Monocyte–Endothelial Cell Interaction in Atherogenesis and Thrombosis

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Summary: Cellular interactions between leukocytes and the endothelium are critical events in vascular biology, such as atherosclerosis and acute coronary syndrome. When monocytes and endothelial cells are activated via direct cell–cell interaction, both types of cells express several biologically active molecules such as adhesion molecules, cytokines, coagulation and fibrinolytic factors, metalloproteinases, and vasoactive substances. All of these molecules could contribute to atherogenesis and thrombosis.

Key words: monocyte, endothelial cell, atherosclerosis, thrombosis, acute coronary syndrome

Introduction

The infiltration of circulating blood monocytes across the vascular endothelium is an early important event in inflammation and atherosclerosis.^{1–3} The first step in monocyte infiltration into the vessel wall is their adhesion to and subsequent migration across the endothelium, which leads to the accumulation of these cells in the intima. When monocytes and endothelial cells are activated via such cell–cell interactions, both types of cells express several biologically active molecules such as cytokines, proteases, vasoactive substances, or coagulation and fibrinolytic factors. All of these molecules could contribute to the pathogenesis of various cardiovascular disorders. In this review article, we focus primarily on this monocyte–endothelial cell interaction in the process of atherogenesis and thrombosis.

Adhesion Molecules

Vascular endothelial cells constitute the interface between the blood stream and tissues. Endothelial cells appear to perform several critical roles in the atherogenic process, including regulation of the adhesion of blood monocytes and their subsequent migration across the endothelium.^{3,4} It is now generally accepted that endothelial surface molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), are responsible for the process of monocyte adhesion and migration.^{5–9} ICAM-1 binds to $\beta 2$ integrins, such as lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18) and Mac-1 (CD11b/CD18), present on all leukocytes. VCAM-1 binds to the integrin very late antigen-4 (VLA-4) present on lymphocytes, eosinophils, and monocytes. *In vivo* immunopathologic studies of both experimental and human atherosclerotic specimens have demonstrated the expression of ICAM-1 and VCAM-1 molecules on the endothelium.^{10–14} Furthermore, it has been shown that patients with unstable angina show increased expression of monocyte $\beta 2$ integrin adhesion molecules that interact with ICAM-1 and VCAM-1 and elevated plasma levels of soluble ICAM-1 and VCAM-1, indicating that an inflammatory reaction takes place within their coronary tree.¹⁵

Previously, we have shown that the addition of unstimulated human monocytes to endothelial cells rapidly induced the expression of ICAM-1 and VCAM-1 protein as well as their mRNA in endothelial cells, whereas the addition of polymorphonuclear leukocytes had no significant effect on their expression.¹⁶ These findings suggest that direct cell–cell contact between monocytes and endothelial cells further promotes their adhesion and interaction through upregulation of endothelial adhesion molecule expression as a positive feedback mechanism.¹⁷ However, at present, the precise signal transduction mechanism involved in this coculture-induced adhesion molecule expression remains to be elucidated.

Cytokines

Direct cell–cell interaction between leukocytes and endothelial cells may induce cytokine synthesis.^{18–20} For example, it has been reported that endothelial cells act as costimulators to augment interleukin-2 (IL-2) synthesis by activated

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CD4⁺ T cells.²¹ The costimulatory activities are dependent upon contact between endothelial cells and the responding T cells, partially mediated by the specific interaction of lymphocyte function-associated antigen-3 (LFA-3) on endothelial cells with CD2 on the T cells. Eierman *et al.*¹⁹ and Sporn *et al.*²² demonstrated that the adhesion of monocytes to cultured endothelial cells resulted in the induction of several cytokine genes, such as those encoding tumor necrosis factor α (TNF α) and colony stimulating factor-1. Haskill *et al.*²³ reported that the adhesion of monocytes induced the expression of IL-1 β in monocytes, suggesting that the adhesive interaction activates the monocytes themselves.

We attempted to determine whether the interaction between monocytes and endothelial cells induces the production of monocyte chemoattractant protein-1 (MCP-1) from these cell types, because this interaction constantly occurs in the recruitment of monocytes, and because production of MCP-1 would affect subsequent monocyte transendothelial migration.²⁴ Migrated monocytes evolve into macrophages. Previous pathologic studies have shown that MCP-1 is expressed in human atherosclerotic plaques^{25, 26} or in small veins of the ischemic and reperfused canine myocardium.²⁷ In our study, coculture of unstimulated human monocytes and endothelial cells induced an increase in the amounts of soluble MCP-1 secreted into the medium.²⁸ Quantification of mRNA levels indicated that augmentation was mediated at the level of MCP-1 synthesis. Anti-IL-1 α/β and anti-TNF α Abs used in combination inhibited MCP-1 production by only 20%, but this inhibition was statistically significant. Immunohistochemical studies showed that adherent and transmigrated monocytes as well as endothelial cells expressed MCP-1 protein, while nonadherent monocytes did not. These findings suggest that monocytes and endothelial cells transmit signals to each other to induce MCP-1 synthesis during the process of cell contact and transmigration. If MCP-1 is produced by monocytes that have migrated into the vascular tissue, it would promote further extravasation of monocytes into the tissue and thus play an important role in atherogenesis.

Cell-cell interactions between monocytes and endothelial cells have also been shown to produce other cytokines such as IL-8,^{29, 30} platelet-derived growth factor,³¹ and granulocyte-macrophage colony-stimulating factor,³² all of which have also been shown to stimulate migration and proliferation of vascular smooth muscle cells or activation of macrophages and thus could promote the development of atherosclerosis.

Coagulation and Fibrinolytic Factors

Endothelial cells are modulators of hemostasis, thrombosis, and fibrinolysis. Lo *et al.*³³ reported that adhesion of monocytes to activated endothelial cells induced the expression of tissue factor, which initiates coagulation by binding to its high affinity ligands, circulating Factors VII and VIIa. Formed thrombi are fibrinolized by the plasminogen-plasmin system, which is inhibited by plasminogen activator inhibitor-1 (PAI-1). PAI-1 is an independent risk factor of coronary heart dis-

ease. Previously, we demonstrated that monocytes decreased the fibrinolytic potential of endothelial cells by increasing PAI-1 production without any effect on tissue plasminogen activator (TPA) production.³⁴ Anti-IL-1 α/β and anti-TNF α Abs used in combination partially but significantly inhibited PAI-1 production under these coculture conditions. However, monoclonal antibodies directed against CD18, VLA-4, ICAM-1, and VCAM-1, which can partially but significantly inhibit monocyte-endothelial cell adhesion, had no effect. Hakkert *et al.*³⁵ reported that conditioned medium of primary cultures of monocytes enhanced endothelial PAI-1 synthesis, although the increase was <43% in the presence of monocytes that were in direct contact with endothelial cells, suggesting that not only cell-cell contact but also secretory products from monocytes contribute to the enhanced production of PAI-1, albeit to a lesser extent. This production of tissue factor and PAI-1 by cell-cell interactions between monocytes and endothelial cells could enhance thrombus formation in acute coronary syndrome.

Metalloproteinases

Mechanical factors that contribute to atherosclerotic plaque rupture include imposed hemodynamic stresses resulting from plaque composition and geometry, and mechanical properties of the fibrous cap tissue.³⁶⁻³⁸ Extracellular matrix molecules are primarily responsible for maintaining the mechanical integrity of the fibrous cap. These matrix components may be degraded by matrix metalloproteinases (MMPs) such as collagenase (MMP-1), gelatinase (MMP-2), and stromelysin (MMP-3), which can be produced by vascular endothelial and smooth muscle cells as well as by macrophages.³⁹ A local excess of these metalloproteinases in the fibrous cap could lead to weakening of the extracellular matrix, rendering the plaque more likely to rupture. Recent studies have demonstrated that greater densities of macrophages appear at rupture-prone sites in plaques and correlate with weaker fibrous cap tissue,^{40, 41} and that increased metalloproteinase activity colocalizes with macrophages in the vulnerable shoulder region of plaques.⁴² Recently, Lee *et al.*⁴³ reported that human monocyte-vascular smooth muscle interaction induced MMP-1 and MMP-3 expression. We also found that human monocyte-endothelial cell interactions induced MMP-1 and MMP-2 expression (unpublished data). Thus, cell-cell interactions between monocytes and endothelial cells might also play crucial roles in the pathogenesis of plaque rupture in acute coronary syndrome.

Nitric Oxide

Endothelial cells are key determinants of vascular tone and intimal integrity through the generation of vasoactive and growth-regulatory substances. Nitric oxide (NO) is generated under basal conditions and in response to a variety of hemodynamic and chemical stimuli from the guanidino nitrogen atom(s) of L-arginine through a reaction catalyzed by endo-

thelial constitutive NO synthase (ecNOS).⁴⁴ Formation and release of NO is a primary mechanism whereby endothelium regulates smooth muscle tone and proliferation.⁴⁵ In addition to its effect on smooth muscle, NO is a potent inhibitor of platelet aggregation and monocyte adhesion.^{46,47} Several lines of evidence from both *in vitro* and *in vivo* studies have recently suggested a role of NO as an antiatherogenic autacoid.⁴⁸ Nitric oxide has also been shown to protect against intracoronary thrombosis and reocclusion after thrombolysis in a canine model.⁴⁹ Recently, Marczin *et al.*⁵⁰ reported that adhesion of monocytes to endothelial cells and monocyte-derived secretory products downregulated steady-state levels of ecNOS, an event associated with attenuated release of biologically active NO, via IL-1 β production. This mechanism might lead to diminished endothelium-dependent and NO-mediated vasodilatation. Decreased endothelial NO production could also enhance smooth muscle proliferation, platelet aggregation, and monocyte adhesion to the endothelium, which could be related to both atherogenesis and thrombosis.

Conclusions

Cellular interactions are critical events in vascular biology. The cellular mechanisms that contribute to the pathogenesis of atherosclerosis or acute coronary syndrome via monocyte-endothelial cell interactions are important subjects for both basic and clinical researchers in cardiovascular medicine.

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