Changes in airway resistance by simultaneous exposure to TNF-\(\alpha\) and IL-1\(\beta\) in perfused rat lungs

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Martin, Christian, Andrea Wohlsen, and Stefan Uhlig. Changes in airway resistance by simultaneous exposure to TNF-\(\alpha\) and IL-1\(\beta\) in perfused rat lungs. Am J Physiol Lung Cell Mol Physiol 280: L595–L601, 2001.—Tumor necrosis factor (TNF)-\(\alpha\) and interleukin (IL)-1\(\beta\) are formed simultaneously under inflammatory conditions such as asthma and acute respiratory distress syndrome. Here we investigated the effects of TNF-\(\alpha\) (10 ng/ml) and/or IL-1\(\beta\) (10 ng/ml) in isolated blood-free perfused rat lungs. In lungs precontracted with methacholine, IL-1\(\beta\) alone and IL-1\(\beta\)/TNF-\(\alpha\) decreased airway resistance 10 min after administration, whereas TNF-\(\alpha\) alone had no effect. In untreated lungs, airway resistance was unaltered by either cytokine alone but started to increase 40 min after treatment with both cytokines together, indicating bronchoconstriction. The bronchoconstriction was accompanied by a steroid-sensitive increase in cyclooxygenase (COX)-2 mRNA expression and thromboxane formation. The cytokine-induced bronchoconstriction was blocked by the thromboxane receptor antagonist SQ-29548, indomethacin, the selective COX-2 inhibitor NS-398, and the steroid dexamethasone. We conclude that IL-1\(\beta\) has an early bronchodilatory effect (after 10 min) that is unchanged by TNF-\(\alpha\). However, at later time points (after 40 min), IL-1\(\beta\) and TNF-\(\alpha\) in concert cause a COX-2- and thromboxane-dependent bronchoconstriction. Our findings show that TNF-\(\alpha\) and IL-1\(\beta\) exert complex and time-dependent effects on lung functions that cannot be predicted by studying each cytokine alone.

Only a few studies have investigated whether TNF-\(\alpha\) and/or IL-1 can cause bronchospasm. There is some evidence that TNF-\(\alpha\) when given directly may cause bronchoconstriction (35) and airway hyperresponsiveness (27). In contrast to TNF-\(\alpha\), IL-1\(\beta\) failed to increase airway resistance (17) and relaxed rather than constricted airway smooth muscle (25). Other studies on the effects of IL-1\(\beta\) treatment on airways reported a decreased response to \(\beta\)-agonists (12) and hyperreactivity to bradykinin (29). However, in these experiments, IL-1\(\beta\) and TNF-\(\alpha\) have always been examined alone, dismissing the fact that under inflammatory conditions, TNF-\(\alpha\) and IL-1\(\beta\) usually occur in concert. The notion that IL-1\(\beta\) and TNF-\(\alpha\) may have synergistic effects is supported by a number of previous studies (reviewed in Ref. 5). However, most of these previous studies examined the synergistic effects on gene expression or cytokine release but only rarely the functional physiological consequences thereof. Using the model of precision-cut lung slices, we have recently shown a thromboxane-dependent airway contraction in the simultaneous presence of TNF-\(\alpha\) and IL-1\(\beta\) but not in the presence of either cytokine alone (14). However, the utilization of precision-cut lung slices to study dynamic airway responses is rather new (13), and it is not known whether mechanisms observed in this model also apply to the whole intact organ, in particular, if events as intricate as those induced by cytokines are under investigation. Therefore, and because of the possible clinical implications of additive or synergistic actions of TNF-\(\alpha\) and IL-1\(\beta\) in the lung, here we investigated the effects of TNF-\(\alpha\) and IL-1\(\beta\) in whole perfused lungs. To gain further insight into the complex pulmonary reactions evoked by these cytokines, we also studied their effects in lungs with precontracted airways. These investigations will help to better understand the consequences of pulmonary inflammation in the course of diseases such as ARDS and asthma.

METHODS

Animals

Lungs were taken from 8-wk-old female Wistar rats (220 ± 20 g) obtained from Harlan Winkelman (Borchen, Germany).

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and kept under controlled conditions (22°C, 55% humidity, 12:12-h day-night rhythm) on a standard laboratory chow.

Chemicals

Rat recombinant (r) IL-1β (rIL-1β) and rat rTNF-α were obtained from R&D Systems (Wiesbaden-Nordenstadt, Germany). Methacholine, indomethacin, and dexamethasone were purchased from Sigma (Deisenhofen, Germany). The selective cyclooxygenase (COX)-2 inhibitor NS-398 and the thromboxane receptor antagonist SQ-29548 were obtained from SPI Bio (Paris, France).

Isolated Perfused Rat Lung Preparation

The rat lungs were prepared and perfused as previously described (30). Briefly, the lungs were perfused with Krebs-Henseleit buffer (37°C; 2% albumin, 0.1% glucose, and 0.3% HEPES) through the pulmonary artery at a constant hydrostatic pressure (12 cmH2O), resulting in a flow rate of ~25 ml/min. The total amount of circulating buffer was 100 ml. The lungs were suspended by the trachea and were ventilated by negative pressure ventilation with 80 breaths/min and a tidal volume between 1.6 and 2 ml. Every 5 min, a sigh (−16 cmH2O) was performed. Artificial thorax chamber pressure was measured with a differential pressure transducer (DP 45-14, Validyne, Northridge, CA), and airflow velocity was measured with a pneumotachograph tube (Fleisch type 0000) connected to a differential pressure transducer (Validyne DP 45-15). The lungs respired humidified air. The perfusate flow (Narcomatic RT 500, Houston, TX) and the arterial and venous pressures (Statham P23BB) were continuously monitored. The pH of the perfusate before it entered the lung was kept at 7.35 by automatic bubbling of the buffer and an automatic pH control system (Hugo Sachs Elektronik). The pH of the perfusate before it entered the lung was kept at 7.35 by automatic bubbling of the buffer and an automatic pH control system (Hugo Sachs Elektronik). The pH of the perfusate before it entered the lung was kept at 7.35 by automatic bubbling of the buffer and an automatic pH control system (Hugo Sachs Elektronik).

Thromboxane Enzyme Immunoassay

Thromboxane release into the supernatant was assessed by measuring the stable metabolite thromboxane B2 with a commercially available enzyme immunoassay (Cayman Chemical, Ann Arbor, MI).

RT-PCR

The lungs were treated with cytokines to induce gene expression. In some experiments, to block gene expression, dexamethasone (10 mM) was added 30 min before cytokine treatment. The lungs were removed from the thoracic chamber and transferred into Eppendorf cups, frozen immediately in liquid nitrogen, and stored at −70°C. Tissue samples from the lungs of individual animals were homogenized with TRIzol Reagent (Gibco BRL, Life Technologies, Karlsruhe, Germany) in a Polytron tissue homogenizer (Kinematica). Total RNA was isolated with TRIzol Reagent. Briefly, 50–80 mg of lung tissue were incubated with 1 ml of TRIzol Reagent. After centrifugation at 12,000 g, the supernatant was transferred into a new cup. RNA was precipitated with isopropanol at room temperature and centrifuged at 12,000 g, and the pellet was then washed with 70% ethanol. The pellet containing RNA was then dissolved in ultrapure water. The RNA yield was assessed with ultraviolet spectrophotometry (GeneQuant Pharmacia Biotech, Cambridge, UK). RNA (4 μg) was reverse transcribed with oligo(dT) primers. The transcribed cDNA was then used for PCR (Uno II Biometra, Gottingen, Germany).

The primers were specific for COX-1 (5′ forward primer, TCA CGT TGG AGA AGG ACT CC), COX-2 (5′ forward primer, CCA GAC AGA TCA GAA GCG AGG and 3′ reverse primer, CCA GCA GTG ATG TGA AGG), and β-actin (5′ forward primer, ATG TAC GTA GCC ATC CAG GC and 3′ reverse primer, TGT GTG GGT GAA GCT GTA GC). These primer pairs were found to yield PCR products of 323, 562, 692, and 216 bp for COX-1, COX-2, TXS, and β-actin, respectively. Amplified cDNA bands were detected by ethidium bromide staining.

The yield of the amplified product was always linear for the amount of input cDNA and PCR cycle number (data not shown). For amplification, 25 cycles were used for COX-1, 20 cycles were used for COX-2 and β-actin, and 30 cycles were used for TXS.

Statistics

Data are expressed as means ± SD. Data were examined by analyzing the maximum values (see Figs. 1 and 2) or the area under the curve (see Fig. 4) with two-sided t-tests. The α error resulting from multiple comparisons was adjusted by the method of Hommel (38). P < 0.05 was considered significant.

RESULTS

Action of Cytokines on Isolated Perfused Lungs

Two different designs were used to determine the effects of IL-1β and/or TNF-α on airway tone in perfused lungs. In the first set of experiments, the lungs were perfused for 40 min under control conditions and were then exposed to the cytokines alone or in combination. In the second set of experiments, the airways were precontracted with methacholine to examine the possible dilatory effects of the cytokines.

Airway resistance and thromboxane release. Under control conditions, the airway resistance in isolated perfused rat lungs remained stable throughout the whole experiment (Table 1). Perfusion with rat rTNF-α alone did not alter airway resistance. Administration of rat rIL-1β alone did not change the airway resistance either. However, perfusion with both cytokines together caused a significant increase in airway resistance over time (Fig. 1A) as well as a decrease in pulmonary compliance and tidal volume (Table 1). There was no effect of either cytokine alone or in combination on vascular resistance or lung weight gain as a measure of edema formation (Table 1).

To elucidate the mechanism by which IL-1β/TNF-α causes bronchoconstriction, we examined the potential contribution of thromboxane because it is known that this lipid mediator is responsible for a similar bronchoconstriction induced by IL-1β/TNF-α in precision-cut lung slices (14) and by endotoxin (32) in perfused rat lungs. Figure 1B shows that compared with control conditions, thromboxane release into the venous effluent was not changed in the presence of either IL-1β or
However, simultaneous treatment with IL-1β and TNF-α resulted in a synergistic increase in thromboxane perfusate levels (Fig. 1B). The time course of thromboxane production was similar to that of the increase in airway resistance (Fig. 1).

Pharmacological interventions. The use of a thromboxane receptor antagonist (10 μM SQ-29548) 10 min before cytokine treatment completely prevented the cytokine-induced bronchoconstriction (Fig. 2A). Moreover, the nonspecific COX inhibitor indomethacin as well as the selective COX-2 inhibitor NS-398 both prevented the increase in airway resistance elicited by IL-1β/TNF-α (Fig. 2A) as well as the release of thromboxane into the perfusate (Fig. 2B). Similarly, the glucocorticoid dexamethasone inhibited both production of thromboxane (Fig. 2B) and cytokine-induced bronchoconstriction (Fig. 2A).

Gene Expression

Because the selective COX-2 inhibitor NS-398 prevented IL-1β/TNF-α-induced bronchoconstriction and thromboxane formation, we investigated the expression of COX-1, COX-2, and TxS mRNAs (Fig. 3). For COX-1 and TxS, there were only small differences in mRNA expression under any condition. With respect to

![Fig. 1](image1.png)

![Fig. 2](image2.png)

Table 1. TNF-α- and IL-1β-induced alterations in lung functions after 180 min of treatment

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>RL, cmH2O·ml⁻¹·s</th>
<th>VT, ml</th>
<th>Cdyn, ml/cmH2O</th>
<th>RV, cmH2O·ml⁻¹·min</th>
<th>ΔW, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>0.248 ± 0.016*</td>
<td>1.928 ± 0.221*</td>
<td>0.317 ± 0.068*</td>
<td>0.275 ± 0.047</td>
<td>0.509 ± 0.178</td>
</tr>
<tr>
<td>IL-1β</td>
<td>3</td>
<td>0.286 ± 0.024*</td>
<td>1.233 ± 0.415†</td>
<td>0.232 ± 0.021*</td>
<td>0.260 ± 0.027</td>
<td>0.339 ± 0.203</td>
</tr>
<tr>
<td>TNF-α</td>
<td>4</td>
<td>0.248 ± 0.012‡</td>
<td>1.923 ± 0.233*</td>
<td>0.379 ± 0.063*</td>
<td>0.256 ± 0.036</td>
<td>0.164 ± 0.099</td>
</tr>
<tr>
<td>IL-1β/TNF-α</td>
<td>4</td>
<td>0.398 ± 0.012‡</td>
<td>0.530 ± 0.145†</td>
<td>0.064 ± 0.004†</td>
<td>0.317 ± 0.043</td>
<td>0.380 ± 0.134</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of experiments. TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; RL, pulmonary resistance; VT, tidal volume; Cdyn, dynamic compliance; RV, vascular resistance; ΔW, weight gain. The cytokines were always added after 40 min of perfusion under baseline conditions. *P < 0.01 vs. IL-1β/TNF-α. †P < 0.05 vs. control.
COX-2 expression, there was little change in TNF-α-treated lungs and a small increase in IL-1β-treated lungs compared with that in control lungs. Simultaneous treatment of the lungs with IL-1β and TNF-α caused the strongest induction in COX-2 mRNA, which was prevented by preincubation with dexamethasone starting 30 min before the cytokine treatment (Fig. 3).

**Action of Cytokines on Methacholine-Contracted Perfused Lungs**

Because a previous study (25) had shown that pre-treatment of bronchial segments with IL-1β decreased the contractile responses to acetylcholine, we investigated whether the cytokines might also have relaxing properties. Therefore, we examined whether rat rIL-1β or rat rTNF-α caused bronchodilation in lungs precontracted with the stable acetylcholine analog methacholine (MCh). To achieve preconstriction, after 30 min of perfusion and ventilation, isolated rat lungs were exposed to 10⁻⁶.5 M MCh (Fig. 4). After an initial peak directly after an injection of MCh, airway resistance declined but, probably as a result of the recirculating perfusion, remained elevated compared with the resistance before the addition of MCh. The addition of TNF-α showed no effect on airway resistance compared with that in control lungs. In contrast, perfusion with IL-1β or IL-1β/TNF-α resulted in a decrease in airway resistance that started ~10 min after cytokine administration (Fig. 4).

**DISCUSSION**

TNF-α and IL-1β are two cytokines present at inflamed sites. The major finding of this study is that in the simultaneous presence of IL-1β and TNF-α, airways may either relax or contract, depending largely on the time scale studied. Thus, almost immediately after administration of TNF-α/IL-1β, a brief relaxation of precontracted airways occurs that is solely due to IL-1β. At later time points, however, starting ~30 min after administration, IL-1β and TNF-α in concert constrict airways. Because the airway relaxation is very short-lived, we speculate that under clinical conditions such as asthma or ARDS, where both cytokines usually occur together, the bronchoconstriction may become more important. Our findings also indicate that the role of cytokines in situ may be difficult to predict from studies with single cytokines only. The present data further suggest that the mechanism responsible for the effects of TNF-α/IL-1β on bronchoconstriction is independent of blood-derived leukocytes and involves induction of COX-2 followed by production of thromboxane.

Synergistic actions between IL-1β and TNF-α are well known in the literature, although the mechanism remained obscure (5). A synergism between IL-1β and TNF-α was reported before with respect to mortality.
(33), pulmonary edema (18, 34), and prostaglandin production (5). In contrast to IL-1β, IL-1α together with TNF-α failed to show such synergism on pulmonary edema (24), thromboxane production (11), and pulmonary vascular resistance (11). Despite the plethora of studies on TNF-α and IL-1β, there is very little information about the effects of these cytokines on airway functions. A direct effect of IL-1β on airway tone has not been described before. Previously, IL-1β was studied in bronchial or tracheal smooth muscle preparations where pretreatment decreased the response to β-agonists (12), induced hyperreactivity to bradykinin (29), and reduced the contractile responses to acetylcholine, histamine, and KCl (25). Here we show that IL-1β relaxes precontracted airways. The mechanism responsible for this airway relaxation is unknown at present but may involve dilatory prostaglandins or NO. The finding that IL-1β can relax precontracted airways extends previous observations made in canine bronchial segments of attenuated airway contractions after preincubation with IL-1β for 150 min (25). It should be noted, however, that these authors failed to observe a direct relaxation with IL-1β in precontracted airway segments. Notable differences between that study and the present one that might explain this discrepancy include the use of human IL-1β instead of homologous IL-1β and the use of bronchial segments in contrast to the whole organ.

In contrast to IL-1β, bronchoconstriction by TNF-α alone in vivo was demonstrated before (35). However, as with IL-1β, most studies were performed in vitro where it was shown that preincubation with TNF-α caused bronchial hyperresponsiveness (1, 20). In general, cytokines are thought to orchestrate the immune response, with indirect rather than direct effects on lung physiology. Therefore, it is important to stress the fact the synergistic bronchoconstriction by IL-1β and TNF-α in otherwise untreated lungs occurred in blood-free perfused lungs, ruling out the participation of blood-derived leukocytes (for confirmation of the absence of blood cells in our model, see Ref. 31). Further evidence for the fact the IL-1β/TNF-α-induced bronchoconstriction does not depend on blood-derived leukocytes is given by the fact that a similar response also occurs in precision-cut lung slices from rats (14). The good agreement of the present findings with those obtained in precision-cut lung slices indicates that the slice model is a good representative of the whole organ. This conclusion is particularly important for studies with precision-cut lung slices from humans (Wohlsen A, Martin C, Vollmer E, and Uhlig S, unpublished observations) in which comparisons between different models cannot be easily made. Of course, as with all such model systems that allow for control of parameters such as cytokine concentration, the caveat should be made that under in vivo conditions, factors such as blood and a functioning nerve supply might modify the responses.

Like in precision-cut lung slices, in perfused lungs, the IL-1β/TNF-α-induced bronchoconstriction is most likely caused by COX-2-dependent thromboxane formation. The evidence for this mechanism is as follows. 1) After 60 min of perfusion with IL-1β/TNF-α, we found significant induction of COX-2 mRNA compared with weak or hardly detectable induction in the presence of IL-1β or TNF-α alone. Previous cell culture studies provide ample evidence that COX-2 expression is inducible in parenchymal lung cells, i.e., lipopolysaccharide (LPS) or IL-1β and TNF-α elicited expression of COX-2 in pulmonary epithelial cells (16), airway macrophages (19), and airway smooth muscle cells (2). In addition, pulmonary COX-2 expression was recently confirmed by immunohistochemical staining with anti-COX-2 antibodies (7). 2) Thromboxane was released into the perfusate with a time course matching that of the bronchoconstriction. As for COX-2 synthase, recent histochemical data (6) have provided evidence that TxB2 is also present in parenchymal lung cells. 3) Inhibition of COX-2 induction by dexamethasone or COX-2 enzyme by NS-398 prevented thromboxane formation as well as bronchoconstriction. 4) Blockade of the thromboxane receptor SQ-29548 completely abrogated the IL-1β/TNF-α-induced bronchoconstriction.

A number of different animal models support the notion that expression of COX-2 and/or thromboxane in the lungs is frequently associated with different forms of lung failure. Perfusion of rat lungs with LPS causes a very similar type of bronchoconstriction, as described here, that is also COX-2 and thromboxane dependent but neutrophil independent (32). In another model, in vivo pretreatment with granulocyte-macrophage colony-stimulating factor or granulocyte colony-stimulating factor resulted in increased bronchial responsiveness to LPS that is neutrophil dependent and, again, causally related to COX-2 expression and thromboxane formation (36). In murine lungs, LPS causes COX-2- and thromboxane-dependent vascular hyperresponsiveness (4, 10). In dogs, priming with endotoxin for oleic acid-induced lung injury was also partly attributed to COX-2 induction (9). Therefore, enhanced expression of COX-2 followed by increased thromboxane formation appears to play an important role in the pathophysiology of many animal models.

The present findings may have bearings on our understanding of the pathophysiology of ARDS and asthma. Several studies have shown increased airway resistance in ARDS patients (e.g., Ref. 37). Because both TNF-α and IL-1β clearly occur in the airways of ARDS patients (15), TNF-α and IL-1β in concert might contribute to the bronchoconstriction in these patients. And also in the case of asthma, there is some evidence for a contribution of IL-1β and/or TNF-α. 1) In the bronchoalveolar lavage fluid and in biopsies from asthmatic patients, IL-1β and TNF-α are present, with the highest concentrations in acute severe asthma (3, 28). 2) In sensitized guinea pigs, an IL-1β receptor antagonist attenuated the infiltration of neutrophils, airway hyperresponsiveness toward substance P, and the late asthmatic bronchoconstriction (17, 23). 3) Th1 cell-derived TNF-α is required for recruitment of inflammatory cells to the airways in a murine asthma model (22). 4) Subsequent to antigen challenge, increased
TNF-α production by mast cells during the early-phase response (3) and by macrophages during the late-phase response has been demonstrated (8, 21). Because, in our model, the effect of IL-1β/TNF-α is mediated by thromboxane, it is of interest to note that a recent study (26) has reported improvements in lung functions in a subpopulation of asthma patients treated with a thromboxane receptor antagonist.

We conclude that IL-1β and TNF-α have time-dependent effects on airway tone that are not mediated by blood-derived leukocytes. An early bronchodilating effect that is mainly caused by IL-1β is followed by a COX-2- and thromboxane-dependent bronchoconstriction. Our findings suggest that IL-1β and TNF-α contribute to the altered lung functions in inflamed lungs where these two cytokines usually occur in concert.

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