Characterization of Vascular Leak Syndrome Induced by the Toxin Component of *Pseudomonas* Exotoxin-based Immunotoxins and Its Potential Inhibition with Nonsteroidal Anti-Inflammatory Drugs

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ABSTRACT

Clinical trials of immunotoxins in cancer patients have been limited in many cases by vascular leak syndrome (VLS). Recently, rats were identified as a model for VLS induced by BR96 sFv-PE40, a carcinoma-reactive single-chain immunotoxin. In this study, the toxin component of this immunotoxin, PE40, was found to be responsible for inducing hydrothorax in rats, thereby demonstrating that direct binding to the BR96 antigen was not essential to the onset of VLS. Mutational analysis of PE40 determined that both ADP ribosylation and proteolytic processing functions innate to *Pseudomonas* exotoxin A (PE) were necessary for PE40 to induce hydrothorax in rats; however, neither function by itself was sufficient for VLS induction. Additionally, nonsteroidal anti-inflammatory agents were found to block VLS in rats receiving BR96 sFv-PE40. These results demonstrate that the toxin component of PE-based immunotoxins induces VLS and suggest agents for clinical management of the toxicity.

INTRODUCTION

The clinical utility of immunotoxins has been hampered by hepatotoxicity, neurotoxicity, and perhaps the most limiting, VLS (1, 2). VLS is manifested following immunotoxin administration as a decrease in serum albumin, an increase in hematocrit, and an accumulation of fluid in the thoracic cavity. Dose escalation and hence effectiveness of immunotoxins, including those prepared with ricin A chain (3, 4), blocked ricin (5) and PE (6), has been limited by VLS, and, in certain cases, it has proven to be lethal (7).

The characterization of immunotoxin-induced VLS has been limited to observations in human trials due to the lack of an appropriate animal model system that closely corresponds to clinical manifestations. Recently, we reported that rats respond to i.v. administration of 2 mg/kg BR96 sFv-PE40, a single-chain immunotoxin that targets a Lewis Y- (Lea) associated carbohydrate antigen, with VLS-like characteristics including fluid in the thoracic cavity and a concomitant increase in hematocrit and decrease in albumin (8).

Although immunotoxins prepared with a variety of antibodies and toxins have been shown to induce VLS in humans, the toxicity has not yet been functionally ascribed to either the cell-binding moiety or the catalytic portion. Investigation of the onset of hydrothorax as well as the cascade of events leading to VLS could help identify inhibitors of this toxicity.

To address this question and to investigate the onset of hydrothorax as well as the cascade of events leading to VLS, we performed a variety of studies. First, the toxin portion of BR96 sFv-PE40 was tested for its ability to induce hydrothorax in rats. PE40 is a mutated form of PE in which domain I (encoding the native cell-binding activity) is genetically deleted, leaving both domain II (encoding proteolytic processing and translocation functions) and domain III (containing the catalytic activity that inhibits protein synthesis) intact (9). In this report, we show that PE40 was sufficient to induce hydrothorax in rats. Functional mutations of PE40 were also generated to investigate the role of catalysis and proteolytic processing of the toxin in the onset of VLS. Studies were also performed to characterize the time course of hydrothorax fluid accumulation in rats, both grossly and microscopically detailing local endothelial cell damage and the presence of specific inflammatory cells in the lungs.

Dexamethasone was previously reported to prevent BR96 sFv-PE40-mediated hydrothorax in rats (8). The testing of anti-inflammatory agents including NSAIDs was performed to identify specific VLS inhibitors and to investigate the cascade of events leading up to the induction of hydrothorax following immunotoxin administration. Last, we investigated the expression of specific proinflammatory cytokines in the lung microenvironment of rats treated with BR96 sFv-PE40. From these data, a model for the cascade of events involved in immunotoxin-induced VLS is proposed.

MATERIALS AND METHODS

Construction and Expression of Mutant PE40 Forms

Expression plasmid pMS 8 F+T (10), encoding LysPE40 (referred to as PE40 herein), was used to generate three mutant PE40 forms. Expression plasmid pSE18.0, containing the catalytic domain of PE (encoded by residues 400–613; Ref. 11),
was constructed by ligation of the 3743-bp fragment resulting from the digest of pMS8 F+T with AarII and NdeI and the oligonucleotide duplex (5'-CGCCGCGGTAAGCTTGGTACCCTGCAGCA-3' and 5'-TATGCTGCAGGGTACCAAGCTTATGGC-3').

Expression plasmid pSE276, in which a glycine was substituted for an arginine residue at amino acid 276, was constructed using the Transformer Site-directed Mutagenesis kit (Clontech, Palo Alto, CA) according to the manufacturer’s instructions using oligonucleotide 5'-AGACTTTCACCCTAGTCCAGGCGGCGACGT-3'.

Isolation of PE4O (pMS8), PE domain III (residues 400–613; pSE18.0), PE4O R276G (pSE276), and PE4O deletion (TSK3000; TosOHas, Philadelphia, PA) was performed as described using wheat germ extract (12).

Isolation of PE4O (pMS8), PE domain III (residues 400–613; pSE18.0), PE4O R276G (pSE276), and PE4O deletion (TSK3000; TosOHas, Philadelphia, PA) was performed as described using wheat germ extract (12).

Toxicity Studies. Six- to 8-week-old female Wistar Furth and Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were given i.v. injections of 2 mg/kg BR96 sFv-PE4O (13), PE4O (1–6 mg/kg), or mutant forms of PE4O (6–12 mg/kg). After 24 h, rats were euthanized by exposure to CO2, and hydrothorax fluid was collected and measured as described (8) using a 5-ml syringe and 21-gauge needle. For certain experiments, 50 mg/kg BR96 IgG (14) were also administered.

Histology and Microscopy of Rat Lungs following Immunotoxin Administration. Rat trachea and lungs were removed 24 h after administration and gently inflated with approximately 2 ml of 10% neutral-buffered formalin delivered via the trachea. Fixed tissues were paraffin embedded, sectioned at 6 μm, and stained with H&E for histological evaluation. For electron microscopic studies, lungs were isolated by osmotic shock with ice-cold H2O, and the protein was isolated by Q-Sepharose (Pharmacia, Uppsala, Sweden) centrifugation with a yield of 0.5–1.0 mg RNA/g lung tissue. Total RNA samples (10–15 μg/ lane) were applied to Hybond-C nylon membranes (Amersham Corp., Arlington Heights, IL).

RESULTS

Hydrothorax Is Induced by Unconjugated PE40. The contribution of the separate components of the BR96 immunotoxin to the toxicity observed in rats was directly evaluated using either the BR96 mAb or recombinant PE40 protein. Rats pretreated with a single dose of 340 Immunotoxin-induced Vascular Leak Syndrome a variety of anti-inflammatory agents 2 h before administration of 2 mg/kg BR96 sFv-PE4O (unless otherwise indicated). Agents tested as inhibitors of immunotoxin-induced hydrothorax were three NSAIDs, indomethacin (15–30 mg/kg s.c.), piroxicam (20 mg/kg i.p. or p.o.), and flurbiprofen (30 mg/kg; i.p.); a phospholipase A2 inhibitor, BMS 181162 (150 mg/kg i.p.); a histamine H2 receptor blocker, cimetidine (1 mg/kg i.v.); and a mast cell stabilizing agent, cromolyn (0.1 mg/kg i.v.). Rats were sacrificed 24 h after immunotoxin administration, and the presence or absence of thoracic fluid was observed as described (8). Induction of mRNA Expression in VLS-damaged Rat Lungs. Induction of mRNA expression for IL-1α, COX-1, COX-2, and GAPDH (used as positive controls for mRNA expression) in normal rat lungs and immunotoxin-induced VLS-damaged lungs was determined with Northern blot analysis. Whole lungs were removed from sacrificed rats at various time points following i.v. administration of 2 mg/kg BR96 sFv-PE4O, rinsed in cold PBS, and weighed, and total RNA was isolated using guanidinium isothiocyanate extraction and cesium chloride centrifugation with a yield of 0.5–1.0 mg RNA/g lung tissue. Total RNA samples (10–15 μg/ lane) were applied to 2.2% formaldehyde containing agarose gels (1%), electrophoresed for 4 h at 50–60 V, and transferred to Hybond-C nylon membranes (Amersham Corp., Arlington Heights, IL).
Catalytic Activity and Proteolytic Processing Are Essential for PE40 Induction of Hydrothorax. The role of catalytic activity and proteolytic processing of the toxin in the onset of VLS was investigated using various PE40 mutant forms. To determine whether the catalytic activity retained by PE40 was essential for induction of VLS, PE40 deletion 553, previously shown to result in the elimination of ADP ribosylation activity (16), was administered to rats at 12 mg/kg. There was no fluid accumulation in the thoracic cavity of rats 24 h after i.v. administration (Table 2). To test whether the enzymatic domain itself was able to induce VLS, PE domain III (residues 400–613), which represents single residue altered in domain II that resulted in the inability to induce VLS, PE domain III (residues 400–613), which represents single residue altered in domain II that resulted in the inability to induce VLS, PE domain III (residues 400–613), which represents single residue altered in domain II that resulted in the inability to induce VLS, PE domain III (residues 400–613), which represents single residue altered in domain II that resulted in the inability to induce VLS, PE domain III (residues 400–613), which represents single residue altered in domain II that resulted in the inability to induce VLS, PE domain III (residues 400–613), which represents single residue altered in domain II that resulted in the inability to induce VLS, PE domain III (residues 400–613), which represents single residue altered in domain II that resulted in the inability to induce VLS, PE domain III (residues 400–613), which represents single residue altered in domain II that resulted in the inability to induce VLS, PE domain III (residues 400–613), which is equivalent to 1250 cpm of [3H]NAD transferred to elongation factor 2. Thoracic fluid was measured via collection from the thoracic cavity. ADP ribosylation activity of PE40 is defined as 100% and is equivalent to 1250 cpm of [3H]NAD transferred to elongation factor 2 (in wheat germ extract; Ref. 12) per 5 ng of PE40.

Time Course for VLS Onset. A time-course study was performed to determine when the onset of thoracic fluid was first apparent. Following administration of 2 mg/kg BR96 sFv-PE40, rats were sacrificed and thoracic fluid was measured. No fluid was present in the thoracic cavities of treated rats through 12 h postadministration of immunotoxin (Table 3). At 16 h, there was a small amount of fluid (<0.8 ml), which increased in volume at 20 h postadministration to as much as 2.5 ml. By 24 h, there was an average of >4.0 ml of thoracic fluid (Table 3), which did not appreciably increase between then and death at approximately 72 h after treatment.

Microscopic evaluation of lungs removed at periodic intervals from the rats following treatment with BR96 sFv-PE40 showed the onset of tissue injury to precede the appearance of hydrothorax. In comparison to normal rat lung tissue (Fig. 1A), by 2 h postadministration, small amounts of fluid in scattered perivascular lymphatics was apparent (Fig. 1B). Between 4 and 8 h, the amount of edema fluid in perivascular lymphatics had increased and surrounded more vessels (Fig. 1C and D). In addition, scant numbers of mixed inflammatory cells were present in some perivascular spaces (Fig. 1D). By 12 h, perivascular edema fluid accumulation was marked (Fig. 1, E and F) and accompanied by alveolar wall thickening and accumulation of macrophages and occasionally fibrin debris within alveolar spaces. Neither thrombosis nor pulmonary parenchymal hemorrhage was observed.

Ultrastructural evaluation of rat lungs collected 20 h after administration of BR96 sFv-PE40 demonstrated distinct morphological differences from the control (Fig. 2). Most significant was the presence of numerous vacuoles or blebs which likely contained fluid along with variable quantities of fibrillar or membranous debris. These vacuoles were present within the cytoplasm of endothelial cells, intercalated between endothelial cells, and within the basall lamina and interstitium. Additional thickening of the alveolar walls was due to focal accumulation of inflammatory cells (primarily neutrophils and monocytes), erythrocytes, and platelets within capillaries. There was no evidence of cellular necrosis, although the presence of debris in some vacuoles is consistent with some cell degeneration or death.

Inhibition of BR96 sFv-PE40-induced VLS in Rats with NSAIDs. Dexamethasone, previously shown to prevent VLS in rats given BR96 sFv-PE40 (8), has many properties including being a potent anti-inflammatory agent. The metabolites of the arachidonic acid pathway, including leukotrienes and prostaglandins, affect many inflammatory processes. Since steroids can ...

Table 1

<table>
<thead>
<tr>
<th>Protein</th>
<th>No. of rats</th>
<th>Dose (mg/kg)</th>
<th>Mean thoracic fluid (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE40</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>BR96 sFv-PE40</td>
<td>12</td>
<td>2-4</td>
<td>2.5 (1.0-4.0)*</td>
</tr>
<tr>
<td>BR96 IgG</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>BR96 IgG +</td>
<td>13</td>
<td>2-4</td>
<td>2.9 (0.8-5.0)</td>
</tr>
<tr>
<td>BR96 sFv-PE40</td>
<td>2</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>

* Protein was administered via the tail vein. Rats were euthanized upon exposure to CO2 after 24 h. Thoracic fluid was measured via collection from the thoracic cavity. Numbers in parentheses, range.

Table 2

<table>
<thead>
<tr>
<th>Protein</th>
<th>Dose (mg/kg)</th>
<th>ADP ribosylation activity (%)</th>
<th>Mean thoracic fluid (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE40</td>
<td>4-6</td>
<td>100</td>
<td>3.4 (2.2-4.6)*</td>
</tr>
<tr>
<td>PE40 deletion 553</td>
<td>12</td>
<td>&lt;1</td>
<td>0</td>
</tr>
<tr>
<td>PE domain III</td>
<td>12</td>
<td>&gt;90</td>
<td>0</td>
</tr>
<tr>
<td>(residues 400-613)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE40 R276G</td>
<td>12</td>
<td>&gt;90</td>
<td>0</td>
</tr>
</tbody>
</table>

* Protein was administered via the tail vein into groups of three to six rats. ADP ribosylation activity of PE40 is defined as 100% and is equivalent to 1250 cpm of [14C]NAD transferred to elongation factor 2 (in wheat germ extract; Ref. 12) per 5 ng of PE40.

Table 3

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>No. of rats</th>
<th>Mean thoracic fluid (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-12</td>
<td>2b</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>0.7 (0.6-0.8)*</td>
</tr>
<tr>
<td>20</td>
<td>4</td>
<td>1.9 (0.5-3.3)</td>
</tr>
<tr>
<td>24</td>
<td>6</td>
<td>4.4 (3.4-5.4)</td>
</tr>
</tbody>
</table>

* BR96 sFv-PE40 was i.v. administered via the tail vein at 2 mg/kg. At various time points thereafter, groups of rats were euthanized with CO2 prior to measurement of thoracic fluid. Numbers in parentheses, range.
block the release of arachidonic acid as well as enzymes involved in generating inflammatory-associated metabolites, specific inhibitors of the arachidonic acid pathway were tested for their ability to block BR96 sFv-PE40-induced hydrothorax. Prophylactic administration of the NSAIDs indomethacin, piroxicam, and flurbiprofen were able to inhibit VLS in rats given 2 mg/kg BR96 sFv-PE40 (Table 4). Prophylactic administration of a phospholipase A₂ inhibitor (BMS 181162; Ref. 20), which blocks the release of arachidonic acid, was also effective at inhibiting BR96 sFv-PE40-induced VLS in rats. Two additional anti-inflammatory agents, cimetidine (histamine receptor H₂ blocker) and cromolyn (mast cell stabilizer), were unable to inhibit BR96 sFv-PE40-induced VLS. These data indicate that metabolites of arachidonic acid are likely to be involved in BR96 sFv-PE40-induced VLS.

**Northern Blot Analysis of Damaged versus Normal Rat Lungs following High-Dose BR96 sFv-PE40 Administration.** NSAIDs have been shown to block the generation of prostaglandins by inhibiting the enzymatic activity of COX-1 and COX-2 (21). Expression of COX-2 has been shown to be

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**Fig. 1** Time-course analysis of BR96 sFv-PE40-damaged rat lungs. Histological sections were taken from rats that were either untreated (A) or treated with 2 mg/kg BR96 sFv-PE40 at 2 (B), 4 (C), 8 (D), or 12 (E and F) h before sacrifice. With time, there is increasing severity of edema fluid accumulation in the perivascular spaces. Arrows indicate the widened, fluid-filled perivascular space (E) and the fluid-distended lymphatics within the swollen perivascular space (F). A–D, ×100; E, ×25; and F, ×50.
Ultrastructural appearance of alveolar wall segments from untreated (A) and BR96 sFv-PE4O-treated (B) rats 20 h after administration. Alveolar walls from a BR96 sFv-PE4O-treated rat contain numerous vacuoles in intravascular, perivascular, and interstitial locations. Vacuoles likely contained fluid prior to processing. Damaged alveoli contained neutrophilic and mononuclear inflammatory cells, and alveolar capillaries were dilated. Cap/rbc, capillary containing a RBC; EC, endothelial cells; Vac., vacuole; Mono. Phag., mononuclear phagocyte within a capillary; Neut., neutrophils within capillaries; Fib., fibroblast; Type 2 Ep., type 2 epithelial cell; Type 1 Ep., type 1 epithelial cell.

Table 4: Prevention of immunotoxin-induced VLS in rats by administration of anti-inflammatory agents

<table>
<thead>
<tr>
<th>Agent</th>
<th>Function</th>
<th>Thoracic fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>COX-1/COX-2 inhibitor</td>
<td>−</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>COX-1/COX-2 inhibitor</td>
<td>−</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td>COX-1/COX-2 inhibitor</td>
<td>−</td>
</tr>
<tr>
<td>BMS 181162</td>
<td>Phospholipase A2 inhibitor</td>
<td>−</td>
</tr>
<tr>
<td>Cromolyn</td>
<td>Mast cell stabilizer</td>
<td>+</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>Histamine H2 blocker</td>
<td>+</td>
</tr>
</tbody>
</table>

*Agents (indomethacin, 15–30 mg/kg; piroxicam, 20 mg/kg; flurbiprofen, 30 mg/kg; BMS 181162, 100 mg/kg; cromolyn, 0.1 mg/kg; and cimetidine, 1 mg/kg) were administered 2 h before BR96 sFv-PE4O (2 mg/kg) in groups of three to six rats. The presence (1.0–5.0 ml) or absence (none detectable) of thoracic fluid was determined 24 h after immunotoxin administration.

Induced by IL-1α (22). To determine whether BR96 sFv-PE40 affected the expression level of IL-1α, COX-1, and COX-2, Northern blot analysis was performed on lung RNA isolated from rats treated with 2 mg/kg BR96 sFv-PE40 and nontreated rats at 2, 4, 8, 12, and 24 h after immunotoxin administration. Blots were probed with radiolabeled cDNAs specific for rat IL-1α, rat COX-1, rat COX-2, and human GAPDH (which cross-reacts with rat GAPDH) used as positive controls for RNA abundance (Fig. 3). The mRNA level for IL-1α increased by 5-fold within 2 h after immunotoxin was administered, reaching a maximum at 4 h (14.5-fold). The mRNA level then slowly decreased over time, although expression was still apparent at the 24-h time period. COX-2 mRNA expression was induced by the administration of BR96 sFv-PE40 starting at 12 h (17.4-fold) and increased further (26.3-fold) at the 24-h time point. The level of both COX-1 and GAPDH expression was relatively unaffected (within 2.5-fold and 1.6-fold of control, respectively) by the immunotoxin, indicative of a constitutive level of expression as has been observed previously (23). Thus, BR96 sFv-PE40 induces the expression of IL-1α and COX-2 in the local lung microenvironment.

**DISCUSSION**

VLS represents the dose-limiting toxicity of many biologics used in the clinic, including immunotoxins (4, 7) and IL-2 (24, 25). The cascade of events that are responsible for inducing VLS are largely unknown. We recently reported the identification of rats as an appropriate model system to study VLS induced by BR96 sFv-PE40, a single-chain immunotoxin targeted to a Leα-associated receptor present in high numbers on a variety of human carcinoma types (8). In this report, we have found that administration of PE40 by itself resulted in hydrothorax at doses comparable to those observed for BR96 sFv-PE40 (Table 1). Large doses of the BR96 antibody (50 mg/kg) were not associated with any VLS-like symptoms in rats, nor could the mAb prevent BR96 sFv-PE40-induced hydrothorax. These data indicate that the toxin component of BR96 sFv-PE40 is responsible for the generation of hydrothorax and VLS in rats. The administration of the catalytic domain (domain III of PE) was unable to induce VLS, and, conversely, administration of a catalytically inactive form of PE40 (deletion 553) did not induce VLS, although it had an intact domain II (Table 2). These data show that although the catalytic domain is required for inducing VLS, it alone does not cause the toxicity. Mutation of a single amino acid residue at position 276 in domain II (PE40 R276G), resulting in a molecule that is not processed (19), also does not induce VLS. Based on these data, it is unlikely that an active form of PE40 that does not induce VLS will be identified.
Immunotoxin-induced Vascular Leak Syndrome

with BR96 sFv-PE40. Probes (cDNAs encoding IL-1α, COX-1, COX-2, and GAPDH) were \[^{32}P\]-labeled and hybridized to total RNA isolated from lungs of rats either left untreated (0) or treated with 2 mg/kg BR96 sFv-PE40 at 2, 4, 8, 12, and 24 h after immunotoxin administration. The molecular sizes of the respective mRNAs are noted. Densitometry was done using ImageQuant software (Molecular Dynamics). Each blot was integrated (minus background). The values below each lane represent the fold increase in mRNA level relative to the untreated control for done using ImageQuant software (Molecular Dynamics). Each blot was integrated (minus background). The values below each lane represent the fold increase in mRNA level relative to the untreated control for each probe.

Fig. 3 Northern blot analysis of lung RNA from rats that were treated with BR96 sFv-PE40. Probes (cDNAs encoding IL-1α, COX-1, COX-2, and GAPDH) were \[^{32}P\]-labeled and hybridized to total RNA isolated from lungs of rats either left untreated (0) or treated with 2 mg/kg BR96 sFv-PE40 at 2, 4, 8, 12, and 24 h after immunotoxin administration. The molecular sizes of the respective mRNAs are noted. Densitometry was done using ImageQuant software (Molecular Dynamics). Each blot was integrated (minus background). The values below each lane represent the fold increase in mRNA level relative to the untreated control for each probe.

![Northern blot analysis of lung RNA from rats that were treated with BR96 sFv-PE40. Probes (cDNAs encoding IL-1α, COX-1, COX-2, and GAPDH) were \[^{32}P\]-labeled and hybridized to total RNA isolated from lungs of rats either left untreated (0) or treated with 2 mg/kg BR96 sFv-PE40 at 2, 4, 8, 12, and 24 h after immunotoxin administration. The molecular sizes of the respective mRNAs are noted. Densitometry was done using ImageQuant software (Molecular Dynamics). Each blot was integrated (minus background). The values below each lane represent the fold increase in mRNA level relative to the untreated control for each probe.](image)

The current observation that PE40 was capable of inducing VLS without an attached antibody-binding specificity (Tables 1 and 2) and prior studies with the ricin A chain (26) emphasize that nonreceptor-mediated modes of interaction with PE or ricin and cells can induce VLS. Additionally, specific antibody-mediated uptake has been implicated in VLS (27), although, since B3 (Fv)-PE38 and not PE38 by itself is shown to induce endothelial cell cytotoxicity, it questions whether the results of in vitro cytotoxicity of endothelial cells caused by B3 (Fv)-PE38 is a meaningful indicator of VLS.

IL-1α has been shown to induce the expression of COX-2 (22), which catalyzes the generation of prostaglandins and has been implicated in a variety of inflammatory conditions (23). The temporal nature of IL-1α and COX-2 expression, which are evident at 2 and 12 h (Fig. 4), respectively, in the lungs of rats following administration of 2 mg/kg BR96 sFv-PE40, also suggests such a relationship and is concurrent with the microscopic appearance of fluid-filled vacuoles (Fig. 2) and the gross accumulation of thoracic fluid (Table 3).

Based on the inhibition of immunotoxin-induced hydrothorax in rats by NSAIDs, it is likely that prostaglandins play a role in the pathogenesis of VLS. Furthermore, dexamethasone, which can inhibit BR96 sFv-PE40-induced VLS if administered prophylactically (8), inhibits cytokine induction of COX-2 (28). Thus, to prevent the onset of hydrothorax, selective inhibitors of COX-2 may be preferred to NSAIDs. The latter inhibit both COX-1 and COX-2 and have gastrointestinal toxicity due to inhibition of physiologically important prostaglandins induced by COX-1 (23).

Based on the data reported here, we propose a model for BR96 sFv-PE40-induced VLS. High concentrations of BR96 sFv-PE40 are achieved in the lung microenvironment because this is the first capillary bed reached for an i.v. administered drug. The PE40 component of the immunotoxin induces vascular permeability through effects on endothelial cells, either indirectly or directly. These effects are likely to include those induced by the expression of proinflammatory mediators such as IL-1α and COX-2.

Future investigations will focus on identifying the specific cell type(s) in the lungs that is affected by PE40, which may help to clarify the basis for organ specificity of VLS damage. Alveolar macrophages, which are scattered throughout the lung and can express IL-1α, COX-2, and a variety of other proinflammatory cytokines, will certainly be among the cell types investigated. Finally, it will be important to determine whether VLS induced by PE40-based immunotoxins is similar to that seen with other immunotoxins, i.e., ricin based (4, 7), as well as that associated with systemic administration of IL-1 (29) and IL-2 (30, 31).

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