Role of macrophage inflammatory protein (MIP)-1α/CCL3 in leukemogenesis

Tomohisa Baba* and Naofumi Mukaida

Division of Molecular Bioregulation; Cancer Research Institute; Kanazawa University; Kanazawa, Ishikawa, Japan

Keywords: MIP-1α/CCL3, leukemia, chemokine, leukemia initiating cell, microenvironment

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; HSC, hematopoietic stem cells; HSPC, hematopoietic stem/progenitor cells; LIC, leukemia initiating cells; LPS, lipopolysaccharide; MIP-1α, macrophage inflammatory protein-1α; SCI, stem cell inhibitor

The biologic function of the CC chemokine macrophage inflammatory protein-1α (MIP-1α/CCL3) has been extensively studied since its initial identification as a macrophage-derived inflammatory mediator. In addition to its proinflammatory activities, CCL3 negatively regulates the proliferation of hematopoietic stem/progenitor cells (HSPCs). On the basis of this unique function, CCL3 is alternatively referred to as a stem cell inhibitor. This property has prompted many researchers to investigate the effects of CCL3 on normal physiologic hematopoiesis and pathophysiologic processes of hematopoietic malignancies. Consequently, there is accumulating evidence supporting a crucial involvement of CCL3 in the physiopathology of several types of leukemia arising from neoplastic transformation of HSPCs. In this review we discuss the roles of CCL3 in leukemogenesis and its potential value as a target in a novel therapeutic strategy for the treatment of leukemia.

Biologic Function of MIP-1α/CCL3 as a Stem Cell Inhibitor

Macrophage inflammatory protein-1α (MIP-1α, also known as CCL3) is a member of the CC chemokine family. MIP-1α cDNA was originally cloned from lipopolysaccharide (LPS)-activated RAW264.7 mouse macrophage cells as a gene encoding an endogenous inflammatory mediator.1 CCL3 and related CC chemokines such as CCL4 and CCL5 are classified as inflammatory chemokines because of their ability to induce chemotactic mobilization of monocyte-lineage cells and lymphocytes into inflammatory tissues. CCL3 also regulates the proliferation of hematopoietic stem/progenitor cells (HSPCs) in the bone marrow (BM).2 However, the contribution of endogenous CCL3 in the BM to normal physiologic hematopoiesis is poorly understood.

Peripheral blood cells are continuously produced in adulthood by differentiation from a limited number of hematopoietic stem cells (HSCs) present in the BM. The HSC pool in the BM is stably maintained by an intricate balance between differentiation, self-renewal, and reversible quiescent cell cycle arrest of HSCs. During the 1970s and 1980s, regulation of the steady-state quiescent status of HSCs was proposed to involve an unknown regulatory factor present in the BM microenvironment termed a stem cell inhibitor (SCI).3,4 Immediately after the cloning of CCL3 cDNA, Graham et al.5 observed that culture supernatant of the J774.2 macrophage cell line contained a factor exhibiting SCI-like activity against colony formation of BM primitive cells. They further demonstrated that this factor was identical to CCL3. Subsequent studies revealed that CCL3 could reversibly inhibit colony formation and proliferation of HSPCs both in vitro and in vivo.5-7 Intriguingly, CCL3 inhibits the proliferation of primitive progenitor cells but activates the proliferation of more mature progenitor cells.7,8 Moreover, CCL3 can maintain a quiescent status in HSCs by blocking cell cycle entry, thereby exhibiting a myeloprotective effect against cell cycle-specific anticancer drugs.6,10 Furthermore, administration of a high dose of CCL3 rapidly induces mobilization of mouse51 and human12 HSPCs from BM to the peripheral blood. Thus, CCL3 potentially contributes to hematopoietic regulation in physiologic and pathologic conditions. However, the BM of CCL3-deficient mice does not exhibit any obvious hematopoietic abnormalities,13 and a major cellular source of CCL3 in steady-state BM has not yet been identified. Thus, the precise regulatory functions of CCL3 in hematopoiesis under physiologic and various pathologic conditions remain elusive.

Chemokines execute their biologic activities through binding to their corresponding receptors, which are G-protein coupled receptors (GPCRs) with seven-span transmembrane (7-TM) portions. Human and mouse CCL3 bind to CCR1, CCR5, and D6 receptors, and mouse CCL3 can additionally bind to CCR3.14 Among these receptors, CCR1 is responsible for CCL3-mediated mobilization of HSPCs, but not SCI activity.15 Moreover, BM cells derived from CCR1−/−, CCR3−/−, CCR5−/−, and D6−/− mice do not exhibit reduced SCI activity in vitro.16 Further investigation of the molecular mechanisms underlying CCL3-mediated SCI
activity, in particular the identity of the receptor involved in this process, is required.

Leukemia is a hematopoietic neoplasm arising from neoplastic transformation of HSPCs and is assumed to involve oligoclonal or heteroclonal cells. Expansion of leukemia cells can arise from a small number of specialized leukemia cells called leukemia initiating cells (LICs).37-40 Furthermore, LICs exhibit phenotypes similar to those of normal HSPCs, such as self-renewal and cellular quiescence. The control of LICs is therefore presumed to involve cellular and molecular mechanisms similar to those regulating normal HSPCs. Thus, CCL3, which has biologic activities in normal HSPC function, might also have a role in leukemogenesis. In this review article we focus on the roles of CCL3 in the multiple processes of leukemogenesis.

Pathologic Role of CCL3 in Chronic Myeloid Leukemia

More than 90% of cases of chronic myeloid leukemia (CML) are associated with the presence of the Philadelphia chromosome that arises from a reciprocal translocation between chromosomes 9 and 22. This chromosomal translocation results in the formation of a breakpoint cluster region and a constitutively activated tyrosine kinase, the BCR-ABL fusion protein.25 BCR-ABL is a pathognomonic protein for CML and its expression transforms normal HSPCs into LICs whose maintenance in BM is indispensable for CML leukemogenesis.26

CML LICs share characteristic capabilities with normal HSCs, including self-renewal and cellular quiescence.19,22 However, in contrast to normal HSCs, LICs are resistant to the SCI activity of CCL3 during in vitro proliferation.27-30 Wark et al.31 revealed that forced activation of the Abl tyrosine kinase in a multipotent stem cell line directly represses the CCL3-mediated increase in cytosolic Ca²⁺ concentration. In contrast, this treatment did not affect expression of the CCL3 receptor or its affinity for CCL3. Consistent with these findings, other independent groups have reported that CCL3 receptors are expressed at similar levels in normal and CML progenitor cells.27-28 Based on this observation, it has been proposed that this Abl tyrosine kinase-mediated unresponsiveness to CCL3 contributes to the preferential expansion of CML LICs in the leukemic BM microenvironment. In contrast, we and other groups have observed decreased expression of CCL3 receptors, especially CCR5, in CML progenitor cells.32-33 Thus, it remains controversial whether the unresponsiveness of LICs to CCL3 arises from decreased CCL3 receptor expression and/or functionality. Nevertheless, CCL3 exposure can preferentially induce quiescent cell cycle arrest in normal HSPCs but not in LICs. Moreover, pretreatment of normal HSPCs with CCL3 confers resistance to cell cycle-specific anti-leukemia drugs such as cytosine arabinoside (Ara-C), whereas cytotoxicity is preserved in LICs.28,31 Thus, combined administration of CCL3 with antileukemia drugs was tested as an approach to selectively kill CML cells28,31 before the advent of molecularly targeted drugs such as the tyrosine kinase inhibitor imatinib.34-36

In the early stages of CML a small number of LICs coexists with a large number of normal hematopoietic cells but over time the LICs gradually accumulate and eventually predominate in the limited space of the BM microenvironment. Several lines of evidence indicate a crucial role of CCL3 in the initiation and progression of CML. Zhang et al.39 demonstrated that CML cells produce high levels of CCL3, which, in combination with other cytokines and chemokines, might confer a growth advantage to LICs over normal HSPCs. Schepers et al.37 further demonstrated that CCL3 induces remodeling of the leukemia niche cell to effectively support LIC proliferation. We observed that BCR-ABL ‘lineage c-kit’ immature leukemia cells are a main source of CCL3.35 Moreover, CCL3 can induce the mobilization of normal HSPCs from BM to peripheral blood and promotes the maintenance of LICs in BM of CML mice. Furthermore, CCL3-mediated maintenance of LICs was also observed in the setting of recurrence after cessation of imatinib treatment. Conversely, normal HSPCs can directly impede the maintenance of LICs in BM when the CML cells lack CCL3 or the normal HSPCs lack CCL3-binding receptors including CCR1 and CCR5.32 Thus, CCL3 might act to expel normal CCR1- or CCR5-expressing HSPCs from the BM, making space available for LIC expansion.32

Pathologic Role of CCL3 in Acute Myeloid Leukemia

It was previously assumed that the outgrowth of acute myeloid leukemia (AML) cells is initiated and maintained from LICs with properties similar to those of normal HSCs,22 as in CML. Based on detailed investigation of BM samples of human AML patients, Goardon et al.18 recently demonstrated that a large number of patients with AML exhibit expansion of cells with LIC potential that are differentiated from HSCs and resemble lymphoid-primed multipotential progenitors or granulocyte-macrophage progenitors. Additionally, there is accumulating evidence that LICs are present among CD34⁺ AML blast cells in human patients with AML.

CD34⁺ AML cells are resistant to the SCI activity of CCL3 when cultured in the presence of CCL3, although the underlying molecular mechanism remains elusive.38 Moreover, as in CML, CCL3 is produced abundantly in the BM of AML patients and contributes to remodeling of the microenvironment.35 It has been proposed that leukemia cell-derived CCL3 inhibits osteoblastic cell functions, thus accelerating the disruption of normal BM microenvironment and hematopoiesis.39 In the NUP98-HOXD13–mediated mouse AML model, the protooncogene Meis1 accelerates AML development and induces CCL3 expression, which is at least partially responsible for the intra-BM survival of LICs by potentiating their repopulation capacity.40 Moreover, binding of the homeodomain of MEIS1
to the regulatory sequence of the CCL3 gene is required for CCL3 expression. However, the detailed molecular and cellular mechanisms by which CCL3 contributes to AML pathophysiology remain unknown.

Pathologic Role of CCL3 in Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL) is a lymphoproliferative disorder in which neoplastic CD5+ B cells clonally expand in the peripheral blood, secondary lymphoid tissues, and BM. Burger et al. recently demonstrated that CLL B cells produce high levels of CCL3 and its related chemokine CCL4 in co-culture with CD68+ nurse-like cells and after B-cell receptor stimulation. They further revealed that the plasma CCL3 level is a reliable prognostic marker in CLL patients. Concomitantly, Zucchetto et al. demonstrated that CD38+CD49d+ CLL cells selectively and aberrantly express CCL3 through interaction with CD38 and CD31 expressed on stromal cells in the BM microenvironment. CCL3 subsequently induces recruitment of macrophage-lineage cells into the leukemia niche. The recruited cells produce inflammatory factors including TNF-α that eventually activate stromal cells to express VCAM-1, a ligand for CD49d that delivers pro-survival signals to CLL cells through its interaction with CD49d. Thus, CCL3 can promote establishment of the leukemia niche, which is essential for CLL cell survival.

Perspective

The above experimental and clinical evidence indicates that CCL3 is crucially involved in multiple pathophysiological processes of leukemogenesis in various types of leukemia. These processes include preferential proliferation of LICs, expulsion of normal HSPCs, and/or establishment of a leukemia-adapted niche in the BM (Fig. 1). Moreover, high levels of CCL3 expression are observed in other malignant hematopoietic neoplasms, such as adult T-cell leukemia and multiple myeloma. Myeloma cell-derived CCL3 can support the proliferation of myeloma cells directly or indirectly through the accelerated formation of osteoclasts, which can provide a pro-myeloma niche within the BM. Thus, CCL3 overexpression may have an important role in the progression of hematopoietic malignancies in general, although malignant transformation of each hematologic disease also involves individual and diverse intrinsic events. Given the crucial role of CCL3 in the leukemic BM microenvironment, we assume that therapeutic blockade of CCL3 activity in leukemia patients would correct the BM microenvironment and eventually inhibit the dominant proliferation of LICs through effects on normal BM cells, rather than through direct killing of LICs.

The development of antineoplastic drugs, including antileukemic drugs, requires identification of causative oncogenes and appropriate molecular targets. However, leukemia cells, especially LICs, crucially depend on the appropriate microenvironment for expansion in the BM, particularly during the initiation phase or chemotherapy-induced remission, when only a small number of LICs is present in the BM. Moreover, the creation of a favorable niche for normal HSPCs may create a disadvantage for growth of leukemia cells. Thus, in parallel with the pursuit of molecular targeted therapy, it is necessary to investigate the pathophysiologic roles of endogenous mediators such as CCL3 that can profoundly affect the proleukemic niche. This may lead to the development of novel antileukemic therapies that supplement molecular targeted therapy.

Conflict of interest

The authors do not have any potential conflicts of financial interest.
References

1. Davatelis G, Tekamp-Olson P, Wolpe SD, Hermsen K, Luedke C, Gallegos C, Cott D, Merryweather J, Cook DN. Characterization of a CDNA for murine macrophage inflammatory protein (MIP), a novel monokine with inflammatory and chemokine properties. J Exp Med 1988; 167:1939-44; PMID:3293082; http://dx.doi.org/10.1084/jem.198.6.1939

2. Cook DN. The role of MIP-1 alpha in inflammation and hematopoiesis. J Leukoc Biol 1996; 59:61-6; PMID:8585696

3. Tokosz D, Dexter TM, Lord BI, Wright EG, Lajtha LG. The regulation of hematopoiesis in long-term bone marrow cultures. II. Stimulation and inhibition of stem cell proliferation. Blood 1980; 55:931-6; PMID:7378583

4. Lord BI, Mori KJ, Wright EG, Lajtha LG. Inhibitor of stem cell proliferation in normal bone marrow. Br J Haematol 1987; 64:441-5; PMID:990183; http://dx.doi.org/10.1111/j.1365-2141.1987.tb03590.x

5. Graham GJ, Wright EG, Hewick R, Wolpe SD, Wilkie NM, Donaldson D, Loriotme S, Pragnell IB. Identification and characterization of an inhibitor of colony formation on colony formation in vitro by murine macrophage inflammatory protein 1 alpha with disrupting effects of recombinant murine macrophage inflammatory protein 1alpha for an inflammatory response to viral infection. Science 1995; 269:1583-5; PMID:7667059

6. Davatelis G, Tekamp-Olson P, Wolpe SD, Hermsen K, Luedke C, Gallegos C, Cott D, Merryweather J, Cook DN. Cloning and characterization of a CDNA for murine macrophage inflammatory protein (MIP), a novel monokine with inflammatory and chemokine properties. J Exp Med 1988; 167:1939-44; PMID:3293082; http://dx.doi.org/10.1084/jem.198.6.1939

7. Broxmeyer HE, Sherry B, Lu L, Cooper S, Oh KO, Akashi K, Kutok JL, Dayaram T, Geary K, Green AR, Rasmussen H, Gordon MS. Myeloid progenitor cell mobilization of early hematopoietic progenitor cells to macrophage inflammatory protein-1alpha, in a phase I clinical trial in patients with relapsed/refractory breast cancer. Blood Cells Mol Dis 1998; 24:14-30; PMID:9563578; http://dx.doi.org/10.1006/bcmd.1998.0167

8. Broxmeyer HE, Ottersbach K, Diouf S, Zhao YQ, Humes B, Lu B, Gerard C, Proudfoot AE, Graham GJ. Macrophage inflammatory protein-lalpha uses a novel receptor for primitive hematopoietic cell inhibition. Blood 2001; 98:3476-8; PMID:11713931; http://dx.doi.org/10.1182/blood.v98.12.3476

9. Vesselinovitch G, Boston K, Nau A, Aljoughi S, Weiser J, Lacy E, Chiao W, Lajtha LG. Demonstration of stem cell inhibition and myeloproliferative effects mediated by chemokine receptor CCR1. J Exp Med 1999; 189:1879-92; PMID:10377195; http://dx.doi.org/10.1084/jem.189.12.1879

10. Eaves CJ, Cashman JD, Wolpe SD, Eaves AC. Unresponsiveness of primitive chronic myeloid leukemia cells to macrophage inflammatory protein 1 alpha, an inhibitor of primitive normal hematopoietic stem cells. Proc Natl Acad Sci U S A 1993; 90:12015-9; PMID:8265663; http://dx.doi.org/10.1073/pnas.90.24.12015

11. Zhang B, Ho YW, Huang Q, Maeda T, Lin A, Lee SU, Hair A, Housalye TL, Huettner C, Bhattacharjee B. Altered microenvironmental regulation of leukemic and normal stem cells in chronic myelogenous leukemia. Cancer Cell 2012; 21:577-92; PMID:22516264; http://dx.doi.org/10.1016/j.ccr.2012.02.018

12. Wark G, Heyworth CM, Spooner E, Czaplewski L, Francis JM, Dexter TM, Wherton AD. Abl protein kinase regulates the response of multipotent hematopoietic cells to the growth inhibitor macrophage inflammatory protein -1 alpha. Oncogene 1998; 16:1319-24; PMID:9546433; http://dx.doi.org/10.1038/sj.onc.1210914

13. Babu T, Naka K, Morishita S, Komatsu N, Hiroa A, Mukaida N. MIP-1a/CCL3-mediated maintenance of leukemia-initiating cells in the initiation process of chronic myeloid leukemia. J Exp Med 2013; 210:2661-73; PMID:24166712; http://dx.doi.org/10.1084/jem.201302102

14. Nicholls SE, Lucas G, Graham GJ, Russell NH, Mottram R, Wherton AD, Buckle AM. Macrophage-inflammatory protein-lalpha receptor expression on normal and chronic myeloid leukemia CD34+ cells. J Immunol 1999; 162:6191-9; PMID:10229684

15. Buchdunger E, Zimmermann M, Meyer T, Muller M, Druker BJ, Lynd NB. Inhibition of the Ab1 protein-tyrosine kinase in vitro and in vivo by a 2-phe-nylaminoypyrimidine derivative. Cancer Res 1996; 56:100-4; PMID:8548747

16. Druker BJ, Tamura S, Buchdunger E, Ohno S, Sagal GS, Fanning S, Zimmermann J, Lydon NB. Effects of a selective inhibitor of the Ab1 tyrosine kinase on the growth of Bcr-Ab1 positive cells. Nat Med 1996; 2:561-6; PMID:8601676; http://dx.doi.org/10.1038/4105

17. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lynd NB, Kantarjian H, Capdeville R, Ohno-Jones S, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. J Exp Med 2005; 201:129-46; PMID:16143870; http://dx.doi.org/10.1084/jem.200513012

18. Schepers K, Piets C, Reynaud D, Flach J, Binnewies MA, Howerth EW, Greaves M, Breeveld H, van der Pol MP, van der Giessen WM, Marinari F, Brandi D, Hengartner H, Spagnoli GC, Akashi K, Kutok JL, Dayaram T, Geary K, Green AR, Rasmussen H, Gordon MS, Myeloid progenitor cell mobilization of early hematopoietic progenitor cells with BB-10010: a genetically engineered variant of human macrophage inflammatory protein 1alpha, in a phase I clinical trial in patients with relapsed/refractory breast cancer. Blood Cells Mol Dis 1998; 24:14-30; PMID:9563578; http://dx.doi.org/10.1006/bcmd.1998.0167

19. Cook DN, Beck MA, Coffman TM, Kirby SL, Sheridan JE, Pragnell IB, Smithies O. Requirement of MIP-1 alpha for an inflammatory response to viral infection. Science 1995; 269:1583-5; PMID:7667639; http://dx.doi.org/10.1126/science.7667639
41. Burger JA, Quiroga MP, Hartmann E, Bürkle A, Wierda WG, Keating MJ, Rosenwald A. High-level expression of the T-cell chemokines CCL3 and CCL4 by chronic lymphocytic leukemia B cells in nurse-like cell cocultures and after BCR stimulation. Blood 2009; 113:3050-8; PMID:19074750; http://dx.doi.org/10.1182/blood-2008-07-170435

42. Sivina M, Hartmann E, Kipps TJ, Rassenti L, Krupnik D, Lerner S, LaPushin R, Xiao L, Huang X, Werner L, et al. CCL3 (MIP-1α) plasma levels and the risk for disease progression in chronic lymphocytic leukemia. Blood 2011; 117:1662-9; PMID:21115978; http://dx.doi.org/10.1182/blood-2010-09-307249

43. Zucchero A, Benedetti D, Tripodo C, Bomben R, Dal Bo M, Marconi D, Bossi F, Lorenzon D, Degan M, Rossi FM, et al. CD38/CD31, the CCL3 and CCL4 chemokines, and CD40/vascular cell adhesion molecule-1 are interchained by sequential events sustaining chronic lymphocytic leukemia cell survival. Cancer Res 2009; 69:4001-9; PMID:19383907; http://dx.doi.org/10.1158/0008-5472.CAN-08-4173

44. Marumoto K, Murao K, Imachi H, Nishimichi T, Cao W, Yu X, Li J, Ahmed RA, Iwama H, Kobayashi R, et al. The role of calcium/calmodulin-dependent protein cascade on MIP-1α gene expression of ATL cells. Exp Hematol 2008; 36:390-400; PMID:18249060; http://dx.doi.org/10.1016/j.exphem.2007.11.013

45. Lentzsch S, Gries M, Jain M, Bargou R, Dirken B, Mapara MY. Macrophage inflammatory protein 1-alpha (MIP-1 alpha) triggers migration and signaling cascades mediating survival and proliferation in multiple myeloma (MM) cells. Blood 2003; 101:3568-73; PMID:12506012; http://dx.doi.org/10.1182/blood-2002-08-2383

46. Vallet S, Raje N, Ishitsuka K, Hideshima T, Podar K, Chhetri S, Pozzi S, Breitkreutz I, Kiziltepe T, Yasui H, et al. MLN3897, a novel CCR1 inhibitor, impairs osteoclastogenesis and inhibits the interaction of multiple myeloma cells and osteoclasts. Blood 2007; 110:3744-52; PMID:17715391; http://dx.doi.org/10.1182/blood-2007-05-093294