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*Review*

# T Cell Integrin Activation by Chemokines in Inflammation

YOSHIYA TANAKA*

The First Department of Internal Medicine, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807-8555, Japan

**Abstract.** The adhesive function of integrins is regulated through cytoplasmic signaling induced by several stimuli, whose process is designated as "inside-out signaling". A large number of lymphocytes are recruited to the sites of inflammation where they form an essential component of the response to infection, injury, autoimmune disorders, allergy, tumor invasion, atherosclerosis and so on. The recruitment of leukocytes into tissue is regulated by a sequences of interactions between the circulating leukocytes and the endothelial cells. Leukocyte integrins play a pivotal role in leukocyte adhesion to endothelial cells. During the process, the activation of integrins by chemokines is essential for integrin-mediated adhesion in which a signal transduced to the leukocyte converts the functionally inactive integrin to an active adhesive configuration. The present review documents the relevance of cytoplasmic signaling and cytoskeletal assembly to integrin-mediated adhesion induced by chemokines during inflammatory processes.

**Key words:** T lymphocytes; recirculation/recruitment; adhesion molecules; integrins; chemokines; inflammation.

## Introduction

The expression and function of adhesion molecules are tightly regulated through intracellular signaling induced by several cellular stimuli, this process being designated "inside-out signaling". Among these stimuli, cytokines are potent inducers of the adhesive function and expressions of several adhesion molecules. Leukocyte function-associated antigen 1 (LFA-1) ($\alpha_L\beta_2$ integrin) and very late antigen 4 (VLA-4) ($\alpha_4\beta_1$ integrin) mediate the adhesion of leukocytes to opposing ligands, the intercellular adhesion molecule 1 (ICAM-1) and the vascular cell adhesion molecule 1 (VCAM-1), respectively, both of which belong to the immunoglobulin superfamily (IgSF), but the adhesive capacity of integrins is tightly regulated. Although integrins expressed on resting cells do not mediate firm adhesion to their ligands, stimulation of these cells results in a rapid increase in integrin function[6, 27, 34]. We here document the mechanism of the integrin-mediated adhesion of circulating T cells to endothelial ligands, shedding light upon chemokine-mediated signaling and the cytoskeletal machinery during inflammatory processes.

## Integrins Require Activation Stimuli to Adhere to Their Ligands

Resting T cells cannot adhere to purified ICAM-1, purified VCAM-1, fibronectin nor endothelial cells,

* Correspondence to: Dr. Yoshiya Tanaka, The First Department of Internal Medicine, University of Occupational and Environmental Health, School of Medicine, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555, Japan, tel.: +81 93 603 16 11 (ex. 2426), fax: +81 93 691 93 34, e-mail: tanaka@med.uoeh-u.ac.jp

whereas T cells activated with phorbol myriacetate ester (PMA) adhere to them very well within 30 min incubation *in vitro*. Thus, activation of integrins is essential for integrin-mediated adhesion, in which a signal transduced to the leukocyte converts the functionally inactive integrin to an active adhesive configuration. In this regard, we have reported that the chemokine macrophage inflammatory proteins 1α (MIP)-1α and MIP-1β trigger integrins and induce adhesion of circulating T cells and leukemic T cells to endothelial cell integrin-ligands[1, 32, 33, 36, 40, 41]. MIP-1α and MIP-1β are members of the chemokine-family, which includes IL-8, platelet factor-4, monocyte chemotactic protein 1 (MCP-1) and the regulated-upon-activation, normal T cell-express and presumably secreted (RANTES). Several recent studies have emphasized the potential importance of chemokines in inflammatory responses; various chemokines produced in large amounts at the site of inflammation activate integrins on leukocytes and result in leukocyte migration and accumulation in inflamed tissues. The mechanisms of integrin-triggering are thought to be such that a conformational change of the ectodomain of the integrins and/or a clustering of integrins on the cell membrane can induce an active, adhesive configuration of integrins, brought about by the cytoskeletal actin-polymerization associated with the endodomain of the integrins[4, 7, 30].

## Chemokines Induce Integrin-Dependent Adhesion of T Cells to Endothelial Cells during Inflammatory Adhesion Cascade

The recruitment of leukocytes into tissues is regulated by a sequences of interactions between the circulating leukocytes and the endothelial cells. An adhesion cascade consisting of tethering, integrin-triggering, firm integrin-mediated adhesion and diapedesis/transmigration has been proposed to explain the mechanism of normal leukocyte migration[6, 27, 34]. Circulating T cells first tether to endothelium through the loose tethering of selectin to its ligand sialomucin, which can effectively lead to the second step of triggering. Efficient triggering brings a high-affinity adhesion of T cells to endothelium and subsequent infiltration into underlying tissues. Thus, the activation of integrins is essential for integrin-mediated adhesion, in which a signal transduced to the leukocyte converts the functionally inactive integrin to an active adhesive configuration (Fig. 1).

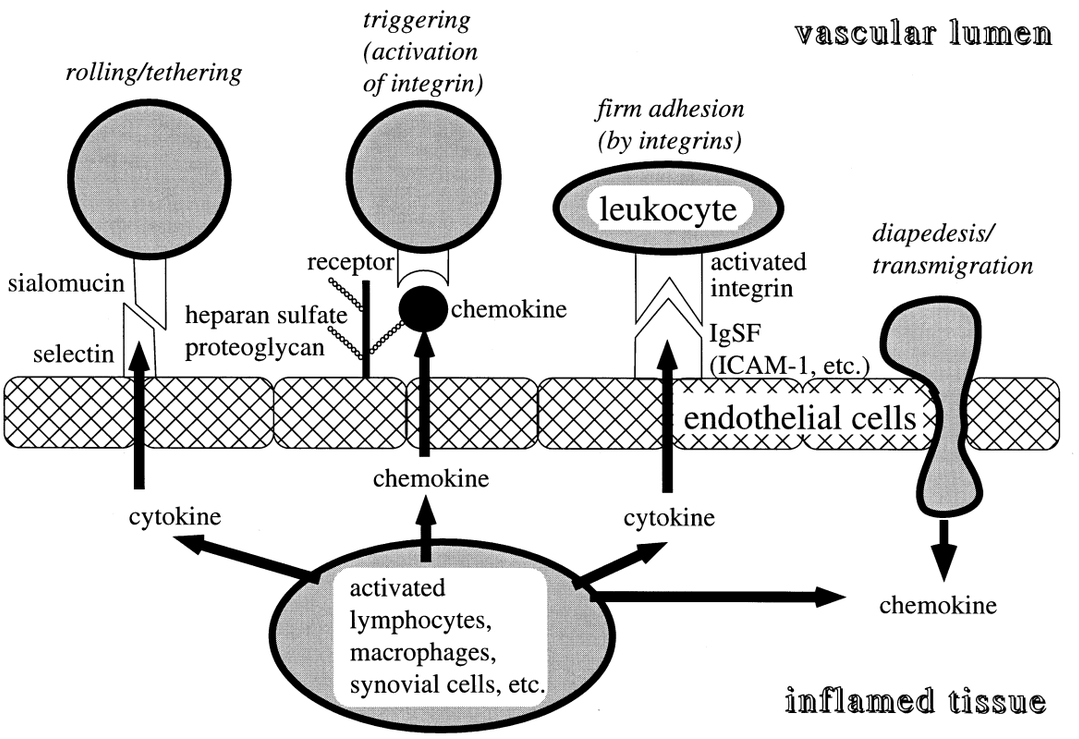


**Fig. 1.** Leukocyte adhesion cascade in the interaction with endothelium in inflamed tissue. Illustrated are the four steps leading to the migration of leukocytes from the blood stream to the inflammatory site. Leukocyte rolling/tethering, activation of integrins, firm integrin--mediated adhesion, and diapedesis/transmigration are mediated by a combinatorial matrix of adhesion and cell signaling molecules

MIP-1α and MIP-1β rapidly trigger T cell integrin and induce integrin-dependent adhesion to ICAM-1, VCAM-1 and fibronectin. Furthermore, when MIP-1α or MIP-1β is immobilized on endothelial cells purified from synovial tissue of patients with rheumatoid arthritis (RA) and subsequently, the soluble chemokines left in the culture supernatant are washed out, T cells adhere to RA-endothelial cells in a concentration-dependent manner[32, 36]. Chemokines including MIP-1α and MIP-1β, are produced in large amounts by activated macrophages and lymphocytes in inflamed tissue or second lymphoid organ, and trigger integrins on leukocytes and induce chemotaxis, which lead to their accumulation there. It is of note that chemokine receptors are expressed on distinctive subsets of leukocytes: receptors for MIP-1α, MIP-1β and RANTES are mainly expressed on T cell subsets and, thereby, preferentially induce T cell adhesion and chemotaxis; receptors for IL-8 and MCP-1 are mainly on neutrophils and monocytes, respectively, and thereby induce accumulation of these cells in certain inflamed tissue. Thus, depending on receptor expression, chemokines provide different and distinctive inflammatory processes.

## Heparan Sulfate Proteoglycan Modulates Chemokine Function

We and others have proposed that the chemokines MIP-1β and IL-8 recruit leukocytes most efficiently when immobilized on the luminal surface of endothelium and that heparan sulfate proteoglycan (HS-PG) on the endothelium immobilizes the chemokines in this process without being washed away by the blood flow[1, 32, 33, 37, 44]. The core protein of HS-PG is post--translationally modified by the addition of glycosaminoglycan side chains, made up of repeating disaccharide subunits, to serine residues of the core protein, some of which are in the extracellular matrix while others are integral membrane proteins[3, 13, 20]. Various cytokines and growth factors, including all the chemokines, fibroblast growth factor and hepatocyte growth factor, possess heparin-binding sites, which allow these proteins to bind to HS-PG.

Cytokines are diffusible and soluble factors. However, HS-PG on either the cell surface or matrices provides the following advantages to the function of these heparin-binding cytokines by immobilizing them and presenting cytokines to their receptors on target cells: 1) HS-PG promotes the accumulation of cytokines at high concentration by binding them on the appropriate location for encountering their target cells; 2) HS-PG protects cytokines from both chemical and physiological stimuli; 3) HS-PG induces conformation-dependent association or the polymerization of the cell surface molecules, including cytokines and cytokine receptors, by binding them[33, 35, 37, 38]. HS-PG thereby plays a pivotal role in the promotion and regulation of the multicrine regulatory mechanisms of particular cytokine functions.

## "Inside-Out Signaling" Stimulated by Chemokines in the Integrin Activation of T Cells

Recent findings indicate that integrin-triggering can be induced by multiple signaling pathways which involve different integrin regulators, including G proteins, tyrosine kinases (TK), protein kinase C (PKC), cAMP pathway and phosphoinositide 3 (PI 3)-kinases[1, 11, 15, 26, 30, 31]. Chemokine receptors belong to the "serpentine" receptor family with seven transmembrane domains and are G-protein-coupled proteins, which are known to activate PI 3-kinases and integrin adhesiveness by ligation of the receptor with fMLP and certain chemokines such as RANTES and MCP-1.

As described, chemokines MIP-1α and MIP-1β efficiently induce integrin-mediated adhesion of resting T cells to IgSF molecules such as ICAM-1 and endothelial cells purified from RA synovium *in vitro*[36]. However, the MIP-1α- and MIP-1β-induced T cell adhesion to RA-endothelial cells is clearly reduced by pretreatment of the T cells with pertussis toxin, which uncouples certain G-proteins from their complex, or wortmannin, a PI 3-kinase inhibitor. However, neither genistein, a TK inhibitor, H7, a PKC inhibitor, nor H89, an A-kinase inhibitor, affect the induced T cell adhesion. Furthermore, pretreatment of T cells with cytochalasin B, a cytoskeleton-disrupting agent, reduces chemokine-induced adhesion of T cells to the endothelial cells. These results suggest that the chemokine-induced integrin-dependent adhesion of T cells to RA-endothelial cells might depend on cytoskeletal rearrangement induced through the G-protein-sensitive PI 3-kinase activation stimulated by these chemokines.

We have further addressed the signaling mechanisms of integrin-mediated adhesion using adult T cell leukemia (ATL) cells[40]. ATL is a unique model for the following reasons: a) ATL is characterized by a malignant expansion of peripheral mature $CD4^+$ T cells caused by HTLV-I infection; b) ATL cells result from monoclonal proliferation and form in each patient a phenotypically and functionally homogeneous population or cell; c) A notable feature of ATL is the re-

markable tendency for malignant cells to infiltrate multiple organs. ATL cells spontaneously bind to purified ICAM-1, and monoclonal antibody (mAb)-blocking studies, in which ATL cell-adhesion to ICAM-1 is inhibited by anti-LFA-1 mAb, indicate that the adhesion is mediated by LFA-1. ATL cells highly produce chemokines MIP-1α or MIP-1β and express receptors for them. The increased adhesion of ATL cells to ICAM-1 is inhibited by pre-treatment of ATL cells with a mixture of anti-MIP-1α and MIP-1β antibodies and is also reduced by transfection of antisense oligonucleotides, but not sense oligonucleotides, for either MIP-1α or MIP-1β. These findings imply that ATL cell adhesion is mediated by endogenous MIP-1α and MIP-1β in an autocrine manner. Furthermore, pretreatment of cells with pertussis toxin, wortmannin or LY294002 reduces LFA-1-dependent adhesion of ATL cells to purified ICAM-1. However, neither genistein, H7, nor H89 affect the ATL cell adhesion. Cytochalasin B and cytochalasin D also reduce the adhesion. Together these data suggest that the increased LFA-1-mediated adhesion of ATL cells to ICAM-1 mainly depends on the activation of G-protein-sensitive PI 3-kinase which is stimulated by the endogenous MIP-1α and MIP-1β.

Since most receptors for chemoattractants are a G-protein-coupled protein, cytoplasmic large G-proteins and/or small G-proteins have been postulated to be involved in the chemokine-induced triggering of integrins[4, 15, 18, 26, 31]. Among the several small G-proteins, Ras is known to play a central role in signal transduction, from which multiple pathways radiate. It is noteworthy that the adhesion of ATL cells expressing the dominant negative H-Ras mutant H-Ras$^{V12}$S17N is markedly reduced to the basal level[39]. LFA-1 requires an active configuration to bind to its ligand, a process that can be induced by a variety of stimuli and can be reported by NKI-L16 mAb, which reacts with a $Ca^{2+}$-dependent activation epitope located on the ectodomain of the α-chain of LFA-1[43]. Resting T cells do not express the activated form of LFA-1, as recognized by the mAb using flow cytometry, whereas ATL cells spontaneously express the activated form of LFA-1. However, the binding of NKI-L16 mAb is inhibited on ATL cells expressing the dominant negative form of H-Ras$^{V12}$S17N. In contrast, expression of the LFA-1 α-chain, as recognized by CD11a mAb TS1/22, is similar on ATL cells and ATL cells expressing the H-Ras$^{V12}$S17N[39]. These results imply that the H-Ras signal pathway followed by PI 3-kinase activation, plays a role in the induction of the active configuration of LFA-1, resulting in enhanced LFA-1-mediated adhesion of T cells and leukemic T cells stimulated by chemokines (Fig. 2).

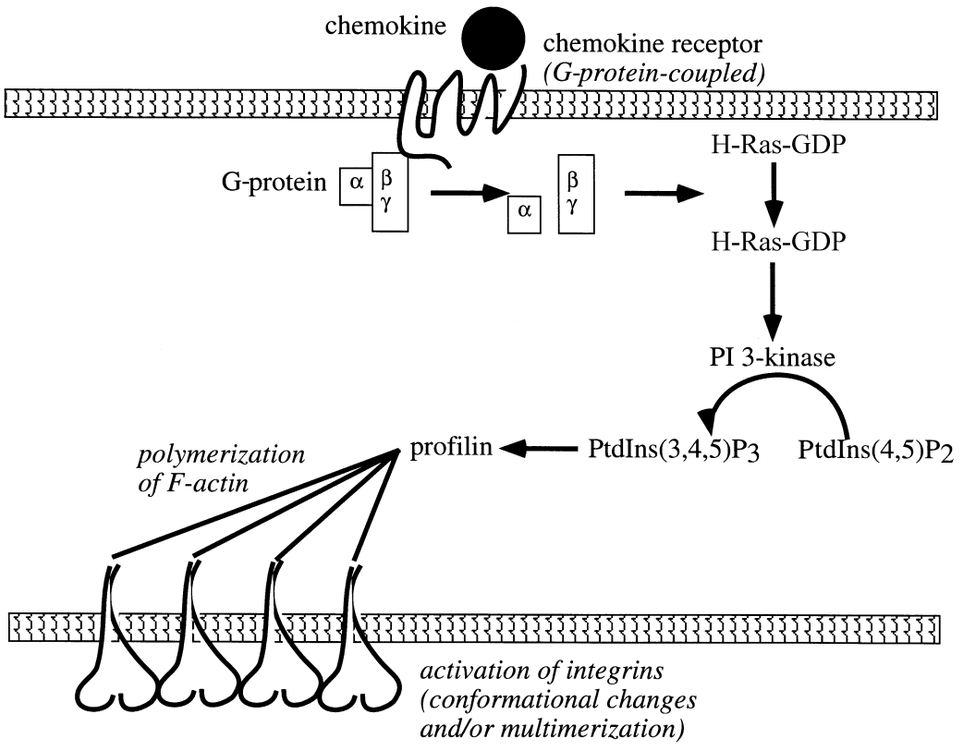


**Fig. 2.** Proposed mechanisms of integrin-activation by chemokines in T cells. We propose that the inflammatory chemokines are involved in triggering integrin LFA-1 through cytoskeletal rearrangement induced by G-protein-dependent activation of PI 3-kinases

In contrast, it was also reported that expression of an active form of H-Ras, and its effector kinase, Raf-1, in CHO cells stably expressing an active chimeric integrin suppressed the function of the chimeric integrin α6A, β1 and β3. Suppression of integrin function correlated with activation of the Ras/Raf/MAP (ERK) kinase pathway[12]. One plausible explanation for such discrepant H-Ras functions is that the active form of H-Ras may exhibit distinct functions in regulating different types of integrins, although cell type-specific functions of H-Ras can also be considered. Alternatively, second signals induced by H-Ras may be differently involved in the "on and off switch" for integrin triggering. Ras is known to be a "hub" which radiates multiple signaling pathways, including Raf-1 and PI 3-kinase[19]. Recently accumulating evidence demonstrates that PI 3-kinase plays a central role in integrin-triggering as well as in cytoskeletal changes[9, 21, 25, 26]. Actually, the H-Ras$^{V12}$Y40C mutant, which binds to PI 3-kinase in T cells, induces the activated form of LFA-1α and LFA-1-dependent adhesion to ICAM-1, and activation of LFA-1 stimulated by chemokines is inhibited by PI 3-kinase inhibitors[39]. These results suggest that H-Ras--sensitive PI 3-kinase activation is involved in an "on switch" for LFA-1 on T cells, while the H-Ras-ERK kinases may function as an "off switch" for integrins.

## Cytoskeletal Machinery during Integrin Activation

PI 3-kinase is thought to be controlled by G-protein--coupled receptors and is involved in cytoskeletal rearrangement associated with localized polymerization of actin filaments and highly cross-linked membrane-associated fibers[4]. The mechanism underlying the activation of integrin involves conformational changes of the ectodomain of the integrins and/or the clustering of integrins on the cell membrane, resulting from cytoskeletal actin-polymerization associated with the cytoplasmic domain of integrins[1, 4, 7, 11, 17, 22, 31, 32, 36, 40, 41]. In resting T cells, F-actin content remains distributed, as observed by confocal microscopy. In contrast, chemokine MIP-1β induces marked rearrangement and polymerization of F-actin in T cells within seconds. Furthermore, the expression of the fully activated H-Ras mutant H-Ras$^{V12}$ or H-Ras$^{V12}$Y40C in T cells show an apparent increase of F-actin in the cell cortex and marked polymerization and rearrangement of F-actin as well as a highly augmented expression of the activation epitope of LFA-1 as recognized by NKI-L16[39].

Profilin, a 12–15 kDa cytoplasmic protein, promotes actin polymerization by converting ADP-actin to ATP-actin, thus stimulating polymerization[8, 14, 24, 29]. Furthermore, profilin is known to be physically associated with $PIP_2$ and PI 3-kinase products and functions as a "linker" between cytoplasmic signaling and actin assembly[5, 10, 28]. By using immunofluorescence with confocal laser-scanning microscopy, T cells expressing the H-Ras$^{V12}$ or H-Ras$^{V12}$Y40C represent increased expression of profilin in their cortex. Furthermore, polymerized F-actin clearly co-localizes with profilin in T cells expressing H-Ras$^{V12}$ or H-Ras$^{V12}$Y40C by double staining of them. Freshly obtained ATL cells also show an increased expression of F-actin in the cell cortex and polymerization of F-actin, which is clearly co-localized with profilin. However, when ATL cells are pretreated with wortmannin, F-actin-polymerization and co-localization with profilin are markedly reduced. Furthermore, cytochalasin B markedly reduces F-actin polymerization, whereas the distribution of profilin remains constant, as observed in non-treated ATL cells. Also, the co-localization of profilin with spontaneously polymerized F-actin in ATL cells is reduced by the expression of dominant negative H-Ras$^{V12}$S17N[39]. These results suggest that G-protein-sensitive PI 3-kinase activation plays a role in the cortical actin assembly composed by profilin, resulting in the triggering of LFA-1-mediated adhesion in T cells and leukemic T cells.

## Relevance of Integrin Activation in Inflammatory Processes

The potential importance of chemokines in inflammatory responses is well accepted. A large number of lymphocytes are rapidly recruited to the sites of inflammation where they form an essential component of the response to infection, injury, autoimmune disorders, allergy, tumor invasion, atherosclerosis and so on. We here document the relevance of integrin activation of T cells, activated T cells, neutrophils and monocytes to inflammatory processes in RA, tumor invasion and atherosclerosis as the followings.

RA is a representative model of a persistent inflammatory process characterized by marked infiltration of the synovium by T cells. It is now generally accepted that T cells locally infiltrating the rheumatoid synovium play an important role as both regulatory and effector cells in the initiation and perpetuation of the inflammatory process of RA synovitis and that various adhesion molecules as well as cytokines are thought to contribute to T cell migration into the tissues. The trig-

ger appears to be essential for the adhesion of T cells and efficient triggering brings a high-affinity adhesion of T cells to the endothelium and subsequent infiltration into the underlying tissues[36]: 1) suitable HS-PG is expressed on endothelial cells purified from RA synovium *in vitro* and vessels in the tissues *in vivo* by immunohistochemical studies; 2) MIP-1α and MIP-1β induce T cell adhesion to RA-endothelial cells more efficiently by immobilizing on them; 3) mAb-blocking studies indicate that the T cell adhesion induced by endothelial immobilized chemokines is mediated by the integrins LFA-1 and VLA-4 on T the immobilization of cells; 4) the addition of anti-MIP-1α and MIP-1β Ab to the endothelial cells during the immobilization of these chemokines on the cells disrupts T cell adhesion to the endothelial cells; 5) the artificially decreased cell surface HS by heparitinase digestion results in a reduced integrin-mediated adhesion of T cells to the endothelial cells; 6) MIP-1α and MIP-1β are produced by infiltrated T cells and are expressed on endothelium in RA synovium *in vivo*. Thus, once "free" chemokines are secreted into the circulation, they would be washed away by the blood flow. However, by binding to the HS, chemokines could be practically accumulated on the cellular surface and presented to the particular receptors efficaciously. These concepts, that HS-PG can bind/post and present/relay cytokines to the specific receptors and induces cellular function, have come into the involvement of HS-PG on RA-endothelial cells in the chemokine-mediated integrin-triggering of tethering T cells along vascular lumen in the tissue in a juxtacrine system.

Tumor-infiltrating lymphocytes (TIL) mediate tumor regression, which depends upon the migration of TIL into tumor-bearing tissue and encountering tumor cells to mediate anti-tumor responses. Other studies reported strong adhesive properties for TIL towards the endothelium, a process that ultimately results in the accumulation of TIL in the appropriate tumor milieu[2]. We have reported that integrins on TIL are spontaneously activated by the endogenous chemokines MIP-1α and MIP-1β in an autocrine manner, which contributes to the highly adhesive characteristics of TIL[41]. Other chemokines, monokine induced by IFN-γ (Mig) and IFN-γ-induced peptide (IP-10), secreted by cells within the tumor, are known to attract T cells to the neoplasm[16, 23]. Thus, chemokines play an important role in the accumulation of tumor-reactive T cells in a tumor milieu.

Atherosclerosis is the principal contributor to the pathogenesis of myocardial and cerebral infarction, gangrene and loss of function in the extremities. In the genesis of the lesions of atherosclerosis, the marked accumulation of macrophages in atherosclerotic plaques has reawakened the interest in inflammatory components. A chemokine, MCP-1, has been postulated as playing an important role in the pathogenesis, since it is highly produced in the tissue and efficiently induces chemotaxis of monocytes to the tissue[42]. A high plasma concentration of low-density lipoprotein (LDL) cholesterol is one of the principal risk factors for atherosclerosis. We have used silent monocytes isolated by counterflow centrifugal elutriation and obtained the following results; 1) oxidized LDL, but not native LDL, induced LFA-1-dependent adhesion of monocytes to endothelial cells which was comparable to MCP-1; 2) MCP-1 and oxidized LDL induced activation epitope of LFA-1 on monocytes and marked polymerization of F-actin in monocytes; 3) MCP-1 and oxidized LDL also induced transendothelial migration of monocytes. Therefore, we now propose that oxidized LDL as well as MCP-1, produced in large amounts in inflamed atherosclerotic plaques, can induce F-actin polymerization and activation epitope of LFA-1 in circulating monocytes and may amplify LFA-1-dependent adhesion and subsequent transendothelial migration of monocytes into the tissue.

## Concluding Remarks

Thus, not only the expression, but also the activation of adhesion molecules are induced by the "inside-out" signaling stimulated by cytokines. Among them, chemokines such as MIP-1, IL-8 and MCP-1 convert the functionally inactive integrin into an active configuration, while inflammatory cytokines such as IL-1, TNF-α and IFN-γ augment the quantity of adhesion molecules such as ICAM-1 and VCAM-1. It is well known that adhesion molecules not only function as a glue, but also transduce extracellular information into the cytoplasm, which leads to T cell activation as well as cytokine production from immune cells and inflamed cells such as rheumatoid synovial cells. Such a busy cross-talking among adhesion molecules and cytokines is most relevant to inflammatory processes by augmenting immune cell migration from the circulation into inflamed tissue and by activating both immune cells and resident cells in the tissue. The concept proposed would bring enormous power and flexibility to clarifying the multiple pathological processes as well as new pharmacological approaches to more specifically control inflammation.

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