The induction of cellular interactions in atherogenesis and their modulation
Joseph Loscalzo
Department of Medicine, Brigham and Women's Hospital, Brockton/West Roxbury VA Medical Center, Harvard Medical School, 75 Francis Street, Boston, Massachusetts, MA 02115, U.S.A.

Interactions between vascular cells and blood cells are central to the development of the atherosclerotic process. Under normal circumstances, blood cells do not associate with the blood vessel; however, in response to atherogenic stimuli, platelets, lymphocytes and mononuclear leukocytes interact with the vessel wall and induce changes in the vascular micro-environment that support atheroma formation. These changes include the enhancement of endothelial permeability, the conversion of the normal endothelium from an anti-thrombotic to a pro-thrombotic phenotype, the induction of endothelial and vascular-smooth-muscle migration, the stimulation of vascular-smooth-muscle-cell replication, and the elaboration of extracellular matrix by the vascular-smooth-muscle cell [1]. The principal effectors that mediate this dramatic modification of vascular properties are soluble intercellular-signaling molecules that are known as cytokines.

Cytokines and atherogenesis
Cytokines are defined as soluble (glyco)proteins that are released by cells to regulate cellular functions [2]. In general, these molecules act in the nano- to picomolar concentration range to regulate cellular responses. Cytokines are themselves grouped into four categories: interleukins, interferons, colony-stimulating factors, and peptide growth factors. Endothelial cells, vascular-smooth-muscle cells, mononuclear cells/tissue macrophages, lymphocytes and platelets can all be induced to elaborate a variety of cytokines, and these molecules ultimately promote endothelial- and vascular-smooth-muscle-cell migration, vascular-smooth-muscle-cell replication, and monocyte activation. Adhesion to matrix molecules, adhesion to other cells, or exposure to cytokines themselves can lead to the elaboration of cytokines by the cells involved in atherogenesis. Cytokines, in turn, exert their effects on cell migration and on replication by inducing cells to produce other cytokines. A list of specific cytokines that have been implicated in atherogenesis follows:

**Interleukins**
- Interleukin-1 α, β
- Interleukin-2
- Interleukin-4
- Interleukin-6
- Interleukin-8
- Tumour necrosis factor-α

**Interferons**
- Interferon-α
- Interferon-β

**Growth factors (GFs)**
- Epidermal GF
- Platelet-derived GF
- Fibroblast GF
- Transforming GF-α
- Transforming GF-β
- Platelet-derived endothelial cell GF
- Monocyte colony-stimulating factor

The historical context within which cytokine biology has been defined, that is, inflammation, leads to an appreciation of the mechanistic underpinning associating atherosclerosis and inflammation, and to a clearer understanding of the regulation of inflammatory-cell responses in atherogenesis [3]. The mechanism by which cytokine growth factors, in particular, induce cell replication has been well-studied. Growth factors bind to their specific growth-factor receptors localized on the surface of the cell responding to that factor. These receptors commonly span the cell-surface membrane and are noncovalently associated with one of several coupling proteins, including guanine-nucleotide-binding (G) proteins and tyrosine kinases, on the inner surface of the membrane. Through common responses supported by these coupling proteins, the surface-binding signal is transduced to increase the intracellular calcium concentration and to activate protein kinases. These latter events ultimately lead to the activation of transcription factors that induce the genomic response(s) that support cell replication.

Vascular-cell replication also depends critically on the modulation of the matrix. Matrix
modification occurs as a consequence of the elaboration of tissue metalloproteases (matrixins) by vascular cells [4], a process that is induced by epidermal GF and by fibroblast GF [5]. By contrast, transforming growth factor-β shows an anti-proliferative action as a consequence of the repression of matrixin activity and the reciprocal induction of an inhibitor of tissue metalloprotease [6].

The expression of adhesion molecules that promote the association of blood cells with vascular cells is yet another effect of cytokine stimulation that is critical for atherogenesis. These adhesion molecules promote the adhesion of vascular cells to the matrix, as well as promoting adhesion to the endothelium and emigration from the blood-vessel lumen into the vessel wall, similarly to their role in inflammation. The complexity of this group of molecules and of the regulation of their expression warrants detailed discussion.

**Adhesion molecules and atherogenesis**

Blood and vascular-cell-adhesion molecules are divided into three classes: the selectins, the integrins, and the immunoglobulin superfamily. Cell-adhesion molecules in each of these classes that are believed to be important in atherogenesis are listed below:

**Selectins**
- E-selectin
- P-selectin
- L-selectin

**Integrins**

- LFA-1 (CD11a/CD18)
- Mac-1 (CD11b/CD18)
- p150, 95 (CD11c/CD18)
- VLA-4 (CD49d/CD29)

**IgG-like molecules**
- Platelet-endothelial cell adhesion molecule (PECAM)
- Intercellular adhesion molecule 1 (ICAM-1)
- ICAM-2

E-selectin (formerly called endothelial leukocyte adhesion molecule, ELAM-1) is synthesized by endothelial cells in response to interleukin-1 and to tumour necrosis factor-α [7]. Maximal surface expression occurs by 4 h, and the expression supports the adhesion of neutrophils, monocytes, eosinophils, and T cells. P-selectin (formerly called GMP-140 or PADGEM) is synthesized by megakaryocytes (and as a result, incorporated into platelets) and by endothelial cells [8]. Stored inside membranes of secreted granules (α-granules of platelets and Weibel–Palade bodies of endothelial cells), P-selectin is rapidly expressed on the surface of these cells after stimulation by comparatively rapidly acting activators, such as thrombin or histamine. Peak expression occurs within 10 min of stimulation, and the expression supports the adhesion of neutrophils, monocytes, eosinophils, T cells and natural killer cells. L-selectin (formerly called Mel 14 or LECAM-1) is constitutively expressed on the surface of neutrophils, monocytes and most lymphocytes; this selectin mediates the adhesion of leukocytes to cytokine-activated endothelial cells [9].

Integrins are heterodimeric molecules that consist of α and β subunits that are associated non-covalently; they are broadly distributed receptors that mediate a number of interactions between cells, and between cells and matrix [10, 11]. Members of the integrin receptor family that are involved in vascular-cell interactions are listed in Table 1.

The three β-2 integrins, or CD11/CD18 molecules, are found only on leukocytes and are associated with one of three α chains (LFA-1, Mac-1, p150, 95); these receptors, in conjunction with VLA-4 (β2α4) and platelet glycoprotein IIb/IIa (αIIbβ3), play a central role in atherogenic responses, largely as a consequence of their facilitating the targeting of leukocytes (and of platelets) to sites of vessel injury.

Integrins on activated leukocytes interact with endothelial counter-receptors that are members of the immunoglobulin superfamily. Each of these proteins contains a series of immunoglobulin-like domains of 90–100 amino acids [12]. LFA-1 binds to ICAM-1 and 2; Mac-1 binds to ICAM-1, but not to ICAM-2; and VLA-4 binds to vascular-cell adhesion molecule 1 (VCAM-1). In addition, another member of the immunoglobulin superfamily, PECAM-1, serves as its own counter-receptor and facilitates early adhesion of mononuclear cells to endothelial cells [13, 14].

The interactions among these adhesion molecules that result in leukocyte adhesion, diapedesis and emigration have been identified recently [15]. Under resting conditions, leukocytes do not adhere to endothelial cells; however, in the presence of inflammatory mediators, a two-phased response is noted. Firstly, within 6–12 h of exposure to acute mediators, such as histamine or thrombin, initial adhesion or rolling is observed, mediated by the selectins and, possibly, by PECAM-1. Cytokine-induced (interleukin-1, interferon-α) tight adhesion occurs at 6–48 h as a consequence of the interaction between integrins and immunoglobulin-like molecules. The precise mechanism(s) by which these
Table I

Vascular integrins

<table>
<thead>
<tr>
<th>β-subunit</th>
<th>α-subunit</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td>γi</td>
<td>α1</td>
<td>Laminin, collagen</td>
</tr>
<tr>
<td>α2</td>
<td>Collagen, laminin</td>
<td></td>
</tr>
<tr>
<td>α3</td>
<td>Fibronectin, laminin, collagen</td>
<td></td>
</tr>
<tr>
<td>α4 (VLA-4)</td>
<td>Fibronectin, VCAM</td>
<td></td>
</tr>
<tr>
<td>α5</td>
<td>Fibronectin</td>
<td></td>
</tr>
<tr>
<td>α6</td>
<td>Laminin</td>
<td></td>
</tr>
<tr>
<td>αv</td>
<td>Fibronectin</td>
<td></td>
</tr>
<tr>
<td>βγ (CD18)</td>
<td>αl (LFA-1)</td>
<td>ICAM</td>
</tr>
<tr>
<td>α4 (Mac-1)</td>
<td>C3bi, fibrinogen</td>
<td></td>
</tr>
<tr>
<td>α4 (α-chain of p150, 95)</td>
<td>C3bi (?)</td>
<td></td>
</tr>
<tr>
<td>βδ (Platelet glycoprotein Ila, vitronectin receptor)</td>
<td>αδ (Platelet glycoprotein llb)</td>
<td>Fibronectin, fibrinogen, vitronectin, von Willebrand factor</td>
</tr>
<tr>
<td></td>
<td>αv</td>
<td>Vitronecin, fibrinogen, von Willebrand factor, thrombospondin</td>
</tr>
<tr>
<td>βδ</td>
<td>α6</td>
<td>Laminin (?)</td>
</tr>
<tr>
<td>βδ</td>
<td>αv</td>
<td>Vitronecin, fibronectin</td>
</tr>
</tbody>
</table>

Interactions support diapedesis and emigration are incompletely understood, but may involve the shedding of selectins from the cell surface and a transient reduction in integrin activity [16]. As a consequence of the rearrangement of integrins to the leading edge of the cells and the subsequent associations with cytoskeletal proteins, migration of cells along chemotactic gradients is promoted [17].

Lipoprotein(a) and its role in atherogenesis: New insights into mechanisms involving cellular adhesion molecules and their ligands

Lipoprotein(a) is a unique lipoprotein that was first identified by K. Berg in 1963 [18] that consists of a low-density-lipoprotein particle linked by a disulphide bridge to apoprotein(a); more recently, elevated plasma levels of lipoprotein(a) have been strongly associated with an increased risk for the development of atherosclerotic vascular disease [19]. Due to the striking homology between apoprotein(a) and human plasminogen [20], several investigators, including our group, hypothesized and demonstrated that lipoprotein(a) could impair plasminogen activation by a variety of mechanisms, including inhibition of fibrin-stimulated plasminogen activation by tissue-type plasminogen activator [19], direct inhibition of tissue-type plasminogen activator [21], inhibition of cell-surface plasminogen activation [22-24], and stimulation of the elaboration of plasminogen-activator inhibitor type 1 by endothelial cells [25].

Given the affinity of apoprotein(a) for fibrinogen and the co-localization of fibrinogen and lipro(apo)protein(a) in atheromatous plaques [26, 27], we hypothesized that lipoprotein(a) is atherogenic not only because it is a ligand for the scavenger pathway after oxidation [28], but also as a result of its impairing the clearance of fibrinogen from atheromatous lesions [29]. Fibrinogen and its proteolytic degradation fragments in the vessel wall show a variety of atherogenic properties, including the induction of vascular-smooth-muscle-cell replication, endothelial migration, and monocyte chemotaxis [29]. This hypothesis was tested by evaluating the ability of lipoprotein(a) to attenuate cellular clearance of fibrinogen. As Altieri and colleagues showed [30] and we confirmed, fibrinogen binds to activated human monocytes through the integrin Mac-1 (CD11b/CD18). We also found, however, that the interaction of this pluripotent integrin with fibrinogen leads to the internalization of the ligand–receptor complex, with subsequent proteolysis of the ligand [31]. We believe that this unique
mechanism of cellular fibrinolysis is probably responsible for the comparatively slow clearance of vascular thrombi during recanalization, as distinct from the rapid plasmin-mediated fibrinolysis. Finally, we showed that lipoprotein(a), when complexed with fibrin(ogen), impaired the uptake and degradation of fibrin(ogen) by the Mac-1 pathway [31]. Very recently, we have taken these observations one step further and shown that the lipoprotein(a)-fibrin(ogen) complex induces the expression of the adhesion molecules E-selectin and ICAM-1 on the endothelial cell. Thus, this lipoprotein has a variety of properties that support atherogenesis by mechanisms that involve the molecules that govern vascular-cell interactions that, in turn, sustain the atherogenic process.

These observations illustrate the molecular mechanisms that govern the complex interactions among the vascular cells and blood cells that are involved in atherogenesis. The induction, regulation and modulation of these interactions between cells, and between cells and vascular matrix, represent potential targets for pharmacologic and, perhaps in the future, genetic intervention.

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