Expression and Probable Roles of Cell Adhesion Molecules in Lung Inflammation

Craig D. Wegner, Ph. D.; Robert H. Gandel, Ph. D.; Robert Rothlein, Ph. D.; and L. Gordon Letts, Ph. D.

Intimate cell-cell contact or "adhesion" is crucial to many aspects of an immune or inflammatory response, including antigen presentation and antibody production, leukocyte margination and migration, leukocyte activation and degranulation, and cell mediated cytotoxicity. The cell surface glycoproteins that specifically mediate adhesion control both the strength of the intercellular contact as well as transducing a signal that controls intracellular activation, degranulation, and probably, motility. Thus, these molecules are principal regulators of inflammatory reactions.

Using monoclonal antibodies (MAbs) to individual adhesion glycoproteins, we have begun to investigate the expression and possible roles of specific cell adhesion molecules in animal models of inflammatory lung diseases. The results not only confirm the importance of cell adhesion in the pathogenesis of lung disease/dysfunction, but also indicate that the contribution of each specific adhesion molecule is determined by the conditions and components of the inflammatory reaction. We begin with an introduction to some of the endothelial, epithelial, and granulocyte adhesion molecules, as well as concepts on how they might regulate inflammatory reactions.

Adhesion: Molecules and Concepts

Adhesion of leukocytes to the microvascular endothelium is primary to their infiltration into tissue. This adhesion is also essential to leukocyte (eg, neutrophil) retention within the pulmonary circulation (margination) and mediation of endothelial damage that characterize many acute lung injuries. Likewise, adhesion of leukocytes to lung tissue components, including airway and alveolar epithelium, is integral to both their retention within and their destruction of lung tissue. The initiation of these adhesions is apparently through the upregulated expression of adhesion molecules on the endothelium or epithelium, and/or the activating of adhesion molecules on leukocytes.

Endothelial/Epithelial Adhesion Molecule Expression

Activation of endothelial cells with inflammatory mediators upregulates or induces the expression of several adhesion "ligands" including the following: granulocyte membrane protein M, of 140 kd (GMP-140), endothelial-leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) (Fig 1). The inducible expression of these molecules seems to be important for directing the focal adhesion of leukocytes to blood vessels at sites of inflammation.

ICAM-1 (CD54) and VCAM-1 are members of the immunoglobulin superfine family. Their induction requires de novo protein synthesis, is slow in onset but prolonged in term (onset 2 to 4 h with a peak of 8 to 24 h poststimulation), and consequently, thought to mediate chronic inflammation/infiltrations. The leukocyte "receptors" for these two ligands are members of the integrin superfine family: LFA-1α (CD11a/CD18) and Mac-1 (CD11b/CD18) for ICAM-1, and VLA-4 (CD49d/CD29) for VCAM-1.

GMP-140 (CD62) and ELAM-1 are members of the selectin family indicative of their amino-terminal carbohydrate-binding (lectin) domain. Their induction is more rapid and transient and consequently thought to mediate more acute infiltrations. The storage of GMP-140 in Weibel-Palade bodies of endothelial cells allows for its mobilization to the surface in less than 5 min of endothelial activation. The leukocyte receptors for GMP-140 and ELAM-1 are not yet well defined but are apparently also selectins, for example LeCAM-1. ELAM-1 binds specifically to the carbohydrate group sialyl Lewis X, a terminal structure found on cell surface glycoproteins and glycolipids of neutrophils, monocytes, and some tumor cells, while GMP-140 binds to the CD15 antigen, lacto-N-fucopentaose III (LNF III), on neutrophils and monocytes.

In addition to endothelium, ICAM-1 is inducible on airway and alveolar epithelial cells by inflammatory stimuli or mediators including cytokines, eosinophil major basic protein, and hyperoxia. In contrast, GMP-140, ELAM-1, and VCAM-1 are not expressed or inducible on lung epithelial cells.

Granulocyte Adhesion Molecules

The major adhesion "receptors" on granulocytes seem to be members of one of two superfine families: (1) the selectins: represented by lectin cell adhesion molecule-1 (LeCAM-1), the human homolog of the mouse MEL-14 antigen, and other yet undefined sialyl Lewis X and LNF III (CD15) containing glycoproteins, and (2) the integrins: represented by the lymphocyte function-associated antigen-1.

Figure 1. Endothelial adhesion molecules, as well as their receptors and time course of expression, that direct the margination, and then diapedesis, of granulocytes to blood vessels at sites of inflammation. Tissues were prepared and stained as described previously.
1 (LFA-1) family (β2, CD18) and the very late antigen (VLA) family (β1, CD29). Besides adhesion, these receptors possess additional properties that aid in their regulation, fine tuning, of immune/inflammatory responses. As the result of neutrophil stimulation with a chemoattractant, or possibly ELAM-1 mediated adhesion, LeCAM-1 is rapidly proteolytically shed from the cell membrane presumably to cause a deadhesion necessary for migration and diapedesis, or demargination. Integrin receptors are transmembrane glycoproteins heterodimers (α and β chains), whose interaction with an intact cytoskeleton is required for adhesion. Their interaction with the cytoskeleton, possibly via talin, is believed to regulate both avidity for ligand binding and signaling (priming or activation) of the leukocyte on ligand binding.\(^1\)\(^{17}\)\(^{20}\)

Granulocyte Infiltration, Activation, and Mediated Tissue Injury

Several lines of evidence suggest that the initial adhesion (rolling or margination) of granulocytes to endothelium is mediated by selectins.\(^{20,31}\) The marginated neutrophil is then activated, via this adhesion or chemoattractants, causing the shedding of LeCAM-1 and activation (increased avidity) of Mac-1 (CD11b/CD18). LFA-1α to ICAM-1 and Mac-1 to ICAM-1 interactions then mediate neutrophil diapedesis.\(^19\) Although less frequently studied, the presence of these same molecules and processes on eosinophils suggests that eosinophil infiltration is similarly regulated.\(^{22,30}\) However, unlike neutrophils, eosinophils also express notable amounts of VLA-4 (CD49d/CD29) that via its binding to endothelial VCAM-1 may also participate in eosinophil diapedesis.\(^{32,33}\)

The adhesion of the activated granulocytes (neutrophils and presumably eosinophils) to the airway/alveolar epithelium, mediated largely by Mac-1 and partial by epithelial ICAM-1, then governs epithelial killing or the retention of the primed granulocytes just adjacent to the air spaces. Adhesion via Mac-1 has been shown to enhance the respiratory burst of neutrophils in vitro.\(^29\)

ANIMALS MODELS

While the paramount importance of cell-cell adhesion in immune/inflammatory responses is now well established, the contribution of each receptor-ligand interaction is not. Can antagonism of a single adhesion molecule lead to a new and novel treatment for inflammatory lung diseases? Will the relative contribution of each adhesion glycoprotein differ between diseases since the components, kinetics, injury, and resulting dysfunction also vary? To answer these questions, we have used animal models to investigate the role of individual cell adhesion molecules (via MAb) specific inflammatory processes.

Airway Hyperresponsiveness in Monkeys

Airway eosinophilia, epithelial desquamation, and hyperresponsiveness are characteristic features of the airway inflammation associated with asthma.\(^{37}\)\(^{40}\) Results from several studies suggest that these features are linked. That is, airway eosinophils, through release of their cytotoxic granule contents (eg, major basic protein), produce damage/desquamation of airway epithelium causing, through a number of possible mechanisms, airway hyperresponsiveness.\(^{41}\) Airway hyperresponsiveness is of particular importance since its severity correlates with the intensity of asthmatic symptoms, diurnal variations in peak flow rates, and therapy required.\(^{42}\)\(^{43}\)

In adult, male cynomolgus monkeys with a naturally occurring hypersensitivity of Ascaris suum extract, we have reported that Ascaris inhalation induces a prolonged airway eosinophilia and that long-term airway eosinophilia is associated with a marked airway hyperresponsiveness.\(^{44}\) Multiple (three alternate day), but not a single, inhalation(s) of antigen were found to produce an increase (usually more than eightfold) in airway responsiveness to inhaled methacholine\(^{45}\) whose severity was correlated to the degree of epithelial desquamation.\(^{46}\) Using MAb to ICAM-1, ELAM-1 and Mac-1 (CD11b/CD18), the contributions of these cell adhesion molecules, of the immunoglobulin, selectin, and integrin superfamilies, respectively, to the airway eosinophilia and hyperresponsiveness induced by repeated antigen were explored.

When we began our studies on ICAM-1, its role on eosinophil adherence to endothelium or its expression on airway epithelium was not known. Therefore, we investi-
gated these features in vitro. The adhesion of plateletactivating factor (PAF) stimulated monkey lung eosinophils to human umbilical vein endothelial cells previously stimulated with lipopolysaccharide (LPS) for 4 h was found to be partially (≤50%) inhibited by a MAb to ICAM-1.16,24 Using an enzyme-linked immunosorbent assay (ELISA), ICAM-1 was found to be constitutively expressed and impressively upregulated by 16 h of stimulation with inflammatory cytokines on confluent monolayers of cultured monkey bronchus epithelial cells.19 Immunohistochemical staining for ICAM-1 confirmed its expression and upregulation on antigen inflamed airways in vivo. A marked increase in ICAM-1 staining was found on both the vascular endothelium and airway epithelium (basal attachment only) of a tracheal section taken 20 min after the third of 3 alternate day Ascaris inhalations compared to a similar section obtained after a single inhalation.19

With these results as background, the role of ICAM-1 on the airway eosinophilia and hyperresponsiveness induced by multiple antigen inhalations was determined using two mouse anti-human MAb: one, R6.5, that inhibits function and another, CL203, that binds to a separate epitope (domain) on ICAM-1 not involved in leukocyte adhesion.19,20 Airway cell composition (assayed by BAL) and responsiveness (inhaled methacholine PC_{20}) were determined 3 days prior to (day 0) and 3 days after (day 10) 3 alternate day (day 3, 5, 7) inhalations of A. suum extract. R6.5 and CL203 were administered intranasally at 1.76 mg/kg daily (days 2 to 9) and compared to bracketing control studies in each animal. The monkeys were rested 5 or more weeks between each study to allow the induced airway inflammation and hyperresponsiveness to resolve. R6.5, but not CL203, attenuated the eosinophil infiltration (≤50% and ≤3±38%, respectively). More importantly, the increase in airway responsiveness was markedly inhibited by R6.5, but not CL203 (92±34 and ≤15±39%, respectively). Like ICAM-1, ELAM-1 expression is enhanced on inflamed endothelium and contributes to both neutrophil and eosinophil adhesion in endothelium.25,26,27 However, ELAM-1 upregulation occurs more rapidly (peak at 4 h) and is more transient (gone by 16 h).25 In addition, while immunohistochemical staining for ICAM-1 is enhanced on airway and alveolar epithelium and vascular endothelium, ELAM-1 staining was only evident on airway vascular endothelium in sections obtained from monkey lungs 4 h after the third of 3 alternate day Ascaris inhalations (Fig 2). The lack of ELAM-1 induction on airway epithelium was confirmed in vitro using the above mentioned ELISA on cytokine (including IL-1β) stimulated confluent monolayers of cultured monkey bronchus epithelial cells.19 Using the mouse anti-human ELAM MAb CL2, as well as the same protocol and dosing regimen as for the anti-ICAM-1 MAbs, the role of ELAM-1 in antigen-induced airway inflammation and hyperresponsiveness was evaluated. CL2 treatment did not significantly inhibit the eosinophil infiltration (∼10^9/ml BAL: 507±118 in control vs 430±114 in CL2 treated) nor the increase in airway responsiveness (change in log PC_{20}: −1.16±0.27 in control subjects vs −1.24±0.29 in CL2 treated).

The role of Mac-1 (CD11b/CD18) in antigen-induced airway eosinophilia and hyperresponsiveness was similarly determined using the mouse antihuman Mac-1 MAb LM2. We have previously reported that LM2 impressively inhibits the adhesion of PAF stimulated monkey lung eosinophils to protein-coated plastic (90 to 100%), and LPS stimulated monolayers of human umbilical vein endothelial cells (∼75%).25 LM2 treatment did not reduce the eosinophil infiltration but significantly inhibited its activation within the airways (BAL eosinophil peroxidase (EPO) activity in OD units: 618±210 in control subjects vs 185±41 in LM2 treated), as well as the increase in airway responsiveness (change in log PC_{20}: −0.99±0.16 in control subjects vs −0.14±0.15 in LM2 treated).

In summary, we have found that both ICAM-1 and Mac-1, but not ELAM-1, contribute to the airway hyperresponsiveness induced by repeated allergen inhalations in primates. Our results are consistent with the following: (1) endothelial ICAM-1 binding to eosinophil LFA-1α (CD11a/CD18) to partially mediate eosinophil diapedesis; and (2) eosinophils Mac-1 binding its ligand(s) within the airways (including epithelial ICAM-1) to mediate eosinophil activation and possibly epithelial desquamation. These postulates are supported by our recent results demonstrating that targeting epithelial ICAM-1 via the daily (day 2 to 9) inhalation of R6.5 (anti-ICAM-1) reduced the antigeninduced increases in eosinophil activation (BAL EPO activity), epithelial desquamation (histopathologic scoring of epithelial shedding), and airway hyperresponsiveness.47

Late Phase Airway Obstruction in Monkeys

Allergen-induced reactions in the skin, naries, eyes, and lungs of atopic individuals are characterized by an IgE-dependent immediate response followed in some individuals 4 to 6 h later by a second "late phase" response (LPR).6 The LPR is poorly reversed by bronchodilators, long lasting, and associated with an infiltration of eosinophils, neutrophils, and monocytes, as well as with an increase in airway responsiveness.68 The similarity of these features with those that characterize and distinguish the airway inflammation found in asthma has led many to suggest that clinical asthma may be the result of repeated/superimposed LPRs. The therapeutic benefit of corticosteroids in both LPRs and clinical asthma is consistent with this supposition.68,30 Like in man, allergen inhalation in A. suum hypersensitive cynomologus monkeys induces an immediate followed by LPR (dual response) in some individuals and only an immediate (single) response in others.4 In these monkeys, dual relative to single responders have significantly more eosinophils and EPO activity in their airways (recovered by BAL) prior to allergen challenge (ie, a baseline, persisting airway inflammation). After antigen inhalation, airway eosinophils acutely decrease, and the severity of the late phase airway obstruction significantly correlates with the intensity of the acute neutrophil influx and increase in airway myeloperoxidase (MPO) activity (BAL at 6 h postantigen).44 Since neutrophil infiltration and activation are regulated by adhesion, we have used this monkey model to determine the governing adhesion molecules.

Immunohistochemical staining with R6.5 and CL2 on biopsy specimens taken prior to and at 6 h postantigen inhalation was performed to study the upregulation of ICAM-1 and ELAM-1, respectively.46 In dual responder
monkeys, ICAM-1 was notably expressed on both the airway epithelium and vascular endothelium prior to antigen and was not consistently upregulated at 6 h postantigen. This baseline (presantigen) expression of ICAM-1 is compatible with the baseline airway inflammation (eosinophilia) in dual responder monkeys. In contrast, ELAM-1 staining was impressively upregulated on the airway vascular endothelium at 6 h postantigen with little or no staining evident prior to antigen. These results agree with the enhanced ELAM-1 expression recently documented in allergen-induced LPR in the skin of patients with respiratory allergy.49

To determine the role of ICAM-1 and ELAM-1 in LPRs, R6.5 (anti-ICAM-1) and CL2 (anti-ELAM-1) were administered intravenously at 2 mg/kg 1 h prior to Ascaris inhalation in dual responder monkeys.50 R6.5 had little if any effect on the neutrophil influx (BAL at +6 h) or the associated late phase airway obstruction. However, CL2 significantly and almost completely inhibited both the neutrophil infiltration and the resulting late phase airway obstruction. Thus, the roles of ICAM-1 and ELAM-1 in the LPR induced by a single allergen inhalation in monkeys with preexisting airway inflammation are strikingly different from those in the induction of airway hyperresponsiveness by multiple allergen inhalations in monkeys without baseline airway inflammation (Table 1). The reasons for these differences deserve further investigation.

**Pulmonary Oxygen Toxicity in Mice**

Bypass surgery, trauma, head injuries, emboli, septic shock, pneumonia, smoke inhalation, and premature birth frequency initiate acute lung injury, edema, and inflammation that result in impaired alveolar gas exchange. In such patients, the use of elevated levels of inhaled oxygen are required to achieve acceptable blood oxygen saturation. However, prolonged exposure to high concentrations of oxygen can precipitate acute edematous lung injury (alveolar septal thickening), followed by fibrosis, pulmonary hyper tension, and/or bronchopulmonary dysplasia.57,58

Results of many investigations indicate that the initiation of pulmonary oxygen toxicity occurs through a direct increase in the intracellular production of partially reduced oxygen species that overwhelm intracellular antioxidant defense mechanisms.59,60 Once these effects are initiated, the lung injury is amplified and morphologic changes become pronounced, exponentially between 48 and 72 h of pure oxygen breathing associated with a marked infiltration of neutrophils.7,8 In addition to its upregulatable expression on antigen-inflamed airway vascular endothelium and epithelium cited above, we found that ICAM-1 is constitutively expressed on the alveolar endothelium and epithelium of mice and that its expression is strikingly enhanced after 48 h of pure oxygen breathing.41 This finding is consistent with a recent report of increases in lung ICAM-1 mRNA induced by hypoxia in mice.41

Thus, to investigate the role of ICAM-1 in pulmonary oxygen toxicity, we evaluated the rat antimeim ICAM-1 monoclonal antibody YNI/1.7 in 2 protocols of oxygen-induced toxicity in adult, male BALB-c mice, first at ≥95% O2 for 84 h, and second, ≥95% O2 for 60 h followed by 48 h at 21% (ambient) O2. YNI/1.7 treatment provided a significant but only partial attenuation of the lung damage (increase lavage LDH activity), lung dysfunction (reductions in dynamic respiratory system compliance [Crs] and Dco), and reduction in body weight induced by exposure to pure oxygen for 84 h (Table 2). The neutrophil influx was also significantly but more markedly attenuated, while a trend for an increase in lung lavage MPO activity was completely blocked. YNI/1.7 completely inhibited the milder lung dysfunction (reductions in Crs and Dco) induced by breathing pure oxygen for 60 h followed by ambient air for 48 h.41

The inhibition of lung damage, dysfunction, and lavage MPO activity by YNI/1.7 may simply be the consequence of the diminished neutrophil infiltration. However, these effects are also consistent with YNI/1.7 reducing an epithelial ICAM-1 dependent stimulation of neutrophil activation and mediated damage. As mentioned above, neutrophil adhesion via Mac-1 (CD11b/CD18; also termed Mo-1), a receptor for ICAM-1, both augments the neutrophils release of hydrogen peroxide46 and killing of alveolar epithelial cells in vitro.47 In either case, these results confirm the contribution of leukocytes in the pathogenesis of pulmonary oxygen toxicity.

**Table 1—Role of ICAM-1, ELAM-1 and Mac-1 in Primate Models of Asthma-like Airway Inflammation and Dysfunction**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Naive</th>
<th>Saline</th>
<th>YNI/1.17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in BW, g</td>
<td>0.1±0.3</td>
<td>-1.1±0.1†</td>
<td>-0.7±0.1†</td>
</tr>
<tr>
<td>Lung lavage:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMNs (×109/ml)</td>
<td>9±1</td>
<td>44±13†</td>
<td>21±4‡</td>
</tr>
<tr>
<td>Protein (μg/ml)</td>
<td>409±32</td>
<td>1573±123†</td>
<td>1248±154†</td>
</tr>
<tr>
<td>LDH (units/l)</td>
<td>86±9</td>
<td>246±14†</td>
<td>193±9†</td>
</tr>
<tr>
<td>MPO (μg/ml)</td>
<td>0.24±0.05</td>
<td>0.42±0.15</td>
<td>0.22±0.06</td>
</tr>
<tr>
<td>Lung function:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crs (μ/l cm H2O)</td>
<td>31.8±2.0</td>
<td>8.9±0.7†</td>
<td>13.2±1.0‡</td>
</tr>
<tr>
<td>Dco (μ/l min/mm Hg)</td>
<td>18.6±0.8</td>
<td>10.3±0.5†</td>
<td>12.0±0.5‡</td>
</tr>
</tbody>
</table>

*Modified from Wegner et al.† Abbreviations: BW, body weight; PMNs, polymorphonuclear leukocytes (neutrophils); LDH, lactate dehydrogenase activity; MPO, myeloperoxidase activity; Crs, dynamic respiratory system compliance; Dco, diffusion capacity of the lungs for carbon monoxide; naive, no O2 exposure; saline, vehicle treated; YNI/1.17, rat antimeim ICAM-1 monoclonal antibody. Values are mean ± S.E.M. (n=8-14).

†Significant from naive animals by Student's t-test (LSD) with p<0.05.
‡Significant from saline treated animals by Student's t-test (LSD) with p<0.05.
oxygen toxicity and indicate that antagonism of ICAM-1 may provide a therapeutic approach to reducing hyperoxic lung injury and dysfunction.

Conclusions

Using animal models of granulocyte-mediated inflammatory lung disease/dysfunction, we have demonstrated that a single cell adhesion glycoprotein, ICAM-1, contributes substantially to the pathogenesis of antigen-induced airway hyperresponsiveness and pulmonary oxygen toxicity. ICAM-1 confers these effects through its upregulated expression on vascular endothelium directing granulocyte margination and managing granulocyte diapedesis, as well as its expression on airway and alveolar epithelium governing granulocyte retention, activation and mediated tissue injury (epithelial damage). In the more acute neutrophil-mediated, antigen-induced LPR, ELAM-1 was found to play a dominant role. Thus, taken together, our findings indicate that antagonism of a single adhesion molecule may lead to a new and novel treatment for inflammatory lung diseases and that the dominant molecule will vary based on the conditions, components, and injury associated with the underlying inflammatory processes.

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References

Cytokine-Mediated Changes in PMN Adherence to Canine Tracheal Epithelial Cells*

Mary K. Schrath, M.D.; and D. Michael Shabtai, M.D., F.C.C.P.

Airway inflammation is often accompanied by increased numbers of polymorphonuclear leukocytes (PMN) in...