Epinephrine promotes pulmonary angiitis: evidence for a \( \beta_1 \)-adrenoreceptor-mediated mechanism

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Jain, Felipe A., Long-hai Zhao, Martin K. Selig, and Richard L. Kradin. Epinephrine promotes pulmonary angiitis: evidence for a \( \beta_1 \)-adrenoreceptor-mediated mechanism. Am J Physiol Lung Cell Mol Physiol 285: L232–L239, 2003; 10.1152/ajplung.00248.2002.—Epinephrine (Epi) increases lymphocyte traffic to lung. We investigated whether Epi also modulates pulmonary cell-mediated immune responses in vivo. C57BL/6 mice were immunized with hen-egg lysozyme (HEL) on day 0, challenged with HEL intratracheally at day 12, and killed at day 15. Mice received Epi (0.5 mg/kg) subcutaneously during the sensitization phase, \( \beta_1 \)-adrenoreceptor antagonist, inhibited airway inflammation and the reduction in CD40-AR blockers inhibited airway inflammation. We conclude that Epi-EP selectively promotes vascular inflammation in vivo via a \( \beta_1 \)-receptor-mediated mechanism.

L-selectin expression (20) and LPS-mediated upregulation of TNF-\( \alpha \) receptors by blood monocytes (11). In rats, Epi administered daily before antigen sensitization and through subsequent antigen challenge inhibits cutaneous tuberculin reactions (12).

In the present study, we examined the effects of Epi on the pulmonary cell-mediated immune response to hen-egg lysozyme (HEL). HEL is a 14-kDa protein with five distinct T-cell epitopes. H-2b strains of mice show strong cell and antibody immune responses to HEL, whereas H-2b strains are weak responders (13). In a previous study, we demonstrated that lymph node cells from C57BL/6 (H-2b) mice immunized with HEL and injected daily with Epi in vivo show increased proliferative responses to HEL and increased IL-2 secretion in vitro compared with controls (21).

Cell-mediated pulmonary immune responses include afferent and efferent phases (28). During the afferent phase, antigen is taken up by antigen-presenting cells and transported from peripheral tissues to regional lymph nodes, where it is presented to naive T lymphocytes. Upon subsequent intratracheal antigen challenge, sensitized immune cells accumulate around the pulmonary microvasculature and to a lesser extent around small pulmonary airways. In the present study, we examined whether Epi might promote the pulmonary cell-mediated immune response by C57BL/6 mice to HEL in vivo and yield differential responses when administered during either the sensitization or effector phases of the immune response.

MATERIALS AND METHODS

Animals. Pathogen-free female C57BL/6 mice (6–8 wk old) were purchased from Charles River Laboratories (Wilmington, MA). Mice were housed four per cage in the Massachusetts General Hospital (MGH) Animal Care Facility and allowed access to food and water ad libitum. All studies were conducted within the guidelines of the MGH for the care and use of laboratory animals.

Complete media and culture conditions. Cells were cultured in RPMI 1640 with l-glutamine (Mediatech, Herndon, VA), 10% heat-inactivated fetal bovine serum (Sigma, St. Louis, MO), 50 \( \mu \)g/ml gentamicin (GIBCO-BRL, Gaithersburg, MD), 0.5% 1 M HEPES buffer (GIBCO-BRL), and 2-mercaptoethanol (5 \( \times \) \( 10^{-5} \) M; Sigma), and incubated at 37°C in a humidified chamber of 95% air and 5% CO2.
HEL immunization and challenge. Mice were immunized subcutaneously at both sides of the tail on day 0 with HEL (200 μg; Sigma RBI) in complete Freund’s adjuvant. On day 12, they were anesthetized with 4% chloral hydrate (400 mg/kg ip; Fisher Scientific, Pittsburgh, PA). The trachea was surgically exposed, and 200 μg of HEL in 100 μl of sterile normal 0.9% saline (Sal) were injected intratracheally. On day 15, mice were anesthetized with chloral hydrate and killed by cardiac puncture.

Epi and AR blocker administration. Mice were injected with Epi (0.5 mg/kg; Abbot Laboratories; North Chicago, IL) or Sal subcutaneously on days 1–7 (sensitization phase (SP)) or days 12–14 (effector phase (EP)). In some experiments, mice were injected intraperitoneally 30 min before Epi with α/β-AR blockers including propranolol (10 mg/kg; Ben Venue Labs, Bedford, OH), phenotamine (10 mg/kg; Ben Venue Labs), atenolol (10 mg/kg; Sigma), or ICI-118551 (10 mg/kg; Sigma). Controls received Sal injections intraperitoneally 30 min before Epi.

Bronchoalveolar lavage. Bronchoalveolar lavage (BAL) was conducted as previously reported (28). Briefly, mice were anesthetized with chloral hydrate, the abdomen was surgically opened, and an incision was made in the diaphragm. The trachea was cannulated with polyethylene tubing and the lungs were lavaged with six to eight 1.0-ml aliquots of normal Sal containing 0.6 mM EDTA. The retrieved BAL cells were washed once with PBS, pH 7.3; hypotonic saline (0.2%) was added to the pellet to lyse red blood cells. Viable cells were enumerated in a hemocytometer by trypan blue exclusion, and cells were washed in PBS, pH 7.3, and resuspended in PBS with 0.5% BSA (Sigma) for immunostaining.

Immunostaining and cytometry of BAL leukocytes. BAL cells were incubated with Fc block (Pharmingen, San Diego, CA) for 10 min and stained in suspension for 30 min at 4°C with conjugated anti-mouse monoclonal antibodies. The fluorochrome-conjugated antibodies (all Pharmingen) included FITC- or phycoerythrin (PE)-conjugated anti-CD3, anti-CD4, anti-CD8, anti-CD19, anti-CD25, anti-NK1.1, anti-class II major histocompatibility complex (Ia), and anti-CD11b. Anti-IgG2a-FITC and anti-IgG1-PE were used as fluorescence controls. Cells were washed in PBS and fixed in PBS with 1% paraformaldehyde (Fisher Biotech, Fairlawn, MO). We used dual color for analysis of the cell surface membrane phenotype in a FACSscan cytometer (Becton-Dickinson, Burlingame, CA) after electrostatically gating on the lymphocyte, monocyte, or granulocyte populations, as judged by forward-angle (0°) and side-angle (90°) light scatter characteristics.

Pulmonary cytokine gene expression. Cytokine and chemokine gene expression was determined by RNase protection assay (RPA). Total cellular RNA was extracted from lung digests with TRIzol (GIBCO Life Technologies, Gaithersburg, MD). RNA was assayed by absorbance at optical density at 260 nM (OD260), and RNA integrity was assessed by the OD260/280 ratio and by direct examination of 28S and 18S bands in 1% agarose gels. RPA was conducted with the RiboQuant Multiprobe RPA system kit (Pharmingen) according to the instruction manual. In brief, a [32P]-labeled anti-sense probe transcribed from a multitemplate cDNA plasmid insert using T7 RNA polymerase was hybridized in excess with target RNA. The protected probe/RNA hybrids were treated with RNase to remove any remaining single-stranded probe and RNA, purified by standard chloroform/phenol extraction and ethanol precipitation techniques, and resolved on 5% denaturing polyacrylamide gels for imaging and quantification by autoradiography and phosphorimaging (Molecular Imager system; Bio-Rad Laboratories, Hercules, CA). GAPDH served as an internal standard for each sample.

Histochemistry. Lungs were harvested, fixed in 10% buffered formalin, sectioned at 5 μm, and stained with hematoxylin and eosin. Airways and vessels were scored for intensity of inflammation in five randomly selected high-power fields under a light microscope. The scoring system was as follows: vessels: 0 = no inflammation, 1 = incomplete leukocyte perivascular cuff, 2 = complete leukocyte perivascular cuff up to two cell layers thick, 3 = complete perivascular leukocyte cuff greater than two cell layers thick; airways: 0 = no inflammation, 1 = peribronchial leukocyte cuff less than two cell layers thick, 2 = peribronchial leukocyte cuff 2–4 cell layers thick, 3 = peribronchial leukocyte cuff greater than four cell layers thick.

Immunohistochemistry. Lungs were rapidly frozen in cryo-embedding medium, sectioned at 5 μm in a cryostat and stained with rat anti-mouse monoclonal antibodies (anti-CD3, anti-CD11b, anti-NK1.1, anti-GR-1, anti-CD31, anti-intracellular adhesion molecule (ICAM)-1; all Pharmingen) by an avidin-biotin immunoperoxidase technique, as previously described (28). Positive cells were scored with a Zeiss light microscope in a minimum of five random high-power (×25 objective) fields.

Electron microscopy. For ultrastructural analysis, lung tissue was finely sectioned and fixed for 3 h at room temperature in modified Karnovsky’s solution (2.5% glutaraldehyde, 2.0% formaldehyde, and 0.0025% CaCl in 0.1 M sodium cacodylate buffer, pH 7.4), transferred to cacodylate buffer, and stored at 4°C until ready for processing. Subsequently, tissue was processed in a Leica Lynx EM tissue processor, postfixed in osmium tetroxide, stained en bloc with uranyl acetate, dehydrated in graded ethanol solutions, infiltrated with propylene oxide/epon, and embedded in epon. Tissue was sectioned at 1 μm, stained with toluidine blue, and examined by light microscopy. Representative areas were chosen, and thin sections were cut with a diamond knife in an LKB Ultrotome III ultramicrotome. Sections were stained with Sato’s lead citrate and examined in a Philips 301 electron microscope.

Cortisone assay. Blood was collected at the time of death by cardiac puncture. Plasma was retrieved and frozen at −80°C until time of assay. Corticosterone was assayed by competitive RIA kit (ICN Pharmaceuticals, Costa Mesa, CA).

Statistical analysis. Data were analyzed by paired or unpaired t-statistics or ANOVA using Statview 4.5 software.

RESULTS

Epi-SP mice show increased vascular and airway inflammation. In previous studies (28), the lungs of HEL-immune mice were demonstrated to develop perivascular lymphoid cuffs around postcapillary venules with minimal airway inflammation following intratracheal challenge with HEL. In the present study, C57BL/6 mice that received Epi during the sensitization phase at days 1–7 (Epi-SP) exhibited increased pulmonary microvascular inflammation (P < 0.05) compared with saline controls (Fig. 1). The pulmonary microvascular inflammation was characterized by immune cell infiltration of the walls of postcapillary venules, with endothelial cell detachment from the basement membrane and subendothelial fibrin deposition (Fig. 2).

To ascertain the specificity of the response to EPI, mice were treated concomitantly with α/β-AR blockers

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Vascular inflammation in situ was reversed by atenolol (P < 0.05), a selective β1-AR antagonist, but not by ICI-118551, a selective β2-adrenoreceptor antagonist, propranolol, or phentolamine (Fig. 3). Epi-SP mice showed increased immune cell cuffing of airways (Fig. 4). Airway inflammation was reversed nonselectively by both α/β-AR blockade (P < 0.05).

When Epi was administered during the effector phase (Epi-EP) at days 12–14, airway inflammation was increased (P < 0.001), but there was no increase in vascular inflammation compared with controls (Fig. 5). These findings suggest that distinct AR-mediated mechanisms regulate the inflammation in pulmonary vessels and airways.

Increased vascular inflammation in Epi-SP mice is characterized by the accumulation of GR-1⁺ granulocytes and CD11b⁺ monocytes. Immunostaining of lung sections revealed no significant increases in CD3⁺ lymphocytes, NK1.1⁺ lymphocytes, or CD31⁺ platelets in the inflamed vessels of Epi-SP mice compared with controls (not shown). GR-1 granulocytes (31 ± 6 vs. 20 ± 5, P = 0.0001) and CD11b⁺ macrophages (76 ± 11 vs. 45 ± 10, P < 0.0001) were both increased in perivascular cuffs compared with controls (Fig. 6).

**Epi induces changes in lymphocyte subsets in the BAL fluid.** Neither Epi-SP- nor Epi-EP-treated mice showed changes in the total number of leukocytes in BAL fluid.

### Table 1. Adrenoreceptor blockers

<table>
<thead>
<tr>
<th>Blocker</th>
<th>Mode of Action</th>
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<tbody>
<tr>
<td>Aten</td>
<td>β1-blockade</td>
</tr>
<tr>
<td>ICI</td>
<td>β2-blockade</td>
</tr>
<tr>
<td>Pro</td>
<td>Nonselective β-blockade</td>
</tr>
<tr>
<td>Phen</td>
<td>Nonselective α-blockade</td>
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Aten, atenolol; ICI, ICI-118551; Pro, propranolol; Phen, phentolamine.
bronchoalveolar lavage fluid (BALF), and there were no differences in the percentages of total lymphocytes, macrophages, or granulocytes compared with controls (data not shown).

cytofluorimetric analysis demonstrated a modest increase in the percentage of CD3+ lymphocytes (Table 2) in Epi-SP mice, and this change was reversed by propranolol. Epi-SP decreased the percentage of NK1.1+ cells by ~50% (P < 0.01). This finding was reversed by both phentolamine and propranolol (P < 0.05, P < 0.01) but not by selective β-AR blockade, suggesting a requirement for the cooperation of both β1- and β2-AR in this response. Epi-SP yielded no change in either the percentages or absolute numbers of CD4+, CD8+, or CD19+ lymphocytes in the BALF (not shown).

Epi-SP reduced both the percentage and total number of CD4+CD25+ lymphocytes in BALF by ~50% (P < 0.05) (Fig. 7). This finding was selectively reversed by the β1-AR blocker atenolol (P < 0.05) but not by ICI-118551, phentolamine, or propranolol.

Epi-SP increased the percentage of CD11b+ monocytes in BALF from 8.4 ± 5.7 to 15.7 ± 9.8 (P < 0.05) paralleling their increased representation in perivascular cuffs within the lung. However, this finding was not reversed by either β-AR blockade. No change was observed in the representation of GR-1+ granulocytes in the BALF compared with controls.

In contrast to the Epi-SP mice, the Epi-EP mice showed decreased percentages of CD3+ lymphocytes (73 ± 3 vs. 67 ± 1) and increased NK1.1+ cells (13 ± 3 vs. 18 ± 2) in the BALF (both P < 0.05).

Epi-SP has no effect on expression of pulmonary chemokine genes. As chemokines expressed in lung tissue play a role in directed leukocyte traffic, lung homogenates were assayed for expression of lymphotactin; regulated on activation, normal T cell expressed, and presumably secreted; eotaxin; macro-
phage inflammatory protein (MIP)-1α; MIP-1β; monocyte chemoattractant protein-1; and T cell activated-3 mRNA by RPA. No differences were found between Epi-SP and Sal groups (not shown).

Epi-EP yields no difference in cortisone levels in the efferent response to HEL. Cytokines including IL-1β, TNF-α, and IL-6 can stimulate both sympathetic arousal and the hypothalamic-pituitary adrenal axis by activating release of corticotropin releasing factor (6). To exclude the possibility that Epi-SP might alter pulmonary cell-mediated immunity by modulating cortisone (Cort) release, plasma Cort was assayed at the time of death. There was no difference in plasma Cort levels between Epi-SP and Sal controls (180 ± 105 vs. 207 ± 99, P = 0.6).

DISCUSSION

In this study, we investigated the effect of Epi on the pulmonary immune response to HEL in C57BL/6 (H-2b) mice, a strain that normally responds weakly to HEL. Epi-SP increased both pulmonary vascular and airway inflammation. Whereas α/β-AR blockers inhibited the increase in airway inflammation, heightened vascular inflammation was reversed only by atenolol, a selective β1-AR blocker, suggesting differential pathways of EPI effects on the pulmonary airways and microcirculation.

Efforts to determine the cellular characteristics of this response showed that Epi yielded modest but sig-

Table 2. Lymphocyte phenotype in BALF

<table>
<thead>
<tr>
<th></th>
<th>CD3⁺</th>
<th>NK1.1⁺</th>
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<tbody>
<tr>
<td>Sal-SP</td>
<td>70.7 ± 6.0</td>
<td>9.0 ± 4.5</td>
</tr>
<tr>
<td>Epi-SP</td>
<td>75.5 ± 5.5*</td>
<td>4.2 ± 2.2†</td>
</tr>
<tr>
<td>Epi + Aten-SP</td>
<td>70.9 ± 1.9</td>
<td>1.7 ± 0.4§</td>
</tr>
<tr>
<td>Epi + ICI-SP</td>
<td>76.0 ± 4.3</td>
<td>2.6 ± 1.2</td>
</tr>
<tr>
<td>Epi + Phen-SP</td>
<td>69.7 ± 3.1</td>
<td>11.3 ± 2.4‡</td>
</tr>
<tr>
<td>Epi + Pro-SP</td>
<td>65.2 ± 10.4§</td>
<td>7.4 ± 3.2§</td>
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C57BL/6 mice were immunized and challenged with hen-egg lysozymes (HEL) and received epinephrine (Epi) ± adrenoreceptor (AR) blockers, saline (Sal), or adrenoreceptor blockers on days 1–7 (sensitization phase (SP)). Bronchoalveolar lavage fluid (BALF) was collected and stained. The lymphocytes were electronically gated and analyzed by cytometry. Data represent the mean percentages of gated lymphocytes ± SD. *P < 0.05 vs. Sal-SP; §P < 0.05 vs. Epi-SP; †P < 0.01 vs. Sal-SP; ‡P < 0.01 vs. Epi-SP.

Fig. 6. Epi-EP increases localization of granulocytes and monocytes in pulmonary perivascular cuffs. C57BL/6 mice were treated as previously described. Lungs were harvested, frozen, sectioned at 5 μm, and stained with anti-GR1 or anti-CD11b by an indirect immunoperoxidase technique. The lungs of the Epi-SP-treated mice show a modest increase in GR-1⁺ cells in the perivascular cuffs and a substantial increase in CD11b⁺ monocytes, both compared with Sal controls. A: Sal-SP (anti-GR1); B: Epi-SP (anti-GR1); C: Sal-SP (anti-CD11b); D: Epi-SP (anti-CD11b). Peroxidase stain. Magnification ×150.

Fig. 7. Effect of Epi-SP and α/β-AR blockers on CD4⁺CD25⁺ lymphocytes in bronchoalveolar lavage (BALF). C57BL/6 mice were treated as described. BALF leukocytes were isolated, costained with FITC anti-CD4 and phycoerythrin anti-CD25, and analyzed by cytometry. Data are the mean percentage ± SD of CD4⁺CD25⁺ cells in the lymphocyte gate. *P < 0.05 vs. Sal; **P < 0.05 vs. Epi.
significantly increased percentages of CD3+ T lymphocytes and concomitantly decreased NK1.1+ lymphocytes in the BALF. These effects were differentially mediated by α- and β-AR. Whereas propranolol, a non-selective β-AR blocker, reversed the increase in CD3+ lymphocytes, the decrease in NK cells was reversed by both propranolol and phentolamine, suggesting a role for both α/β-AR regulation. The effects of β-AR in the reduction of NK cells appeared to require the cooperation of both β1-AR and β2-AR, because neither atenolol nor ICI-118551 alone yielded this effect. Administration of both Epi and atenolol, a selective β1-AR blocker, decreased NK cells below levels observed with Epi alone. This suggests that β1-AR may regulate NK cell motility in the context of increased adrenergic tone. Neither propranolol nor phentolamine diminished the increased vascular inflammation yielded by Epi-Sp, suggesting that this change was not directly related to the representation of CD3+ and NK1.1+ lymphocytes in the BALF.

Epi-Sp yielded decreased percentages of CD4+ CD25+ lymphocytes in BALF, and this was specifically reversed by atenolol, a selective β1-AR blocker. Because vascular inflammation was also specifically reversed by atenolol, CD4+CD25+ lymphocytes may play a direct role in this response. Propranolol, a nonselective β-AR blocker, did not reverse the decline in CD4+CD25+ lymphocytes or vascular inflammation. This may be due to differential effects of selective β1-AR blockade vs. blockade of both β1-AR and β2-AR. No conclusive role for either receptor subtype in vascular inflammation has previously been described.

The changes produced by Epi-EP were distinct from those observed in the SP. Whereas Epi yielded increased airway inflammation in both phases, vascular inflammation was not increased above controls in response to Epi-EP. Furthermore, Epi-EP decreased the percentage of CD3+ lymphocytes but increased the percentage of NK1.1+ cells in the BALF, in contrast to Epi-Sp.

Both norepinephrine and Epi mediate their effects on immune cells by stimulating α- and β-AR. At least three subtypes of β-AR (β1, β2, β3) and two α-AR (α1, α2) have been identified (8). Lymphoid cells express β-AR, and both T and NK lymphocytes express β2-AR. Maisel and colleagues (17) have suggested that β2-AR density differs among leukocytes with NK cells > CD14 monocytes > T cytotoxic cells > Th cells. Sanders and coworkers (23) have demonstrated that unlike Th1 lymphocytes, Th2 lymphocytes do not express β2-AR. However, β2-AR are not hypothesized to play a critical role in the observed vascular inflammation because ICI-118551, a selective β2-AR blocker, did not reverse it. Although β1-AR appear to play a critical role in Epi-induced pulmonary vascular inflammation, the representation of β1-AR on leukocytes is controversial (1).

The mechanisms by which α-AR modulate airway inflammation and trafficking of NK1.1+ cells induced by Epi-SP are uncertain. Increased granulocyte release from marginal pools in bone marrow, blood vessels, and lung is mediated by α2-AR (1). Platelets also express α2-AR but the expression of α-AR by monocytes and lymphocytes has not been convincingly established (2).

Reduced representation of CD4+CD25+ lymphocytes in BALF was a consistent finding in the response to Epi-SP. CD4+CD25+ lymphocytes include a quantitatively small immunoregulatory cell subset that has been demonstrated to prevent the induction of a variety of autoimmune diseases in mice, including gastritis, insulin, adrenalitis, and polyarthritis (22). Decreasing the frequency of CD4+CD25+ cells in murine pancreatic lymph nodes yields greater induction of autoimmune diabetes (9). As vascular inflammation is a feature of most autoimmune disorders, the reduction in CD4⁺CD25⁺ lymphocytes may contribute to heightened vascular injury via as yet uncertain mechanisms. Currently, no information exists concerning the expression of AR by the CD4⁺CD25⁺ lymphoid subset. However, the ability to modulate the representation of CD4⁺CD25⁺ lymphocytes with atenolol suggests that these cells may be distinguished by their expression of β1-AR.

The acute administration of catecholamines in murine species yields a transient increase in circulating lymphocytes with small increases in CD3+ and CD8+ lymphocytes and large increases in NK cells (1, 24). But repeated challenges with the β2-AR agonist terbutaline yields decreased numbers of circulating NK lymphocytes without significant changes in CD3+ lymphocytes (18). The mechanisms underlying the difference in the acute and chronic effects of β-adrenergic stimulation have not been determined but may include the acute mobilization of NK cell depots in the spleen and the marginalizing blood pool (24) that are exhausted by prolonged β-adrenergic administration. In the present study, the larger total dosage and duration of Epi administration to Epi-SP mice may explain why NK cells were decreased compared with Epi-EP mice. However, the absence of correlation between NK1.1+ lymphocyte representation and vascular inflammation suggests that NK cells are not the primary mediator of the Epi-heightened immune response in this model.

Catecholamines can inhibit Th1 responses and favor the development of Th2 responses (7). β-AR stimulation inhibits IL-12 production by monocytes and dendritic cells (27) and blocks TNF-α release by LPS-treated monocytes and glial cells in vitro. However, the effects of catecholamines on inflammation may be organ specific and compartmentalized. For example, α2-AR stimulation augments LPS-stimulated release of TNF-α by peritoneal macrophages (25) and increases expression of TNF-α and IL-1 by lung macrophages. β-AR also potentiate the release of IL-8 from human monocytes and lung epithelial cells (15), effects that would be expected to augment pulmonary Th1 inflammation. Some studies suggest that Th1 responses are increased by catecholamines. Dhabhar and McEwen (5) demonstrated that stress augments Th1 antigen-mediated cutaneous cellular immunity in vivo.

Catecholamines can modulate chemokine expression. As noted, β2-AR stimulation promotes expression of IL-8 by human lung monocytes (15). Hasko et al. (10)
demonstrated that MIP-1α, a proinflammatory chemokine that participates in the recruitment of leukocytes to the lung, is downregulated by endogenous and exogenous β-AR stimulation. But in the present study, we were unable to detect significant differences in the expression of MIP-1α and other proinflammatory cytokines by RPA.

The increased pulmonary vascular inflammation observed in the present study may be mediated primarily by nonimmune factors. Epi increases cardiac output by promoting cardiac rate and myocardial contractility via β1-AR stimulation (16). In addition, Epi also vasoconstricts peripheral blood vessels. These hemodynamic effects can potentially contribute to shear stress to the pulmonary endothelial lining (3), which can injure endothelial cells directly and/or upregulate their expression of immunomodulatory adhesion molecules (19), including ICAM or vascular adhesion molecules. Increased adhesion molecule expression promotes adherence of circulating leukocytes, including monocytes and granulocytes. This could account for why the increased pulmonary vascular inflammation in the present study was inhibited by atenolol, a β1-AR antagonist. However, this does not fully explain why vascular injury was not also inhibited by propranolol, a nonselective β-AR blocker.

We conclude that the effects of Epi on cell-mediated vascular and airway inflammation are dissociable and may reflect selectively patterned interactions with α/β-receptors distributed in tissues. The present findings suggest that Epi promotes vascular injury via β1-AR mechanisms in vivo. They do not support a role for a β2-AR mechanism. The findings in these studies raise potentially important questions concerning the use of catecholamines in medical practice, particularly in asthma and shock states where new exogenous or endogenous antigen challenges may occur. We speculate that Epi-mediated microvascular injury is exacerbated by pulmonary cell-mediated immunity. Future studies will examine the expression of α/β-AR by CD4+CD25+ lymphocytes, the mechanisms of β1-AR-mediated vascular injury, and AR-mediated airway inflammation.

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