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Invited Review

Integrin activation by chemokines: Relevance to inflammatory adhesion cascade during T cell migration

Y. Tanaka

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

Summary. The adhesive function of integrins is regulated through cytoplasmic signaling induced by several stimuli, whose process is designated "inside-out signaling". A large number of leukocytes 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. The recruitment of leukocytes into tissue is regulated by a sequence 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 various chemoattractants, especially 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. We have proposed that H-Ras-sensitive activation of phosphoinositide 3 (PI 3)-kinase and subsequent profilin-mediated actin polymerization, can be involved in chemokine-induced integrin-dependent adhesion of T cells. The present review documents the relevance of cytoplasmic signaling and cytoskeletal assembly to integrin-mediated adhesion induced by chemoattractants including chemokines during inflammatory processes. In contrast, various adhesion molecules are known to transduce extracellular information into cytoplasm, which leads to T cell activation and cytokine production from the cells, designated "outside-in signaling". Such a bi-directional "cross-talking" among adhesion molecules and cytokines is most relevant to inflammatory processes by augmenting immune cell migration from circulation into inflamed tissue such as rheumatoid arthritis, tumor invasion, Behçet's disease and atherosclerosis.

Key words: T lymphocytes, Recirculation/Recruitment, Adhesion molecules, Chemokines, Inflammation

Offprint requests to: Dr. Yoshiya Tanaka, The First Department of Internal Medicine, University of Occupational and Environmental Health, Japan, School of Medicine, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu 807-8555 Japan. Fax: +81-93-691-9334. e-mail: tanaka@med.uoeh-u.ac.jp

Introduction

Definition of the molecular basis of cellular adhesion and its importance in cell-cell and cell-matrix interactions have progressed during the last decade. Adhesion molecules are involved in signaling in multiple physiological and pathological processes. The expression and function of adhesion molecules are tightly regulated through intracellular signaling induced by several cellular stimuli, whose process is designated "inside-out signaling". Among these stimuli, cytokines are potent inducers of adhesive function as well as expression of several adhesion molecules. Leukocyte function-associated antigen (LFA)-1 ($\alpha_L\beta_2$ integrin) and very late antigen-4 (VLA)-4 ($\alpha_4\beta_1$ integrin) mediate adhesion of leukocytes to opposing ligands, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, respectively, both of which belong to 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 (Butcher, 1991; Shimizu et al., 1992; Tanaka et al., 1992).

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 protein (MIP)-1α and MIP-1ß trigger integrins and induce adhesion of circulating T cells and leukemic T cells to endothelial cell integrin-ligands (Tanaka et al., 1993a,b, 1998a,c,d; Adams et al., 1994). Several recent studies have emphasized the potential importance of chemokines in inflammatory responses; various chemokines including MIP-1ß 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 that conformational change of ectodomain of integrins and/or clustering of integrins on the cell membrane can induce active, adhesive configuration of integrins, which is brought about by the cytoskeletal actin-polymerization

associated with endodomain of integrins (Bokoch, 1995; Springer, 1995; Camphell et al., 1996). We here document the mechanism of integrin-mediated adhesion of circulating T cells to endothelial ligands, shedding light upon chemokine-mediated signaling and cytoskeletal machinery during inflammatory processes.

Integrin activation in adhesion cascade during migration of circulating T cells

The recruitment of leukocytes into tissues is regulated by a sequence 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 (Butcher, 1991; Shimizu et al., 1992; Tanaka et al., 1992). 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. The efficient triggering brings high affinity adhesion of T cells to endothelium and subsequent infiltration into underlying tissues. 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 chemokines such as MIP-1α and MIP-1β trigger T cell integrin functions (Tanaka et al., 1993a,b, 1998a,c,d; Adams et al., 1994) (Fig. 1). Resting T cells cannot adhere to purified ICAM-1, purified VCAM-1, fibronectin nor endothelial cells, whereas T cells activated with phorbol myriacetate ester (PMA) adhere to them very well within 30 minutes incubation *in vitro*. MIP-1ß rapidly triggers T

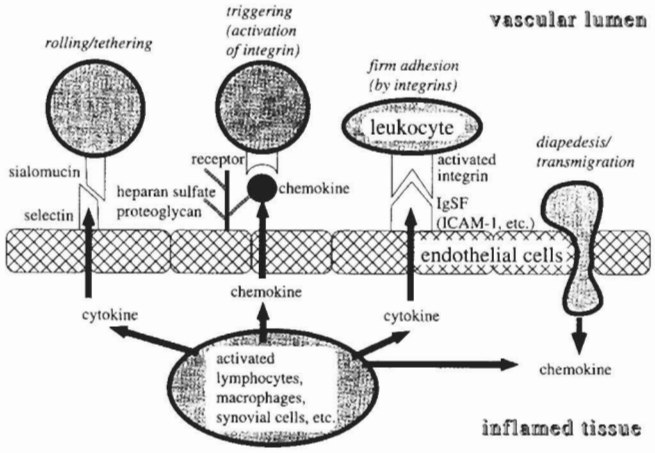

Fig. 1. Leukocyte adhesion cascade in the interaction with endothelium in inflamed tissue. Illustrated are the four steps leading to the migration of leukocytes from the blood stream to the inflammatory site. Leukocyte rolling/tethering, activation of integrins, firm integrin-mediated adhesion, and diapedesis/transmigration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

cell integrin and induces 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 in patients with rheumatoid arthritis (RA) and subsequently soluble chemokines left in culture supernatant are washed out, T cells adhere to RA-endothelial cells in a concentration-dependent manner. MIP-1α and MIP-1β are members of chemokine family, including IL-8, platelet factor (PF)-4, monocyte chemotactic protein (MCP)-1 and regulated upon activation, normal T-cell expressed and presumably secreted (RANTES). These chemokines are produced in a large amount from activated macrophages and lymphocytes in inflamed tissue or second lymphoid organ, trigger integrins on leukocytes and induce chemotaxis, resulting in leukocyte accumulation there. 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 they 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 inflammed tissue. Thus, depending on receptor expression, chemokines provide different and distinctive inflammatory processes.

Furthermore, we and others have proposed that 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 immobilize the chemokines in this process without being washed away by the blood flow (Tanaka et al., 1993a,b, 1998b; Webb et al., 1993; Adams et al., 1994). 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 (Bernfield et al., 1992; Jackson et al., 1995; Mertens et al., 1996) . Various cytokines and growth factors including all the chemokines, fibroblast growth factor and hepatocyte growth factor (HGF) 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 where they encounter with their target cells; 2) HS-PG protects cytokines from both chemical and physiological stimuli; and 3) HS-PG induces conformation-dependent association or the polymerization of the cell surface molecules including cytokines and cytokine receptors by binding them (Tanaka et al., 1993b, 1996, 1998b; Tanaka and Aso, 1998). 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 integrin activation of T cells

We have investigated the relevance of cytoplasmic signaling and cytoskeletal assembly to chemokine-induced adhesive function of integrins, with special emphasis on signaling through H-Ras and profilin-mediated actin polymerization in 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 (Adams et al., 1994; Springer, 1995; del Pozo et al., 1996; Laudanna et al., 1996; Shimizu and Hunt III, 1996; Stewart et al., 1996) . 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* (Tanaka et al., 1998a). However, the MIP-1α and MIP-1β-induced T cell adhesion to RA-endothelial cells is clearly reduced by pretreatment of 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 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 (Tanaka et al., 1998c). 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; and c) a notable feature of ATL is the remarkable 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 Abs 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 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 chemokine-induced triggering of integrins (Bokoch, 1995; Laudanna et al., 1996; Mackay, 1996; Shimizu and Hunt III, 1996; Stewart et al., 1996). Among several small G-proteins, Ras is known to play a central role in signal transduction, from which multiple pathways radiate. For instance, activated H-Ras induces expression of LFA-1 by modulating the transcription of LFA-1α-chain on B cells (Endo et al., 1991). Similarly, expression of active form of R-Ras, which is related to H-Ras, was found to enhance cell adhesion to extracellular matrix via activation of several integrins (Zhang et al., 1996). ATL cells which are pre-cultured in the serum-free medium weakly adhere to purified ICAM-1. However, the adhesion of ATL cells, transfected with the fully activated H-Ras mutant H-Ras^{V12} or H-Ras^{V12}Y40C mutant which selectively binds to PI 3-kinase, is further increased and the induced adhesion of ATL cells is completely inhibited by the addition of anti-LFA-1 mAb. In contrast, the adhesion of ATL cells expressing the dominant negative H-Ras mutant H-Ras^{V12}S17N is markedly reduced to the basal level (Tanaka et al., 1999).

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 α-chain of LFA-1 (van Kooyk et al., 1994). Resting T cells do not express the activated form of LFA-1 as recognized by the mAb using flow cytometry. However, the expression of the activated form of LFA-1 is clearly observed on most of T cells expressing H-$RasV^{12}$ or H-Ras^{V12}Y40C, although they express comparable levels of the epitope to resting T cells (Tanaka et al., 1999). The 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 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. These results imply that the H-Ras signal pathway followed by PI 3-kinase activation, plays a role in the induction of active configuration of LFA-1, resulting in enhanced LFA-1-mediated adhesion of T cells and leukemic T cells stimulated by chemokines (Fig. 2).

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, ß1 and ß3. Suppression of integrin function correlated with activation of the Ras/Raf/MAP (ERK) kinase pathway (Hughes et al., 1997). One plausible explanation for such a discrepant nature of 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 "on and off switch" for integrin triggering. Ras is known to be a "hub" which radiates multiple signaling pathway including Raf-1 and PI 3-kinase (Marshall, 1996). Recently accumulating evidence demonstrates that PI 3-kinase plays a central role in integrin-triggering as well as cytoskeletal changes (Shimizu, 1996; Shimizu and Hunt III, 1996; Chan et al., 1997; Metzner et al., 1997). As described, 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. These results suggest that H-Ras-sensitive PI 3-kinase activation is involved in "on switch" for LFA-1 on T cells, while the H-Ras-ERK kinases may function as an "off switch" for integrins.

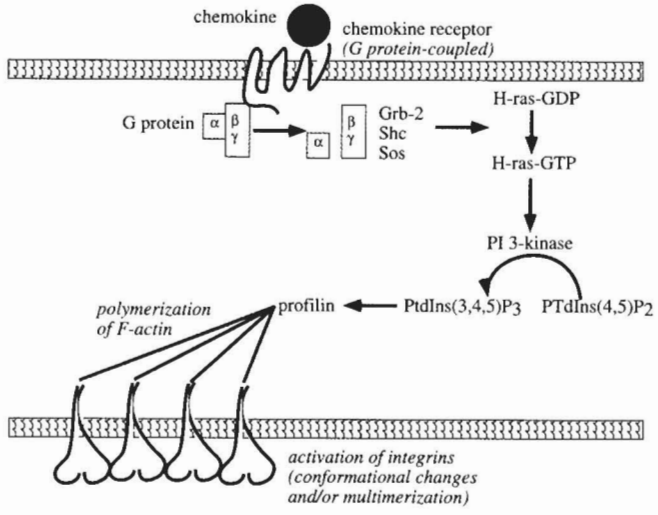

Fig. 2. Proposed mechanisms of integrin-activation by chemokines in T cells. We propose that the inflammatory chemokines are involved in triggering integrin LFA-1 through cytoskeletal rearrangement induced by G-protein-dependent activation of PI 3-kinases.

Involvement of cytoskeletal machinery in 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 crosslinked membrane-associated fibers (Bokoch, 1995). The mechanism underlying activation of integrin involves conformational changes of the ectodomain of integrins and/or clustering of integrins on the cell membrane, resulting from cytoskeletal actin-polymerization associated with cytoplasmic domain of integrins (Tanaka et al., 1993a, 1998a,c,d: Adams et al., 1994; Bokoch, 1995; Camphell et al., 1996; del Pozo et al., 1996; Luque et al., 1996; Stewart et al., 1996; Newton et al., 1997). 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 H-Ras^{V12} or H-Ras^{V12}Y40C in T cells shows an apparent increase of F-actin in the cell cortex and marked polymerization and rearrangement of F-actin as well as highly augmented expression of the activation epitope of LFA-1 as recognized by NKI-L16. The epitope of LFA-1 is thought to be induced by a conformational change of LFA-1 due to multimerization of the LFA-1 molecules. It has also been suggested that multimerization of integrins, mediated by F-actin polymerization, results in the induction of an active conformation of integrins (Miyamoto et al., 1995) .

Profilin, a 12-15 kDa cytoplasmic protein, promotes actin polymerization by converting ADP-actin to ATP-actin, thus stimulating polymerization (Sohn and Goldschmidt-Clermont, 1994; Carlier and Pantaloni, 1997; Schluter et al., 1997; Korenbaum et al., 1998). Furthermore, profilin is known to be physically associated with PIP2 and PI 3-kinase products and to function as a "linker" between cytoplasmic signaling and actin assembly (Sohn et al., 1995; Bubb et al., 1998; Chaudhary et al., 1998). PI 3-kinase products exhibit much higher affinity for the profilin-actin complex than the primary products, PIP and PIP_2, and activated PI 3-kinase initiates massive actin polymerization through profilin. It is also reported that integrin-mediated adhesion of endothelial cells to fibronectin was increased by profilin overexpression (Moldovan et al., 1997). 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, whereas resting T cells show constant and slight distribution of profilin (Tanaka et al., 1999). Furthermore, polymerized F-actin clearly co-localizes with profilin in T cells expressing H-Ras^{V12} or H-Ras^{V12}Y40C by the double staining of them. Freshly obtained ATL cells also show 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 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 expression of dominant negative H-Ras^{V12}S17N. These results suggest that G-protein sensitive PI 3-kinase activation plays a role in 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: crosstalking between cytokines and adhesion molecules

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, Behçet's disease and atherosclerosis as follows.

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 both as 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 trigger appears to be essential for the adhesion of T cells and the efficient triggering brings high affinity adhesion of T cells to endothelium and subsequent infiltration into underlying tissues (Tanaka et al., 1998a): 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 RA-endothelial cells; 3) mAb-blocking studies indicate that the T cell adhesion induced by endothelial immobilized chemokines is mediated by integrins LFA-1 and VLA-4 on T cells; 4) the addition of anti-MIP-1α and MIP-1β Ab to the endothelial cells during immobilizing 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 the reduced integrin-mediated adhesion of T cells to the endothelial cells; and 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 circulation, they would be washed away by the blood flow. However, by the binding to the HS, chemokines could be practically accumulated on the cellular surface and be 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 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 appropriate tumor milieu (Adams et al., 1997). We have reported that integrins on TIL are spontaneously activated by endogenous chemokines MIP-1α and MIP-1β, which contribute to highly adhesive characteristics of TIL (Tanaka et al., 1998d): 1) TIL produce high quantities of both MIP-1α and MIP-1β in the culture supernatant as well as in the cytoplasmic fraction of TIL; 2) a clear increase of F-actin in the cell cortex and a marked polymerization are shown in TIL; 3) TIL spontaneously express the activated form of integrin LFA-1, as recognized by NKI-L16 mAb; and 4) TIL spontaneously adhere to endothelial cells through the activated LFA-1. Based on these results, we suggest that endogenous MIP-1α and MIP-1β activate integrins on TIL in an autocrine manner. Other chemokines, monokine induced by IFN-γ (Mig) and γ-interferon-induced peptide (IP-10), secreted by cells within the tumor, are known to attract T cells to the neoplasm (Liao et al., 1995; Proost et al., 1996) . Thus, chemokines play an important role in the accumulation of tumor-reactive T cells in tumor milieu.

Recruitment of neutrophils into tissue occurs in initial phase of inflammation, infection, thrombosis and ischemia. Neutrophil adherence to endothelium depends on neutrophil integrins. Chemoattractants, such as IL-8 and fMLP, may be the best candidates to functionally activate neutrophil LFA-1 (Webb et al., 1993; Springer, 1995). We have reported that HGF activates neutrophil integrin LFA-1 and enhances neutrophil adhesion to endothelial cells and transmigration through the endothelium *in vitro* and that HGF is detected in acute inflammatory skin lesions associated with marked accumulation of neutrophils in patients of Behçet's disease. HGF is originally described as a potent mitogen for hepatocytes and causes proliferation and scattering of epithelial cells through its tyrosine kinase receptor c-Met. HGF possesses heparin-binding sites and is present on the vessel walls at the site of inflammation by binding to cell surface HS-PG in a manner similar to chemokines (Adams et al., 1994). Our results showed that; 1) HGF induces LFA-1-mediated adhesion of neutrophils to endothelial cells and also their transmigration; 2) HGF

transforms neutrophil integrin LFA-1 to active form; 3) HGF induces F-actin polymerization within seconds; 4) genistein or wortmannin inhibits both F-actin polymerization and LFA-1-mediated adhesion of neutrophils to endothelial cells; 5) histamine induces the production of HGF in neutrophils; and 6) neutrophils in cutaneous inflamed tissue express HGF in patients with Behçet's disease (Mine et al., 1998). Thus, other than chemokines, HGF also plays a pivotal role in integrin-mediated adhesion and transmigration of neutrophils to sites of acute inflammation through cytoskeletal rearrangement activated by TK and PI3-kinase.

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 T cells and macrophages in atherosclerotic plaques has reawakened the interest in inflammatory components. T cells and monocytes/macrophages contribute to the development of cell-mediated responses not only to lipoproteins but also endothelial cells and smooth muscle cells, which display "chronic inflammation patterns". Monocytes are representative in atherosclerotic area and differentiated to lipid-filled foamy macrophage at subendothelial space. A chemokine MCP-1 has been postulated to play an important role in the pathogenesis since it is highly produced in the tissue and efficiently induces chemotaxis of monocytes to the tissue (Terkeltaub et al., 1998). High plasma concentration of low-density lipoprotein (LDL) cholesterol is one of the principal risk factors for atherosclerosis. When LDL particles become trapped in an artery, they can undergo progressive oxidation and be internalized by macrophages by means of the scavenger receptors on the surfaces (Han et al., 1997). Oxidized LDL, which is produced by endothelium or macrophage, has been identified as a potent chemoattractant for monocytes and T cells (McMurray et al., 1993). 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) oxidized LDL induced activation epitope of LFA-1 on monocytes and marked polymerization of F-actin in monocytes; 3) oxidized LDL also induced transendothelial migration of monocytes; and 4) expression of activated epitope of LFA-1 was inhibited by PKC inhibitors. Therefore, we now propose that oxidized LDL produced in large amounts in inflamed atherosclerotic plaques can induce F-actin polymerization and activation epitope of LFA-1 through the activation of PKC in circulating monocytes and may amplify LFA-1-dependent adhesion and subsequent transendothelial migration of monocytes into the tissue.

Conclusion

Thus, not only expression but also activation of adhesion molecules are induced by "inside-out" signaling stimulated by cytokines. Among them, chemokines such as MIP-1, IL-8 and MCP-1 converts the functionally inactive integrin to 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 cytoplasm, which leads to T cell activation as well as cytokine production from immune cells and inflammed 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 circulation into inflammed tissue and by activating both immune cells and resident cells in the tissue. The concept proposed would bring enormous power and flexibility to clarify multiple pathological processes as well as the new pharmacological approaches to more specifically control inflammation.

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