the rabbits in each group were treated with mAb IB4 (1 mg protein/kg) administered as an intravenous bolus dose.

Acute Inflammation. Rabbits were challenged intracisternally (i.c.) with one of the following: 10^7 CFU of washed living *S. pneumoniae*, *H. influenzae*, or *N. meningitidis*, 50 μg of pneumococcal cell wall, or 25 ng of LPS. mAb was administered intravenously 40 min before intracisternal challenge. CSF samples were tested for leukocyte density, protein concentration, histology of leukocyte infiltrate, and bacterial density (23).

To study the effect of alterations in the regimen of therapy with the antibody on the course of acute meningeal inflammation, a highly reproducible inflammation was induced by challenge with 10^8 CFU equivalents of heat-killed pneumococci. In one set of experiments, rabbits were rendered complement deficient by treatment with Cobra venom factor (*Naja haja*; Cordis Laboratories, Miami, FL) 75 U/kg s.c. at 24, 18, and 12 h and intravenously 0.5 h before intracisternal challenge (25). In a second set of experiments, blood brain barrier permeability was quantitated by following accumulation in CSF of intravenously infused ^{125}I-human serum albumin (HSA) (14). HSA (Armour Pharmaceuticals, Kankakee, IL) was iodinated by the iodogen procedure (26) and 100 μCi/kg was infused at 5 h after pneumococcal challenge; control animals received saline intracisternally. Permeability was calculated as cpm CSF/cpm blood $\times$ 100 at 2 h after infusion of ^{125}I-HSA.

Established Meningitis. In a second set of experiments, 10^8 cell equivalents of heat-killed pneumococci were administered intracisternally at 0, 3, 6, 24, 27, and 30 h and the course of CSF inflammation, survival, and brain edema (dry versus wet weight of cerebral hemispheres and cerebellum excised immediately at death [6]) were assessed. mAb IB4 was given intravenously at 0 h (1 mg/kg) and 24 h (0.1 mg/kg) to half of the rabbits.

The impact of the mAb on the outcome of therapy of active pneumococcal meningitis was

tested in animals receiving intravenous ampicillin (30 mg/kg) 14 h into established infection. mAb IB4 was given intravenously simultaneously with the antibiotic (1 mg/kg) and 6, 18, and 24 h later (0.1 mg/kg).

Results

Reduction of Acute Meningeal Inflammation by mAb IB4. Animals receiving mAb IB4 demonstrated a dramatic reduction in CSF leukocyte density following challenge with living bacteria, endotoxin, or cell wall ($p < 0.001$ by analysis of variance; Fig. 1). In the first few hours, leukocytes were >90% polymorphonuclear, but thereafter, mononuclear cells also appeared in control animals. mAb IB4 blocked influx throughout the time course studied (Table I), suggesting it was effective in inhibiting migration of both major classes of leukocytes. The few leukocytes that did appear in the subarachnoid space despite administration of mAb were 90% mononuclear.

mAb IB4 appeared to act by blocking migration of circulating cells into CSF, not by removing leukocytes from the circulation. A peripheral leukocytosis developed in control animals receiving intravenous mAb IB4, consistent with the release of leukocytes from a marginated pool. Before mAb administration, leukocyte density was 8,960 ± 810 cells/μl; 2, 4, and 7 h later, values increased to 33,720 ± 1,324; 25,200 ± 2,203; and 98,490 ± 3,901, respectively. The circulating leukocytes remained viable as assessed by exclusion of trypan blue.

Effect of Variation in Therapeutic Regimen. After standard challenge of heat-killed pneumococci (Table I), blockade of leukocytosis was equally effective at doses of mAb of 1.0 and 0.1 mg/kg administered 30–60 min before the bacterial challenge. The mAb remained effective even if given only 10 min before bacterial challenge but not if given 24 h before the bacterial challenge. Thus, blockade of leukocyte movement by the mAb was transient. Intracisternal rather than intravenous administration of mAb did not protect against CSF leukocytosis, suggesting that the antibody must interfere with leukocyte adherence on the systemic, not the cisternal, side of the blood brain barrier in order to be effective.

Since there is a delay in leukocyte accumulation in animals decomplemented with cobra venom factor (Table I; reference 25), complement components appear to participate in the early phase of recruitment of leukocytes into cerebrospinal fluid (27).

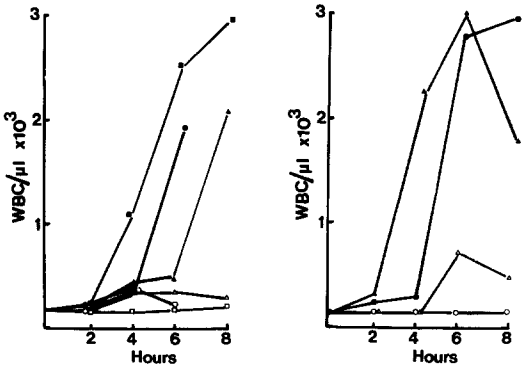

FIGURE 1. The effect of mAb IB4 on leukocytosis in cerebrospinal fluid in response to intracisternal challenge with bacteria or purified bacterial products. The density of leukocytes in cerebrospinal fluid was measured in control rabbits (*closed symbols*) or rabbits pretreated with mAb IB4 (*open symbols*) after challenge with living bacteria (*left*) or purified bacterial components (*right*). (*Left*) Pneumococcus (*circle*), *H. influenzae* (*triangle*), meningococcus (*square*). (*Right*) Cell wall (*circle*), endotoxin (*triangle*).

TABLE I

Effect of Variation in Regimen of Treatment with mAb on the Development of Leukocytosis in CSF

Treatment			Mean CSF leukocyte density (cells/ul)		
Bacteria (i.c.)	mAb	Other	4 h	6 h	11 h
–	–	–	34 ± 16	38 ± 14	47 ± 26
–	1 mg/kg i.v.	–	21 ± 10	45 ± 30	
+	–	–	659 ± 231	2,532 ± 362	
+	1 mg/kg i.v.	–	109 ± 24*	168 ± 43*	
+	0.1 mg/kg i.v.	–	56 ± 34*	82 ± 29*	
+	1 mg/kg i.c.	–	192 ± 31*	1,085 ± 371	
+	1 mg/kg i.v. 10 min before bacteria	–	25 ± 6*	112 ± 4*	
+	1 mg/kg i.v. 24 h before bacteria	–	378 ± 58	2,950 ± 367	
+	–	CoVF	120 ± 12	300 ± 40	2,780 ± 930
+	1 mg/kg	CoVF	131 ± 33	306 ± 15	613 ± 246‡

Animals received mAb IB4 30–60 min before intracisternal (i.c.) challenge with 8×10^7 heat-killed unencapsulated pneumococci R6 suspended in pyrogen-free saline (23). Values for groups designated bacteria alone and bacteria plus mAb IB4 1 mg/kg i.v. are the mean ± SD of leukocyte densities from six animals/group. All other values are the mean ± range of leukocyte densities from pairs of animals.

* Significantly different from bacteria alone.

‡ Significantly different from bacteria + CoVF. $p < 0.001$ by one-way analysis of variance.

However, decomplemented animals eventually do show a dramatic leukocytosis that is presumed to result from the generation of metabolites of arachidonic acid (25). mAb IB4 strongly blocked both the early complement-dependent phase of chemotaxis of leukocytes in normal animals and the late complement-independent phase of recruitment in complement-depleted animals (Table I).

The specificity of the effects of mAb IB4 is indicated by the observation that an additional anti-CD18 mAb, 60.3, also blocked meningeal inflammation (≤22 ± 13 cells/μl at 6 h in mAb 60.3–treated animals vs. 2,800 ± 607 cells/μl in control animals; $p < 0.001$ by analysis of variance). In contrast, mAb OKM1 directed against CD11b/CD18 did not interfere with the development of leukocytosis in CSF (mean leukocyte density 1,453 ± 265 cells/μl at 6 h). Though raised against human leukocytes, mAbs IB4, 60.3, and OKM1 each reacted strongly with rabbit leukocytes (data not shown).

Protective Activity of IB4 Against Blood Brain Barrier Injury, Cerebral Edema, and Mortality. Animals pretreated with intravenous mAb IB4 showed a substantial decrease in blood brain barrier injury as measured by CSF protein concentration (Table II). This finding was further substantiated by measurements of the penetration of ^{125}I-HSA from blood into CSF (saline i.c.: 1.8%; pneumococcus i.c.: 3.9%; pneumococcus i.c. + mAb IB4 i.v.: 1.5%; $p < 0.01$). For as yet unknown reasons, animals receiving mAb alone showed a small but significant elevation in CSF protein concentration.

The inflammatory response in meningitis is strongly enhanced by treatment with antibiotics, presumably because the killed bacteria disintegrate into numerous fragments, each of which serves as a new focus for inflammation (7). To test the efficacy of mAb IB4 in this context, pneumococcal meningitis was treated with ampicillin so as to achieve >10 times the minimum inhibitory concentration in the CSF.

TABLE II

Effect of Treatment with mAb IB4 on CSF Protein Concentration (mg/100 ml) 4 h After Challenge with Inflammatory Bacterial Components

Stimulus	No mAb	+ mAb
None	50 ± 10	128 ± 16
8×10^7 Heat-killed pneumococci R6	352 ± 12*	111 ± 12
10^8 Cell equivalents of cell wall	300 ± 60*	136 ± 20
10^7 Cell equivalents of endotoxin	330 ± 36*	113 ± 37

Values are the mean ± range of determinations made on pairs of rabbits challenged intracisternally with high doses of bacterial components, either intact unencapsulated pneumococcus R6 or purified cell wall (50 μg) or endotoxin (25 ng). 1 mg/kg of mAb IB4 was administered intravenously 30 min before challenge. Protein determinations were made using the BCA protein assay test kit (Pierce Chemical Co., Rockford, IL).

* Significantly different from control (no stimulus/no mAb) and from + mAb paired value at $p < 0.01$ by two-way analysis of variance and Dunnett control group comparison test. All + mAb values are statistically equivalent to control (no stimulus/no mAb).

Ampicillin-induced bacterial lysis caused the expected rapid increase in leukocyte density over that seen in a third group of animals which received no drug (Table III). This ampicillin-induced burst of leukocytosis was prevented by intravenous (but not intracisternal) mAb IB4 (Tables III and IV).

The concentration of ampicillin in the CSF of infected animals was lowered by treatment with mAb IB4 (Table IV). This result was predictable in view of the protection from blood brain barrier permeability afforded by the antibody (Table II). CSF concentrations of ampicillin were above the minimum inhibitory concentration in all animals, and killing of bacteria in antibody-treated animals was equivalent ($p > 0.1$) to that in untreated animals (Tables III and IV).

Upon clearance of a single dose of ampicillin from serum, the residual bacterial population resumes growth, and after a period of time bacteria escape from the CSF

TABLE III

Effect of Tratment with mAb IB4 on Burst of Leukocytosis in CSF During Therapy of Pneumococcal Meningitis with Ampicillin

	Median CSF leukocyte density (cells/μl)			Δ log mean bacterial density
Treatment (*n*)	0 h	4 h	6 h	(CFU/ml CSF) 4 h after antibiotic
None (4)	1,317	2,223	2,523	+ 1.2
Ampicillin (4)	529	7,051	9,194	- 1.6
Ampicillin + mAb IB4 iv (7)	224	246	2,142	- 1.0

At least four rabbits/group (*n*) were inoculated with 10^{3-4} CFU/ml of pneumococci strain SIII and meningitis was established over 14 h. Ampicillin was administered as an intravenous bolus (6 mg/kg) and a burst of leukocytosis was documented in comparison to untreated controls. Some animals received mAb IB4 (1 mg/kg) intravenously 30 min before antibiotic. Two-way analysis of variance shows a significant difference across treatments ($p < 0.01$), time ($p < 0.01$), and a significant interaction ($p < 0.05$) between the time of onset of leukocytosis and treatment with antibiotic with or without antibody.

into the blood. Bacteremia was first detected in animals treated with ampicillin at 37 h after intracisternal inoculation (Table IV). However, in animals receiving mAb IB4 plus ampicillin, detection of bacteremia was delayed until 60 h of meningeal infection. The density of bacteria in CSF in the two groups was the same at all times, i.e., bacterial multiplication in CSF before ampicillin and ampicillin-induced killing in CSF was equivalent in the presence or absence of CSF leukocytosis (Table IV). Bacterial denisities in blood were not affected by the presence of antibody, as evidenced by identical titers in animals challenged with 10^5 pneumococci intravenously with and without antibody (data not shown). Thus, mAb IB4 does not affect the rates of bacterial growth or killing in blood or CSF, but does delay dissemination of the infection. In an additional meningitis experiment similar to that in Table IV, only 1 of 4 control animals recovered without sequelae, while all 10 animals treated with four doses of mAb (12, 18, 24 and 30 h) experienced complete recovery by 5 d after ampicillin (30 mg/kg at 12 h).

The contribution of leukocytes to brain damage was tested using a protocol in which pathology was all intracranial (i.e., inflammatory challenge known to remain in the subarachnoid space [25]). Six intracisternal doses of 10^8 heat-killed pneumococci resulted in dramatic CSF inflammation (Fig. 2) and between 36 and 40 h all control animals died. In contrast, animals receiving mAb IB4 at 0 and 24 h were protected against the development of CSF inflammation and death (Fig. 2). 72 h after intracisternal challenge with a lethal dose of bacteria, rabbits treated with mAb IB4 were not only alive but appeared perfectly well. The mAb also protected against the development of brain edema. Brain weight in control animals dying of meningitis was 415.7 ± 3.0 g water/100 g dry brain weight versus 394 ± 4.2 for mAb-treated animals ($p < 0.01$). Brain weight in normal rabbits was 396 ± 2.7, a value not different from mAb-treated groups ($p > 0.1$).

The effect of mAb IB4 on inflammation appeared to be transient since leukocytes became detectable in CSF at 24 h after the first dose of antibody (Fig. 2). However, the leukocyte density again decreased sharply after a second dose of mAb. This result also suggests that leukocytes have a half-life in CSF of the order of hours. In a similar fashion, penetration of protein into CSF was inhibited for >6 h by the first dose of mAb and increased by 24 h. After the second dose of antibody, protein concentrations remained stably high.

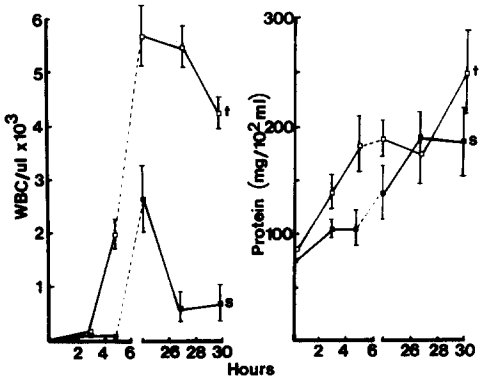

FIGURE 2. Prevention of CSF leukocytosis, influx of protein into CSF, and death by mAb IB4 following a lethal dose of pneumococci. Eight rabbits were challenged intracisternally with 10^8 cell equivalents of heat-killed pneumococci at 0,3,5,24,27, and 30 h. Five rabbits received mAb IB4 intravenously (1 mg/kg at 0 h; 0.1 mg/kg at 24 h) (*closed symbols*); three rabbits served as controls (*open symbols*). (*Left*) Mean leukocyte density in CSF; (*right*) mean CSF protein concentration. *t*, all animals died at 36–48 h; *s*, all animals alive and well at >60 h.

TABLE IV

Effect of Treatment of mAb IB4 on Parameters of CSF Inflammation Bacterial Density, Drug Penetration into CSF, and Outcome of Meningitis due to S. pneumoniae SIII

Antibody	Mean (SD) leukocyte density in CSF (cells/μl)			Mean (SD) protein concentration (mg/100 ml)			Mean (range) ampicillin concentration (μg/ml	Log mean (SD) bacterial density (CFU/ml)					Time of death
								CSF		Blood			
(*n*)	12 h	18 h	37 h	12 h	18 h	37 h	2 h after dose)	12	18	19	37	60	(*h*)
− mAb (3)	284 (63)	1,040 (360)	13,060 (2,769)	186 (21)	224 (83)	238 (87)	0.38 (0.2 to 0.5)	6.1 (1.4)	3.2 (0.9)	0	2.3 (0.2)	3.3 (1.3)	75§
+ mAb (5)	149 (105)	518* (158)	2,169* (3,064)	95 (21)	162* (21)	270 (67)	0.20 (0.05 to 0.5)	5.8 (1.3)	2.6 (0.7)	0	0*	2.9‡)0.2)	75§

Rabbits were injected with 10^3 CFU of living *S. pneumoniae* III intracisternally. At intervals, CSF was sampled for leukocyte density, bacterial density, protein concentration, ampicillin concentration. Rabbits treated with mAb IB4 received 1 mg/kg i.v. at 12 h and 0.1 mg/kg at 24 h.

* Two-way analysis of variance shows significant difference from control ($p < 0.01$).

‡ Only two of fiver rabbits were bacteremic.

§ All animals moribund and euthanized at 75 h.

Discussion

At least two mechanisms are known by which leukocytes interact with endothelium. One mechanism uses the CD18 complex of receptors on leukocytes (16, 18), and enables stimulated leukocytes to adhere to unstimulated, naive endothelium. A second mechanism depends on the synthesis of novel molecules on the endothelium, such as endothelial-leukocyte adhesion molecule (ELAM) in response to specific stimuli (LPS, TNF, or IL-1) (28). Here we show that nearly all migration across the blood brain barrier is blocked by anti-CD18 antibodies. In our animal model, the endothelium has ample exposure to bacterial products that engender CD18-independent adhesion in vitro (28–31), yet little or no CD18-independent migration was observed. It is not known whether ELAM can be expressed on endothelium of the central nervous system.

This study provides clear evidence that leukocytes play a significant role in causing several types of intracranial pathology that are sufficiently severe so as to induce death following challenge with both live bacteria and inflammatory bacterial surface components. Cerebral edema was absent in mAb-treated animals, confirming previous evidence that activated leukocytes contribute to this damage (11, 32). Limitation of adherence of leukocytes to endothelial cells decreased three additional parameters of blood brain barrier permeability in meningitis: (*a*) influx of serum proteins and ^{125}I-HSA into CSF, (*b*) penetration of antibiotics from serum into CSF, and (*c*) onset of bacteremia resulting from intracisternal bacterial growth.

Neither the course of bacterial growth in blood nor CSF was adversely affected by mAb-induced functional neutropenia. This was observed previously in cyclophosphamide-treated neutropenic rabbits (12) and may be explained by the inefficiency of phagocytosis in liquid spaces (33). The observation that bacteremia arising from bacterial multiplication in CSF was delayed in the absence of CSF leukocytosis suggests leukocytes may be important in spreading infection between central and peripheral spaces either as carriers of bacteria or as causative agents of disrupted endothelial integrity.

Studies of the treatment of meningitis with several antiinflammatory agents (steroids and nonsteroidal) in combination with antibiotics in humans and animal models have demonstrated an association between inhibition of inflammation in CSF and improved outcome of disease (5–9). Our studies indicate that at least part of the mechanism of such beneficial effects arises from limitation of the accumulation of leukocytes in the subarachnoid space. While antileukocyte mAbs have been under investigation for the therapy of autoimmune diseases in humans (34) and for protection against ischemia/reperfusion injury in animal models (35, 36), the deployment of such mAbs in infectious diseases has not been explored. Our data demonstrate that there exists an important rationale for their use in this new context.

Summary

We tested if specific inhibition of recruitment of leukocytes across the blood brain barrier from the vascular compartment to the cerebrospinal fluid (CSF) space reduced tissue damage and improved the outcome of infection in a rabbit model of experimental meningitis. The CD11/CD18 complex of receptors on leukocytes promotes adhesion of these cells to endothelia, a process required for egress of cells into the extravascular space. Intravenous injection of the anti-CD18 mAb IB4 effectively

blocked the development of leukocytosis in the CSF of animals challenged intracisternally with living bacteria, bacterial endotoxin, or bacterial cell wall. This effect was associated with protection from blood brain barrier injury as measured by exclusion of serum proteins from CSF in mAb-treated animals. The densities of bacteria in CSF and the degrees of bacterial killing due to ampicillin were not affected by the antibody. Animals receiving the antibody experienced a delay in the development of bacteremia and a significantly reduced inflammatory response during ampicillin-induced bacterial killing. Therapy with mAb IB4 prevented development of brain edema and death in animals challenged with lethal doses of *Streptococcus pneumoniae*. These studies indicate that the major mechanism of leukocyte migration across the blood brain barrier involves the CD11/CD18 receptors and that inflammatory leukocytes recruited by this mechanism are a major cause of blood brain barrier injury and cerebral edema during meningitis.

We thank A. R. Hill, P. Detmers, A. Aderem, Z. Cohn, and A. Tomasz for critical review of the manuscript. Statistical calculations were performed by Mr. Ping Li, SUNY Health Sciences Center, Brooklyn, NY.

Received for publication 12 April 1989 and in revised form 10 May 1989.

References

1. Centers for Disease Control. 1978. *Morbid. Mortal. World Rep.* 28:277.
2. Dodge, P. R. 1986. Sequelae of bacterial meningitis. *Pediatr. Infect. Dis. J.* 5:618.
3. Smith, A. L. 1988. Neurologic sequelae of meningitis. *N. Engl. J. Med.* 319:1012.
4. Kaplan, S. L., and M. A. Fishman. 1987. Supportive therapy for bacterial meningitis. *Pediatr. Infect. Dis. J.* 6:670.
5. Syrogiannopoulos, G. A., K. D. Olsen, J. S. Reisch, and G. H. McCracken, Jr. 1987. Dexamethasone in the treatment of experimental *Haemophilus influenzae* type B meningitis. *J. Infect. Dis.* 155:213.
6. Tauber, M. G., H. Khayam-Bashi, and M. A. Sande. 1985. Effects of ampicillin and corticosteroids on brain water content, cerebrospinal fluid pressure, and cerebrospinal fluid lactate levels in experimental pneumococcal meningitis. *J. Infect. Dis.* 151:528.
7. Tuomanen, E., B. Hengstler, R. Rich, M. A. Bray, O. Zak, and A. Tomasz. 1987. Nonsteroidal anti-inflammatory agents in the therapy for experimental pneumococcal meningitis. *J. Infect. Dis.* 155:985.
8. Kadurugamuwa, J. L., B. Hengstler, E. Tuomanen, M. A. Sande, and O. Zak. 1987. Effect of a cyclo-oxygenase inhibitor on the course and outcome of experimental meningitis in rabbits. *Proc. Interscience Conf. Antimicrobial Agents Chemotherapy, 27th, Atlanta.* American Society of Microbiology, Washington D.C., Abstr. no. 619.
9. Lebel, M. H., B. J. Freij, G. A. Syrogiannopoulos, D. F. Chrane, M. J. Hoyt, S. M. Stewart, B. D. Kennard, K. D. Olsen, and G. H. McCracken Jr. 1988. Dexamethasone therapy for bacterial meningitis. Results of two double-blind, placebo-controlled trials. *N. Engl. J. Med.* 319:964.
10. McAllister, C. K., J. M. O'Donoghue, and H. N. Beaty. 1975. Experimental pneumococcal meningitis. II. Characterization and quantitation of the inflammatory process. *J. Infect. Dis.* 132:355.
11. Tauber, M., U. Borschberg, and M. A. Sande. 1988. Influence of granulocytes on brain edema, intracranial pressure, and cerebrospinal fluid concentrations of lactate and protein in experimental meningitis. *J. Infect. Dis.* 157:456.
12. Ernst, J. D., J. M. Decazes, and M. A. Sande. 1983. Experimental pneumococcal menin-

gitis: role of leukocytes in pathogenesis. *Infect. Immun.* 4:275.
13. Quagliarello, V. J., W. J. Long, and W. M. Scheld. 1987. Human Interleukin-1 modulates blood-brain barrier injury *in vivo. Proc. Interscience Conf. Antimicrobial Agents Chemotherapy, 27th, Atlanta.* American Society for Microbiology, Washington, D.C., Abstr. no. 614.
14. Lesse, A. J., E. R. Moxon, A. Z.vahlen, and W. M. Scheld. 1989. The role of cerebrospinal fluid pleocytosis and *Haemophilus influenzae* type b capsule on blood brain barrier permeability during experimental meningitis in the rat. *J. Clin. Invest.* 82:102.
15. Petersdorf, R. G., and C. N. Luttrell. 1962. Studies on the pathogenesis of meningitis. I. Intrathecal infection. *J. Clin. Invest.* 41:311.
16. Wright, S. D., and P. A. Detmers. 1988. Adhesion promoting receptors on phagocytes. *J. Cell Sci.* 9(Suppl.):99.
17. Wright, S. D., P. E. Rao, W. C. Van Voorhis, L. S. Craigmyle, K. Iida, M. A. Talle, E. F. Westberg, G. Goldstein, and S. C. Silverstein. 1983. Identification of the C3bi receptor of human monocytes and macrophages by using monoclonal antibodies. *Proc. Nat. Acad. Sci. USA.* 80:5699.
18. Todd, R. F., III, and D. R. Freyer. 1988. The CD11/CD18 leukocyte glycoprotein deficiency. *In* Phagocytic Defects. I: Abnormalities Outside of the Respiratory Burst. J. T. Curnette, editor, W.B. Saunders, Philadelphia. 13–31.
19. Arfors, K.-E., C. Lundberg, L. Lindbom, K. Lundberg, P. G. Beatty, and J. M. Harlan. 1987. A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage *in vivo. Blood.* 69:338.
20. Rosen, H., and S. Gordon. 1987. Monoclonal antibody to the murine type 3 complement receptor inhibits adhesion of myelomonocytic cells *in vitro* and inflammatory cell recruitment *in vivo. J. Exp. Med.* 166:1685.
21. Lo, S. K., P. A. Detmers, S. M. Levin, and S. D. Wright. 1989. Transient adhesion of neutrophils to endothelium. *J. Exp. Med.* 169:1779.
22. Harlan, J. M., P. D. Killen, F. M. Senecal, B. R. Schwartz, E. K. Yee, R. F. Taylor, P. G. Beatty, T. H. Price, and H. D. Ochs. 1985. The role of neutrophil membrane glycoprotein GP-150 in neutrophil adherence to endothelium *in vitro. Blood.* 66:167.
23. Tuomanen, E., A. Tomasz, B. Hengstler, and O. Zak. 1985. The relative role of bacterial cell wall and capsule in the induction of inflammation in pneumococcal meningitis. *J. Infect. Dis.* 151:535.
24. Tuomanen, E., H. Liu, B. Hengstler, O. Zak, and A. Tomasz. 1985. The induction of meningeal inflammation by components of the pneumococcal cell wall. *J. Infect. Dis.* 151:859.
25. Tuomanen, E., B. Hengstler, O. Zak, and A. Tomasz. 1986. The role of complement in inflammation during experimental pneumococcal meningitis. *Microb. Pathog.* 1:15.
26. Fraker, P. J., and J. C. Speck. 1978. Protein and cell membrane iodination with sparingly soluble chloramides: 1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril. *Biochem. Biophys. Res. Commun.* 80:849.
27. Ernst, J. D., K. T. Hartiala, I. M. Goldstein, and M. A. Sande. 1984. Complement (C5)-derived chemotactic activity accounts for accumulation of polymorphonuclear leukocytes in cerebrospinal fluid of rabbits with pneumococcal meningitis. *Infect. Immun.* 46:81.
28. Bevilacqua, M. P., J. S. Pober, D. L. Mendrick, R. S. Cotran, and M. A. Gimbrone Jr. 1987. Identification of an inducible endothelial leukocyte adhesion molecule. *Proc. Natl. Acad. Sci. USA.* 84:9238.
29. Pohlman, T. H., K. A. Stanness, P. G. Beatty, H. D. Ochs, and J. M. Harlan. 1986. An endothelial cell surface factor(s) induced *in vitro* by lipopolysaccharide, interleukin-1, and tumor necrosis factor-alpha increases neutrophil adherence by a CDw18-dependent mechanism. *J. Immunol.* 136:4548.
30. Zimmerman, G. A., and T. M. McIntyre. 1988. Neutrophil adherence to human en-

dothelium *in vitro* occurs by CDw18 (Mol, MAC-1/LFA-1/GP 150,95) glycoprotein-dependent and -independent mechanisms. *J. Clin. Invest.* 81:531.

31. Luscinskas, F. W., M. P. Bevilaqua, A. F. Brok, M. A. Arnaout, and M. A. Gimbrone Jr. 1989. Endothelial-leukocyte adhesion molecule-1-dependent and leukocyte (CD11/CD18)-dependent mechanisms contribute to polymorphonuclear leukocyte adhesion to cytokine-activated human vascular endothelium. *J. Immunol.* 142:2257.
32. Fishman, R. A., K. Sligar, and R. B. Hake. 1977. Effects of leukocytes on brain metabolism in granulocytic brain edema. *Ann. Neurol.* 2:89.
33. Smith, M. R., W. D. Perry, J. W. Berry, and W. B. Wood, Jr. 1951. Surface phagocytosis *in vivo*. *J. Immunol.* 67:71.
34. Clarkson, S. B., J. B. Bussel, R. P. Kimberley, J. E. Valinsky, R. L. Nachman, and J. C. Unkeless. 1986. Therapy of refractory immune thrombocytopenic purpura with an anti-Fc gamma-receptor antibody. *N. Engl. J. Med.* 314:1236.
35. Simpson, P. J., R. F. Todd III, J. C. Fantone, J. K. Mickelson, J. D. Griffin, B. R. Luccesi, M. D. Adams, P. Hoff, K. Lee, and C. E. Rogers. 1988. Reduction of experimental canine myocardial reperfusion injury by a monoclonal antibody (anti-Mol, anti-CD11b) that inhibits leukocyte adhesion. *J. Clin. Invest.* 81:624.
36. Vedder, N. B., R. K. Winn, C. L. Rice, E. Y. Chi, K.-E. Arfors, and J. M. Harlan. 1988. A monoclonal antibody to the adherence-promoting leukocyte glycoprotein, CD18, reduces organ injury and improves survival from hemorrhagic shock and resuscitation in rabbits. *J. Clin. Invest.* 81:939.