Recombinant Human Interleukin-1 Induces Meningitis and Blood-Brain Barrier Injury in the Rat

Characterization and Comparison with Tumor Necrosis Factor

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Abstract

The diversity of infectious agents capable of inducing meningitis and blood-brain barrier (BBB) injury suggests the potential for a common host mediator. The inflammatory polypeptides, IL-1 and TNF, were tested in an experimental rat model as candidate mediators for induction of meningitis and BBB injury. Intracisternal challenge of rIL-1β into rats induced neutrophil emigration into cerebrospinal fluid (CSF) and significantly increased BBB permeability to systemically administered ^{125}I-BSA as early as 3 h later ($P < 0.05$). This injury was reversible, dose dependent and significantly inhibited by prior induction of systemic neutropenia (via intraperitoneal cyclophosphamide) or preincubation of the rIL-1β inoculum (50 U) with an IgG monoclonal antibody to rIL-1β. Similar kinetics and reversibility of CSF inflammation and BSA permeability were observed using equivalent dose inocula of rIL-1 alpha. rTNF-α was less effective as an independent inducer of meningitis or BBB injury over an inoculum range of 10^1 U (0.0016 μg/kg)–10^6 U (160 μg/kg) when injected intracisternally, but inoculum combinations of low concentrations of rTNFα (10^3 U) and rIL-1β (0.0005–5.0 U) were synergistic in inducing both meningitis and BBB permeability to systemic ^{125}I-BSA. These data suggest that in situ generation of interleukin-1 within CSF (with or without TNF) is capable of mediating both meningeal inflammation and BBB injury seen in various central nervous system infections. (*J. Clin. Invest.* 1991. 87:1360–1366.) Key words: meningitis • cytokine • interleukin • blood-brain barrier

Introduction

Bacterial meningitis remains a substantial health problem accounting for worldwide morbidity and mortality. As such, it represents a unique dichotomy in human infectious disease since available antimicrobial agents achieve bactericidal concentrations within the central nervous system (CNS)[1] capable of microbiologic cure. This observation supports the hypothesis that the pathophysiologic sequelae of the disease beyond the leptomeninges, but within the CNS, progress despite bacterial eradication and effect permanent neuronal injury (1).

The blood-brain barrier (BBB), ultrastructurally localized at the cerebral microvascular endothelium (2), represents a vital extrameningeal focus of injury in meningitis. Indeed, BBB injury, with its associated alteration in albumin transcytosis represents the major precipitant of vasogenic brain edema observed in many forms of CNS infections in humans and experimental animals. Previous investigations in an experimental rat model of meningitis have demonstrated consistent ultrastructural and functional alterations of the BBB after intracisternal challenge with live bacteria as well as bacterial cell surface constituents (3, 4). This observation of diverse challenge inocula eliciting a uniform host injury suggests that one or more endogenous inflammatory mediators might be involved.

The polypeptide IL-1, an inflammatory cytokine and immunomodulator, represents such a candidate mediator of meningitis and BBB injury given its stimulation by various infectious agents, its ability to enhance neutrophil adherence to endothelium in vitro (5), and the presence of its gene within resident cells of the central nervous system.

The goals of this inquiry were to ask: (*a*) does intracisternal challenge with IL-1 generate an inflammatory response in the cerebrospinal fluid; (*b*) is there a time/dose-dependent injury to the BBB similar to that observed in challenge with live bacteria or bacterial surface components; (*c*) is the BBB injury observed dependent upon neutrophil exudation into CSF; (*d*) does intracisternal challenge with TNF, an inflammatory cytokine with similar in vivo and in vitro effects as IL-1, elicit a similar injury pattern; and (*e*) is there a synergistic effect observed upon intracisternal challenge of IL-1 with TNF?

Methods

Challenge inocula. Recombinant human IL-1β (isoelectric point [pI 7.0]) and IL-1α (pI 5.0), the translation products of the cloned complementary DNA isolated from a macrophage cDNA library, were purchased from Cistron (Pine Brook, NJ). They were diluted in PBS with 2 μM dithiothreitol (DTT) (Bio-Rad Laboratories, Richmond, CA) and 0.1% BSA. Each contained 1,000 U/ml of thymocyte costimulation activity (1 ng protein/U) and < 0.1 ng endotoxin activity/μg protein by Limulus lysate. Mouse monoclonal IgG antibody to the recombinant human IL-1β was also purchased from Cistron. Recombinant human TNF-α (19.9 pg/U) was a generous gift of Genentech (South San Francisco, CA), and was diluted in 0.5% BSA. It contained < 0.06 pg of endotoxin activity/μg protein. Anti-murine TNF monoclonal antibody (250 μg/5,000 U) was obtained from Genzyme (Boston, MA). Polymyxin B was purchased from Sigma Chemical Co. (St. Louis, MO).

Induction of meningitis. As previously reported (3, 4, 6), adult 125-g Wistar rats were anesthetized with ketamine (Parke-Davis, Morris

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1. *Abbreviations used in this paper:* BBBP, blood-brain barrier permeability; CNS, central nervous system; CSF, cerebrospinal fluid; DTT, dithiothreitol.

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Plains, NJ) and xylazine (Miles Laboratories, Shawnee, KS) intramuscularly at a dose of 100 mg/kg and 8 mg/kg, respectively. After removal of CSF using a 25-gauge butterfly needle (Abbott Inc., North Chicago, IL), 50 μl of the challenge inoculum was injected into each experimental group and 0.1% BSA with 2 μM DTT inoculated into simultaneous matched controls. 1 h before the second cisternal puncture, rats had intracardiac administration of 10 μCi ^{125}I-BSA (ICN Radiochemicals, Irvine, CA). At the time of the second cisternal puncture, simultaneous CSF and blood samples were obtained for assay of ^{125}I cpm in a Gamma 300 counter (Beckman Instruments, Inc., Irvine, CA) as well as CSF leukocyte concentration using a hemocytometer. Only CSF samples without visible blood contamination were evaluated. The percentage of CSF penetration of ^{125}I-BSA was determined from the equation: cpm per ml CSF/cpm per ml blood × 100.

Experimental groups consisted of: group I, inoculated with rIL-1β; group II, inoculated with rIL-1α; group III, inoculated with rIL-1β preincubated (12 h at 4°C) with mouse IgG monoclonal Ab (1:50 dilution) to rIL-1β; group IV, inoculated with rIL-1β preincubated with polymyxin B (2 μg/ml); group V, inoculated with rIL-1β four days after induction of leukopenia with 100 mg/kg cyclophosphamide (Adria Laboratories, Columbus, OH) intraperitoneally (only animals with blood WBC < 1,000/mm^3 were used); group VI, inoculated with rTNFα; group VII, inoculated with rIL-1β combined with rTNFα. Control groups were inoculated with 0.1% BSA with 2 μM DTT in PBS.

Statistical analysis. The percentage of CSF penetration of ^{125}I-BSA and the concentration of CSF leukocytes in experimental and control groups were compared using the Student's *t* test (two-tailed unpaired).

Results

1. Influence of rIL-1β and rTNFα on CSF exudation of leukocytes

(a) Time dependence. After intracisternal inoculation of 50 U (0.4 μg/kg) of rIL-1β there was a significant CSF pleocytosis (> 95% neutrophils) beginning 3 h later (mean±SE WBC = 4.5±1.4 × 10^3/mm^3) that peaked at 6 h (mean±SE WBC = 19.3±3.4 × 10^3/mm^3) and was reversible by 24 h with (mean±SE WBC = 0.09±0.04 × 10^3/mm^3). This was significant compared with controls, over the same time period, as well as animals inoculated with 10^5 U (16 μg/kg) of rTNFα (P < 0.05) (see Fig. 1).

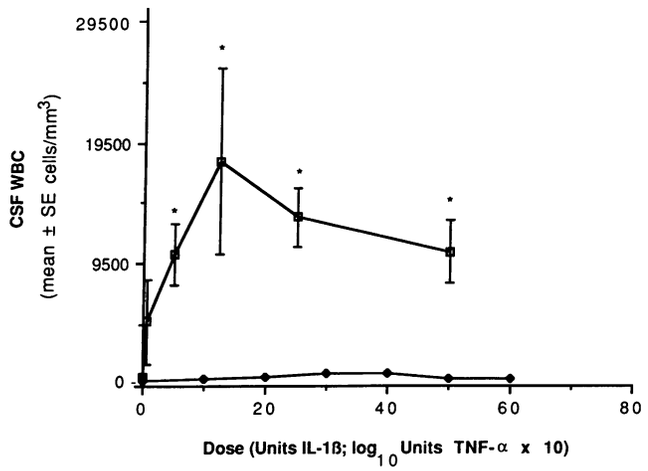

Figure 2. Dose response effect on CSF WBC concentration after intracisternal inoculation with rIL-1β (— □ —) and rTNFα (— ♦ —). All CSF samples were obtained 3.5 h postinoculation. *P < 0.05.

(b) Dose dependence. Similarly, when CSF was sampled at 3.5 h post-inoculation (a time of significant CSF exudation of leukocytes) dose dependence was observed. Over an inoculum range of 0 to 50 U of rIL-1β injected intracisternally, the mean concentration of CSF leukocytes was maximal (mean±SE WBC = 18.0±7.7 × 10^3/mm^3) with 12.5 U (0.1 μg/kg), but was significantly greater than controls (P < 0.05) with as little as 5 U of rIL-1β (mean±SE WBC = 10.2±2.5 × 10^3/mm^3). Conversely, CSF exudation of leukocytes after challenge with intracisternal rTNFα (inocula ranging from 10^1 to 10^6 U) was no different from controls and significantly less than with rIL-1β (P < 0.005) (see Fig. 2).

2. Influence of rIL-1β and rTNFα on blood-brain barrier permeability (BBBP) to systemic administration of ^{125}I-BSA

(a) Time dependence. As demonstrated in Fig. 3, 3 h after intracisternal inoculation of 50 U rIL-1β (0.4 μg/kg) there was a significant increase in ^{125}I-BSA penetration into CSF

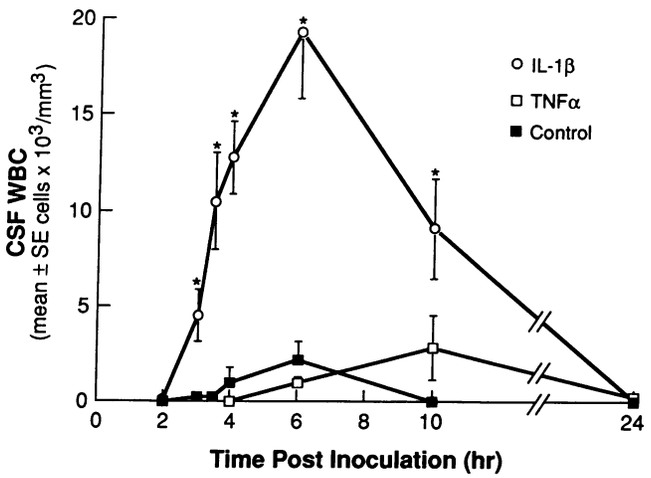

Figure 1. Kinetics of changes in CSF WBC concentration after intracisternal inoculation with rIL-1β (— o —), rTNFα (— □ —), and controls (— ■ —). *P < 0.05.

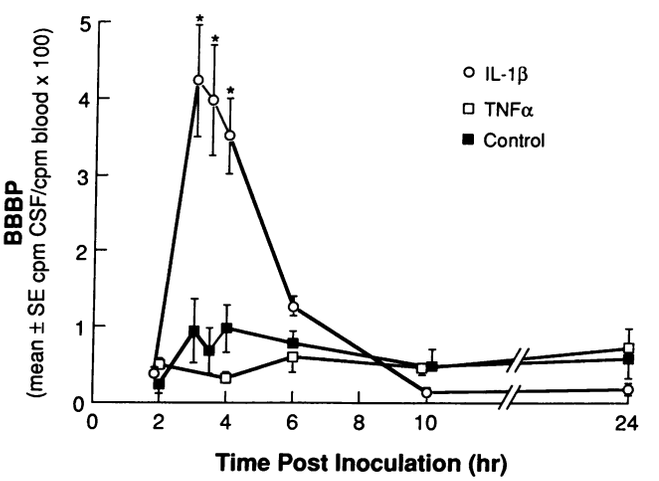

Figure 3. Kinetics of changes in CSF traversal of systemically administered ^{125}I-BSA after intracisternal inoculation with rIL-1β (— o —), rTNFα (— □ —), and controls (— ■ —). *P < 0.05.

(mean±SE ^{125}I-BSA penetration, 4.23±0.73%), compared to controls (mean±SE ^{125}I-BSA penetration, 0.94±0.42%; $P < 0.05$), that was reversible by 6 h and remained so for the 24-h observation period. This effect was in contrast with 10^5 U rTNFα as the intracisternal inoculum which elicited no significant ^{125}I-BSA CSF penetration for as long as 24 h later (mean±SE ^{125}I-BSA penetration, 0.73±0.25%), an observation that paralleled the minimal CSF inflammation it provoked.

(b) Dose dependence. As noted for leukocyte exudation, when the CSF was sampled 3.5 h after intracisternal inoculation with rIL-1β, the effect on BBBP to ^{125}I-BSA was dose dependent. The mean (±SE) ^{125}I-BSA penetration into CSF was maximal (4.29±0.83%) compared with controls (0.60±0.15%; $P < 0.005$) with a 25-U (0.2 μg/kg) inoculum, but was significantly increased ($P < 0.05$) with as low as a 5-U inoculum (2.07±0.51%). Conversely, intracisternal inoculation with rTNFα doses ranging from 10^1 U (0.02 μg/kg) to 10^6 U (160 μg/kg) elicited no significant change in BSA penetration into CSF (mean±SE ^{125}I-BSA CSF penetration = 0.64±0.17% using a 10^3-U inoculum) (see Fig. 4).

3. rIL-1α vs. rIL-1β induction of CSF pleocytosis and BBB permeability

To assess whether IL-1α (pI 5.0) (which shares many biological properties and recognizes the same receptor as IL-1β) could induce a similar in vivo response, the time course of CSF inflammation and BBB injury was determined using 50 U of IL-1α as an intracisternal inoculum. As shown in Table I, intracisternal inoculation of rIL-1α induced very similar kinetics of CSF inflammation (mean±SE WBC = 11.3±2.6 × 10^3/mm^3) and CSF ^{125}I-BSA penetration (mean±SE ^{125}I-BSA = 2.8±0.8%) that were reversible 4 h later, analogous to that seen with rIL-1β.

4. rIL-1β-induced CSF pleocytosis and BBB injury: influence of leukopenia, polymyxin B and monoclonal antibody to rIL-1β

As noted in Table II, the induction of systemic leukopenia (via intraperitoneal cyclophosphamide, 100 mg/kg) before intracisternal inoculation of 50 U of rIL-1β significantly reduced both CSF leukocyte exudation and BBB permeability to ^{125}I-BSA previously described in nonleukopenic animals ($P < 0.001$). In addition, Table II describes the influence of preincubation of the rIL-1β inoculum (50 U) with either polymyxin B (2 μg/ml)

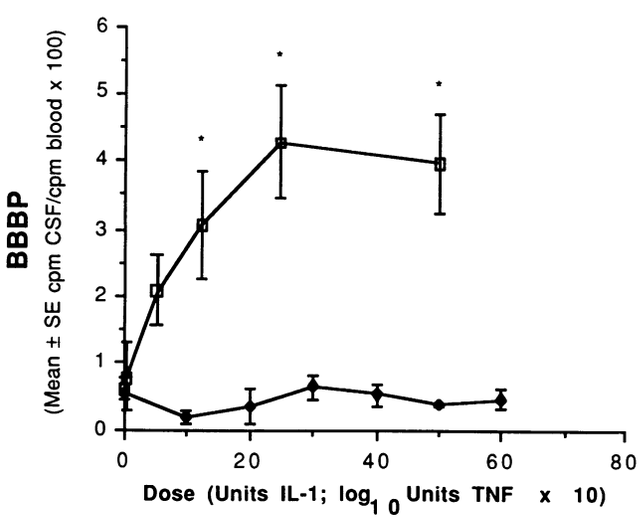

Figure 4. Dose-response effect on CSF traversal of systemically administered ^{125}I-BSA after intracisternal inoculation with rIL-1β (— □ —) and rTNFα (— ♦ —). *$P < 0.05$.

Table I. Comparison of rIL-Iβ to rIL-Iα Induction of CSF Pleocytosis and BBB Permeability to ^{125}I-BSA

Time	Inoculum (*n*)	CSF WBC concentration*	Percentage of BBB permeability‡
h			
2	rIL-1β (12)	0.05±0.01	0.39±0.08
	rIL-1α (4)	0.06±0.02	0.30±0.20
	Control (4)	0.01±0.01	0.25±0.13
3	rIL-1β (8)	4.50±1.38§	4.23±0.73§
	rIL-1α (5)	0.10±0.03	0.22±0.11
	Control (5)	0.22±0.19	0.94±0.42
4	rIL-β (12)	12.75±1.90§	3.51±0.49§
	rIL-1α (5)	11.29±2.55§	2.76±0.77§
	Control (3)	0.95±0.78	0.98±0.31
6	rIL-1β (9)	19.27±3.41§	1.27±0.13
	rIL-1α (3)	4.43±1.98§	0.85±0.46
	Control (5)	0.03±0.12	0.59±0.24
10	rIL-1β (5)	9.05±2.61§	0.15±0.05
	rIL-1α (3)	8.27±3.05§	0.06±0.04
	Control (4)	0.04±0.02	0.49±0.22
24	rIL-1β (4)	0.09±0.04	0.19±0.08
	rIL-1α (4)	0.06±0.04	0.18±0.07
	Control (3)	0.02±0.02	0.58±0.25

* Mean±SE WBC × 10^3/mm^3; ‡ mean + SE ^{125}I cpm CSF/^{125}I cpm blood × 100; and § $P < 0.05$ compared with controls.

Table II. Changes in CSF Leukocyte Concentration and BBB Permeability with rIL-1β: Influence of Leukopenia, Polymyxin B and a Monoclonal IgG Antibody to rIL-1β

n	Inoculum*	CSF leukocyte concentration‡	BBB permeability§
9	Control	0.25±0.08	0.60±0.15
12	rIL-1β (50 U)	10.48±2.55	3.97±0.72
9	rIL-1β (50 U; Leukopenic)	0±0‖	0.42±0.05‖
6	rIL-1β + polymyxin B (2 μg/ml)	6.11±1.46¶	3.59±1.09¶
4	rIL-1β + monoclonal Ab to rIL-1β	1.00±0.57**	1.12±0.62**
6	rIL-1β + control monoclonal Ab‡‡	7.55±0.20	3.42±1.17
5	rIL-1β + monoclonal Ab to TNF	0.26±0.44‖	0.43±0.43‖

* Duration of intracisternal inoculum was 3.5 h in all groups; ‡ mean±SE white blood cells/mm^3 × 10^3; § mean±SE ^{125}I cpm CSF/^{125}I cpm blood × 100; ‖ $P < 0.001$ compared with rIL-1β alone; ¶ $P > 0.05$ compared with rIL-1β alone and $P < 0.05$ compared with controls; ** $P < 0.01$ compared with rIL-1β alone or rIL-1β inoculated with control Ab; and ‡‡ mouse anti-rat transferrin receptor monoclonal IgG.

or a mouse IgG monoclonal antibody (1:50 dilution) to rIL-1β. Preincubation with polymyxin B (for 15 min at 37°C) did not significantly alter either the CSF exudation of leukocytes or BBB permeability to ^{125}I-BSA compared with rIL-1β inoculum alone ($P > 0.05$). However, preincubation with the IgG monoclonal Ab to rIL-1β significantly reduced both the CSF pleocytosis and the traversal of ^{125}I-BSA into the CSF ($P < 0.01$) compared with the rIL-1β alone or rIL-1β inoculated with a control monoclonal Ab. To assess the possibility that endogenous rodent TNF may be induced by the rIL-1β inoculum and account for some of its biologic activity, rIL-1β was inoculated intracisternally with a hamster anti-murine TNF monoclonal IgG. As seen in Table II, this anti-TNF antibody significantly reduced both the CSF WBC (mean±SE = $0.3\pm0.4 \times 10^3/mm^3$) and ^{125}I-BSA penetration (mean±SE = 0.4±0.4%) compared with the rIL-1β inoculum alone ($P < 0.01$).

5. *Synergism of rIL-1β and rTNFα in influencing CSF leukocyte exudation and BBB permeability to systemic ^{125}I-BSA*

As noted previously, intracisternal inoculation of rTNFα at concentrations of 10^1–10^6 U caused minimal CSF pleocytosis and negligible changes in BBB permeability to systemically administered ^{125}I-BSA. However, to test the possibility that the combination of rIL-1β and rTNFα might exhibit a synergistic biologic response, various combinations of low dose rIL-1β plus rTNFα were inoculated intracisternally with CSF inflammation and ^{125}I-BSA penetration assayed 3.5 h later (Table III). As shown, when an inoculum of rTNFα that elicited no independent response (10^3 U) was combined with rIL-1B at low doses (0.0005–5.0 U) a synergistic effect was observed in both CSF leukocyte exudation and BBB permeability to ^{125}I-BSA.

Discussion

The major focus of this investigation was to determine whether an inflammatory cytokine could independently induce meningitis and effect BBB injury in vivo. The salient results suggest that intracisternal challenge with human rIL-1β induced both CSF pleocytosis and BBB injury in a time- and dose-dependent manner. Furthermore, the BBB injury is reversible and preventable by prior induction of systemic leukopenia. Human rIL-1α showed similar kinetics of both CSF inflammation and BBB injury when intracisternally inoculated in similar doses. Intracisternal inoculation with human rTNFα conversely caused minimal independent CSF pleocytosis or BBB injury but showed a synergistic effect with rIL-1β.

From a clinical and experimental perspective, the diversity of infectious agents capable of inducing meningitis and BBB injury has always suggested the potential for an endogenous host mediator. Specifically, previous investigations of experimental meningitis in the rat have demonstrated a reproducible delay of at least 2 h after intracisternal inoculation with diverse live bacteria (*Haemophilus influenzae, Streptococcus pneumoniae, Escherichia coli*) as well as bacterial surface components (i.e., lipooligosaccharide of *H. influenzae, S. pneumoniae* cell wall) (3, 4, 6–8). Hence, one logical hypothesis is that the uniform timing and extent of BBB injury stems from host synthesis and release of one or more common inflammatory mediators by in situ cells of the CNS.

Interleukin-1, a multifunctional polypeptide inflammatory cytokine (9), represents a potential common mediator of diverse causes of meningitis and BBB injury for several reasons. First, several cell types within the CNS are capable of in situ release of IL-1 including astrocytes, microglial cells, vascular endothelium, as well as macrophages lining the leptomeninges (10). Second, IL-1 has been shown to increase adhesiveness of neutrophils to vascular endothelium in vitro via inducible leukocyte adhesion glycoproteins (i.e., ELAM-1, ICAM-1) on the endothelial cell membrane (5, 11–13). Hence, in situ synthesis of IL-1 within the CSF as a consequence of meningeal infection could serve as a tropic factor localizing neutrophil adherence and emigration through the cerebral microvasculature with subsequent BBB injury.

As the initial investigation into this concept, we sought to characterize the potential of intracisternal IL-1 as an inducer of meningitis and BBB injury using a well characterized rat model. Recombinant human IL-1β (pI 7.0) was used to facili-

Table III. Synergism between Intracisternal rIL-1β and rTNFα in CSF Leukocyte Exudation and BBB Permeability

n	rIL-1β inoculum*	rTNFa inoculum‡	CSF leukocyte concentration§		BBB permeability‖	
6	0	1,000	0.08±0.05		0.64±0.17	
4	0.0005	1,000	0.34±0.19	$P = 0.41$	1.04±0.18	$P = 0.04$
4	0.0005	0	0.15±0.11		0.39±0.18	
3	0.05	1,000	12.32±3.56	$P = 0.08$	4.00±0.19	$P = 0.001$
3	0.05	0	0.48±0.13		0.68±0.38	
4	0.5	1,000	27.70±4.38	$P = 0.004$	3.95±0.45	$P = 0.003$
5	0.5	0	4.75±3.43		0.78±0.52	
10	5.0	1,000	20.31±4.57	$P = 0.03$	6.24±0.81	$P < 0.001$
10	5.0	0	8.46±1.96		2.64±0.53	
3	12.5	1,000	34.50±5.88	$P = 0.17$	4.68±0.96	$P = 0.25$
4	12.5	0	17.99±7.70		3.06±0.80	
3	25.0	1,000	31.83±8.01	$P = 0.009$	4.83±0.83	$P = 0.77$
12	25.0	0	13.31±2.40		4.29±0.83	

* Concentration in Units of rIL-1β; ‡ concentration in Units of rTNFα; § mean±SE leukocytes $\times 10^3/mm^3$; and ‖ mean±SE ^{125}I cpm CSF/^{125}I cpm blood × 100.

tate purity of preparation and was selected as the major test inoculum because IL-1β is the predominant extracellular form of IL-1 in vivo (10, 14–16). As shown in Figs. 1 and 2, intracisternal inoculation of human rIL-1β induced both a time- and dose-dependent meningitis by emigration of leukocytes (>95% neutrophils) into CSF. Significant CSF pleocytosis began 3 h postinoculation, peaked at 6 h, and was reversible by 24 h. Significant BBB injury, as measured by CSF traversal of systemically administered ^{125}I-BSA, was concomitantly observed 3 h postinoculation, peaked between 3–4 h, and was reversible by 6 h. Both of these effects were dose dependent and were significant compared with controls with an inoculum as low as 5 U (5 ng) of rIL-1β. The reversibility of BBB injury is intriguing and suggests either rapid clearance of rIL-1β, generation of a soluble adhesion inhibitor (17), or tachyphylaxis to its effect on the microvasculature similar to that described in other systems (18).

This dose-response effect of human rIL-1β in the rat model is similar to that recently reported by Ramilo et al. using 5–200 ng of recombinant rabbit IL-1β to induce experimental meningitis in rabbits (19). Interestingly, recent preliminary data from our laboratory have documented that during *H. influenzae* lipooligosaccharide-induced experimental meningitis, IL-1 activity can be detected in CSF in a similar nanogram range. Specifically, after 20 ng of *H. influenzae* lipooligosaccharide intracisternal inoculation, up to 4.5 ng/ml of CSF IL-1 activity can be detected 30 min later (20). This is corroborated in recent human studies where IL-1β concentrations in the nanogram range were detectable in the CSF of children with bacterial meningitis (21). However, precise correlation between detectable CSF concentrations of IL-1β during natural disease and the concentration necessary to induce experimental meningitis may be unrealistic for several reasons. First, there may be some species-specific ligand receptor interaction mandating a higher concentration of human rIL-1β inoculum to induce experimental rat meningitis and BBB injury. Second, the IL-1β within the human CSF documented at clinical presentation has been present for hours or days and is likely continuously synthesized and secreted as long as bacteria (and their surface components) are present. This contrasts with the experimental models in which the rIL-1β inoculum is given as a single pulse dose, an experimental design in which a higher rIL-1β inoculum concentration may be necessary to induce disease. Third, the IL-1β measured within CSF during natural disease is likely acting in concert with other inflammatory peptides (e.g., TNF, macrophage inflammatory protein (MIP), GM-CSF) to induce the observed meningeal inflammation. Thus, one can expect that a higher concentration of IL-1β would be necessary to induce meningitis when given exogenously as the sole stimulus.

As noted previously, IL-1β is the predominant form of IL-1 as observed in vitro and in vivo. The amount of IL-1β mRNA in activated cells is up to 50-fold greater than IL-1α and culture supernatents of human body fluids contain more IL-1β than IL-1α (16). Nonetheless, despite different amino acid sequences, IL-1α and IL-1β are structurally related by crystallographic analysis and recognize the same receptor. Hence, parallel experiments were done using rIL-1α as an intracisternal inoculum and its time course of inducible CSF inflammation and BBB injury were compared with rIL-1β. As shown in Table I, 50 U of rIL-1α inoculated intracisternally induced a CSF pleocytosis and ^{125}I-BSA permeability similar to rIL-1β. However, the IL-1α response was slower in onset, its induced CSF inflammation less intense, and its induced BBB permeability more transient than that seen with IL-1β. These observations parallel other experimental observations showing that both forms of IL-1 are capable of inducing systemic acute phase responses, sleep, as well as augmentation of B, T, and natural killer cell responses (16).

To elucidate whether the injury to the cerebral microvasculature allowing for ^{125}I-BSA penetration was an effect of the inflammatory neutrophil response or an independent effect of IL-1 on the endothelium, experiments were repeated in leukopenic animals. As shown in Table II, when rats rendered systemically leukopenic were inoculated with 50 U of rIL-1β into a CSF pleocytosis was prevented and BBB injury was not observed. This observation is similar to recent findings in the same model using *H. influenzae* lipooligosaccharide as an inoculum (4), and supports the hypothesis that the BBB injury due to a pulse inoculum of IL-1 is neutrophil dependent. Limitations of this model prevent observations of the effect of a sustained IL-1 inoculum in CSF over several hours (i.e., by continuous infusion); such experiments are worth pursuing since IL-1 may effect an independent injury to endothelium with a more prolonged exposure. Nonetheless, the precise mechanism in which IL-1 and emigrating neutrophils interact to induce cerebral microvascular injury and ^{125}I-BSA transcytosis is unknown but may be related to generation of additional neutrophil-activating peptides (e.g., c5a, GM-CSF) within the CSF (22).

Although endotoxin activity was virtually undetectable in the rIL-1β preparation, control experiments were done to ensure that the biological effect was indeed due to the cytokine. As shown in Table II, preincubation of the rIL-1β inoculum (50 U) with polymyxin B did not significantly alter either CSF emigration of neutrophils or BBB permeability to ^{125}I-BSA. However, preincubation with a mouse monoclonal IgG antibody to human rIL-1β significantly reduced both CSF pleocytosis as well as BBB permeability to ^{125}I-BSA; an effect not seen with a control monoclonal antibody. These observations reinforce that it was indeed the rIL-1β in the test inoculum that was inducing the observed meningitis and BBB injury.

TNFα, a cytokine produced by the monocyte/macrophage in response to lipopolysaccharide (23), induces similar biologic effects ascribed to IL-1 including pyrogenicity (24), alteration of endothelial hemostasis (25, 26), and induction of leukocyte-endothelial cell adhesion in vitro (27). Since it thus represented an additional potential mediator of meningitis and BBB injury, we compared the effect of intracisternal challenge with human rTNFα to the effect of rIL-1β. As demonstrated in Fig. 1–4, intracisternal inocula as high as 10^6 U of rTNFα (160 μg/kg) were much less effective in independently inducing either a significant neutrophil emigration into CSF or BBB traversal of systemic ^{125}I-BSA. This demonstrated relative impotency of human rTNFα to independently induce experimental meningitis is corroborated by recent reports showing a similar finding when human rTNFα is inoculated into rabbit CSF at physiologic concentrations (< 100 ng) by Saukkonen et al. (28), as well as its ineffectiveness in inducing meningitis when inoculated into rabbits in concentrations up to 200 μg by Mustafa et al. (29).

There are several potential explanations for the relative impotency of human rTNFα in this model, as well as others. First, the biologic response of a TNF ligand-receptor interaction may be species specific (as with IL-1), making human recombinant

TNFα less effective in the rat. Although previous reports have documented the ability of human rTNFα to induce septic shock with end organ damage in the rat (30), it is possible that as a heterologous cytokine it is less effective within the unique physiologic milieu of the CSF than a homologous cytokine. The recently detailed report by Ramilo et al. (19), investigating homologous rabbit cytokines in the rabbit model, strongly support this notion. Specifically, intracisternal inoculation of purified rabbit TNFα (10^2–10^5 IU) as well as recombinant rabbit IL-1β (5–200 ng) produced significant CSF inflammation, and antibodies to each rabbit cytokine reduced the CSF inflammation induced by *H. influenzae* lipooligosaccharide. A second possibility is that the oligimerization and molecular conformation of rTNFα is different after inoculation into CSF (than when given intravenously) preventing the full expression of biologically active epitopes. Therefore, as an indirect inquiry into a potential role for TNF in meningeal injury, we examined whether part of the IL-1 biological effect might be facilitated by induction of TNF synthesis in vivo. To do this, rats were inoculated intracisternally with rIL-1β (50 U) combined with hamster anti-murine TNF monoclonal antibody. Indeed, when compared with rIL-1β alone, the anti-TNF antibody caused a significant reduction in both CSF pleocytosis and ^{125}I-BSA permeability. This suggests that the biologic effect of IL-1β may be related to endogenous CSF production of TNF which may synergistically participate in the observed in vivo effects. Such a concept is supported by recent observations that interleukin-1 can induce TNF synthesis in human mononuclear cells in vitro as well as circulating TNF activity in rabbits in vivo (31). However, recently reported observations by Ramilo et al. studying the CSF inflammatory effects of recombinant rabbit cytokines could not detect CSF TNF activity after recombinant rabbit IL-1β intracisternal inoculation (19). These contrasting observations are difficult to explain but may relate to different dose responses of endogenous TNF generation when heterologous and homologous Il-1β are used in experimental models.

To assess directly whether the combination of TNF and IL-1 might exhibit a synergistic inflammatory effect, the final experiment combined rTNFα and rIL-1β as an intracisternal inoculum. As shown in Table III, although an rTNFα inoculum of 10^3 U was without independent effect, the addition of rIL-1β as low as 0.05 U to the rTNFα induced a synergistic effect in both CSF neutrophil emigration and BBB permeability to systemic ^{125}I-BSA. The synergism of these cytokines in this response is similar to that observed in other experimental systems including the induction of shock (32), the Shwartzman reaction (33), as well as muscle catabolism in the rat (34). Additionally, the recent report by Ramilo et al. (19) revealed similar observations using a single dose combination of homologous cytokines in the rabbit model of meningitis. Specifically, simultaneous inoculation of 10^3 U of rabbit TNFα and 5 ng of recombinant rabbit IL-1β elicited a synergistic effect both in the rapidity and degree of CSF inflammation. Clearly then, although exogenous rIL-1β appears more independently potent as an inducer of meningitis and BBB injury in this model, there is evidence for a cytokine induction network resulting in a synergistic biological response in vivo between both IL-1 and TNF.

In summary, this investigation as well as those recently reported in the rabbit model can be objectively assessed to support the following concepts regarding cytokine participation in meningeal inflammation. First, interleukin-1 (both α and β) is capable of inducing both CSF inflammation and BBB injury as evidenced by exogenous intracisternal administration of homologous and heterologous preparations. Second, TNFα also participates in the induction of CSF inflammation as evidenced by exogenous administration of the homologous cytokine in the rabbit model, and demonstration of a synergistic inflammatory effect with IL-1β in both rat and rabbit models. Support of both IL-1β and TNFα as endogenous mediators is evidenced by their detection within CSF during experimental (both rat and rabbit) and human disease as well as the ability to modulate the CSF inflammatory response using specific antibodies to each cytokine.

In total, these experimental observations strongly support a critical biological role of these cytokines in the induction of CSF inflammation and BBB injury and suggest their potential as specific targets for therapeutic intervention in humans.

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