REVIEW ARTICLE

Integrins, selectins and CAMs — the 'glue of life'

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The ability of cells to adhere to one another and to surrounding structural tissue proteins is essential to the life process of all multicellular organisms. Specific receptors, their ligands and their counter-receptors, collectively referred to in *Time*[1] as the 'glue of life', play a major role in maintaining organ and tissue integrity. Progress in molecular and cell biology has advanced knowledge of these cellular adhesion molecules (CAMs) to a point where they will soon be part of the everyday vocabulary of practising doctors. CAMs, first recognised for their role in the organisation of developing embryological neural tissue, respond to neural, endocrine and paracrine stimuli and in turn influence immune and inflammatory cell function, cell repair processes and the integrity of specialised organs and tissues. The development of therapeutic agents which influence cell adhesion processes offers a new and exciting approach to old diseases such as tuberculosis and cancer.

CAMs in diseases rare to common

As is so often the case in medicine, the importance of CAMs was first appreciated when scientists found a small group of patients in whom they were deficient. The condition, leucocyte adhesion deficiency (LAD), is characterised by recurrent bacterial infections, poor wound healing, lack of pus formation, chronic peripheral blood granulocytosis and abnormal leucocyte function. These unfortunate patients were shown to have a rare inherited deficiency of the β_2-integrins found on leucocyte membranes.[2] Depending on the severity and extent of the CAM deficiency, death can occur in the first 2 years unless curative bone marrow transplantation is performed.

The discovery that a deficiency of CAMs was associated with increased susceptibility to infection led scientists to examine their role in a variety of common conditions. Subjects included normal neonates and premature infants who were shown to have neutrophils with a blunted ability to adhere to, and migrate through, vascular endothelium in order to reach sites of infection and inflammation.[3] More recently we have found similarly impaired neutrophil-endothelial cell interactions in patients with chronic liver disease.[4] Other workers have shown low levels of essential CAMs on β-cells in patients with Burkitt's lymphoma.[5] Because adhesion between leucocytes is central to the regulation of lymphocyte proliferation, abnormal adhesiveness caused by dysfunctional CAMs is thought to result in poor immune surveillance and an increased risk of cancer. Certain inflammatory processes are associated with acquired leucocyte adhesion defects and impaired neutrophil adhesion to endothelium. Patients with sepsis, adult respiratory distress syndrome and myocardial infarction are among those affected.[6]

The nature and function of CAMs

The search for the processes which mediate cell-cell recognition and interaction was based on the assumption that, in order to establish and maintain specialised tissues and organs, multicellular organisms must have a mechanism by which cells interact with each other and with constituents of the extracellular matrix within which they exist. The initial evidence to support this hypothesis was the identification of a specific neuronal cell surface protein which mediated early embryonic events such as the orderly layering of neural tissue.[7] Apart from their role in ontogeny, cellular interactions are critical for the differentiation and function of the immune system. Leucocytes achieve continual immune surveillance of the body by circulating in the bloodstream and, when called upon, bind to the vascular endothelium and migrate into tissues where they either become local effector cells or perform other functions before returning to the circulation via the lymphatic system. The interaction of leucocytes with endothelial cells is the primary and essential step in leucocyte migration into any tissue. This process is particularly well illustrated by neutrophil recruitment into sites of inflammation and by lymphocyte development, recirculation and migration to predestined sites within the body.

The classification of CAMs

Pro-adhesive protein receptors are found on leucocytes and both resting and stimulated endothelium. These receptors have now been classified and characterised into three main superfamilies, the immunoglobulin superfamily, the integrins and the selectins.[8-12]

The immunoglobulin superfamily

Members of the immunoglobulin superfamily are characterised by one or more immunoglobulin-like domain(s), a transmembrane region and a cytoplasmic tail (when the receptor member is membrane-bound). Examples of these proteins include intercellular adhesion molecules (ICAM-1 and 2) and vascular cell adhesion molecules (VCAM-1) which are found on endothelium and are critical for leucocyte binding to, and migration through, endothelial tissue.

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The integrins

The second superfamily, the integrins, are heterodimeric transmembrane glycoproteins which consist of two non-covalently associated α- and β-subunits. The receptor family is made up of varying combinations of one of 14 distinct α-subunits (α_1-α_{14}) and one of 8 β-subunits (β_1-β_8). The ligand-binding site of integrins is formed by sequences from both subunits but is named after the contributing β-chain which confers its specificity. The integrin structure is stabilised by its cytoplasmic domain which connects it to the cytoskeleton.[13] Integrins are found on a large variety of cells. Individual cells express more than one type of integrin and these in turn often differ in their state of activation. Integrins may bind to cell surface adhesion proteins or to counter-receptors on other cells, allowing for cell-cell interactions and/or adhesion between cells of the same type (homotypic) or of different types (heterotypic). These counter-receptors may be members of the immunoglobulin superfamily (ICAM-1, ICAM-2, VCAM-1). Several integrins bind to extracellular matrix proteins, both soluble in the form of glycoprotein or peptide sequences, and fixed solid-phase moieties; this allows for cell-extracellular matrix interactions. Examples of such extracellular matrix ligands include fibronectin, fibrin, vitronectin and laminin. The recognition site for many of the integrins that bind to the extracellular matrix is often a short peptide sequence held in a specific conformational state. The first to be defined was the tripeptide Arg-Gly-Asp (RGD) which is found in the adhesion domains of fibronectin, fibrinogen and a number of other adhesive proteins.[9] Further sequences have since been recognised, and these include Lys-Glu-Ala-Gly-Asp-Val (KQAGDV) and others with no RGD sequences.

The selectins

The selectins are another superfamily of structurally related molecules that mediate adhesive interactions of endothelium, leucocytes and platelets. These reactions are necessary for vascular homeostasis and inflammation but if disregulated, contribute to inflammatory tissue injury and pathological thrombosis. Selectins recognise sialylated glycans attached to target cells. P-selectin is an integral membrane protein present in subcellular granules of platelets and endothelial cells and rapidly translocated to the membrane following specific stimulation. E-selectin, an endothelial cell selectin, is synthesised and expressed only after stimulation of endothelial cells by cytokines and

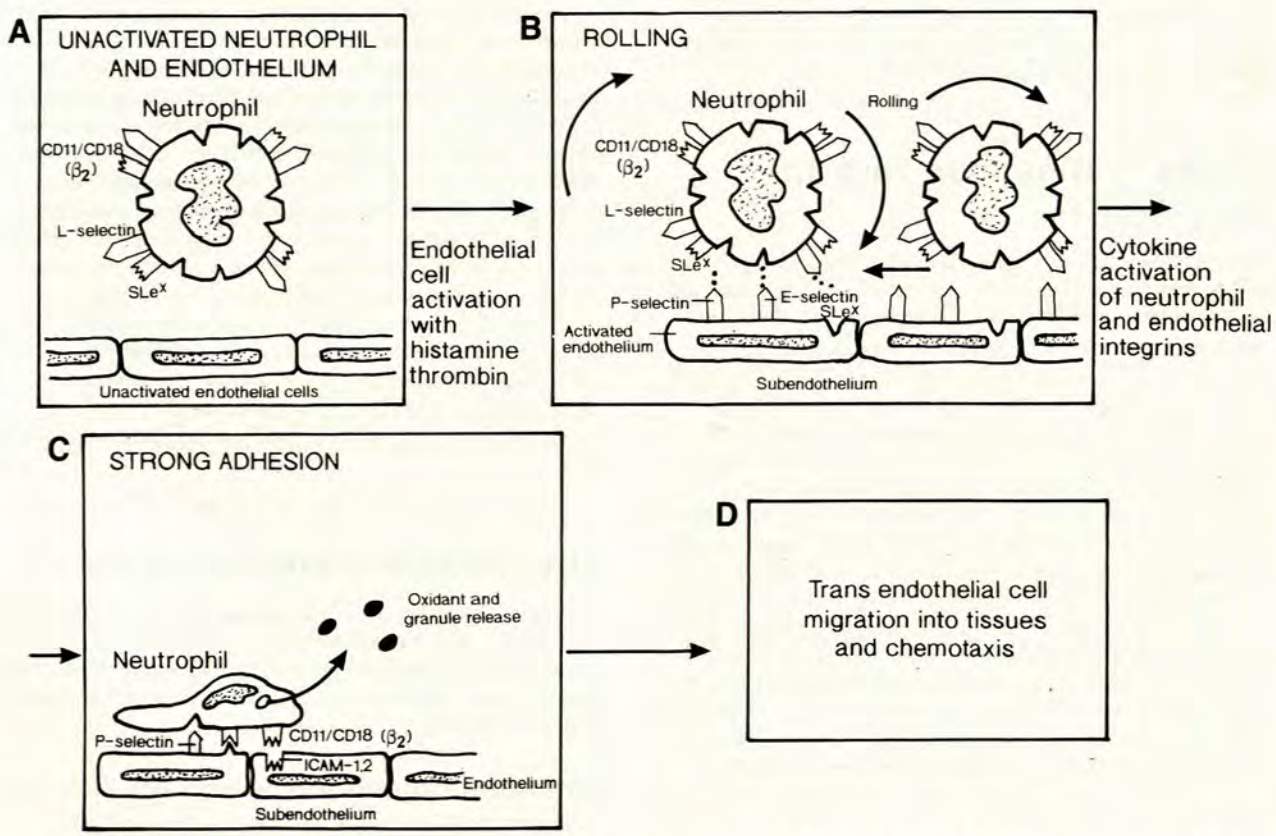

Fig. 1. Schematic representation of neutrophil adherence and migration through vascular endothelium. Under normal conditions, the blood flow presents neutrophils to the endothelium but no interaction occurs (a). At sites of inflammation (b), the vascular endothelial surfaces express the counter-receptor to selectins normally present on neutrophil surfaces, and the resultant interaction allows the neutrophil to 'roll' along the vessel. This process slows the white cell enough to allow stronger adhesion or 'tethering' to occur between the cell types (c). The tethering action is mediated by selective upregulation of integrins on the neutrophil, a process requiring further signalling which, if not present, reverses the adhesion and allows the neutrophil to rejoin the circulation. If signalling occurs, neutrophil surface selectins are immediately shed, further integrins are recruited towards the adhesion process and tight adhesion follows. Following tight adhesion, transmigration between endothelial cells occurs through co-ordinated interactions between various groups of adhesion molecules. The migration along a chemotactic gradient allows the neutrophil to enter inflammatory sites and phagocytose bacterial organisms and cellular debris (d). Pictures are not drawn to scale.

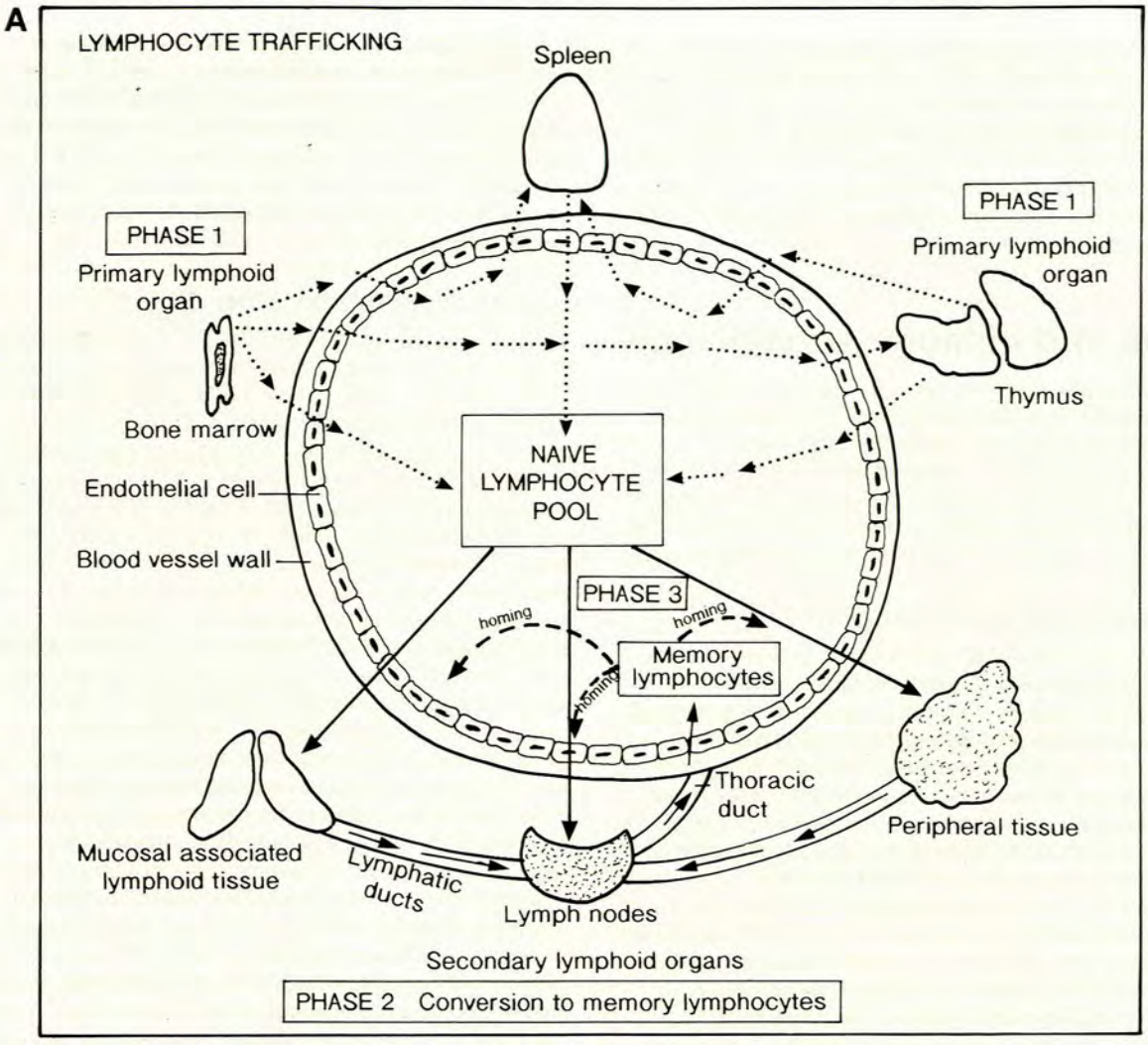

Fig. 2(a). Schematic representation of lymphocyte trafficking. In the first phase, immature lymphocytes are initially released from the bone marrow from where they travel to the thymus to undergo primary differentiation into T-cell subsets. They are released from the thymus into the circulation as naive lymphocytes bearing high numbers of selectins on their surface. This configuration allows them to be preferentially taken up into lymphoid tissue, as seen in phase 2, where they encounter antigen through adhesive contact with resident antigen-processing cells. Further activation and differentiation occur and the lymphocyte is re-released into the circulation via the thoracic duct as a 'memory' lymphocyte expressing fewer selectins and a higher number of integrins on its surface. This lymphocyte, depicted in phase 3, is now able to perform immune surveillance by being primed to a particular antigen which, if re-encountered by the lymphocyte, will initiate an integrated host response. Pictures are not drawn to scale.

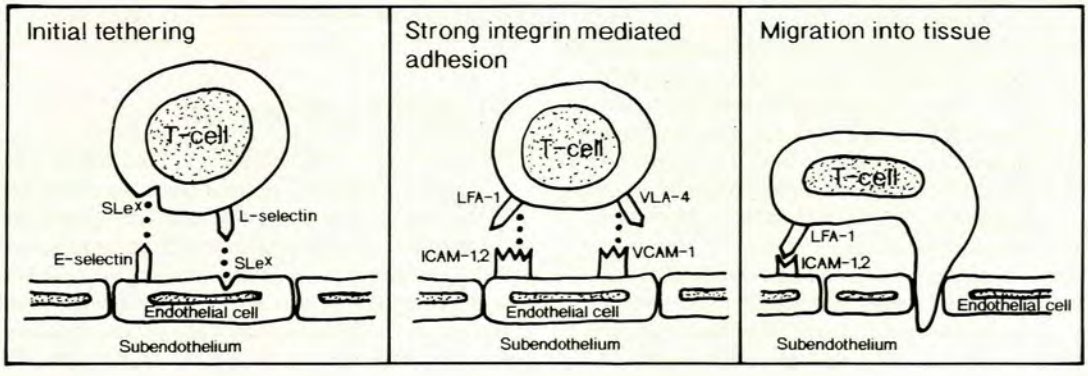

Fig. 2(b). A schematic representation showing the adhesion receptors predominantly involved in co-ordinating lymphocyte interactions with endothelial cells during the various phases of trafficking shown in Fig. 2(a).

mediates lymphocyte and granulocyte endothelial cell interactions. L-selectin is constitutively expressed on granulocytes, lymphocytes and monocytes and is shed from their membranes after cellular activation. An endothelial glycoprotein acts as a ligand for L-selectin.[14-17] The complex, selective recognition and binding processes which involve each of the three CAM superfamilies are described in detail in a recent review.[18]

CAMs and immune surveillance

An integrated immune response capable of maintaining constant body surveillance is dependent to a large degree on adhesive proteins. This concept is well illustrated by leucocyte recruitment into inflamed tissue and by lymphocyte trafficking. In both cases leucocyte-endothelial cell recognition is essential for adhesion which, in turn, is an obligatory prerequisite for migration of cells to their target.

Leucocyte recruitment (Fig. 1)

Leucocyte-endothelial cell recognition is an active process requiring three sequential events for attachment of leucocytes to occur. These steps, which include a phase of reversible adhesion, leucocyte activation and activation-dependent binding, are most commonly seen in neutrophil endothelial interactions in venules soon after tissue injury. Reversible adhesion, which slows the flow of circulating neutrophils and causes them to 'roll' along the margins of blood vessels, depends on activated endothelium expressing functional selectins which bind loosely to carbohydrate moieties on neutrophils and the simultaneous expression on the neutrophils of functional selectins which bind to endothelial counter-receptors. This process is transient unless followed by further leucocyte and endothelial activation. Tissue damage results in macrophage and lymphocyte synthesis of an array of cytokines, including IL-1, IL-4, IL-6 and tumour necrosis factor (TNF). Exposure to these cytokines converts the endothelial surface from an anticoagulant to a procoagulant surface. This results in the generation of thrombin, fibrin deposition and adhesion of granulocytes to the endothelium. TNF and interleukins 1 and 8 stimulate neutrophils to express β_2-integrins which are normally stored in intracellular adhesomes, a form of neutrophil granule; together with endothelial-derived platelet-activating factor these ensure that the integrins are in a functionally activated state.

The activation of β_2-integrins by these chemotactic factors constitutes the third step in the binding process. Activation changes the configuration of the receptor and results in stable and sustained attachment of neutrophils to counter-receptors expressed by the activated endothelial cells. Thus attachment, the first step of leucocyte migration, is a tightly controlled process which can be modulated at any of the three stages described above.[18]

Leucocytes circulating in intact blood vessels express inactive forms of CAMs on their surface as do normal endothelial cells, but both must, upon stimulation, be able, through signalling events, to upregulate selectively the activation state of those same CAMs in order to allow for adhesion and targeting. Signalling may occur via cytokines as well as by the presence of ligand in the receptor site of the surface CAM. Both lead to changes in intracellular tyrosine kinase/phosphorylase pathways which initiate further activation of the cell surface proteins. In the case of already activated cells, inactivation may be mediated by both pathways. In this way leucocytes are able to interact with a host of targets by varying the status of individual groups of surface adhesion receptors in response to appropriate signals.[19]

Lymphocyte trafficking (Fig. 2)

Lymphocytes are unique in their ability to patrol the body by continuously migrating from the blood into tissue, organs and lymphoid systems, utilising surface CAMs to facilitate recognition and response to antigen. There may be two distinct phases in the life cycle of lymphocytes. In the first phase lymphocytes are considered to be naive while in the second they are endowed with 'memory'. Naive cells are immature lymphocytes which have not yet encountered antigen. These cells leave the thymus and enter lymphoid tissue where they come into contact with antigen through adhesive interactions with antigen-processing cells. In order to enter lymphoid tissues these naive cells must express a high number of L-selectins on their surface (as opposed to memory cells). These facilitate adherence to lymphatic postvenular vascular endothelium and migration into lymphatic tissue. On contact with foreign antigen, the naive lymphocyte is stimulated to express different adhesion molecules on its surface and is transformed into a memory cell which enters the circulation via the thoracic duct. Memory cells express various integrins, including β_1 and β_2 integrins, which facilitate enhanced binding to their respective ligands, ICAM-1, VCAM-1 and extracellular matrix proteins. The site at which the conversion from naive to memory cells occurs is important in that each tissue, e.g. skin, gut, has particular antigens which are present in local lymphatic tissue. After re-entering the circulation, memory cells preferentially return to the tissue where the initial stimulating response originated, a phenomenon thought to be brought about by specific and differential surface expression of CAMs on the lymphocyte and local endothelium. This process is termed homing. With the increased levels of CAMs on their surface, memory cells can also migrate into inflamed non-lymphoid tissue for surveillance. When stimulated by contact with foreign antigen to which it is 'primed,' a memory lymphocyte becomes an effector cell and is able to bring about local and systemic host responses.

Platelet adhesion

Platelets, unlike lymphocytes and neutrophils, do not adhere to normal vasculature. However, any disruption of the endothelial surface caused by damage, inflammation, complement or atherosclerotic plaque, exposes collagen and other extracellular matrix proteins which interact with specific platelet receptors and mediate platelet adhesion. The extent of vascular injury determines whether platelets ensure normal haemostasis or cause abnormal thrombotic events. Platelet plugs at sites of vascular injury limit blood loss but, when associated with atherosclerotic vessels, impair blood flow. Thus adhesion of platelets to the

subendothelium may initiate either haemostasis or thrombosis. The interaction of platelets with damaged endothelium is via platelet-associated α_{1-6}-, β_1- and β_3-integrins and specific extracellular matrix proteins, laminin, collagen, fibronectin and vitronectin. This process activates platelets and is characterised by a conformational change in several platelet-associated receptors (e.g. the fibrinogen receptor GpIIb/IIIa, β_3 family), translocation of P-selectin to the membrane and thrombin generation. Platelet plugs are formed through sequential steps of adhesion, activation and aggregation. Thrombin generated as described above, or released from platelets, initiates fibrin formation. Neutrophil and monocyte accumulation in thrombi are mediated via cellular adhesion through neutrophil and monocyte β_2-integrin interaction with fibrin and with cell surface carbohydrate binding to platelet-expressed P-selectin. Thus CAMs mediate a variety of reactions which not only allow cells to reach their target but to exert effects upon arrival in the tissues.

Bacterium host cell interactions occur via integrins

It is now well known that adhesion molecules play a major role in host-pathogen interactions. Viral, bacterial and protazoan pathogens utilise a wide range of host cell surface receptors, including the integrin receptors, to gain entry into cells. Poliovirus and rhinovirus infect cells via the ICAM-1 molecule; *Bordetella pertussis, Legionella pneumophilia* and *Leishmania donovani* all bind to the CD11b/CD18 receptor, while *Salmonella typhimurium* appears to gain entry through the epidermal growth factor receptor.[20] Enteropathogenic *Yersinia pseudotuberculosis* interacts with mammalian cells through the interaction of the outer membrane proteins, invasin and adhesin YadA, with several members of the β_1-integrin family.[21] By attachment to β_1-integrins, these species appear to exploit existing signal transduction pathways intimately, to attach themselves to and enter the host cell. Several species of Gram-negative and Gram-positive bacteria, including *Mycobacterium tuberculosis,* contain cell surface proteins capable of binding to the extracellular matrix protein, fibronectin.[22] This possibly intensifies their virulence by promoting adherence to mucosal surfaces. Since fibronectin binding to β_1-integrins is important for transduction of cell signals, fibronectin-binding bacterial constituents may alter the functions of many cell types and their interaction with the extracellular matrix.

Therapeutic modulation of CAMs

The recognition that cell adhesion plays an important role in various disease states has raised the exciting prospect of therapeutic intervention via modulation of CAMs. Various methods of therapy are currently available or are being tested. In some conditions the focus is directed at increasing the ability to express CAMs. This currently includes bone marrow transplantation in LAD. In other conditions, notably sepsis, where excessive expression of certain CAMs leads to leucocyte aggregation and intravascular sludging, therapy aims at reducing functional CAMs. This includes the use of monoclonal antibodies directed against CAMs, and the administration of soluble adhesion receptor proteins (known natural *in vivo* inhibitors) and peptides which bind to CAMs or to their counter-receptors. Anti-adherence therapy has proved useful in some forms of infection, such as meningitis and malaria, as well as in sepsis-associated acute respiratory distress syndrome where leucocyte accumulation in the lung contributes to organ injury.[23] Antibodies against integrins, selectins and immunoglobulin adhesion receptors have been therapeutically beneficial in animal models of asthma and arthritis.[24,25]

The localisation and accumulation of neutrophils in reperfused ischaemic tissue following myocardial infarction appears to be of critical importance in determining the incidence of complications as well as the degree of damage. Studies in animal models have shown that antibodies against CAMs markedly decrease ischaemic injury and the resultant complications. Antibodies specific to platelet CAMs also inhibit thrombosis *in vivo* and have potential use in myocardial infarction, unstable angina and cerebrovascular disease.[26,27]

T-lymphocyte accumulation mediates the damage caused by rejection of transplanted organs. Antibodies directed against these T-lymphocytes and their specific receptors, e.g. OKT3, are routinely used in this situation.

Cell adhesion is an important process in leucocyte function. An increased understanding of the structure and function of CAMs, as well as their modulation by various cytokines, is rapidly leading to a new approach to the management of everyday diseases.

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REFERENCES

1. Thompson D. The glue of life. *Time* 1992; **38:** 58-59.
2. Anderson DC, Springer T. Leukocyte adhesion deficiency: an inherited defect in the Mac-1, LFA-1, and p150,95 glycoproteins. *Ann Rev Med* 1987; **38:** 175.
3. Anderson DC, Rothlein R, Martin SD, Krater SS, Wayne Smith C. Impaired transendothelial migration by neonatal neutrophils: abnormalities of mac-1 (CD11b/CD18)-dependent adherence reactions. *Blood* 1990; **76:** 2613-2621.
4. Froese S, Robson SC, Kirsch RE. Neutrophil adhesion abnormalities in chronic liver disease (manuscript in preparation).
5. Makgoba MW, Bernard A, Saunders ME. Cell adhesion/signalling: biology and clinical applications. *Eur J Clin Invest* 1992; **22:** 443-453.
6. Fein AM, Grant MM, Niederman MS, Kantrowitz N. Neutrophil-endothelial cell interaction in critical illness. *Chest* 1991; **99:** 1456-1462.
7. Edelman GM. Cell adhesion molecules. *Science* 1983; **219:** 450-462.
8. Springer TA. Adhesion receptors of the immune system. *Nature* 1990; **346:** 425-434.
9. Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. *Science* 1987; **238:** 491-497.
10. Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987; **48:** 549-554.
11. Patarroyo M, Makgoba MW. Leukocyte adhesion to cells in immune and inflammatory responses. *Lancet* 1989; **2:** 1139-1141.
12. Harlan JM. Leukocyte-endothelial cell interactions. *Blood* 1985; **65:** 513-525.
13. Ruoslahti E. Integrins. *J Clin Invest* 1991; **87:** 1-5.
14. Bevilacqua MP, Stengeln S, Gumbrone MA, Seed B. ELAM-1: an inducible receptor for neutrophils related to complement regulatory proteins and lectins. *Science* 1989; **243:** 1160-1165.
15. Geng J, Bevilacqua MP, Moore KL, *et al.* Rapid neutrophil adhesion to activated endothelium mediated by GMP-140. *Nature* 1990; **343:** 757-760.
16. Stoolman LM. Adhesion molecules controlling lymphocyte migration. *Cell* 1989; **56:** 907.
17. Kishimoto TK, Wanock RA, Jutila MA, *et al.* Antibodies against human neutrophil LECAM-1 and ELAM-1 inhibit a common CD 18-independent adhesion pathway *in vitro. Blood* 1991; **78:** 805-811.
18. Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 1991; **67:** 1033-1036.
19. Hynes RO. Integrins: versatility, modulation, and signalling in cell adhesion. *Cell* 1992; **69:** 11-25.

20. Brett SJ, Mazuvov AV, Charles IG, Tite JP. The invasin protein of *Yersinia* spp. provides co-stimulatory activity to human T cells through interaction with β1 integrins. *Euro J Immunol* 1993; **23:** 1608-1614.
21. Bliska JB, Copass MC, Falksow S. The *Yersinia pseudotuberculosis* adhesin YadA mediates intimate bacterial attachment to and entry into Hep-2 cells. *Infect Immun* 1993; **61:** 3914-3921.
22. Abou-Zeid C, Garba T, Lathigra R, *et al.* Genetic and immunological analysis of *Mycobacterium tuberculosis* fibronectin binding proteins. *Infect Immun* 1991; **59:** 2712-2718.
23. Saez-Harens X, Jafari HS, Severier C, *et al.* Enhanced attenuation of meningeal inflammation and brain edema by concomitant administration of anti-CD18 monoclonal antibodies and dexamethasone in experimental haemophilus meningitis. *J Clin Invest* 1991; **88:** 2003-2011.
24. Wegner CD, Grundel RH, Reilly P, Haynes N, Letts G, Rothlein R. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science* 1990; **247:** 456-459.
25. Vedder NB, Winn RK, Rice CL, Chi EY, Arforf KE, Hanan JM. A monoclonal antibody to the adherence-promoting leukocyte glycoprotein CD18, reduces organ injury and improves survival from haemorrhagic shock and resuscitation in rabbits. *J Clin Invest* 1988; **81:** 939-944.
26. Falanga PB, Butcher EC. Late treatment with anti-LFA-1 (CD11a) antibody prevents cerebral malaria in a mouse model. *Eur J Immunol* 1991; **21:** 2259-2263.
27. Ma X, Tsao PS, Lefer AM. Antibody to CD18 exerts endothelial and cardiac protective effects in myocardial ischaemia and reperfusion. *J Clin Invest* 1991; **88:** 1237-1243.

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Dokter en digter

Vreemde liefde

(in offerande aan: Anairda)

Jou liefde is 'n helder vlam
Wat stil en suiwer voor my brand
En elke nag sy wierook hef.

Ek hoop eendag
Oor my sal die Suiderkruis
se kalm sterre skyn.

Hoe kan jy weet, in jou vroeë jare
Dat hierdie wonder aan jou sou geskied
Draaikolk van sterre en rooi duiseling

O liefde, liefde wat my alles gee
Uit watter dieptes het jy my gehaal!
Ek min jou, soos die vlakpatryse die veld

Saam sal ons twee nie meer die vuur
Van sterre sien nie, maar daar bly
'n Gloedvolle herinnering vir jou en my

O liefde dieper as die diepste see
en ruimer as die sterbestrooide nag
Straal tot waar die verste sterre bewe

Ek wonder of jy soms
Of jy nog aan dié nagte dink
O welbeminde?

Ek het my aan jou oorgegee
So onvoorwaardelik dat ek soms vrees
Vernietiging is al wat uiteindelik vir my kan wees

Hier voor my oor die breë helling hang
Die trae skemering van verdonkerde lande
Hoe kan my ryk alleen wees jou vervang?

In die verhongering
van 'n liefdelose nag
Kan 'k veiling ween
Die sterre dans en vrolik skyn
Stil my hart se bittere pyn
Middernagtelike ure kan die skim 'n tydjie keer.

Vergeefs strek ek die hande om te pluk!
want onbereikbaar aan die takke hang
Die ryp rooi vrugte van jou sielsgeluk
Al wat ek het, is joune as jy terug wil keer
Al wat bly is die bleek wit rose van verdriet

Maar net die wysheid van die hart
Kan wit ontrafel van die swart
Hoe mooi is alles nou vir my!

Uit die swart leegtes van die Niet
wat ruis deur die soet goue lied
Uit die groot harp van GOD

'Ekuphumleni'