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Eicosanoid Regulation of Hematopoiesis and Hematopoietic Stem and Progenitor Trafficking

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Abstract

Hematopoietic stem cell (HSC) transplantation is a potentially curative treatment for numerous hematologic malignancies. The transplant procedure as performed today takes advantage of HSC trafficking; either egress of HSC from the bone marrow to the peripheral blood, i.e. mobilization, for acquisition of the hematopoietic graft, and/or trafficking of HSC from the peripheral blood to bone marrow niches in the recipient patient, i.e. HSC homing. Numerous studies, many of which are reviewed herein, have defined hematopoietic regulatory mechanisms mediated by the 20-carbon lipid family of eicosanoids, and recent evidence strongly supports a role for eicosanoids in regulation of hematopoietic trafficking, adding a new role whereby eicosanoids regulate hematopoiesis. Short-term exposure of HSC to the eicosanoid prostaglandin E_2 (PGE_2) increases CXCR4 receptor expression, migration and *in vivo* homing of HSC. In contrast, cannabinoids reduce hematopoietic progenitor cell (HPC) CXCR4 expression and induce HPC mobilization when administered *in vivo*. Leukotrienes have been shown to alter $CD34^+$ cell adhesion, migration, and regulate HSC proliferation, suggesting that eicosanoids have both opposing and complimentary roles in the regulation of hematopoiesis. Since numerous FDA approved compounds regulate eicosanoid signaling or biosynthesis, the utility of eicosanoid based therapeutic strategies to improve hematopoietic transplantation can be rapidly evaluated.

Keywords

Eicosanoid; homing; mobilization; stem cell; prostaglandin; cannabinoid; leukotriene

Introduction

Stem cell migration is a common feature of hematopoiesis, occurring both during development and throughout life. Hematopoietic stem (HSC) and progenitor (HPC) cells continuously traffic to and from their bone marrow niche (1), and the egress of hematopoietic cells from bone marrow into the periphery can be facilitated by various agents, such as cytokines or chemotherapy, a process termed mobilization (2, 3). Circulating HSCs can home to the bone marrow and lodge within specific microenvironmental niches that support their survival, self-renewal and differentiation, and only HSC homing to this bone marrow niche are able to sustain hematopoiesis long-term (4, 5). These trafficking processes form the basis of modern hematopoietic transplantation, which is routinely used to treat patients with leukemias, cancer, and hematologic and genetic diseases. Sources of HSC for transplant include bone marrow, mobilized peripheral blood (MPB) and umbilical cord blood (UCB). While bone marrow was the traditional source of HSC for transplantation,

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MPB has become the preferred source for hematopoietic grafts (6, 7), and the use of UCB for transplants is steadily increasing (8). Hematopoietic reconstitution after transplantation is a multi-step process, but with some sources of HSCs, efficacy is limited by inadequate HSC number, inability to migrate/home to marrow niches, and poor engrafting efficiency and self-renewal (9–11).

Considerable research, much of which is discussed in this issue of *Leukemia*, has focused on pathways governing HSC trafficking, with the goal of understanding and translating these findings to improve hematopoietic transplantation. Research strategies designed to increase HSC mobilization from bone marrow to peripheral blood in order to obtain an enhanced MPB product, or to enhance homing of HSC into the recipient bone marrow for enhanced hematopoietic engraftment are being evaluated in pre-clinical and clinical models. While cytokines, chemokine/chemokine receptor interactions and adhesion molecules have been the major targets of attention for improvement of HSC mobilization and homing, recent evidence has begun to elucidate the role of bioactive lipids as regulators of HSC migration. In particular, an emerging role for the 20-carbon lipid eicosanoids in hematopoietic trafficking suggests new avenues and therapeutic strategies for clinical improvements in hematopoietic transplantation. Here we review what is known about eicosanoids and regulation of hematopoiesis and hematopoietic cell trafficking, present novel effects of eicosanoids on cell trafficking, and discuss potential strategies for improvement in hematopoietic stem cell transplantation based on modulation of eicosanoid signaling and/or synthesis.

Eicosanoids

The eicosanoid family of lipids include the prostaglandins along with prostacyclins and thromboxanes, leukotrienes and endocannabinoids, most of which are formed by oxidation of 20-carbon essential fatty acids. Eicosanoids affect all organs, tissues, and cells (12). Prostaglandin E_2 (PGE_2) is the primary metabolite of arachidonic acid and the most abundant eicosanoid. PGE_2 is a known mediator of cancer, fever, inflammation, atherosclerosis, blood pressure and strokes, ovulation and numerous other physiological systems (reviewed in (13, 14).

All nucleated cells can synthesize prostaglandins (14), which occurs in 3 steps: cleavage of arachidonic acid from phospholipids by phospholipase A_2; oxidation by the cyclooxygenase enzymes (COX1 and COX2) forming the unstable intermediate PGG_2, with subsequent reduction to form PGH_2; and isomerization to mature prostaglandins by specific synthases (15–17) (Figure 1). Non-steroidal anti-inflammatory drugs (NSAIDs) have been developed that inhibit COX enzymes with selective specificities for COX1 and COX2 (18–20) and their primary therapeutic effect is due to reduced PGE_2 biosynthesis. Based upon its chemical/metabolic instability, PGE_2 is thought to act locally in autocrine or paracrine fashion (21). Within the bone marrow microenvironment, osteoblasts are a major source of PGE_2 (22–24) and due to their physical proximity to hematopoietic cells in the niche, are likely a primary source of PGE_2 for paracrine regulation of stem and progenitor function. Macrophages and monocytes also possess strong PGE_2 biosynthetic capacity and we have previously shown that monocyte/macrophage-derived PGE_2 plays a physiological role in hematopoiesis (25–27).

The leukotrienes are biosynthesized by oxygenation of arachidonic acid by 5'-Lipoxygenase (LOX) and conversion to the unstable intermediate leukotriene A_4 (LTA_4), which is enzymatically hydrolyzed to LTB_4 or conjugated to glutathione forming the cysteinyl leukotriene LTC_4. Leukotriene C_4 is subsequently converted to LTD_4 and LTE_4 (Figure 1). Leukotriene formation occurs predominantly in inflammatory cells, including granulocytes,

mast cells and macrophages, dendritic cells, and B lymphocytes (28), however 12'-LOX in platelets can convert LTA_4 produced by granulocytes to LTB_4 and lipoxins (29). LTB_4 is produced at sites of inflammation and stimulates inflammatory leukocyte function (30, 31). Like prostaglandins, the leukotrienes have a short half-life and are primarily involved in localized signaling.

The two main endocannabinoids, anandamide and 2-arachidonoyl glycerol (2-AG), are derivatives of arachidonic acid and are synthesized on demand (32–34). However, since they are structurally similar to arachidonic acid, they are also substrates for COX enzymes (35–37), resulting in alternative prostaglandins, with similar and novel effects (35, 38). In addition, alternative prostaglandins can be readily metabolized into traditional prostaglandins via esterases or by dehydration (38). Endocannabinoids can also be metabolized back to arachidonic acid through the action of fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL) (39) (Figure 1). Recycling of endogenous endocannabinoids for new endocannabinoid biosynthesis can also occur (40). Cannabinoids can stimulate the production of prostaglandins (41), and likewise, PGE_2 can stimulate synthesis of 2-AG intermediates (42). In summary, it is clear that eicosanoid biosynthesis is highly interactive and changes in biosynthesis or cell signaling pathways can alter overall eicosanoid balance and produce eicosanoids with similar or opposing functions.

Prostaglandin E_2: Inhibitory or Stimulatory to Hematopoiesis?

Numerous studies by us and others spanning a period of more than 20 years from the early 1970s to the mid 1990s demonstrated that PGE_2 inhibits mouse and human myeloid progenitors cells, defined as colony forming units-granulocyte macrophage (CFU-GM) and macrophage (CFU-M), *in vitro* (25, 26, 43–46). A physiological role for PGE_2 in regulation of hematopoiesis, particularly as a negative feedback regulator of myelopoiesis was further defined by studies in mice differing in PGE synthetic capacity (27, 47); demonstration of abnormal PGE_2 responses in patients with leukemia (43, 44, 46, 48, 49); prognostic association of disordered PGE_2 response in patients with myelodysplastic syndromes (MDS) (50); abnormal hematopoietic progenitor cell response in patients cured of germ cell tumors but progressing to acute leukemia (51); and association of HPC response to PGE_2 with clinical response to Interferon-γ in patients with chronic myelogenous leukemia (CML), chronic lymphocytic leukemia (CLL), and Hodgkin's disease (52, 53).

Not all studies, however, demonstrated an inhibitory effect of PGE_2. Studies by Fehrer and Gidali in 1974 showed that short-term PGE_2 treatment of murine marrow cells *in vitro* increased the number of day 9 colony forming unit – spleen (CFU-S) in cell cycle (54). An increase in CFU-GM in S-phase was also seen after PGE pulse exposure of human marrow (55). However, early studies evaluating *in vivo* dosing of PGE_2 in mice led to little or no increase in hematopoiesis (56). We later demonstrated that repetitive *in vivo* PGE_2 administration inhibits CFU-GM frequency and cell cycle rate, and decreases marrow and spleen cellularity (57–60); whereas, short-term *in vitro* exposure to PGE_2 stimulated proliferation and cell cycle of quiescent cells and an increase in HPC, suggesting a stimulatory effect of PGE_2 on HSC (61, 62). Further studies identified that dose, timing and duration of exposure were critical factors that determined positive or negative effects of PGE_2.

Hematopoietic Stem Cell Regulation by Prostaglandin E_2

Interest in the regulatory roles of PGE_2 on hematopoiesis has recently increased and new studies have provided further insights into hematopoietic regulation and possible therapeutic applications with PGE_2. Studies by North et al. demonstrated that zebrafish embryos treated

with a long-acting derivative of PGE_2, 16,16 dimethyl-PGE_2 ($dmPGE_2$), had an increase in HSC production, while treatment with an NSAID or COX knockout decreased HSC number during embryogenesis (63). In a zebrafish kidney marrow irradiation-recovery assay, $dmPGE_2$ increased kidney marrow repopulation, and *ex vivo* pulse exposure to $dmPGE_2$ increased the repopulating capacity of murine bone marrow cells in a competitive repopulation assay.

While these studies demonstrated that PGE_2 affects HSCs, the mechanisms of action of PGE_2 on HSC were not determined. Wnt signaling, particularly in the contexts of hematopoietic stress, ageing or disease, play an important regulatory role on HSCs (reviewed in 64). Prostaglandin E_2 signaling through the EP2 and EP4 receptors results in phosphorylation of GSK-3 and increased β-catenin signaling (65), which is downstream of the Wnt pathway and signaling specifically through the EP4 receptor has been shown to directly increase β-catenin suggesting synergistic cross-talk between COX2 and Wnt pathways (66). In a follow-up study to those by North et al., PGE_2 signaling was shown to stabilize β-catenin in HSC and promote survival and proliferation during embryogenesis (67) demonstrating the *in vivo* significance of PGE_2/Wnt interactions. Recently, we demonstrated that PGE_2 increases survival and proliferation of HSC (68), and exposure to PGE_2 increased the anti-apoptotic protein Survivin, which we have previously reported to be required for HSC to enter and progress through cell cycle (69, 70), providing further mechanistic insights into the regulation of HSC by PGE_2. In the context of pulse exposure to enhance hematopoietic transplantation, an important clinical translation of these described studies, we suggest a model in which PGE_2 facilitates enhancement of engraftment by a multipart mechanism. PGE_2 increases survival and self-renewal of HSC, through regulation of Wnt/ β-catenin, Survivin and possibly unidentified pathways, and increases CXCR4 expression enhancing homing and tethering of HSC in the bone marrow. The effect of PGE_2 on CXCR4 expression is critical to its ability to enhance HSC homing and engraftment and is described in further detail below.

Clinical trials are currently exploring the utility of *ex vivo* exposure to PGE_2 to enhance HSC engraftment. However, the ability to clinically modulate PGE_2 signaling *in vivo* may provide future therapeutic applications. An early study by Gidali and Feher in 1977, primarily evaluating CFU-S, suggested that *in vivo* dosing of PGE_2 in mice led to little or no increase in hematopoiesis (56). Extensive studies by our laboratory in the 1980s showed that repetitive *in vivo* PGE_2 administration inhibits CFU-GM frequency and cell cycle rate, and decreases marrow and spleen cellularity (57–60), however utilization of competitive repopulation assays, definitive assays of HSC function, were not performed. In a recent study by Frisch et al., repeated administration of PGE_2 for 16 days *in vivo*, expanded short-term, but not long-term, HSC in transplantation models (71). This regimen resulted in changes in the bone marrow niche, suggesting that the *in vivo* effects of PGE_2 on HSC and HPC may include indirect mechanisms. However, high doses of PGE_2 (~120 μg/mouse) were administered for extended periods of time, and control vehicle alone showed a ~2-fold increase in the percentage of $Lineage^{neg}$ $Sca\text{-}1^+$ $c\text{-}kit^+$ (LSK) cells after 16 days, while PGE_2 treatment showed ~3-fold increase over the same time period (71). The effects of vehicle may be suggestive of inadvertent stress effects on hematopoiesis in this system. Moreover, the effects of PGE_2 on hematopoiesis at physiological concentrations may be different than those observed using pharmacologic doses, as previously suggested (56). Additional studies evaluating *in vivo* modulation of PGE_2 signaling, both at a receptor level and on biosynthesis, are necessary to fully explore the therapeutic potential of the prostaglandin pathway.

Hematopoietic Regulation by other Eicosanoids

The cannabinoids have been implicated in positive and negative effects on mature cells of the immune system (72, 73); however, little is known about their effects on early hematopoietic cells. Anandamide can act as a synergistic growth factor for HPC (74) and has a pro-apoptotic effect on erythrocytes (75). The endocannabinoid 2-AG also stimulates proliferation of HPC (76), and has recently been shown to increase CFU-GEMM colony formation and cell migration (77). Furthermore, activation of cannabinoid CB receptors on murine embryonic stem cells promotes hematopoietic differentiation (78). In addition, Non-Hodgkin's lymphoma cells have abnormally high levels of CB_2 receptor expression (79), suggesting a potential proliferative role for cannabinoids.

Leukotrienes, like other eicosanoids, are produced in the marrow microenvironment (80), and 5'-LOX is found in HPC (81). While PGE_2 inhibits HPC proliferation and COX inhibitors enhance HPC production *in vitro*, the leukotrienes LTB_4, LTC_4 and LTD_4 increase mouse and human HPC (82–84), which is inhibited by 5'-LOX inhibitors (82, 83, 85). Moreover, PGE stimulates erythropoiesis (86, 87), while LTB_4 and LTC_4 inhibit early and late erythroid progenitor cells (88). Similarly, LOX inhibitors enhance erythropoiesis. In mice, dual COX inhibition enhances HPC recovery (85), while selective 5'-LOX inhibitors decrease CFU-GM and blast colony-forming cells (CFU-BL) (82). The addition of LTB_4 to UCB cells cultured with growth factors enhances HPC proliferation with concomitant reduction in total $CD34^+$ cells, while the selective LTB_4 receptor antagonist CP105696 enhances production of $CD34^+$ cells and blocks HPC proliferation (89). Overall, the available data suggest that LTB_4 signaling decreases HSC self-renewal and increases differentiation, while blocking LTB_4 receptor signaling increases HSC self-renewal and blocks their differentiation. This is in contrast to PGE_2 which enhances self-renewal (68), and blockade of PGE_2 biosynthesis enhances HPC production (90). Whether enhanced HPC production observed upon inhibition of PGE_2 synthesis *in vivo* is a direct consequence of lack of PGE_2-mediated HSC self-renewal remains to be determined. In any case, the use of PGE_2 or a leukotriene receptor antagonist or LOX inhibitor in the post-transplant setting may favor self-renewal. In addition, since blockade of COX enzymes makes more arachidonic acid available to the LOX pathway (84, 91), the use of a COX inhibitor post-transplant could promote HSC differentiation via direct inhibition of PGE_2 synthesis and/or increased synthesis of leukotrienes.

It is clear that prostaglandins, cannabinoids and leukotrienes have important roles in hematopoietic homeostasis, and evaluating their responses is critical to understanding eicosanoid function and development of eicosanoid-based therapeutic strategies for improvements in hematopoietic transplantation. The remainder of this article will focus on experimental systems that highlight the effects of eicosanoids on HSC and HPC in the context of how they can be used to affect trafficking to bone marrow, i.e. homing, in order to improve HSC delivery, and directed egress from bone marrow, i.e. mobilization, in order to obtain more HSC and HPC for transplant.

Prostaglandin E_2 Increases Hematopoietic Homing

Homing is a description of the ability of HSC and HPC to traffic from the peripheral blood, where they are injected intravenously for hematopoietic transplantation, to the bone marrow hematopoietic niche, where they can lodge, self-renew and differentiate to successfully repopulate the host's blood forming system. Homing is a rapid process, which is measured in hours (or at most 1–2 days) (92), and should be separated from the concept of "engraftment", which is more a description of the culmination of events pre- and post-homing of HSC. Homing of HSC to bone marrow appears to be regulated by the same

processes responsible for HSC retention within the bone marrow niche, many of which are discussed in this issue of *Leukemia*. Adhesion molecules aid in trafficking, leukocyte rolling, transendothelial migration, and ultimate tethering in the marrow, and several adhesion molecules have been implicated as contributing to HSC and HPC tethering in the bone marrow hematopoietic niche; notably the integrins α4β1 – very late antigen-4 (VLA-4) (93–96), α5β1 – very late antigen-5 (VLA-5) (93, 95–97), α4β7 – lymphocyte Peyer's patch adhesion molecule-1 (LPAM-1) (98), the alpha 6 integrins (Laminins) (99, 100), CD44 (96, 101), E-selectins (102–104), and others, and are essential for proper bone marrow homing of HSC (95, 97–100, 105, 106). Similarly, the CXCR4/SDF-1α axis is a critical component of HSC homing to the bone marrow (107), and increases in CXCR4 receptor expression on HSC either with growth factors (105), or by gene overexpression (108, 109) significantly increases HSC homing and engraftment.

PGE_2 increases CXCR4 receptor expression on murine (68) and human (68, 110) HSC and HPC. In light of this increase in CXCR4 mediated by PGE_2, we evaluated the ability of a short *in vitro* treatment with PGE_2 to increase the migration of HSC and HPC to SDF-1α. Mouse bone marrow cells were FACS sorted for the SKL population that is enriched for HSC, and $CD34^+$ cells from human UCB and G-CSF MPB samples were acquired by magnetic antibody isolation. After short-term treatment with PGE_2 *in vitro*, the ability of SKL, UCB $CD34^+$ and MPB $CD34^+$ cells to migrate to SDF-1α was evaluated using an *in vitro* transwell assay. Treatment with PGE_2 significantly increased transwell migration of each of these hematopoietic cell populations (Figure 2A). To further evaluate if increased chemotaxis observed with PGE_2- treated cells led to a functional increase in HSC bone marrow homing, we utilized a novel head- to-head homing assay, where FACS purified SKL cells from C57Bl/6 mice (CD45.2) and B6.SJL-PtrcAPep3B/BoyJ (BOYJ) (CD45.1) mice were treated with either PGE_2 or vehicle and transplanted competitively into lethally irradiated hybrid C57Bl/6 / BOYJ hybrid mice (CD45.1/CD45.2). Analysis of homed SKL cells in recipient bone marrow demonstrated that PGE_2 treatment enhanced SKL cell homing compared to cells treated with vehicle (Figure 2B).

Cannabinoids Increase Hematopoietic Progenitor Cell Mobilization

Recent evidence suggests that cannabinoids, signaling through the peripheral CB_2 receptor, inhibit SDF-1α induced and CXCR4 mediated chemotaxis of Jurkat T-cells (111) and activated human peripheral T-cells (112). In addition, hematopoietic cell lines transfected with cannabinoid receptor migrate in response to the endogenous cannabinoid, 2-AG (113), and 2-AG can act as a chemoattractant for dendritic cells (114). Stimulation of cannabinoid receptors reduces adhesion molecule expression (115, 116), transendothelial migration of monocytes (116), and regulates myeloid progenitor trafficking by alterating chemokines and chemokine receptors (116, 117). Cannabinoids have also been reported to affect matrix metalloproteinase-9 (MMP-9) production (118) in a number of cell lines and can modulate neutrophil function (119, 120). Taken together, these data suggest that cannabinoids could play a role in hematopoietic mobilization, through interference in the SDF-1α/CXCR4 axis, reduction in adhesion and/or release of MMP-9, which can degrade adhesion molecule interactions between HSC and HPC with the bone marrow niche (121), or through other undefined mechanisms.

While many of the above effects attributed to cannabinoids have been defined in mature blood cells, little research has explored their function on HSC and HPC. Using a number of different antibodies and flow cytometry, we were able to clearly detect CB receptors on immunophenotypically defined mouse and human HSC (not shown). We utilized the dual CB_1 and CB_2 agonist CP55940 (122) and examined its effects on expression of CXCR4 and the adhesion molecule VLA-4 on SKL cells *in vitro*, compared to cells treated with

$dmPGE_2$. As we previously reported, short term *in vitro* treatment with $dmPGE_2$ increased expression of CXCR4 on treated SKL cells (Figure 3A). In addition, $dmPGE_2$ also increased SKL VLA-4 expression. In contrast, SKL cells treated with cannabinoids demonstrated significant reduction in both CXCR4 and VLA-4, suggesting that cannabinoids may be able to facilitate release of hematopoietic stem and progenitor cells from bone marrow niches.

In order to directly evaluate the effects of cannabinoid receptor agonists on HPC mobilization, BALB/c mice were treated with single injections of the CB_1 selective agonist ACEA (123), the CB_2 selective agonist GP1a (124), or the dual CB_1 and CB_2 agonist CP55940 and CFU-GM in peripheral blood were quantitated after 2 hours. Single administration of the CB_2 selective agonist GP1a and the dual CB_1/CB_2 agonist CP55940 mobilized CFU-GM to peripheral blood to a significant degree (Figure 3B); whereas, the CB_1 selective agonist ACEA had only marginal mobilizing activity. In separate groups of mice, we also evaluated combination mobilization with G-CSF and the dual CB_1/CB_2 agonist CP55940. Mice were mobilized with a standard 4-day regimen of G-CSF alone (50 μg/kg, *bid* X 4 days) or the G-CSF regimen plus a single dose of CP55940 administered on day 5, 16 hours after the last dose of G-CSF and 2 hours prior to sacrifice. The addition of a single dose of CP55940 to the G-CSF regimen significantly increased CFU-GM mobilization compared to G-CSF alone (Figure 3C). These data suggest that CB receptor ligation, particularly CB_2, rapidly mobilizes CFU-GM, and that CB receptor activation enhances mobilization by G-CSF, likely by an effect on inhibition of CXCR4 signaling, or reductions in integrin adherence, since the kinetics of mobilization are consistent with the kinetics of mobilization by the CXCR4 antagonist AMD3100 and/or VLA-4 inhibitors. It should be noted that these effects of cannabinoids on CFU-GM mobilization are in contrast to a previously published study that showed that the CB_2 receptor mediates retention of immature B cells in bone marrow sinusoids (125), perhaps suggesting cell-type specific mobilization responses. While these studies clearly indicate mobilization of HPC, further analysis of the full repertoire of HPC and HSC mobilized by cannabinoids, will aid in the potential future development of cannabinoid/cannabinoid receptor based mobilization strategies.

A Role for Leukotrienes in Hematopoietic Trafficking?

Leukotrienes are known mediators of allergic inflammation and asthma, however there is little information on their role in hematopoietic stem and progenitor trafficking. LTB_4 has been shown to increase the migratory capacity of dendritic cells (126, 127), and T-cell subsets (126). It is also a potent chemoattractant for polymorphonuclear leukocytes and regulates transendothelial migration (128, 129), which is at least partially mediated by regulation of adhesion molecules. While LTB_4 can induce migration of mature blood cells, only the cysteinyl leukotriene LTD_4 can induce chemotaxis and transendothelial migration of $CD34^+$ progenitors (130), which is mediated in part by regulation of VLA-4 and VLA-5 adhesion (131). However, *in vivo* treatment with a LTD_4 receptor antagonist does not result in mobilization of $CD34^+$ cells (131) possibly due to low baseline levels of LTD_4 in steady state bone marrow. Evaluation of the use of leukotriene antagonism in combination with other mobilizing agents has not yet been explored. It remains to be determined if modulation of leukotriene activity either *in vivo* or *ex vivo* will have any efficacy in enhancing hematopoietic transplantation.

The Yin and Yang of Eicosanoid Regulation

In many physiological systems, prostaglandins, leukotrienes and endocannabinoids exhibit compensatory or opposing roles (reviewed in Table 1). The action of many NSAIDs, which block the production of PGE_2, may also act by increasing signaling via endocannabinoids

(132). PGE_2-glycerol has been shown to mobilize calcium, activate signal transduction pathways (133) and have neurological effects opposite of those induced by 2-AG (134). Furthermore, prostaglandins and leukotrienes have been shown to have numerous opposing roles in pulmonary fibrosis (135) and in other systems they may act in a coordinate fashion (136). It is particularly noteworthy that cannabinoid ligands block CXCR4 signaling (111, 112) and neutrophil migration (120, 137), which one would expect to negatively affect homing, but enhance spontaneous release from marrow facilitating mobilization as we have demonstrated. In contrast, PGE_2 enhances CXCR4 expression and signaling, which facilitates hematopoietic homing, but may result in dampening of mobilization responses. Future studies evaluating combination eicosanoid therapies, which take into account these opposing functions, are likely to lead to novel approaches to regulate hematopoietic trafficking and improve transplantation protocols. The current availability of numerous FDA approved pharmaceuticals that specifically regulate biosynthesis and signaling of prostaglandins, leukotrienes and cannabinoids will facilitate rapid translation of eicosanoid based therapeutic research, both at the level of graft isolation or measured in terms of graft performance.

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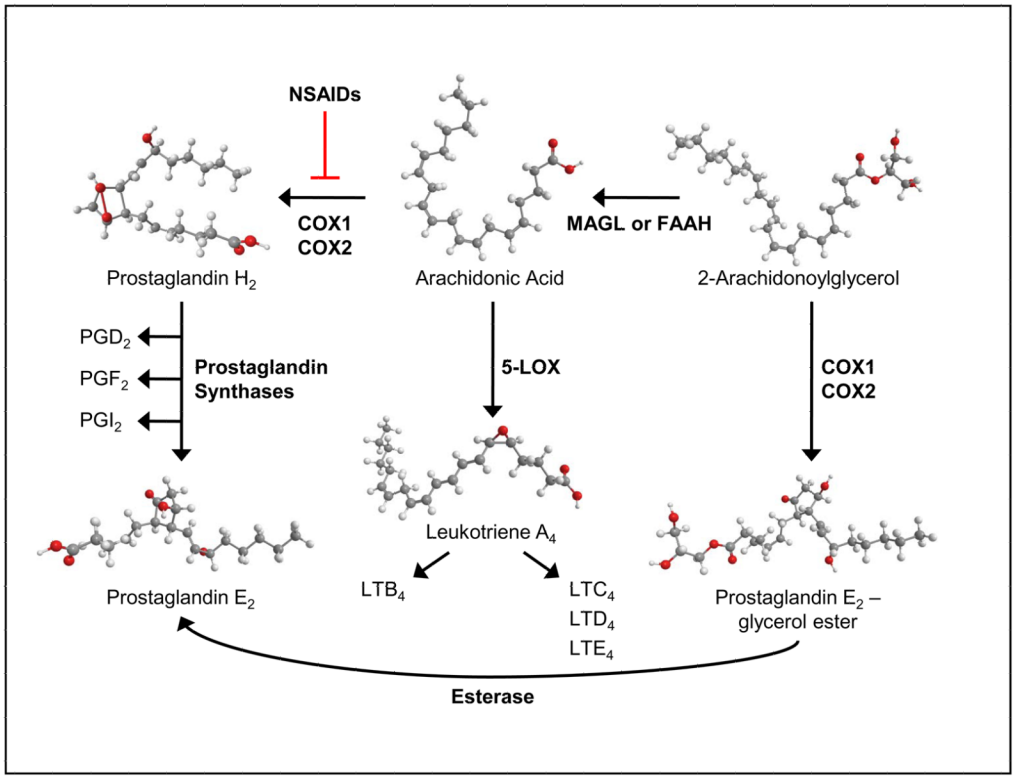

Figure 1. Schematic of eicosanoid biosynthesis

Activation of c-phospholipase A_2 releases arachidonic acid from phospholipids. Cyclooxygenase (COX) enzymes, which can be inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), convert arachidonic acid into the Prostaglandin H_2 intermediate, which is then converted to the various prostaglandins by distinct prostaglandin synthases . Similarly, 5- lipoxygenase (5-LOX) converts arachidonic acid into Leukotriene A_4 (LTA_4) and then synthesis proceeds to the various leukotrienes. Endocannabinoid (2-Arachidonoylglycercol) is catabolized by fatty acid amide hydrolase (FAAH) or monoacylglycerol lipase (MAGL) and can be converted into alternate prostaglandin forms.

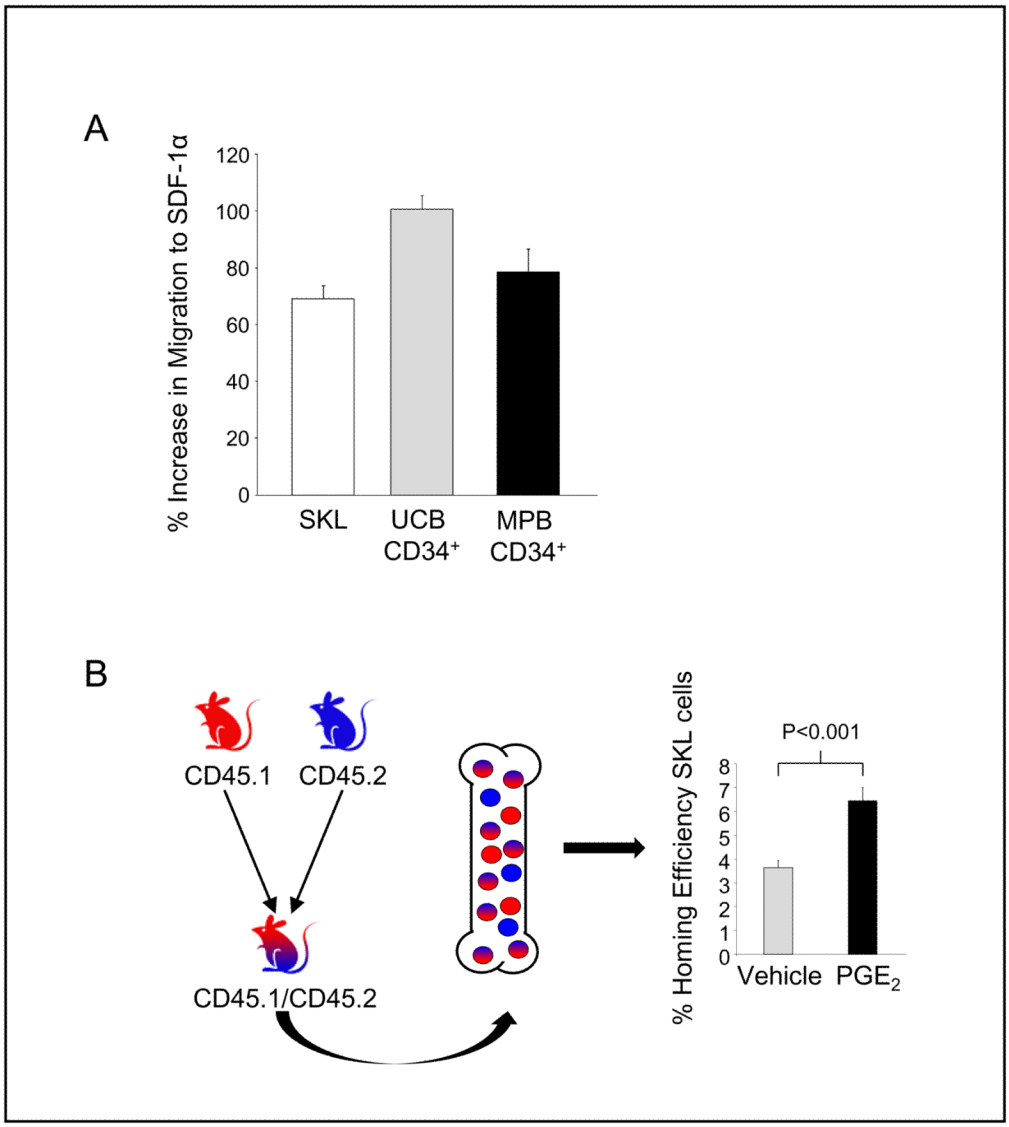

Figure 2. PGE_2 increases hematopoietic migration and homing

A.) Mouse SKL cells or human $CD34^+$ cells were treated on ice with 1 μM 16,16-dimethyl PGE_2 ($dmPGE_2$) or vehicle control for 2 hours, washed, and then re-suspended in media with 10% heat inactivated fetal bovine serum and cultured at 37 °C for 16 hours. After incubation, cells were washed, re-suspended in RPMI/0.5% BSA, and allowed to migrate to 100 ng/ml recombinant mouse or human SDF-1α through a two-chamber, 6.5 mm diameter, 5 μm pore transwell. Cells migrating to the bottom chamber were enumerated by flow cytometry, and the % increase in migration over vehicle control determined.

B.) SKL cells from CD45.1 and CD45.2 mice were isolated by FACS sorting and treated with either $dmPGE_2$ or vehicle. Treated SKL cells were then transplanted competitively, head-to-head into lethally irradiated (1100 cGys) CD45.1/CD45.2 hybrid mice, allowing for distinction of vehicle treated, $dmPGE_2$ treated and recipient SKL cells within the same animal. Sixteen hours post-transplant, hind limb bones were isolated and homed SKL cells determined by flow cytometry. Mean ± SEM, N=10 mice, each assayed individually, are shown.

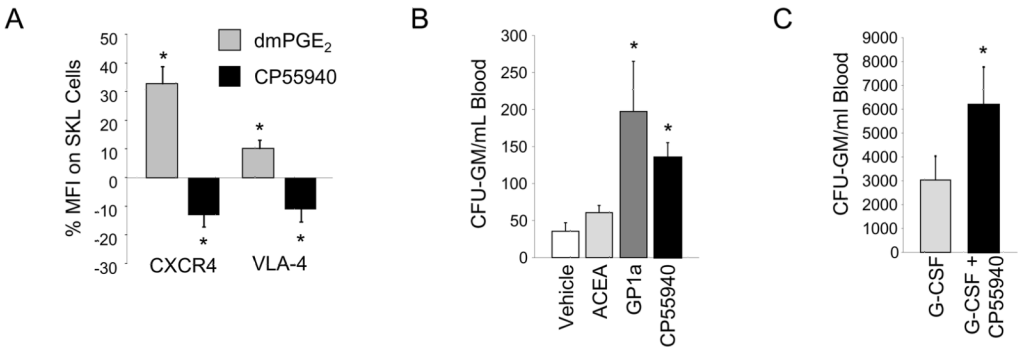

Figure 3. Cannabinoid agonism rapidly and synergistically mobilizes HPC

A.) Mouse Lineage^{neg} bone marrow was treated with $dmPGE_2$, the cannabinoid agonist CP55940, or vehicle control. Sixteen hours post-treatment, cells were stained for CXCR4 receptor or VLA-4, and SKL phenotypic markers, and % mean fluorescence intensity (MFI) compared to control treated cells determined. Data are Mean ± SEM, N=5 mice, each assayed individually. * P<0.05 compared to vehicle control.

B.) CFU-GM mobilization 2 hours post single administration of 10 mg/kg ACEA, 5 mg/kg GP1a or 10 mg/kg CP55940. Data are expressed as Mean ± SEM, N=3 mice per group, each assayed individually. * P<0.05 compared to vehicle control.

C.) Mobilization by G-CSF (50 μg/kg, *bid* X 4 days), or G-CSF for 4 days followed by a single dose of CP55940 on day 5. Data are expressed as Mean ± SEM, N=3 mice per group, each assayed individually. * P<0.05 compared to G-CSF.

Table 1

Opposing roles of cannabinoids, leukotrienes and prostaglandins.

System Affected	Cannabinoids	Leukotrienes	Prostaglandin E_2
CXCR4/CXCL12	↓(111, 112)	----------	↑(68, 138, 139)
cAMP	↓(140, 141)	↓(142)	↑↓(143)
Neutrophil Migration	↓(120, 137)	↑(128, 136)	↑(128, 144, 145)
Inflammation	↓(72, 140, 146)	↑(31, 147)	↑(16, 148, 149) ↓(150)
Myleopoiesis	↑(74, 76, 77)	↑(151–153)	↓(25, 26, 62)
Erythropoiesis	↓(75)	↓(88)	↑(86, 87, 154, 155)

Summary of several physiological processes relevant to hematopoiesis and hematopoietic cell trafficking that are regulated by the eicosanoid system. In most cases, cannabinoids act in an opposing role to prostaglandins, while leukotrienes regulate similar or opposing functions to prostaglandin. The dual prostaglandin effects shown for some physiological processes are the result of EP receptor subtype differences.