Multi-functional Roles of Granulocyte Macrophage Colony-Stimulating Factor in Atherosclerosis

Yasuyuki Sasaguri*, and Akihide Tanimoto**

Granulocyte macrophage colony-stimulating factor (GM-CSF) is a growth factor for hematopoietic progenitor cells, and is one of major pro-inflammatory cytokines. In the atherosclerotic lesion, the cells constituting the arterial intima such as endothelial and smooth muscle cells, and monocytes/macrophages can produce GM-CSF in response to other cytokines, modified low-density lipoprotein and cell-cell interaction. GM-CSF exhibits multi-functional effects on the intimal cells and closely related to the pathogenesis of atherosclerosis. In particular, the monocytes/macrophages are present in all stages of atherosclerosis and play a central role in atherogenesis. Monocytic differentiation, macrophages proliferation, expression of adhesion molecules, matrix-degrading enzymes and cytokines are regulated by GM-CSF. Among these, monocytic migration and matrix metalloproteinase expression would be one of critical functions for monocytes/macrophages. GM-CSF is also related to angiogenesis and vasculogenesis by enhancing endothelial cell mobilization from progenitor cells and monocyte adhesion.（J. Jpn. Coll. Angiol., 2003, **43**: 715-723）

Key words: GM-CSF, Atherosclerosis, Monocytes/macrophages, Migration, Matrix metalloproteinase (MMP)

Introduction

Inflammatory events are involved in the pathogenesis of atherosclerosis[1~2], and T-lymphocytes, monocytes/macrophages, and monocyte-derived dendritic cells are abundant in the atherosclerotic lesion[3~5]. These inflammatory cells are also functional to regulate Th-1 and Th-2 balance in the lesion environment of atherosclerosis[6~7]. Especially, monocytes/macrophages are present in all stages of atherosclerosis, playing an important role in atherogenesis; and they have multiple functions, which include migration and secretion of growth factors, cytokines, and matrix-degrading enzymes, and the uptake of modified lipoproteins[8~9]. During the initiation of atherosclerosis or inflammation, circulating monocytes adhere to endothelial cells via specific cell-adhesion molecules, and subsequently migrate into the subendothelial space[10]. These processes take place in the micro-environment formed by endothelium, intimal smooth muscle cells, migrating lymphocytes and macrophages, and extracellular matrixs, as well as being regulated by many biologically active substances secreted from the constituent[1].

In this review we describe the multi-functional roles of granulocyte macrophage colony-stimulating factor (GM-CSF) in relation to atherosclerosis. Especially, we focus on GM-CSF effects for monocyte/macrophage functions, including migration and matrix metalloproteinase (MMP) expression. Furthermore, the importance of GM-CSF in monocyte/macrophage functions is more emphasized than that of platelet-derived growth factor (PDGF), which is a predominant factor for the functions of smooth muscle cells (SMC).

1. Multi-functional Role of GM-CSF

GM-CSF is a growth factor required for the survival, growth, and differentiation of hematopoietic proenitor cells[11], and is produced by inflammation-related cells including activated T-cells, monocytes/macrophages, and endothelial cells[12]. GM-CSF is involved in a cytokine/growth factor network present in the atherosclerotic lesion[1, 13~14]. With regards to monocyte/macrophage functions, GM-CSF plays an essen-

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tial role in monocyte differentiation[12] and macrophage proliferation[15~16] in relation to the pathogenesis of atherosclerosis. In addition, the expression of adhesion molecules, MMPs, plasminogen-activator inhibitor, urokinase-type plasminogen activator, and myeloperoxidase is regulated by GM-CSF in monocytes/macrophages[14, 17~20]. These findings indicate that GM-CSF modulates the conditions of the lesion environment of atherosclerosis.

2. Rho activation and GM-CSF

The Rho family GTPases, including Rho (A, B and C), Rac, and Cdc42, have been found to mediate cell adhesion, migration, and invasion. Several lines of evidence have suggested that the Rho is directly linked to the migratory or invasive phenotypes of epithelium-derived tumor cells[21]. In inflammation, the first step of leukocyte/monocyte transmigration is an interaction with endothelial cells; and the activated endothelial cells express adhesion molecules including E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1)[22]. The clustering of these cell-surface molecules induced by monocyte adhesion is regulated by the Rho signaling pathway[23]. RhoA is also activated after the stimulation with GM-CSF (unpublished data).

The signals for monocyte chemoattractant protein-1 (MCP-1)-dependent integrin activation via Rho are mediated by MCP-1 receptor CCR2, a member of the family of transmembrane-domain, G protein-coupled receptors[24]. On the other hand, the growth factor receptors of receptor tyrosine kinase family, such as receptors for epidermal growth factor (EGF), hepatocyte growth factor (HGF), and PDGF-BB, activate Rho small G-protein via Ras activation[21]. Our results demonstrate that GM-CSF also stimulated the monocytes to activate RhoA and integrin clustering just like MCP-1 and platelet-derived growth factor-BB (PDGF-BB). At present, the molecular mechanism of the signaling pathway for GM-CSF-induced Rho activation is unknown. However, the GM-CSF-induced activation of a number of signaling molecules, including JAK2, Ras, Raf, ERK, and RSK[25] indicate a possible involvement of one of these signaling molecules as a mediator for Rho activation.

3. Integrin and GM-CSF

The recruitment of monocytes from the blood stream is one of the critical steps for atherosclerosis, and is regulated by various adhesion molecules expressed on both endothelial and monocytes[1]. As shown in **Fig 1**, in monocytes/macrophages, very late antigen-4 (VLA-4) and lymphocyte function-associated antigen (LFA-1) are essential for facilitating lateral migration and diapedesis across the endothelial cells in the vascular wall, respectively[26~27]. This monocyte-endothelial cell interaction is an initial event for atherogenesis. By stimulation with GM-CSF, both integrins are clustered on the cell surface (**Fig. 2**), indicating that GM-CSF, as well as PDGF-BB and monocyte MCP-1, is an important factor for the initial step of monocyte transendothelial migration.

4. Matrix metalloproteinase production and GM-CSF

A close linkage between MMP production and cell migratory activity of many types of cells may be implied; and the secretion of matrix-degrading enzymes, such as MMP, from monocytes is one of the key events to enhance the migratory action of the monocytes[28~29]. GM-CSF has been reported to enhance MMP-12 expression in monocytes/macrophages, and the MMP-12 is localized in the atherosclerotic lesion[17, 30]. Addition to MMP-12, MMP-1 and MMP-9 expression is also induced by GM-CSF in U937 monocytes (unpublished data) (**Fig. 3**). The expression of these MMPs in the atherosclerotic lesion is seen in commercially available antibodies (MMP-1, -7, and -9). For example, **Fig 4** shows the expression of MMP-1 and MMP-9 in monocyte-derived foam cells in the atherosclerosis. An overall review on MMP expression and atherogenesis is discussed in our previous review[31].

GM-CSF induces MMP-9 expression in human peripheral blood monocytes[32], but the molecular mechanism for gene regulation by GM-CSF has not been clarified. The results of an earlier study using U937 monocytic cells indicated that the AP-1 motif in the MMP-12 promoter region was involved in gene regulation by GM-CSF, and that the AP-1 binding complex consisted of multiple fos/jun isoforms including junD, c-jun, fosB, c-fos, and fra-1[17]. The AP-1

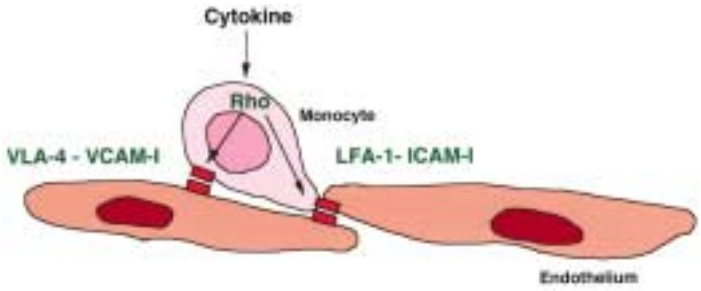

Figure 1 Rho activation and integrin clustering.

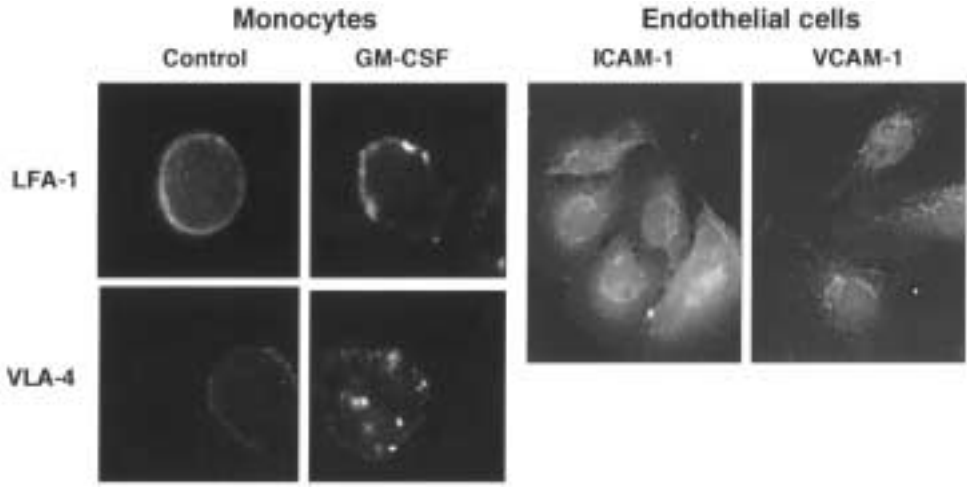

Figure 2 Immunofluorescence of integrin in monocytes (U937) and in human aortic endothelial cells.
Left: After treatemtnt with GM-CSF, clustering of LFA-1 and VLA-4 (granular immunofluorescence) is taken place on the surface of monocytes.
Right: Upon stimulation with inflammatory stimuli (histamine), the expression of ICAM-1 and VCAM-1 is enhanced in the endothelial cells.

site is involved in many types of MMP gene expression[33] (**Fig. 5**). Recent report demonstrates an expression of extracellular matrix metalloproteinase inducer (EMMPRIN) in human atheroma, and GM-CSF-induced macrophages show enhanced expression of the EMMPRIN as well as MMP-9[34]. GM-CSF may play a central role monocytic MMP expression and its regulation in relation to atherogenesis.

PDGF, which has multilfunctional effects on SMC, does not enhance MMP-1, MMP-9 and MMP-12 expression in U937 monocytes (unpublished data).

5. Cytokine network and GM-CSF in atherosclerosis

In the atherosclerotic lesion environment, a cytokine/

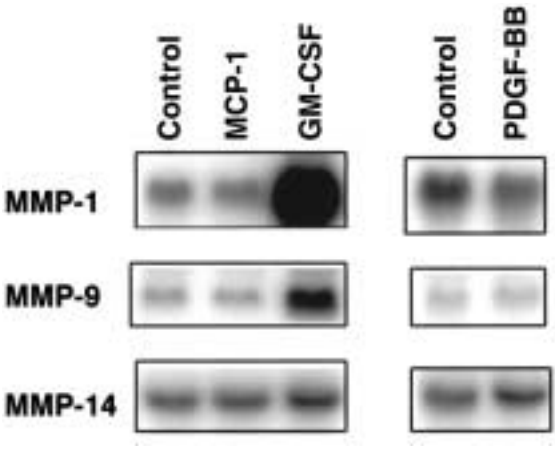

Figure 3 Northern bot analysis of MMPs mRNA.
Unlike PDGF-BB, GM-CSF stimulates monocytes to up-regulated the transcrition of MMP-1 and MMP-9.

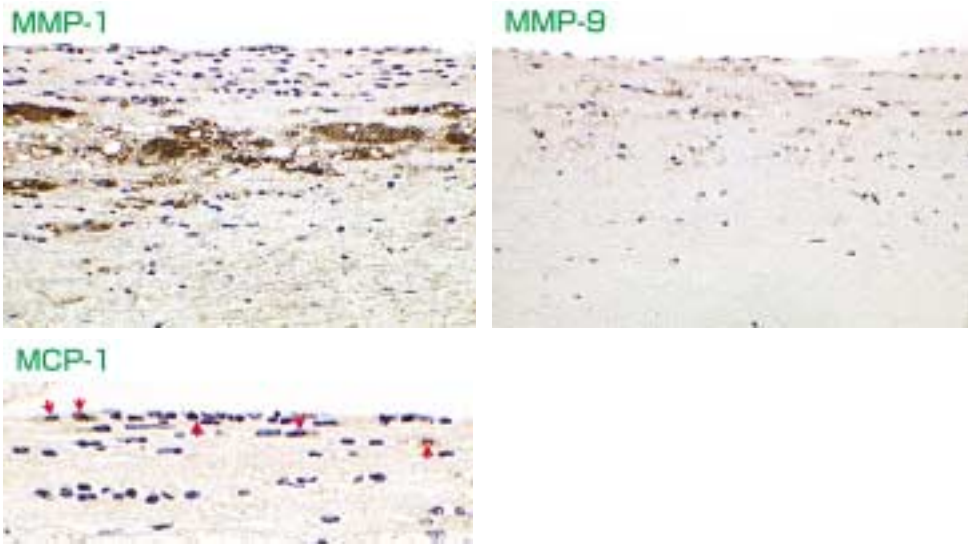

Figure 4 Immunohistochemical detection of MMP-1, MMP-9 and MCP-1 in human atherosclerotic lesions. MMP-1 and MMP-9 expresse in macrophages (form cells) in atherosclerotic thickened intima. MCP-1 is detected in endothelial cells and mononuclear cells beneath the endothelium (arrows).

growth factor network is constructed by various cytokines, growth factor, chemokines, and other biologically active substances[35~36]. In this cytokine/growth factor network, "cross talk" between various active substances determines the net effects of the cytokines. Among these cytokines or growth factors, we focus on the effects of GM-CSF in the monocytes, because some earlier reports demonstrated that GM-CSF stimulated peritoneal macrophages or a monocytic cell line to express MCP-1[37]. Our data also shows that GM-CSF, but not PDGF, enhances MCP-1 production at transcription, as well as mRNA and protein levels (unpublished data) (**Fig. 6**). GM-CSF may enhance monocytic functions via stimulation of MCP-1 production. GM-CSF has been reported only to prime monocytes for enhanced migration in response to MCP-1 through LFA-1 activation[18]. The mononuclear cells in human atherosclerotic lesion express MCP-1 (**Fig. 4**).

Other than GM-CSF, many other pro-inflammatory cytokines such as TNF-α, interleukine-1β, and IL-6 have been reported to enhance the MCP-1 expression in monocytes, endothelial cells, and fibroblasts[38]. In addition, bacterial LPS stimulated monocytes/macrophages to produce MCP-1[39]. Interestingly, LPS has also been reported to enhance Rho-mediated LFA-1 activation and AP-1-mediated MMP-1 production in monocytes[40~41]. This MMP-1 gene transcription stimulated by LPS is not NF-kB (located at -20 to -10) dependent[40], and in our study mutation at the NF-kB-like site did not affect either basal activity or induction of the transcription by GM-CSF (unpublished data). There may thus exist a similar molecular mechanism in the signal transduction pathway of GM-CSF and LPS.

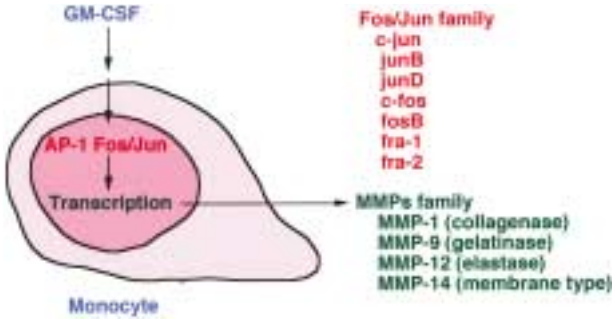

Figure 5 Transcriptional regulation of MMPs.

6. Expression of scavenger receptors and GM-CSF

A few reports describe GM-CSF regulation of scavenger receptor expression in monocytes. Treatment with GM-CSF

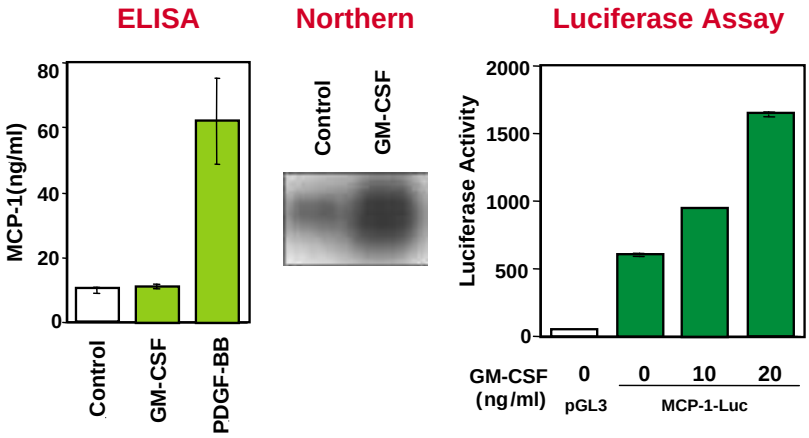

Figure 6 MCP-1 expression analysis by GM-CSF in monocytes.

down-regulates a number of binding sites for modified low density lipoprotein (LDL), and mRNA expression of type I and II scavenger receptor[42]. The scavenger receptor CD163 in monocytes is down-regulated by treatment with GM-CSF and IL-4[43~44]. In contrast, we found that inflammatory stimuli (histamine) stimulate monocytes to expression of lectin-like oxidused low-density lipoprotein receptor-1 (LOX-1)[45]. We also found that transcriptional activity of Lox-1 is up-regulated by GM-CSF and IL-4, but not by PDGF-BB, in U937 monocytic cells (unpublished data). Since monocytic treatment with GM-CSF and IL-4 induces dendritic differentiation[46~47], GM-CSF may have a potential role of scavenger expression in monocytes and also in dendritic cells. Interestingly, monocyte-derived dendritic cells are activated by oxidized-LDL to increase HLA-DR, CD40 and CD86 expression[48].

7. GM-CSF effects on vascular smooth muscle cell

SMC are one of major constituents of vascular wall and participate in local inflammatory, immune responses[49] and MMP production[50]. However, most studies have demonstrated PDGF effects on SMC and endothelial cells or fibroblasts in terms of MMP expression, migration and proliferation[51~53] (**Fig. 7**). Based on our data, PDGF, but not GM-CSF, predominantly contributes to the functions of SMC to expression of MMP-12[54], while GM-CSF, but not PDGF, up-regulates monocytic MMP-12 expression[17].

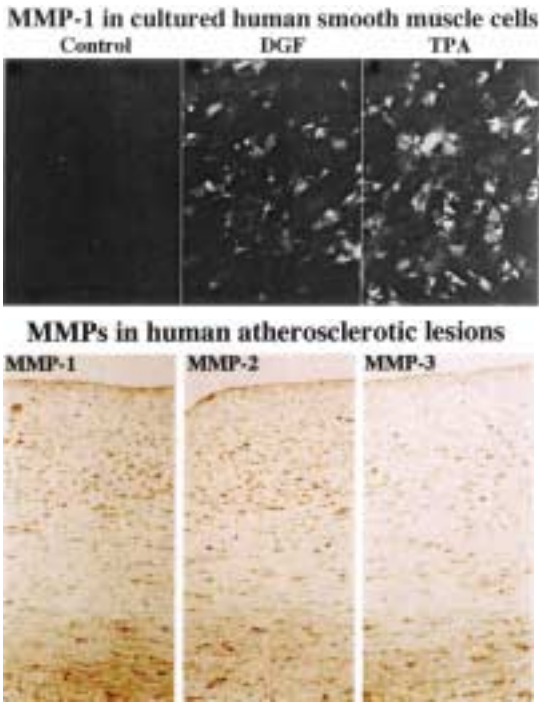

Figure 7 Expression of MMPs in SMC.
Immunofluorescence staining:Synthesis of MMP-1 is stimulated in cultured human aortic smooth muscle cells by PDGF and TPA. Immunohistochemical staining: Expression of MMP-1, -2, and -3 is detected in human atherosclerotic intima.

One notable report is that GM-CSF stimulates SMC to express type VIII collagen[55], which is present in neonatal aorta rather than in adults' and is expressed at high levels in atherosclerotic lesion[56].

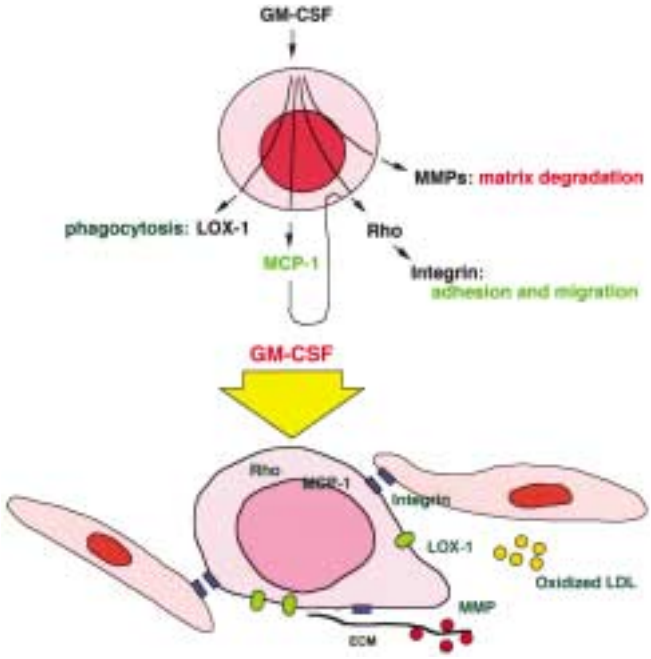

Figure 8 Multi-functional effects of GM-CSF in lesion environment of early stage of atherogenesis.

8. Source of GM-CSF in atherosclerotic lesion

In addition to the presense of GM-CSF in peripheral blood, monocytes/macrophages, endothelial, fibroblasts and smooth muscle cells can produce GM-CSF[57~58]. Monocyte and endothelial interaction, which is an initinal step of atherogenesis, stimulates both types of cells to express GM-CSF[13]. Modified-LDL induces GM-SCF expression in endothelial cells[59]. Cultured human coronary SMCs constitutively express GM-CSF[60], and SMCs from internal mammary artery and aorta increase GM-CSF expression by treatment with IL-1 and TNF-α[49]. GM-CSF localization in human atherosclerotic lesion is shown. Based on our data, multi-functions of GM-CSF are summarized in **Fig. 8**.

9. GM-CSF effects on angiogenesis and vasculogenesis

GM-CSF influences the migration and proliferation of human endothelial cells[58]. Recent reports demonstrate that GM-CSF enhances the endothelial cells mobilization from bone marrow- or peripheral blood-derived progenitor cells[61~62]. This event is closely related to angioenesis and vasculogenesis[63~64]. Especially in the situation of arteriogenesis (collateral development from pre-existing arterioles) circulating monocytes adhere to the endothelium and play an important role in remodeling of the arterioles[64]. Furthermore, GM-CSF is a strong arteriogenic factor, which regulates monocytes proliferation and life span[64~65]. The monocytes are also the predominant source of growth factors and proteolytic enzymes like MMP that regulate migration of SMC[65]. In fact, promotion of collateral growth by GM-CSF has been reported in human coronary artery disease[66].

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