Leukocyte and Endothelial Cell Adhesion Molecules in Inflammation Focusing on Inflammatory Heart Disease

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Abstract. In multicellular organisms the development of adhesion bonds, either among cells or among cells and components of the extracellular matrix, is a crucial process. These interactions are mediated by molecules which are named adhesion molecules and play a main role both at the early stages of the development of tissue integrity and later. Cell adhesion molecules (CAMs) have a key role in several pathologies such as cancer and inflammatory diseases. Selectins, integrins and immunoglobulin gene superfamily of adhesion receptors mediate different steps of leukocyte migration from the bloodstream towards the inflammatory foci. Leukocyte interactions with the vascular endothelium are highly orchestrated processes that include the capture of free-flowing leukocytes from the blood with subsequent leukocyte rolling, arrest, firm adhesion and ensuing diapedesis. These interactions occur under high shear stresses within venules and depend on multiple families of adhesion molecules. As a response to infection mediators, leukocyte gathering is considered to be crucial for the adequate defence of the organism to any kind of injury or infection. Endothelial activation contributes significantly to the systemic inflammatory response to bacteraemia and increased expression. Release of soluble endothelial markers into the circulation has been demonstrated together with elevated plasma levels of CAMs and has been reported in bacteraemic patients. It has been proposed that infection of endothelial cells with Staphylococcus aureus, Streptococcus sanguis, or Staphylococcus epidermidis induces surface expression of ICAM-1 and VCAM-1 and monocyte adhesion. In general, leukocyte/endothelial cell interactions such as capture, rolling, and firm adhesion can no longer be viewed as occurring in discrete steps mediated by individual families of adhesion molecules but rather as a series of overlapping synergistic interactions among adhesion molecules resulting in an adhesion cascade. These cascades thereby direct leukocyte migration, which is essential for the generation of effective inflammatory responses and the development of rapid immune responses.

Adhesion is a vital property of cells. It provides a stable environment for cell growth and differentiation and allows cells to migrate. The interaction between cells and their extracellular matrices is also an important factor in the regulation of further protein deposition. Likewise, matrix proteins can influence cellular function, thus creating a complex feedback mechanism. The adherence of cells to each other, their extracellular matrices and endothelial surfaces is mediated by a variety of membrane proteins collectively known as cell adhesion molecules (CAMs). Thus, CAMs are responsible for those cellular interactions belonging to a complex mechanism which come into play at the receptors on the cell surface. In this mechanism, apart from cell adhesion molecules, many other soluble cell mediators, such as cytokines, and components of the tissue matrix, such as fibronectin and collagen, play a crucial role (1, 2). Disturbance of one of these systems may induce a pathological condition. The physiological state of the individual therefore depends on the balance of all these components.

Cell adhesion molecules are substances with a protein character expressed on the cell surface of all tissues. They function as receptors that trigger intracellular pathways and participate in the control of basic vital processes such as embryogenesis, migration, cellular growth and differentiation,
and cell death, ensuring the interaction of cells with the environment (3, 4). Specifically, adhesion molecules are membrane receptors that mediate several interactions, recognized to play a major role in a variety of normal and pathological phenomena related to traffic and interactions between cells, cell-matrix contact and in determining, the specificity of cell-cell binding (4-7). A variety of recently identified glycoproteins have been implicated in cell-cell interactions that are critical for normal homeostasis, immune surveillance and vascular wall integrity. These CAMs are known to mediate blood cell (leukocyte, platelet)-endothelial cell interactions that can occur in all segments of the microvasculature under certain physiological (e.g. homeostasis) and pathological (e.g. inflammation, immune responses, cancer) conditions (8-12). From the immunological point of view, they are involved in virtually every process of cell interaction, involving thymic selection and antigen priming, antigen recognition and cell activation, cytotoxicity and lymphocyte recirculation (13). On the other hand, adhesion molecules play an essential role in the development of inflammation. In general, cytoadhesion molecules play an important role in the pathophysiology of cardiovascular, neoplastic, infectious and skin diseases. Some cardiovascular diseases are associated with pathological impairment of the structure and function of endothelial cells with the appearance of endothelial dysfunction (14). In particular, they are involved in the endothelial dysfunction and activation processes, and are related to, for example, the pathogenesis of atherosclerosis, coronary artery disease, reperfusion injury, allograft vasculopathy, myocarditis, hypertrophic myocardial hypertrophy (5, 15). At present, the main classes of cytoadhesion molecules known are integrins, cadherins, selectins, members of the immunoglobulin gene superfamily (IgSF) and CD44 (16, 17). Among these, the selectins, integrins, CD44 and immunoglobulin (Ig) gene superfamily of adhesion receptors (IgSF) mediate the different steps of the migration of leukocytes from the bloodstream towards inflammatory foci (5, 18).

The vascular endothelium plays a key role in the regulation of the inflammatory response. Adhesion molecules such as selectins, immunoglobulin receptors, integrins and cadherins as well as connexins expressed on the endothelial cell surface, participate in several interactions. In normal circumstances, they mediate endothelial cell matrix interactions and regulate vascular permeability. The surface expression of adhesion molecules changes during the process of inflammation. Subsequently, these receptors participate in interactions between leukocytes and activated endothelium surface, in the process of leukocyte activation and extravasation. The vascular endothelium, a governing barrier for the exchanges between blood and tissues, plays an active part in regulation of the transcapillary permeability, control of proliferation of haematopoietic cells and the phases of the inflammatory response (1, 19). Soluble forms of several adhesion molecules are released into the circulating blood. Thus, plasma levels of soluble adhesion molecules may be a diagnostic marker of systemic endothelial injury (19).

Leukocyte–Endothelial Cell Soluble Cell Adhesion Molecules

Some members of the selectins and IgSF as well as the CD44 adhesion molecule represent the most studied adhesion molecules in this area.

Figure 1. Structure of some selectin family members.
Selectins. Selectins are lectin-like binding protein molecules that mediate the initial low affinity leukocyte-endothelial cell interaction that is manifested as leukocyte rolling (Figure 1, Table I). This transient binding results in further leukocyte activation and subsequent firm adhesion and transendothelial migration of leukocytes (8, 20-22). Specifically, selectins are implicated in heterotypic interactions between blood cells and endothelial cells during leukocyte migration and firm adhesion (23). Their role is manifested during the initial adherence of the circulating leukocytes to the vascular wall that follows their rolling as a response to an infective or a carcinogen mechanism. Additionally, as a response to infection mediators, leukocyte gathering is considered to be crucial for the adequate defence of the organism to any kind of injury or infection.

Table I. Soluble cell adhesion molecules (sCAMs) involved in leukocyte-endothelial cell adhesion. Localization and function of sCAMs.

<table>
<thead>
<tr>
<th>Adhesion molecule</th>
<th>Localization</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td><strong>Selectin family</strong></td>
<td>All leukocytes</td>
<td>Rolling</td>
</tr>
<tr>
<td>L-Selectin</td>
<td>Endothelial cells and platelets</td>
<td>Rolling</td>
</tr>
<tr>
<td>P-Selectin</td>
<td>Endothelial cells</td>
<td>Rolling</td>
</tr>
<tr>
<td><strong>Ig gene Superfamily</strong></td>
<td>Endothelium and monocytes</td>
<td>Adherence/migration</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>Endothelium</td>
<td>Adherence/migration</td>
</tr>
<tr>
<td>ICAM-2</td>
<td>Endothelium</td>
<td>Adherence</td>
</tr>
<tr>
<td>ICAM-3</td>
<td>All resting leukocytes</td>
<td>Adherence/migration</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>Endothelium</td>
<td>Adherence/migration</td>
</tr>
<tr>
<td>PECAM-1</td>
<td>Endothelium, leukocytes, platelets</td>
<td>Adherence/migration</td>
</tr>
<tr>
<td>MAAdCAM-1</td>
<td>Endothelium (intestine)</td>
<td>Adherence/migration</td>
</tr>
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</table>

In contrast to most of the other CAMs the role of selectin is strictly restricted to the interactions between leukocytes and the vascular endothelium. In general, selectins share an important role in human physiology. In leukocyte adhesion deficiency II syndrome (LAD II), where selectin ligands are absent there is an inability to recruit neutrophils into sites of inflammation so that they cannot fulfill their role as effector cells in the immune system (26). Soluble circulating forms of the selectins can be detected in plasma, where elevated levels have been reported in serum of animals and patients with inflammatory diseases (27).

P-selectin (also known as CD62P or GMP-140 or PADGEM) is stored in specific granules present in platelets and endothelial cells (Weibel-Palade bodies) from where it can be rapidly mobilized to the cell surface in response to a variety of inflammatory agents such as thrombin, histamine, complement factors, free radicals and cytokines (25, 28). Cell surface expression of P-selectin is generally short-lived (minutes), which makes it an ideal candidate for mediating early leukocyte-endothelial interactions. Ligand for P-selectin is considered to be the P-selectin glycoprotein ligand-1 (PSGL-1) (29, 30). PSGL-1 undergoes special glycosylation in order to function as a ligand. P-selectin also mediates neutrophil and monocyte adherence to stimulated thrombocytes and stimulated endothelial cells. Additionally, it mediates the in vitro capture of stimulated B-cells together with a subpopulation of T-cells in the stimulated endothelium. E-selectin (CD62E, ELAM-1) is expressed by cytokine-activated endothelial cells (29, 30).

E-selectin mediates the adhesion of neutrophils, monocytes and some memory T-cells to the vascular endothelium and may function as a tissue specific homing receptor for T-cell subsets (25). It is broadly expressed within the vasculature at sites of inflammation. Additionally, it is found in arthritic joints, in heart and renal allograft undergoing rejection, and in cutaneous vessels of inflamed skin with psoriasis, contact dermatitis and delayed type hypersensitivity reactions (25). E-selectin is found in a biologically active form in serum, as a result of proteolytic cleavage from the cell surface (31, 32). Many ligands for E-selectin have been reported and are expressed by neutrophils, monocytes and lymphocytes, such as ESL-1 ligand (E-selectin ligand-1)(30) and PSGL-1 (P-selectin glycoprotein ligand-1) (30). Although there is no preformed
surface glycans that possess a specific sialyl-LewisX-type cells. The major ligands for all three selectins are cell distress syndrome (ARDS). Levels are reported in patients with adult respiratory syndrome, leukemias and malignant tumors. Decreased levels are reported in patients with acquired immunodeficiency syndrome, leukemias and malignant tumors. Decreased levels are reported in patients with adult respiratory distress syndrome (ARDS).

Cytokines, bacterial toxins and oxidants are known to promote the synthesis of E- and P-selectin in endothelial cells. The major ligands for all three selectins are cell surface glycans that possess a specific sialyl-LewisX-type structure (34); L-selectin may also serve as a ligand for P- and E-selectin (8, 26, 35).

Immunoglobulin gene superfamily (IgSF). The IgSF is the most abundant family of cell surface molecules, accounting for 50% of all leukocyte surface glycoproteins. Their structure is characterized by repeated domains, similar to those found in immunoglobulins, built from a tightly packed barrel of β strands (Figure 2). By mutation and selection, the Ig domain has evolved to serve many different functions including: acting as receptors for growth factors and for the Fc region of Ig, and as adhesion molecules, which now seems to be a function of the majority (8, 36). Some members of this family that are of relevance to vascular diseases include the intercellular cell adhesion molecules-1 and -2 (ICAM-1, ICAM-2), vascular cell adhesion molecule-1 (VCAM-1), platelet-endothelial cell adhesion molecule (PECAM)-1, and the mucosal addressin cell adhesion molecule-1 (MAdCAM-1) (Table 1). Leukocyte rolling is a prerequisite for eventual firm adhesion to blood vessels. However selectin-mediated adhesion of leukocytes does not lead to firm adhesion and transmigration unless members of the IgSF are involved. Additionally, IgSF members undergo increased expression in chronic immunological inflammatory processes (37, 38). For endothelial cell–T-cell interactions, the most important members of this family are ICAM-1, ICAM-2 and VCAM-1, which serve as surface ligands for the LFA-1 and VLA-4 integrins (39).

ICAM-1 is basally expressed on many cell types, but its expression is regulated on endothelial cells (29, 40), where it exhibits remarkable heterogeneity between vascular beds (41-43). The most important ligands for ICAM-1 are considered to be the β2-integrins LFA-1 and Mac1 (CD11B/CD18) that are expressed in leukocytes. Subsequently, ICAM-1 mediates the leukocyte ICAM-1 presenting cell adherence. ICAM-1 is found in a biologically active form in serum, probably as a result of proteolytic cleavage from the cell surface, being elevated in patients with various inflammatory syndromes such as septic shock, LAD, cancer and transplantation (32). ICAM-1 is expressed on endothelial and epithelial cells, lymphocytes, monocytes, eosinophils, keratinocytes, dendritic cells, ancestral haemopoietic cells, liver cells and fibroblasts. Deregulation of ICAM-1 expression leading to increased levels is triggered by infectious cytokines (tumor necrosis factor-alpha, TNF-α; interferon-γ, INF-γ; interleukin-1, IL-1), while decreased expression is observed when inflammatory factors such as glycocorticoids are induced. The immune cell circulation related function of ICAM-1 is the most well studied to date. Inflammatory cytokines increase the expression of ICAM-1 in vascular endothelial cells while on the other hand activating the leukocytic integrins LFA-1 and Mac-1 at the site of inflammation. Subsequently, this leads to leukocytic adherence to the regional endothelium which is considered to be a necessary step for leukocyte migration at the site of inflammation. In general, increased serum levels of soluble ICAM-1 are related to different inflammatory conditions caused by bacteria, viruses, autoimmune diseases and various kinds of neoplasm. Additionally, different levels of ICAM-1 have been monitored in ARDS. Corticosteroids that are used therapeutically in ARDS inhibit the secretion of ICAM-1 and endothelial leukocyte adhesion molecule-1 (ELAM-1) (44). Several studies have demonstrated that in some cases of septicaemia, soluble forms of ICAM-1 exhibit fluctuations that are related to the levels of particular endotoxins, tumor necrosis factor and different types of cytokines (45, 46). Moreover, in the literature we find proof for the importance of ICAM-1 molecule in the migration of leukocytes to the brain being implicated in cases of encephalitis and other immunological type disorders of the central nervous system (47).

ICAM-2 is a truncated form of ICAM-1 that is basally expressed on endothelial cells (48). In contrast to ICAM-1, ICAM-2 expression is not increased on activated endothelial cells and is not triggered by cytokine activation (49). Moreover, it is found at low levels in leukocytes, epithelial cells and generally in latent phase cells while on the other hand it is stimulated by IFN-γ, TNF-α, IL-1 and lipopolysaccharide (LPS) (50, 51). ICAM-3 is considered a ligand for the leukocytic integrins LFA-1 (CD11a/CD18, αLβ2).
ICAM-3 is constitutively expressed at high levels by all resting leukocytes, such as monocytes, lymphocytes and neutrophils, as well as antigen-presenting cells, showing a pattern of expression clearly distinct from that of ICAM-1 and ICAM-2 (52). During the latent status of T-cells the ICAM-3 is considered the ligand for LFA-1. It is possible that ICAM-3 has a very important role in the activation cascade of the immunological response, cellular adhesion and signal transduction if we take into account the fact that it causes increased adhesion through the β1- and β2-integrin pathways (53, 47). Additionally, it has been postulated that ICAM-3 is related to lymphomas and myelomas considering that the vascular endothelium in such conditions secretes increased amounts of ICAM-3 (54, 55).VCAM-1, which exhibits low to negligible expression on unstimulated endothelial cells, can be profoundly up-regulated after cytokine challenge. This molecule is expressed on the surface of activated endothelium and a variety of other cell types including bone marrow fibroblasts, tissue macrophages and dendritic cells. It can be up-regulated by inflammatory mediators such as interleukinβ (IL-β), IL-4, CD44, TNFα and IFN-γ. PECAM-1, also known as CD31 or endoCAM, is constitutively expressed on platelets, monocytes and neutrophils, and in large amounts on endothelial cells at intercellular junctions and on T-cell subsets (56-58). PECAM-1 can mediate adhesion through either homophilic or heterophilic interactions (59). It is produced by platelets, monocytes and neutrophils in lower doses (57, 58). PECAM-1 is highly implicated in the migration of leukocytes through the vascular endothelium via intercellular junctions (57). Furthermore, it is implicated in the cross reactions of CD8+ and T-cells with intercellular adhesion site molecules via the integrin adhesion process (39).

The mucosal addressin MAdCAM-1 is found on high endothelial venules (HEV) and mainly expressed on HEVs of Peyer’s patches, on venules in small intestinal lamina propria, on the marginal sinus of the spleen, and on HEVs of embryonic lymph nodes (60). It is involved in tissue-specific homing of lymphocytes in lymph nodes and mucosal lymphoid tissues (8, 61).

CD44. The CD44 proteins belong to a highly heterogenous family of hyaluronan-binding type I transmembrane glycoproteins, involved in cell-cell and cell-matrix interactions. This family of transmembrane glycoproteins is identified as a large number of isoforms with a common stable molecular part. The main structure of the CD44 molecule consists of an N-terminal extracellular domain, a membrane proximal region, a transmembrane domain and a cytoplasmic tail (Figure 3) (17, 62). The extracellular domain of the standard form consists of a 270 amino acid chain, folded into a globular tertiary structure by disulfide bonds between three pairs of cysteine residues on its
N-terminus. These cysteine residues are very important for molecular stability and seem to be required for correct folding and hyaluronan binding. The CD44 gene is unique for all various isoforms and consists of 20 exons, only 10 of which are normally expressed, encoding CD44H (hemopoietic) also known as CD44s (standard), the most abundant form (Figure 3). The variable regions of CD44 molecules are inserted in an alternative splicing site, encoded by exons v1-v10. The CD44 gene has been mapped in chromosome 11p13. Normal tissues in which CD44 variant isoforms have been detected at low levels include tonsils, thyroid, breast, prostate, cervix, tongue, esophagus, epithelium and skin (17, 63). CD44s (s for standard) is ubiquitously present in hematopoietic tissue and gastrointestinal tract epithelium. In general, CD44 has been implicated in processes such as lymphocyte homing, hematopoiesis, tumor progression, lymphocyte activation, pattern formation in embryogenesis, signal transduction and inflammation (64, 65). Hyaluronan, a ubiquitously expressed polysaccharide, is the principal ligand for CD44 (66). Together, CD44 and hyaluronan may mediate a number of physiological and pathophysiological processes, including the inflammatory response (67-70).

The hallmark feature of acute inflammation is neutrophil infiltration into tissues; however, a role for neutrophil CD44 has not been elucidated. Neutrophil recruitment into tissues particularly during acute inflammation has been well studied (71, 72). In the following paragraphs we will describe the mode of action of a series of adhesion molecules that act in an interdependent fashion to allow fast flowing cells in the mainstream of the blood to leave the circulation and enter the adjacent tissue.

As already mentioned, selectins mediate the initial tethering and rolling event. The rolling process brings neutrophils into close proximity with chemokines on the surface of the endothelium, which activates the rolling leukocytes to firmly adhere via integrins and members of the IgSF. Subsequently, the adherent neutrophils emigrate through the endothelial wall via PECAM-1, ICAM-1, and almost certainly other less well-established mechanisms (72, 73). CD44 has also been proposed to be an important neutrophil adhesion molecule, but is primarily found in activated lymphocytes. Despite extensive expression on neutrophils, the role of CD44 on these cells is not well established. The strategic position of the CD44 ligand, hyaluronan, on the endothelium raises the possibility that CD44/hyaluronan could mediate neutrophil-endothelial cell interactions. Indeed, cross-linking of neutrophil CD44 induces cellular activation (74-76) an important step in the recruitment of leukocytes in inflammation. Thus, numerous studies in the literature have indirectly implicated CD44 as an important molecule in neutrophil motility and recruitment (77-79). In particular, one study has systematically examined the role of CD44 on neutrophils as a molecular mediator of the recruitment cascade. This study suggested that rolling of the neutrophils in postcapillary venules was not mediated by CD44, and circulating neutrophils did not interact with the CD44 ligand, hyaluronan, in vitro (80). The same data also suggested a very limited role for CD44 in the migration of neutrophils through tissue in an vivo model system. However, CD44 was found to play an important role in the adhesion of neutrophils to the endothelium, and this process did require hyaluronan. Finally, the transmigration of neutrophils across the venular endothelium was dependent upon both hyaluronan and CD44 (80). Many other studies demonstrated that integrins were the dominant molecules for neutrophil recruitment. The mechanism of action by which CD44 contributes to neutrophil recruitment could be as an accessory molecule for integrins. For example, CD44 may influence integrin function: cross-linking of CD44 in cancer cells has been shown to activate LFA-1 (81). In T-cells, CD44 has been shown to complex with the VLA-4 integrin, and this association is important for T-cell extravasation to an inflammatory site (82-84). It is possible that similar CD44-integrin associations occur on neutrophils.

Physiological Functions of CAMs. The trafficking of leukocytes within the microcirculation is critical for normal immune surveillance of tissues. In the development of inflammation, adhesion molecules play an essential role in the localization of the inflammatory response. At this level, the vascular endothelium, a governing barrier for the exchanges between blood and the tissues, plays an active part in regulation of the transcapillary permeability, control of proliferation of haematopoietic cells and the phases of the inflammatory response (1). The process of leukocyte recruitment is tightly regulated by the sequential expression and activation of specific adhesion molecules on the surface
of leukocytes and endothelial cells (Figure 4). These adhesion molecules mediate distinct steps in the recruitment of leukocytes in the microcirculation. Selectins mediate leukocyte rolling, whereas glycoproteins belonging to the integrin and immunoglobulin supergene families enable leukocytes to firmly adhere and migrate in venules. The leukocyte-endothelial cell adhesion that is mediated by these adhesion molecules has been shown to alter the function of endothelial cells in all parts of the vasculature (i.e. in arterioles, capillaries, and venules) (22). In vivo observations of the behaviour of leukocytes in venules has led to a model of leukocyte-endothelial cell interactions that predicts three sequential and coordinated steps for leukocyte recruitment: rolling, firm adhesion (adherence), and finally migration of leukocytes (8).

In the dormant state, leukocytes and endothelial cells do not interact. Selectin-binding sites are present on leukocytes but dormant endothelial cells do not express selectins. In cases of damage or inflammatory processes of the blood vessels due to the action of released cytokines, increased expression of adhesion molecules of the integrin, selectin and immunoglobulin groups occurs and subsequently increased adhesion and migration of inflammatory cells across the vascular wall is observed (3). Specifically, to establish an adhesive interaction with endothelial cells, circulating leukocytes must first move from the central stream of flowing blood towards the vessel wall (85). It is now well accepted that endothelial cell activation results in selectin expression and the subsequent interaction of selectins with their ligands thus mediating the weak (low-affinity) adhesive interactions that are manifested as leukocyte rolling (8, 21, 86). Once leukocyte activation is fulfilled leukocyte integrins bind with IgSF glycoproteins such as ICAM-1 and VCAM-1, permitting firm adhesion. Although the quantitative significance of CAMs remains unclear some of them (e.g. VLA-4, VCAM-1, MadCAM-1, and members of the β7 subfamily of integrins) have been implicated in transient leukocyte binding (tethering) and rolling (85).

The tethered leukocytes are then exposed to low concentrations of chemoattractants/inflammatory mediators that result in leukocyte activation and subsequently elicit integrin-Ig-dependent leukocyte adherence, with a simultaneous down-regulation (shedding) of L-selectin. Leukocyte activation is also associated with an increased affinity of the integrins, which can be elicited by chemokines, bacterial peptides, platelet-activating factor (PAF) and leukotriene B4 (21, 87). After they have marginalized, the active cells migrate by diapedesis towards the site of inflammation by the creation of chemotactic signals, as the adhesion between the cells is insufficient to induce their migration (1).

Leukocyte migration is an important mechanism in the pathogenesis of inflammatory diseases, the regulation of hematopoiesis and hemostasis. The transendothelial migration of leukocytes begins with locomotion of adherent leukocytes toward the endothelial cell junctions. Transendothelial migration is mediated by additional IgSF members such as PECAM-1 (8). During this process, the cell steadily establishes new adhesive contacts at the migration front while reducing adhesive interactions at the tail (8, 88). It was shown that CD11/CD18 (alpha L, M, X/beta 2) integrins have an important role in subsequent steps of leukocyte migration into tissues (21).

It is evident that cell adhesion molecules provide the foundation for cell communication, trafficking and immune surveillance central to host defence. These soluble adhesion molecules (selectins, integrins, CD44 and members of the IgSF), provide a recognition system between leukocytes, endothelial cells and matrix molecules. The activation and increased expression of these adhesion glycoproteins have been attributed to excessive production of cytokines and oxidants (22). Additionally, the adherence phenomena depend on a process that is strictly controlled by the cytokines and enables intervention of cell-cell reactions and cell-protein recognition of the extracellular matrix. Cytokines play a key role in control of the expression and/or avidity of membrane receptors for ligands (1). Deregulation of these adhesion and signal transduction pathways can contribute to continued recruitment and persistent leukocyte activation with unresolved inflammation.

CAMs and Inflammatory Heart Diseases

*Infective endocarditis.* Experimental data and pathological observations support the assumption that endothelial cells play a fundamental role in the development of inflammatory
processes and various stimuli result in endothelial activation and endothelial leukocyte interactions including adhesion and extravasation. These interactions are mediated by augmented expression of adhesion molecules, such as E-selectin, ICAM-1, and VCAM-1 (89-93). The attachment of pathogenic microorganisms to vascular endothelial cells (EC) or sites of vascular injury is considered a critical initiating event for many types of intravascular infections.

In bacterial endocarditis (BE), the microbial infection is localized on the endocardial surface of the heart and, depending on the bacterial species, may cause an inflammatory reaction that in most cases affects the mural endocardium and the mitral and aortic valves (94). Providing a brief definition, infective endocarditis is a microbial infection of the endothelial surface of the heart. The characteristic lesion is a variably sized amorphous mass of platelets and fibrin in which abundant microorganisms and moderate inflammatory cells are enmeshed. Acute infective endocarditis is caused typically, although not exclusively, by *Staphylococcus aureus*, whereas the subacute syndrome is more likely to be caused by *Viridans streptococci*, enterococci, coagulate-negative staphylococci, or gram negative coccobacilli. Endothelial activation contributes significantly to the systemic inflammatory response to bacteraemia. Increased expression and release of soluble endothelial markers into the circulation have been demonstrated. Elevated plasma levels of E-selectin have been reported in bacteraemic patients (95, 96). It has also been proposed that the release of E-selectin is related to the degree of vascular or endothelial injury caused by the sepsis (95, 96). Increased plasma and serum levels of VCAM-1 and ICAM-1 have been shown in bacteraemic patients. E-selectin as well as ICAM-1 has also been found to be associated with multiple organ dysfunction, septic shock and death (97). It has been proposed that infection of endothelial cells with *Staphylococcus aureus*, *Streptococcus sanguis*, or *Staphylococcus epidermidis* induces surface expression of ICAM-1 and VCAM-1 and monocyte adhesion (98).

Several studies have demonstrated that during endocarditis the formation of circulating immune complexes, which are identified as containing bacterial components, may contribute directly or indirectly to the stimulation of endothelial cells. Expression of adhesion molecules in vivo can be maintained for several days after stimulation (95-100). It has been suggested in the literature that patients with *Staphylococcus aureus* bacteraemia and endocarditis, which represents a sustained endothelial involvement, showed significantly higher E-selectin and VCAM-1 concentrations on admission than those with *Staphylococcus aureus* bacteraemia without endocarditis which might reflect a more extensive activation of endothelial cells (99-101). It is known that in BE intravascular infection with *Staphylococcus aureus*, *Streptococcus sanguis*, or *Staphylococcus epidermidis* can lead to formation of a fibrin clot on the inner surface of the heart and cause heart dysfunction. In addition, the same study demonstrated that infection of endothelial cells with these three pathogens induces surface expression of ICAM-1 and VCAM-1 as well as monocyte adhesion (102). Furthermore, using immunohistochemistry, the CAM expression of endothelial cells on degenerative, mostly calcified heart valves and on heart valves with florid endocarditis was characterized. As expected, the constitutively expressed molecules (ICAM-1, CD34, CD31) were found both on degenerative and on inflamed valves. Moreover, marked expression of E-selectin and VCAM-1 was found not only on inflamed valves but also on larger portions of the degenerative valves with no morphological evidence of inflammation. This striking finding might help to explain why patients with fibrotic heart valves are susceptible to recurrent endocarditis (103). Another perspective was given from a relatively recent study regarding the role of soluble adhesion molecules E-selectin and P-selectin. Specifically, the mean plasma concentrations of P-selectin were elevated in patients with embolic events as compared to both patients without embolic events and control subjects. Similarly, the patients with embolic events had increased plasma levels of E-selectin compared to those without embolic events and the control group (104). This reflected enhanced platelet activation, which has a direct impact on thrombus generation. Moreover, the increased expression of the endothelial activation markers E-selectin and VCAM-1 on degenerative heart, the CAM expression of endothelial cells on degenerative, mostly calcified heart valves and on heart valves with florid endocarditis as well as the constitutively expressed molecules (ICAM-1, CD34, CD31) both on degenerative and on inflamed valves suggest that adhesion molecule-mediated leukocyte recruitment or activation of the endothelium may constitute a critical role in the pathogenesis of endocarditis and in the manifestation of its major complications such as thromboembolism (99, 100, 102, 103). However, it remains to be clarified by future studies whether the elevated adhesion molecule levels result from focal release of adhesion molecules at the site of endocardial involvement or from the systemic effects of severe bacteraemic disease. Any potential clinical value of establishing the diagnosis of endocarditis by measuring serum adhesion molecule concentrations is diminished by the presence of an overlap between the groups of bacteraemic patients with and without endocarditis (99). CAMs nevertheless constitute relevant diagnostic targets and after additional elaborate studies might be considered as future diagnostic criteria of endocarditis in the future.
Myocarditis. Myocarditis is one of the most challenging diagnoses in cardiology. The entity is rarely recognised, the pathophysiology is poorly understood, there is no commonly accepted diagnostic gold standard and all current treatment is controversial. Primary myocarditis is presumed to be due to either an acute viral infection (e.g. coxackie, cytomegalovirus, adenovirus, influenza, rubella virus) or a postviral autoimmune response. Secondary myocarditis is myocardial inflammation caused by a specific pathogen including bacteria, protozoa, rickettsia, spirochetes, fungi, drugs, chemicals, physical agents or other inflammatory diseases such as lupus erythematosus (15). Briefly, in viral myocarditis, the virus enters the body through the respiratory or gastrointestinal tract stimulating a systemic immune response. Immune response cells, such as dendritic cells and macrophages release cytokines, interleukins, perforin, reactive oxygen species, tumour necrosis factor and regulatory growth factors. Once these products activate nuclear factor B, the production of cytokines, ICAM, and inducible nitric oxide is induced. In parallel, antigen-presenting cells expressing major histocompatibility complex and associated with co-receptors CD 40 and ICAM couple with infected myocytes displaying antigen (epitope) on their surface (105-107). This involvement of CAMs in the pathophysiology of myocarditis is supported and further enhanced by several studies found in the literature. Specifically, cell-mediated autoimmunity has been strongly implicated in the pathogenesis of the myocardial cell damage involved in viral myocarditis. To investigate the cellular and molecular bases of both target cells and effector cells for cell-mediated cytotoxicity involved in viral myocarditis, scientists attempted to examine the expression of major histocompatibility complex (MHC) antigens and ICAM-1 in myocardial cells of a murine model of viral myocarditis and in patients with acute myocarditis and dilated cardiomyopathy with rather interesting results. Both MHC and interestingly ICAM-1 were clearly expressed in the hearts of these patients (108, 109). In general, the importance of VCAM-1 and ICAM-1 in controlling cell adhesion and migration is well established (110). Moreover, studies have clearly suggested that the elevated levels of ICAM-1 and VCAM-1 are often found in the serum of patients with non-ischemic heart failure. These raised serum levels correlate with inflammatory infiltrates in the myocardial tissue (111). Expression of ICAM-1 and VCAM-1 on endothelial cells of capillaries in myocardium are increased in patients suffering from eosinophilic myocarditis or coxackie virus B3 acute myocarditis (108, 111, 112). In this context, it has been shown that expression of endothelial ICAM-1 is a prerequisite for target organ recognition by autoreactive T-cells (105). In addition, VCAM-1 expression has been demonstrated in mice that developed myosin-induced CD4-mediated myocarditis (113, 114). Consistently, up-regulation of VCAM-1 expression has been observed following early acute Trypanosoma cruzi infection (115,116). Relatively recently, in the literature it was revealed that during chronic T. cruzi infection, a remarkable increase in the expression of VCAM-1 was observed on cardiac vascular endothelium (117). The same phenomenon was also found in coxackie virus B3-induced myocarditis (108, 117). Most importantly, it seems that ICAM-1 and VCAM-1 blood vessels associated with CD8+ infiltrating T-cells were also detected in the cardiac tissue of chagasic patients with severe cardiomyopathy (118). It is more than evident that the participation of CAMs in the establishment of autoimmune and infectious myocarditis is an important matter of investigation, providing a broad wide field of investigation which may have promising diagnostic and therapeutic implications regarding myocarditis in the future.

Pericarditis. Although it is evident from general knowledge that CAMs should have a role in the molecular pathology of inflammatory pericarditis, there is still no direct proof in the literature concerning this issue. It is more than likely that future studies are or will be conducted towards understanding CAM involvement in the development of inflammatory pericarditis.

Conclusion

Scientific evidence is rapidly accumulating to support the view that leukocyte and endothelial cell-associated CAMs are critical participants in the vascular dysfunction and tissue injury that is associated with a wide variety of inflammatory and cardiovascular diseases. Advances in this field have largely resulted from the marriage of novel immunological and molecular biological approaches to traditional experimental strategies in cardiovascular physiology. This effort has led to a new appreciation of the difficulties in distinguishing cardiovascular from inflammatory diseases and provides hope that therapeutic interventions and new diagnostic and prognostic perspectives targeting CAM may be of some benefit in the treatment of inflammatory as well as cardiovascular diseases.

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