Expression of the Complement Anaphylatoxin C3a and C5a Receptors on Bronchial Epithelial and Smooth Muscle Cells in Models of Sepsis and Asthma

Scott M. Drouin, Jens Kildsgaard, Joie Haviland, Joseph Zabner, Hong Pen Jia, Paul B. McCray, Jr., Brian F. Tack and Rick A. Wetsel

*J Immunol* 2001; 166:2025-2032; doi: 10.4049/jimmunol.166.3.2025
http://www.jimmunol.org/content/166/3/2025
Expression of the Complement Anaphylatoxin C3a and C5a Receptors on Bronchial Epithelial and Smooth Muscle Cells in Models of Sepsis and Asthma

Scott M. Drouin,* Jens Kildsgaard,* Joie Haviland,* Joseph Zabner,† Hong Pen Jia,§ Paul B. McCray, Jr.,§ Brian F. Tack,‖ and Rick A. Wetsel*‡†‡

The presence of the complement-derived anaphylatoxin peptides, C3a and C5a, in the lung can induce respiratory distress characterized by contraction of the smooth muscle walls in bronchioles and pulmonary arteries and aggregation of platelets and leukocytes in pulmonary vessels. C3a and C5a mediate these effects by binding to their specific receptors, C3aR and C5aR, respectively. The cells that express these receptors in the lung have not been thoroughly investigated, nor has their expression been examined during inflammation. Accordingly, C3aR and C5aR expression in normal human and murine lung was determined in this study by immunohistochemistry and in situ hybridization. In addition, the expression of these receptors was delineated in mice subjected to LPS- and OVA-induced models of inflammation. Under noninflamed conditions, C3aR and C5aR protein and mRNA were expressed by bronchial epithelial and smooth muscle cells of both human and mouse lung. C3aR expression increased significantly on both bronchial epithelial and smooth muscle cells in mice treated with LPS; however, in the OVA-challenged animals only the bronchial smooth muscle cells showed increased C3aR expression. C5aR expression also increased significantly on bronchial epithelial cells in mice treated with LPS, but was not elevated in either cell type in the OVA-challenged mice. These results demonstrate the expression of C3aR and C5aR by cells endogenous to the lung, and, given the participation of bronchial epithelial and smooth muscle cells in the pathology of diseases such as sepsis and asthma, the data suggest a role for these receptors during lung inflammation. The Journal of Immunology, 2001, 166: 2025–2032.

The respiratory airways of the lung undergo constant exposure to pathogens and other potentially inflammatory agents. One potential mechanism by which the lung can protect itself from infection is through in situ activation of the complement system (reviewed in Ref. 1). The complement system helps defend the host by initiating inflammatory and immunological responses and by promoting the cell lysis and death of invading microorganisms (reviewed in Ref. 2). Upon activation of the complement system, cleavage of the third and fifth components of the complement system (C3 and C5, respectively) generates the peptides C3a and C5a, both of which are potent anaphylatoxins (reviewed in Ref. 3). The human C3a and C5a peptides are 77 and 74 aa in length, respectively, and have a carboxyl-terminal Arg residue that is important for biological activity. Removal of the terminal Arg by carboxypeptidase N generates the C3adesArg and C5adesArg peptides and markedly reduces their ability to elicit inflammatory responses (reviewed in Ref. 4).

C3a and C5a can trigger contraction of smooth muscle, increase the permeability of small blood vessels, and regulate vasodilation (reviewed in Refs. 3 and 5). In addition, C3a and C5a can stimulate respiratory burst in macrophages (6, 7), neutrophils (8, 9), and eosinophils (10); stimulate the release of histamine from basophils (11, 12) and mast cells (13, 14); and regulate the synthesis of eicosanoid cationic protein and adhesion to endothelial cells by eosinophils (15–17). C3a can also stimulate serotonin release from platelets (18) and modulate synthesis of IL-6 and TNF-α by β lymphocytes and monocytes (19, 20). C5a is a potent chemotactic molecule for macrophages (21), neutrophils (8, 22), T lymphocytes (23), and basophils (24). Both C3a and C5a can induce chemotaxis of eosinophils (25) and mast cells (26, 27).

The C3a and C5a peptides regulate inflammatory functions by interacting with their receptors, C3aR and C5aR, both of which belong to the rhodopsin family of seven transmembrane G protein-coupled receptors (28–30). Traditionally, C3aR and C5aR were thought to be present only on myeloid cells such as macrophages (28, 31), neutrophils (28, 31), eosinophils (32, 33), basophils (11, 34), and mast cells (34, 35). However, recent studies have demonstrated these receptors on nonmyeloid tissue cells. C5aR has been found in kidney tubular epithelial and mesangial cells (36) and in hepatocytes (37, 38), and both receptors have been detected on neurons and glial cells of the CNS (39–44). In the lung, Northern blot analysis of RNA from human (29, 45), baboon (37), mouse (37, 46, 47), rat (48, 49), and guinea pig (50) lungs revealed the presence of C3aR and C5aR mRNA transcripts. Although no studies have yet defined the distribution of C3aR in the lung, studies have documented the presence of C5aR on bronchial and alveolar epithelial cells as well as on vascular endothelial and smooth muscle cells (37).
The functional importance of C3a and C5a has been established in studies examining the inflammatory effects of intrabronchial instillation of C3a or C5a in guinea pig lungs. Instillation of the anaphylatoxins induces respiratory distress characterized by contraction of the smooth muscle walls in bronchioles and pulmonary arteries and aggregation of platelets and leukocytes in pulmonary vessels (51, 52). Synthetic peptides, based on the carboxyl-terminal sequence of C3a, can also mimic these properties (53), and the addition of inhibitors to carboxypeptidase N potentiates the respiratory distress stimulated by instillation of C3a and C5a (54, 55). Previous studies have suggested that these properties may be mediated in part by expression of leukotrienes, histamine, or platelet-activating factor (55, 56). Whether the pulmonary changes are the direct result of C3a or C5a or are controlled through mediators regulated by these anaphylatoxins remains to be fully determined.

In this report we demonstrate C3αR and C5αR in normal human and mouse lung by immunohistochemistry and in situ hybridization. Furthermore, we show elevated expression of these receptors on bronchial epithelial and smooth muscle cells in mouse lung during conditions of endotoxemia and asthma. These studies suggest a role for these receptors in C3α- and C5α-mediated regulation of bronchial epithelial and smooth muscle cell functions during lung inflammation.

Materials and Methods

**Mice**

C3αR knockout mice were generated as described (57). For the LPS studies, C57BL/6J mice were injected i.p. with saline alone or saline containing LPS (20 mg/kg LPS from *Escherichia coli* 0111:B4, phenol extract; Sigma, St. Louis, MO). Twenty-four hours later, the animals were euthanized.

For the asthma studies, C57BL/6J mice were sensitized and challenged according to the method of Foster et al. (58). Briefly, animals were sensitized on days 0 and 12 with an i.p. injection of saline/uline alone or saline/uline containing 50 μg OVA (Sigma). On day 24, mice were challenged with aerosolized saline alone or saline containing 1% OVA for three 1-h sessions every other day for 8 days. Twenty-four hours after the last challenge, animals were euthanized, and blood and bronchoaveolar lavage fluid was collected to assess IgE, IL-4, and IL-5 levels and eosinophil recruitment into the lung.

**Tissue preparation.**

Mouse lungs were perfused with 10% neutral buffered formalin (Sigma). The perfused lung was placed in a MegaCassette (Tissue-Tek, Torrance, CA) and fixed in formalin overnight at 4°C. Lungs were dehydrated with increasing concentrations of ethanol and then embedded in paraffin for sectioning. Paraffin-embedded human lung tissue was obtained from normal human donors.

**Culture of human airway epithelia.**

Airway epithelia were isolated from bronchi and grown at the air-liquid interface as previously described (59). All preparations used were well-differentiated (>2 wk old, transepithelial resistance >1000 Ω × cm²) (59, 60). This study was approved by the Institutional Review Board at the University of Iowa.

**Antibodies.**

The mouse monoclonal anti-α-actin Ab, clone 1A4, was purchased from Sigma. For preparation of polyclonal rabbit anti-mouse C3αR Abs or rabbit anti-human C3αR Abs, peptides corresponding to amino acid residues 240–258 in the third extracellular domain of mouse C3αR (CSPDSPF SLDSANQPHYGG) or amino acid residues 307–325 in the third extracellular domain of human C3αR (CESELPQGQODYNLGQFTD) were synthesized. The peptides were coupled to keyhole limpet hemocyanin, and antisera was raised in New Zealand rabbits as described (Alpha Diagnostic International, San Antonio, TX). By ELISA, it was determined that the rabbit anti-serum raised recognizes C3αR peptides. The specificity of the C3αR antisera was demonstrated by flow cytometry using HEK293 cells transfected with either mouse or human full-length C3αR cDNA. In these experiments, the antisera did not bind to nontransfected cells, nor did the mouse or human anti-C3αR antisera cross-react with cells transfected with the other species’ cDNA. Moreover, the anti-mouse C3αR Ab did not exhibit nonspecific binding to tissues or cells obtained from C3αR-deficient mice.

The rabbit anti-human C5αR antisera used in these studies has been described previously (37). Its specificity was demonstrated by ligand blocking experiments (37) and by Western blot analysis, which identified a single band from HL-60 cells of the expected Mr for C5αR of 45,000. In addition, the specificity of the antisera was shown by flow cytometry using HEK293 cells transfected with human C5αR cDNA. For preparation of polyclonal rabbit anti-mouse C5αR Abs, a peptide corresponding to amino acid residues 7–24 in the ammino-terminal domain of mouse C5αR (SSFEINYHDGYTMVPN) was synthesized. The peptide was injected with Freund’s adjuvant (Quality Control Biochemicals, Hopkinton, MA) into New Zealand rabbits as described. By ELISA, it was determined that the rabbit anti-serum raised recognizes the C5αR peptide. The rabbit anti-mouse C5αR IgG was purified by protein A chromatography. The specificity of the anti-mouse C5αR Ab was determined by flow cytometry using HEK293 cells transfected with mouse C5αR cDNA and by immunoprecipitation of a single Mr band of 45,000 from iodinated RAW 264.7 mouse macrophage cells.

**Immunohistochemistry.**

Immunohistochemistry was performed on paraffin-embedded lung sections.Serial 7-μm sections were mounted on glass slides, de waxed, and rehydrated with PBS (pH 7.4). Endogenous peroxidase was blocked with 10% H₂O₂ in PBS (pH 7.4), and nonspecific Ig-binding sites were blocked with 10% goat serum in PBS (pH 7.4). The sections were then analyzed using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions. Briefly, sections were stained with either rabbit anti-human C3αR anti-serum (1/2000 dilution), rabbit anti-mouse C5αR anti-serum (1/1000 dilution), rabbit anti-human C5αR anti-serum (1/2000 dilution), anti-rabbit IgG (10 μg/ml), the corresponding preimmune sera or rabbit IgG as a negative control, or anti-α-actin Ab (1/2000 dilution) as a positive control. Sections were incubated with either biotinylated goat anti-rabbit IgG (1/200 dilution; Vector Laboratories) to detect binding of rabbit anti-C3αR or rabbit anti-C5αR Abs, or biotinylated goat anti-mouse IgG (1/200 dilution; Sigma) to detect binding of the anti-α-actin Ab. All Abs were diluted in PBS (pH 7.4) containing 5% goat serum. Sections were then incubated with Vectastain ABC reagent and developed with 3,3′-diaminobenzidine (Vector Laboratories). Sections were counterstained with Harris Hematoxylin (Fisher Scientific, Pittsburgh, PA), mounted in Permount (Fisher Scientific), and examined by light microscopy.

Staining intensity of the LPS- and OVA-challenged mouse experiments (see Fig. 6) was standardized to saline controls that were incubated together with the corresponding LPS or OVA experimental sample. In these experiments, lungs from three mice were analyzed from each group (OVA or LPS), and at least three separate sections were stained from each lung. Independent examination by four different investigators was used to assess qualitative changes in expression of C3αR and C5αR after LPS or OVA challenge (see Fig. 6 and Table I).

**Riboprobes.**

Mouse C3αR riboprobes were transcribed from a 327-bp DNA fragment corresponding to nucleotides 553–879 of mouse C3αR cDNA (46), and human C3αR riboprobes were transcribed from a 333-bp DNA fragment corresponding to nucleotides 553–885 of human C3αR cDNA (46). Mouse C5αR riboprobes were transcribed from a 214-bp DNA fragment corresponding to nucleotides 793–1014 of mouse C5αR cDNA (61), and human C5αR riboprobes were transcribed from a 205-bp DNA fragment corresponding to nucleotides 202–225 of human C5αR cDNA (28). Both C3αR and C5αR fragments were cloned into pSP72 (Promega, Madison, WI). The C3αR plasmid was linearized with *Bam*II or *Hind*III endonucleases (Roche Molecular Biochemicals, Indianapolis, IN), and the C5αR plasmid was linearized with *Xho*I or *Bgl*II endonucleases (Roche Molecular Biochemicals) for generation of sense and antisense riboprobes, respectively. In vitro transcription was performed using reagents from Roche Molecular Biochemicals’ digoxigenin kit (SP6/T7) according to the manufacturer’s instructions. Briefly, probes were labeled with DIG by incubating digested template DNA with 10× DIG RNA Labeling (DIG) 11 RNA Labeling (DIG) 11 RNase inhibitor, 10× transcription buffer (400 mM Tris-HCl (pH 8.0), 60 mM MgCl₂, 100 mM DTT, 20 mM spermidine), and SP6 or

---

3 Abbreviation used in this paper: DIG, digoxigenin.
T7 RNA polymerases at 37°C for 2 h. The resulting transcripts were then analyzed on a 5% acrylamide/8 M urea gel to assess quantity of transcript before use with the in situ hybridization experiments.

In situ hybridization

In situ hybridization was performed on paraffin-embedded lung sections. Serial 7-μm sections were mounted on glass slides, dried, and de-waxed extensively by soaking with xylene. Sections were then prepared and stained according to the method of Breitschopf and Suchanek (62). Sections were hybridized overnight at 42°C with DIG-labeled sense or antisense riboprobes diluted 1/50 in hybridization buffer (2× SSC, 10% dextran sulfate, 0.02% SDS, and 50% formamide). Sections were washed once with 2× SSC at room temperature, three times with 1× SSC containing 50% formamide at 42°C, and twice with 1× SSC at room temperature.

**FIGURE 1.** Staining of normal mouse lung for C3aR and C5aR protein expression by immunohistochemistry. Lung sections from wild-type mice were stained with rabbit anti-mouse C3aR serum, rabbit anti-mouse C5aR IgG, or an anti-α-actin mAb as a positive control for smooth muscle cells. Lung sections from C3aR knockout mice were stained with rabbit anti-mouse C3aR serum as a negative control for the C3aR immunostaining, and lung sections from wild-type mice were stained with non-specific rabbit IgG as a negative control for the C5aR immunostaining. The sections shown are ×63 magnification. BE, Bronchial epithelial cells; SM, bronchial smooth muscle cells; AE, alveolar epithelial cells.

**FIGURE 2.** Staining of normal mouse lung for C3aR and C5aR mRNA expression by in situ hybridization. Lung sections from wild-type mice were hybridized with mouse C3aR or C5aR antisense riboprobes or with mouse C3aR or C5aR sense riboprobes as negative controls. The sections shown are ×63 magnification.
Sections were blocked with TBS (pH 7.4) containing 10% goat serum and incubated with an alkaline phosphatase-conjugated anti-DIG Ab (Roche Molecular Biochemicals) diluted in TBS (pH 7.4) containing 5% goat serum. Sections were developed with nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Roche Molecular Biochemicals) according to the manufacturer’s instructions. Sections were counterstained with Nuclear Fast Red (Vector Laboratories), dehydrated, mounted in Permount (Fisher Scientific), and examined by light microscopy.

Results

C3aR and C5aR expression in mouse lung

To examine in vivo the expression and distribution of C3aR and C5aR in the lung, sections from normal mouse lung were analyzed by immunohistochemistry and in situ hybridization. Staining by immunohistochemistry showed expression of C3aR and C5aR protein in unstimulated mouse lung (Fig. 1). Expression was predominant in the bronchioles (Fig. 1, middle), with bronchial epithelial cells staining positive for both receptors. C3aR was also found along the basolateral surface of the bronchiole. Staining of these sections for α-actin localized to the basolateral surface of the bronchiole (Fig. 1, bottom), indicating that these C3aR-positive basolateral cells are bronchial smooth muscle cells. Although mouse bronchial smooth muscle cells may also express C5aR, staining along the basolateral surface of the bronchiole was not as intense as the staining for C3aR in this region. No signal was observed for C3aR staining in lungs from C3aR knockout mice or in lungs from wild-type mice stained with nonspecific rabbit IgG (Fig. 1, top) used as negative controls.

In situ hybridization also revealed expression of both C3aR and C5aR mRNA in unstimulated mouse lung, primarily in the bronchioles (Fig. 2, bottom), and signal for each receptor was present on the bronchial epithelial cells. Bronchial smooth muscle contribution to the C3aR or C5aR signal within the bronchioles could not be determined due to the signal intensity. C3aR and C5aR mRNA was also seen in alveolar epithelial cells and on pulmonary blood vessels (data not shown). No hybridization was noted in lungs from wild-type mice probed with C3aR or C5aR sense riboprobes (Fig. 2, top), indicating the specificity of the probes used.

C3aR and C5aR expression in human lung

To determine whether human lung expresses C3aR and C5aR, sections from normal human lung were analyzed by immunohistochemistry and in situ hybridization. Staining by each method revealed expression of C3aR or C5aR protein and mRNA in the bronchioles (Figs. 3 and 4). By immunostaining, signal for C3aR and C5aR protein was localized to the apical surface of the bronchial epithelial cells, consistent with the staining patterns observed in mouse lung (Fig. 3, middle). In addition, C3aR and C5aR were detected along the basolateral surface of the bronchiole. Staining of these sections for α-actin localized to the basolateral surface of the bronchiole (Fig. 3, bottom), indicating that these smooth muscle cell types express both receptors. C3aR and C5aR protein were also found on alveolar epithelial cells and pulmonary blood vessels (data not shown). Similarly, analysis by in situ hybridization demonstrated positive signal for C3aR and C5aR mRNA in the bronchioles (Fig. 4, bottom) in addition to alveolar epithelial cells and pulmonary blood vessels (data not shown). No staining was present in lung sections stained with rabbit serum (Fig. 3, top) or in lung sections probed with human C3aR or C5aR sense riboprobes (Fig. 4, top).

C3aR and C5aR expression in human bronchial epithelial cells

To support the observations found with normal human lung, paraffin-embedded sections of primary cultures of bronchial epithelial cells were stained for the expression of C3aR and C5aR protein and mRNA. Analysis of these cells by immunohistochemistry and in situ hybridization demonstrated staining for C3aR and C5aR protein and mRNA (Fig. 5). No staining was observed on cells stained with rabbit preimmune serum or probed with human C3aR or C5aR sense riboprobes.

C3aR and C5aR expression in LPS-stimulated and OVA-challenged mouse lungs

To determine whether lung expression of C3aR and C5aR increases during inflammation, the receptors were evaluated in lungs from mice subjected to models of endotoxemia and asthma. For the endotoxemia model, mice were injected i.p. with saline alone or with LPS. Lungs were then removed after 24 h, sectioned, and stained by immunohistochemistry. Analysis by immunohistochemistry demonstrated increased signal for C3aR and C5aR in the bronchioles from LPS-stimulated lungs (Fig. 6). In both immunostaining experiments, bronchial epithelial cells from LPS-treated
lungs exhibited higher staining intensity for both C3aR and C5aR compared with the saline-treated control lungs (Fig. 6, middle). Furthermore, C3aR signal intensity also increased along the basolateral surface of the bronchiole and had a similar staining pattern compared with the staining for α-actin (data not shown), indicating elevated smooth muscle expression of C3aR. Bronchial smooth muscle expression of C5aR was difficult to differentiate from the intense signal for this receptor on the bronchial epithelial cells. Alveolar epithelial cells and pulmonary blood vessels also exhibited elevated C3aR and C5aR expression in LPS-stimulated lung compared with saline controls (data not shown). No staining was present in lungs stained with either preimmune serum or nonspecific rabbit IgG (Fig. 6, top).

To determine whether C3aR and C5aR expression increases in a mouse model of asthma, mice were sensitized and then challenged with aerosolized saline alone or OVA. Consistent with this model, mice exposed to aerosolized OVA developed massive eosinophilia in the lung (1.13 ± 0.33 eosinophils/ml of bronchoalveolar lavage fluid) and exhibited increased IgE (saline, 261 ± 60.3 ng/ml; OVA, 1191 ± 244 ng/ml), IL-4 (saline, 11.54 ± 2.4 pg/ml; OVA, 77.71 ± 5.8 pg/ml), and IL-5 levels (saline, 78 ± 12.9 pg/ml; OVA, 225 ± 12.9 pg/ml). Lungs were removed after 24 h, sectioned, and stained by immunohistochemistry to assess C3aR and C5aR protein levels. Immunohistochemistry analysis demonstrated increased signal for C3aR on bronchial smooth muscle cells in OVA-challenged mice relative to the saline controls (Fig. 6, bottom). In contrast to mice treated with LPS, C3aR and C5aR expression in OVA-challenged lungs did not change on bronchial (Fig. 6, bottom) and alveolar epithelial cells (data not shown). Staining for both receptors on pulmonary blood vessels from OVA-challenged mice was difficult to assess due to the massive influx of granulocytes and macrophages (data not shown). However, C3aR and C5aR staining was detected on granulocytes and macrophages recruited into the lung as a result of the OVA challenge (data not shown). No staining was present in lungs stained with either preimmune serum or nonspecific rabbit IgG (Fig. 6, top).

**Discussion**

This study documents for the first time the expression of C3aR by lung cells and confirms previous reports that cells endogenous to mouse and human lungs express C5aR. Moreover, we have established that both receptors are up-regulated in two distinct models of lung inflammation: endotoxemia and OVA-induced asthma.

**FIGURE 4.** Staining of normal human lung for C3aR and C5aR mRNA expression by in situ hybridization. Lung sections were hybridized with human C3aR or C5aR antisense riboprobes or with human C3aR or C5aR sense riboprobes as negative controls. The sections shown are ×63 magnification.

**FIGURE 5.** Staining of unstimulated primary cultures of human bronchial epithelial cells for C3aR and C5aR expression by immunohistochemistry and in situ hybridization. For immunohistochemistry, sections were stained with rabbit anti-human C3aR serum, rabbit anti-human C5aR serum, or rabbit preimmune serum as negative controls. For in situ hybridization, sections were hybridized with human C3aR or C5aR antisense riboprobes or with human C3aR or C5aR sense riboprobes as negative controls. The sections shown are ×63 magnification.
C3aR and C5aR protein and mRNA were demonstrated on normal mouse and human lung bronchioles by immunohistochemistry and in situ hybridization, respectively (summarized in Table I). Both receptors are predominantly seen on mouse and human bronchial epithelial cells. C3aR and C5aR protein and mRNA expression was further confirmed by analysis of primary cultures of human bronchial epithelial cells. Although both receptors were present on bronchial smooth muscle in human lung, only C3aR was strongly detected on bronchial smooth muscle cells in the mouse lung. Differences in expression of these receptors may be explained by considering the section location along the bronchioles in human vs mouse lung. C5aR staining was demonstrated in sections of upper airway in human lung, but C5aR immunostaining was not apparent in sections of lower airway in mouse lung. To clarify this inconsistency, sections from mouse trachea were stained for C5aR, and the receptor was detected on smooth muscle as well as on epithelial cells in the upper airways (data not shown).

In addition to the bronchial epithelial and smooth muscle cell expression in normal human lung, C3aR and C5aR was found on alveolar epithelial cells and pulmonary blood vessels. Although the distribution of C3aR in the lung has not been documented previously, the C5aR staining pattern described in this study was consistent with previously documented results of C5aR immunostaining in human lung with cystic fibrosis (37). Namely, C5aR

Table 1. Summary of C3aR and C5aR protein expression in the lung

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Mouse Lung LPS</th>
<th>OVA</th>
<th>Human Lung Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3aR expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial epithelial cells</td>
<td>+a</td>
<td>+++</td>
<td>+</td>
<td>+a</td>
</tr>
<tr>
<td>Bronchial smooth muscle cells</td>
<td>+a</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Alveolar epithelial cells</td>
<td>+a</td>
<td>+++</td>
<td>+</td>
<td>+a</td>
</tr>
<tr>
<td>Pulmonary blood vessels</td>
<td>NDb</td>
<td>+</td>
<td>ICb</td>
<td>+</td>
</tr>
<tr>
<td>C5aR expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial epithelial cells</td>
<td>+a</td>
<td>+++</td>
<td>+</td>
<td>+a</td>
</tr>
<tr>
<td>Bronchial smooth muscle cells</td>
<td>IC</td>
<td>IC</td>
<td>IC</td>
<td>+</td>
</tr>
<tr>
<td>Alveolar epithelial cells</td>
<td>+a</td>
<td>+++</td>
<td>+</td>
<td>+a</td>
</tr>
<tr>
<td>Pulmonary blood vessels</td>
<td>NDb</td>
<td>+</td>
<td>ICb</td>
<td>+</td>
</tr>
</tbody>
</table>

a Receptor mRNA detected by in situ hybridization.
b Inconclusive.
expression was found on bronchial epithelial and smooth muscle cells, alveolar epithelial cells, and pulmonary smooth muscle and endothelial cells. However, these findings conflict with studies by Fayazy et al. that detected C5aR protein and mRNA expression only on alveolar macrophages in normal human lung (63, 64). These differences may be explained by the types of reagents used for the immunohistochemistry and in situ hybridization experiments and the level of sensitivity required to detect basal C5aR expression in normal human lung.

In the mouse models of sepsis and asthma, lung expression of C3aR and C5aR changed in a cell- and disease-specific manner. In the mouse model for sepsis, both C3aR and C5aR expression increased on bronchial epithelial cells, alveolar epithelial cells (types I and II), and pulmonary blood vessels. C3aR expression was also increased on bronchial smooth muscle cells. In contrast to mice treated with LPS, C3aR and C5aR expression did not change on bronchial and alveolar epithelial cells from OVA-challenged lungs. However, bronchial smooth muscle expression of C3aR was increased in OVA-challenged mice relative to the saline controls. Although further study is required to determine the role of each receptor in these inflammatory models, these results suggest that both receptors contribute to lung function in the endotoxemia model, whereas C3αR may play a more significant role in lung inflammation in the asthma model. Recent observations that OVA-challenged C3αR-deficient guinea pigs (65) and mice (66) have reduced bronchial hyperreactivity support the concept that C3αR may regulate bronchial smooth muscle function in this disease.

Interestingly, bronchoalveolar lavage fluid from human patients with asthma (67, 68) or respiratory distress syndrome (69–71) have been shown to contain C3α and C5α anaphylatoxins. These peptides might potentially originate from complement components produced by cells in the lung and then cleaved upon complement activation. Previous studies have demonstrated that resident alveolar macrophages and type II alveolar epithelial cells can synthesize the necessary components for complement system activation (72). Although the exact functions that C3α and C5α regulate in human lung disease require further examination, C3α and C5α have been shown to elicit functional responses in the lung. In guinea pigs, instillation of C3α or C5α into the lungs induces respiratory distress characterized by contraction of the smooth muscle in bronchioles and pulmonary arteries and recruitment of platelets and leukocytes in pulmonary vessels (51, 52).

The importance of C3αR or C5αR during lung inflammation becomes apparent when C3α- and C5α-mediated functions are blocked using Abs or animals deficient for these receptors. Septic primates or rats treated with anti-C5α Abs have reduced pulmonary edema and lung injury (73, 74). With immune complex-mediated lung injury, intratracheal administration of anti-C5α reduces lung inflammation in rats (75). In addition, C3αR-deficient guinea pigs that are challenged with immune complexes have delayed bronchoconstriction compared with controls (76). Similarly, C3αR-deficient guinea pigs (65) and mice (66) have reduced bronchial hyperreactivity when challenged with aerosolized OVA. Finally, mice deficient in C5αR were unable to clear intrapulmonary-instilled Pseudomonas aeruginosa and succumbed to pneumonia (77).

The studies presented here demonstrate for the first time expression of C3αR in cell types indigenous to mouse and human lungs and confirm previous reports of C5αR expression in human lung. These studies also suggest specific roles for each receptor in the lung in diseases such as sepsis and asthma and suggest that C3αR and C5αR may regulate as yet unknown functions in bronchial epithelial and smooth muscle cells during inflammation.

Acknowledgments
We gratefully acknowledge Dr. Irma Gigli, Dr. David Haviland, and Kirstin Matthews for critical evaluation of the data and text, and Dr. Pam Beck and Mei-Fang Lu for technical assistance with the immunohistochemistry and in situ hybridization experiments.

References

Downloaded from http://www.jimmunol.org/ by guest on October 22, 2017