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Research Article

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Differing Roles for Platelet-activating Factor during Inflammation of the Lung and Subarachnoid Space

The Special Case of *Streptococcus pneumoniae*

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Abstract

Although well-characterized in the lung, the role of platelet-activating factor (PAF) in inflammation in the central nervous system is undefined. Using rabbit models of meningitis and pneumonia, PAF was found to induce significant blood-brain barrier permeability and brain edema at doses five times lower than those required to generate leukocyte recruitment to the subarachnoid space. Both leukocytosis and increased vascular permeability occurred in response to PAF in the lung. Antibody to the CD-18 family of leukocyte adhesion molecules inhibited leukocyte recruitment in response to PAF in the brain (> 80%); a similar level of inhibition in the lung required treatment with a combination of a PAF receptor antagonist (L-659,989) and anti-CD18 antibody. Treatment with L-659,989 decreased abnormal cerebrospinal fluid cytochemical values induced by intracisternal challenge with pneumococci but not *Haemophilus influenzae*, indicating a special role for PAF in pneumococcal disease. Antibodies directed at phosphorylcholine, a unique, shared determinant of bioactivity of PAF and pneumococcal cell wall, obviated the inflammatory potential of both agents. However, no evidence for a direct PAF-like activity of pneumococcal cell wall components was detected in vitro by bioassay using platelets or neutrophils. It is concluded that PAF can induce inflammation in the subarachnoid space. In brain, PAF effects appear to be mediated through CD-18–dependent events, while in lung, PAF effects independent of CD-18 are also evident. At both sites, PAF is of particular clinical importance during inflammation induced by pneumococci apparently due to a unique proinflammatory relationship between the pneumococcal cell wall teichoic acid and PAF. (*J. Clin. Invest.* 1992. 90:612–618.) Key words: meningitis • pneumococci • *Haemophilus influenzae*

Introduction

Platelet activating factor (PAF)[1] is a mediator of inflammation produced by macrophages, neutrophils, platelets, and endothelial cells in response to injury (1, 2). PAF induces cellular activation by binding to a receptor that is a member of the superfamily of G proteins (3). The sequelae of PAF–receptor interactions are varied but have been most clearly defined in the lung. They include bronchoconstriction, increased vascular permeability with protein exudation, and platelet aggregation. PAF has been implicated in the release of cytokines such as tumor necrosis factor (4) and putatively plays a role in the pathophysiology of anaphylaxis, shock, and adult respiratory distress syndrome (2, 5). We sought to define the role of this potent mediator in the inflammatory response elicited during bacterial meningitis.

It is widely recognized that invasive pneumococcal infection is characterized by particularly intense inflammation. In pneumococcal meningitis this leads to the highest rates of mortality and morbidity of common meningeal pathogens (6). Studies of pneumonia also indicate that pneumococcal infection triggers leukocyte recruitment mechanisms that are more complex than those seen with Gram-negative infection (7). We have demonstrated that inflammation during pneumococcal infection of both the subarachnoid space and the lung is induced in large part by the pneumococcal cell wall (8–10) and thus hypothesize that special features of the pneumococcal cell wall confer its enhanced inflammatory potential.

Pneumococcal cell wall is a complex macromolecule composed of two major polymers: a peptidoglycan and a teichoic acid of unusually complex structure that contains a constituent unique among pathogens: phosphorylcholine (11). In addition to the wall-bound teichoic acid, pneumococci also harbor a lipid-linked form of the teichoic acid, the lipoteichoic acid or Forssman antigen (12) (Fig. 1). Although many pneumococcal cell wall subcomponents are inflammatory, teichoic acid–containing cell wall fragments have the highest inflammatory activity: these components trigger the alternative pathway of complement activation in vitro (13), induce leukocytosis and increased vascular permeability in models of meningitis and pneumonia (8–10), and, at concentrations 100-fold lower than endotoxin, induce secretion of IL-1 from macrophages (14) and induce procoagulant activity on endothelial cells (15). These findings suggest that the pneumococcal teichoic acid and lipoteichoic acid have unusual inflammatory potential. This is further supported by the findings that neutralization of the inflammatory activity of the teichoic acid and lipoteichoic acid by antiphosphorylcholine antibodies or C-reactive protein is protective in murine models of sepsis (16, 17).

Phosphorylcholine is not found in the cell walls of pathogens other than the pneumococcus and this moiety is a critical determinant of the inflammatory activity of the pneumococcal

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1. *Abbreviations used in this paper:* BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; PAF, platelet-activating factor; PPP, platelet poor plasma; PRP, platelet rich plasma.

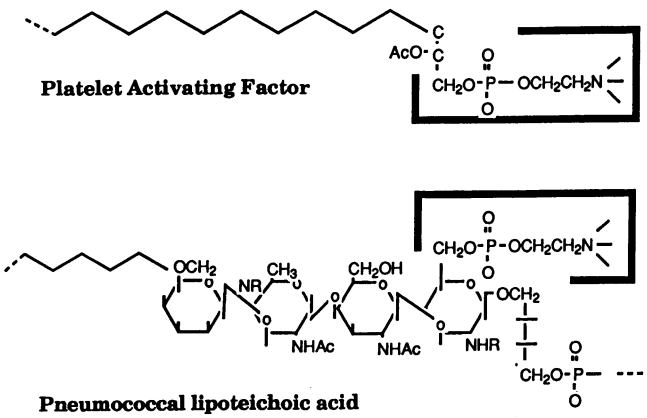

Figure 1. Partial chemical structures of platelet-activating factor and the pneumococcal lipoteichoic acid highlighting the common phosphorylcholine moiety in the box (the presence of lipid is indicated by the zigzag line).

teichoic and lipoteichoic acids (8, 9, 14). Phosphorylcholine is also a critical determinant of the biological activity of PAF (1) (Fig. 1). Like the pneumococcal teichoic and lipoteichoic acids, PAF reacts with the acute phase reactant, C-reactive protein, but rather than neutralizing its bioactivity, this complex enhances PAF-induced platelet aggregation (18). These relationships between the phosphorylcholine-dependent inflammatory activities of pneumococcal cell wall and those of PAF suggested the possibility that PAF might play a special role in pneumococcal disease.

The aims of our study were threefold: (*a*) to determine the ability of PAF to induce inflammation and altered physiology in the subarachnoid space and the lung; (*b*) to determine if PAF is activated during pneumococcal meningitis or pneumonia; and finally (*c*) to assess if the pneumococcal teichoic acid or lipoteichoic acid can act as agonists at the PAF receptor.

Methods

Preparation of platelet-activating factor, bacterial components, and antibodies. PAF (1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine; Sigma Chemical Co., St. Louis, MO) was diluted in chloroform/methanol (9:1) and stored at −20°C. Immediately before use, aliquots were removed and dried under a stream of air. PAF was rediluted in 0.2 ml of saline, sonicated for 2 min, and 100 μg was instilled in 0.2 ml of saline intracisternally or intratracheally.

Unencapsulated *Streptococcus pneumoniae* strain R6 or *Haemophilus influenzae* type b strain Eagan were grown to mid-logarithmic phase as described (6, 19), washed, and resuspended in pyrogen-free saline. 10^5 cell equivalents were administered intracisternally or intratracheally in a volume of 0.2 ml. In some experiments, pneumococci were boiled for 20 min in growth medium before introduction into the animal models; this preparation of killed cells is known to induce a highly reproducible inflammatory response similar to natural disease.

Pneumococcal cell wall was prepared according to an established protocol from strain R6 (8). Crude cell wall was treated with DNAse, RNAse, and trypsin, extracted with boiling SDS, washed and lyophilized. A dose of 50 μg in 200 μl of saline was used in animal experiments. Pneumococcal lipoteichoic acid was prepared from a 16-liter culture of strain R6 using a modification (12) of the original procedure (20). A dose of 100 μg in water was used in animal experiments.

L-659,989, trans-2-[3-methoxy-5-methylsulfonyl-4-propoxyphenyl]-5-[3,4,5-trimethoxyphenyl]tetrahydrofuran (Merck Sharp and Dohme Research Laboratories, Rahway, NJ), a competitive PAF receptor antagonist highly active in the rabbit (21, 22), was prepared as a 2-mg/ml (5-mM) solution in dimethylsulfoxide. Immediately before use, 50-μl aliquots were diluted into 0.5 ml isotonic saline preheated to 55°C, and 100 μl was injected intravenously or intracisternally per rabbit. This dose of 10 μg/kg (25 μM/kg) was previously shown to be at least 1,000-fold greater than the ED_{50} for inhibition of PAF-induced effects on rabbit platelets and neutrophils in vitro and equal to the ED_{50} to inhibit PAF-induced bronchoconstriction in guinea pigs (22). In pilot experiments using the meningitis model, doses of 0.1 or 2 μg/kg were less effective while two doses of 10 μg/kg 1 h apart were no more effective than the single dose of 10 μg/kg. The diluent was noninflammatory in both animal models.

Monoclonal antibody IB4 (Merck Sharp and Dohme Research Laboratories), directed against the CD18 epitope of the CD11/CD18 family of leukocyte adhesion molecules, was administered intravenously at 0.5 mg purified protein/kg. TEPC-15 (Hazleton Laboratories, Rockville, MD), a monoclonal antibody recognizing phosphorylcholine, was injected intracisternally or intratracheally at 0.1 mg purified protein/kg.

Rabbit model of meningitis. The rabbit model was performed according to an established protocol (6, 23). For all experiments, rabbits were challenged in groups of at least four. In brief, 2 kg, specific pathogen-free, New Zealand white rabbits (Hare Marland, Nutley, NJ) were anesthetized with ketamine and xylazine, and a dental acrylic helmet was affixed to the calvarium. 24 h later, the animals were anesthetized with urethane and pentobarbital and placed in a stereotaxic frame. A spinal needle was introduced into the cisterna magna and 300 μl of cerebrospinal fluid (CSF) was withdrawn. Live bacteria, heat-killed *S. pneumoniae* R6, pneumococcal cell wall, lipoteichoic acid, or PAF were instilled into the subarachnoid space in 200 μl of pyrogen-free saline. In some experiments, L-659,989 (10 μg/kg) was administered intravenously or intracisternally at the same time as the bacterial challenge. Similarly, mAb IB4 (0.5 mg/kg) was administered intravenously at the same time as the bacterial challenge. CSF samples were withdrawn over 6 h and tested for leukocyte density using a counter (Coulter Electronics Inc., Hialeah, FL). The CSF samples were centrifuged at 10,000 *g* for 5 min, and the supernatant fluid was frozen at −70°C until assayed for protein with the BCA method (Pierce Chemical Co., Rockford, IL). After 6 h, animals were euthanized, and brains were removed immediately for quantitation of brain edema by comparing wet/dry wt (g H_2O/100 g dry wt) (24).

Rabbit model of pneumonia. Using a model adapted from that of Onofrio and coworkers (13, 25), intratracheal instillation of bacteria or PAF was undertaken in groups of at least four rabbits. New Zealand white rabbits (2 kg, specific pathogen free; Hare Marland) were anesthetized with ketamine and xylazine and a 2-cm incision was made over the trachea. The catheter from a 19 G 7/8 butterfly infusion set (Abbott Hospitals, Inc., North Chicago, IL) was introduced into the right mainstem bronchus, wedged in place, and then withdrawn 1 cm. The inoculum was introduced into the catheter in 0.2 ml of saline followed by 5 ml of air, and the incision was stapled. In some animals L-659,989 (10 μg/kg) or mAb IB4 (0.5 mg/kg) or both were administered intravenously simultaneous with the bacterial challenge. 1 h later in animals challenged with PAF and 3 h later in animals challenged with bacteria, bronchoalveolar lavage (BAL) was performed with saline supplemented with 5% heparin and 0.1% methylene blue (2 × 10 ml). Lavage fluid volume was recorded and the fluid was centrifuged at 1,000 *g* for 10 min. The pellet was resuspended in 10 ml of isotonic buffered saline and the number of leukocytes determined using a counter (Coulter Electronics Inc.). The protein content of the supernatant was determined with the BCA method (Pierce Chemical Co.). The protein and leukocyte counts were corrected to reflect values in the original lung fluids by an adaptation (26) of the method of Baughman et al. (27) using the methylene blue as an external marker.

Platelet-activating factor assays. The ability of pneumococcal cell wall to mimic PAF was tested in an assay of induction of aggregation of guinea pig platelets in platelet rich plasma (22, 28). Cell wall or lipoteichoic acid was tested in two ways: directly for PAF-like effects along-

side a PAF control and indirectly for the ability to competitively inhibit PAF-induced effects. Guinea pig (male Dunkin-Hartley, 400–800 *g*) blood was obtained by heart puncture using a 20 G needle and plastic syringe from animals under light urethane anesthesia. Blood was immediately added to plastic tubes containing 10% (vol/vol) trisodium citrate (0.13 *M*) and mixed by inversion. After centrifugation of whole blood (1,000 *g*; 4 min; 20°C), the supernatant platelet-rich plasma (PRP) was aspirated using plastic pipettes and stored in stoppered plastic containers at 37°C for the duration of the experiment. Residual blood was centrifuged (8,000 *g*; 5 min; 20°C) to obtain platelet-poor plasma (PPP). The platelet concentration in PRP was estimated using a cell counter (model ZM; Coulter Electronics) and was adjusted to 4 $\times 10^8$ cells/ml by addition of autologous PPP. Platelet aggregation was monitored by standard photometric techniques using a PAP4 Platelet Aggregation Profiler (Bio-Data, Inc., Horsham, PA), where maximum light transmission was set using PPP.

The pneumococcal components were also compared with PAF for the ability to induce degranulation of human neutrophils. Human blood, obtained by antecubital venipuncture from consenting volunteers who denied taking medication within the preceding seven days, was collected into plastic tubes containing sodium heparin (50 U/ml final concentration). PMNs were isolated by Ficoll-Hypaque (Histopaque; Sigma Chemical Co., St. Louis, MO) separation, followed by hypotonic lysis of contaminating erythrocytes, essentially as described previously (28). PMN were resuspended at 10^6 cells/ml in Hepes (15 mM) buffered HBSS, pH 7.4 and used within 1 h of isolation. Degranulation was monitored as human leukocyte elastase–catalyzed cleavage of the fluorogenic substrate methoxysuccinyl-Ala-Ala-Pro-Val-aminomethylcoumarin, by a modification of the assay originally described by Dewald and Baggiolini (29). Briefly, assays were performed at 37°C in a final volume of 250 μl in 96-well plates (Costar Corp., Cambridge, MA). To each well was added fluorogenic substrate (10 nmol in 125 μl HBSS). Thereafter, PMN (10^5 cells in 100 μl HBSS) previously treated with cytochalasin B (1.2 μM; 37°C; 10 min) were added, and plates were allowed to incubate for 2 min at 37°C before addition of PAF (1 μM final concentration), cell wall constituents, or vehicle (control) added in a volume of 25 μl. Fluorescence of the cleavage product aminomethylcoumarin (excitation = 355 nm; emission = 460 nm) was monitored over a 60-min period in a Fluoroskan II plate-reading fluorimeter (Flow Laboratories, Rockville, MD) thermostatted to 37°C. Preliminary studies established that PAF-induced elastase release was complete within 30 s of agonist addition. Data are expressed as arbitrary fluorescence units.

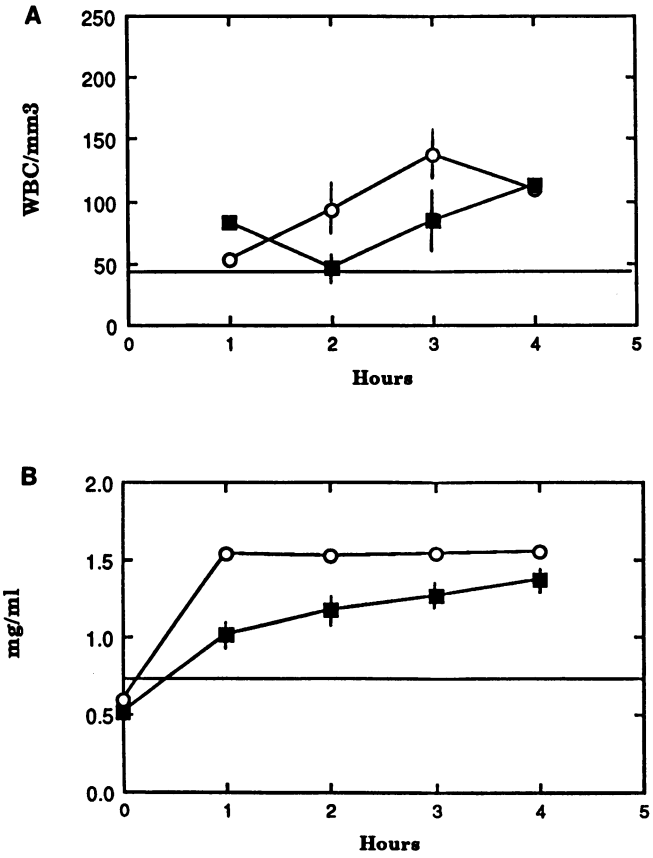

Figure 2. Induction of inflammation in CSF by intracisternally administered PAF (100 μg) and modulation by L-659,989 intracisternally (10 μg/kg) (mean of four rabbits±SEM). PAF (*open symbols*); PAF plus L-659,989 (*closed symbols*). (*A*) Leukocyte number (*WBC*); (*B*) protein concentration (the horizontal line shows the upper limit of normal).

Table I. Inflammatory Activity of Platelet-activating Factor in Bronchoalveolar Lavage

	Leukocyte density	Protein concentration
	$\times 10^3$/ml lung fluid	*mg/ml lung fluid*
PAF (100 μg)	93.6±48	1.00±0.10
PAF + L-659,989 (10 μg/kg intravenously)	58.3±7	0.41±0.02

Upper limit of normal: 0.4 cells $\times 10^3$/ml lung fluid; 0.1 mg protein/ml lung fluid. Values represent peak values and were taken 1 h after challenge.

Results

Inflammatory activity of PAF in the brain and lung. Intracisternal inoculation of 100 μg PAF into 15 rabbits caused a marked increase in protein concentration in the CSF over a period of 1 h which persisted for at least 4 h (Fig. 2). This index of abnormal blood-brain barrier permeability was accompanied by the rapid appearance of brain edema: mean brain wt at 4 h of 409±6 *g* H_2O/100 *g* dry wt (upper limit of normal = 400; $P < 0.01$). However, PAF induced only a mild leukocytosis (peak 150 cells/μl) (Fig. 2). Administration of a lower dose of PAF (20 μg; $n = 6$) resulted in the same peak value of protein (1.34±0.24 mg/ml CSF) at 4 h but the rate of rise of protein was slower (2-h value of 1.11±0.36). Brain edema was present in 75% of these animals at 4 h. With the administration of doses of PAF ≥ 500 μg, greater leukocytosis was observed (values > 2,000 cells/mm^3 at 4 h for each of three animals). Intracisternal administration of the PAF receptor antagonist, L-659,989 delayed the influx of protein into CSF in response to 100 μg PAF (Fig. 2) and prevented brain edema (396±4 g H_2O/100 *g* dry wt at 4 h). Intravenous L-659,989 was ineffective. These results suggested that the threshold for induction of leukocytosis by PAF was higher than that for blood-brain barrier permeability, but that PAF could induce both aspects of inflammation.

The inflammatory activity of PAF in the lung is shown in Table I. PAF induced a rapid increase in protein concentration in BAL fluid similar to that seen in CSF but, in contrast to the brain, also induced leukocytosis in the lung. L-659,989, administered intravenously (but not intratracheally), reduced the influx of protein by 60%±5; a diminution of leukocytosis

by 38%±3 also occurred but did not achieve statistical significance.

To determine if PAF-dependent leukocytosis proceeded through the participation of CD18 family of leukocyte adhesion molecules, animals were challenged with PAF in the presence and absence of intravenous anti-CD18 antibody. All leukocytosis in the CSF was inhibited by CD18 (> 98±6% decrease), while inhibition of leukocytosis in the lung was reduced by half (53±12% decrease). More substantial inhibition of leukocytosis in lung required administration of L-659,989 in combination with the anti-CD18 mAb IB4 (Table II).

Role of PAF in pneumococcal inflammation. Having demonstrated inflammatory activity of PAF in the subarachnoid space and lung, the contribution of PAF to inflammation during natural bacterial infection was determined. Rabbits were challenged with killed *S. pneumoniae* intracisternally or intratracheally. Control animals developed a marked leukocytosis and influx of protein in CSF (Fig. 3). L-659,989 partially suppressed the leukocytosis in CSF (7,168±660 vs 1,523±1,168 mean cells/μl at 6 h, $P < 0.001$) and delayed the increase in protein concentration by ~ 2 h. This effect was evident upon challenge with live pneumococcus also, but could not be demonstrated for infection with *H. influenzae* (Table III). In the pneumonia model (Table II), administration of L-659,989 maximally reduced the BAL protein concentration to 43%±3 at 1 h but leukocytosis persisted at > 69%±4 of control levels even at 3 h. Results from both models suggested PAF contributed to leukocyte influx and endothelial permeability during *S. pneumoniae* infection. However, the greater ability of L-659,989 to prevent leukocytosis in the brain than in the lung, suggested PAF-independent effects were active in lung.

Cross-neutralization of PAF and wall-induced inflammation by antiwall and PAF antagonists. The antiphosphorylcholine mAb TEPC-15 binds to the teichoicated components of the pneumococcal cell wall. Administration of the antibody intracisternally neutralized inflammation induced by *S. pneumoniae,* pneumococcal cell wall, or lipoteichoic acid (Fig. 4). mAb TEPC-15 also reduced brain edema induced by PAF (409±6 vs 393±4 g H_2O/100 g dry wt, $P < 0.01$) but not the PAF-induced increase in CSF protein concentration (1.6±0.3 vs 1.8±0.4 mg/ml, $P > 0.5$). Since the antiwall antibody cross-neutralized inflammation caused by PAF, we tested the converse, i.e., could L-659,989 neutralize inflammation induced by cell walls. Treatment with L-659,989 decreased CSF leukocytosis and protein concentrations induced by pneumococcal cell wall or lipoteichoic acid (Fig. 4).

This cross-neutralization could occur by two mechanisms. In one case, the cell wall or lipoteichoic acid could be acting directly as a PAF analogue, or, in the second case, the wall could generate endogenous PAF. To distinguish these possibilities, cell wall components were tested for the ability to mimic

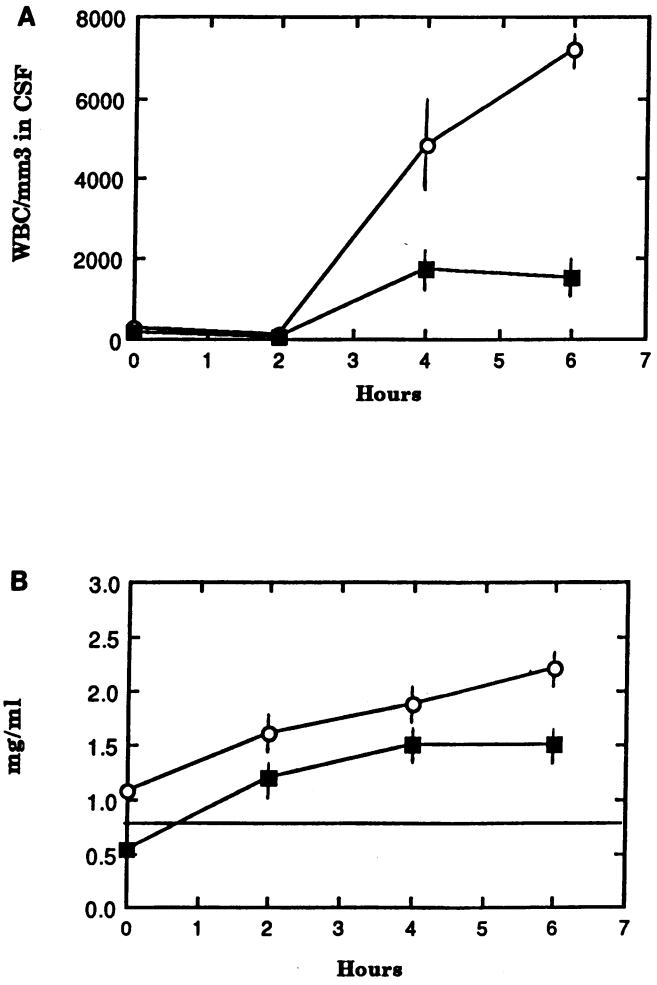

Figure 3. Modulation of CSF inflammation due to *S. pneumoniae* by L-659,989. *S. pneumoniae* (*open symbols*); *S. pneumoniae* plus L-659,989 (10 μg/kg intracisternally) (*closed symbols*). (*A*) Leukocyte number (*WBC*); (*B*) protein concentration (the horizontal line shows the upper limit of normal).

Table II. Inflammatory Activity of S. pneumoniae in Bronchoalveolar Lavage and Modulation by Platelet-activating Factor Antagonist or Anti-CD18 mAb IB4

	Leukocyte density	Protein concentration
	×10³/ml lung fluid	*mg/ml lung fluid*
Pn	92±3	0.56±0.01
Pn + L-659,989	64±17*	0.24±0.04*
Pn + L-659,989 + IB4	35±9*	0.16±0.10*
Pn + IB4	74±2*	0.46±0.24

Upper limit of normal: 0.4 cells ×10³/ml lung fluid; 0.1 mg protein/ml lung fluid. Values represent peak values and were taken 3 h after challenge. Pn, pneumococcus; * $P < 0.01$ compared to Pn.

Table III. Effect of Platelet-activating Factor Antagonist on Leukocytosis in Colony-stimulating Factor

	S. pneumoniae	*H. influenzae*
Bacteria alone	2,257±359	2,766±228
+ L-659,989	335±68	2,614±365

Animals were challenged intracisternally with 10^5 CFU of living bacteria. Half the animals received L-659,989 (10 μg/kg intravenously) at the time of bacterial challenge and sampling of CSF for all animals was made at 2-h intervals. Values are peak values taken at 6 h after inoculation.

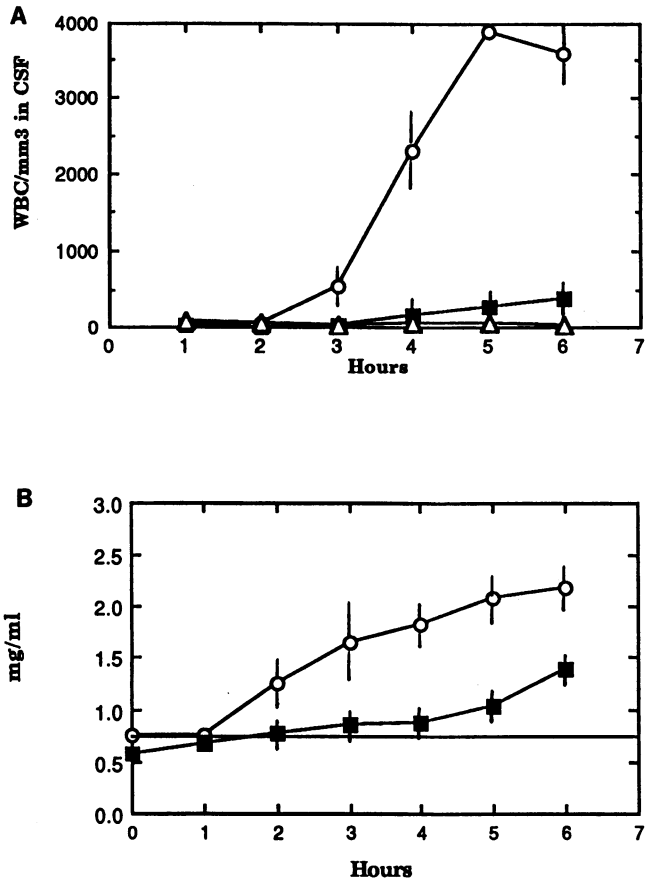

Figure 4. Modulation of CSF inflammation due to pneumococcal cell wall by L-659,989 or antiwall antibody. Pneumococcal cell wall (*open circle*); cell wall plus L-659,989 (*closed square*); cell wall plus TEPC-15 mAb (*open triangle*). (*A*) Leukocyte number (*WBC*); (*B*) protein concentration (the horizontal line shows the upper limit of normal). Protein determinations for the TEPC-15–treated group were not interpretable since the presence of the antibody itself led to spuriously high values.

PAF in assays of platelet aggregation and neutrophil degranulation in vitro. PAF induced platelet aggregation in a concentration-dependent manner while cell wall and lipoteichoic acid were inactive (Table IV). Based on these results, 20 μg cell wall contains < 1 nM PAF equivalents. Furthermore, neither cell wall derivative inhibited PAF-induced aggregation of platelets, in contrast to the effects of L-659,989 (EC_{50} 31 nM). Similar results were obtained using a neutrophil degranulation assay in that neither direct induction of degranulation nor inhibition of PAF-induced degranulation could be demonstrated using cell wall or lipoteichoic acid (Table V).

Discussion

PAF is the first well-characterized phospholipid to be considered a mediator in acute inflammation (30). PAF is produced by many cells and is rapidly metabolized to the inactive precursor lyso-PAF, suggesting that its biological activities are tightly regulated. PAF acts on a very wide range of cell types (neutrophils, eosinophils, monocytes, muscle cells, fibroblasts, endothelial cells, type II alveolar cells, and neuronal cell lines) and induces increased vascular permeability with protein exudation and accumulation of platelets and leukocytes (2). More recently, PAF has also been implicated as a contributor to circulatory disturbances such as pulmonary hypertension (31) or sytemic hypotension during endotoxic shock (32). Our study suggests that PAF is inflammatory in the central nervous system, causing significant blood-brain barrier permeability and brain edema; at higher doses, these effects are accompanied by leukocytosis. This is consistent with the findings of Humphrey et al. (33) in which the vasoactive properties of PAF were found to be dissociable from the leukotactic properties in a rat model of dermal inflammation. However, PAF induced both an increased protein exudation and leukocytosis in BAL fluid. The more prominent leukotactic effect of PAF in lung than in the brain suggests that leukocyte recruitment in response to this mediator in the lung differs from that in the brain. This is substantiated by the ability of mAb IB4 directed against the CD18 leukocyte adhesion molecules to prevent virtually all CSF leukocytosis caused by intracisternal *S. pneumoniae* (34) or by PAF, but to reduce only partially the leukocytosis in BAL fluids of rabbits challenged with *S. pneumoniae* (2, confirmed in this study) or PAF intratracheally. Since the combination of mAb IB4 and L-659,989 greatly inhibited BAL leukocytosis, we suggest that PAF-dependent leukotaxis can occur independent of CD18 in the lung; such a CD18-independent mechanism has been described for peritoneal inflammation also (35). In contrast to the lung and peritoneum, PAF appears to induce

Table IV. Ability of Platelet-activating Factor and Cell Wall Components to Induce Aggregation of Guinea Pig Platelets

Stimulus	Concentration	Percent maximal response (SD)
PAF	0.03 nM	3.1±4
	1.0	16.3±23
	3.0	46.3±63
	10.0	79.8±11
Cell wall	2 μg/ml	0
	20	3.1±4
Lipoteichoic acid	2 μg/ml	0
	20	2.7±4

Samples (0.5 ml) of citrated PRP were challenged with PAF, cell wall, or lipoteichoic acid at the concentrations indicated. Platelet aggregation was measured as changes in light transmission through the stirred PRP. Maximum aggregation was defined as the difference in light transmission between PRP and PPP. Data are mean values±SD of triplicate determinations.

Table V. Ability of Platelet-activating Factor and Cell Wall Components to Induce Neutrophil Degranulation

Stimulus	Elastase release
	Fluorescene U±SD
Saline	217±4
PAF (1 μM)	1,687±6
PAF + L-659,989 (1 mM)	207±24
Cell wall (1 μg/ml)	200±3
Cell wall + PAF	1,651±1

only CD18-dependent leukotaxis in the central nervous system.

PAF is detectable in the CSF of children with bacterial meningitis, and the concentration of PAF correlates with the outcome of disease (36). To prove the clinical significance of the ability of PAF to induce protein influx and leukocytosis in lung and brain, a PAF antagonist was administered to animals challenged with pneumococci. The antagonist greatly decreased inflammation in the brain, but only partially reversed inflammation in the lung suggesting that PAF-independent modes of inflammation also contribute to pulmonary inflammation in response to infectious agents. The fact that the PAF-independent pulmonary inflammation was extinguished by addition of the anti-CD18 antibody strengthens the conclusion that PAF and CD18 work together in brain but independently in lung.

In contrast to inflammation induced by *H. influenzae,* PAF played a prominent role in pneumococcal disease. PAF is most commonly generated as part of an inflammatory cascade and would be expected to appear after the interaction of pneumococcal cell walls with host receptors. Since the intense inflammatory activity of the pneumococcal cell wall resides in the teichoicated subcomponents and these components share with PAF the requirement for the phosphorylcholine moiety for bioactivity, we investigated: (*a*) if the phosphorylcholine determinants were cross-neutralizing and therefore participated in related biological response pathways; and (*b*) if these wall components might act directly as PAF analogues and exert agonist activity after binding to PAF receptors. No direct PAF-like agonist activity was demonstrable by cell walls for two target cells, platelets and neutrophils. However, two findings in the animal model of meningitis supported cross-neutralization by inhibitory antibodies or drugs. The PAF receptor antagonist neutralized wall-induced inflammation and, conversely, anti-wall antibodies neutralized PAF-induced brain edema. Anti-wall antibodies are found in sera from patients with pneumococcal pneumonia and a high titer has been associated with a poor clinical outcome (37). Our findings suggest that anti-pneumococcal wall antibodies could diminish the effectiveness of PAF during inflammation in the lung and thereby adversely affect the course of disease. We conclude that the intensity of inflammation associated with pneumococcal infection in brain and lung is augmented by a unique participation of endogenous PAF in the inflammatory response to *S. pneumoniae* cell wall components.

Acknowledgments

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