EDITORSIAL

Adhesion molecule antagonists: future therapies for allergic diseases?

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In the past 20 yrs, substantial progress has been made in the identification of cells and mediators involved in allergic inflammation. As a result, an increased emphasis has been placed on the role of inflammation in allergic disease. Extensive efforts have focused on the study of allergic inflammation, often employing experimental allergen challenge to elicit late phase responses. Despite years of intensive investigation, the exact mechanisms responsible for this inflammatory response remain a puzzle. One of the fascinating characteristics of both late phase reactions and chronic allergic diseases, including rhinitis and asthma, is the preferential accumulation of eosinophils and the TH2 subpopulation of T-lymphocytes at mucosal sites.

Many laboratories, including our own, have attempted to understand the mechanisms of selective leucocyte recruitment in allergic disease, with the belief that this may lead to novel treatments that prevent the accumulation of these cells within the airways. Within the past 10 yrs, scientists have witnessed an explosive growth in the knowledge of cell adhesion molecules, an ever increasing class of molecules grouped into several distinct families (e.g. integrins, selectins, and members of the immunoglobulin supergene family) based on functional and structural similarities. These molecules are critical in mediating essentially every step in cell recruitment, including endothelial adhesion, diapedesis (transendothelial migration) and chemotaxis. In a recent issue of the Journal, Moezzi et al. [1] provided an excellent review of this rapidly advancing field and included a discussion of the role of adhesion molecules in airway disease. These authors are amongst the leaders in the study of the expression of adhesion molecules in vivo at sites of human allergic inflammation. The challenge for these and other investigators in the field is to elucidate the pattern of regulated expression, function, and co-ordinated interactions of these molecules. It appears, based on the existence of numerous adhesion molecules with varied specificities and regulatory patterns, that the answer will be quite complex. Nevertheless, numerous pharmaceutical companies have already begun to focus on adhesion molecules as relevant targets in trying to design novel anti-inflammatory agents that might be useful in the treatment of a wide variety of diseases including asthma. Clearly, if and when such specific reagents become available, they will be extremely important tools in dissecting, and ultimately defining, the true importance of each adhesion molecule during the inflammatory response. The purpose of this brief review is to discuss the rationale for attempting to block adhesion molecules as a possible therapeutic strategy, and to summarize a number of approaches being taken to achieve this.

Functions of adhesion molecules during various inflammatory reactions

An important theme in the recruitment of circulating leucocytes to inflammatory sites is the cascade of steps orchestrated by a succession of adhesion molecules. The initial step in inflammation is rolling of leucocytes along the endothelial wall, as first described in 1889 by Cornheim [2] with the use of intravitral microscopy. This crucial first step occurs despite the shear forces of blood flow, and has been convincingly shown to be mediated by the selectin family of adhesion molecules [3]. Studies using artificial lipid bilayers containing purified P-selectin, which will support leucocyte rolling, confirm that non-selectins such as intercellular adhesion molecule-1 (ICAM-1) are neither necessary nor sufficient to mediate initial rolling [4]. In addition, monoclonal antibodies against β2-integrins do not inhibit rolling, whilst monoclonal antibodies to L- and P-selectin do, further documenting the importance of selectins in mediating the initial attachment of leucocytes under conditions of flow.

Many different kinds of adhesion molecules can participate during cell adhesion. Known ligand binding pairs for endothelial cell and leucocyte adhesion molecules include CD11a with ICAM-1 and ICAM-2, CD11b with ICAM-1, very late activation antigen-4 (VLA-4) (α4β1) with vascular cell adhesion molecule-1 (VCAM-1), and the carbohydrate structure sialyl-Lewis X (sLeX) with E-selectin and P-selectin [5, 6]. L-selectin binds to a sulphated 50 kD glycoprotein termed GlyCAM-1 and to another 90 kD molecule [7]. The ligand binding pairs for other β2-integrins include extracellular matrix proteins, such as collagen, laminin and fibronectin [8].

A salient point is the specificity of the selectins for carbohydrate moieties. Although leucocyte adhesion to all three selectins is dependent on sLeX [6], important differences in binding characteristics have been noted. Firstly, HL60 cells treated with various proteases retain the ability to bind E-selectin but not P-selectin [9]. Secondly, growth of HL60 cells in tunicamycin, which blocks synthesis of oligosaccharide precursors, inhibits E-selectin binding to a greater extent than P-selectin [9]. Thirdly, cells which do not bind to the selectins can be transfected with the enzyme α1-3 fucosyltransferase, and will then bind to E-selectin but not P-selectin [9]. Finally, soluble E-selectin completely inhibits P-selectin binding, but soluble P-selectin only partially inhibits E-selectin binding [9]. It therefore appears that differences in the sLeX supporting

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structures (e.g. glycoprotein vs glycolipid) and subtleties in the carbohydrate configuration may be important in determining selectin ligand specificities.

The importance of distinct adhesion molecules and cytokines in mediating eosinophil recruitment during allergic inflammatory reactions

In asthma, there is now strong evidence that selective recruitment of eosinophils is a critical event. The number of eosinophils is strikingly increased in sputum, blood, and bronchoalveolar lavage fluid from asthmatic individuals [10]. The importance of these cells is also supported by the finding that inhalation of eosinophil major basic protein causes increased airway reactivity in animals, and by the correlation between eosinophil number and clinical severity, as measured by lung function, airway responsiveness, and symptom scores. The fact that eosinophils accumulate in large numbers, despite being present in peripheral blood at much lower levels, suggests that pathways must exist that selectively permit their local influx and survival.

One level of specificity may occur via the interaction between the eosinophil and the vascular endothelium. There has recently been demonstrated that both eosinophils and neutrophils bind to cytokine-activated endothelium under conditions of shear stress [11]. These adhesive interactions are due in part to L-selectin on the surface of the leucocyte, since monoclonal antibodies recognizing L-selectin inhibit adhesion. However, one unique anti-L-selectin antibody only inhibited eosinophil adhesion, suggesting that eosinophils but not neutrophils may use a distinct epitope on the L-selectin molecule during adhesion under flow conditions. Thus, eosinophils, like neutrophils, use L-selectin to bind to activated endothelium under nonstatic conditions, but potential differences in the epitopes used by eosinophils and neutrophils, as well as differences in chemoattractants and other stimuli that induce shedding of L-selectin, may play a role in situations where preferential eosinophil recruitment is observed.

Binding of eosinophils to activated endothelium under static conditions might also differ among granulocyte subtypes. For example, the sLe^ structure-containing carbohydrate ligands for E-selectin on eosinophil and neutrophils appear to be different [12], and eosinophil adhesion to endothelial cells can involve VCAM-1, a ligand for the integrin molecule VLA-4 (CD49d/CD29) on eosinophils but not neutrophils [13]. IL-4 increases VCAM-1 expression on cultured human umbilical vein endothelial cells, without altering expression of E-selectin or ICAM-1, resulting in conditions that support adhesion of eosinophils but not neutrophils [14]. Other reports confirm the importance of interleukin-4 (IL-4) driven eosinophil recruitment. IL-4 transgenic mice have allergic-like eosinophilic inflammation [15], and inoculation with a tumour cell line transfected with the IL-4 gene induces marked eosinophil accumulation at the tumour site [16].

The role of chemotactic factors in cell adhesion has attracted more attention since the discovery of the platelet factor 4/interleucin/chemokine superfamily of 8-10 kD cytokines, which appear to have restricted target cell specificity [17]. While members of the C-X-C branch of chemokines (C-X-C referring to the position of the first two cysteines in the conserved motif) are relatively specific for neutrophils, several members of the C-C branch of chemokines, such as RANTES (and to a less extent macrophage inflammatory protein-α (MIPα/2)) are chemotactic for eosinophils but not neutrophils [18, 19].

Another important step in the recruitment of leucocytes involves the process of transendothelial migration. Treatment of endothelial monolayers with IL-1 or tumour necrosis factor (TNF), which induces the expression of E-selectin, ICAM-1, and VCAM-1, was shown to increase eosinophil transendothelial migration [20]. Transendothelial migration through IL-1-treated endothelium was almost completely inhibited by antibodies to CD18 (β1 integrin) on the leucocyte, whilst anti-β2-integrin antibody had little or no effect [20]. However, when eosinophil transendothelial migration across IL-1 activated endothelium was examined in the presence of various leucocyte chemotactic factors, both β1- and β2-integrins were found to mediate eosinophil transendothelial migration [21]. These data suggest that chemokine ligands can promote VLA-4/VCAM-1 interactions in vitro, and may, thereby, activate eosinophil but not neutrophil transendothelial migration responses.

Recent work examining the interaction between extracellular matrix proteins and eosinophils suggests another avenue for the selective accumulation of eosinophils, again with special attention to the β2 family of integrins. The adhesion molecule heterodimer α6β1 (CD49f/CD29) has been shown to enhance eosinophil adhesion to laminin [22]. Binding of eosinophils via these receptors may help to immobilize them within tissues. Binding of leucocytes to fibronectin is mediated by α5β1, and for eosinophils, adhesion to fibronectin has been shown to increase their survival, an effect blocked by anti-α5 antibodies or by antibodies to IL-3, suggesting that α5β1 acts as a receptor to stimulate autocrine generation of cytokines [23].

VLA-4 is expressed on other cell types, suggesting additional mechanisms for selective eosinophil recruitment. An alternative hypothesis for the selective accumulation of eosinophils is that they may experience enhanced survival under some circumstances, such as in the presence of certain cytokines (e.g. IL-3, IL-5 or granulocyte macrophage colony stimulating factor (GM-CSF)). IL-5 selectively enhances adhesion of eosinophils, but not neutrophils, in a β2-integrin-dependent fashion [24], and neutralizing antibody to IL-5 prevents lung eosinophilia in vivo [25]. Therefore, selective eosinophil-activating cytokines may play an important role in eosinophil recruitment responses by prolonging the survival of recruited eosinophils once they have entered a tissue site.

One final piece of evidence for the existence of independent mechanisms of leucocyte recruitment is the observation that in patients with leucocyte adhesion deficiency type 1 (a disease where leucocytes are congenitally deficient in the expression of β2-integrins) neutrophils cannot be recruited into inflamed or infected skin sites, whilst eosinophils and mononuclear cells do accumulate [26]. The existence of a β2-independent pathway is also supported by the finding that anti-β2-integrin antibodies are ineffective at preventing neutrophil infiltration into the lung during experimental pneumonias caused by Streptococcus pneumoniae [27]. Interestingly, patients with leucocyte adhesion deficiency type 1 do not suffer from frequent pneumonias and are able to mobilize neutrophils into the lungs. These data illustrate important differences between the function of adhesion molecules on various leucocyte subtypes,
and suggest that differences may also exist in the types of adhesion molecules responsible for cell recruitment into different tissues.

Possible pharmacological approaches to interrupt cell adhesion events

Based on the potential importance of cell adhesion molecules in allergic and other inflammatory reactions, various approaches are being developed to generate specific antagonists. One such approach is to interrupt airways inflammation at the early steps involving leucocyte-endothelial interactions. In theory, leucocyte-endothelial interactions might be blocked with soluble, recombinant forms of the adhesion molecules, or oligopeptides derived from these molecules. However, the biological half-life and affinity for their respective ligands may limit the usefulness of this approach. Therefore, most initial studies have used blocking monoclonal antibodies developed in mice. In monkeys, infusion of monoclonal antibodies against E-selectin inhibited the influx of neutrophils associated with the pulmonary late phase response [28], whilst the eosinophil influx that occurs following repeated antigen challenge was attenuated by infusion of anti-ICAM-1 antibody [29]. In a rat model of pulmonary inflammation mediated by neutrophils, another anti-E-selectin antibody was found to attenuate lung injury [30]. Early studies using an anti-CD antibody revealed inhibition of chemotactic factor-induced accumulation of eosinophils in guinea-pig skin [31]. Since these antibodies are produced in a different species, their half-life is relatively short, and antibodies against these immunoglobulins may be produced by the host. In an attempt to avoid these problems, many companies are “humanizing” antibodies. In this approach molecular biological methods are employed to replace the mouse Fc portion of the immunoglobulin G (IgG) antibody with a human Fc portion, which preserves the structure and function of the mouse anti-human antigen binding (Fab) domains. Such reagents have already been produced, and clinical trials are underway to determine their safety and efficacy in a number of inflammatory diseases.

Since the primary amino acid structures for most adhesion molecules are known, scientists at some companies are using a different strategy. Several investigations have produced chimeric molecules containing a human Fc fragment linked in the Fab position to two molecules of the functional lectin domains of either P-, E- or L-selectin [6, 32]. These selectin-immunoglobulin fusion proteins can bind to the carbohydrate ligands present on cells recognizing the selectins expressed on cells in various tissue sites [33]. Both humanized antibodies and engineered chimeric molecules should be metabolized slowly, like human IgG, with a half-life of several weeks. This may translate into significantly prolonged therapeutic usefulness.

Analogs of the selectins may be used, not only to block cell adhesion but also to alter the functional status of leucocytes. For example, P-selectin binding has been shown to diminish superoxide production [34], and pretreatment with a soluble form of P-selectin can actually reduce neutrophil binding to endothelium [35]. These studies indicate that therapy with soluble forms of the adhesion molecules themselves has the potential to modify the immune response and suppress inflammation in a multifaceted fashion. Another approach is to develop soluble carbohydrate-based counterligands which bind to the selectins, preventing them from attaching to carbohydrate structures in vivo. Several companies are developing glycosylated structures, which act as competitive inhibitors by binding selectins and blocking selectin-mediated adhesion.

A recent trend in asthma therapy has been to focus on the inflammatory aspect of the disease, resulting in increased use of anti-inflammatory agents, such as corticosteroids. One of the major pharmacological effects of corticosteroids, which has been recognized for years, is the inhibition of cell recruitment in vivo [36]. Although the mechanisms by which this occurs is not entirely clear, it appears to be the result of the ability of corticosteroids to inhibit the production and/or release of many cytokines, some of which (e.g. IL-1, IL-4, TNF, IL-5) are probably responsible for cell recruitment in allergic inflammation [37]. Therefore, strategies that are being employed to modulate cytokine activity have the potential to prevent allergic cell recruitment, by reducing the number of circulating eosinophils or by preventing cytokine-induced expression of adhesion molecules, especially on the surface of the endothelium. Many such reagents are already in various stages of development or in clinical trials. For example, antibodies against TNF blocked mast cell-mediated neutrophil infiltration in a mouse model [38], whilst a combination of anti-IL-1 and anti-TNF antibodies blocked allergen-induced E-selectin expression in human skin explants [39]. Antibodies to cytokine receptors, soluble forms of the receptors themselves, have been used to block various proinflammatory effects of cytokines. For example, preliminary results from a phase I human trial with a soluble form of the IL-1 receptor showed remarkable efficacy in preventing the cutaneous late phase response [40]. Therefore, soluble human cytokine receptors, receptor antagonists, or cytokine-neutralizing agents may prove useful in preventing many adhesion molecule-dependent leucocyte recruitment responses.

Conclusions

The recent explosion of information in cell adhesion has dramatically improved our understanding of the mechanisms involved in leucocyte accumulation. Only when specific cytokine and adhesion molecule antagonists are developed and used in well-designed human trials will we truly understand their individual roles and myriad interactions in allergic inflammation. Whilst the potential for such agents is enormous, there are many obstacles. We still do not fully understand how various tissues differ with respect to adhesion molecules, whether the functional participation of an adhesion molecule can be altered, based on the initiating cause of inflammation, and whether tissue-specific homing mechanisms exist in humans. In addition, for the successful treatment of chronic diseases, such as asthma, effective antagonists must exist in a form that permits chronic administration (perhaps in a topically active form) and yet does not cause excessive immunosuppression. The road ahead is a long one, but through the development of adhesion molecule antagonists, investigators will finally have the necessary tools to study the role of these molecules in inflammatory diseases including asthma and allergic rhinitis.
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