

Platelet Activating Factor Mediates Interleukin-2-induced Lung Injury in the Rat

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Abstract

Interleukin-2 was recently shown to cause acute lung injury characterized by microvascular permeability defect, interstitial edema, and leukosequestration. Similar responses can also be produced by platelet activating factor (PAF). Thus, the present study aimed to examine whether PAF plays a key role in the development of IL-2-induced lung injury in the anesthetized rat. Intravenous infusion (60 min) of recombinant human IL-2 at 10^5 – 10^6 U/rat ($n = 7$ – 9) dose-dependently elevated lung water content ($27 \pm 1\%$, $P < 0.01$), myeloperoxidase activity ($+84 \pm 23\%$, $P < 0.05$), and serum thromboxane B_2 ($990 \pm 70\%$, $P < 0.01$), but failed to alter blood pressure, hematocrit, serum tumor necrosis factor- α , and circulating leukocytes and platelets. Pretreatment (-30 min) with a potent and specific PAF antagonist, BN 50739 (10 mg/kg, intraperitoneally, $n = 6$) prevented the pulmonary edema ($P < 0.05$) and thromboxane B_2 production ($P < 0.01$), and attenuated the elevation of lung myeloperoxidase activity ($+18 \pm 16\%$, $P < 0.05$) induced by IL-2. These data suggest that PAF is involved in the pathophysiological processes leading to IL-2-induced lung injury, and point to the potential therapeutic capacity of PAF antagonists in preventing pulmonary edema during IL-2 therapy. (*J. Clin. Invest.* 1992. 89:1669–1673.) Key words: pulmonary edema • cytokines • phospholipids • thromboxane • myeloperoxidase

Introduction

Human recombinant interleukin-2 has been shown to mediate tumor regression in animals (1) and humans (2) and is currently under investigation as a new treatment modality for patients with advanced metastatic cancer (3). In these patients, continuous infusion with systemic IL-2 is frequently associated with lung microvascular injury and pulmonary edema which might develop within 24 h after initiation of therapy (4, 5). These adverse effects are often part of a sepsis-like clinical syndrome characterized by generalized multisystem edema and hypotension (2, 3, 6, 7). In animals, IL-2 infusion can cause

acute permeability defect with fluid extravasation. For example, IL-2 (10^6 U) given intravenously (for 1 h) to anesthetized rats produced lung edema at 6 h along with pulmonary leukosequestration and elevated serum thromboxane B_2 (8). Also, studies in sheep with lung lymph fistula detected increased microvascular permeability as early as 2–3 h after the intravenous bolus injection of IL-2 at 10^5 U/kg (9–11). The mechanism(s) of IL-2-induced lung injury is still obscure. Nevertheless, it is believed that lung edema which follows continuous prolonged IL-2 infusion may result, at least in part, from lymphocyte activation several days after drug therapy (12–14). In contrast, recent studies suggested that IL-2-induced production of inflammatory mediators such as interleukin-1 (15), tumor necrosis factor- α (TNF α ,¹ 16, 17), and TXB $_2$ (8, 10, 18), or IL-2-induced activation of humoral systems such as the complement cascade (19), might account for the acute pulmonary toxicity associated with IL-2 infusion. These mediators might in turn activate neutrophils to further augment tissue injury (18, 19). The possibility that IL-2 might inflict tissue injury indirectly through the production of inflammatory mediators is supported by studies showing that IL-2 itself has no demonstrable effects on microvascular permeability in vitro (9).

Like IL-2, platelet activating factor (PAF), a potent alkyl-ether-glycerophosphorylcholine inflammatory mediator (for review, see reference 20), has been shown to increase pulmonary microvascular permeability (21–23), lung edema (21–23), pulmonary leukosequestration (24), and serum TXB $_2$ levels (25). In addition, relationships between PAF and IL-2 has been shown in studies where spleen mononuclear cells taken from rats subjected to long-term (seven days) administration of PAF demonstrated increased Con A-induced IL-2 production (26). This response was markedly inhibited by pretreatment with a PAF antagonist (26). Taken together, these data led us to hypothesize that PAF might be involved in the pathophysiology of IL-2-induced lung injury. The present study was designed to test this hypothesis by monitoring the capacity of BN 50739, a highly selective, potent, and long-acting PAF antagonist (27), to prevent lung injury produced by IL-2 infusion.

Methods

Drugs

IL-2. Recombinant human IL-2 (Hoffmann-La Roche Inc., Nutley, NJ) was reconstituted just before use with 1 ml of 0.9% NaCl per 10^6 U

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1. Abbreviations used in this paper: MAP, mean arterial pressure; MPO, myeloperoxidase; PAF, platelet activating factor; TNF α , tumor necrosis factor- α .

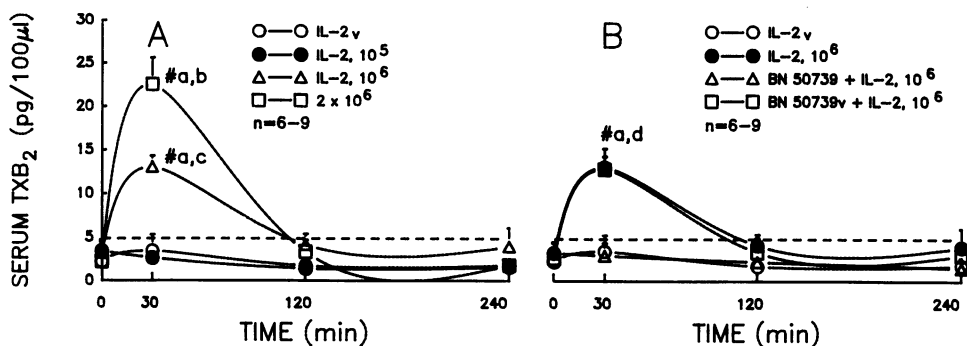


Figure 1. Serum TXB₂ response to IL-2 (A) and effect of pretreatment with BN 50739 on this response (B). IL-2, interleukin-2; IL-2v, IL-2 vehicle; BN 50739v, BN 50739 vehicle; **P* < 0.01; a, vs. basal value; b, vs. all other groups; c, vs. IL-2v and IL-2 10⁵ groups; d, vs. IL-2v and BN 50739 + IL-2 groups. IL-2 doses are units per animal. The dashed line represents the sensitivity of the assay (5 pg/100 μl).

IL-2. For the 10⁵ U dose, the stock solution of 10⁶ was diluted 10 times so that each rat received 1 ml vol.

BN 50739. BN 50739, kindly provided by Dr. P. Braquet (Institut Henri Beaufour, Paris), was solubilized in 64% DMSO in 0.9% NaCl to reach a concentration of 5 mg/ml.

Animals

Male Sprague-Dawley rats ranging from 220 to 280 gm were studied. All animals were housed in groups of three in standard cages, and kept with food and water ad lib. in a temperature-controlled room (22°C) on a 12-h dark/light cycle, until surgery.

Experimental protocols

Following anesthesia with pentobarbital (30 mg/kg, intraperitoneally [i.p.]), catheters (PE-50) were introduced into the femoral vein for drug infusion and femoral artery for blood pressure measurements and blood sampling. Basal mean arterial pressure (MAP) was recorded, and a blood sample for TNFα, TXB₂, hematocrit, leukocyte, and platelet determinations was collected (0.4 ml, exchanged with an equivalent volume of 0.9% NaCl). The animals were then randomly assorted into four experimental groups. In the first two groups, IL-2 at 10⁵ U/rat (*n* = 7), 10⁶ U/rat (*n* = 9), or vehicle (*n* = 6) was infused intravenously for 1 h (Syringe Infusion Pump 22; Harvard Apparatus, South Natick, MA). Blood pressure was continuously monitored and blood samples were repeated at 0.5, 2, and 4 h. At the end of the observation period both lungs were removed and used to determine lung myeloperoxidase (MPO) activity, lung wet weight, and tissue water content. In the third group, a similar protocol was repeated but with IL-2 (10⁶ U/rat) intravenous bolus injection followed immediately by IL-2 (10⁶ U/rat) infusion for 1 h (*n* = 6). Vehicle-treated animals served as controls (*n* = 6). The last group consisted of animals given IL-2 (10⁶ U/rat) infusion for 1 h, 30 min after pretreatment with BN 50739 (10 mg/kg, i.p., *n* = 6) or BN 50739 vehicle (*n* = 6). This dose of BN 50739 was selected based on our previous studies which characterized its pharmacological profile (28). In these latter studies, BN 50739 provided maximal protection against PAF-induced hypotension and platelet aggregation when administered at 10 mg/kg, i.p., 30 min before PAF challenge.

To exclude the possibility that BN 50739 exerts its protective effect through inhibition of thromboxane A₂ synthesis, the effect of BN 50739 on TXB₂ production by clotting blood was tested *in vitro* as follows: 2 ml of blood was directly aspirated from the femoral vein of enflurane (2% in 100% oxygen)-anesthetized rats (*n* = 3) using ice-cooled tubes containing 10 μl of 4 mg/ml indomethacin (in 0.1 M NaHCO₃, pH = 8). Blood samples were then allowed to clot for 20 min on ice and centrifuged at 14,000 rpm for 30 s (Microfuge B; Beckman Instruments, Palo Alto, CA). Plasma was transferred to another tube (triplicates) and frozen on dry ice until assayed. The same protocol was repeated using: (a) plasma incubated with BN 50739 at 1, 10, or 100 μM; (b) plasma taken from unclotted blood, i.e., blood was immediately centrifuged; and (c) plasma taken from clotted blood and incubated with indomethacin at 10⁻⁶–10⁻⁵ M.

Assays and techniques

TNFα. Plasma levels of TNFα were measured using a "sandwich" ELISA (29) employing a hamster monoclonal anti-mouse TNFα (Genzyme Corp., Cambridge, MA) as the capture antibody and a polyclonal rabbit anti-murine TNFα (Genzyme) as the detecting antibody. TNFα levels in rat samples were calculated from a standard curve generated with recombinant murine TNFα (Genzyme). TNFα levels determined by ELISA correlated with levels detected by the L-929 bioassay (30), with 1 U of activity in the bioassay corresponding to 5 pg of TNFα in the ELISA. The ELISA detected levels of TNFα down to 25 pg/ml.

TXB₂. TXB₂ was determined by radioimmunoassay (sensitivity of 5.0 pg/100 μl) as previously described (31).

MPO. MPO activity in the lung was assayed as described previously (32) based on a modification of Bradley's method (33) adapted for rat lung MPO assay (34).

Pulmonary water content. At the end of the experimental period the left lung was removed and immediately frozen on dry ice until assayed. When defrosted the lung was weighed to determine wet weight. Dry weight was determined after the lung was dried at 80°C for 36 h, and the pulmonary water content was calculated by subtracting the lung dry weight from the wet lung weight.

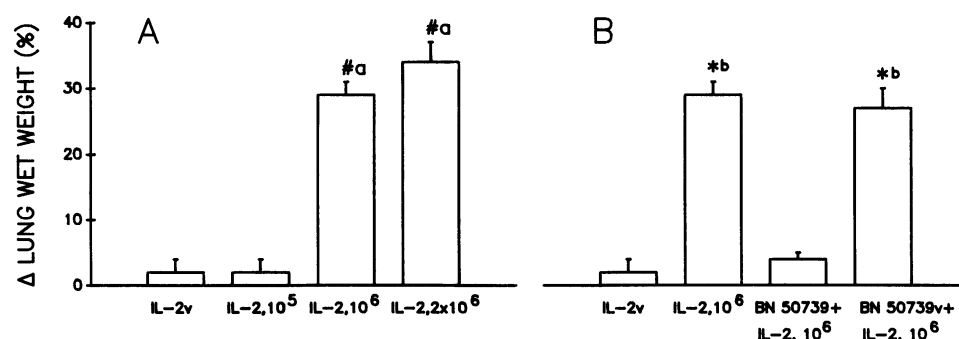


Figure 2. Lung wet weight response to IL-2 (A) and effect of pretreatment with BN 50739 on this response (B). IL-2, interleukin-2; IL-2v, IL-2 vehicle; BN 50739v, BN 50739 vehicle; **P* < 0.05; **P* < 0.01; a, vs. IL-2v and IL-2 10⁵ groups; b, vs. IL-2v and BN 50739 + IL-2 groups. IL-2 doses are units per animal.

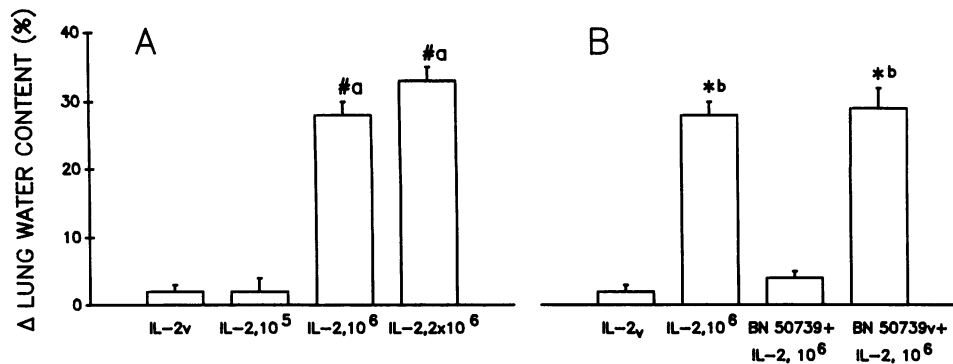


Figure 3. Lung water content response to IL-2 (A) and effect of pretreatment with BN 50739 on this response (B). IL-2, interleukin-2; IL-2v, IL-2 vehicle; BN 50739v, BN 50739 vehicle; * $P < 0.05$; # $P < 0.01$; a, vs. IL-2v and IL-2 10⁵ groups; b, vs. IL-2v and BN 50739 + IL-2 groups. IL-2 doses are units per animal.

Data analysis

Data in text and figures are mean \pm SEM for the indicated number of animals. One-way analysis of variance followed by Student-Newman-Keuls test was used for statistical analysis. $P < 0.05$ was considered significant.

Results

Effect of BN 50739 on IL-2-induced TXB₂ and TNF α responses. Basal serum TXB₂ and TNF α levels were below the sensitivity of the assays employed as previously reported (28, 35, 36). IL-2 dose-dependently increased serum TXB₂ ($P < 0.01$, Fig. 1 A), but failed to affect serum TNF α (data not shown). Pretreatment with BN 50739 completely prevented the IL-2-induced elevation of serum TXB₂ ($P < 0.01$, Fig. 1 B) and had no effect on serum TNF α .

Effect of BN 50739 on IL-2-induced lung weight response. The wet (485 \pm 4 mg), dry (92 \pm 4 mg), and wet-dry (pulmonary water content, 393 \pm 4 mg) lung weight did not differ among the control groups. IL-2 increased wet ($P < 0.05$, Fig. 2 A), dry, and wet-dry ($P < 0.01$, Fig. 3 A) lung weight in a dose-dependent manner. The wet-dry:dry ratio (4.25 \pm 0.01), however, remained unchanged. Pretreatment with BN 50739 prevented these responses ($P < 0.05$, Fig. 2 B and Fig. 3 B).

Effect of BN 50739 on IL-2-induced lung MPO response. Control animals had lung MPO activity of 12.7 \pm 1.7 U/g wet lung weight. IL-2 at 10⁵ U elevated MPO activity by 36 \pm 22%

but no statistical significance was reached. IL-2 at 10⁶ U elevated MPO activity by 84 \pm 23% ($P < 0.05$, Fig. 4). These responses were significantly attenuated ($P < 0.05$) by pretreatment with BN 50739 (Fig. 4).

Effect of BN 50739 on IL-2-induced hemodynamic and hematologic responses. Basal mean arterial pressure (107 \pm 9 mmHg), platelet (803 \pm 76 $\times 10^3/\mu\text{l}$), and leukocyte count (7.4 \pm 0.8 $\times 10^3/\mu\text{l}$), and hematocrit (40.2 \pm 1.7%) did not differ among groups. These variables were not significantly affected by IL-2 infusion or pretreatment with BN 50739 (data not shown).

Effect of BN 50739 on TXB₂ production by clotting blood. TXB₂ level in plasma of clotted blood was 884 \pm 183 pg/100 μl . BN 50739 at all doses tested did not affect this level, whereas indomethacin dose-dependently decreased TXB₂ concentration ($P < 0.01$, Fig. 5). TXB₂ level in plasma taken from unclotted blood was below the minimal detection level of the assay (5.0 pg/100 μl).

Discussion

The present data suggest for the first time that IL-2-induced lung injury is PAF-dependent. The mechanism(s) by which IL-2 provokes PAF to play a key role in the genesis of lung injury is still unknown. It is possible, however, that IL-2, a known activator of T lymphocytes (for review, see reference

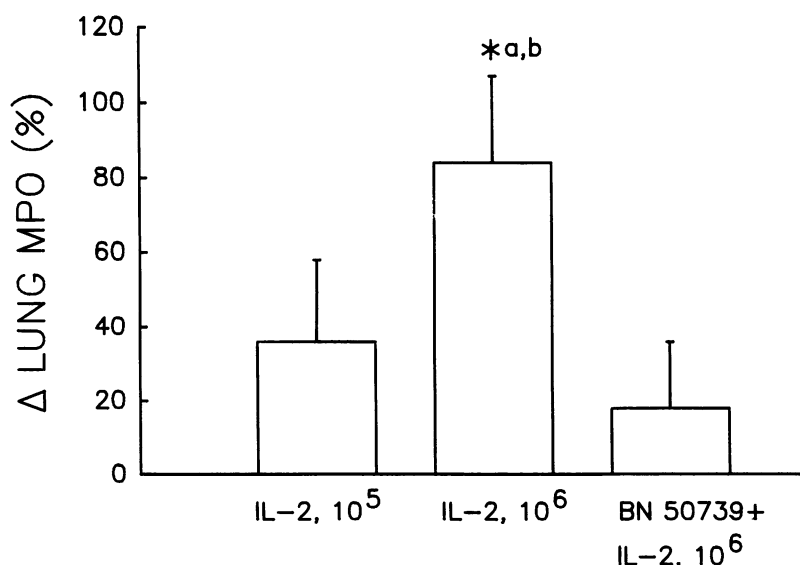


Figure 4. Effect of pretreatment with BN 50739 on lung MPO activity response to IL-2. Percent change is versus the respective vehicle control group. * $P < 0.05$; a, vs. respective vehicle control group; b, vs. BN 50739 + IL-2 10⁶ group. IL-2 doses are units per animal.

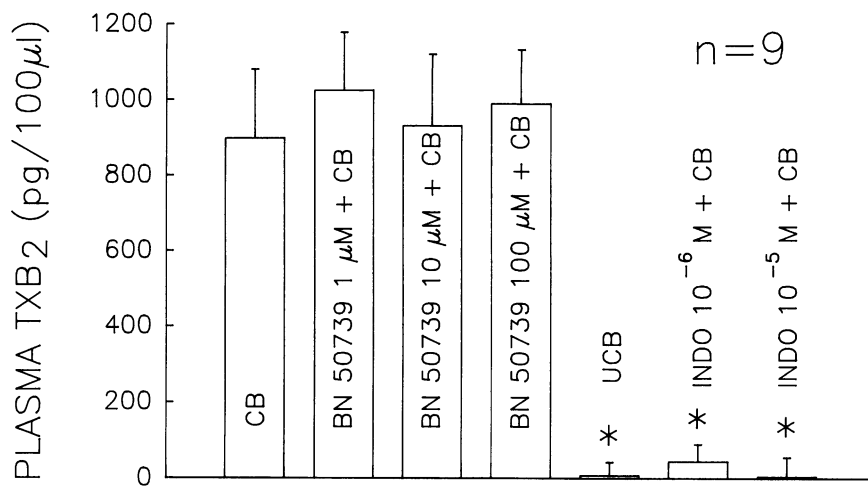


Figure 5. Effect of BN 50739 on TXB₂ production by clotting blood. CB, clotted blood; UCB, unclotted blood; indo, indomethacin; *, $P < 0.01$ vs. CB and all BN 50739 groups.

37), stimulates PAF release from these cells. While lymphocytes were considered by some investigators as incapable of producing PAF (38), recent studies reported that large granular cell lymphocytes, human leukemic cell lines of B and T origin, and lymphoblastoid cell lines can produce PAF-like substances in response to several stimuli (39, 40). Moreover, other reports demonstrated that lymphocytes are capable of producing lyso-PAF (an inactive intermediate substance in the metabolism of PAF) when stimulated with the Ca^{++} ionophore, A23187 (41); the failure of resting lymphocytes to produce PAF might be attributed to the lack of intracellular acetyltransferase, which catalyzes the production of PAF from lyso-PAF. However, the latter study also suggested that in response to proper stimuli circulating lymphocytes may also release PAF. IL-2 may be the appropriate stimulant for expression of genes which ultimately result in the synthesis of acetyltransferase, the vital enzyme required for PAF generation from lyso-PAF. An alternative mechanism by which IL-2 induces PAF activity might involve IL-1, which has been shown to be released following IL-2 infusion (15) as well as to stimulate PAF production in cultured human endothelial cells (42). However, these possible mechanisms should await further investigation.

Once produced in response to IL-2 stimulation, PAF can precipitate lung injury through several mechanisms. PAF might directly cause microvascular permeability defect (21–23), pulmonary edema (21–23), and leukosequestration (24). Alternatively, PAF may inflict tissue injury indirectly through triggering the release/production of other inflammatory mediators. For example, PAF might act through the release of thromboxane, a key prostaglandin mediator of inflammation (for review, see reference 43). This possibility is strongly supported by the inhibitory effect of BN 50739 on IL-2-induced elevation of serum TXB₂. However, it is important to note that the inhibitory effect of BN 50739 was not the consequence of direct inhibition of TXA₂ synthetase or the cyclooxygenase system; this conclusion is based on data showing no inhibition of arachidonate, ATP, or A23187-induced rabbit platelet aggregation in platelet rich plasma (Dr. P. Braquet, personal communication) and the lack of inhibition of TXA₂ production by clotting rat blood in vitro (Fig. 5). Also, BN 50739 was directly evaluated in our laboratory in various cellular systems and was found to be completely specific to PAF (44, 45); to our knowledge, no data contrary to this fact have ever been presented or published.

PAF might also trigger rat spleen mononuclear cells to release interleukin-1 (26), a highly potent inflammatory mediator (for review, see reference 46) reported to be released also by direct IL-2 stimulation (15). In that respect, TNF α , a highly potent mediator of tissue injury and inflammation (for review, see reference 47), known to be released by both IL-2 (17) and PAF (34), might also be involved in mediating IL-2-induced lung injury. However, our data do not support this possibility since no significant changes in serum TNF α were observed. PAF may induce tissue injury also by promoting neutrophil adherence and migration through vascular endothelium (48), a process which was recently suggested to mediate IL-2-induced multisystem organ edema (8, 18). Indeed, our data which show increased lung MPO activity in IL-2-treated animals support this concept.

Of special interest is the possibility that PAF produced in response to IL-2 stimulation amplified endogenous IL-2 production. This possibility draws credence from somewhat conflicting reports on the immunoregulatory role of PAF; on one hand, PAF has been shown to inhibit human lymphocyte proliferation and IL-2 production in vitro in response to phytohemagglutinin (49), while on the other hand, spleen mononuclear cells taken from rats exposed to prolonged PAF infusions in vivo demonstrated increased Con A-induced IL-2 production (26).

In summary, the present data suggest that IL-2 exerts its pulmonary toxic effect in part through PAF and point to the potential therapeutic capacity of PAF antagonist in preventing or attenuating this common and severe complication of IL-2 therapy.

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