Differential role of CD97 in interleukin-8-induced and granulocyte-colony stimulating factor-induced hematopoietic stem and progenitor cell mobilization

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ABSTRACT

CD97 is broadly expressed on hematopoietic cells and is involved in neutrophil migration. Since neutrophils are key regulators in HSC/HPC mobilization, we studied a possible role for CD97 in interleukin-8 and granulocyte-colony stimulating factor-induced HSC/HPC mobilization. Mobilization was absent in mice receiving CD97 mAb followed by interleukin-8, while granulocyte-colony stimulating factor-induced mobilization remained unaltered following anti-CD97 administration. Furthermore, combined administration of CD97 mAb and IL-8 induced a significant reduction in the neutrophilic compartment. We hypothesize that the absence of interleukin-8-induced HSC/HPC mobilization after CD97 mAb administration is due to its effect on neutrophil function.

Key words: stem cell mobilization, CD97, interleukin-8, granulocyte-colony stimulating factor, animal models.

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Introduction

Mobilized hematopoietic stem- and progenitor cells (HSC/HPC) have become the primary source of HSC/HPC for transplantation in humans.[1] Mobilization of HSC/HPC to the peripheral blood can be achieved by the administration of various growth factors such as granulocyte colony-stimulating factor (G-CSF) and interleukin (IL)-8.[2-4]

We have previously demonstrated that a single injection of IL-8 induces rapid mobilization of hematopoietic HSC/HPC in mice and monkeys.[4,5] Neutrophils play an essential role in this process, since IL-8-induced HSC/HPC mobilization is prevented in neutropenic mice and could be restored by infusion of neutrophils.[6] Mobilization induced by G-CSF results in increased HSC/HPC numbers in the blood, peaking at levels of 10 to 100 times above baseline 4-7 days after initiation of daily G-CSF administration. CD97 is a member of the EGF-TM7 family of seven-span transmembrane (TM7) receptors[7-9] and is expressed on a broad array of hematopoietic cells.[10] The structure of CD97 is characterized by an extended extracellular region comprising several tandemly arranged EGF-like domains coupled to the TM7 region by a spacer. CD55 (decay accelerating factor) and chondroitin sulfate have been identified as cellular ligands for CD97.[9,11,12]

Recently, a role for CD97 in neutrophil migration was demonstrated.[13] In experimental colitis, application of CD97 mAb to adoptively transferred neutrophils caused a delay in homing to the colon. In mice intranasally inoculated with *Streptococcus pneumoniae*, treatment with CD97 mAb impaired the recruitment of neutrophils to the lungs leading to lethal pneumonia. Since neutrophils are indispensible for IL-8-induced HSC/HPC mobilization, and since CD97 plays a role in neutrophil migration, we investigated the role of CD97 in IL-8 – and G-CSF-induced HSC/HPC mobilization using a mAb directed against mouse CD97. Administration of this mAb selectively blocked IL-8-induced mobilization while G-

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CSF-induced mobilization was not affected. Combined administration of CD97 mAb and IL-8 induced a significant reduction in the neutrophilic compartment. We hypothesize that the absence of HSC/HPC mobilization after CD97 mAb administration is due to its effect on neutrophil function.

Design and Methods

Eight to 12 week old male Balb/c mice purchased from Charles River (Maastricht, The Netherlands) were used in all mobilization studies. The animals were fed commercial rodent chow and acidified water *ad libitum* and were maintained in the animal facilities of the Leiden University Medical Center under conventional conditions. All experimental protocols were approved by the institutional ethics committee on animal experiments.

Recombinant human IL-8 was purified from *Escherichia coli* expressing a synthetic gene[14] and generously provided by Dr. I.J.D. Lindley (Novartis Research Institute, Vienna, Austria). The concentration of endotoxin was less than 0.05 unit/mg as determined by the limulus amebocyte lysate assay. IL-8 was diluted in endotoxin-free phosphate-buffered saline (PBS) with 0.1% bovine serum albumin (BSA) immediately before use. Non-depleting hamster anti-murine CD97 mAb (clone 1B2)[13] directed against the first EGF-like domain of mCD97 was used for *in vivo* administration. Commercially available hamster immunoglobulin (Ig) was used as control (control IgG; Rockland, Gilbertsville, PA, USA). Phycoerythrin (PE)-conjugated anti-CD3ε and FITC-conjugated anti-GR-1 (clone RB6-8C5) were all obtained from Pharmingen (San Diego, CA, USA).

Mobilization of HSC/HPC was induced as previously described.[15] In blocking experiments, mice were pretreated with an i.p. injection of 500 µg CD97 mAb or control IgG 24 hrs. prior to IL-8 injection. Alternatively, mobilization was induced by injecting mice with 10 Ìg G-CSF i.p. for two consecutive days. In blocking experiments, 500 µg CD97 mAb was given 24 hrs. before the first G-CSF injection and 30 mins. prior to each G-CSF administration. Twenty-four hours after the last G-CSF injection, mice were sacrificed and colony-forming unit-granulocyte macrophages (CFU-GM) were analyzed in peripheral blood, bone marrow and spleen. Control mice received PBS supplemented with 0.1% BSA in all mobilization experiments. CFU-GM were cultured as previously described.[16]

Neutrophils were determined by flowcytometry. Following lysis of erythrocytes, 2×10^5 cells were incubated with anti-CD3ε, anti-CD45R/B220 and anti-GR-1. The GR-1^{HI}CD3^{NEG}B220^{NEG} cell population was determined to represent mature neutrophils. Differences were evaluated using the Student's *t*-test. *p* values of <0.05 were considered statistically significant.

Results and Discussion

To investigate the role of CD97 in IL-8-induced HSC/HPC mobilization, Balb/c mice were injected with CD97 mAb 24 hrs. prior to IL-8 injection. A significant decrease in HSC/HPC mobilization was observed in mice that received CD97 mAb prior to IL-8 administration compared with mice that received control IgG and IL-8 or IL-8 alone ($p<0.01$; Figure 1A). Neither administration of CD97 mAb nor control IgG nor PBS alone induced HSC/HPC mobilization *in vivo*. Combined administration of CD97 mAb or control IgG and IL-8 did not affect the number of HSC/HPC in bone marrow (Figure 1B).

To assess whether CD97 is involved in G-CSF-induced HSC/HPC mobilization, CD97 mAb was administered 24 hrs. prior to G-CSF-induced mobilization. In contrast to IL-8-induced HSC/HPC mobilization, treatment with CD97 mAb prior to G-CSF administration had no effect on HSC/HPC mobilization compared with animals treated with control IgG followed by G-CSF or G-CSF alone ($p>0.7$; Figure 2A). In addition, administration of control IgG, CD97 mAb or PBS alone did not induce HSC/HPC mobilization. Combined administration of CD97 mAb or control IgG and G-CSF did not affect HSC/HPC counts in bone marrow (Figure 2b). Analysis of peripheral blood plasma and bone marrow extracellular extracts indicated that free circulating CD97 mAb were present in both the peripheral blood and the bone marrow extracellular fluid at the time the mice were sacrificed for CFU-GM analysis (data not shown), indicating that saturating levels of mAb were present.

Our previous research showed that neutrophils are indispensible for IL-8-induced HSC/HPC mobliliza-tion.[6] To explore whether the inhibition of IL-8-induced HSC/HPC mobilization upon anti-CD97 antibody administration may be due to a defect in the neutrophilic compartment, the percentage of GR-1^{HI} cells was assessed in the peripheral blood of mobilized mice. We have previously shown that administration of IL-8 induces instant neutropenia, followed by a profound neutrophilia.[5] Combined administration of CD97 mAb and IL-8 led to a significant decrease in GR-1^{HI} cells in the peripheral blood 20 min after administration compared with administration of control IgG and IL-8 or CD97 mAb and PBS ($p<0.03$; Figure 3A). In addition, we studied the kinetics of neutrophil counts during combined administration of IL-8 and anti-CD97 mAb. From 5-60 mins. after CD97 mAb and IL-8 administration, neutrophil counts were significantly decreased ($p<0.001$) compared with the combined administration of control IgG and IL-8. However, at 120 mins. after administration, the percentage of neutrophils increased 2.7 times to levels similar to those of the control group. At 480 mins. after administration, all

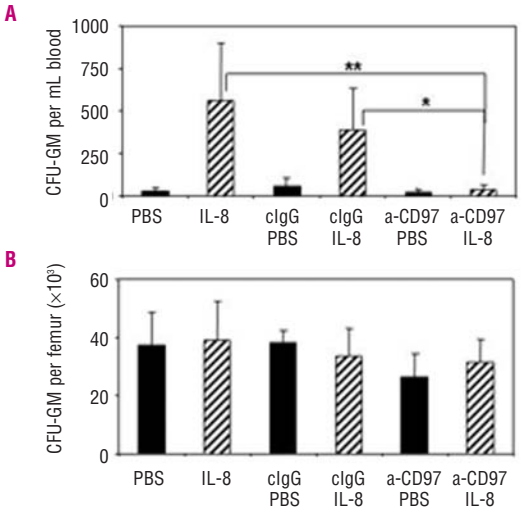

Figure 1. Pre-treatment with CD97 mAb prevents IL-8-induced HSC/HPC mobilization. Mice were pre-treated with a single i.p. injection of 500 μg anti-CD97 (clone 1B2), hamster control IgG or PBS. The following day, mobilization was induced by administration of IL-8 (30 μg/mouse; n=7), or PBS (n=5) as a control. Colony-forming capacity of (A) peripheral blood and (B) bone marrow are shown. Results are expressed as mean ± SD. **p*=0.036 ***p*=0.023

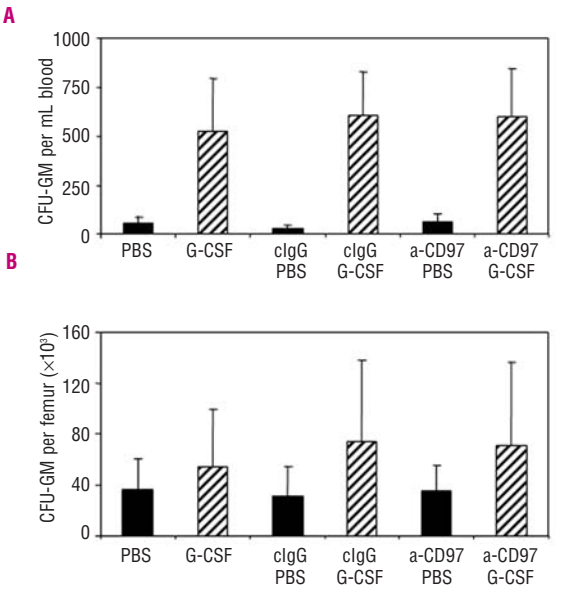

Figure 2. CD97 mAb has no effect on G-CSF-induced HSC/HPC mobilization. Mice were mobilized with 10 μg G-CSF (n=6) i.p. for two days or PBS (n=4) as a control. Before the first G-CSF injection and 30 mins. prior to each G-CSF administration 500 μg CD97 mAb (clone 1B2), hamster control IgG or saline was given. Twenty-four hours after the last G-CSF injection, mice were sacrificed and CFU-GM were analyzed in (A) peripheral blood and (B) bone marrow. Results are expressed as mean ± SD.

neutrophil counts had returned to basal levels (Figure 3B). These data indicate that anti-CD97 treatment affects neutrophil migration.

There is still no conclusive evidence to demonstrate the involvement of neutrophils in the inhibition of IL-8-induced HSC/HPC mobilization following CD97 mAb administration. However, we hypothesize that the abrogation of IL-8-induced HSC/HPC mobilization is due to functional blockade of CD97 expressed on neutrophils and there is evidence to support this. Firstly, IL-8-induced HSC/HPC mobilization has been shown to be dependent on neutrophils, since neutropenic mice failed to mobilize HSC/HPC following IL-8 administration.[6] Secondly, CD97 is critically involved in the migration of neutrophils.[13,17] Thirdly, preliminary data obtained from progenitor cell assays showed that CD97 is not expressed on colony forming cells (*data not shown*), indicating the involvement of an intermediate cell type in IL-8-induced HSC/HPC mobilization. Finally, 20 mins. after administration of IL-8, there was a significant decrease in neutrophil counts in the peripheral blood of CD97 mAb/IL-8-treated mice, compared with IL-8 mobilized mice or CD97 treated controls. Therefore, reduced neutrophil counts may be responsible for the absence of IL-8-induced HSC/HPC mobilization in CD97 mAb/IL-8 treated mice. Together, these data support the hypothesis that CD97 expression on neutrophils is responsible for the inhibition of IL-8-induced HSC/HPC mobilization by CD97 mAb.

The exact molecular function of CD97 in neutrophil

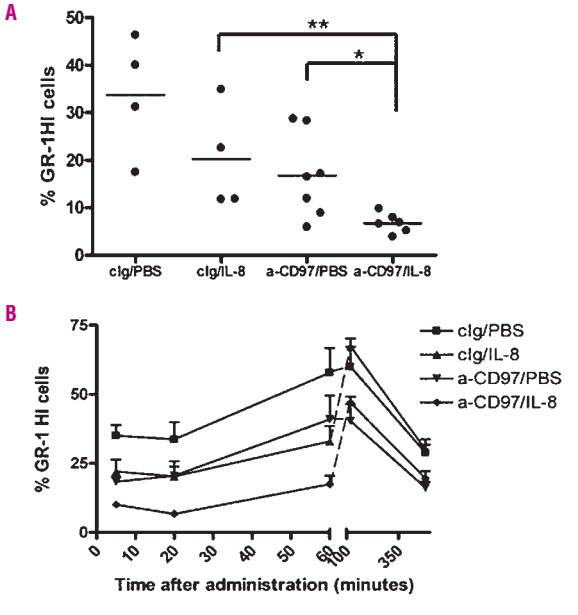

Figure 3. There was a significant decrease in neutrophils following combined administration of CD97 mAb and IL-8. Mice were pre-treated with a single i.p. injection of 500 μg anti-CD97 (clone 1B2) or hamster control IgG. The following day, mobilization was induced by administration of IL-8 (30 μg/mouse), or PBS as a control. (A) Twenty minutes after IL-8 administration, the mice were sacrificed, peripheral blood was harvested, and the percentage of GR-1^{HI} CD3^{NEG}B220^{NEG} cells was measured by FACS analysis. **p*=0.0214; ***p*=0.0162 (B) Peripheral blood was drawn at several time points following IL-8 administration and the percentage of GR-1^{HI} CD3^{NEG}B220^{NEG} cells was measured by FACS analysis. Each data point represents mean ± SEM of 4-6 mice.

migration must still be investigated. Neutrophils and the proteases that are released by these cells have already been identified as important mediators of G-CSF-induced HSC/HPC mobilization.[18,19] Although these findings suggest that activation of PMNs and subsequent release of proteases may represent a final common pathway in IL-8- and G-CSF-induced stem cell mobilization, their kinetics differ remarkably. While IL-8 induces HSC/HPC mobilization within 20 mins., G-CSF-induced mobilization requires several days. Furthermore, G-CSF-induced HSC/HPC mobilization depends upon bone marrow resident neutrophils, whereas peripheral blood neutrophils are important for IL-8-induced HSC/HPC mobilization. As CD97 mAb inhibits IL-8- but not G-CSF-induced HSC/HPC mobilization, only the circulating neutrophils seem to be affected by CD97 mAb treatment, while the bone marrow resident neutrophils remain untouched.

In conclusion, we show that CD97 mAb differentially affects IL-8- and G-CSF-induced HSC/HPC mobilization. Clarifying the molecular function of CD97 will provide important clues about the pathways engaged in HSC/HPC mobilization following IL-8 and G-CSF administration.

Authorship and Disclosures

MvP conceived and designed the study, collected, analyzed and interpreted data, and wrote the paper; MJK and HH collected, analyzed and interpreted the data and wrote the paper; JH conceived and designed the study, and critically revised the manuscript; WEF conceived and designed the study, interpreted data and critically revised the manuscript. All authors approved the final version of the manuscipt. The authors reported no potential conflicts of interest.

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