# Sulfite Induces Release of Lipid Mediators by Alveolar Macrophages*

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Air pollutants are supposed to modulate physiological responses of alveolar macrophages (AM). This study was addressed to the question whether at neutral pH sulfur(IV) species in comparison to sulfur(VI) species cause AM to release proinflammatory mediators and which pathways are involved in their generation. Supernatants obtained from canine AM treated with sulfite (0.1 mM to 2 mM) enhanced the respiratory burst of canine neutrophils, measured by lucigenin-dependent chemiluminescence, whereas supernatants derived from AM treated with sulfate (1 mM) did not. The neutrophil-stimulating activity released by sulfite-treated AM consisted of platelet-activating factor (PAF) and leukotriene $B_4$ ($LTB_4$) as shown by desensitization of the corresponding receptors. Inhibitors of phospholipase $A_2$ substantially suppressed release of neutrophil-stimulating activity by sulfite-treated AM. Inhibition of 5-lipoxygenase in sulfite-treated AM also reduced neutrophil-stimulating activity, while inhibition of cyclooxygenase had no effect. In conclusion, sulfite induces AM to release lipid mediators via phospholipase $A_2$- and 5-lipoxygenase-dependent pathways. These mediators activate neutrophils via the receptors for PAF and $LTB_4$.

## Introduction

Sulfur dioxide is a fairly ubiquitous air pollutant which forms sulfite when adsorbed to water droplets. Sulfur dioxide and sulfite are known inducers of bronchoconstriction (Balmes *et al.* 1987; Fine *et al.,* 1987; Gong *et al.,* 1995). We have recently shown that long-term exposure of dogs to a neutral sulfite aerosol representing high ambient levels of sulfur dioxide (corresponding to 0.2 ppm sulfur dioxide) induced moderate inflammatory reactions in the lungs (Maier *et al.,* 1992). Reactions included an altered permeability of the alveolar-capillary membrane for proteins, an increased release of the lysosomal enzyme β-N-acetylglucosaminidase as well as an increase in the number of neutrophils (PMN), lymphocytes and eosinophils in the epithelial lining fluid (Maier *et al.,* 1992). These alterations are assumed to be triggered by inflammatory mediators including lipid mediators (Evans *et al.,* 1987; O'Donnell and Barnett, 1987; Hayashi *et al.,* 1991; Pinckard *et al.,* 1992; DeLima *et al.,* 1995; Longphre and Kleeberger, 1995). Alveolar macrophages (AM) play an important role as part of the primary pulmonary defense system releasing such mediators. However, the mechanisms of interaction between 4-valent sulfur species (sulfur dioxide, sulfite) and cellular components of the lungs remain undefined.

Our previous *in vitro* studies on the interaction of sulfite with cells and tissues indicated adverse effects on energy metabolism dependent on the level of sulfite oxidase activity which detoxifies sulfite by oxidation to sulfate (Beck-Speier *et al.,* 1985). We have further demonstrated that sulfite modulates the oxidative metabolism of human PMN which are endowed with a very low level of

*Abbreviations:* CL, chemiluminescence; PBS, phosphate buffered saline; AM, alveolar macrophages; PMN, polymorphonuclear neutrophils; PAF, platelet-activating factor; $LTB_4$, leukotriene $B_4$; IL-8, interleukin-8; C5a, complement factor 5a; PMA, phorbol 12-myristate 13-acetate; $PLA_2$, phospholipase $A_2$; 5-LO, 5-lipoxygenase; COX, cyclooxygenase.

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the sulfite-detoxifying enzyme (Beck-Speier *et al.*, 1993; Beck-Speier *et al.*, 1994; Mishra *et al.*, 1995). AM and other phagocytic cells exhibit low sulfite oxidase levels, and therefore have a relatively low protective capacity against sulfite (Beck-Speier *et al.*, 1985). Recently we have found that AM release a PMN-stimulating activity with a PAF-like character upon treatment with sulfite (Beck-Speier *et al.*, in press).

To extend our previous studies we investigated the effect of sulfite on the release and generation of proinflammatory mediators by AM which modulate PMN functions. Freshly isolated AM were treated with sulfite and for comparison with sulfate, and the resulting supernatants containing putative mediators were analyzed for their ability to stimulate the respiratory burst of PMN. Mechanisms of mediator release were studied by inhibiting several pathways of lipid metabolism in AM.

## Materials and Methods

### *Materials*

Phorbol 12-myristate 13-acetate (PMA), lucigenin, leukotriene $B_4$ ($LTB_4$), complement factor 5a (C5a), and quinacrine were purchased from Sigma (Deisenhofen, Germany); PBS buffer with or without $Ca^{2+}/Mg^{2+}$ was from Biochrome (Berlin, Germany); Polymorphprep was from Nycomed (Oslo, Norway); carrier-free human interleukin 8 (IL-8) was from Biermann (Bad Nauheim, Germany); platelet-activating factor (PAF), cytochalasin B, $AACOCF_3$, OBAA, and MK 886 were from Calbiochem-Novabiochem (Bad Soden, Germany); indomethacin was from LC Laboratories/Alexis Deutschland (Grünberg, Germany).

### *Isolation of alveolar macrophages*

Canine AM were obtained by bronchoalveolar lavage of healthy beagles according to Maier *et al.* (1992). After bronchoalveolar lavage the cells were obtained by centrifugation (400 x *g* for 20 min) and resuspension in PBS-buffer (without $Ca^{2+}/Mg^{2+}$). Viability was more than 95% as determined by trypan blue exclusion. Cells were characterized by microscopic examination after May Grünwald Giemsa staining of cytospin preparations. 85 to 90% of the cells were identified as AM.

### *Isolation of neutrophils*

Canine PMN were isolated from citrate-anticoagulated venous blood of healthy beagles by density-gradient centrifugation with Polymorphprep. Blood (5 ml) was layered over Polymorphprep (5 ml) diluted with 1.5% (w/v) NaCl to a density of 1.106 g/ml. After centrifugation at 450 x *g* for 30 min PMN were separated from lymphocytes and monocytes. PMN suspension was washed once with $Ca^{2+}$- and $Mg^{2+}$ -free PBS buffer and contaminating erythrocytes were removed by hypotonic lysis according to Beck-Speier *et al.* (1988). The pure population of PMN was resuspended in PBS-buffer (without $Ca^{2+}/Mg^{2+}$) containing 0.1% glucose, and viability was more than 95% as determined by trypan blue exclusion. Human PMN were obtained from citrate-anticoagulated venous blood of healthy donors by density-gradient centrifugation with Polymorphprep according to Mishra *et al.* (1995).

### *Incubation of alveolar macrophages with sulfite or sulfate*

Canine AM (1 x $10^6$/ml) were incubated for 30 min at 37 °C in the absence (control) and presence of sulfite (0.1–2.0 mM) or sulfate (1 mM), respectively, in PBS buffer, pH 7, containing 0.1% glucose. Sulfite solutions, pH 7, were freshly prepared by dissolving sodium sulfite in PBS buffer, pH 7, containing 0.1% glucose, and by readjusting the pH value to pH 7 with HCL (Mishra *et al.* (1995)). Sulfate solutions, pH 7, were prepared by dissolving sodium sulfate in the same buffer as sulfite. After centrifugation at 400 x *g* for 10 min the supernatants were dialyzed against PBS-buffer in a dialysis tubing with a molecular weight cut-off of 500 Da (Roth, Karlsruhe, Germany) for 20 h to remove residual sulfite or sulfate, respectively. The elimination of the concentration of sulfite by this dialysis membrane was more than 97%, that of PAF less than 5% and that of $LTB_4$ less than 25%.

### *CL-measurements of neutrophils with alveolar macrophage-derived supernatants*

The supernatants obtained from incubations of AM with sulfite or sulfate were assayed for their

ability to stimulate the respiratory burst of PMN by measuring lucigenin-dependent CL (Allen, 1981; Allen, 1982; Allen, 1986; Gyllenhammar, 1987). PMN ($1.5 \times 10^4$) were preincubated in 0.25 ml PBS buffer, pH 7, containing 0.1% glucose and 0.8 mM lucigenin for 10 min at 37 °C in a six-channel Biolumat LB9505 (Berthold, Wildbad, Germany). CL measurements were started, the AM-derived supernatants (50 µl) were added, and CL signals of PMN were recorded for 20 min. Immediately afterwards PMN were stimulated by PMA (50 ng), and CL was measured again for 20 min at 37 °C using the Biolumat analyzer. Each AM-derived supernatant of an individual canine donor was analyzed at least in duplicate with PMN.

*Desensitization of neutrophil receptors*

Desensitization of PMN receptors was achieved by preincubation of human PMN ($1 \times 10^5$ cells/0.25 ml PBS-buffer) with PAF ($1 \times 10^{-6}$ M), $LTB_4$ ($1 \times 10^{-7}$ M), IL-8 (0.5 µg/ml) or C5a ($1 \times 10^{-6}$ M), respectively, for 10 min at 37 °C in the Biolumat. Cytochalasin B ($4 \times 10^{-6}$ M) was added and cells were incubated for further 5 min. CL measurements were started, AM-derived supernatants were added, and CL response of PMN was recorded for 10 min at 37 °C. Because PAF, $LTB_4$, IL-8 and C5a are weak elicitors of the respiratory burst, CL-measurements were performed in the presence of cytochalasin B ($4 \times 10^{-6}$ M) (Honeycutt and Niedel, 1986) with $1 \times 10^5$ PMN/0.25 ml PBS-buffer.

*Inhibition of phospholipase $A_2$*

To study inhibition of phospholipase $A_2$ ($PLA_2$)-dependent pathways, canine AM were treated with several inhibitors of $PLA_2$, AA-$COCF_3$ ($1 \times 10^{-4}$ M) (Bartoli *et al.*, 1994), quinacrine ($1 \times 10^{-4}$ M) (Tsunawaki and Nathan, 1986) or OBAA ($1 \times 10^{-5}$ M) (Köhler *et al.*, 1992), respectively, for 25 min at 37 °C prior to incubation with 1 mM sulfite. The resulting supernatants were dialyzed and analyzed for their ability to stimulate the respiratory burst of PMN.

*Inhibition of 5-lipoxygenase and cyclooxygenase*

To investigate inhibition of 5-lipoxygenase (5-LO)- and cyclooxygenase (COX)-dependent pathways, canine AM were preincubated for 20 min at 37 °C with MK 886 ($1 \times 10^{-6}$ M), an inhibitor of 5-LO (Rouzer *et al.*, 1990), or with indomethacin ($1 \times 10^{-6}$ M), an inhibitor of COX (Shams *et al.*, 1989), followed by incubation with sulfite for 30 min at 37 °C. The resulting supernatants were dialyzed and estimated for their ability to activate the respiratory burst of PMN.

*Statistical analysis*

Statistical significance was determined by analysis of variance and two-sample t-test (STAT-SAK, Version 2.12 by G. E. Dallal, 1986). Changes with $p \leq 0.05$ were considered significant.

## Results

*Release of a neutrophil-stimulating activity by sulfite-treated alveolar macrophages in comparison to sulfate-treated alveolar macrophages*

To compare the effect of sulfite on the secretion of mediators by AM with that of sulfate, canine AM were incubated with sulfite or sulfate, respectively. Control AM were treated simultaneously. The resulting supernatants were analyzed for their ability to stimulate the respiratory burst of canine PMN by lucigenin-dependent CL measuring superoxide anion production (Allen, 1981; Allen, 1982; Allen, 1986; Gyllenhammar, 1987).

As seen in Fig. 1, supernatants (conditioned supernatant) of sulfite-treated canine AM significantly increased CL of resting and PMA-stimulated canine PMN as compared to supernatants of control AM. The PMN-stimulating activity was dependent on the sulfite concentration used for AM treatment showing the strongest response with 1 mM sulfite. In contrast, supernatants of AM treated with sulfate (1 mM) did not increase CL-response of resting or PMA-stimulated canine PMN compared to control supernatant. These findings indicate that sulfite specifically induces AM to release an activity that stimulates the respiratory burst of PMN. Since the strongest response was obtained with 1 mM sulfite, this sulfite concentration was used in subsequent experiments.

*Characterization of neutrophil-stimulating activity by desensitization of neutrophil receptors*

To identify the mediators of the PMN-stimulating activity, PMN receptors for PAF, $LTB_4$, IL-8

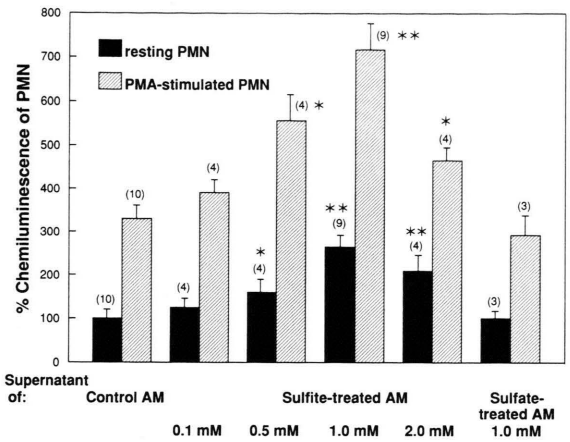


Fig. 1. Comparison of the effects of supernatants derived from sulfite-treated, sulfate-treated and control alveolar macrophages on the respiratory burst of neutrophils. Supernatants of canine AM were incubated in PBS buffer, pH 7, in the absence (control) and presence of sulfite (0.1 mM – 2 mM) or sulfate (1 mM), respectively, and analyzed for their ability to stimulate the respiratory burst activity of canine PMN by measuring CL. CL was recorded following incubation of PMN with AM-derived supernatants and subsequent stimulation with PMA (for details see Materials and Methods). Values (means ± SEM) are given as percentages of CL-response of resting PMN incubated with control supernatant corresponding to 1.804 ± 0.240 x $10^6$ CL-counts integrated during 20 min in 1.5 x $10^4$ cells ($n$=10). Numbers in parentheses represent the number of experiments with AM-derived supernatants of different dogs. Asterisk indicates a significant difference by $p \leq 0.05$ (*) or $p \leq 0.001$ (**) between CL of PMN incubated with control supernatant or supernatant of sulfite-treated AM.

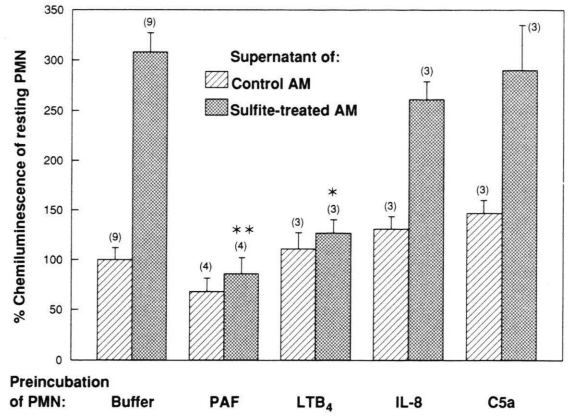


Fig. 2. Desensitization of neutrophil receptors for PAF, $LTB_4$, IL-8, and C5a with supernatant of sulfite-treated canine alveolar macrophages.
Human PMN were desensitized with PAF, $LTB_4$, IL-8, or C5a, subsequently incubated with supernatant of sulfite-treated canine AM, and analyzed for respiratory burst activity by measuring CL. Values (mean ± SEM) are given as percentages of CL-response of resting PMN incubated with control supernatant corresponding to 1.288 ± 0.154 x $10^6$ CL-counts integrated during 10 min in 1 x $10^5$ cells ($n$=9). Numbers in parentheses represent the number of experiments with AM-derived supernatants of different dogs. Asterisk indicates a significant difference by $p \leq 0.05$ (*) or $p \leq 0.001$ (**) between CL of PMN incubated with supernatant of sulfite-treated AM without or with desensitizing pretreatment of PMN with PAF or $LTB_4$.

or C5a were desensitized with their corresponding agonists. The addition of supernatant of sulfite-treated AM should then result in a decreased CL-response when the target receptor for the PMN-stimulating activity is identical with one of the agonists. Since supernatants of sulfite-treated AM activated not only canine but also human PMN (Beck-Speier *et al.*, in press), these studies were performed with human PMN which in addition showed a stronger response to IL-8, $LTB_4$ or C5a than canine PMN. The optimal concentrations of PAF, $LTB_4$, IL-8 and C5a for desensitization of their corresponding receptors were determined in separate experiments (data not shown). Fig. 2 shows that the PMN-stimulating activity was strongly reduced when the cells had been preincubated with PAF or $LTB_4$, whereas preincubation with IL-8 or C5a did not affect the stimulating activity. This suggests that the PMN-stimulating activity interacts with the PAF receptor and the $LTB_4$ receptor during activation of PMN.

### *Effect of phospholipase $A_2$, 5-lipoxygenase and cyclooxygenase on the release of the neutrophil-stimulating activity*

Since the PMN-stimulating activity seems to contain lipid mediators such as PAF and $LTB_4$, the activity should be reduced by inhibition of $PLA_2$ which is involved in the biosynthesis of both mediators. We used three inhibitors of $PLA_2$, $AACOCF_3$ (Bartoli *et al.*, 1994), quinacrine (Tsunawaki and Nathan, 1986) and OBAA (Köhler *et al.*, 1992), respectively. AM were pretreated with either one of these $PLA_2$ inhibitors prior to incubation with sulfite. As demonstrated in Fig. 3, inhibition of $PLA_2$ in sulfite-treated AM significantly reduced the release of the PMN-stimulating activity. This indicates that the PMN-stimulating

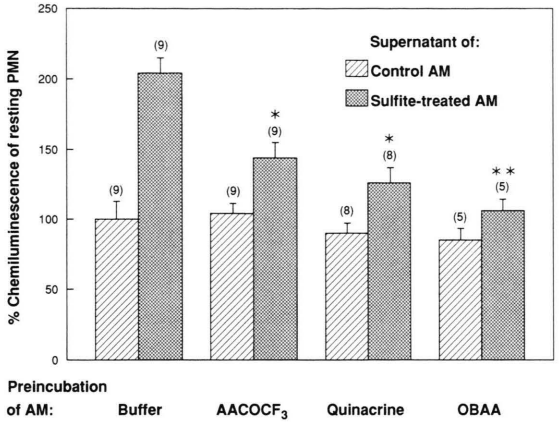


Fig. 3. Effect of phospholipase $A_2$ inhibitors $AACOCF_3$, quinacrine and OBAA, on release of neutrophil-stimulating activity.
Canine AM were preincubated with $AACOCF_3$ ($1 \times 10^{-4}$ M), quinacrine ($1 \times 10^{-4}$ M) and OBAA ($1 \times 10^{-5}$ M), and subsequently incubated with sulfite. The resulting supernatants were analyzed for ability to activate respiratory burst activity of canine PMN by measuring CL. Values (mean ± SEM) are given as percentages of CL-response of resting PMN incubated with control supernatant corresponding to $1.546 \pm 0.247 \times 10^6$ CL-counts integrated during 20 min in $1.5 \times 10^4$ cells ($n$= 9). Numbers in parentheses represent the number of experiments with AM-derived supernatants of different dogs. Asterisk indicates a significant difference by $p \leq 0.05$ (*) or $p \leq 0.001$ (**) between CL of PMN with supernatant of sulfite-treated AM incubated in the absence or presence of $PLA_2$ inhibitors.

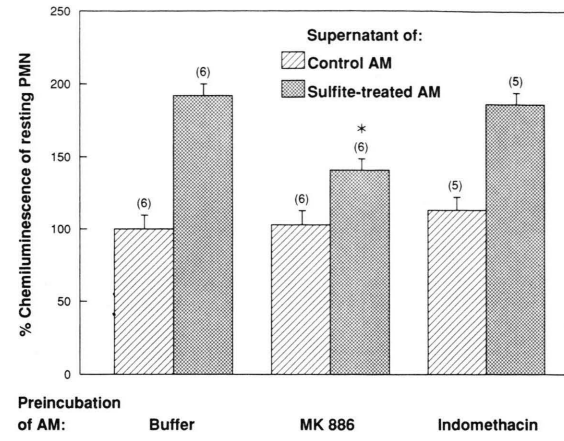


Fig. 4. Effect of 5-lipoxygenase inhibitor MK 886 and cyclooxygenase inhibitor indomethacin on release of neutrophil-stimulating activity.
Canine AM were preincubated with MK 886 ($1 \times 10^{-6}$ M) or indomethacin ($1 \times 10^{-6}$ M) respectively, and subsequently incubated with sulfite. The resulting supernatants were analyzed for ability to activate respiratory burst activity of canine PMN by measuring CL. Values (mean ± SEM) are given as percentages of CL-response of resting PMN incubated with control supernatant corresponding to $1.910 \pm 0.203 \times 10^6$ CL-counts integrated during 20 min in $1.5 \times 10^4$ cells ($n$=6). Numbers in parentheses represent the number of experiments with AM-derived supernatants of different dogs. Asterisk indicates a significant difference by $p \leq 0.05$ (*) between CL of PMN with supernatant of sulfite-treated AM incubated in the absence or presence of MK 886.

activity is generated by $PLA_2$-dependent pathways.

$PLA_2$ hydrolyzes phospholipids to lyso-phospholipids and arachidonate, which is further metabolized to leukotrienes by 5-LO, prostaglandins by COX and other metabolites of arachidonic acid (Denzlinger, 1996; Holtzman, 1991). To study which pathway is involved in the generation of the PMN-stimulating activity, prior to incubation of AM with sulfite 5-LO was inhibited by MK 886 (Rouzer *et al.*, 1990) and COX was inhibited by indomethacin (Shams et al., 1989) in separate experiments. As shown in Fig. 4, inhibition of 5-LO by MK 886 in sulfite-treated AM resulted in a significant reduction of the PMN-stimulating activity, whereas inhibition of COX showed no effect. This indicates that part of the PMN-stimulating activity is generated via 5-LO-dependent pathways.

## Discussion

PAF and $LTB_4$ are potent lipid mediators involved in a number of pathophysiological reactions. In inflammatory reactions PAF is mainly synthesized by the remodeling pathway (Snyder, 1995). This pathway results in generation of lyso-PAF through action of $PLA_2$ on phosphatidylcholine. Lyso-PAF is converted to PAF by acetyl-CoA:lyso-PAF acetyltransferase. When lyso-PAF is produced from phosphatidylcholine species containing arachidonic acid at the *sn*-2 position, the arachidonic acid released is available for leukotriene synthesis (Ramesha and Pickett, 1986; Suga *et al.*, 1990). Arachidonic acid is converted to leukotriene $A_4$ ($LTA_4$) by 5-LO acting together with 5-lipoxygenase-activating protein (Denzlinger, 1996; Abramovitz *et al.*, 1993). $LTA_4$ can be metabolized to $LTB_4$ by $LTA_4$ hydrolase or to the cysteinyl leukotrienes by leukotriene $C_4$ synthase ($LTC_4$) (Denzlinger, 1996; Minami *et al.*, 1990).

$LTB_4$ is the major leukotriene product of rat and human AM (Lee and Lane, 1992). Recently Shamsuddin *et al.* (1995) showed that PAF and leukotrienes can be regulated differently in rat AM.

Our present study shows that, in contrast to sulfate, sulfite induces AM to release an activity which modulates PMN function by stimulating the respiratory burst indicating that the release of a PMN-stimulating activity is specific for sulfite (Fig. 1). Desensitization of PMN receptors showed that the PMN-stimulating activity elicits the respiratory burst of PMN via receptors for PAF and $LTB_4$ (Fig. 2). These data provide indirect evidence that the two mediators secreted by sulfite-treated AM are identical to PAF and $LTB_4$. The existence of PAF in the PMN-stimulating activity was confirmed by PAF antagonists and by the PAF-like CL-kinetic of the PMN-stimulating activity after extraction of apolar lipids (Beck-Speier *et al.*, in press). Our studies with inhibitors of $PLA_2$ and 5-LO support the conclusion about the release of PAF and $LTB_4$ induced by sulfite. Among the inhibitors of the $PLA_2$ pathway, OBAA was most efficient in suppressing PMN-stimulating activity by more than 90% (Fig. 3). Inhibition of $PLA_2$ downregulates both the remodeling pathway for PAF and arachidonic acid metabolism. Looking downstream the arachidonic acid pathway, inhibition of 5-LO by MK 886 diminished the PMN-stimulating activity in the conditioned supernatant by about 60% (Fig. 4). This finding confirms that the formation of leukotrienes is not exclusively responsible for the activation of PMN, and suggests the presence of a second mediator in the supernatant of sulfite-treated AM which does not derive from a 5-LO metabolite but which appears to be identical with PAF.

Since sulfite unlike sulfate is able to activate the respiratory burst of PMN even at very low concentrations (Beck-Speier *et al.*, 1994), the supernatants of sulfite-treated AM being tested with PMN were dialyzed against PBS buffer in a dialysis membrane with a molecular weight cut-off of 500 Da to remove sulfite. The dialysis eliminated the concentration of sulfite by more than 97%, and reduced that of PAF by less than 5% and that of $LTB_4$ by less than 25%.

The respiratory burst activity of PMN was determined by superoxide dismutase-inhibitable lucigenin-dependent CL which is generated by reductive dioxygenation of lucigenin by superoxide anions (Allen, 1981; Allen, 1982; Allen, 1986; Gyllenhammar, 1987). Recently it was shown that lucigenin itself can reduce oxygen to superoxide anion in the presence of NADH/NADPH and reducing enzyme systems (Liochev and Fridovich, 1997; Vasquez-Vivar *et al.*, 1997). However, these in-vitro studies have not yet been proofed with cellular systems. The stimulation of PMN by PMA leads to an activation of NADPH oxidase which produces superoxide anions (Bellavite, 1988). This correlates with a strong increase of lucigenin-dependent CL compared to the CL-level of resting control cells measured prior to addition of PMA into the assay system (Beck-Speier *et al.*, 1993). PMN of patients with chronic granulomatous disease having genetic defects in their NADPH oxidase system cannot produce superoxide anions (Curnutte and Babior, 1987; Dinauer *et al.*, 1987; Volpp *et al.*, 1988). Since the PMN of these patients show no increase in lucigenin-dependent CL during activation by PMA in contrast to normal PMN (Beck-Speier *et al.*, 1993), we conclude that lucigenin only detects superoxide anions produced by PMN. Prevention of NAPDH oxidase activation in PMN by inhibitors of protein kinase C completely abolished lucigenin-dependent CL which confirms that lucigenin can be used as a probe for detecting cell-related superoxide anion release in our system (data not shown). Therefore, although lucigenin produces superoxide anions in in-vitro systems we suggest that the lucigenin-dependent CL can be used as a differential measurement for NADPH oxidase activity by comparing the CL-levels of resting and activated cells.

PAF has been reported to be an effective mediator in triggering an increase in the alveolar-capillary permeability (Evans *et al.*, 1987; O'Donnell and Barnett, 1987; DeLima *et al.*, 1995; Longphre and Kleeberger, 1995). Recent findings have shown that PAF induces the transcription of vascular endothelial growth factor in vascular smooth muscle cells which increases vascular permeability in-vivo (Nauck *et al.*, 1997). In addition, PAF is also known as a potent mediator for the secretion of lysosomal enzymes (Hayashi *et al.*, 1991; Pinckard *et al.*, 1992). $LTB_4$ is a potent chemotactic agent for PMN and is involved in the induction of PMN-endothelial cell adhesion (Henderson, 1994; Palmblad and Lerner, 1992). It is also involved in

increased vascular permeability (Björk *et al.*, 1982) and in PMN degranulation (Denzlinger, 1996). There is also evidence that PAF and $LTB_4$ interact in mediating their effects (Denzlinger, 1996).

Chronic exposure of dogs to a neutral sulfite aerosol alters the alveolar-capillary permeability as well as the lysosomal enzyme secretion as assessed by determination of the content of protein and albumin and the extracellular activity of β-N-acetyl-glucosaminidase in the bronchoalveolar lavage fluid (Maier *et al.*, 1992). Both capillary permeability and lysosomal enzyme secretion significantly increased in the second half of the exposure period of one year. The data from the present in-vitro study suggest that PAF and $LTB_4$ released by sulfite-treated AM may significantly contribute to the altered permeability and the increased release of lysosomal enzyme activity during exposure of dogs to a neutral sulfite aerosol. The concentration of particulate sulfite in our in-vivo study corresponds to sulfur dioxide levels occurring during smog episodes (0.2 ppm). In our in-vitro model 0.5 to 2 mM sulfite were required to stimulate AM for the release of mediators. These concentrations are high but may be of biological relevance. Association of the 4-valent sulfur with particles favors elevated deposition in the lung periphery, while gaseous sulfur dioxide is predominantly adsorbed in the upper airways. Studies with a rat model have shown that inorganic particles are preferentially deposited in the alveolar duct bifurcations (Warheit *et al.*, 1984; Osornio-Vargas *et al.*, 1991). From our in-vivo study with neutral sulfite and acidic sulfate aerosols we have indirect evidence that sulfur-related particles are enriched in this lung compartment indicated by epithelial type II cell proliferation in the alveolar ducts (Takenaka *et al.*, 1996). Accumulation of these particles in the alveolar ducts might result in high local concentrations of sulfur-related compounds. In addition, the local concentration of sulfite is dependent on the activity of the sulfite-detoxifying enzyme sulfite oxidase present in tissues or cells at the sites of particle deposition. Lung tissue as well as AM have very low activities of sulfite oxidase and are therefore sensitive to sulfite toxicity (Beck-Speier *et al.*, 1985).

In summarizing the results from our in-vivo and in-vitro studies, we suppose that inhalation of sulfur dioxide/sulfite-aerosols can activate AM to release lipid mediators thus contributing to initiation or amplification of inflammatory responses in the lungs.

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