CAMs and anti-CAMs
The clinical potential of cell adhesion molecules

The survival of any complex multicellular organism depends on the ordered and controlled interaction of its various specialised cells with one another and with the surrounding extracellular matrix (ECM). In the case of leukocytes, migration into and within the tissues requires the co-ordinated expression and function of cell adhesion molecules (CAM) which mediate adhesion to other cells and to components of ECM. The application of monoclonal antibody and molecular biological techniques has considerably increased our understanding of the mechanisms of leukocyte adhesion. This in turn has given new insights into the pathogenesis not only of inflammation but also of other processes such as viral infection and tumour spread.

Whilst much of the work to date has been at a basic scientific level, appreciation of the central role played by CAMs in determining leukocyte function is increasingly bringing this subject into the clinical arena. This article reviews some of the recent developments, with particular attention to possible clinical applications.

Classification of leukocyte adhesion molecules

The classification and detailed description of leukocyte adhesion molecules and their ligands has been the subject of recent reviews [1, 2]. A problem faced by many non-immunologists is the puzzling number of synonyms and abbreviations that are used to describe the various molecules. Where possible we have therefore included in this article the cluster of differentiation (CD) designations agreed by international typing workshops. The molecules of particular interest for leukocyte traffic fall into the following gene families.

Selectins and carbohydrates

The selectins are a recently described group of three single-chain cell surface glycoproteins (LECAM-1, GMP-140 and ELAM-1)* which have in common an N-terminal lectin domain (Fig. 1). In each case the lectin domain is thought to bind specific carbohydrate residues present on glycoproteins or perhaps glyco-lipids on the surface of certain other cell types. Selectin–carbohydrate interactions can occur at low temperatures (4°C) and are relatively independent of intracellular metabolic events. Each of the three selectins is therefore thought to play a part in the tethering of unstimulated leukocytes to the luminal surface of endothelial cells (EC) prior to transmigration into the tissues.

During physiological lymphocyte recirculation, lymphocytes pass from the blood into fixed lymphoid tissues via specialised blood vessels known as 'high endothelial venules' (HEV) because of their distinctive cuboidal or columnar endothelial cells. There is evidence that the surface molecules on HEV to which lymphocytes bind vary between lymphoid organs, giving rise to the term 'addressin' to describe endothelial–leukocyte adhesion molecules of restricted anatomical distribution [3]. LECAM-1 was initially identified and characterised in the mouse as a surface molecule involved in the 'homing' of recirculating lymphocytes to HEV in peripheral lymph nodes but not in Peyer's patches. Since then it has emerged that LECAM-1 is found on other leukocyte types besides lymphocytes and probably has a more general role in leukocyte adhesion to EC than originally proposed. There are differences in the size of LECAM-1 obtained by immunoprecipitation from neutrophils and lymphocytes, probably reflecting differential post-translational modification. There may therefore be different LECAM-1 ligands, depending upon leukocyte lineage.

Whereas LECAM-1 is found on leukocytes, GMP-140 (CD62) and ELAM-1 are endothelial molecules. GMP-140, which is also a constituent of platelet α granules, is stored within intracellular organelles known as Weibel–Palade bodies and is translocated within minutes to the cell surface after activation by agonists such as thrombin or histamine [4]. In contrast, synthesis of ELAM-1 does not occur constitutively but requires gene induction by cytokines (see below). Both GMP-140 and ELAM-1 bind neutrophils, eosinophils and monocytes. In addition, recent reports indicate that ELAM-1 also binds a subset of peripheral blood memory T lymphocytes [5, 6].

Understanding of the carbohydrate ligands for the selectins is in its infancy. A 50 kd sulphated endothelial

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* Added in proof. There has been a recent consensus to change the names of the three members of the selectin family to L-selectin (LECAM–1), E-selectin (ELAM–1) and P-selectin (GMP-140) (Bevilacqua M, Butcher EC, Harlan J, *et al.* Selectins: a family of adhesion receptors. *Cell* 1991; **67:**223).

Fig. 1. *Selectins.*

glycoprotein which binds LECAM-1 has recently been isolated from mouse lymph nodes [7], whilst the sialylated and possibly also unsialylated forms of the poly-lactosamine blood group determinant 3-fucosyl-*N*-acetyl-lactosamine (Lewis X) have been identified as ligands for ELAM-1 and GMP-140 [8].

Integrins

The integrins derive their name from their essential function of integrating the intracellular cytoskeleton with the extracellular environment. Integrins are therefore intimately involved in regulating spatial orientation and cellular movement.

The integrin family comprises a large group of glycoproteins found in one form or another on most animal cell types. Each integrin is a heterodimer composed of non-covalently linked heavy (α) and light (β) chains (Fig. 2). At present there are at least 16 recognised integrins, made up from 11 known α chains and 7 known β chains. The integrins of most importance for leukocyte function are those involving the β_1 (CD29) and β_2 (CD18) chains (Table 1).

The six molecules in the β_1 family [otherwise known as the very late antigen (VLA) family] mainly function as receptors for components of ECM, such as fibronectin (VLA-3, 4, 5), collagen (VLA-1, 2, 3) and laminin (VLA-1, 2, 3, 6), although VLA-4 additionally binds the cell surface molecule VCAM-1 (see below). Whilst the VLA molecules are widely distributed on different cell types, their expression by leukocytes is largely restricted to monocytes and lymphocytes.

Recently VLA-4 has also been described on eosinophils [9].

In contrast to the β_1 integrins, expression of the β_2 family (also called the Leu-CAM family) is confined to leukocytes. LFA-1 ($\alpha_L\beta_2$; CD11a/CD18) is present on the surface of virtually all circulating leukocytes, whilst Mac-1 ($\alpha_M\beta_2$; CD11b/CD18) and p150,95 ($\alpha_X\beta_2$; CD11c/CD18) have more limited distributions on neutrophils, monocytes and natural killer cells. β_2 integrins function as accessory molecules in leukocyte interactions with other cells by binding the immunoglobulin molecules ICAM-1 (LFA-1 and Mac-1) and ICAM-2 (LFA-1 only) (see below) [10]. Mac-1 and p150,95 also

Fig. 2. *Integrin groups.*
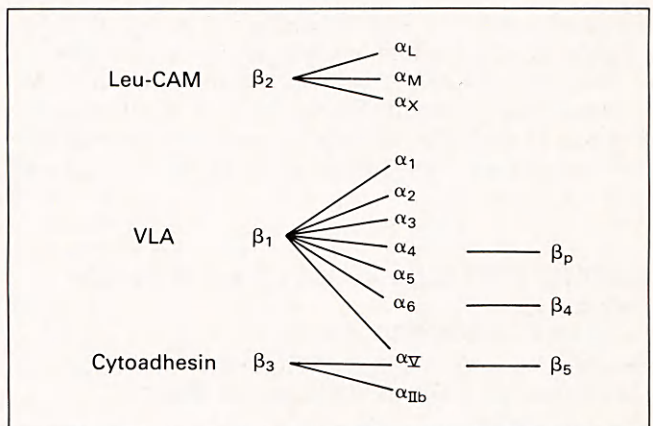
function as receptors for the inactivated complement protein C3b (iC3b) and are therefore involved in phagocytosis of complement coated bacteria.

In contrast to selectins, the capacity of some (and possibly all) integrins to bind their ligands depends on cellular metabolism in response to chemotactic and other factors. Integrins undergo rapid fluctuations in avidity for their ligands, allowing these molecules to play a key role in the adhesion/de-adhesion events required for cell migration. β_2 integrin avidity changes probably involve altered $\alpha\beta$ conformation in response to phosphorylation and require a critical segment of the β chain cytoplasmic tail [11].

In the case of myeloid cells, preformed Mac-1 and p150,95 can be recruited from intracellular granules resulting in increased surface expression. Although this may lead to a general increase in cell adhesiveness it is not critical for changes in adhesion. Immunostaining of the cell surface for integrins with most available monoclonal antibodies is therefore not a precise indication of the adhesive function of the cell.

Clinical significance of integrins. Dramatic evidence for the importance of β_2 integrins comes from a rare congenital disease, leukocyte adhesion deficiency (LAD), characterised by recurrent bacterial infections [12]. Several molecular defects have been shown to result in LAD, the common denominator being a failure of CD18 synthesis with an inability to assemble the $\alpha\beta$ integrin complex into the cell membrane. Although the peripheral blood neutrophil count is high (up to 150/μl), infected tissues contain few neutrophils, demonstrating a defect in leukocyte emigration.

The gene encoding the CD18 is situated on chromosome 21. In trisomy 21 (Down syndrome) there is an increased expression and function of LFA-1 on lymphocytes. It is therefore possible that disregulated intercellular adhesion may play a role in the impaired immune response in Down syndrome [13].

Immunoglobulins

The immunoglobulin supergene family is a large group of molecules which includes a number of cell membrane glycoproteins with structural homology to antibodies [14]. Characterisation of the ligands which bind leukocyte integrins has led to the discovery of three single-chain glycoproteins which function as ligands for integrins. These are intercellular adhesion molecule-1 (ICAM-1, CD54), ICAM-2 and vascular adhesion molecule-1 (VCAM-1) (Table 2). The immunoglobulin family includes several other adhesion molecules, including CD2 and its ligand LFA-3 (CD58).

Besides functioning as an adhesion molecule, ICAM-1 is now known to be a cell surface receptor for the major group of rhinovirus serotypes responsible for the common cold and also for *Plasmodium falciparum* infected erythrocytes [15, 16]. The rhinovirus

Table 1. β_1 and β_2 integrins

β subunit	α subunit	Usual title	Ligand(s)
β_1	α_1	VLA-1	Laminin, collagen
β_1	α_2	VLA-2	Collagen, laminin
β_1	α_3	VLA-3	Laminin, fibronectin, collagen
β_1	α_4	VLA-4	Fibronectin, VCAM-1
β_1	α_5	VLA-5	Fibronectin
β_1	α_6	VLA-6	Laminin
β_2	α_L	LFA-1	ICAM-1, ICAM-2
β_2	α_M	Mac-1	ICAM-1, iC3B, fibrinogen
β_2	α_X	Gp 150/95	iC3B, fibrinogen

binding site on ICAM-1 has been mapped to the amino terminal C2 domain and is distinct but overlapping with the site for LFA-1 attachment [17].

Role of leukocyte adhesion molecules in inflammation

Direct inspection of the microvasculature shows that circulating leukocytes normally 'roll' along the surface of the vessel wall [18]. In inflamed tissues the rolling leukocyte becomes tethered to endothelium and subsequently transmigrates through the vessel wall into the tissues. To a large extent these different events in leukocyte emigration depend upon expression and activation of appropriate adhesion molecules on endothelial cells and leukocytes in response to the mediators of the various forms and stages of inflammation.

Endothelial cells and other resident cells

The critical mechanism localising inflammatory lesions is probably the activation of adhesion molecule expression on endothelial cells and resident cells within the tissues. Endothelial cells can undergo different phases of activation, each associated with the appearance of different adhesion molecules for leukocytes. In very early inflammatory lesions translocation of preformed GMP-140 to the endothelial cell surface allows binding of neutrophils, monocytes and probably eosinophils. This is followed after 4–6 hours by a subacute phase of endothelial activation, governed by the

Table 2. Adhesion molecules in the immunoglobulin family

Ligands	Where found	Receptor(s)
ICAM-1	Many cell types	LFA-1, Mac-1
ICAM-2	Many cell types	LFA-1
VCAM-1	Endothelial cells, macrophages, dendritic cells	VLA-4
CD2	Lymphocytes	LFA-3
LFA-3	Many cell types	CD2

induction of a number of genes in response to interleukin-1 and/or tumour necrosis factor and involving the *de novo* synthesis and expression of ELAM-1, ICAM-1 and VCAM-1 [19]. The expression of these three molecules is differentially regulated by the lymphokines interferon gamma (IFNγ) and interleukin-4 (IL-4), potentially resulting in alterations in their relative densities in different forms of immune-mediated inflammation and thereby predisposing to the migration of lymphocytes and monocytes [20]. The nature of the endothelial adhesion molecules responsible for lymphocyte traffic in established chronic inflammatory lesions is not yet clear but could include 'addressins' similar to those involved in lymphocyte recirculation.

Cytokines also regulate the expression of adhesion molecules on other resident cells such as keratinocytes, fibroblasts and synoviocytes, with the optimal stimuli for expression of individual molecules varying with cell type.

Regulation of leukocyte adhesiveness

The successful transmigration of leukocytes from the luminal surface of endothelium into the tissues is likely to depend upon stimulation of leukocyte adhesion molecule function during the initial tethering event. Activation of neutrophils by chemotactic stimuli leads to the shedding of LECAM-1, allowing de-adhesion from endothelial ligands. At the same time there is upregulation of leukocyte integrin expression and function, and this allows integrin mediated migration to commence [21]. The precise stimuli which mediate these events are not well defined but may be secreted either by activated endothelial cells themselves or by other cells in the perivascular environment. An important principle is that chemotactic factors may have specific effects on the adhesion molecule function of particular leukocyte types, resulting in different patterns of cell migration. For example, interleukin-8 and macrophage chemotactic and activating factor are selectively chemotactic for granulocytes and monocyte/macrophages respectively. Other factors that may be involved include leukotriene B4, f-Met-Leu-Phe, and granulocyte–macrophage colony stimulating factor.

The availability of markers for unsensitised 'naive' and sensitised 'memory' T lymphocytes has led to fresh insights into which types of lymphocytes predominantly recirculate through fixed lymphoid tissue and which enter sites of inflammation. Whilst there are approximately equal numbers of naive (CD45RA+) and memory (CD45RO+) T cells in peripheral blood, the large majority of T cells in inflammatory tissues are of the memory phenotype, indicating that memory T cells have a greater capacity than naive cells to enter non-lymphoid tissues, particularly during inflammation [22]. This may in large part be due to the increase in surface density of several adhesion molecules (including LFA-1, ICAM-1, CD2, LFA-3, VLA-4, VLA-5, VLA-6 and CD44) that occurs upon lymphocyte sensitisation.

It is important to stress that the role of adhesion molecules in regulating leukocyte function is much greater than merely allowing a cell to stick to and spatially orientate on cells and other surfaces. In many instances adhesion is a prerequisite for successful leukocyte activation, as with the respiratory burst of neutrophils in response to TNF [23]. Furthermore, receptor occupancy of adhesion molecules can itself contribute to cellular activation, as with lymphocyte proliferation in contact with fibronectin or laminin [24], or the induction of cytokine genes in monocytes [25].

Role of leukocyte adhesion molecules in tumour spread

Local extension of tumours depends at least in part upon adhesion to components of extracellular matrix. Whilst different tumours use different molecules for adhesion to ECM, poorly differentiated tumours often have few or undetectable fibronectin and collagen receptors [26]. On the other hand, overexpression of fibronectin receptors has been shown to suppress anchorage independent growth of tumour cells *in vitro* [27]. The capacity to adhere to ECM may therefore limit tumour growth rate and local spread. Conversely, the loss of adhesion receptors may play a central role in enhancing malignancy.

As with leukocyte migration in inflammation, the first step in the metastatic process of blood-borne tumour cells is adhesion to endothelial cells in the target organ. There is a mounting body of evidence that some tumour cells may utilise leukocyte adhesion mechanisms to adhere to endothelial cells; for example, colon carcinoma and melanoma cell lines respectively bind ELAM-1 and VCAM-1 [28]. Since both haematopoietic and non-haematopoietic tumour cells can secrete IL-1 and/or TNF, it is possible that vascular activation by cytokines released from tumour cells is important in conditioning endothelium to express adhesion molecules involved in the process of metastasis. In support of this hypothesis, administration of IL-1 to athymic mice results in an augmentation of experimental metastases from human melanoma [29].

The endothelial determinants involved in lymphocyte recirculation may also be used for tumour dissemination to lymph nodes. In the mouse, cloned lymphoma cells can demonstrate specificity of adhesion to HEV of either peripheral or mucosal lymph node. Furthermore, the capacity of murine lymphomas to bind HEV *in vitro* predicts the ability of the tumour cells to disseminate via the blood to distant lymph nodes *in vivo* [30].

Besides influencing the capacity to spread locally or to metastasise, the expression of adhesion molecules may determine the susceptibility of tumour cells to immunosurveillance. For example, reduced expres-

sion of LFA-1, LFA-3 and ICAM-1 is associated in Burkitt's and other lymphomas with reduced suscepti-bility to killing by cytotoxic T cells [31].

Identification of cytokine inducible leukocyte adhe-sion molecules *in vivo*

The cytokine inducible molecules ELAM-1, ICAM-1 and VCAM-1 were each initially identified using cul-tured cells. Monoclonal antibodies against these molecules have been used to determine with immuno-cytochemical techniques their presence in the tissues during inflammatory responses. To a large extent this has confirmed the relevance of the *in vitro* experi-ments to events taking place *in vivo*.

Experimental inflammation

Analysis of the kinetics of adhesion molecule expres-sion after inducing cutaneous inflammation provides a useful technique for comparison with the responses of isolated cultured cells and allows an analysis of the relation between adhesion molecules and the localisa-tion of leukocytes in particular forms or phases of inflammation [32, 33].

As predicted from its low expression on resting cells *in vitro*, ELAM-1 is minimally expressed in uninflamed skin, but is observed 6–24 hours after initiating a delayed hypersensitivity response, after UV-B irradia-tion, or after local injection of interleukin-1, tumour necrosis factor or endotoxin. Recently Redl *et al.* have shown that ELAM-1 is induced in baboons on endothelium of lung, liver and kidneys during experi-mentally induced septic shock but not in hypo-volaemic shock [34].

Endothelial cells are the predominant cell type expressing ICAM-1 in uninflamed skin. During inflam-matory responses ICAM-1 is also found on infiltrating mononuclear cells, dermal dendritic cells and ker-atinocytes. The expression of ICAM-1 on keratinocytes is not an invariable feature of cutaneous inflammation since it has been reported to be absent in primary irri-tant dermatitis and following exposure to UV-B irradi-ation. A key mechanism involved in the induction of keratinocyte ICAM-1 may be release of interferon γ from activated T cells [35].

As with ELAM-1, there is little expression of VCAM-1 in uninflamed skin, although occasional endothelial cells and tissue macrophages are positive. VCAM-1 is induced on endothelium and dermal cells of dendritic morphology during the delayed hypersensitivity response to tuberculin but not during the normal inflammatory response to UV-B irradiation [33]. It is therefore possible that, unlike ELAM-1, expression of VCAM-1 in human skin may depend upon an immune-mediated element to the inflammatory response.

This experimental approach can also be applied to study mechanisms of inflammation in specific forms of inflammation. Thus, UV-irradiated skin of patients sus-ceptible to polymorphic light eruption shows expression of ICAM-1 on keratinocytes and VCAM-1 on endothelial cells, suggesting an abnormal immune response to light (P. Norris *et al.*, submitted for publi-cation). Further, allergen challenge to skin of allergic individuals results after 4–6 hours in the induction of ELAM-1 and enhanced expression of ICAM-1 on der-mal endothelium, consistent with involvement of these molecules in the recruitment of leukocytes during the late-phase reaction [36].

Clinical inflammation

In the more complex forms of inflammation present-ed by clinical pathological material, expression of adhesion molecules can give insight into pathophysio-logical mechanisms and may even have diagnostic sig-nificance [37]. Expression of ELAM-1 on vascular endothelium has been detected in a number of clini-cal pathological settings, indicating cytokine-mediated endothelial activation. These include the vascular leak syndrome due to systemic administration of inter-leukin-2 [38], Kawasaki disease [39], chronic der-matoses [40], and graft versus host disease [41]. A sur-prising finding from looking at chronic inflammatory lesions is the persistence of ELAM-1 beyond that anticipated from the kinetics of expression *in vitro*. The mechanism whereby ELAM-1 expression is pro-longed *in vivo* is not yet understood.

Expression of ICAM-1 in pathological skin has been the subject of great interest. ICAM-1 is increased on endothelium and induced on keratinocytes and other resident cells in cutaneous inflammation characterised by the presence of activated T cells in the tissues such as allergic contact dermatitis, lichen planus, psoriasis, atopic dermatitis and graft versus host disease [42]. As discussed above, the expression of ICAM-1 by ker-atinocytes may be a consequence of interferon γ (IFNγ) released by infiltrating lymphocytes and proba-bly facilitates lymphocyte migration in the epidermis. The subsequent immune interactions between T cells and keratinocytes may be fundamental to the patho-physiology of inflammatory dermatoses. The reduced capacity to release IFNγ and induce keratinocyte ICAM-1 expression may underlie the development of the leukaemic stage (Sezary syndrome) of mycosis fun-goides in which malignant lymphocytes cease to localise in the epidermis [43].

Targeting leukocyte adhesion molecules in animal models

The recent advances in understanding of leukocyte migration have led to great interest in the possibility that inflammatory responses might be manipulated therapeutically by agents directed at adhesion molecules. Monoclonal antibodies and recombinant adhesion proteins are powerful research tools with which to explore the consequences of inhibiting adhe-

sion molecule function *in vivo* and will allow the testing of hypotheses generated by *in vitro* observations.

Integrins

A number of studies have shown that it is possible to mimic the effects of leukocyte adhesion deficiency by infusing monoclonal antibodies against the CD11/CD18 complex [44]. Anti-CD18 mAb inhibit neutrophil emigration into rabbit skin in response to chemotactic factors or endotoxin-soaked sponges. Furthermore, neutrophil migration into the inflamed peritoneal cavity of mice is inhibited by anti-CD11a or anti-CD11b. It is of interest that leukocytes are not inhibited by anti-CD18 mAb from 'rolling' on the luminal surface of endothelium, supporting the view that this process, which facilitates leukocyte tethering at inflammatory sites, is not governed by integrins. Besides inhibiting neutrophil emigration into inflammatory lesions, anti-CD18 mAb also block neutrophil dependent oedema formation.

An exciting application of anti-β_2 integrins has been the successful prevention of neutrophil-mediated tissue injury during bacterial meningitis [45] and in various models of hypoxic reperfusion injury, such as that following reperfusion of the ischaemic heart [46]. A more diffuse form of hypoxic reperfusion injury occurs upon resuscitation from shock. Here too anti-adhesion therapy may have an application: Vedder *et al.* observed that an anti-CD18 mAb led to significant reduction in organ injury and increased survival upon resuscitating hypovolaemic rabbits [47].

Immunoglobulins

Another approach to inhibiting integrin-mediated adhesion is to block the ligands to which they bind. At present ICAM-1 is the only integrin ligand that has been studied with *in vivo* techniques. The anti-ICAM-1 mAb R6.5, which inhibits adhesion to ICAM-1 of both LFA-1 and Mac-1, inhibits the binding of activated neutrophils to EC in rabbit mesentery [48], and reduces the migration of neutrophils into phorbol ester stimulated rabbit lungs [49]. Recently anti-ICAM-1 mAb has been shown to inhibit eosinophil migration and bronchial hyper-reactivity in primate asthma [50] and to prolong the survival of renal and cardiac allografts [51–53].

Selectins and carbohydrates

The *in vivo* manipulation of selectin-mediated adhesion has been relatively little studied compared with integrins and immunoglobulins. In the mouse, inhibition of LECAM-1 with monoclonal antibody MEL-14 results in reduced neutrophil migration into endotoxin-soaked subcutaneous sponges or peritoneal exudates [54]. Recently, Watson *et al.* have demonstrated

similar effects using recombinant LECAM-1 rather than a monoclonal antibody [55].

Targeting adhesion molecules for therapy

The observation that patients with leukocyte adhesion deficiency often accept HLA-mismatched bone marrow in spite of having T and B lymphocytes led to the use of anti-CD11a or anti-CD18 as an adjunct to other conditioning protocols. The results of inhibiting leukocyte adhesion in this context have been variable and may depend upon the disease state. In one series, improved bone marrow survival was noted in patients with primary immunodeficiency or with osteopetrosis, but not in patients with Fanconi's anaemia [56]. In another series anti-CD18 did not improve bone marrow transplant survival in patients with leukaemia [57].

Prospects for the future

The quest for more selective and less toxic anti-inflammatory agents has identified leukocyte adhesion molecules as important potential targets for pharmacological intervention. Research in this area is now concentrating on increasing our understanding of the relative importance of individual molecules in different pathogenic processes. This applies to the contribution of adhesion molecules to viral infection and tumour spread as well as to inflammation. In parallel, the molecules are being closely analysed to improve our understanding of the control of their adhesive function. The possibilities for the development of drugs which block adhesion by receptor occupancy or inhibition of activation have now become realistic.

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