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Bacteria Induce Release of Platelet-Activating Factor (PAF) from Polymorphonuclear Neutrophil Granulocytes: Possible Role for PAF in Pathogenesis of Experimentally Induced Bacterial Pneumonia

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The role of platelet-activating factor (PAF) as mediator of the endotoxin shock and endotoxin-dependent tissue injury has been examined. The ability of opsonized bacteria to stimulate the release of PAF from human polymorphonuclear neutrophil granulocytes was evaluated by measuring both the activity and the amount of the mediator released in the supernatant of the cell-bacteria reaction in vitro. There was no significant difference between gram-positive and gram-negative bacteria in the ability to release PAF from neutrophils. However, preincubation of the cells with the specific PAF receptor antagonist WEB 2170 decreased release of PAF from the cells. Furthermore, a possible protective effect of the PAF antagonist was examined during experimentally induced pneumonia with *Klebsiella pneumoniae* in NMRI mice. Oral treatment of mice with WEB 2170, followed by infection with the microorganisms, resulted in a considerable increase in the animals' survival (53 to 73%) compared with the control group (40%); this increase corresponded with a decrease in the CFU per gram of lung tissue. These findings indicate an important role of PAF in the pathogenesis of pneumonia in mice.

Platelet-activating factor (PAF) is a potent autacoid (4) lipid mediator with a unique spectrum of diverse biological and pharmacological activities. In vitro, PAF induces aggregation and degranulation of platelets (2, 3), contraction of vascular smooth muscle cells (17), and negative inotropic and arrhythmogenic cardiac effects (1, 28) and stimulates chemotaxis and degranulation in polymorphonuclear leukocytes and monocytes (7, 31, 37). In vivo, PAF provokes vasodilation and increased vascular permeability (22, 23), edema, systemic hypotension (12), and alteration of lung function such as bronchoconstriction (30).

It is well known that PAF is produced by various cell types that are crucially involved in the initiation of inflammatory changes in the tissue (6, 26, 32), such as polymorphonuclear neutrophil granulocytes (PMN). Although microbial products have been shown to induce the release of PAF (5, 24), it is presently unknown whether actual contact between viable microorganisms and PMN leads to the release of the mediator. The aim of this study was to evaluate in vitro whether (i) live opsonized bacteria are capable of stimulating the release of PAF from PMN, (ii) there are differences between gram-positive and gram-negative microorganisms in the ability to induce the release of the mediator from PMN, and (iii) the generation of PAF can be inhibited by pretreatment of the cells with a specific PAF receptor antagonist. Furthermore, we examined whether treatment of animals with a specific PAF antagonist, in an experimentally induced bacterial pneumonia model, is reflected in a change of the pathogenicity of microorganisms in vivo.

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MATERIALS AND METHODS

Bacteria. The bacterial strains used were *Klebsiella pneumoniae* 476, *Haemophilus influenzae* 1447, *Streptococcus pyogenes* 68, and *Streptococcus pneumoniae* 179 (capsular type 14). All microorganisms were isolated from clinical specimens from the Department of Infectious Diseases and Chemotherapy. Bacteria were kept frozen in liquid nitrogen (−190°C) at a concentration of 5×10^8 CFU/ml in Trypticase soy medium supplemented with 2.5% (vol/vol) glycerin until used (18).

Preparation of PRP, PMN, and opsonized bacteria. Peripheral blood of healthy adult volunteers, drawn into 1/10 volume 3.8% citrate, was taken as the source of platelet-rich plasma (PRP) and PMN. Volunteers were previously screened for antibacterial antibodies and found to be negative. PRP was collected after centrifugation of blood at 160 × *g* for 20 min at 23°C (26). The platelet concentration in PRP was adjusted to 2.8×10^8 cells per ml, using platelet-poor plasma obtained by centrifugation of PRP (2,500 × *g*, 15 min, 23°C). Human PMN were separated according to the following technique. Twenty-five milliliters of blood was layered carefully over 15 ml of Mono Poly resolving medium–Ficoll-Hypaque (1.114 g/ml; Flow Laboratories) and centrifuged at 700 × *g* for 40 min at 23°C. The PMN band, which can be seen as a single layer within Ficoll-Hypaque, was collected, and the cells were washed twice in Hanks' buffered salt solution without Ca and Mg (GIBCO). Pelleted cells were resuspended in erythrocyte-lysing solution, centrifuged (210 × *g*, 10 min, 23°C), and washed twice in Tyrode's solution supplemented with 0.25% bovine serum albumin (Tyrode's BSA) (26). Cells were resuspended to a concentration of 2×10^7 cells per ml in Tyrode's BSA. Cell suspensions thus prepared contained 96 to 99% PMN, 93 to 97% of which were viable, as shown by staining with trypan blue.

Bacteria were opsonized by rapidly thawing the frozen (−190°C) bacterial suspensions to room temperature and, after addition of fresh homologous human serum (10%, vol/vol), incubating the suspensions at 37°C for 30 min (33). After incubation, opsonized bacteria were washed thoroughly in Hanks' balanced salt solution without Ca and Mg and resuspended to a concentration of 10^9 CFU/ml in Tyrode's BSA.

Preparation of supernatants. In the standard reaction mixture, PMN (10^7 cells per ml) were incubated with opsonized bacteria (5×10^8 CFU/ml) for 60 min or with lipopolysaccharides (LPS) from *Escherichia coli* (serotype O55:B5; Sigma) and *K. pneumoniae* (Sigma) for 18 h at 37°C under conditions of high humidity and with gentle shaking. When PMN were stimulated with LPS (36) from *K. pneumoniae*, cells were incubated with the endotoxin 30 min before addition of the microorganism itself. When PMN were treated with the specific PAF receptor antagonist WEB 2170 (kindly provided by Boehringer, Ingelheim, Germany), cells were incubated with the agent for 5 min before addition of *K. pneumoniae*.

The reaction was stopped by centrifugation at $4,000 \times g$ for 15 min. The supernatant was removed carefully and filtered through a membrane filter (Millex-GS; pore size, 0.22 μm; Millipore). An aliquot of the filtered supernatant was immediately assayed in the bioassay; the remainder was diluted with an equal volume of 20% acetic acid and kept frozen at −80°C until used for the PAF radioimmunoassay (RIA).

Bioassay and characterization of PAF. Aggregation measurements were performed in an impedance dual-channel aggregometer (Fresenius Pharmazeutica) linked to a double-pen recorder. A siliconized cuvette containing 450 μl of PRP was transferred into the heating block of the aggregometer (37°C), where the sample was stirred at 1,000 rpm with a Teflon-coated bar. Fifty microliters of the supernatant or a standard solution, prepared from commercially available PAF (Sigma) in a concentration range of 10^{-9} to 10^{-6} M, was added to PRP to stimulate aggregation. The PAF antagonist WEB 2170 (10^{-5} M) was added to the platelets 1 min before addition of the supernatant or PAF standard.

The PAF content in the supernatants was quantitatively determined by an RIA [Platelet Activating Factor (^{125}I) RIA kit; NEN Research Products; Du Pont]. The samples were handled as recommended in the instruction manual for the RIA kit. Recovery of the assay was between 70.9 and 86%, depending on the PAF content of the samples. The levels of intraassay and interassay reproducibility were 88.1 to 93.2% and 92.9 to 94.4%, respectively.

Animal experiment. Female NMRI mice (specific pathogen free) weighing between 28 and 32 g were obtained from Charles River, Munich, Germany. The animals were kept under standard conditions at room temperature and fed ad libitum.

K. pneumoniae 476 was used for the experimental pulmonary infection. Under thiopental anesthesia and after a small incision was made in the regio colli medialis, the animals were infected intratracheally below the larynx (19) with 25 μl of a bacterial suspension containing 10^4 CFU/ml. Preliminary experiments showed that this inoculum was the one most suitable for the desired degree of infection. The animals were treated per os in four groups ($n = 15$) with three different doses of the specific PAF antagonist WEB 2170 (1 plus 3 [group 1], 3 plus 10 [group 2], and 10 plus 30 [group 3] mg/kg of body weight per day) (20) or placebo for the control group (group 4). The first treatment took place 1 h before infection, the second, third, and final treatments took place 6, 24, and 30 h, respectively, after the first application of WEB 2170 or placebo. The same amounts of WEB 2170 were administered for the first and third treatments in each respective group of animals (1 [group 1], 3 [group 2], and 10 [group 3] mg/kg of body weight). This was also the case for the second and fourth treatments, which were approximately three times higher than the first and third treatments because of the correspondingly longer time interval following the former (3 [group 1], 10 [group 2], and 30 [group 3] mg/kg of body weight). Seventy-two hours after the first treatment, the lungs of the surviving animals were aseptically removed and homogenized, and the CFU per gram of lung tissue was determined by agar plating.

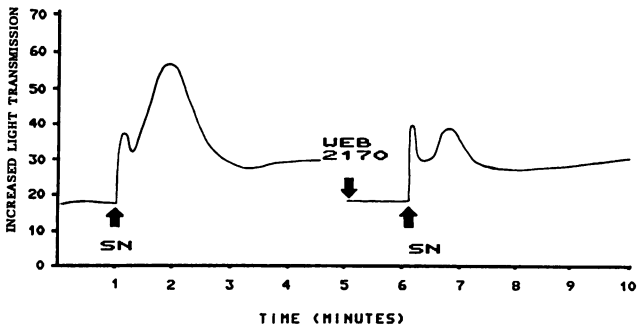

FIG. 1. Aggregatory activity of the supernatant (SN) from the reaction of PMN (10^7 cells per ml) with *H. influenzae* (5×10^8 CFU/ml) on human PRP (2.5×10^8 cells per ml) and inhibition caused by treatment of PRP with WEB 2170 (10^{-5} M) 1 min before addition of the supernatant.

Statistical analysis. Data are presented as mean ± standard deviation (SD). Statistical evaluations were made on all measured values, using Student's *t* test, variance analysis, and the Tukey test. Probability values of $P < 0.05$ were considered statistically significant.

RESULTS

Bioassay. PAF activity in the supernatants was determined in a semiquantitative manner by aggregation measurements. The supernatants of the PMN-bacteria reaction showed an aggregatory activity in the case of all strains used in this study. This activity was dependent on the bacterial concentration and the incubation time. Preliminary studies showed that when increasing concentrations of bacteria were added to 10^7 PMN per ml of Tyrode's BSA (final concentration), the highest aggregatory activity was achieved by using 50 times more bacteria than cells (data not shown). Furthermore, the aggregatory activity of the supernatants was nearly at the maximum level as soon as 1 h after the start of incubation (data not shown).

The aggregatory activity of the supernatants could be due not only to PAF but also to other platelet-active mediators released during the incubation. To determine PAF activity, PRP was treated with the PAF receptor antagonist WEB 2170 (10^{-5} M) for 1 min before addition of the supernatant to inhibit the effects of the mediator. As seen in Fig. 1 (aggregation was caused by the supernatant of the reaction between PMN [10^7 cells per ml] and *H. influenzae* 1447 [5×10^8 CFU/ml]), there was a remaining aggregatory activity, although WEB 2170 at this concentration completely inhibited

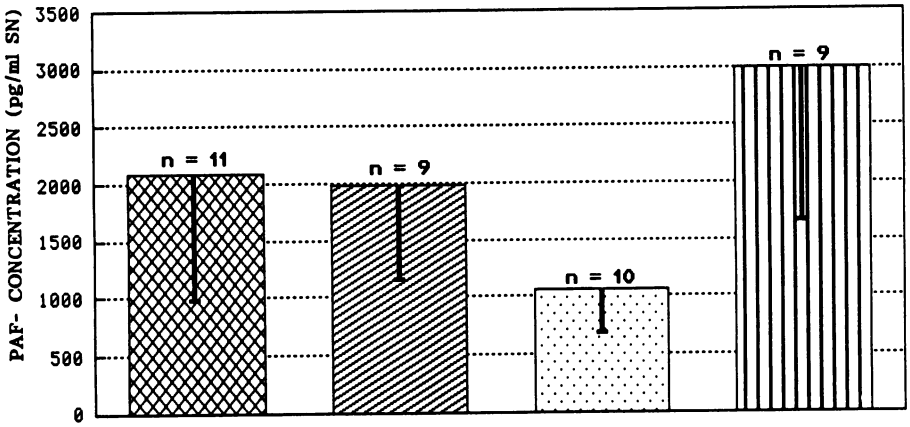

FIG. 2. Amount of PAF released in supernatants (SN) of the PMN (10^7 cells per ml)-bacteria (5×10^8 CFU/ml) reaction after 1 h of incubation with the four bacterial strains used. Symbols: ▩, *K. pneumoniae*; ▨, *S. pyogenes*; ▦, *H. influenzae*; ▥, *S. pneumoniae*.

the aggregation caused by the highest PAF standard (10^{-6} M).

Characterization of PAF by RIA. To characterize the specificity of the several PAF-induced changes observed at the semiquantitative level, the presence of PAF was determined with an RIA, testing the same supernatants as in the bioassay.

The results of the RIA confirm the findings of the bioassay. The platelet aggregation activity of PAF in the cell supernatant coincides with its appearance as measured directly by the RIA. In all experiments, no PAF could be measured in the supernatants of the negative controls (PMN incubated without bacteria or LPS). The PAF contents of the supernatants of the cells incubated with *S. pneumoniae* 179, *K. pneumoniae* 476, *S. pyogenes* 68, and *H. influenzae* 1447 were 2,996 ± 1,338, 2,092 ± 1,130, 1,990 ± 850, and 1,053 ± 384 pg/ml, respectively (Fig. 2). The PAF concentration in the supernatants of the cells incubated with *S. pneumoniae* was significantly higher ($P < 0.05$) than that in the supernatant of the PMN-*H. influenzae* reaction but not than that from the incubation with *K. pneumoniae* or *S. pyogenes*. The PAF concentrations measured in the supernatants of PMN with *K. pneumoniae*, *S. pyogenes*, and *H. influenzae* were not significantly different.

LPSs from *E. coli* and *K. pneumoniae* proved to be much weaker stimuli of PAF production and release from the PMN than were bacteria. Cells were incubated with four different concentrations (0.1, 1, 10, and 50 μg/ml) of each endotoxin (Fig. 3). When PMN were stimulated with 0.1 μg of LPS per ml, no PAF release could be detected. Upon stimulation of PMN with 1, 10, and 50 μg of LPS per ml, the PAF concentrations in the supernatants were 322 ± 83, 321 ± 58, and 281 ± 12 pg/ml for *E. coli* and 366 ± 164, 480 ± 181, and 311 ± 62 pg/ml for *K. pneumoniae*, respectively. The highest PAF content (480 ± 181 pg/ml) was measured in the supernatant of the cells incubated with LPS (10 μg/ml) from *K. pneumoniae*. This value was significantly lower ($P < 0.01$) than that in the supernatants of PMN incubated with live *K.*

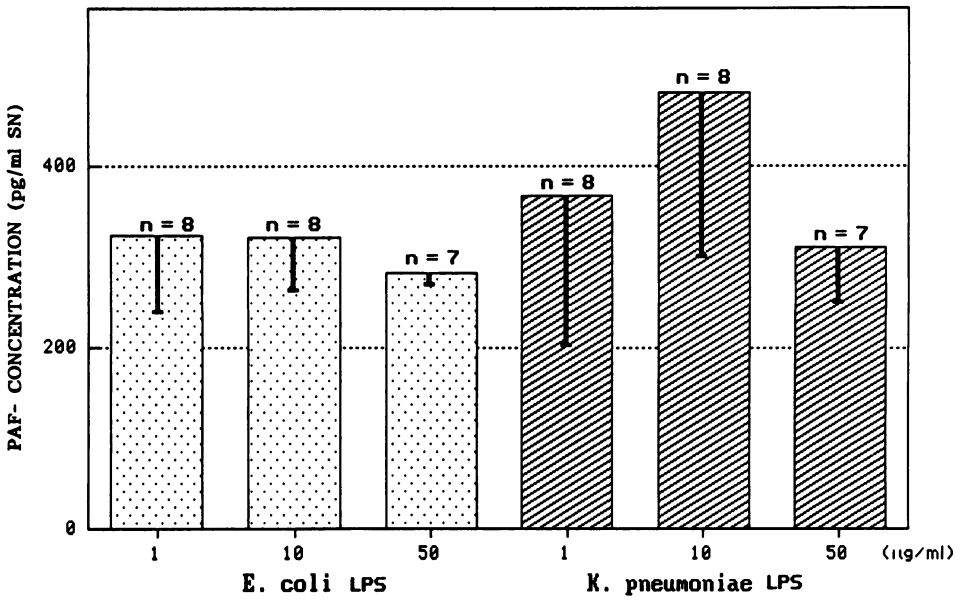

FIG. 3. Amount of PAF released in supernatants (SN) after 18 h of incubation of PMN (10^7 cells per ml) with LPS from *E. coli* and *K. pneumoniae* at different concentrations (1, 10, and 50 μg/ml).

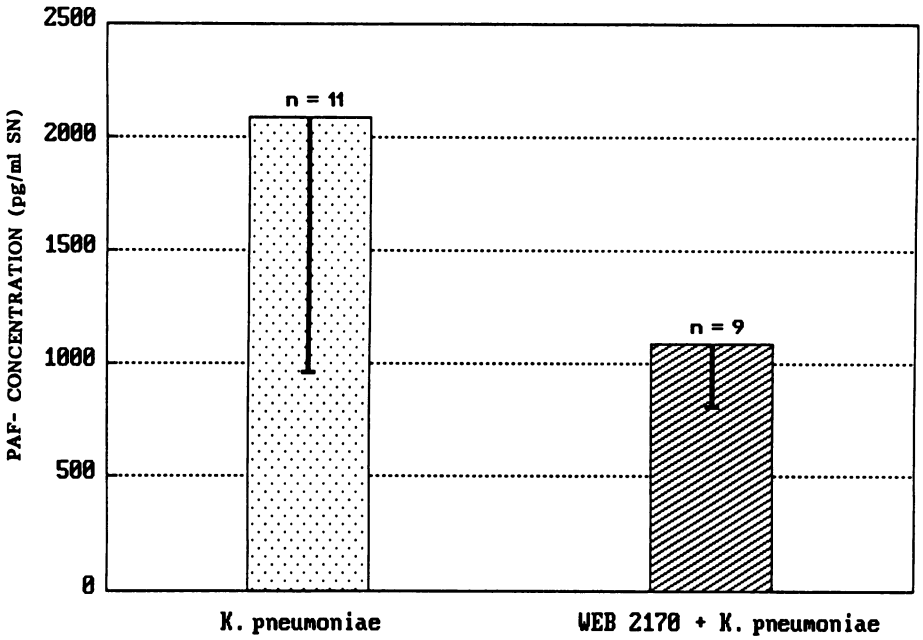

FIG. 4. Significant reduction of the amount of PAF released in supernatants (SN) of PMN (10^7 cells per ml), pretreated for 5 min with WEB 2,170 (10^{-5} M), after 1 h of incubation with *K. pneumoniae* (5×10^8 CFU/ml) compared with that of untreated cells incubated under the same conditions with the microorganism ($P < 0.05$).

pneumoniae (2,092 ± 1,130 pg/ml). Priming of neutrophils with the same four concentrations of LPS from *K. pneumoniae* before addition of the live microorganism did not alter the cells' ability to release the mediator (data not shown). As shown in Fig. 4, treatment of PMN with WEB 2170 (10^{-5} M) 5 min before addition of *K. pneumoniae* to the incubation mixture resulted in a significant decrease ($P < 0.05$) in the amount of PAF in the supernatant (1,088 ± 281 versus 2,092 ± 1,130 pg/ml). The decrease in the amount of the released mediator corresponded with a decrease of the aggregatory activity of the supernatants.

Animal experiment. Treatment of mice with the PAF antagonist resulted, depending on the dose, in a considerable increase of animal survival (Fig. 5). From 15 mice, 9 animals (60%) in the first group, 11 (73.3%) in the second group, 8 (53.3%) in the third group, and only 6 (40%) in the control group survived.

With respect to bacterial growth in the lungs of the surviving animals, all three WEB 2170-treated groups showed a reduction of the CFU per gram of lung tissue compared with the control group (Fig. 6).

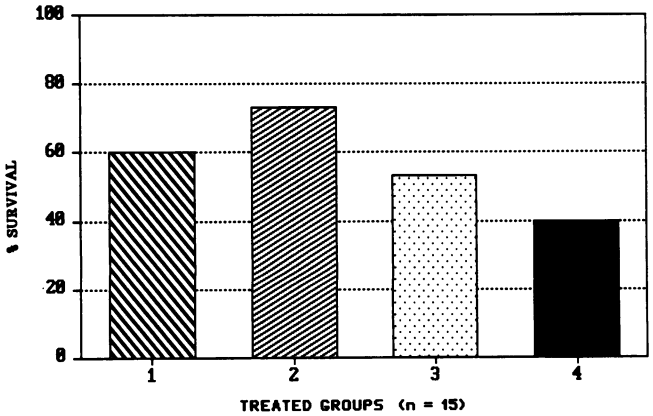

FIG. 5. Percentage survival in *K. pneumoniae*-infected mice treated with WEB 2170 (1 plus 3 [group 1], 3 + 10 [group 2], and 10 + 30 [group 3] mg/kg of body weight per day) and placebo-treated controls (group 4).

DISCUSSION

This study demonstrates that live opsonized bacteria are effective in releasing PAF from PMN. Evidence of PAF activity in the supernatants was obtained semiquantitatively by a bioassay, which is the standard method for measuring PAF. The RIA used proved to be an accurate and sensitive method of detection, even when only low concentrations of the mediator were present.

In previous studies (10, 14, 16, 34), PAF has been reported to be a mediator of endotoxin shock. In this study, we found no significant difference between gram-negative and gram-positive strains in the ability to release PAF from PMN. Stimulation of the cells with LPS resulted in the release of small amounts of mediator, which could be detected only immunochemically. This result confirms the findings of Worthen et al. (36). Incubation of PMN with LPS from *K. pneumoniae* 30 min before addition of the microorganism itself revealed no marked difference in the amount of PAF released compared with the control. According to these findings, the presence of LPS does not appear to have a major effect on the release of PAF from PMN under bacterial stimulus. It is more likely that the amount of the mediator released in the supernatant of the cell-bacteria reaction is related to the virulence of the microorganisms used (i.e., membrane components and/or bacterial products).

WEB 2170 is reported to be a specific PAF receptor antagonist of the hetrazepine type (21). This agent is a potent

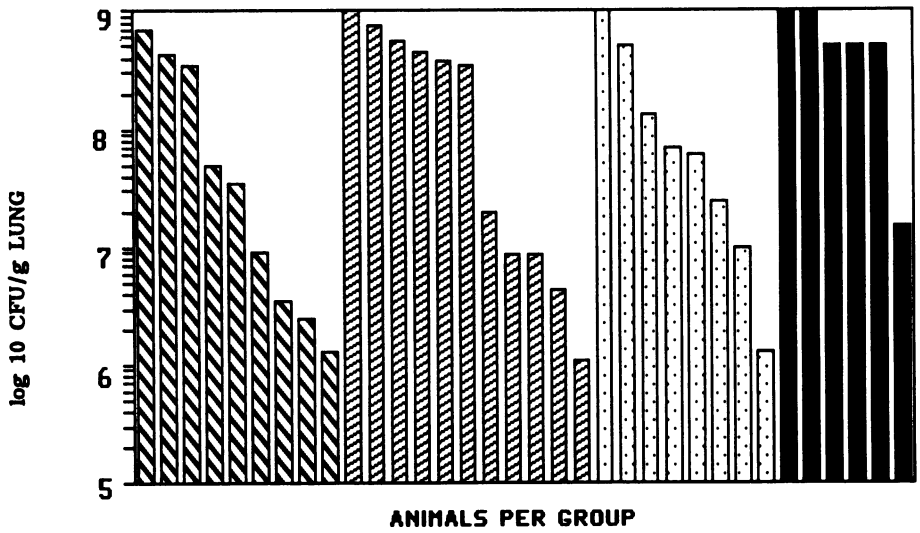

FIG. 6. Bacterial counts in lung tissue of individual animals surviving infection in all groups tested. Symbols: ▨, group 1; ▨, group 2; ▩, group 3; ■, group 4.

inhibitor of the aggregatory effects of PAF on human platelets. For our study, it was important to determine whether WEB 2170 has any antimicrobial activity and any immunomodulatory action with respect to the function of PMN. Previous studies (data not shown) have demonstrated that WEB 2170 lacks antimicrobial activity. Furthermore, the main parameters of PMN function, such as chemotaxis, respiratory burst (luminol-dependent chemiluminescence assay by stimulating cells with either opsonized zymosan or *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine), and intracellular killing, are virtually unaffected by treatment of the cells with WEB 2170. Treatment of PMN with the PAF receptor antagonist before addition of *K. pneumoniae* resulted in a significant decrease in the amount of released PAF compared with untreated controls. This finding suggests that WEB 2170 is capable of interfering with the autacoid action of PAF released from PMN after challenge with bacteria.

These findings indicate a possible beneficial action of PAF antagonists during inflammatory processes occurring in infected tissues. In the infected lung, two sets of phagocytic cells primarily are available to defend the lung against inhaled bacteria: resident alveolar macrophages and infiltrated granulocytes from the circulation. However, the relative contribution of these two groups of cells appears to be dependent on the bacterial species involved (27). Rehm and coworkers (27) showed in experimental lung infections in mice that neutropenic animals cleared in 4 h only 10.0 ± 7.0% of an inoculum of *K. pneumoniae*, compared to 33.0 ± 4.0% clearance in normal animals. This finding indicates that PMN play a major role in the clearance of this organism from murine lungs.

Casals-Stenzel showed that treatment of mice with the hetrazepine WEB 2086 (another specific PAF receptor antagonist closely related to WEB 2170) per os 1 h before challenge with PAF blocked the effects of the mediator in a dose-dependent manner (8, 9). Furthermore, Heuer et al. demonstrated that in vivo oral administration of WEB 2170 is, depending on the species, about 5- to 40-fold more potent against PAF-induced alterations than is administration of WEB 2086 (21). Therefore, in the present study in a well-standardized model, mice were treated with WEB 2170 1 h before inoculation with *K. pneumoniae* to achieve extensive protection against the effects of a possible PAF release during the experimentally induced lung infection.

As shown in Fig. 5, treatment with the PAF antagonist leads, depending on the dose, to a considerable increase in survival of the animals. In the animal group treated per os with 3 plus 10 mg of WEB 2170 per kg per day (group 2), 73.3% of the animals survived, compared with 40% survival observed in the control group (group 4). This finding corresponds with a reduction of the CFU per gram of lung tissue in all WEB 2170-treated animal groups and indicates a role of PAF in the infected lung during bacterial pneumonia. In our hands, addition of the PAF antagonist or PAF to cultures of the various microorganisms did not alter bacterial growth. This finding excludes the possibility of a direct action of the mediator on the bacterial microorganisms.

Thus, these observations and the fact that the PAF antagonist in vitro reduces the release of PAF from PMN, when challenged with bacteria, suggest that the decrease in microbial growth is achieved by blocking the action of the active PAF component. In this context, besides living bacteria as shown in the in vitro experiments, microbial products themselves have been shown (5) to stimulate mammalian cells to release PAF and also arachidonic acid metabolites, all of which are stimulated by PAF (11, 15, 25) and should be inhibited by PAF antagonists, since the mediator has been shown to act through membrane receptors on its target cells (35). Moreover, it has been reported (13) that some microorganisms are capable of synthesizing PAF from its inactive precursor lyso-PAF, which can be released in larger amounts from alveolar macrophages (29).

In conclusion, both gram-positive and gram-negative microorganisms are effective in releasing PAF from PMN in vitro. This activity seems to be independent of the LPS content of the bacteria and can be blocked by the specific PAF receptor antagonist WEB 2170. In vivo, in experimentally induced bacterial pneumonia in mice, treatment with the PAF antagonist apparently protects animals against the pathogenicity of the microorganism. Further investigations will be necessary to determine the possible therapeutic

consequences of these studies for bacterial pneumonia in humans.

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REFERENCES

1. **Benveniste, J., C. Boullet, C. Brink, and C. Labat.** 1983. The actions of PAF-aceter (platelet-activating factor) on guinea pig isolated heart preparations. Br. J. Pharmacol. **80**:81–85.
2. **Benveniste, J., P. M. Henson, and C. G. Cochrane.** 1972. Leukocyte-dependent histamine release from rabbit platelets. The role of IgE, basophiles, and platelet activating factor. J. Exp. Med. **136**:1356–1377.
3. **Block, L. H., W. M. Abraham, P. Groscurth, B. Y. Qiao, and A. P. Perruchoud.** 1989. Platelet-activating factor (PAF)-dependent biochemical, morphologic, and physiologic responses of human platelets: demonstration of translocation of protein kinase C associated with protein phosphorylation. Am. J. Respir. Cell Mol. Biol. **1**:277–278.
4. **Braquet, P., M. Paubert-Braquet, R. H. Bourgain, F. Bussolino, and D. Hosford.** 1989. PAF/cytokine auto-generated feedback networks in microvascular immune injury: consequences in shock, ischemia and graft rejection. J. Lipid Mediators **1**:75–112.
5. **Bremm, K. D., W. König, J. Brom, M. Thelestam, F. J. Fehrenbach, and J. E. Alouf.** 1988. Release of lipid mediators (leukotrienes, PAF) by bacterial toxins from human polymorphonuclear granulocytes. Zentralbl. Bakteriol. **17**(Suppl.):103–104.
6. **Cammusi, G., M. Aglietta, R. Coda, F. Bussolino, W. Piacibello, and C. Tetta.** 1981. Release of platelet-activating factor (PAF) and histamine. II. The cellular origin of human PAF: monocytes, polymorphonuclear neutrophils and basophils. Immunology **42**:191–199.
7. **Cammusi, G., C. Tetta, F. Bussolino, F. Caligaris-Cappio, C. Masera, and G. Segoloni.** 1981. Mediators of immune complex induced aggregation of polymorphonuclear neutrophils. II. Platelet-activating factor as the effector substance of immune-induced aggregation. Int. Arch. Allergy Appl. Immunol. **64**:25–41.
8. **Casals-Stenzel, J.** 1987. Effects of WEB 2086, a novel antagonist of platelet activating factor, in active and passive anaphylaxis. Immunopharmacology **13**:117–124.
9. **Casals-Stenzel, J., J. Franke, T. Friedrich, and J. Lichey.** 1987. Bronchial and vascular effects of PAF in the rat isolated lung are completely blocked by WEB 2086, a novel specific PAF antagonist. Br. J. Pharmacol. **91**:799–802.
10. **Chang, S. W., S. Fernyak, and N. V. Voelkel.** 1990. Beneficial effect of a platelet activating antagonist, WEB 2086, on endotoxin-induced lung injury. Am. J. Physiol. **258**:H153–H158.
11. **Chilton, H. F., J. T. O'Flaherty, C. E. Walsh, M. J. Thomas, R. L. Wykle, L. R. DeChatelet, and B. M. Waite.** 1982. Platelet activating factor. Stimulation of the lipoxygenase pathway in polymorphonuclear leukocytes by 1-O-alkyl-2-O-acetyl-sn-glycero-3-phosphocholine. J. Biol. Chem. **257**:5402–5407.
12. **Darius, H., D. J. Lefer, B. Smith, and A. M. Lefer.** 1986. Role of platelet activating factor in mediating guinea pig anaphylaxis. Science **232**:58–60.
13. **Denizot, Y., E. Dassa, H. Y. Kim, M. J. Bossant, N. Salem, Y. Thomas, and J. Benveniste.** 1989. Synthesis of paf-acether from exogenous precursors by the prokaryote Escherichia coli. FEBS Lett. **243**:13–16.
14. **Diez, F. L., M. L. Nieto, S. Fernandez-Gallardo, M. A. Gijon, and M. S. Crespo.** 1989. Occupancy of platelet receptors for platelet-activating factor in patients with septicemia. J. Clin. Invest. **83**:1733–1740.
15. **Doebber, T. G., and M. S. Wu.** 1987. Platelet-activating factor (PAF) stimulates the PAF-synthesizing enzyme acetyl-CoA:1-alkyl-sn-glycero-3-phosphocholine O-acetyl transferase and PAF synthesis in neutrophils. Proc. Natl. Acad. Sci. USA **84**:7557–7561.
16. **Etienne, A., F. Hecquet, C. Soulard, C. Touvay, F. Clostre, and P. Braquet.** 1986. The relative role of PAF-acether and eicosanoids in septic shock. Pharm. Res. Commun. **18**(Suppl.): 71–79.
17. **Findley, S. R., L. M. Lichtenstein, D. J. Hanahan, and R. N. Pinckard.** 1981. The contraction of guinea pig ileal smooth muscle by acetyl glyceryl ether phosphorylcholine. Am. J. Physiol. **241**:C130–C133.
18. **Georgopoulos, A.** 1978. Tiefgefrierkonservierung von Pilzen in flüssigem Stickstoff als Grundlage für standardisierte Inokula. Mykosen **21**:19–23.
19. **Georgopoulos, A., S. M. Feistauer, M. Georgopoulos, H. Walzl, and W. Graninger.** 1991. Chemotherapeutic activity of difloxacin, sarafloxacin, and ciprofloxacin against experimentally induced pneumonia in rats. Int. J. Exp. Clin. Chemother. **4**:73–77.
20. **Heuer, H. O.** 1989. Action of the new hetrazepinoic PAF-antagonist WEB 2170 on shock states induced by PAF, antigen or endotoxin/TNF. Arch. Pharmacol. **339**(Suppl.):R78.
21. **Heuer, H. O., J. Casals-Stenzel, G. Muacevic, and K. H. Weber.** 1990. Pharmacologic activity of Bepafant (WEB 2170), a new and selective hetrazepinoic antagonist of platelet activating factor. J. Pharmacol. Exp. Ther. **255**:962–968.
22. **Humphrey, D. M., L. McManus, D. J. Hanahan, and R. N. Pinckard.** 1984. Morphologic basis of increased vascular permeability induced by acetyl glyceryl ether phosphorylcholine. Lab. Invest. **50**:16–25.
23. **Humphrey, D. M., L. McManus, K. Satouchi, D. J. Hanahan, and R. N. Pinckard.** 1982. Vasoactive properties of acetyl glyceryl ether phosphorylcholine and analogs. Lab. Invest. **46**:422–427.
24. **Kasimir, S., W. Schönfeld, J. E. Alouf, and W. König.** 1990. Effect of *Staphylococcus aureus* delta toxin on human granulocytes functions and platelet-activating factor metabolism. Infect. Immun. **58**:1653–1659.
25. **Lin, A. H., D. R. Morton, and R. R. Gorman.** 1982. Acetyl glyceryl ether phosphorylcholine stimulates leukotriene B_4 synthesis in human polymorphonuclear leukocytes. J. Clin. Invest. **70**:1058–1065.
26. **Lotner, G. Z., J. M. Lynch, S. J. Betz, and P. M. Henson.** 1980. Human neutrophil derived platelet activating factor. J. Immunol. **124**:676–684.
27. **Rehm, S. R., G. N. Gross, and A. K. Pierce.** 1980. Early bacterial clearance from murine lungs. Species-dependent phagocyte response. J. Clin. Invest. **66**:194–199.
28. **Robertson, D. A., D. Y. Wang, C. O. K. Lee, and R. Levi.** 1988. Negative inotropic effect of platelet-activating factor: association with a decrease in intracellular sodium activity. J. Pharmacol. Exp. Ther. **245**:124–128.
29. **Robinson, M., and H. F. Snyder.** 1985. Metabolism of platelet-activating factor by rat alveolar macrophages: lyso-PAF as an obligatory intermediate in the formation of alkylarachidonoyl glycerophosphocholine species. Biochim. Biophys. Acta **873**: 52–56.
30. **Rubin, A. H., L. J. Smith, and R. Patterson.** 1987. The bronchoconstrictor properties of platelet-activating factor in humans. Am. Rev. Respir. Dis. **136**:1145–1151.
31. **Shaw, J. O., R. N. Pinckard, K. S. Ferrigni, L. McManus, and D. J. Hanahan.** 1981. Activation of human neutrophils with 1-O-hexadecyl/octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (platelet activating factor). J. Immunol. **127**:1250–1252.
32. **Sisson, J. H., S. M. Prescott, T. M. McIntyre, and G. A. Zimmerman.** 1987. Production of platelet-activating factor by stimulated human polymorphonuclear leukocytes. Correlation of synthesis with release, functional events, and leukotriene B4 metabolism. J. Immunol. **138**:3918–3926.
33. **Southwick, F. S., and T. P. Stossel.** 1986. Phagocytosis, p. 326–331. *In* N. R. Rose, H. Friedman, and J. L. Fahey (ed.), Manual of clinical laboratory immunology, 3rd ed. American Society for Microbiology, Washington, D.C.
34. **Terashita, Z., Y. Imura, K. Nishikawa, and S. Sumida.** 1985. Is

platelet activating factor (PAF) a mediator of endotoxin shock? Eur. J. Pharmacol. **109:**257–261.

35. **Valone, F. H., and E. J. Goetzl.** 1983. Specific binding by human polymorphonuclear leucocytes of the immunological 1-O-hexadecyl/octadecyl-2-acetyl-sn-glycero-3-phosphorylcholine. Immunology **48:**141–148.
36. **Worthen, G. S., J. F. Seccombe, C. L. Clay, L. A. Guthrie, and R. B. Johnston, Jr.** 1988. The priming of neutrophils by lipopolysaccharide for production of intracellular platelet-activating factor. J. Immunol. **140:**3553–3559.
37. **Yasaka, T., L. A. Boxer, and R. L. Baehner.** 1982. Monocytes aggregation and superoxide anion release in response to formyl-methyionyl-leucyl-phenyalanine (FMLP) and platelet activating factor (PAF). J. Immunol. **128:**1939–1944.