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IL-33–Induced Hematopoietic Stem and Progenitor Cell Mobilization Depends upon CCR2

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IL-33 has been implicated in the pathogenesis of asthma, atopic allergy, anaphylaxis, and other inflammatory diseases by promoting the production of proinflammatory cytokines and chemokines or Th2 immune responses. In this study, we analyzed the in vivo effect of IL-33 administration. IL-33 markedly promoted myelopoiesis in the bone marrow and myeloid cell emigration. Concomitantly, IL-33 induced hematopoietic stem and progenitor cell (HSPC) mobilization and extramedullary hematopoiesis. HSPC mobilization was mediated mainly through increased levels of CCL7 produced by vascular endothelial cells in response to IL-33. In vivo treatment of IL-33 rapidly induced phosphorylation of ERK, JNK, and p38, and inhibition of these signaling molecules completely blocked the production of CCL7 induced by IL-33. Consistently, inhibitor of CCR2 markedly reduced IL-33–mediated HSPC mobilization in vivo and migration of HSPCs in response to CCL7 in vitro. IL-33–mobilized HSPCs were capable of homing to, and of long-term reconstitution in, the bone marrow of irradiated recipients. Immune cells derived from these recipients had normal antifungal activity. The ability of IL-33 to promote migration of HSPCs and myeloid cells into the periphery and to regulate their antifungal activity represents a previously unrecognized role of IL-33 in innate immunity. These properties of IL-33 have clinical implications in hematopoietic stem cell transplantation. The Journal of Immunology, 2014, 193: 000–000.

Interleukin-33 is a recently described member of the IL-1 superfamily that signals through ST2, a heterodimer receptor complex comprising an ST2L and IL-1R accessory protein (1, 2). Recent studies suggest that IL-33 acts as an alarmin, similar to HMGB1 (high-mobility group protein 1) and IL-1α, to alert cells of the innate immune system in response to tissue damage during trauma or infection (3, 4). Even though the secretory mechanism of IL-33 is unknown, IL-33 is released into the extracellular matrix after cell damage, mechanical injury, or necrosis (3). Under apoptotic conditions, IL-33 undergoes cleavage into a less bioactive form by caspase-3 and -7, and the cleaved IL-33 remains in the cytoplasm (3). Under normal conditions, IL-33 is localized in the nucleus of endothelial and epithelial cells (5) and has chromatin-binding activity (6). IL-33 targets multiple cells driving distinct inflammation behind the pathological basis of various diseases. Since early studies revealed the function of ST2L, an IL-33–specific subunit of ST2, in Th2 activation (7, 8), an explosion of research has shown that IL-33 acts on a variety of innate type 2 immunity cells. In addition to Th2 cells, IL-33 has been shown to act on macrophages (9), dendritic cells (10), mast cells (11), basophils (12), eosinophils (13), natural helper cells (14), neutrophils (15), and multipotent progenitors (16). Even though IL-33 functions as a key mediator for Th2-mediated asthma and fibrosis (17), IL-33 is also a crucial cytokine for chronic inflammatory arthritis that integrates the activation of fibroblasts and Th1 and Th17 effector T cells operating through a mast cell–dependent pathway (18).

Bone marrow (BM) cavities are the anatomical site where hematopoietic stem and progenitor cells (HSPCs) reside and their hematopoietic activities occur (19). However, HSPCs are not entirely sessile in BM niches; rather, some HSPCs recirculate constantly between the BM and the periphery (20, 21). Although it is unknown why this propensity of HSPCs is required for homeostasis, extensive experimental evidence has shown that the regulation of chemokine gradients between the BM and blood is integral to their migration and repopulation (19). In stressful situations such as inflammation, massive hematopoiesis occurs in the BM, followed by massive mobilization of HSPCs and immature leukocytes into circulation. Studies on G-CSF, which is clinically used to induce the mobilization of HSPCs, have provided clues to the mechanism of HSPC mobilization. For example, mobilization with repeated G-CSF stimulation triggers a transient increase in the secretion of CXCL12, massive progenitor proliferation and differentiation, activation of polymorphonuclear neutrophils (PMNs), secretion of membrane-bound stem cell factor (22), and increased secretion of proteolytic enzymes, including cathepsin G, elastase and MMP-9 (matrix metallopeptidase-9) (23). These are followed by the degradation of CXCL12, the increased expression of CXCR4, and the mobilization of maturing leukocytes and HSPCs (24, 25).
On the basis of the properties of IL-33 as a cytokine that can induce the production of proinflammatory cytokines and chemokines linked to acute inflammation, we predicted that IL-33 would be important for regulating hematopoiesis, HSPC mobilization, and enhancing innate immunity against infection. Our results indicate that IL-33 was able to induce mobilization of functional HSPCs that rapidly reconstituted the immune system after transplantation into irradiated recipients and subsequently protected the host against opportunistic Candida albicans infection.

Materials and Methods

**Mice**
Female C57BL/6 and BALB/c mice, 6–8 wk of age, were purchased from Orient (Seoul, Korea), MyDB8−/− C57BL/6, GFP transgenic C57BL/6, Thy1.1 congenic C57BL/6, and CXCR2−/− BALB/c mice were purchased from The Jackson Laboratory and bred under pathogen-free conditions at the University of Ulsan (Ulsan, Korea) animal facility. The University of Ulsan Animal Care Committee approved these studies. All experiments were performed using the C57BL/6 strain if not mentioned otherwise.

**Production of rIL-33 protein**
The rIL-33 protein was produced as previously described (26). In brief, the cDNA for mouse IL-33 was subcloned into the expression vector pET3a, starting with amino acid 109 of the full-length protein. DL21(D3) (Merck, Whitehouse Station, NJ) was transformed and expression was induced with isopropyl β-D-thiogalactoside (Thermo Fisher Scientific, Waltham, MA). Harvested bacteria were lysed by sonication, and the lysate was purified using HiTrap Q column (Amersham Biosciences, Piscataway, NJ). LPS was removed using the Thermo Scientific Detoxi-Gel Endotoxin Removing Gel (Thermo Fisher Scientific). The Linumus Amelocyte Lysate (Lonza, Basel, Switzerland) confirmed that LPS contamination was low (<0.05 endotoxin unit per microgram).

**Peripheral blood cell mobilization and hematopoietic stem cell transplantation**
To induce HSPC mobilization, mice were i.p. injected with rIL-33 (1 µg/mouse) one time per day for 4-consecutive days, as previously described (26). PBS was used as the control. At 1 d after the last injection, the mice were sacrificed, splenocytes were prepared, and 2 × 10^7 splenocytes were i.v. transplanted into lethally irradiated (9 Gy) recipient mice. In some experiments, 1 × 10^7 PBMCs were used as mobilized HSPCs.

**Flow cytometry**
The following FITC-, PE-, PE-Cy5 (PE-cytochrome 5), PerCP- or allophycocyanin-conjugated mAbs to mouse proteins were purchased from BD Biosciences (San Diego, CA) or eBioscience (San Diego, CA) and used for cell staining: B220, CD3, CD19, CD11b, CD11c, CD49d, CD115 (CSF-1R), CD144 (VE-cadherin), c-Kit, CCL2, CCL7, c-Kit, CXCR2, CXCR4, FcεRl, F4/80, Gr1, Ly6G, Sca-1, Ter119, and Thy1.1. Purified cells were reinfused into a blocking buffer (PBS containing 2.4G2 mAbs/0.2% BSA/0.1% sodium azide), incubated with the relevant mAbs at 4°C for 30 min, and then washed twice with staining buffer. Flow cytometric analysis was performed using a FACScan Skylab II (BD) cytometer, and data were analyzed using FACSDiv software (BD Biosciences) and FlowJo software (TreeStar, Ashland, OR).

**Blood cell counts**
Mice were injected with PBS or 1 µg IL-33 for 4-consecutive days. At 1 d after the last injection, blood was harvested and blood leukocytes were counted using a Hemavet Moscot hematology analyzer (CDC Technologies, Oxford, CT).

**Colony-forming assay**
Colony-forming assays were performed according to the manufacturer’s recommendations (StemCell Technologies, Vancouver, BC, Canada). In brief, BM cells, splenocytes, and PBMCs were harvested into PBS-containing tubes. Cells were then incubated in cold erythrocyte lysis buffer (145 mM NaCl and 17 mM Tris-HCl, pH 7.2) for 3 min at room temperature and washed once. They were counted and resuspended in 1% IMDM prior to plating at a density of 2 × 10^5 cells per 0.1 ml. The cultures were incubated in MethoCult M3534 complete medium containing cytokine mixtures at 37°C, and colony numbers were counted for CFU-GM, CFU-G, and CFU-M at the indicated times. To count CFU-pre-B, BM cells and splenocytes (2 × 10^5 cells per well) were cultured in MethoCult M3530 medium containing IL-7 (StemCell Technologies).

**Homing assay**
To examine the homing capacity of IL-33–mobilized HSPCs, 2 × 10^7 splenocytes of GFP+ or Thy1.1 C57BL/6 mice that received PBS or IL-33 were transferred into Th1.2 congenic recipient mice. Donor-derived LK (lineage c-Ki+ or LKS (lineage c-Ki+ Sca-1)+) cells in the BM were enumerated using flow cytometry 14 h after the transfer.

**ELISA**
Levels of CXCL1 (R&D Systems, Minneapolis, MN), CXCL2, CXCL12, CCL2 (Abcam, Cambridge, UK), and CCL7 (PeproTech, Rocky Hill, NJ) were determined using ELISA according to the manufacturer’s instructions.

**Culture of aortic fragments**
Aortic fragments (4–5 mm in length) were removed and immediately placed in cold HBSS, and incubated in cold erythrocyte lysis buffer (144 mM NaCl and 17 mM Tris-HCl, pH 7.2) for 3 min at room temperature and washed in cold HBSS. Washed aortic fragments were placed in RPMI 1640 medium without serum and incubated in the presence of 100 ng/ml IL-33 in combination with 5 µM inhibitors (FR180204, SP600125, or SB203580; Abcam) at 37°C for 4 h.

**In vitro migration**
BM LK cells were enriched by removing lineage+ cells using mixtures of microbead-conjugated Abs to lineage markers (Miltenyi Biotec, Auburn, CA), followed by sort purification using a BD FACSAria cell sorter. Sorted LK cells were preincubated with 500 ng/ml nonpeptide CCR2 antagonist RS 504393 (TOCRIS, Bristol, UK) for 1 h, washed, and resuspended in RPMI 1640 containing 10% FBS. Conditioned culture medium of aortic fragments or PBS was preincubated with 50 µg/ml neutralizing anti-CCL7 (R&D Systems) or rat IgG for 1 h. The cells (3.6 × 10^5 in 0.1 ml assay medium) were seeded in the upper chamber of 8-µm Transwells (Corning, Corning, NY), and 0.1 ml medium containing CCL2, CCL7, or 40 µl conditioned culture medium of aortic fragments or PBS was added to the bottom chamber. The cells were then allowed to migrate into the lower chamber for 6 h at 37°C. Migrated cells were enumerated using a hemacytometer. In some experiments, PBS-injected or IL-33–injected mouse serum was used for LK cell migration.

**Western blotting**
Aortic fragments were placed in radioimmunoprecipitation assay buffer (Tris 50 mM, NaCl 150 mM, 0.1% SDS, 0.5% Na-deoxycholate, and 1% Nonidet P-40, pH 8.0), homogenized, incubated for 15 min at 4°C, and centrifuged at 13,000 × g for 15 min. The protein concentration in the supernatant was determined by BCA kit (Thermo Scientific). From each sample, 20 µg protein was resolved using 10% SDS-PAGE before transferring to nitrocellulose membrane (Whatman, Dassel, Germany). The blots were blocked in 5% skimmed milk in TBST [20 mM Tris-HCl (pH 7.4), 250 mM NaCl, and 0.05% Tween 20] for 1 h before probed for 1 h using the appropriate primary Ab. The blots were washed with TBST for 10 min three times, before being incubated with the appropriate secondary Ab raised against ERK or phosphorylated ERK, JNK, or phosphorylated JNK, p38 or phosphorylated p38, IkB-α, or GAPDH (Santa Cruz Biotechnology, Dallas, TX) for 1 h. Following three additional washes in TBST-T, they were developed using the ECL detection system (Amersham Biosciences, Amersham, U.K.).

**Immunohistochemistry**
The femur and tibia were harvested from IL-33–injected mice. The harvested tissues were decalcified in 0.07% EDTA, placed in B-5 fixative overnight, and embedded in paraffin. The 5-µm-thick serial sections were cut on a charged slide. Paraffin sections were deparaffinized and rehydrated in graded alcohol series. The sections were then washed in distilled water and boiled in a microwave oven for epitope retrieval in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6) for 20 min. Slides were equilibrated in PBS and incubated with blocking solution for 1 h at room temperature, followed by overnight incubation with FITC-conjugated anti-CD144 and anti-CCL2 or FITC-conjugated anti-CD144 and anti-CCL7 Ab (all from eBioscience) in staining buffer (PBS containing 0.3% BSA) at 4°C. After washing, sections were incubated with...
IL-33 promotes myelopoiesis in a MyD88-dependent manner

We monitored changes in hematopoietic cell numbers in the BM and spleen after IL-33 treatment to understand involvement of IL-33 in hematopoiesis. Mice that were treated daily with IL-33 for 4 consecutive days displayed a transient decrease in total BM cell numbers. The decreased number of BM cells was accompanied by a marked decrement in CD11b+Gr-1hi neutrophils and B220+ B cells (Fig. 1A, 1B). Unlike these two subsets, numbers of CD11b+Gr-1int/lo cells, which include monocytes and other granulocytes such as eosinophils and basophils, were markedly increased by IL-33 injection (Fig. 1A, 1B). Consistent with the results shown by others (26), IL-33 increased the number of total splenocytes, which was due to increased numbers of all subsets, including CD11b+ myeloid cells and lymphocytes (Fig. 1B). Representative FACS plots used to analyze these immune cell subpopulations were provided in Supplemental Fig. 1. Changes in the number of hematopoietic cells occurring after IL-33 treatment was abrogated in the BM and spleen due to increased numbers of all subsets, including CD11b+ myeloid cells and lymphocytes (Fig. 1B). Representative FACS plots used to analyze these immune cell subpopulations were provided in Supplemental Fig. 1. Changes in the number of hematopoietic cells occurring after IL-33 treatment was abrogated in the BM and spleen.
of MyD88 knockout (KO) mice (Fig. 1C, 1D), suggesting that IL-33–induced hematopoiesis is completely dependent upon MyD88. This observation was consistent with results showing that MyD88 is required for the ST2 signaling transduction pathway (27). Taken together, our results indicate that IL-33 differentially regulates myelopoiesis and lymphopoiesis in the BM and spleen.

**IL-33 promotes myeloid cell emigration**

The results shown in Fig. 1A suggested that IL-33 might decrease the number of CD11b+Gr-1hi neutrophils in the BM because of their rapid emigration, as seen during inflammation (28). IL-33 injection markedly increased the number of CD11b+CD115+ monocytes, CD11b+CD49d+c-Kit+FcεRI+ basophils, and CD11b+Ly6G+Ly6C+F4/80+ eosinophils in the BM, while decreasing CD11b+Gr-1hiSSCint neutrophils (Fig. 2A). However, significantly increased numbers of these myeloid subsets were present in the peripheral blood after IL-33 treatment, except for monocytes (Fig. 2B). We presented FACS plots used to analyze these myeloid cell types in Supplemental Fig. 2. These results suggest that IL-33 treatment promoted the generation of monocytes, basophils, and eosinophils and their subsequent emigration in the BM with different kinetics. In the case of neutrophils, their massive emigration following IL-33 treatment appeared to precede their generation in the BM.

**IL-33 induces HSPC mobilization**

The observation that IL-33 was active in expanding both myeloid cells and lymphocytes in the spleen suggested that IL-33 could induce HSPC mobilization, followed by extramedullary hematopoiesis. To test this prediction, we examined the frequency of colony-forming cells in the BM and in the periphery after IL-33 treatment. IL-33–treated mice showed a 5-fold decrease in CFU–pre-B frequency in the BM compared with PBS-treated control mice (Fig. 3A), whereas no difference in the frequencies of CFU-M, CFU-G, and CFU-GM was observed between the two groups of mice (Fig. 3B). There was a marked increase in the CFU frequency for pre-B cells in the spleen and for myeloid cells in the peripheral blood and spleen after IL-33 treatment (Fig. 3A, 3B). Taken together, our results demonstrated that IL-33 regulated HSPC mobilization and suggested that the inhibitory effect of IL-33 on lymphopoiesis in the BM might be due to massive emigration of lymphoid progenitor cells.

**IL-33–induced HSPC mobilization depends upon CCR2**

IL-33–injected mouse sera were more potent in inducing the migration of LK cells than PBS-injected mouse sera (Fig. 4A). We predicted that chemokines whose expression was rapidly upregulated in the blood after IL-33 injection would mediate HSPC mobilization. We investigated the concentration of chemokines...
whose receptors are known to be involved in HSPC mobilization (24, 29, 30), including CXCR12 (CXCR4 ligand), CXCL1/2 (CXCR2 ligands), and CCL2/7 (CCR2 ligands), at the various time points after injection with IL-33. Levels of all chemokines except CXCL12 rapidly increased in 1 or 2 h after IL-33 injection and declined thereafter with a different kinetics and extent (Fig. 4B). There were marginal changes in the levels of CXCL1, CXCL2, CXCL12, and CCL7 in the BM (Fig. 4B). PBS injection increased significant levels of CCL2 in the BM, suggesting that CCL2 might be upregulated by stress. Overall, these results suggested that CXCR2 and/or CCR2 might be responsible for IL-33-mediated HSPC mobilization. In vivo blocking assays demonstrated that the CCR2 antagonist RS504393, but not the CXCR2 antagonist SB225202, markedly inhibited HSPC mobilization induced by IL-33 (Fig. 4C, 4D). We confirmed that CXCR2 is not involved in IL-33–mediated HSPC mobilization using CXCR2 KO and control BALB/c mice (Fig. 4C). There was a higher ability of HSPC mobilization in CXCR2 KO mice for an unknown reason (Fig. 4C).

**CCL7 is responsible for IL-33–induced HSPC mobilization**

Our data indicated that the rapid production of CCL7 in response to IL-33 might form the CCL7 concentration gradient for HSPC mobilization toward vessels. We hypothesized that endothelial cells would be the main cell type responding to IL-33. Immunohistochemical analysis demonstrated positive staining for CCL7 in VE-cadherin (CD144)–positive endothelial cells of BM vessels (Fig. 5A). A similar pattern of staining for CCL2 was observed, but its intensity was weaker (Fig. 5A). We next examined whether

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**FIGURE 4.** IL-33–induced HSPC mobilization depends upon CCR2. (A) Migration of LK cells in response to PBS- or IL-33–treated serum (40 μl per well). Results are representative of three experiments and are presented as the mean ± SEM (n = 3 per group). **p < 0.01 between the two indicated groups. (B) Levels of chemokine (CXCL12, CXCL1, CXCL2, CCL2, and CCL7) were measured in BM plasma and serum at various time points after PBS or IL-33 injection. Results are representative of two to three experiments and are presented as the mean ± SEM (n = 4 per group). *p < 0.05, **p < 0.01, ***p < 0.001 between 0 h and the indicated time point. (C and D) Colony-forming cell assay was performed using peripheral blood of C57BL/6 mice that received PBS or IL-33 in combination with SB225002 (CXCR2 antagonist) (C) or RS504393 (CCR2 antagonist) (D). Experiments were repeated using blood of wild-type and CXCR2−/− mice that were injected with IL-33 (C). Results are representative of two experiments and are presented as the mean ± SEM (n = 6 per group). ***p < 0.001 between the two indicated groups.
aortic fragments cultured in the presence of IL-33 would secrete CCL7 and CCL2. As shown in Fig. 5B, IL-33 triggered the production of CCL7 by the aortic fragments from 3 h after IL-33 treatments, and its levels further increased at 4 h. In our culture condition, the effect of IL-33 on levels of CCL2 was not so evident, as the PBS control group showed a similarly increased pattern of CCL2 secretion at 3 and 4 h after treatment, even though IL-33–treated aortic fragments produced higher levels of CCL2 at 4 h (Fig. 5B). Consistent with the in vivo results shown in Fig. 5B, CCL2 production seems to be sensitive to stress. Migration assays showed that the conditioned medium of IL-33–treated aortic fragment culture induced potent migration of LK cells compared with that of PBS-treated aortic fragment culture (Fig. 5C). The increased portion of LK cell migration in the former group was completely abrogated by neutralization of CCL7 (Fig. 5C), suggesting that CCL7 produced by aortic fragments following IL-33 treatment might be in large part responsible for IL-33–mediated HSPC mobilization. Addition of RS504393 in culture medium further decreased migration of LK cells in both groups to the same level (Fig. 5D). This result indicates that the contribution of CCL2 to their migration was similar in both groups. We repeated a migration assay using recombinant CCL2 and CCL7 and found that CCL7 was by far more potent in inducing migration of LK cells (Fig. 5D). In vivo neutralization of CCL7 also was shown to completely block HSPC mobilization induced by IL-33 (Fig. 5E). Taken together, these data suggest that CCL7 is a major type of chemokine that mediates IL-33–induced HSPC mobilization.

**IL-33 signaling leading to the production of CCL7 in vessels**

IL-33 binding to its receptor complex activates downstream signaling molecules through recruiting MyD88 to exert biological

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**FIGURE 5.** CCL7 produced by endothelial cells is responsible for IL-33–mediated HSPC mobilization. (A) Immunohistochemical staining for CD144 and CCL2 or CD144 and CCL7 in BM sections harvested 4 h after IL-33 injection. Original magnification, ×100. (B) Aortic fragments were cultured in the presence or absence of IL-33 (20 ng/ml) for the indicated times. CCL2 and CCL7 levels in the culture supernatant were measured using ELISA. Results are presented as the mean ± SEM (n = 4–9 per group). **p < 0.01, ***p < 0.001 between the two indicated groups at the indicated time point. (C) Migration of LK cells in response to aortic fragment culture medium. LK cells were preincubated with RS504393 for 1 h, and migration was allowed for 6 h. Results are representative of at least three experiments and are presented as the mean ± SEM (n = 4 per group). (D) Migration of LK cells in response to CCL2 or CCL7. (E) Effect of in vivo neutralization of CCL7 on IL-33–mediated HSPC mobilization. Experiments were done as described in Materials and Methods (n = 3 per group). CIg, control Ig Ab. Results are representative of three experiments and are presented as the mean ± SEM (n = 4 per group). *p < 0.05 between the indicated groups.
activities. Through a poorly defined mechanism, MyD88 complexes activate the transcription factor NF-κB and the MAPK pathway, which is mediated by the activation of ERK, p38, and JNK to produce cytokines and chemokines in a variety of cells (1). Because it is not known how IL-33 signaling results in the production of CCL7 in vessels, we wanted to identify which signaling pathway is involved in this process. We harvested aortic fragments at various time points after injection of IL-33 and examined the phosphorylation status of ERK, JNK, and p38 and the degradation of IκB-α. Western blot analysis showed that although IL-33 did not induce degradation of IκB-α, it increased phosphorylation of ERK, JNK, and p38 (Fig. 6A). Inhibitor of ERK (FR180204), JNK (SP600125), and p38 (SB203580) completely abrogated IL-33–induced production of CCL7 by aortic fragments (Fig. 6B), suggesting that the MAPK pathway is responsible for CCL7 production by vessels following IL-33. More importantly, in vivo injection of these inhibitors markedly blocked IL-33–induced HSPC mobilization with a similar potency (Fig. 6C).

**Immune cells derived from IL-33–mobilized HSPCs have an effective response to C. albicans infection**

We investigated whether IL-33–mobilized HSPCs could reconstitute the immune system of lethally irradiated recipients. We used PBMCs or cells of the spleen as reservoirs for large quantities of IL-33–mobilized HSPCs (Fig. 3). There were distinct populations of donor GFP+ LK and LKS in the BM of irradiated recipients 14 h after transfer of donor splenocytes. The numbers of donor LK and LKS cells of mice that received IL-33–injected mouse splenocytes were significantly higher than those of mice that received PBS-injected mouse splenocytes (Fig. 7A), indicating that IL-33–mobilized HSPCs had a normal homing capacity. We next compared the colony-forming ability of LK cells purified from the PBS-treated BMs or the IL-33–treated spleens using in vitro colony-forming assays. As shown in Fig. 7B, the colony-forming capacity of IL-33–mobilized HSPCs was approximately half that of BM HSPCs (Fig. 7B). To examine whether the PBMCs of mice that received IL-33 contained HSPCs that were capable of long-term reconstitution in the BM, irradiated recipients received, i.v., 1 × 10⁶ PBMCs of IL-33–injected or PBS-injected mice. A majority of mice that received PBMCs of IL-33–injected mice survived until the experiment termination (9 mo after PBMC transfer), whereas all mice that received PBMCs of PBS-injected mice died within 20 d after PBMC transfer (Fig. 7C). Taken together, these results suggest that IL-33–mobilized HSPCs were capable of homing and long-term reconstitution in the BM of irradiated recipients.

Next, we investigated whether immune cells were rapidly generated from IL-33–mobilized HSPCs in recipients irradiated with 9 Gy. Immune cells were counted in the BM and spleen 7 d after transplantation with 5 × 10⁶ splenocytes containing PBS- or IL-33–mobilized HSPCs. There were markedly higher numbers of CD11b+Ly6Ghi neutrophils, CD11b+F4/80+ monocytes, CD11b+SinglecF+ eosinophils, CD3+ T cells, and B220+ B cells in the BM of mice that received IL-33– versus PBS-mobilized HSPCs (Fig. 7D). At the same time, the spleens of mice that received IL-33–mobilized HSPCs also contained a significantly higher number of neutrophils, monocytes, and eosinophils, compared with the spleens of mice that received PBS-mobilized HSPCs. No difference was found in the number of lymphocytes in the spleens between the two groups (Fig. 7D). To address whether this rapid reconstitution of myeloid cells after transplantation with IL-33–mobilized HSPCs could result in a protective response against microbial infection, we used a C. albicans sepsis model. Mice were i.p. inoculated with a lethal dose of C. albicans (5 × 10⁶ CFUs), an opportunistic pathogen known to infect immunocompromised patients, at 7 d post transplantation. Mice transplanted with IL-33–mobilized HSPCs displayed a markedly higher survival rate than did mice transplanted with PBS-mobilized HSPCs (Fig. 7E). The higher survival rate correlated with a lower number of C. albicans in various organs (Fig. 7E). In sum, our findings suggest that IL-33–mobilized HSPCs were fully functional in immune reconstitution and that their progenies played a protective role in C. albicans infection.

**IL-33 treatment after IL-33–mobilized HSPC transplantation further enhances host defense against C. albicans infection**

During the pre-engraftment period, promotion of myelopoiesis for donor HSPCs and the host defense against infection is essential for rescue from mortality caused by infection. To investigate whether

![Figure 6](http://www.jimmunol.org/)

**Figure 6.** Signaling pathways involved in IL-33–mediated production of CCL7 in aorta. (A) Western blot analysis for phosphorylation of ERK, JNK, and P38 and degradation of IκB-α. Aorta extracts were prepared from mice at various time points after injection with 1 μg of IL-33. (B) Aortic fragments were cultured for 4 h in the presence of IL-33, 100 ng/ml, plus inhibitors: 5 μM of FR180204 (for ERK), SP600125 (for JNK), or SB203580 (for P38). Levels of CCL7 were measured by ELISA. (C) Effects of inhibitor of ERK, JNK, or P38 on IL-33–mediated HSPC mobilization. Experiments were done as described in Materials and Methods (n = 3 per group). **p < 0.01, ***p < 0.001 between the IL-33–treated mice and the other groups.
IL-33 could play a protective role in *C. albicans* infection after transplantation with IL-33–mobilized HSPCs, mice were injected with IL-33 on day 6 after HSCT (1 d before *C. albicans* infection). A significantly higher percentage of mice that received IL-33 were protected from a highly lethal dose of *C. albicans* infection (8 × 10⁶ CFUs) compared with that of mice receiving PBS (Fig. 8A). Consistent with this result, pretreatment of IL-33 resulted in a decrease in fungal burden in various organs (Fig. 8B). In addition, there were rapidly increased levels of CXCL1 and CXCL2 in the peritoneal cavity and blood in mice that received IL-33 (Fig. 8C). This increase was accompanied by a rapid recruitment of neutrophils at the site of infection (Fig. 8D) and a higher number of CXCR2⁺ neutrophils in the peritoneum (Fig. 8E).

Furthermore, IL-33–sensitized neutrophils were more active in phagocytizing *C. albicans* than were PBS-sensitized neutrophils (Fig. 8F). As a whole, these results suggest that IL-33 acted to increase the responsiveness of neutrophils to *C. albicans* infection.

**IL-33 protects mice receiving autologous bone marrow transplantation from *C. albicans* infection**

We evaluated the effect of IL-33 on the host defense against *C. albicans* infection in mice that received autologous BM transplantation (BMT). Two daily injections of IL-33 before *C. albicans* infection did not affect the number of myeloid cells or lymphocytes in the BM and spleen except for a 2-fold increase in BM eosinophils (Fig. 9A). Nonetheless, IL-33 injection in-
increased the survival rates of mice infected with a lethal dose of *C. albicans* (3.3 × 10^7 CFUs) (Fig. 9B). It seemed that IL-33 injection 24 h before infection was critical to protecting mice from *C. albicans* infection. Consistent with the survival data, IL-33 injection significantly diminished fungal burden in various organs (Fig. 9C) and enhanced the phagocytic activity of neutrophils (Fig. 9D, 9E) but lowered that of macrophages (Fig. 9F). Taken together, these results suggest that IL-33 could play a protective role against *C. albicans* infection after autologous BMT.

**Discussion**

In this study, we demonstrated a novel function of IL-33 that can be exploited to obtain HSPCs for stem cell transplantation. Our results indicate that vessel endothelial cells were the cellular targets of IL-33 and might be responsible for HSPC mobilization through CCL7, based on the following observations. First, CCL7 was the most markedly increased in serum, compared with BM plasma, among chemokines known to mobilize HSPCs after IL-33 injection (Fig. 4B). Second, vessel endothelial cells in the BM produced CCL7, and aortic fragments did so in response to IL-33 (Fig. 4A, 4B). Third, IL-33–treated mouse serum or conditioned medium of aortic fragment culture was able to induce the migration of LK cells in a CCR2-dependent manner (Fig. 4C, 5C). Fourth, neutralization of CCL7 completely blocked IL-33–mediated HSPC mobilization (Fig. 5E).

The CXCL2–CXCR2 axis is involved in hematopoietic stem cell mobilization and its homing and engraftment (29). Even though CXCL2 was detected in serum after IL-33 injection, HSPC mobilization was enhanced rather than decreased by either CXCR2 inhibitor or CXCR2 gene deletion (Fig. 4C). This observation indicates that CXCR2 signaling should negatively regulate CCR2-mediated HSPC mobilization. In addition, CXCR2 KO mice maintained higher levels of HSPC mobilization in normal conditions compared with wild-type mice (Fig. 4C), strongly suggesting that CXCR2 signaling is a key negative checkpoint in the step of emigration from the BM for HSPCs to patrol the periphery. This interesting observation ensures further study.

Overall, our results indicate that IL-33 supported acute inflammatory events in a coordinated fashion. In the BM, IL-33 fulfilled the need for sufficient numbers of inflammatory cells through promoting myelopoiesis and inhibiting lymphogenesis. Despite active emigration of HSPCs following IL-33 treatment, the frequency of myeloid, but not lymphoid, progenitors was maintained in the BM, which may explain how IL-33 promoted myelopoiesis and inhibited lymphopoiesis in the BM. In vessels, IL-33 stimulated the release of CCL7 and CXCR2 chemokines by endothelial cells and subsequently induced mobilization of HSPCs and neutrophils, respectively, into the periphery. IL-33 also induced migration of monocytes and other granulocytes from the BM to the blood (Fig. 2). In the peritoneum, IL-33 sensitized peritoneal macrophages to produce CXCL1 and CXCL2 in response to *C. albicans* infection (31), suggesting that IL-33 can induce neutrophil migration from the blood to organs. Finally, the observation that IL-33 enhanced the phagocytic activity of neutrophils suggests that IL-33 regulates the effector phase of inflammation (31). Taken together, our results warrant further studies to explore the function of IL-33 as a key regulator in orchestrating acute inflammation.

Even though IL-33 induced active myelopoiesis in the BM and extramedullary hematopoiesis in the spleen in normal mice (Fig. 1),
IL-33 increases the antifungal activity in mice that receive autologous BMT. (A) Lethally irradiated BALB/c mice were transplanted with 5 × 10^6 autologous BM cells. IL-33 (1 μg) was administered twice at days 5 and 6, and C. albicans was i.p. injected at day 7 post transplantation. Cell populations were analyzed in the BM and spleen at day 7. (B) BALB/c mice were administered IL-33 at the indicated time points after BM transplantation and infected with C. albicans (3 × 10^7 CFUs) at day 7 post transplantation, and survival was monitored for 7 d. (C) BALB/c mice were administered IL-33 at days 5 and 6 after BM transplantation, and fungal burden in the organs was analyzed 24 h post infection with C. albicans (1 × 10^7 CFUs). (D–F) BALB/c mice were transplanted with autologous BM cells, and IL-33 (1 μg) was administered twice at days 5 and 6 after BM transplantation, followed by injection with FITC-labeled heat-killed C. albicans (2 × 10^6 CFUs). At 1 h later, phagocytosis was analyzed on a gated population of neutrophils (CD11b^+Ly6G^hi). Phagocytosis was expressed as the mean fluorescence intensity of FITC^+ neutrophils (D) or the percentage of FITC^+ neutrophils (E). Phagocytosis was similarly analyzed for macrophages (D11b^+F4/80^hi). Results are presented as the mean ± SEM (n = 5–7 per group). *p < 0.05, **p < 0.01, ***p < 0.001.

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Disclosures
The authors have no financial conflicts of interest.

References


Supplemental Information

Figure S1. Representative original FACS plots used to analyze results shown in Figs. 1A and B.
Figure S2. Representative original FACS plots used to analyze results shown in Fig. 2A.

Gating strategies were shown for neutrophils, basophils, and eosinophils.