

**REVIEW**

## Leucocyte-endothelial adhesion molecules and their role in bronchial asthma and allergic rhinitis

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*Leucocyte-endothelial adhesion molecules and their role in bronchial asthma and allergic rhinitis. S. Montefort, S.T. Holgate, P.H. Howarth. ©ERJ Journals Ltd 1993.*  
**ABSTRACT:** Leucocyte-endothelial adhesion molecules are involved in the initial stages of the recruitment and migration of inflammatory leucocytes from the circulation to sites of inflammation. There is accumulating evidence for their involvement in the pathophysiology of airway mucosal allergic inflammation, such as that found in asthma and rhinitis. The best characterized adhesion molecule families are the integrins, the immunoglobulin supergene family and the selectins. This review article describes some of the characteristics and properties of these families. We also discuss the situations in which these adhesion molecules might be involved in inflammatory airway diseases, and how evidence for this role might lead to future modes of therapy for these common conditions.  
*Eur Respir J, 1993, 6, 1044-1054.*

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Keywords: Adhesion molecules  
allergic rhinitis  
asthma

Received: January 11 1993

Accepted after revision April 24 1993

Mucosal inflammation is a feature of both bronchial asthma and allergic rhinitis, with immunohistochemical evidence of tissue eosinophilia, ultrastructural evidence of mast cell and eosinophil activation, and bronchoalveolar lavage analysis revealing T-lymphocyte activation [1-3]. The tissue eosinophil recruitment and the policing of the airways by T-lymphocytes requires selective cell recruitment from the circulation. The initial phase in this process is the margination and adhesion of leucocytes to the endothelium, prior to their transendothelial migration under a directed chemotactic stimulus. This adhesion, which is transient, so as to allow leucocyte movement, occurs through specific ligand-receptor couplets, involving leucocyte-endothelial adhesion molecules [4, 5]. There is now accumulating evidence that these molecules are important in the pathophysiology of asthma and allergic rhinitis.

Cell adhesion molecules (CAMs) are involved in several of the immunological processes relevant to the inflamed airways (fig. 1). Antigen-presenting cells, such as the macrophage, utilize adhesion molecules pairs, e.g. CD2 - LFA-3 (lymphocyte function associated antigen-3) and LFA-1 - ICAM-1 (intercellular adhesion molecule-1), interactions to bind to T-cells. Cell-to-cell contacts activate the T-cell to generate and release cytokines [6] within the local environment, the cytokine profile being dependent on the T-cell subtype [7]. Several cytokines, including interleukin (IL)-1, IL-4, tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) and interferon- $\gamma$  (IFN- $\gamma$ ), which may have T-lymphocyte or mast cell origins, promote induction and upregulation of adhesion molecules, both on the endothelium and on leucocyte surfaces. Induction of these adhesion molecules by either upregulation of basal expression or confor-

mational changes of the molecule concerned, with a consequent increased avidity of the molecule for its receptor, results in an increased adherence of the leucocyte to the vessel wall, and eventual transendothelial migration into the airway submucosa. Once there, the leucocyte further uses the adherence/de-adherence properties of the same molecules to migrate from one cell to another, until it reaches its target, one of which is the respiratory epithelium, where yet again adhesion molecules are expressed and aid in retaining the inflammatory cell at this site, in order to impart its destructive or protective effects. Important properties of these adhesion molecules are the specificity of the ligand-receptor pairs, their different time-courses of upregulation, their sensitivity to upregulation by different sets of cytokines, and also their differential involvement with adhesion of different types of leucocytes.

These CAMs are of interest to clinicians and immunopharmacologists alike, because their specificity and their involvement in the early stages of cell recruitment make them good candidates as possible therapeutic targets in the immunomodulation of inflammatory diseases. The rapid pace at which these adhesion molecules are being discovered dictates that this is an expanding area of research, the full implications and therapeutic potential of which are as yet incompletely defined. At present, three major groups of adhesion molecules are described, the integrins, the immunoglobulin supergene family and the selectins. This review will discuss the current classification and basic knowledge of the differing adhesion molecules and the evidence, to date, for their relevance to asthma and allergic rhinitis.

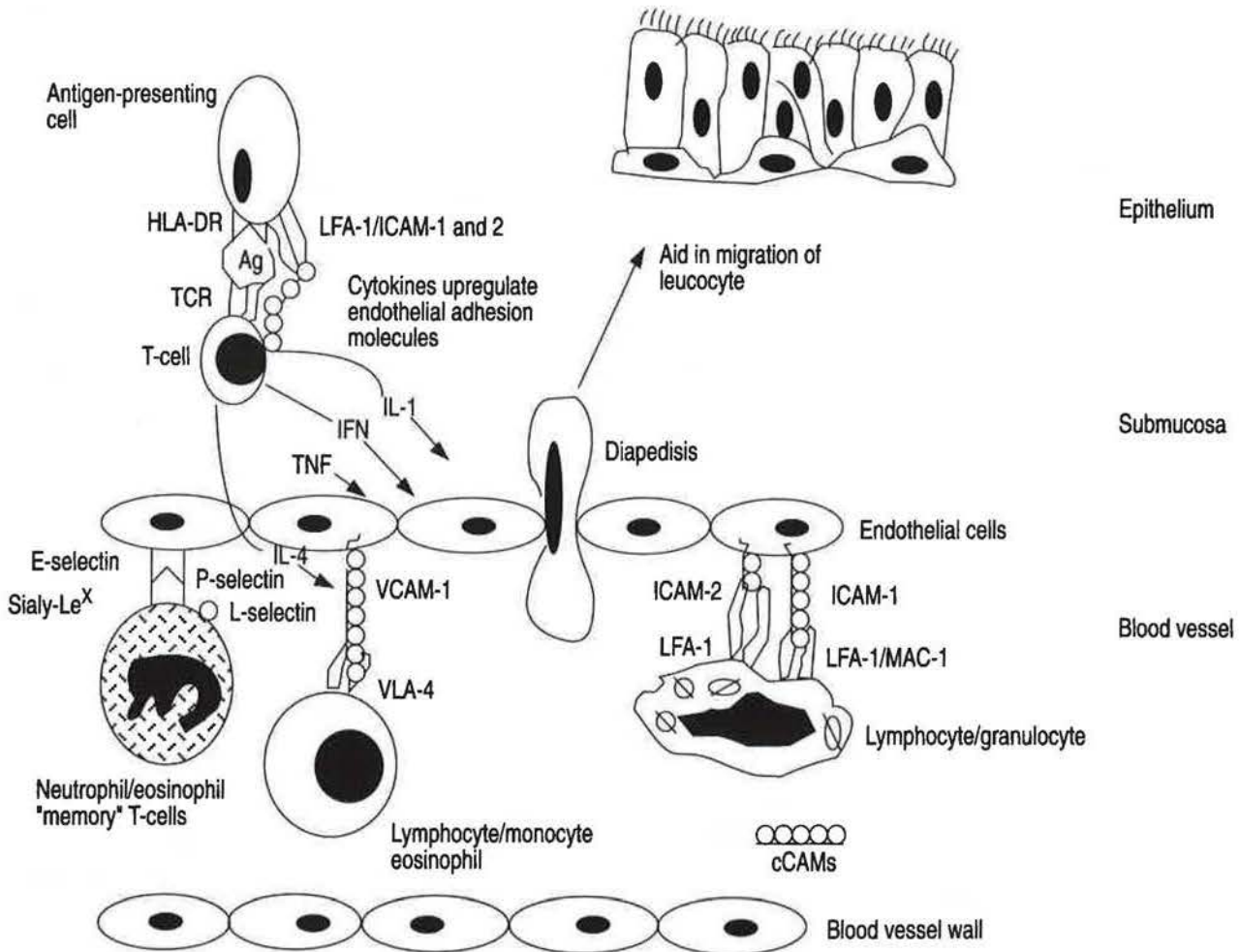


Fig. 1. - Diagrammatic representation of the main leucocyte-endothelial adhesive events in asthma/allergic rhinitis. HLA-DR: human leucocyte antigen-DR; TCR: T-cell receptor; LFA: lymphocyte function-associated antigen; ICAM: intercellular adhesion molecule; IL: interleukin; IFN: interferon; TNF: tumour necrosis factor; VCAM: vascular cell adhesion molecule; VLA: very late antigen; cCAMs: circulating cell adhesion molecules. Ag: antigen.

### The integrins

These leucocyte adhesion molecules represent a collection of heterodimeric receptors, expressed on cell surfaces, that integrate extracellular environment to cytoskeletal structure (thus the name integrins), and are involved in cell-to-cell adhesion [8]. Each member is made up of a non-covalently associated pair of subunits, designated  $\alpha$  and  $\beta$ . HYNES [9] originally subdivided the integrins into three major subfamilies. Each subfamily had a common  $\beta$  subunit, with the individual members of every subfamily having a different  $\alpha$  subunit. The subfamilies were originally referred to as  $\beta 1$ ,  $\beta 2$  and  $\beta 3$ . It is now appreciated, however, that there are at least a further five  $\beta$  subunits [8], designated  $\beta 4$ ,  $\beta 5$ ,  $\beta 6$ ,  $\beta 8$  and  $\beta p/\beta 7$  [10]. In addition, the original classification has been further complicated by the realization that the newly recognized  $\beta$  subunits also associate with  $\alpha$  subunits, already assigned to the original three integrin subfamilies. Thus, a revised classification of the integrins is now developing, in association with an improved recognition of their structure. The basic structure of each of the integrin  $\alpha$  and  $\beta$  subunits consists of a sizeable extracellular domain at the amino-terminus, a

membrane-spanning segment and a small cytoplasmic domain at the carboxy-terminus (fig. 2). Twelve of the 14 known human  $\alpha$  subunits have been deoxyribonucleic acid (DNA) sequenced, and demonstrate 25–63% homology. They can be divided into two main types. The first type undergoes post-translational cleavage into heavy and light chains, with the two parts remaining bound to each other through a disulphide bond. The second group (three of the four members of which were associated with the  $\beta_2$  integrin subfamily) contain 180–200 amino acid-long domains, and is designated the I (inserted) domain. The binding between the integrins and their respective ligands requires the presence of divalent cations ( $Mg^{++}$  and/or  $Ca^{++}$ ), with their binding site being localized to the  $\alpha$  subunit [11]. The three major human  $\beta$  subunits have also now been DNA-sequenced, and exhibit 44–47% homology, with each containing 57 cysteines, situated largely in four cysteine-rich repeat domains.

The integrins bind to specific amino acid sequences found on their ligand, and use them as cell-recognition sites. The best-known receptor sequence of this type is the arginine-glycine-aspartic acid (RGD) found on various integrin ligands (e.g. type I collagen, vitronectin and

fibronectin). Although a number of integrins use the RGD sequence for binding, most of them bind to only one specific ligand. The  $\beta 1$  (CD29) integrin family used to be referred to as the very late antigens (VLA), as the *in vitro* expression of two of its members, VLA 1 and 2 [12], increased dramatically on lymphocytes after some weeks of mitogen stimulation. The members of this subfamily most relevant to allergic airway disease are VLA-1 and VLA-4. The VLA-1+ T-cells in the lower airways are predominantly localized to the epithelium, suggestive of a homing receptor role [13]. Very late antigen-4 is the receptor for the endothelially expressed vascular cell adhesion molecule-1 (VCAM-1), and is found on monocytes, lymphocytes, basophils and eosinophils but, interestingly, not on neutrophils. The VLA-4-VCAM-1 interaction may thus contribute to the selective

eosinophilic leucocyte tissue migration observed in allergic diseases [14, 15]. As the  $\beta 1$  integrins recognize ligands, such as the three extracellular matrix (ECM) proteins, collagen, laminin and fibronectin [16], they are considered to be involved in leucocyte cell-to-cell migration. The lymphocyte-ECM interactions occurring through these integrin adhesion molecules, together with antigen stimulation, act as costimuli to the leucocyte [17]. These adhesion molecules also play a significant role in epithelial-ECM adhesion [18]. This role may be critical to the integrity of the bronchial epithelium, and a defect could contribute to the ease of epithelial disruption which characterizes the asthmatic state.

The  $\beta 2$  integrin subfamily have been termed leucocyte integrins (LeuCAM subfamily), as their expression is confined to leucocytes. They comprise three glycoproteins, namely lymphocyte function-associated antigen-1 (LFA-1), Mac-1 and p150.95 (named because of the size of its polypeptides) (table 1). These integrins are involved in leucocyte-to-leucocyte, leucocyte-to-endothelium and leucocyte-to-epithelium interactions. The common 95 kD  $\beta$  subunit in this leucocyte-adhesion molecule group has been designated CD18, and is genomically localized to the long arm of chromosome 21. The  $\alpha$  subunits of the respective LeuCAM molecules are designated as CD11a ( $\alpha L$ ) for LFA-1 (180 kD), CD11b ( $\alpha M$ ) for Mac-1 (165 kD) and CD11c ( $\alpha X$ ) for p150.95 (150 kD), all of which have been encoded on chromosome 16 [19]. Mac-1 and p150.95 molecules are present on macrophages and granulocytes, and on a subset of lymphocytes [9], while LFA-1 is present on T and B lymphocytes, macrophages, granulocytes, lymphoid tissue and bone marrow elements [20, 21].

The clinical importance of leucocyte integrin-mediated cell adhesion is emphasized by the "leucocyte adhesion deficiency (LAD) syndrome", in which an affected individual has a congenital deficiency of the CD18 ( $\beta 2$ ) subunit. This autosomal recessive immunodeficiency syndrome, which can vary in severity, is characterized by the failure of expression of all LeuCAM members and, as a result, leucocytes cannot adhere to target cells or migrate into tissue sites of inflammation. It is characterized by progressive soft tissue infections, diminished pus

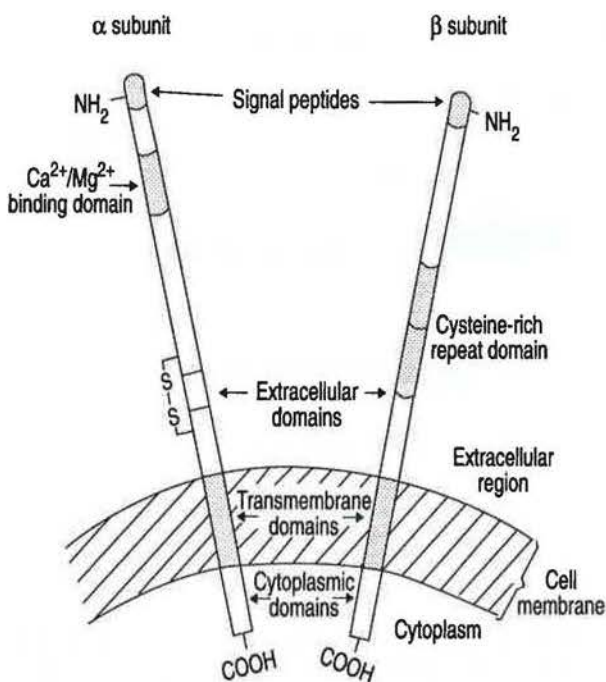


Fig. 2. - Basic structure of integrins.

Table 1. - Leucocyte-endothelial integrin adhesion molecules

Receptor	$\beta$ subunit/CD (MW)	$\alpha$ subunit/CD (MW)	$\alpha$ subunit		Ligands	RGD	Distribution	Chromosome	
			I	C				$\alpha$	$\beta$
LFA-1	$\beta 2$ /CD18 (95 kD)	$\alpha L$ /CD11a (180 kD)	+	-	ICAM-1/2/3	-	All leucocytes	16	21
Mac-1 CR3	$\beta 2$ /CD18 (95 kD)	$\alpha M$ /CD11b (170 kD)	+	-	ICAM-1 FX, LPS C3bi, FB	-	Mon, Gran NK lymph	16	21
p150.95	$\beta 2$ /CD18 (95 kD)	$\alpha X$ /CD11c (150 kD)	+	-	?	?	Mon, Gran	16	21

I: (inserted) domain C: post-translational cleavage; FB: fibrinogen; FX: factor X; LPS: lipopolysaccharide; NK: lymph: natural killer lymphocytes; Mon: monocytes; Gran: granulocytes; CD: cluster designation; LFA: lymphocyte function-associated antigen; ICAM: intercellular adhesion molecule; RGD: arginine-glycine-aspartic acid.

formation, granulocytosis and delayed umbilical cord separation [22, 23]. The granulocytosis that exists in this syndrome (5–20 times normal levels) is thought to be due to the inability of these leucocytes to migrate through the endothelium to sites of inflammation. Lymphocytes are able to migrate using CD18-independent adhesion.

Mac-1, which is also the macrophage ligand for complement receptor C3bi, and to a lesser extent p150.95, is primarily involved in myeloid cell adhesion. These LeuCAMs are stored in intracellular granules and are recruited to the cell surface following cell exposure to mediators such as C5a, platelet-activating factor (PAF) and leukotriene B<sub>4</sub> (LTB<sub>4</sub>) [24]. In addition, Mac-1 has also been demonstrated to be upregulated on the surface of eosinophils *in vitro* after stimulation with PAF, IL-3, IL-5 and granulocyte macrophage colony stimulating factor (GM-CSF) [25], and by IL-8 on the surface of neutrophils. It has been proposed that ICAM-1, which is the ligand for LFA-1 is also the ligand for Mac-1 [26], with this integrin interacting with a distinct and weaker binding site than LFA-1, the more established ICAM-1 related integrin [27].

LFA-1, which is found on all leucocytes, is heterogeneous in its expression, with a 2–3 fold higher expression on memory T-cells (CD45Ro+) than on antigen-naïve cells (CD45RA+) and, thus, adheres more readily to other cells. As a consequence, this may render them more suited for binding to the endothelium and for migrating into sites of inflammation [28]. Apart from differences in the level of expression of LFA-1 on the cell surface, LFA-1 also exists in activated and inactivated forms, this difference being related to a conformational change in the molecule, rather than an alteration in the level of cell surface expression [29]. The LFA-1, which is present on resting T-cells, is rapidly "switched on" (within minutes) when the T-cell encounters an antigen on another cell, and will serve to augment adhesion by stimulating receptors such as CD2 and CD3 molecules on T-cells [30]. Activation of LFA-1 by CD3 lasts for about 30 min, whilst

activation *via* CD2 is longer-lived. The induced conformational change in the LFA-1 molecule increases its avidity to bind the ligands ICAM-1 and ICAM-2 [29]. A further ligand for LFA-1 has recently been described, and is named ICAM-3 [31].

### The immunoglobulin supergene family

The members of this superfamily all bear a close similarity in a 90–100 amino acid domain, which was originally observed in the constant regions of the light and heavy chains of immunoglobulins [32]. This family includes as diverse a range of members as the T-cell receptors CD3, CD4 and CD8, the major histocompatibility complexes (MHC) I and II, and adhesion molecules from various systems, such as the nervous system (NCAM) and a number of leucocyte-endothelial adhesion molecules. The adhesion molecules belonging to this family, which have mainly been investigated in conjunction with inflammatory airway disease, are intercellular adhesion molecules 1 and 2 (ICAM-1 and 2), and vascular cell adhesion molecule 1 (VCAM-1) (table 2). Other adhesion molecules belonging to this family are CD2 and its ligand LFA-3.

#### Intercellular adhesion molecule 1 (ICAM-1)

The major ligand for LFA-1 and Mac-1 is ICAM-1 (CD54), which is a 76–114 kD single chain glycoprotein, with a core polypeptide of approximately 55 kD [33]. The ICAM-1 molecule is composed of a short cytoplasmic, a hydrophobic transmembranous and five immunoglobulin-like extracellular domains (fig. 3). The gene for ICAM-1 has been located on chromosome 19, but since no ICAM-1 deficiency state has been described, gene deletion is probably lethal. ICAM-2, another ligand for LFA-1 [34], differs from ICAM-1 in possessing only two immunoglobulin (Ig)-like extracellular domains, which resemble the two N-terminal domains of ICAM-1.

Table 2. – Immunoglobulin supergene family of leucocyte-endothelial adhesion molecules

Receptor	Other names	MW kD	Ligands	Distribution	Promotes adhesion of	Induced by	Upregulation
ICAM-1	CD54	76–114	LFA-1 Mac-1	Endothelium - constitutive and activated Epithelium - tonsil, thymus, renal tubules, respiratory tract Fibroblasts & dendritic cells Leucocytes & mast cells	All leucocytes	IL-1, TNF, IFN, LPS, MBP	Depends on cytokine, usually 2–4 h
ICAM-2			LFA-1	Endothelium Leucocytes	All leucocytes	Nil	
VCAM-1	INCAM-110	110	VLA-4	Endothelium - activated ? Dendritic cells	Lymphocytes Monocytes Eosinophils	IL-1, IL-4, TNF	2–4 h

VCAM: vascular cell adhesion molecule; INCAM: inducible cell adhesion molecule; NCAM: neural cell adhesion molecule; VLA: very late antigen; IL: interleukin; TNF: tumour necrosis factor; IFN: interferon; LPS: lipopolysaccharide; MBP: major basic protein. For further abbreviations see legend to table 1.

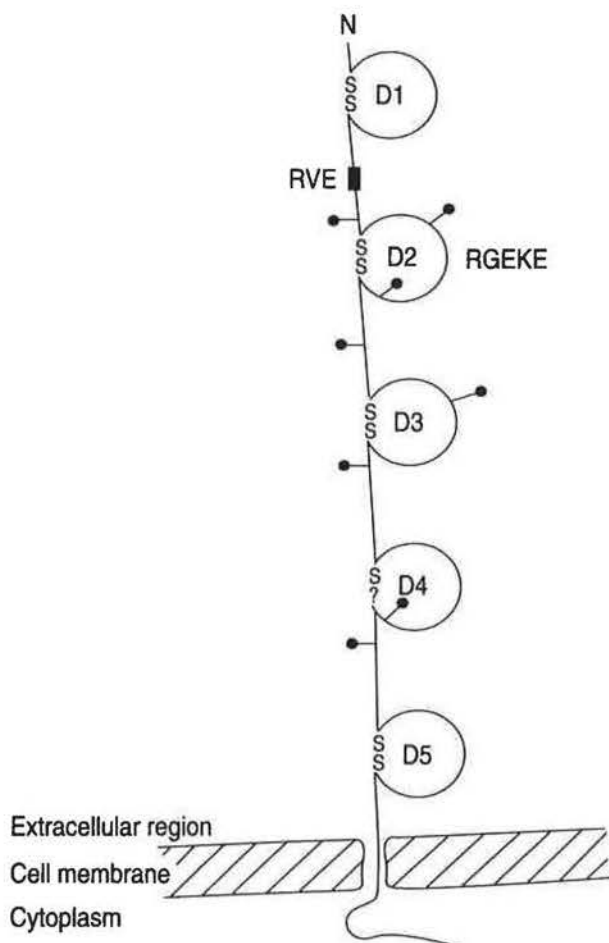


Fig. 3. — Structure of intercellular adhesion molecule-1 (ICAM-1).

ICAM-1 is constitutively expressed in a variety of tissue sites, but this expression is more widespread in inflamed tissue [35, 36]. Immunohistochemical staining of frozen sections of normal tissue have identified ICAM-1 in the skin, kidney, liver, thymus, tonsil, lymph nodes, airways and intestine. At these sites, the most prominent expression of ICAM-1 is on the vascular endothelium. ICAM-1 expression has, however, also been identified on reticular cells, macrophages in the germinal centres of secondary lymphoid follicles, on fibroblast-like and dendritic cells, in thymic epithelial cells, in the mucosal epithelium of the tonsil [37], on mast cells [38], and occasionally, to a low degree, on peripheral blood leucocytes.

This adhesion molecule is involved in T-cell migration, as evidenced by its strong expression on endothelial cells in areas exhibiting delayed hypersensitivity in the skin [39]. Neutrophil migration through endothelial cell monolayers is blocked by ICAM-1 monoclonal antibodies (MoAbs) [40, 41], suggesting a role for ICAM-1 in the passage of this granulocyte type to sites of tissue inflammation [42]. The eosinophilia observed in the epithelium and submucosa of tracheal sections from allergen-challenged sensitized monkeys has also been shown to be attenuated following an ICAM-1 MoAb inhalation or intravenous infusion [43, 44], implying that

ICAM-1 is also important in eosinophilic-endothelial interactions. This, and the fact that the monkeys' allergen-induced airway hyperresponsiveness was also blocked, suggests a role for this adhesion molecule in asthma.

WALSH *et al.* [25] have shown that IL-5 enhances eosinophil adhesion selectively *in vitro*, but has no effect on neutrophil adhesion. The same group has shown that this IL-5 induced upregulation was Mac-1- and to a lesser extent LFA-1-dependent, thereby implying a relationship between ICAM-1 and its ligands to selective eosinophil adhesion. Another interaction between ICAM-1 and eosinophils has been reported by AYARS *et al.* [45], in showing that physiological concentrations of eosinophil granule-derived major basic protein (MBP) upregulated ICAM-1 expression on human cultured nasal cells by up to 50%. This provides a mechanism by which eosinophils, at the site of airway inflammation, further increase adhesion and chemotaxis for other cells of the same type.

In addition to its role in allergic inflammation, ICAM-1 is also likely to be a key factor to viral induced exacerbations of airways disease, as this molecule is also the surface receptor for the major group of rhinoviruses [46, 47], which comprises about 80% of rhinoviruses, and cause about 50% of common colds. The additional observation that rhinovirus colonization of the epithelium leads to increased IFN- $\gamma$  production, which in turn upregulates ICAM-1 expression, suggests that this might serve to amplify the vulnerability of the bronchial epithelium for virus infection. It is important to note that the rhinovirus utilizes a distinct but overlapping binding site on the most distal immunoglobulin-like domain of ICAM-1 from that used by LFA-1 [48]. It is interesting to note that ICAM-1 is also the receptor for *Plasmodium falciparum* infected erythrocytes [49].

ICAM-1 is expressed basally in a number of cell types. However, its levels of expression, and the number of cell types on which it is expressed, increases on exposure of cells to cytokines *in vitro*, and by inflammatory processes *in vivo*. IFN- $\gamma$ , IL-1, TNF $\alpha$  and lipopolysaccharide (LPS) upregulate ICAM-1 expression to varying degrees on different cell types [39]. The time-course for the initiation and complete expression of ICAM-1 starts at 2–4 h, reaching a plateau at 24 h, but with continuing expression for a further 24–72 h while in the presence of the cytokines [50, 51]. In contrast to ICAM-1, the related molecule ICAM-2 does not appear to be inducible by a range of cytokines on a number of cell types so far tested [34], and seems to be the main LFA-1 ligand on resting endothelium.

#### Vascular cell adhesion molecule-1

VCAM-1 is the most recently discovered member of the supergene Ig family. There are two known forms of this 110 kD glycoprotein adhesion molecule, one with six and the other with seven extracellular unpaired Ig-like domains. The seven domain form, which has an extra domain spliced between two similar triads of three domains each, is the major form of the two [52]. VCAM-1, as its name suggests, is potentially found on vascular

endothelial cells. There is basal expression on 5–20% of vessels in lymphoid tissue, and under resting conditions it is absent at other tissue sites, such as skin [53]. It is induced by IL-1, TNF $\alpha$  [54, 55], LPS and IL-4 [56], but unlike ICAM-1 not by IFN- $\gamma$ . Increased expression can be identified within 2 h of stimulation, and this upregulation lasts for 72 h. VCAM-1 has been shown to adhere lymphocytes, monocytes and eosinophils in a CD11/18-independent fashion. Its receptor on these leucocytes is the lymphocyte-homing receptor, the  $\beta$ 1 integrin, VLA-4. This integrin is also a receptor for fibronectin, but ELICES *et al.* [57] reported that VCAM-1 interacts with a determinant on VLA-4 which is distinct from that involved in interacting with fibronectin. As already mentioned, VLA-4 is on the surface of eosinophils and a MoAb to this integrin has been shown to specifically inhibit eosinophil, but not neutrophil, adhesion to IL-1 stimulated human umbilical vein endothelial cells (HUVECS) [15, 16]. The identification of the fact that IL-4 selectively upregulates VCAM-1 on vascular endothelium [15, 56], and the specificity of the VCAM-1-VLA-4 interaction provides a potential explanation for the eosinophil airway recruitment in allergic rhinitis and asthma.

### The selectins

The selectins are a family of adhesion molecules (table 3), each containing: an amino terminal C-type lectin domain, followed by an epidermal growth factor-like module, and a variable number of repeating units similar to those in complement-binding proteins, a transmembrane domain and a short cytoplasmic tail [58] (fig. 4).

E-selectin (previously known as endothelial-leucocyte adhesion molecule-1 (ELAM-1)) is a cell-surface glycoprotein, with a MW of 113 kD, which *in vitro* is found on vascular endothelial cells only when the latter are "activated" [59]. E-selectin is inducible by bacterial endotoxin and cytokines IL-1 and TNF $\alpha$ . Cytokine-induced upregulation of this selectin occurs within 30 min, peaks at 2–4 h and declines back to basal levels by 24 h.

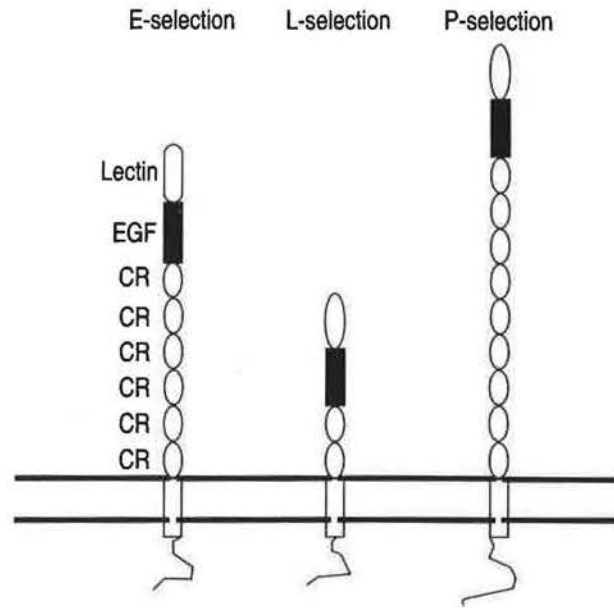


Fig. 4. – The structures of the selectin subfamily members. EGF: epidermal growth factor-like molecule; CR: complement-regulatory domains.

E-selectin mediates neutrophil migration and their adhesion to endothelium [60], with a role in the recruitment of memory T-lymphocytes [61] and monocytes. In addition to the blocking neutrophil adhesion, KYAN AUNG *et al.* have observed that an anti-E-selectin MoAb partially inhibited eosinophil adhesion to stimulated endothelial cell monolayers [62]. This might suggest that E-selectin also contributes to eosinophil adhesion and migration in allergic processes. Consistent with this, E-selectin, along with ICAM-1, has been shown to be expressed in skin biopsies from atopic subjects 6 h after intradermal allergen challenge, at a time when tissue eosinophil recruitment was evident [62, 63]. The role of E-selectin in eosinophil airway recruitment has, however, been questioned, as an anti-E-selectin MoAb has not been found to inhibit eosinophil airway infiltration induced by repeated challenges

Table 3. – The selectin/LEC-CAM family of leucocyte-endothelial adhesion molecules

Receptor	Other names	MW kD	Ligands	Distribution	Promotes adhesion of	Induced by	Upregulation
E-selectin	ELAM-1	110–115	Sialyl-Le <sup>x</sup>	Endothelium	Granulocytes, "memory" T-cells	IL-1, TNF, LPS	30 min
P-selectin	GMP-140 CD62 PADGEM	140	?Le <sup>x</sup> CD15	Endothelium Platelets	Neutrophils, (platelets-leucocytes)	Thrombin, histamine, PAF, LTC <sub>4</sub>	5 min
L-selectin	LECAM-1 LAM-1, Leu 8 gp90 <sup>me114</sup>	90	?E-selectin ?P-selectin	Lymphocytes, monocytes, granulocytes			Shed upon cell activation

LEC-CAM: lectin-cell adhesion molecule; ELAM: endothelial leucocyte adhesion molecule; GMP: granule-membrane protein; PADGEM: platelet activation-dependent granule-external membrane protein; LECAM: lectin adhesion molecule; LAM: leucocyte adhesion molecule; PAF: platelet activating factor; LTC<sub>4</sub>: leukotriene C<sub>4</sub>. For further abbreviations see legend to tables 1 and 2.

with *Ascaris* antigen [64], but does block late phase bronchoconstriction and neutrophil influx after a single inhalation challenge, in a primate model of asthma [65]. The ligand for E-selectin has recently been identified as a sialyl-fucosyl pentasaccharide determinant on granulocyte surface glycoproteins, named Sialyl-Le<sup>x</sup> [66].

Another member of the selectin family is P-selectin (formerly known as granule-membrane protein 140 (GMP-140) or platelet activation-dependent granule-external membrane protein (PADGEM), (CD62) [58, 67]. This is a 140 kD glycoprotein found in the  $\alpha$  secretory granules of platelets and the Weibel-Palade bodies of endothelial cells. Its main characteristic is its ability to be mobilized to the cell surface in less than 5 min by  $\alpha$ -thrombin, LTC<sub>4</sub> and other rapidly-acting mediators, such as histamine and PAF, to promote the rapid onset of adhesion between the endothelium and leucocytes and platelets [67], and thus is important in the initial stages of this phenomenon, referred to as "rolling". The counterligand for P-selectin is possibly also Sialyl-Le<sup>x</sup>. In the interactions between platelets and monocytes/neutrophils, P-selectin binds to CD15.

The third selectin in the series is the lymphocyte-homing receptor, which in the mouse is recognized by Mel-14 antibody, and is known as L-selectin (formerly lectin adhesion molecule-1 (LECAM-1)). It is expressed by monocytes, lymphocyte subsets and granulocytes [68]. Using MoAbs recognizing neutrophil L-selectin and anti-CD11a, CD11b, CD18 antibodies, SMITH *et al.* [69] have shown *in vitro*, that this adhesion molecule determines the adhesion of granulocytes to HUVECS when they are exposed to high wall shear stress forces, whilst the integrins dominate when the neutrophils are chemotactically stimulated. L-selectin is downregulated and shed from the leucocyte surface once the cell is adherent to the endothelium or becomes activated. The level of L-selectin is also reduced after unstimulated neutrophils are in contact with IL-1 activated endothelium for 30 min - a phenomenon repeated when neutrophils are exposed to supernatant produced by these endothelial cells, after exposure to PAF and endothelial IL-8 [70]. Thus, immobilization of granulocytes on the endothelium by L-selectin is terminated by endothelial factors, which thereby contribute to the transient nature of the process. L-selectin contains some of the cellular Sialyl-Le<sup>x</sup> on its surface and might actually bind to the other selectins.

Thus the selectins seem to be most prominent in the early stages of adhesion, especially in the case of neutrophils. Once the endothelium is stimulated, this induces leucocytes to marginate and roll on the vessel wall. This rolling involves adherence/de-adherence with the endothelium and the weak and rapidly induced adhesive properties of selectins are suited for this phenomenon [71]. Once the leucocyte is activated or is adherent to stimulated endothelium, the L-selectin molecules are shed off [70]. At this stage, if the inflammatory process is to proceed, other adhesion molecules, such as ICAM-1 and its ligands, become involved and this results in firmer adhesion of the leucocyte. These molecules then aid the leucocyte to migrate between the endothelial cells into the tissues [72].

### Other adhesion molecules

A number of adhesion molecules have been described, but not allocated to specific families. The first of these is intercellular adhesion molecule-3, which as already mentioned is a recently discovered ligand to LFA-1 [31]. This molecule was thought to exist, due to the fact that MoAbs to ICAM-1 and ICAM-2 could not completely block the CD18 dependent adhesion between many lymphoid cell lines, especially the SKW3 cell line [31]. In contrast to ICAM-1 and ICAM-2, ICAM-3 is not expressed on endothelium, even when the latter is stimulated. It is only found on leucocytes, especially on lymphoid and monocytic lines. It is expressed to a higher degree than ICAM-1 or ICAM-2 on resting leucocytes, but on activated lymphocytes it is not upregulated to the same extent as ICAM-1 [31, 37]. Thus, ICAM-3 seems to determine LFA-1 dependent adhesion on resting lymphocytes.

Other classes of adhesion molecules are vascular addressins and lymphocyte homing receptors, which together aid lymphocytes to home onto and adhere to specific types of endothelium on specific lymphoid organs - either peripheral or mucosal [73, 74]. The integrins, LPAM-2 and LFA-1, and the selectin, L-selectin, have been implicated in lymphocyte homing, as has the hyaluronic acid receptor, CD44 [75].

### Circulating forms of adhesion molecules

Recently, circulating forms of these adhesion molecules have been identified in the peripheral blood and other body fluids of normal subjects and in various diseases [76]. Circulating ICAM-1 (cICAM-1) contains at least four out of the five extracellular domains of the endothelial-attached form, and has been identified in serum and the supernatants from mononuclear cell cultures [77]. Serum levels of cICAM-1 have been found to be increased in patients with gastrointestinal malignancies, especially those which have metastasized [78], and in serum of subjects with the leucocyte adhesion deficiency (LAD) syndrome in which the  $\beta$ 2 chain (CD18) is genetically deficient, resulting in the absence of the ligands for ICAM-1. Circulating ICAM-1, cVCAM-1 and cE-selectin have also been detected in bronchoalveolar lavage of patients with interstitial lung disease [79]. The levels of cE-selectin [80], and of cICAM-1 [81] were also increased in bronchoalveolar lavage (BAL) from ragweed sensitive asthmatics, 19 and 48 h post-segmental allergen challenge, respectively. The origin and function of these soluble forms of adhesion molecules is still unknown, although by binding to ligands on leucocyte surfaces they may serve to block adherence to tissue ligands and, thereby, limit the inflammatory process.

### Evidence for adhesion molecules in asthma and rhinitis

WEGNER and co-workers [43, 44, 64, 65] have carried out a number of studies on a primate model of asthma, to

study the role of adhesion molecules in allergic airway inflammation. A blocking anti-ICAM-1 MoAb, both when administered intravenously [44], and by inhalation [43], to these *Ascaris*-sensitized monkeys, attenuated the intense tracheal mucosal eosinophilia and the accompanying airway hyperresponsiveness induced by multiple allergen inhalation. This group also showed that, in primates with chronically inflamed airways, this anti-ICAM-1 MoAb did not reduce the pre-existing airway inflammation, but when administered after 7 days of dexamethasone therapy it prevented the recurrence of allergic airway inflammation [82]. These findings suggest that ICAM-1 is predominantly involved in the primary influx of inflammatory cells, including eosinophils, into the airway submucosa, but that inhibition of this pathway does not resolve existing inflammation. A MoAb acting against E-selectin failed to reduce either acute allergen-induced [64] or chronic allergen exposure-induced eosinophilia in these primates, but did prevent the late-phase bronchoconstriction and neutrophilia occurring 6 h post-allergen challenge [65]. Thus, E-selectin seems to be primarily involved in neutrophil, rather than eosinophil, airway recruitment.

Bronchial allergen challenge studies in human asthma have identified challenge related increases in ICAM-1 and E-selectin at 5–6 h post-challenge [83], and VCAM-1 24 h following allergen exposure [84]. This upregulation of ICAM-1 and E-selectin in the airways is comparable to findings in the skin [63], and conjunctiva [85], at similar time-points following local allergen challenge. Within the airways, the upregulation of ICAM-1 and E-selectin was associated with an increase in neutrophils, eosinophils, T-lymphocytes and mast cells in comparison to a saline-challenged control site [83]. Thus, the enhanced expression of ICAM-1 and E-selectin, while promoting tissue inflammation, does not confer specificity for the characteristic airway inflammation of asthma. The later upregulation of VCAM-1 at 24 h post-allergen challenge was, however, associated with a tissue eosinophilia and an accumulation of T-lymphocytes, suggestive of the relevance of this immunoglobulin adhesion molecule to chronic airway inflammation in asthma, through interaction with VLA-4 positive leucocytes.

Consistent with this, immunohistochemical investigation of nasal biopsies in perennial and seasonal allergic rhinitis have identified enhanced expression of VCAM-1, with, in addition, ICAM-1 in perennial rhinitis [86]. Within the normal nose there is constitutive expression of ICAM-1 and to a lesser extent E-selectin, but not VCAM-1. The enhanced expression of VCAM-1 in allergic rhinitis can be linked to the local release of the cytokines TNF $\alpha$  and IL-4, both of which have been co-localized to airway mast cells, and the release of which would be anticipated as mast cell degranulation is also a feature of this disease [87, 88]. Although mast cell degranulation is also a feature of allergic asthma, early immunohistochemical studies have failed to reveal any difference in the expression of ICAM-1 or E-selectin in airway biopsies from non-asthmatic and allergic asthmatic subjects [89]. These studies, in contrast to the nasal studies which were undertaken in methacrylate embedded tissue allowing better quantification

of CAM expression, were undertaken in snap frozen biopsies in which the morphology is poorly preserved and the immunostaining less distinct. It is thus possible that disease-related abnormalities could have been underestimated in mild asthma, due to the insensitivity of the method of detection. Consistent with this, upregulation of CAM expression has been reported in endobronchial biopsies from the lower airways of patients with non-atopic asthma, a disease group often characterized by more florid airways inflammation than that encountered in mild atopic asthma [84].

Thus, in order to address dynamic changes within the airways in patients with asthma, we have investigated changes in the levels of cCAMs in association with acute exacerbations of their disease requiring hospitalization [90]. Although the function and origin of the circulating forms of these adhesion molecules is unknown, increased levels have been noted in various conditions such as gastrointestinal malignancies [78], and pulmonary interstitial fibrosis [79]. Utilizing sandwich enzyme-linked immunosorbent assays (ELISAs), we measured the levels of these adhesion molecules in the peripheral blood of asthmatics on days 1, 3 and 28 after an acute asthmatic episode, stable asthmatics and atopic and non-atopic normal volunteers [90]. As in the case of the biopsies we found no significant difference between the stable asthmatics and normals, but cICAM-1 and cE-selectin levels were increased during the three acute asthma days.

The lack of a complementary increase in cVCAM-1 could be due to a cytokine profile in acute asthma which is more suited to upregulation of cICAM-1 and cE-selectin. Despite clinical improvement, however, there was a lack of downregulation of these two circulating adhesion molecules within the 28 days post-acute asthmatic episode, even though all patients received conventional high dose corticosteroid therapy. The failure to identify a reduction in levels over this time period, despite lower levels in chronic persistent asthma, might suggest the subclinical persistence of airway inflammation. If so, cICAM-1 or cE-selectin may prove valuable markers for indirectly monitoring acute airway inflammation. Further studies are, however, required before this can be recommended.

In addition, the prevalence of cICAM-1 in acute convalescent asthma could relate to a viral exacerbation of the disease. ICAM-1 is the surface receptor for the major group of rhinoviruses, which have been recovered in nasal washings from 70% of school children with acute episodes of clinical deterioration in their asthma [91], and rhinovirus colonization of epithelium promotes IFN- $\gamma$ , which in itself promotes epithelial ICAM-1 expression. The expression of ICAM-1 in the airway epithelium, which is enhanced in the basal cell layer [89], provides an explanation for the tendency for upper respiratory tract infections to exacerbate asthma, and in experimental investigation, to lead to physiological deterioration for 4–6 weeks post airway inoculation.

There have been no studies in human asthma and rhinitis investigating the modulating effect of anti-CAM MoAbs on disease expression, either in relation to acute disease exacerbation or in chronic disease. Such studies, mirroring those performed by GUNDEL *et al.* [65] in their



primate model, will allow dissection of the contribution of CAMs to airway inflammation and disease expression. The evidence to date, however, suggests the relevance of these molecules to both asthma and rhinitis, and the regulation of their expression or the antagonism of their actions offers novel future potential methods for disease regulation in both the upper and lower airways.

**Acknowledgements:** The authors would like to thank C. Martin for typing this manuscript.

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