Liver-Derived Matrix Metalloproteinase-9 (Gelatinase B) Recruits Progenitor Cells from Bone Marrow into the Blood Circulation

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Matrix metalloproteinases (MMPs) are involved in invasive cell behavior, embryonic development and organ remodeling. In this report, we investigated the role of liver-derived MMP-9 in the in vivo system at liver injury. Liver injury induced MMP-9 expression in the liver 3 to 12 h after intravenous administration of anti-Fas antibody, followed by the expression of the activity and the protein detected by zymography and Western blotting, respectively, in the blood circulation. Interestingly, the MMP-9 expression was accompanied by the recruitment of hematopoietic progenitor cells from bone marrow into the circulation. The recruitment was blocked by a specific MMP-9 inhibitor, R94138, which did not affect the Fas-mediated liver injury or induced expression of MMP-9. Compulsive expression of mutant active MMP-9 in the liver also recruited the progenitor cells into the circulation. In contrast, partial hepatectomy, which treatment does not directly injure hepatocytes, did not recruit progenitor cells despite the increased expression of MMP-9 in the circulation. These results suggest that liver-derived MMP-9 induced by liver injury plays an essential role in the recruitment of hematopoietic progenitor cells from bone marrow into the blood circulation.

Key words matrix metalloproteinase (MMP)-9; hematopoietic progenitor cell; recruitment; bone marrow; liver; hepatitis

Liver regeneration is one of the most remarkable and complicated tissue remodelings occurring in an adult body. It is thought that the process is regulated by the orchestration of various cell types, cytokines, extracellular matrix (ECM) and other molecules such as adhesion molecules.2,3) MMPs (matrix metalloproteinases) are also considered to be one family of these players in the tissue remodeling of the liver. We and others reported in previous studies that among MMPs MMP-9 rather than MMP-2 is possibly involved in the process4,5) since the expression is regulated by cytokines regulating the regeneration and MMP-9 regulates the proliferation and migration of hepatocytes.6) However, the role of MMP-9 in the in vivo systematic macroenvironment is still unclear. Recently, it has been reported that the enzyme is involved in the recruitment of hematopoietic progenitor cells from bone marrow into the blood circulation under certain conditions.6–10) Therefore, we examined in this study whether liver-derived MMP-9 contributes to the recruitment of progenitor cells into the circulation. Liver injury induced MMP-9 in the blood circulation, accompanied by the recruitment of progenitor cells. The recruitment was inhibited by a MMP-9 inhibitor. Gene transfection of active MMP-9 in the liver also induced the recruitment. These results indicate the possible involvement of MMP-9 in the recruitment of progenitor cells from bone marrow.

MATERIALS AND METHODS

Reagents and Animals Polyclonal anti-mouse MMP-9 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.). Anti-mouse Fas antibody (Jo2) was obtained from Pharmingen. A selective inhibitor of MMP-9 was generously provided by Dr. K. Tanzawa (Sankyo Co. Ltd., Tokyo, Japan). Other reagents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Female BALB/c (6—12 weeks) mice used in the present experiments were purchased from Charles River Japan, Inc. (Kanagawa, Japan).

Gelatin Zymography Sera from blood samples were electrophoresed on 7.5% SDS (sodium dodecyl sulfate)-polyacrylamide gel containing 0.1% gelatin. Liver tissue homogenates were prepared from 0.5 g of liver tissues homogenized in extraction buffer (50 mM PIPES–NaOH (pH 7.5), 50 mM KCl, 5 mM EGTA, 2 mM MgCl2, 1 mM DTT, 10 mM PMSF, 1 mg/ml aprotinin, leupeptin, and pepstatin) at 4 °C. The supernatants were obtained from the homogenates by centrifugation. In some cases, the gelatinolytic activity was concentrated by a gelatin-Sepharose column (Pharmacia, Uppsala, Sweden) before being subjected to SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis). The gel was washed with 0.1% Triton X-100 solution for 30 min and then incubated at 37 °C overnight in the activation buffer (50 mM Tris–HCl, pH 7.4, 0.2 mM NaCl, 5 mM CaCl2, and 0.02% NaN3). After staining with CBB (Coomassie Brilliant Blue) R-250, the gelatinolytic activities were detected as clear bands against the blue background.

Western Blotting After SDS-PAGE on a 7.5% gel, proteins were electroblotted onto PVDF membranes (Amer sham, Illinois) in cold transfer buffer for 3 h at 60 V. The filters were incubated with anti-MMP-9 antibodies for 1 h at room temperature after blocking with 3% skim milk, washed with TBS, and then incubated with the peroxidase-labeled second antibodies for 1 h. Bands were detected with an ECL kit (Amer sham, Illinois).

Colon-forming Unit (CFU) Assay Bone marrow-derived hematopoietic progenitor cells were measured by CFU assay as described.12) Briefly, PBMCs (peripheral blood mononuclear cells) after deletion of erythrocytes from blood samples by APC solution were cultured in IMDM medium containing 1.2% methylcellulose, 20% FCS, 5×10−3 M 2-mercaptoethanol, recombiant GM-CSF (granulocyte macrophage-colony stimulating factor) (10 ng/ml), G-CSF (granulocyte-colony stimulating factor) (10 ng/ml), stem cell factor (10 ng/ml), erythropoietin (2 U/ml), and rmIL-3 (recombinant mouse interleukin-3) (50 ng/ml). Colonies (>50 cells) were scored after 8 d.

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Jo2 Treatment and Partial Hepatectomy Mice were intravenously injected with anti-Fas antibody (Jo2) in 200 μl of PBS to induce acute hepatitis or subjected to 75% hepatectomy. After these treatments of mice, the activities of GOT (glutamic oxaloacetic transaminase) in the blood samples at indicated times were measured using GOT-UV (Wako Chemicals Co.) assay kits. The protocol recommended by the manufacturer was strictly followed in these assays. Measurements were repeated three times in each sample.

Liver Specific Gene Transfection in Vivo In vivo transcription into the liver was performed as described. Briefly, the gene of active MMP-9 in the expression vector, pTracer (100 μg/mouse) was intravenously injected into mice with 6 ml Ringer solution, which results in the specific expression of transfected genes in hepatocytes. After the transduction, the serum and liver tissue lysates were subjected to zymography at the indicated time.

RESULTS AND DISCUSSION

Jo2 Antibody Induced MMP-9 Expression in the Liver and Serum with the Liver Injury, Accompanied by the Recruitment of Hematopoietic Progenitor Cells into the Circulation. It has been reported that partial hepatectomy induces MMP-9 in the liver. However, whether pathological liver injury such as hepatitis induces the same response is unknown. In addition, whether liver injury induces MMP-9 activity in the blood circulation is also to be examined. Thus, we first investigated the expression of MMP-9 by Fas-mediated hepatitis in the liver and serum. A moderate amount of Jo2 administration induced acute hepatitis measured by GOT level in mice 9 h at peak after the administration (Fig. 1A). The stimulation also induced MMP-9 protein expression with the activity, measured by zymography and Western blotting, respectively (Fig. 1B). The majority of the induced MMP-9 was proMMP-9 according to the result of zymography. The kinetics was corresponding to that of liver injury (GOT level). Interestingly, Jo2 administration induced the increased rate of hematopoietic progenitor cells in the blood circulation (Fig. 1C). The kinetics is in parallel with both liver injury and MMP-9 expression. We observed the peaks of GOT, MMP-9 expression (both activity and protein) and the increase rate of progenitor cells in the circulation 6 to 9 h at peak after Jo2 administration. However, the kinetics could be changeable by the administered Jo2 amount.

MMP-9 Plays an Essential Role in the Recruitment of Progenitor Cells into the Circulation. We next examined the role of liver-derived MMP-9 in the recruitment of progenitor cells using a synthetic selective inhibitor of MMP-9, R94138(12,13) (IC₅₀ MMP-9; 1.2 nm, MMP-2/-13; 38 nm, MMP-3; 120 nm from ref. 13). As shown in Fig. 2, R94138 did not inhibit the Jo2-mediated hepatitis (Fig. 2A) or MMP-9 expression in the circulation (Fig. 2B). The higher position in the zymography is thought to be the dimer form of proMMP-9. In contrast, this inhibitor almost completely blocked the recruitment of progenitor cells into the circulation (Fig. 2C). These data strongly suggest the essential role of liver-derived MMP-9 activity in the recruitment of progenitor cells from bone marrow into the circulation. However, although the inhibitor has the selectivity to MMP-9 which is strongly induced in the liver injury, we cannot rule out the possibility that other MMPs are also involved in this process. We next compulsively induced the gene of active mutant MMP-9 in the liver to confirm the role of liver-derived MMP-9 in the progenitor recruitment. The gene-transduced cells express endogenously activated soluble MMP-9 in the supernatant. Therefore, the system is suitable to examine the function of active MMP-9 since the cleavage process to activate MMP-9 protein, which is still unclear in vivo, is not required in this system. Intravenous injection of the solution containing the plasmid resulted in the rapid expression of the molecule in the serum Fig. 3A). The expression of the MMP-9 in the serum was observed 3 to 9 h after the transduction. Since the transduction method induced liver injury, the early expression of MMP-9 may be attributed to the stimulation of transduction. However, the progenitor recruitment into the circulation was in parallel with the expression. These results confirmed the involvement of liver-derived MMP-9 in the recruitment of progenitor cells into the circulation. Furthermore, the result by Masure et al. that systemic administration of recombinant MMP-9 into mice increased the rate of white blood cells in the circulation also supports our conclusion.
They did not describe the necessity of liver injury for the recruitment of leukocytes. They used rabbits for their experiments instead of mice in this study. The discrepancy can be attributed to species differences or other unknown mechanisms.

Partial Hepatectomy Did Not Induce the Progenitor Cell Recruitment

As described, the involvement of MMP-9 in the liver regeneration was originally reported in the model of partial hepatectomy. Therefore, we compared this model to the Jo2-hepatitis model in the aspect of the progenitor cell recruitment. Partial hepatectomy induced MMP-9 expression in both activity and protein levels in the liver. The stimulation also induced the molecule in the circulation (Fig. 4A). However, interestingly, we did not observe any significant increase of the progenitor cell rate in the circulation (Fig. 4B). These results do not deny the involvement of liver-derived MMP-9 in the progenitor cell recruitment. However, overall results suggest that the liver-derived MMP-9 by injury is essential but not sufficient to recruit the hematopoietic progenitor cells from bone marrow into blood circulation. In addition, the mechanisms of liver injury by Jo2 antibody and partial hepatectomy are different. The critical difference is that partial hepatectomy does not directly destroy parenchymal cells in the liver while the target cell population of Jo2 is hepatocytes. Therefore, the difference in the progenitor cell recruitment of these models is not contradictory. To recruit the progenitor cells may require unknown factors besides MMP-9 such as some types of chemokines from hepatocytes.

Physiological roles of MMP-9 in the liver are suggested by several reports. For example, it is suggested that MMP-9 promotes cell proliferation and cell migration of hepatocytes by degrading extracellular matrix around hepatocytes. The expression is derived from parenchymal cells and regulated by the cytokines such as TNFα/HGF involved in liver regeneration. However, there are still enigmas about the roles of liver-derived MMP-9 despite MMP-9 knockout mice being prepared. Since MMP-9 is a soluble protein and the producing cells (hepatocytes) constitute a large population in vivo, it is natural to think that the liver-derived MMP-9 has systemic physiological functions in vivo. In this report, we have suggested the systemic role of liver-derived MMP-9 in the recruitment of hematopoietic progenitor cells from bone marrow at liver injury and the following regeneration.

There are some studies demonstrating the involvement of MMP-9 in the progenitor cell recruitment from bone marrow. These studies insist that MMP-9 promotes the recruitment of hematopoietic progenitor cells from bone marrow by cleaving the interaction of VACM-1/VLA-4 between progenitor cells and stromal cells in bone marrow or by processing membrane-type Kit ligand (mKitL) to soluble-type Kit ligand (sKitL) which accelerates the migration/proliferation of progenitor cells. Our study suggests that liver-derived MMP-9 is systemically involved in these processes via the circulation. Therefore, the response occurring in the liver is not restricted to that organ but affects the systemic macroenvironment to promote the regeneration. In contrast, some investigators reported that MMP-9 is dispensable in the progenitor cell recruitment. Thus, it is likely that the mecha-
anism to recruit the progenitor cells into the circulation is dependent on the type of stimulation. In view of the variety of the molecules such as cytokines and adhesion molecules involved in the recruitment, it would be consistent for there to be plural mechanisms in the recruitment system.

It is unlikely that recruited progenitor cells by liver-derived MMP-9 differentiate into hepatocytes in the injured liver, because the rate of differentiated cells supplied by progenitor cells in the regenerating organ is too low, although a number of investigators have reported that progenitor cells from bone marrow have the capacity to differentiate into hepatocytes in vitro and in vivo. In addition, the likelihood has recently been reported that bone marrow-derived stem cells do not differentiate into mature cells but only fuse with differentiated cells. Therefore, we rather think that the recruited progenitor cells contribute not to the regeneration of parenchymal cells but to that of nonparenchymal cells in the injured liver. First, hematopoietic progenitor cells from bone marrow differentiate into endothelial cells. In addition, reconstruction of liver sinusoidal endothelial cells depends on bone marrow-derived cells. We have also previously demonstrated that Kupffer cells (liver specific macrophages) are reconstructed by bone marrow cells in vivo. Second, it has been reported that hepatocytes and nonparenchymal cells are independently regulated in the regenerating liver. Thus, it is likely that the sources of regeneration in these cells are different.

In conclusion, we have proposed the systemic involvement of MMP-9 in the recruitment of hematopoietic progenitor cells in this study. The recruited progenitor cells are assumed to participate in liver regeneration via the reconstruction of nonparenchymal cells.

REFERENCES AND NOTES


Fig. 4. Partial Hepatectomy Did Not Recruit Hematopoietic Progenitor Cells into the Circulation
Blood samples and PMBC were collected at indicated times after 70% hepatectomy was performed, then zymography and Western blotting (A), and CFU assay (B) were performed as described.


