Cannabinoid receptor 2 and its agonists mediate hematopoiesis and hematopoietic stem and progenitor cell mobilization

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Running title: Regulation of hematopoiesis by CB2 cannabinoids
Abstract

Endocannabinoids are arachidonic acid derivatives and part of a novel bioactive lipid signaling system, along with their G-coupled receptors (CB₁ and CB₂) and the enzymes involved in their biosynthesis and degradation. However, their roles in hematopoiesis and hematopoietic stem and progenitor cell (HSPC) functions are not well characterized. Here, we show that bone marrow stromal cells express endocannabinoids, (anandamide and 2-arachidonylethanolamide), while CB₂ receptors are expressed in human and murine HSPCs. Upon ligand stimulation with CB₂ agonists, CB₂ receptors induced chemotaxis, migration and enhanced colony formation of bone marrow cells, which were mediated via ERK, PI3-kinase and Gαi-Rac1 pathways. In vivo, the CB₂ agonist AM1241 induced mobilization of murine HSPCs with short- and long-term repopulating abilities. In addition, G-CSF-induced mobilization of HSPCs was significantly decreased by specific CB₂ antagonists and was impaired in Cnr2⁻/⁻ cannabinoid type 2 receptor knockout mice. Taken together, these results demonstrate that the endocannabinoid system is involved in hematopoiesis and that CB₂/CB₂ agonist axis mediates repopulation of hematopoiesis and mobilization of HSPCs. Thus, CB₂ agonists may be therapeutically applied in clinical conditions, such as bone marrow transplantation.
**Introduction**

Endocannabinoids and exogenous cannabinoid ligands bind to and activate the cannabinoid receptors CB₁ and CB₂. Endocannabinoids are endogenous lipid mediators generated by many cell types both in the brain and peripheral tissues, which exert broad range of biological effects such as cardiovascular, neurological and anti-inflammatory effects. Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the two most widely studied endocannabinoids. The endocannabinoid system represents a pivotal neuroprotective mechanism both in acute forms of neuronal injury (such as a stroke and traumatic brain injury) and chronic neurodegenerative disorders. The synthetic and natural ligands of cannabinoid receptors exert various anti-inflammatory and neuroprotective effects by several mechanisms that include inhibiting the generation and release of proinflammatory cytokines, activation of cytoprotective signaling pathways, modulation of calcium homeostasis and excitability via interactions with Ca⁺², K⁺ and Na⁺ channels as well as antioxidant properties of endocannabinoids.

Both the CB₁ and CB₂ receptors are seven transmembrane Gα/ᵢ protein-coupled receptors (GPCRs), and are highly conserved during evolution. The CB₂ receptor is predominantly expressed in the immune system, such as B and T cells, natural killer cells, monocytes and neutrophils, where cannabinoids can modulate cytokine release and immune cell migration. While the expression and function of cannabinoid receptors in mature hematologic and immune cells was reported, the effect of cannabinoids on HSPCs has not been investigated in depth. Interestingly, CB₂ receptors were expressed in neural progenitor cells, and cannabinoid
agonists stimulated progenitor proliferation (15), whereas the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) promoted the growth of primary murine marrow progenitor cells (16, 17). Further, CB2 was reported to control myeloid progenitor trafficking (18, 19), and we recently reported the role of cannabinoid receptors during hematopoietic differentiation of murine embryonic stem cells (20).

Hematopoiesis is a tightly regulated life-long process, in which HSPCs differentiate into all mature blood cells through the production of progenitor cells (21, 22). Hematopoiesis is regulated in the bone marrow through the microenvironment, cytokines, cellular interactions, transcription and metabolic factors (21). HSPCs move from their main site of production in the bone marrow (BM) when stimulated with cytokines such as granulocyte colony-stimulating factor (G-CSF) and other agents (23, 24). The molecular mechanisms that contribute to the release of HSPCs, from the marrow and their trafficking in the circulation are not completely understood. The VLA4-VCAM-1 pathway and the CXCR4-CXCL12 axis play important roles in adhesion of HSCs, neutrophils and B cells to the BM (26, 27, 35). Recently, it was also been shown that HSCs depend on Gαs-mediated signaling for HSCs BM engraftment (28) and prostaglandin E2 enhanced hematopoietic stem cell homing, survival and proliferation (29).

Here, we examined the expression of endocannabinoids in the BM niche and the expression and function of CB2 cannabinoid receptors in human and murine HSPCs. This study demonstrates that the CB2 / CB2 agonist axis is involved in hematopoiesis and HSPC mobilization. Thus, pharmacological intervention targeted towards the endocannabinoid system can be a novel modality for HSPCs based therapies in patients.
Material and Methods

Mice

C57BL/6J and SJL-Ptprca Pep3b/BoyJ mice were purchased from Jackson Laboratories (Bar Harbor, ME, USA) at 8 weeks of age and were used in the experiments at between 12 and 13 weeks of age. Cnr1−/− and Cnr2−/− knockout mice were a generous gift from Dr. S. K. Dey (30). All animal experiments were approved by the BIDMC Institutional Animal Care and Use Committee. Cnr1−/− and Cnr2−/− mice were in the background of C57BL6/129 as described previously (31, 32). The control animals for experiments involving cannabinoid receptor knockout mice were generated by interbreeding between Cnr1−/− and Cnr2−/− strains, then breeding between heterozygous Cnr1+/− and Cnr2+/− offspring, and then selecting the wild-type (i.e. Cnr1+/+ and Cnr2+/+) animals for further breeding as controls.

Antibodies, chemical and biological compounds

Rabbit polyclonal anti-CB2 antibodies (ABR-Affinity BioReagents, Golden, CO) were used for immunofluorescent staining. For Western blot analysis a rabbit polyclonal anti-CB2 antibody was purchased from Cayman Chemical Company (Ann Arbor, MI). Anti-actin antibody was obtained from Chemicon (Temecula, CA). The human CD34 and CD133 isolation kits were products of Miltenyi Biotec (Auburn, CA). PE-conjugated anti-CD34 antibody was from Pharmingen (San Diego, CA). The cannabinoid ligands JWH133 and CP55940 were obtained from Sigma. AM1241 cannabinoid ligand was synthesized in Center for Drug Discovery and Department of Chemistry and Chemical Biology, Northeastern University, Boston. The cannabinoid CB2
receptor antagonist AM630 and the CB$_1$ receptor antagonist AM251 were purchased from Tocris (Ellisville, MO). G-CSF (Neupogen) was obtained from Amgen Inc. (Thousand Oaks, CA).

**Cells**

Mononuclear cells (MNCs) from human bone marrow and human G-CSF-mobilized blood were obtained from Cambrex Corp. (East Rutherford, New Jersey). Human CD34-positive cells were isolated from mononuclear cells derived from human bone marrow (Cambrex) by using the CD34 immunomagnetic bead separation method of the mini-MACS system following the manufacturer’s guidelines (Miltenyi Biotec). Isolated CD34$^+$ cells derived from mobilized blood were purchased from Cambrex.

**Chemotaxis of human CD34$^+$ cells and murine Lin$^-$Sca-1$^-$c-kit$^+$ cells**

CD34$^+$ cells ($1 \times 10^4$), murine BM cells ($1 \times 10^5$) or LSK ($1 \times 10^3$) in DMEM$^+$ media were placed onto the filters of the Transwell using a 5-$\mu$m thick polyvinylpyrrolidone-free poly-carbonate filters. Cells were seeded in the upper wells while medium containing cannabinoid agonists were added to the lower wells: CP55940 (10 nM), JWH133 (10nM) CXCL12 (100 ng/ml) or AM1241 (10 nM). Agonist, antagonist and inhibitors were added to the upper chambers as indicated. Cells were allowed to migrate for 4 h at 37$^\circ$C in a humidified atmosphere with 5% CO$_2$. Filters were then removed from the chambers and stained with Diff. Quick (Baxter.) Each condition was performed in triplicate.

**Mobilization of bone marrow HSPCs**

For *in vivo* mobilization experiments, mice received: G-CSF (125 $\mu$g/kg) i.p. twice daily (morning and evening) for 4 consecutive days. The cannabinoid ligands were dissolved in
ethanol/cremophor/saline (1:1:18) vehicle and were administered i.p. once daily (in the morning) for four consecutive days: CP55940, JWH133, AM1241 (all ligands at a dose of 10 mg/kg) either alone or in the presence of cannabinoid receptor CB₂ antagonist AM630 (5 mg/kg), as indicated. Twenty-four hours following the last injection, mice were sacrificed; dissected and peripheral blood was collected by heart puncture. Control mice received i.p. injections of vehicle alone once daily for 4 days, (administered in the morning), and twenty-four hours following the last injection, mice were sacrificed, dissected, and peripheral blood was collected by heart puncture. Effects due to circadian regulation were controlled by restricting experimental procedures to morning schedule. White blood cells (WBC) (0.6 ml from each mice) were isolated by Ficoll separation. Briefly, blood was diluted to 6 ml and was overlaid on the 5 ml Ficoll layer. Tubes were centrifuged at 2000 rpm at 18°C for 30 min. The top layer was removed slowly with a Pasteur pipette and the WBC layer was transferred into 8-9 ml DPBS⁺ (DPBS containing 2% FBS) in a 15 ml conical tube. Tubes were spun at 1500 rpm at 4°C for 5 to 7 min and then the pellet was resuspended in DPBS⁺. The PBCs cells were collected and used for the colony-formation assays using MethoCult media, as detailed below.

**Colony Formation assays**

PBCs (1x 10⁵ cells/ml) were cultured in MethoCult GF M3434 (Stem Cell Technologies, Vancouver, BC, Canada) containing a cocktail of cytokines to enumerate colony-forming unit granulocyte-macrophage (CFU-GM), CFU-GEMM(granulocyte-erythrocyte-monocyte-megakaryocyte), CFU-E (erythrocyte), and burst forming unit erythrocyte (BFU-E). Cultures were
incubated for 10-14 days at 37°C with 5% CO₂ and 37°C O₂ in a fully humidified incubator. Total colonies per ml blood were determined by multiplying the CFU frequencies by the number of low-density cells per ml blood. Triplicate assays were performed for each sample. Following the incubation period, the numbers of colonies were determined by light microscopy. Positive colonies were scored on the basis of an accumulation of 40 or more cells. For human colony cultures, we employed MethoCult methylcellulose based assays (Stem Cell Technologies), based on the protocol provided by the company.

Murine transplantation assays

Transplantation assays were performed using the Ly5 congenic mouse system. Nine mice were included in each experimental group. 8 × 10⁵ mobilized cells from B6-Ly5.2 mice were mixed with 2 × 10⁵ BM cells from B6-Ly5.1 and were transplanted into lethally irradiated B6-Ly5.1 (950 rads) mice. Long-term engraftment was determined 20 weeks after transplantation by analyzing peripheral blood and bone marrow by flow cytometry. Cells were stained with a mixture of biothynylated anti-Ly5.1, allophycocyanin (APC)-conjugated anti-Ly5.2, FITC-conjugated anti-CD4, FITC-conjugated anti-CD8, PE-conjugated anti-Gr1, PE-conjugated anti-mac1, and APC-Cy7-conjugated anti-B220 (BD Bioscience). Secondary staining was performed using PE-Cy7-streptavidine. Flow cytometry analysis was performed on a BD LSR II cell analyzer (BD Bioscience), and data was analyzed by FlowJo 7.2.4 software.
Statistical analysis

The results are presented as the mean ± SD. The statistical significance of the results reported hereby was determined by a two-tailed t test. $P < 0.01$ and $P < 0.05$ were considered significant, as indicated.

Results

Expression of endocannabinoids in bone marrow-stromal cells

To study the role of the endocannabinoid system in bone marrow (BM)-stromal cells, we examined the expression of endocannabinoids, 2-AG and AEA in BM-stromal cells. As shown in Table 1, both 2-AG and AEA were detected with AEA at 35.2pg/10^7 cells and 2-AG at 75.2ng/10^7 cells. The expression levels of AEA and 2-AG in BM stromal cells is similar to those reported in brain (37), a major organ for synthesis of endocannabinoids. In response to the stress inducer endotoxin (LPS), the expression levels of both 2-AG and AEA were increased (Table 1). Interestingly, LPS induced-mobilization of HSPCs, which was impaired in Cnr2^{-/-} mice (Table 2). Thus, these results suggest that BM stromal cells express endocannabinoids, which are upregulated following an immune challenge. Increased endocannabinoids may facilitate the release of HSPCs from the BM niches to the peripheral blood circulation for repopulation of hematopoiesis.

CB2 receptors are expressed on human and murine HSPCs

Next, we assess the expression of CB2 in human and murine HSPCs. Using human CB2 specific antibodies we analyzed the cell surface expression of cannabinoid receptors on human BM
mononuclear cells (MNC), and detected CB$_2$ in MNC (Fig.1A). We then determined the expression of cannabinoid receptors on CD34$^+$ and CD133$^+$ expressing cells. CB$_2$ was expressed in CD34$^+$ and CD133$^+$ cells as determined by RT-PCR analysis (Fig. 1B). In addition, we detected CB$_2$ protein in CD34$^+$ BM cells by Western blot analysis (Fig. 1C). We also examined the expression of CB$_2$ in CD34$^+$ cells isolated from human BM and from mobilized blood. CB$_2$ was expressed in most of the CD34$^+$ cells (Fig. 1A, 1D), indicating that CB$_2$ receptors are abundantly expressed in CD34$^+$ cells. CB$_2$ is also expressed in CD34$^+$ CD38$^-$ cells that contain the most primitive human HSPCs (Fig. 1E).

We then evaluated the expression of CB$_2$ in murine BM MNCs. 54.2% of total MNC expressed the CB$_2$ receptors (Fig. 2A). We assessed the expression of CB$_2$ in BM SP cells (side population) as well as LSK (Lin$^-$Sca-1$^+$c-Kit$^+$) cells, enriched for stem cell populations. Both SP cells and LSK cells expressed CB$_2$ receptor (Fig. 2B). RT-PCR analysis showed the expression of CB$_2$ in the whole bone marrow population and in FACS sorted LSK as well as in the enriched SLAM(CD150$^+$, CD48$^+$) LSK cells for primitive repopulating HSCs (Fig. 2C). Together, these analyses indicate that CB$_2$ receptors are expressed in HSPCs and in the more immature stem cell fractions.

**Cannabinoid ligands induce colony formation and migration of human and murine HSPCs in vitro**

To study the direct actions of cannabinoids on HSPCs, we first assessed the effects of various cannabinoid compounds on colony formation by human and murine bone marrow-derived cells in vitro. Treatment of human MNCs cells with CB$_2$-specific agonist JWH133 or with CP55940, a
ligand for both $\text{CB}_1$ and $\text{CB}_2$, yielded a significant increase in colony numbers as compared to control non-stimulated cells (Fig. 3A). Similarly, treatment of WT murine bone marrow cells with CP55940 or JWH133, yielded an increase in colony number (Fig. 3B). None of the cannabinoids had any effect on the colony formation of cells derived from the Cnr2$^{-/-}$ mice (Fig. 3B), indicating that stimulation of colony formation can only be transduced via the $\text{CB}_2$ receptor.

Next, we assessed the effect of cannabinoid ligands on the chemotaxis of human CD34$^+$ cells and murine LSK cells. Cannabinoid agonists induced a significant increase in the migration of human CD34$^+$ cells (Fig. 3C) and murine LSK cells (Fig. 3D). Cannabinoid-induced migration of bone marrow cells from Cnr2$^{-/-}$ mice was severely impaired, as compared to WT mice (Fig. 3E). These results demonstrate that $\text{CB}_2$ cannabinoid ligands induce migration of human CD34$^+$ cells and murine HSPC bone marrow cells via $\text{CB}_2$ receptors.

**Cannabinoids induce hematopoietic stem and progenitor cell mobilization in vivo**

Since endocannabinoids induced mobilization of HSPCs and since $\text{CB}_2$ cannabinoids induced chemotaxis of human CD34$^+$ cells and murine bone marrow cells *in vitro*, this prompted us to examine their effects on the mobilization of HSPCs *in vivo*. For these studies, we employed a mouse model of HSPCs mobilization from the bone marrow to the circulation, in which the number of circulating HSPCs in blood is assessed by the number of colonies formed in semi-solid medium. We did not observe any significant changes in total numbers of WBC in peripheral blood from mice following treatment with cannabinoid compounds, (Supplemental Table 1). Treatment of WT mice with CP55940 for up to 4 hours resulted in modest increase in the number of hematopoietic colonies (Supplemental Figure 1), indicating that short term
mobilization by cannabinoids is not effective. Regarding response timeline, the short term exposure of HSPCs to CB2 agonists produced similar, although less pronounced effects as those observed with AMD3100 (for 2-3 hrs only) (data not shown). Further, no significant effects on HSPC mobilization was observed when G-CSF was administered twice daily for 4 days followed by CB2 agonist administration at day 5 for 2 hr, as compared to G-CSF alone (data not shown).

Treatment of WT mice with either JWH133 (CB2 specific) or CP55940 daily for 4 days resulted in a significant \( P < 0.01 \) increase in the number of hematopoietic colonies in peripheral blood (Fig. 4A), suggesting that HSPC mobilization by cannabinoids can be induced via CB2 receptors. Knockout of CB2 \((Cnr2^{--})\) significantly \( P < 0.05 \) reduced the effects of CP55940 (Fig. 4B), and these effects were nearly abolished in the \(Cnr2^{--}\) mice. HSPCs mobilized by CB2 cannabinoid agonists yielded CFU-GM, CFU-GEMM as well as BFU-E types of colonies \textit{in vitro}, similar to those obtained with G-CSF suggesting that cannabinoids can mobilize multipotent progenitor cells from the bone marrow (Supplemental Table 2).

**CB2 inhibition impaired G-CSF induced mobilization of HSPCs**

Next, we investigated whether CB2 could modulate G-CSF-induced mobilization. In a model involving G-CSF, a significant reduction in the number of G-CSF-induced HSPC-derived colonies was observed following the administration of CB2 (AM630) receptor antagonists (Fig. 4C). Similarly, \(Cnr2^{--}\) mice showed a significant reduction of HSPCs-derived colonies upon G-CSF treatment. Since the effects in the knockout mice may not represent the full assessment due to potential compensation by the other cannabinoid receptor, we treated \(Cnr2^{--}\) knockout mouse with the CB1 receptor antagonist for the other cannabinoid receptor. In \(Cnr2^{--}\) mice
treated with G-CSF plus AM251 (CB₁ antagonist), the HSC mobilization was markedly reduced as compared to the G-CSF only treated mice (Fig. 4D).

The Cannabinoid receptor CB₂ agonist AM1241 induces mobilization of LT-HSC in vivo

To assess if stimulation of CB₂ by AM1241 is capable of mobilizing early progenitors as well as the long-term (LT) repopulating cells, we used the CAFC assay. First we determined that 10 mg/kg of AM1241 as the optimal dose for mobilization of HSPCs in vivo (Fig. 5A). Administration of AM1241 induced mobilization of both progenitors as well as HSC with potency comparable to G-CSF as determined by CAFC assays (data not shown). Furthermore, mice treated with AM1241 during 4 days showed a significant reduction in the number of LT-HSCs, defined as lin⁻c-kit⁺Sca-1⁺CD48⁻ and CD150⁺, (38) in bone marrow (vehicle = 2398 ± 147 versus AM1241 = 1670 ± 284, \( P = 0.029 \)), suggesting a potential mobilization of these cells.

To investigate whether AM1241-mobilized cells contain LT-HSCs capable to engraft and reconstitute lethally irradiated recipient mice, a competitive repopulating assay was carried out, in which vehicle-, G-CSF- or AM1421-mobilized test cells were mix with bone marrow competitor cells and transplanted into lethally irradiated mice (Fig. 5B and Methods for details). 20 weeks after transplantation, recipient mice were sacrificed and the BM and blood were analyzed for the presence of Ly5.2 positive cells. BM analysis showed that the LT engraftment of the AM1241-mobilized cells was significantly higher than the engraftment of the vehicle-mobilized cells (26.83% versus 0.36%, \( P < 0.01 \)) (Table S3). To investigate whether the engrafted cells retained stem cell properties, we analyzed their contribution into the formation of B-, T-, and myeloid cells. Mice transplanted with AM1241-mobilized cells showed Ly5.2
derived B220, CD8, CD4, mac1 and Gr1 positive cells, suggesting that the AM1241-mobilized cells were capable of giving tri-lineage reconstitution (Fig. 5C and Table S3). None of the vehicle-mobilized transplanted mice reconstituted all three lineages with Ly5.2 cells. Interestingly, the effect of AM1241 in HSC mobilization, engraftment and reconstitution was similar to that observed by G-CSF (Fig. 5C and Table S3). In conclusion, our data strongly suggest that the cannabinoid agonist AM1241 induces mobilization of LT-HSCs, which retain transplantation characteristics.

**Cannabinoids induce HSPC migration and mobilization via and G\(\alpha\)i-Rac1 pathways**

To elucidate the signaling pathways by which the CB\(_2\) agonists can induce HSPC migration and mobilization, we performed chemotaxis assays. First, we demonstrated that CP55940 and AM1241 induced a significant increase in the migration of human CD34\(^+\) cells and murine LSK cells (Figs. 3C-D, and 6A). Next, we examined the effects of specific inhibitors on AM1421-induced migration. As shown in Fig. 6A, AM1241- induced significant migration of HSPCs, which was inhibited by the CB\(_2\) specific antagonist AM630 and by the inhibitors LY294002 (inhibitor for PI3K), U0126 (inhibitor for ERK) and PTX (G\(\alpha\)i inhibitor). Further, AM1241- induced the activation of both Erk and AKT, an upstream target of PI3-kinase (data not shown). These results suggest that CB\(_2\)/AM1241- induced migration of HSPCs is dependent on PI3-Kinase, Erk and G\(\alpha\)i pathways. Since Rac1 was shown to play a central role in the regulation of the actin-based cytoskeleton and cell movement (41, 42), and Rac1 activation is mediated by G\(\alpha\)i protein, we therefore examined Rac1 activity in human MNC. As shown in Fig.
6B, AM1241 induced Rac1 activation, which was inhibited by PTX inhibitor. Thus, based on these results, AM1241-induced HSPC migration via G\(\alpha_i\)-Rac1 pathway.

To further address the mechanism by which cannabinoids induce mobilization of HSPCs, we examined the effects of AM1241 on CXCL12-mediated migration of HSPCs. We observed that CXCL12-induced migration of CD34\(^+\) cells (Fig. 6C) and LSK cells (Fig. 6D) was inhibited by CP55940, suggesting that cannabinoid stimulation may interfere with the CXCL12-induced migration. We also assessed the combinatory effects of AM1241 and AMD3100 (a specific inhibitor of CXCR4) in HSPC mobilization \textit{in vivo}. As shown in Fig. 6E, the combination of AM1241+AMD3100 significantly enhanced HSPC mobilization, suggesting a “cross-talk” between CB\(_2\) and CXCR4. However, when we assessed if CB\(_2\) agonist stimulation resulted in changes in CXCR4 expression in HSCs, no significant differences were observed in CXCR4 expression in HSCs mobilized by CB\(_2\) agonist (data not shown).

Next, we evaluated whether AM1241 competed with SDF1\(\alpha\) for binding to CXCR4, following simultaneous stimulation, by a live cell-binding assay (43). To quantify the affinity and \(B_{\text{max}}\) of SDF1\(\alpha\) for CXCR4 on the surface of living cells a competition assay was used using MDA-MB-231 cells known to express CXCR4. SDF1\(\alpha\) and AM1241 were radiolabel, \([^{99}\text{Tc-MAS3}]-\text{SDF1\(\alpha\)}\) and \([^{99}\text{Tc-MAS3}]-\text{AM1241}\), respectively; we used 293T human embryonic kidney cells as a control, as they do not express CXCR4. \([^{99}\text{Tc-MAS3}]-\text{SDF1\(\alpha\)}\) exhibited a high specificity for CXCR4, an affinity of \(K_D= 2.4\pm 0.2\text{nM (mean \pm SD)}\), and a \(B_{\text{max}}\) of \(1.21 \times 10^4\) binding sites per cell. The affinity of AM1241 for CXCR4 was non-specific, with an affinity of \(K_D= 16.3\pm 1.4\text{nM (mean \pm SD)}\), and a \(B_{\text{max}}\) of \(1.21 \times 10^4\) binding sites per cell (Fig.
The measured affinities were consistent with previously published values for SDF1α for CXCR4 (44). We then assessed the affinity and specificity of \([^{99}\text{mTc}-\text{MAS3}]-\text{SDF1}\alpha\), simultaneously with unlabeled AM1241, for CXCR4 on the surface of living cells. The affinity of SDF1α for CXCR4 in the presence of AM1241 was \(K_D= 3.01\pm1.02\text{nM}\), and a \(B_{\text{max}}\) of \(1.36 \times 10^4\) (Fig. 6G). Thus, CB2 did not simultaneously compete with SDF1α for CXCR4 binding and therefore the “cross-talk” of CB2 with CXCR4 pathway is not on the level of the receptor or receptor-ligand.

**Discussion**

The physiological balance between self-renewal and differentiation is essential for HSC function and hematopoiesis. In this report, we provide evidence that endocannabinoids are expressed in BM stromal cells (Table1), while CB2 receptors are expressed on human and murine HSPCs (Figures 1+2). CB2, upon binding to its cannabinoid agonists, play a critical role in hematopoiesis and mobilization of HSPCs, through ERK/PI3-kinase and G\(\alpha_i\)-Rac1 pathways (Figures 4-6). Thus, we proposed that cannabinoids are novel molecules for the mobilization of HSPCs \textit{in vivo}.

The endocannabinoid system participates in neuroendocrine control of homeostasis (6). Endocannabinoids are involved in the innate immune response and in homeostasis maintenance (5). Further, the anti-inflammatory actions of endocannabinoids represent a pivotal protective mechanism both in acute and chronic disorders such as in neurodegenerative diseases (7-8). Marked increase of endocannabinoid production (AEA and 2-AG) was reported in various
tissues (myocardial, cerebral, hepatic and immune cells such as platelets and activated macrophages) (45), which correlated with the degree of tissue injury and inflammation. Here we show that LPS as an endotoxin stimuli and immune challenge (46) can increase endocannabinoid levels in BM stromal cells (Table 2), resulting in mobilization of HSPCs from the BM niche to the blood circulation for proper hematopoiesis. LPS as an inflammatory stimuli enhanced endocannabinoid levels, mediated by cytokines including TNF-α (46). Therefore, BM stromal cells may represent a very significant source of endocannabinoids produced in various pathological conditions associated with increased inflammation, in addition to the previously reported activated macrophages (4).

Understanding of the signals that regulate HSPCs development and the intrinsic and extrinsic mechanisms that are involved in maintenance of HSC in the bone marrow niches are crucial for proper hematopoiesis. Hematopoiesis is a life long process in which HSPCs differentiate into mature blood cells. These HSPCs are valuable in a clinical setting for patients requiring hematopoietic repair (21). The current treatment involves hematopoietic stem cell transplantation with HSPCs obtained from mobilized peripheral blood or umbilical cord blood. Repopulation of hematopoiesis is a multistep process that is regulated by the ability of HSPCs to migrate, home to the appropriate marrow niches and differentiate to mature blood cells. Hence, insights into the physiological stimuli as well as external signals that induce HSPC exist from the bone marrow and traffic to peripheral blood is important for proper hematopoiesis repair. In this regard, we provide new evidence on the involvement of the endocannabinoid system in hematopoiesis by inducing migration and mobilization of HSPCs from the BM niches to the
blood circulation following exposure to stress inducer such as LPS, or to exogenous cannabinoid agonists. The migration of HSPCs to the peripheral circulation may limit tissue damage and contribute to hematopoietic repair. Physiological levels of endocannabinoids may regulate hematopoietic homeostasis in the BM by maintaining important HSPC functions such as survival and retaining HSCs in the BM stromal niches. However, following stress-induced inflammation there is increased in endocannabinoids levels, which may facilitate the release of HSPCs from their niches to the peripheral blood circulation. The mechanisms for endocannabinoids mediated mobilization of HSPCs could be due to either changes in the expression and secretion of inflammatory cytokines in the BM niches, or via activation of CXCR4 signaling and/or changes in the interactions of HSPCs with BM-stroma niches via integrins.

Endocannabinoids have been reported as positive or negative factors in hematopoietic cell migration and differentiation (45, 47-50). Endocannabinoids were shown to directly modulate hematopoietic cell migration and differentiation as noted by increased of CFU-GEMM. Further, they play important role in endotoxic shock and inflammation (14). The level of AEA in brain was reported to be 35 ± 8 pmol/g and 2-AG levels were in brain was reported to be 62 ± 1.8 nmol/g as compared to the expression in blood of AEA (2.5 ± 0.7 pmol/g) and 2-AG (10^{-3} nmol/g) (37). Here, we report that 2-AG and AEA are found in similar levels to those reported in brain. The presence of endogenous cannabinoids in immune cells, hematopoietic cells and BM niches suggest that they play a critical physiological role in hematopoietic system and immunoregulation, although the precise nature of which remains to be characterized. Increased elevated levels of AEA and 2-AG may further protect HSPCs from endotoxic shock and
apoptosis and induce their migration from the BM niches to the peripheral blood circulation following insult, by untethering HSPCs from the BM niches, and facilitating their trafficking to the peripheral blood circulation.

The cannabinoid receptors are seven transmembrane G protein-coupled receptors (GPCR). Cannabinoid receptors specifically bind to the subtype G\(\alpha\) \(\beta\gamma\) proteins, characterized for inhibiting adenyl cyclase activity and reducing cAMP levels upon activation (47-50). Binding of 2-AG to CB\(_2\) stimulated Ca\(^{2+}\) transients and activated ERK-MAPK (47-48). Here, we observed that AM1241-induced cell migration of HSPC was dependent on the ERK/PI3-kinase and on G\(\alpha\)–Rac 1 pathways (Fig. 6), indicating that CB\(_2\) promoted HSPC migration via activation of ERK/PI3-Kinase/Rac1, and leading to the changes in actin dynamics that facilitate the changes necessary for HSPC motility.

HSCs move from their main site of production in the bone marrow when stimulated with cytokines such as G-CSF or after myelosuppression with chemotherapy. Application of G-CSF to induce mobilization of HSCs is the main procedure currently used in clinical medicine (51). However, there is some degree of variability in the responsiveness of normal donors and patients to the HSC-mobilizing effects of G-CSF. Here, we demonstrate that inhibition of CB\(_2\) receptors resulted in inhibition of G-CSF-induced HSPC mobilization (Fig. 4C). Further, G-CSF-induced-mobilization of HSPCs was significantly decreased in Cnr2\(^{-/-}\) mice (Fig. 4D). These observations suggest that the CB\(_2\) expression and activation can affect the G-CSF-response obtained in donors/patients. G-CSF mobilized peripheral blood stem cells are used to reconstitute hematopoiesis. Understanding the mechanisms of G-CSF-induced
mobilization of HSPCs should provide new regimens for enhanced engraftment capabilities and expanding the utility of hematopoietic transplantation. The mechanism by which CB$_2$ receptors modulate G-CSF-induced HSPC is unclear. CB$_2$ may modulate G-CSF induced mobilization of HSPCs via chemokines action, and/or enhancing CXCR4 signaling or by modulation of integrins. Further studies are required to better understand the mechanisms by which CB$_2$ affects G-CSF mobilization and the potential link of G-CSF to the endocannabinoid system.

Mobilization of HSPCs is a complex and incompletely understood process (24). We observed inhibition of CXCL12-induced chemotaxis of human CD34$^+$ cells and murine HSPCs by cannabinoid agonists (Fig. 6), in agreement with previous publication (11). As the CXCL12/CXCR4 axis is responsible for the retention of HSPCs in the bone marrow niches (35), the effects of cannabinoids on HSPC mobilization can be partially explained by activities of the cannabinoid system through activation of common downstream targets of CXCL12/CXCR4 axis that results in inhibition of CXCR4 signaling and/or through heterologous desensitization between CB$_2$ and CXCR4 receptors. 

In vivo, AM1241 augmented the mobilization of HSPC induced by the antagonist of CXCR4, AMD3100 (Fig. 6). The modulation of CXCL12/CXCR4 axis by cannabinoids during HSPC migration and mobilization is mediated by activation of downstream targets (Fig.6) and not through regulation of CXCR4 expression level on HSPCs (data not shown).

The current study implicates CB$_2$ and CB$_2$ agonists in stem cell function, by inducing HSPC-migration, mobilization and engraftment for repopulation of hematopoiesis. The CB$_2$ agonist AM1241 has direct and stable effects on long-term repopulating HSPCs as determined
by HSPC transplantation (Fig.5). CB₂ agonist AM1241 induced HSPC frequency similar to those obtained with G-CSF in a 20 week period (Fig. 5). CB₂ receptors participate in the control proliferation and differentiation for cell fate decisions (53-56). CB₂ receptor activation controls proliferation and differentiation in neural cells (18), and B-cells (from virgin B-cells to centroblasts (55). CB₂ receptor activation and overexpression has been reported to block neutrophil cell differentiation (12). Changes in CB₂ expression were reported to control myeloid progenitor trafficking (18). However, we have not observed changes in CB₂ expression level in mobilized HSPC. Thus, the effects of CB₂ on hematopoiesis are probably mediated via the interaction of CB₂ with its ligands (exogenous cannabinoids and endocannabinoids), and their level of expression can be modulated during stress-induced inflammation or insult.

Taken together, our findings support a novel physiological role for the cannabinoid system hematopoietic homeostasis. Accordingly, while it was recently shown that CB₂ mediates the retention of immature B cells in bone marrow sinusoids (56), our results suggest that physiological levels of endocannabinoids are important for retention of HSPC in the BM niches. However, under stress situations, when endocannabinoid expression is elevated or following exposure to exogenous CB₂ agonists, HSPCs are then release from their BM-niches. Our studies have also important implication of the endocannabinoid system in HSC homing and engraftment of HSCs in the bone marrow. Although a very large number of HSPCs are used in the clinical procedure, the homing efficiency of HSPC following stem cell transplantation is low. Recently, Goαs was found to govern specific aspects of HSC localization and engraftment to the BM (28). In addition, Prostaglandin E2-Prostaglandin receptors and adrenergic receptors were also
reported to enhance stem cell transplantation in mice (29). Here, we show that the CB2 receptor, a component of the endocannabinoid system, and its specific agonists are also important in stem cell transplantation (Fig.5). Thus, identification of additional physiological systems that can increase homing efficiency and/or increase the nurturing capacity of the niche or increase the number of cells that can be mobilized for in vivo transplantation is crucial for HSPCs based therapies. Pharmacological intervention targeted towards the endocannabinoid system could represent a novel modality for HSPC-based therapies in patients.

**Acknowledgement**

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**Authorship Contributions**

Shuxian Jiang: Conception and design, collection and assembly of data, data analysis and interpretation, final approval of manuscript.

Meritxell Alberich Jorda: Conception and design, collection and assembly of data, data analysis and interpretation, final approval of manuscript.

Radoslaw Zagozdzon: Conception and design, collection and assembly of data, data analysis and interpretation, final approval of manuscript.

Kalindi Parmar: Collection and/or assembly of data, data analysis and interpretation, final approval of manuscript.

Peter Mauch: Data analysis and interpretation, final approval of manuscript.

Naheed Banu: Collection and/or assembly of data, final approval of manuscript.

Yigong Fu: Data analysis and interpretation, final approval of manuscript.

Alexandros Makriyannis: Data analysis and interpretation, final approval of manuscript.

Daniel G. Tenen: Data analysis and interpretation, final approval of manuscript.

Shalom Avraham: Data analysis and interpretation, final approval of manuscript.

Jerome E. Groopman: Data analysis and interpretation, financial support, final approval of manuscript.

Hava Karsenty Avraham: Conception and design, financial support, administrative support, provision of study material or patients, Collection and/or assembly of data, data analysis and interpretation, final approval of manuscript.
Conflict of Interest Disclosures

The authors declare that have no competing interests.

Abbreviations:

2-AG: 2-arachyldonylglycerol
AEA: Anandamide
CB1: Cannabinoid receptor 1
CB2: Cannabinoid receptor 2
E CBS: Endocannabinoids
FAAH: Fatty acid amide hydrolase
HSPC: Hematopoietic stem and progenitor cells
HSC: Hematopoietic stem cells
LPS: Lipopolysaccharide
MNCs: Mononuclear cells
MGL: Monoacylglycerol lipase
Cnr1−/−: Cannabinoid receptor type 1 knockout mice
Cnr2−/−: Cannabinoid receptor type 2 knockout mice
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marrow-repopulating and spleen colony-forming hematopoietic stem cells in the mouse. 


<table>
<thead>
<tr>
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<th>AEA (pg/10^7 cells)</th>
<th>2-AG (ng/10^7 cells)</th>
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<tbody>
<tr>
<td>BM stroma cells</td>
<td>35.2</td>
<td>75.2</td>
</tr>
<tr>
<td>BM stroma cells/LPS</td>
<td>75.6**</td>
<td>98.8 *</td>
</tr>
</tbody>
</table>

**Table 1:** Endocannabinoid levels determined for murine-stroma cells, either untreated or exposed to LPS. Note differences in units (pg vs ng).

* p< 0.05 as compared to BM stroma cells for AEA.

** p< 0.001 as compared to BM stroma cells for 2-AG.

<table>
<thead>
<tr>
<th>PB Progenitors</th>
<th>WT</th>
<th>Cnr2^-</th>
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<tbody>
<tr>
<td>Vehicle</td>
<td>5 ± 1</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>LPS</td>
<td>40 ± 7 *</td>
<td>18 ± 4 **</td>
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**Table 2:** Induction of HSPC mobilization in wild-type (WT) versus Cnr2^- mice following LPS administration. LPS was injected i.p. at a dose of 2μg/kg. Mice were sacrificed five days after injection of LPS and the incidence of PB-HSPC was evaluated using colony-formation assay as described in the Methods section. These are representative experiments out of duplicate experiments involving 5 mice per treatment group. *P<0.01 versus control; **P<0.05 versus LPS alone;
Figure Legends

Figure 1: Expression of cannabinoid receptors CB$_2$ in human bone marrow HSPC by flow cytometry.

A) Analysis of the surface expression of CB$_2$ in human bone marrow (BM) mononuclear cells and CD34$^+$ cells isolated from human bone marrow. This is a representative experiment out of 5 experiments.

B) Analysis of CB$_2$ mRNA expression in bone marrow mononuclear cells (MNCs) as compared to isolated CD34$^-$, CD34$^+$, CD133$^-$ or CD133$^+$ cells. RNA was isolated from the indicated populations of bone marrow cells and the expression of cannabinoid receptor mRNA was evaluated using the RT-PCR technique. GAPDH was used as an internal control.

C) Western blot analysis of CB$_2$ expression in CD34$^+$ cells derived from human bone marrow. Jurkat cell line was used as a positive control of CB$_2$ expression.

D) Analysis of surface expression of CB$_2$ in total peripheral blood mononuclear cells and CD34$^+$ cells isolated from G-CSF-mobilized peripheral blood. This is a representative experiment out of 5 experiments.

E) Expression of CB2 in human CD34$^+$CD38$^-$ cells: A representative FACS profile of the CB$_2$ surface receptor expression in human bone marrow CD34$^+$CD38$^-$ cells is shown.

Figure 2: Expression of CB$_2$ in murine HSPCs
A) Expression of CB2 in mouse bone marrow cells. The surface expression of cannabinoid receptors was analyzed in total cells, non-SP (side population) cells and SP cells. This is a representative experiment out of 5 experiments.

B) Expression of CB2 receptors on mouse Lin−Sca-1−c-Kit+ (LSK) cells. Total bone marrow cells were gated for lineage negative cells (Lin-), followed by selection of c-kit/sca1 double positive cells. CB2 expression was determined in the LSK population. This is a representative experiment out of 4 experiments.

C) Analysis of CB2 mRNA expression in bone marrow mononuclear cells (MNCs) as compared to isolated Lin− c-kit+ and Sca1+ cells (LSK) and SLAM (LSK CD150+ CD48−) cells. RNA was isolated from the indicated murine populations of bone marrow cells and the expression of cannabinoid receptor mRNA was evaluated using the RT-PCR technique. Actin was used as an internal control.

**Figure 3: Colony formation and migration of human and murine hematopoietic stem and progenitor cells upon cannabinoid ligand stimulation**

A) Effects of cannabinoid ligands on the *in vitro* colony formation by human hematopoietic progenitor cells. $1 \times 10^4$ of human MNCs were seeded onto Petri dishes with or without the addition of cannabinoid ligands, as indicated. On Day 10, colonies were counted under a light microscope. *P < 0.05. Values are means ± s.d. (N = 3).

B) Effects of cannabinoid ligands on the *in vitro* colony formation by mouse hematopoietic progenitor cells. $1 \times 10^4$ WT or Cnr2−/− BM cells were seeded onto Petri dishes suspended in colony formation-supporting medium with or without the addition of cannabinoid ligands, as
indicated. On Day 10, colonies were counted under a light microscope. *$P < 0.01$. Values are means ± s.d. ($N = 6$).

C) Human bone marrow CD34+ cells were exposed to CP55940 or JWH133 in a transwell assay. Y-axis shows percentage of migration from an input of $1 \times 10^4$ human CD34+ cells. Values are means ± s.d. ($N = 6$). *$P < 0.01$

D) Induction of chemotaxis of murine LSK cells by CP55940. Transwell inserts were used to evaluate the migration of LSK cells in distinct CP55940 concentrations. Cells were allowed to migrate for 4 hours and cells in the bottom chambers were then counted under a light microscope. The data are the mean ± s.d. ($N = 9$). *$P < 0.001$.

E) Murine wt or Cnr2−/− bone marrow cells were added to the upper chamber in a migration assay. Cells were exposed to CP55940 and AM1241, present in the lower well, and migrated cells were counted. Y-axis represents percentage of migration from an input of $1 \times 10^5$ total murine BM cells. Values are means ± s.d. ($N = 6$). *$P < 0.01$ as compared to control untreated mice. **$P < 0.01$ as compared to WT treated with the cannabinoid agonist, as indicated.

**Figure 4: Effects of cannabinoids on mobilization of hematopoietic stem and progenitor cells in C57BL/6J mice.**

A) Effects of intraperitoneally (i.p.) injections of cannabinoid agonists (10 mg/kg, once daily, for 4 days) on the number of circulating PB-HSPC in WT mice. 24 hours following the last injection, peripheral blood (PB) was collected and an in-vitro colony formation assay was done. Y-axis indicates the number of colonies per $1 \times 10^5$ blood cells. The data are the mean ± s.d. ($N = 18$). *$P < 0.05$, **$P < 0.01$
B) Effect of CP55940 (as described above) on mobilization of wt and Cnr2<sup>−/−</sup> HSPC. *P < 0.05 versus control; **P < 0.05 versus WT. Values are means ± s.d. (N = 18).

C) Wild-type mice received i.p. injections of G-CSF twice daily for 4 consecutive days. Cannabinoid antagonists were injected i.p. into mice 30 min prior to each G-CSF injection. The antagonists AM630 (for CB2) was applied at a concentration of 5 mg/kg. Frequency of PB-HSPC was assessed as described above. Values are means ± s.d. (N = 12). *P < 0.01 versus control; **P < 0.05 versus G-CSF alone.

D) Effects of Cnr2<sup>−/−</sup> knockout on the G-CSF-induced mobilization of PB-HSPC. WT and Cnr2<sup>−/−</sup> mice received i.p. injections of G-CSF and cannabinoid antagonist as indicated. The data are the mean ± s.d. (N = 12). **P < 0.05 versus G-CSF in WT mice.

**Figure 5: Long-term engraftment of mobilized HSPC in vivo.**

A) Dose-dependent effects of AM1241 in HSPC mobilization. Mice were injected i.p. with AM1241 at the indicated doses once daily for 4 consecutive days. 24 hours following the last injection, mice were sacrificed, PB collected and MNC isolated and seeded (1 × 10<sup>5</sup> cells). 10 days following seeding colonies were counted. *P < 0.05 versus control (N = 9).

B) Schematic representation of the transplantation experiment. B6-Ly5.2 mice were treated with AM1241, G-CSF, or vehicle control (i.p. injection). 8 × 10<sup>5</sup> mobilized-PB MNC were mixed with 2 × 10<sup>5</sup> BM cells from B6-Ly5.1 mice and transplanted into lethally irradiated Ly5.1 mice.

C) Triple-lineage reconstitution by mobilized HSPC in vivo 20 weeks after transplantation. Contribution of the vehicle-, AM1241-, and G-CSF-mobilized cells (Ly5.2+) into the distinct lineages was determined by B220-APC-Cy7, CD4/CD8-FITC, and Gr1/Mac1-PE labeling and
flow cytometry analysis. The percentages of B-cells, T-cells, and myeloid cells are in relation to
the total MNC in the recipient mice. The figure represents one representative animal in each
group (N = 9 per group). See supplementary Table 3 for details.

Figure 6: Molecular mechanisms for AM1241/CB2-induced HSPC migration and
mobilization.

A) Effect of distinct inhibitors in AM1241-induced migration of human CD34+ cells. 1 × 10^4
cells were placed in the upper well of transwell inserts and AM1241 (1 μM) or vehicle control
were added to the bottom compartments. The system was then incubated for 4 h at 37°C and the
cells that migrated to the lower compartment were counted. As indicated, the following
inhibitors were added to CD34+ cells 45 minutes at 37°C in a CO₂ incubator before AM1241
treatment: 10 μM LY294002 (LY), 25 μM U0126 (U), 100 ng/ml of pertussis toxin (PTX) or 1
μM AM630. Results are presented as mean ± s.d. (N = 4). *P < 0.01 is statistically significant as
compared to control cells.

B) Effects of AM1241 on Rac1-GTPase activity. Human MNC were either untreated or
pretreated with 100 ng/ml of pertussis toxin (PTX) for 1 h followed by stimulation with AM1241
(1 μM) for 4 minutes. Rac1-GTP levels were then measured using Rac1-GTPase pull down
assay. The results are normalized to the density of total Rac1 band in the corresponding samples
as analyzed by GST pull down assay, followed by Western blot analysis (N = 3). *P < 0.01, as
compared to control untreated cells.

C-D) Effect of CP55940 on the CXCL12 (SDF-1) induced migration of CD34+ cells (C) and
murine LSK cells (D). Cells were placed in the upper chamber with or without 1 μM AMD3100
(CXCR4 antagonist) or 10 nM CP55940. 100 ng/ml CXCL12 were placed the lower chamber as indicated. Cells were allowed to migrate for 4 hours and then cells in the lower chamber were counted. Values are mean ± s.d ($N = 12$). $p<0.01$ as compared to control.

E) Synergistic effects of AM1241 and AMD3100 treatment. Mice were injected i.p. with AM1241 at a dose of 10 mg/kg once daily for four consecutive days. Twenty-four hours following the last injection of AM1241, mice were injected i.p. with AMD3100 at a dose of 5 mg/kg. One hour following injection of AMD3100 mice were sacrificed, their PB was collected and mononuclear cells were isolated and seeded ($1 \times 10^5$ cells per dish). Ten days following seeding, colonies were counted under a light microscope. The data are the mean ± s.d. ($N = 6$) *$P$ < 0.05 versus vehicle control, **$P$ < 0.05 versus each drug alone.

(F-G): The affinity binding of AM1241 and SDF-1 alpha to CXCR4. 293T cells and MDA-MB-231 cells were grown to a density of 75% in 96 well puncher plates. See methods for more details to assign absolute affinity of each ligand for CXCR4, a competitive displacement assay was employed as described (43-44). To avoid internalization of the radioligand due to constitutive endocytosis, live cell binding was performed at 4°C. Well contents were counted on a model 1470 Wallac Wizard (Perkin Elmer, Wellesley, MA) detector gamma counter.
Figure 2

A

Hoechst red vs. Hoechst blue for SP and Non-SP cells.

Total

Non-SP

SP

FSC vs. CB2-PE

44.8  54.2

44.8  54.1

48.6  49

0.48  0.46

0.53  0.61

1.63  0.81

B

Total BM cells gated for Lin-

Lin- cells gated for LSK

CB2 staining on LSK cells

FSC vs. C-Kit-APC

FSC vs. Sca-PE

FSC vs. CB2-FITC

5.95

8.48

14.1

C

Total BM

LSK

LSK/SAM

CB2

actin
Figure 3

A

**Total MNCs**

Number of human CFU (colonies per $1 \times 10^6$ cells)

![Bar graph showing the number of human CFUs in different conditions](image)

Control, CP55940, JWH133

B

**Number of murine CFU (colonies per $1 \times 10^5$ BM cells)**

![Bar graph showing the number of murine CFUs in different conditions](image)

Control, CP55940, JWH133

WT, Cnr2−/−

C

**% Migration of human CD34+ cells**

![Bar graph showing the % migration of human CD34+ cells](image)

Control, CP55940, JWH133

D

**Chemotaxis of LSK cells (total cells in 5 fields)**

![Bar graph showing chemotaxis of LSK cells](image)

CP55940 (nM)

E

**% Migration**

![Bar graph showing % migration](image)

- CP55940
- AM1241

WT, Cnr2−/−

*Significant difference
Figure 4

A

Number of colonies (per 1 x 10⁵ blood cells)

Vehicle  JWH133  CP55940

B

Number of colonies (per 1 x 10⁵ blood cells)

Vehicle  WT  Cnr2⁻/⁻  CP55940

C

Number of colonies (per 1 x 10⁵ blood cells)

Vehicle  G-CSF  G-CSF+/AM630

D

Number of colonies (per 1 x 10⁵ blood cells)

Vehicle  G-CSF  Vehicle  G-CSF  G-CSF/AM251

WT  Cnr2⁻/⁻
Figure 5

A

![Graph showing number of colonies (per 1x5 blood cells) vs. AM1241 (mg/kg)]

B

![Diagram of experiment design]

C

![Flow cytometry analysis]

Counts

Ly5.2 APC

Ly5.1 PE-Cy7

B220 APC-Cy7

CD4/CD8 FITC

Gr1/Mac1 PE

Counts

Counts

Counts

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Figure 6

A

Migration of CD34+ cells (per 5 fields, magnif. 100x)

B

GTP-bound Rac1 (fold/basal)

C

Number of CD34+ cells (per 5 fields, magnif. 100x)

D

Number of LSK cells (per 5 fields, magnif. 100x)

E

Mobilization of PB-HSPC (colonies per 1x 10^5 PB-WBC)

F

293T Cells expressing CXCR4

G

Counts (cpm)

**K_0 = 3.00 ± 1.02 nM**

B_max = 1.36 x 10^4
Cannabinoid receptor 2 and its agonists mediate hematopoiesis and hematopoietic stem and progenitor cell mobilization

Shuxian Jiang, Meritxell Alberich-Jorda, Radoslaw Zagozdzon, Kalindi Parmar, Yigong Fu, Peter Mauch, Naheed Banu, Alexandros Makriyannis, Daniel G. Tenen, Shalom Avraham, Jerome E. Groopman and Hava Karsenty Avraham