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PLAG alleviates chemotherapy-induced thrombocytopenia via promotion of megakaryocyte/erythrocyte progenitor differentiation in mice

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ABSTRACT

Previously, PLAG (1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol, acetylated diglyceride) was reported to have an effect on the proliferation of hematopoietic stem cells (HSCs) or to contribute to the prevention of chemotherapy-induced neutropenia. In this study, we examined the role of PLAG in the differentiation of bone marrow cells from HSCs into progenitor cells in mice. After 15 days, the lineage-negative cells, especially megakaryocyte/erythrocyte progenitors (MEP), were significantly increased in mice that received daily PLAG administration compared to those in the untreated mice. Furthermore, we explored the possibility that the PLAG-induced increase in MEP will contribute to reduction of chemotherapy-induced thrombocytopenia (CIT) in a thrombocytopenia mouse model. Mice were administrated 5-fluorouracil (5-FU) and PLAG. After 7 days, bone marrow cells were analyzed. Treatment with 5-FU powerfully decreased myeloid precursor populations and treatment with 5-FU/PLAG resulted in reduction of decreased myeloid progenitor cell numbers. In addition, numbers of circulating platelets were also increased by PLAG treatment. Taken together, PLAG plays a role in differentiating HSCs toward MEP and alleviating chemotherapy-induced bone marrow cell reduction. Thus PLAG shows its potential to augment the therapeutic effect of anti-cancer drugs-induced thrombocytopenia.

1. Introduction

Chemotherapy frequently causes various complications that limit the both the dose intensity and the effectiveness of cancer treatment [1]. Bone marrow injury is a major adverse side effect of chemotherapy and induces hematopoietic cell apoptosis [2,3]. Following chemotherapy, multipotent progenitor and hematopoietic progenitor cells undergo damages such as cell apoptosis and rapid proliferation [4]. Then, hematopoietic stem cells (HSCs) are generated as multipotent progenitor and hematopoietic progenitor cells through their self-renewal and differentiation [5]. HSCs classically and gradually differentiate into lymphoid progenitors or myeloid progenitors, including the common myeloid progenitors (CMPs), the granulocyte-monocyte progenitors (GMPs), and the megakaryocyte/erythrocyte progenitors (MEPs) [6,7]. Megakaryocytes arise from MEP and give rise to circulating thrombocytes (platelets) [8]. Chemotherapy drugs decrease the numbers of bone marrow cells and inhibit the differentiation of HSCs, resulting in the exhaustion of bone marrow cells via a strong attenuation of a feed-back mechanism [9].

This depleted bone marrow is considered to be the main cause of chemotherapy-induced thrombocytopenia (CIT), which is a condition with decreased levels of thrombocytes related to the progression of disease [10]. Because thrombocytes are integral for the clotting of blood to prevent excessive bleeding when a blood vessel is injured, decreased thrombocyte counts in peripheral blood indicates a high risk for bleeding and bruising [11]. Such bleeding, which may lead to pain, hemodynamic instability, and death, may require interventions, such as transfusion or pharmacological treatment [12]. Platelet transfusion from a healthy donor is the most common treatment for thrombocytopenia patients [13,14]; however, this approach carries the risk of infection. Administration of recombinant interleukin (IL)-11 (Oprelvekin) has also been prescribed for thrombocytopenia patients in order to stimulate platelet production [15,16], despite the significant side effects, tolerance, and the limited effects of this agent [17]. Although CIT has been recognized as a significant risk to cancer treatment, no specific therapy is available.

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PLAG (1-palmitoyl-2-linoleoyl-3-acetyl-rac-glycerol, acetylated diglyceride, Supplementary data 1) has been purified from the antlers of Sika deer and chemosynthesized equally, and confirmed to be identical to the naturally occurring protein [18]. PLAG has been reported to exert a stimulatory effect on hematopoietic stem cells and megakaryocytes or reduce the incidence of gemcitabine-induced neutropenia in pancreatic cancer patients by PLAG oral administration [19,20]. Furthermore, PLAG treated mice were shown to have reduced chemotherapy-induced neutropenia and cachexia symptoms [21,22]. In this study, mice were treated with PLAG daily, and their HSC and progenitor cell populations were analyzed according to specific lineage markers using flow cytometry [23]. 5-FU-induced MEP decrease was significantly alleviated following PLAG administration. These data indicate that PLAG may be useful as a supplementary agent for chemotherapy-induced thrombocytopenia.

2. Materials and methods

2.1. Animal experiments and reagents

C57BL/6 male mice were obtained from Koatech Co. (Pyongtaek, Republic of Korea) and maintained under specific pathogen-free conditions. The mice were 6–8 weeks of age and 20–22 g at the time of the experiments. Each mouse was administrated orally with 50 mg/kg body weight of PLAG (Enzychem Lifesciences Co., Daejeon, Republic of Korea) in phosphate buffered saline (PBS, Wellgene, Daegu, Republic of Korea) using feeding needle catheter every day. Control and 5-FU injected groups were administrated orally with the same PBS daily.

For chemotherapy-induced thrombocytopenia model, 5-fluorouracil (5-FU, 150 mg/kg; Sigma Aldrich, MO, USA) was injected intraperitoneally. Control mice were introduced with DMSO (Dimethyl sulfoxide, Sigma Aldrich) the same way. Each group contained five mice in all experiments.

2.2. Ethics statement

All animal experimental procedures were performed in accordance with the Guide and Use of Laboratory Animals (Institute of Laboratory Animal Resources). The study was approved by the Institutional Review Committee for Animal Care and Use of KRIBB (Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea, Approval number KRIBB-AEC-16031).

2.3. Bone marrow cell and peripheral blood analysis

To obtain bone marrow cells, femurs and tibias were removed from each mice and were dissected free of muscle and tendons. After both ends of the bones were cut, bone marrow was flushed with 2 ml RPMI 1640 media (Life Technologies, Karlsruhe, Germany). The resulting cell suspension was filtered through a 70 μm mesh and washed in PBS.

Whole blood was collected from the orbital sinuses of the mice using heparinized capillary tubes (Kimble Chase Life Science and Research Products LLC, FL, USA) and collection tubes (Greiner Bio-One International, Frickenhausen, Germany). The collected cells were counted by complete blood count analysis using a BC 5300 Mindray analyzer (Shenzhen Mindray Bio-medical Electronics, China).

2.4. Flow cytometric analysis

For flow cytometric analysis, additional steps were required to remove erythrocytes from the collected cells. After incubation with ACK Lysing Buffer (Life Technologies, Karlsruhe, Germany), the collected cells from each mouse sample were washed with PBS and subsequently subjected to specific antibody staining. For staining of the lineage-negative cell population, the isolated bone marrow cells were incubated with a lineage marker antibody cocktail, containing antibodies to CD3, CD45R (B220), GR1, CD11b, and TER-119 (BD Biosciences, NJ, USA). The hematopoietic progenitor populations were analyzed using PE-Cy7-conjugated rat anti-mouse Sca-1, PE-conjugated rat anti-mouse c-Kit, FITC-conjugated rat anti-mouse CD34, APC-Cy7-conjugated rat anti-mouse CD16/32, and PE-CF594 conjugated rat anti-mouse CD127. To assess the platelet or erythrocyte population, whole blood cells were stained with PE-conjugated rat anti-mouse CD41 or APC-Cy7-conjugated rat anti-mouse TER119, respectively. These antibodies were purchased from BD Biosciences. Cells were analyzed with a FACSAria or FACSCalibur flow cytometer (BD Biosciences), and data were processed with FlowJo software (Tree Star, OR, USA).

2.5. Statistical analysis

Results are presented as mean ± standard error of the mean (s.e.m.). The level of significance, assumed at the 95% confidence limit or greater ($p < 0.05$), was calculated with one-way analysis of variance (ANOVA) followed by Duncan's *post hoc* test using SPSS 18.0 program. Different letters (*a*, *b*, *c*, *d*, *e*) show values having significant difference at $p < 0.05$. The same letter represents groups that are not statistically significant.

3. Results

3.1. PLAG stimulates HSC differentiation toward MEP

To elucidate the effect of PLAG on differentiation of progenitor cells, bone marrow cells were isolated from mice treated with PLAG and analyzed with specific markers as indicated in Table 1. The absolute cell numbers per mouse were represented in figures and the frequencies of total bone marrow cells from tibias and femurs of each mouse were displayed in tables. The total cell number of bone marrow cells was not increased significantly, but the lineage-negative progenitor cell populations of bone marrow was slightly increased in the PLAG-treated mice after 15 days (Fig. 1A and B). PLAG alone did not affect the differentiation of common lymphoid progenitors but did induce effects on the differentiation of myeloid progenitors (Fig. 1C and D). In particular, MEP population was significantly increased in both the absolute cell numbers and the frequency of total bone marrow cells by PLAG administration (Fig. E–G and Table 2A).

The increased population of platelet and erythrocyte were confirmed using the specific antibodies in whole blood cells (Fig. 1H and I). A complete blood count analysis of whole blood in PLAG-treated mice revealed that the circulating platelet was significantly increased by PLAG treatment (Table 2B). Along with this, the circulating erythrocyte was also upregulated. Therefore, PLAG administration increases the circulating platelet and erythrocyte through stimulating differentiation of HSCs toward MEP.

3.2. PLAG alleviates the damage from 5-FU-induced MEP decrease

To determine whether PLAG has a potential to relieve

Table 1
Cell surface phenotypes of various progenitor cell population.

Progenitor cell type	Marker
Lineage negative cell	$CD3^-$ $CD45R\ (B220)^-$ $GR1^-$ $CD11b^-$ $TER\text{-}119^-$
Myeloid Progenitor	Lin^- $Sca1^-$ $c\text{-}Kit^+$
Common lymphoid progenitor	Lin^- $Sca1^{low}$ $c\text{-}Kit^{low}$ $CD127^+$
Common myeloid progenitor (CMP)	Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD34^{low}$ $CD16/32^{low}$
Granulocyte-monocyte progenitor (GMP)	Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD34^+$ $CD16/32^+$
Granulocyte-monocyte progenitor (MEP)	Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD34^-$ $CD16/32^-$

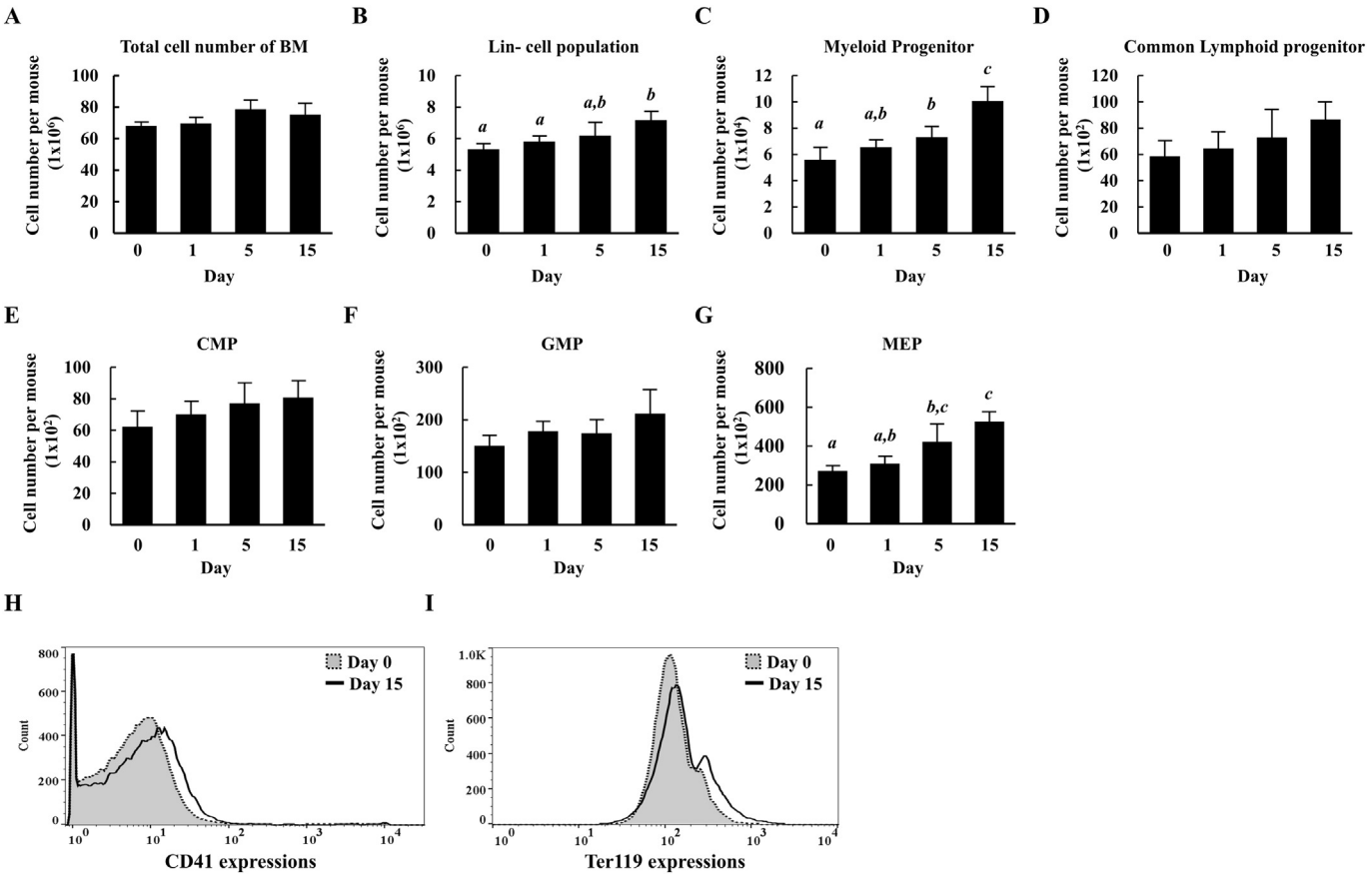

Fig. 1. PLAG exhibits an increased differentiation potential toward the megakaryocyte/erythrocyte progenitors lineages (n = 5 per group).
Mice were treated with 50 mg/kg PLAG for 1, 5, and 15 days. After sacrifice, bone marrow-derived cells were collected from femurs and tibiae of mice (A). To identify the hematopoietic lineage-negative cells (Lin^-) population, we used a cocktail of antibodies to lineage-specific markers, including anti-mouse CD3, CD11b, CD45R, Gr-1, and TER-119 (B). The populations of myeloid progenitor (Lin^- $Sca1^+$ c-Kit^+), CLP (Lin^- $Sca1^{low}$ c-Kit^{low} $CD127^+$), CMP (Lin^- $Sca1^-$ c-Kit^+ $CD34^{low}$ $CD16/32^{low}$), GMP (Lin^- $Sca1^-$ c-Kit^+ $CD34^+$ $CD16/32^+$), and MEP (Lin^- $Sca1^-$ c-Kit^+ $CD34^-$ $CD16/32^-$) were determined (as described in Table 1). Absolute numbers of progenitors from each of the populations were analyzed in (C–G). Numbers represent cells retrieved from tibias and femurs of each mouse. Data represent one experiment performed in triplicate. Results are presented as mean ± s.e.m. Each group contained five mice. Statistical analysis was performed by one-way ANOVA, followed by Duncan's *post hoc* test. Different letters (*a*, *b*, *c*) show values having significant difference at $p < 0.05$. The same letter represents groups that are not statistically significant. After mice were treated with 50 mg/kg PLAG for 15 days, flow cytometry analysis was performed using PE-conjugated rat anti-mouse CD41 (for platelet) or APC-Cy7-conjugated rat anti-mouse TER119 (for erythrocyte), respectively (H and I). The control showed as the gray-shaded area, and PLAG-treated mice represented as the bold line. Data represent one experiment performed in triplicate.

Table 2
Hematological indices and absolute blood cell counts during PLAG administration (n = 5 per group).

(A)

Frequency of total BM cells per mouse	Control	PLAG for 1 days	PLAG for 5 days	PLAG for 15 days
Lineage negative (%)	7.82 ± 0.58 *a*	8.40 ± 0.06 *a*	7.98 ± 1.06 *a*	9.58 ± 0.76 *b*
CLP (%)	0.11 ± 0.02	0.12 ± 0.01	0.11 ± 0.02	0.13 ± 0.01
Myeloid progenitor (%)	1.05 ± 0.13 *a*	1.12 ± 0.08 *a,b*	1.19 ± 0.08 *b*	1.40 ± 0.09 *c*
CMP (%)	0.12 ± 0.00	0.12 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
GMP (%)	0.29 ± 0.03	0.31 ± 0.03	0.28 ± 0.03	0.30 ± 0.06
MEP (%)	0.52 ± 0.05 *a*	0.53 ± 0.08 *a*	0.69 ± 0.07 *b*	0.74 ± 0.08 *b*

(B)

Cell numbers per mouse	Control	PLAG for 1 days	PLAG for 5 days	PLAG for 15 days
Platelet in blood (10^3/μl)	986.67 ± 157.83 *a*	1133.33 ± 305.75 *a,b*	1274.00 ± 111.62 *b*	1293.00 ± 96.19 *b*
RBC in blood (10^6/μl)	9.54 ± 0.26 *a*	9.59 ± 0.40 *a*	10.52 ± 0.75 *b*	10.83 ± 0.37 *b*
Neutrophil in blood (10^3/μl)	0.70 ± 0.13	0.77 ± 0.19	0.80 ± 0.10	0.68 ± 0.07

After mice were treated with 50 mg/kg PLAG for 1, 5, and 15 day, the hematopoietic lineage-negative cells (Lin^-) and the progenitor population was analyzed using specific markers as described in Table 1. The frequency of hematopoietic progenitor subsets was analyzed (A). The whole blood cells were counted by a complete blood count analysis (B). Data represent one experiment performed in triplicate. Results are presented as mean ± s.e.m. Each group contained five mice. Statistical analysis was performed by one-way ANOVA, followed by Duncan's *post hoc* test. Different letters (*a*, *b*, *c*) show values having significant difference at $p < 0.05$. The same letter represents groups that are not statistically significant.

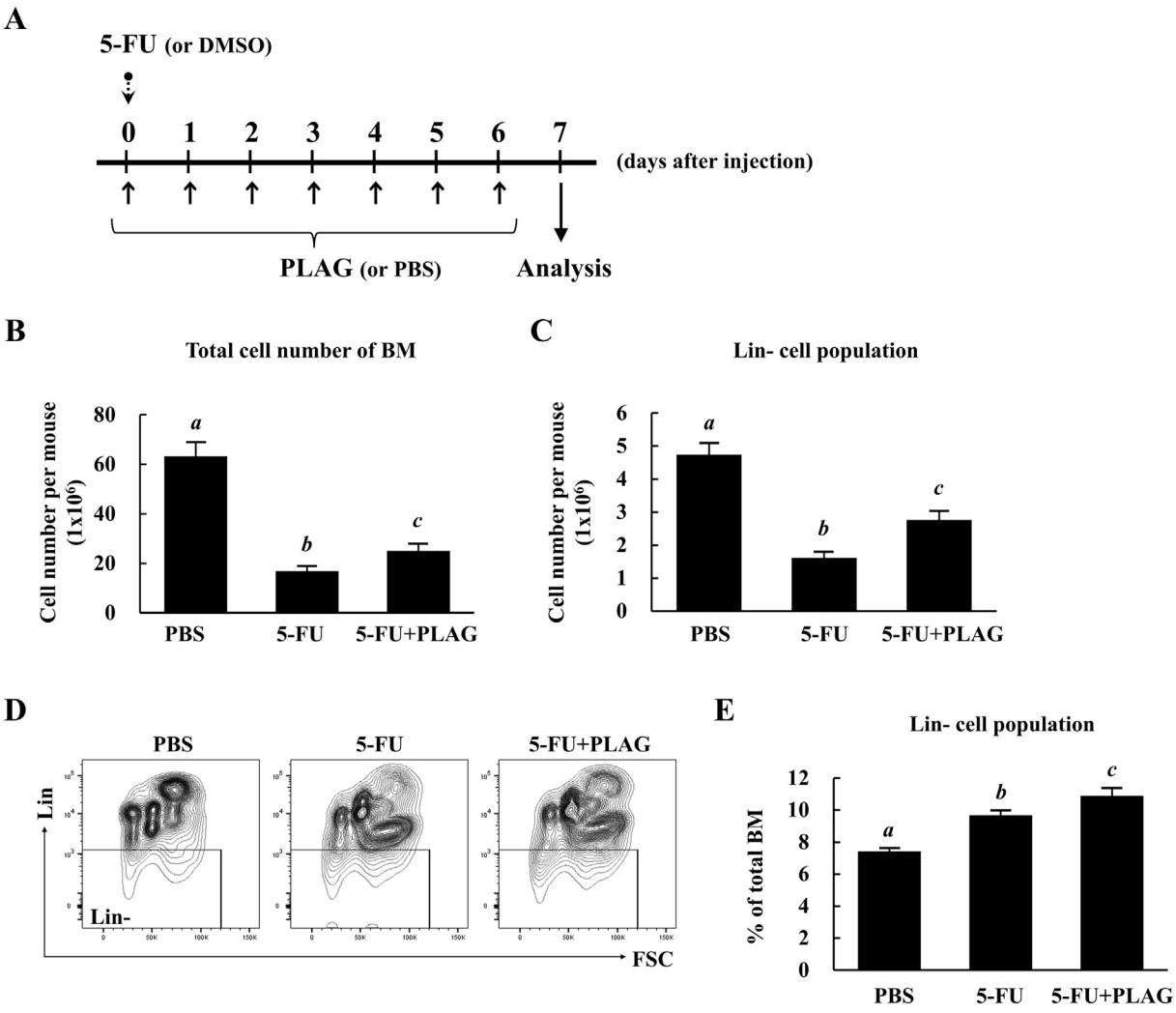

Fig. 2. A decrease in 5-FU-induced bone marrow cells is reduced by PLAG treatment (n = 5 per group).
Mice were divided into three group: 1) PBS-treated group, 2) 5-FU-treated group, 3) PLAG and 5-FU-treated group. 5-FU was administered intraperitoneally at a dose of 150 mg/kg, and PLAG were administered orally at a dose of 250 mg/kg/day (A). After 7 days, cells were collected from femurs and tibiae of mice and stained for hematopoietic lineage-negative cells (Lin^-) populations (as described in Table 1). The acquired total BM cell number was counted (B). Absolute numbers of hematopoietic lineage-negative cells (Lin^-) were analyzed in (C). Numbers represent cells retrieved from tibias and femurs of each mouse. Data represent one experiment performed in triplicate. Results are presented as mean ± s.e.m. The frequency of hematopoietic lineage-negative cells (Lin^-) was analyzed (D) and represented as a bar graph (E). Data represent one experiment performed in triplicate. Results are presented as mean ± s.e.m. Each group contained five mice. Statistical analysis was performed by one-way ANOVA, followed by Duncan's *post hoc* test. Different letters (*a*, *b*, *c*) show values having significant difference at $p < 0.05$. The same letter represents groups that are not statistically significant.

chemotherapy-induced thrombocytopenia, 5-FU in the presence or absence of PLAG was administrated to C57BL/6 mice (Fig. 2A). After 7 days, total bone marrow cells and lineage negative progenitors were significantly decreased in 5-FU-treated mice, and PLAG was found to diminish the 5-FU-induced bone marrow cell reduction (Fig. 2B and C). Because the frequency of lineage negative cells was shown to display moderate growth, the absolute cell numbers of lineage negative population were slightly increased in both the 5-FU- and 5-FU/PLAG-treated mice (Fig. 2D and E).

Next, we analyzed the effect of PLAG on subsets of damaged hematopoietic progenitor cells by the 5-FU. 5-FU treatment resulted in a severely decreased myeloid progenitor population, but not in CLP population (Fig. 3A–C). PLAG treatment diminished 5-FU-induced reduction of myeloid progenitors and slightly upregulated CLP population. Because PLAG alone had no effect on CLP differentiation (Fig. 1D), the increased lineage negative population may have influenced CLP cell numbers. PLAG administration alleviated 5-FU-induced damage in all myeloid progenitor subsets (Fig. 3D–G). Most interestingly, MEP population exhibited a larger proportion with PLAG treatment among myeloid progenitor subsets. These data indicate that chemotherapy-induced progenitor cell loss was partially protected by PLAG treatment. (See Table 3)

3.3. PLAG reduces the 5-FU-induced decrease of circulating platelets

Because chemotherapy-induced bone marrow cell loss was reduced by PLAG treatment, we performed a complete blood count analyses of whole blood in 5-FU/PLAG-treated mice. Administration of 5-FU diminished the numbers of circulating platelets, and the addition of PLAG alleviated the decreased numbers of circulating platelets in these mice (Fig. 4A). Flow cytometric analysis for CD41-positive platelets confirmed these results on day 7 (Fig. 4B). Erythrocytes were slightly decreased in 5-FU-treated mice, but no additional effects were observed in the presence of PLAG (Fig. 4C). 5-FU-induced neutropenia was also significantly reduced by PLAG administration (Fig. 4D). Taken together, PLAG decreased chemotherapy-induced damages of bone marrow cells and circulating platelets.

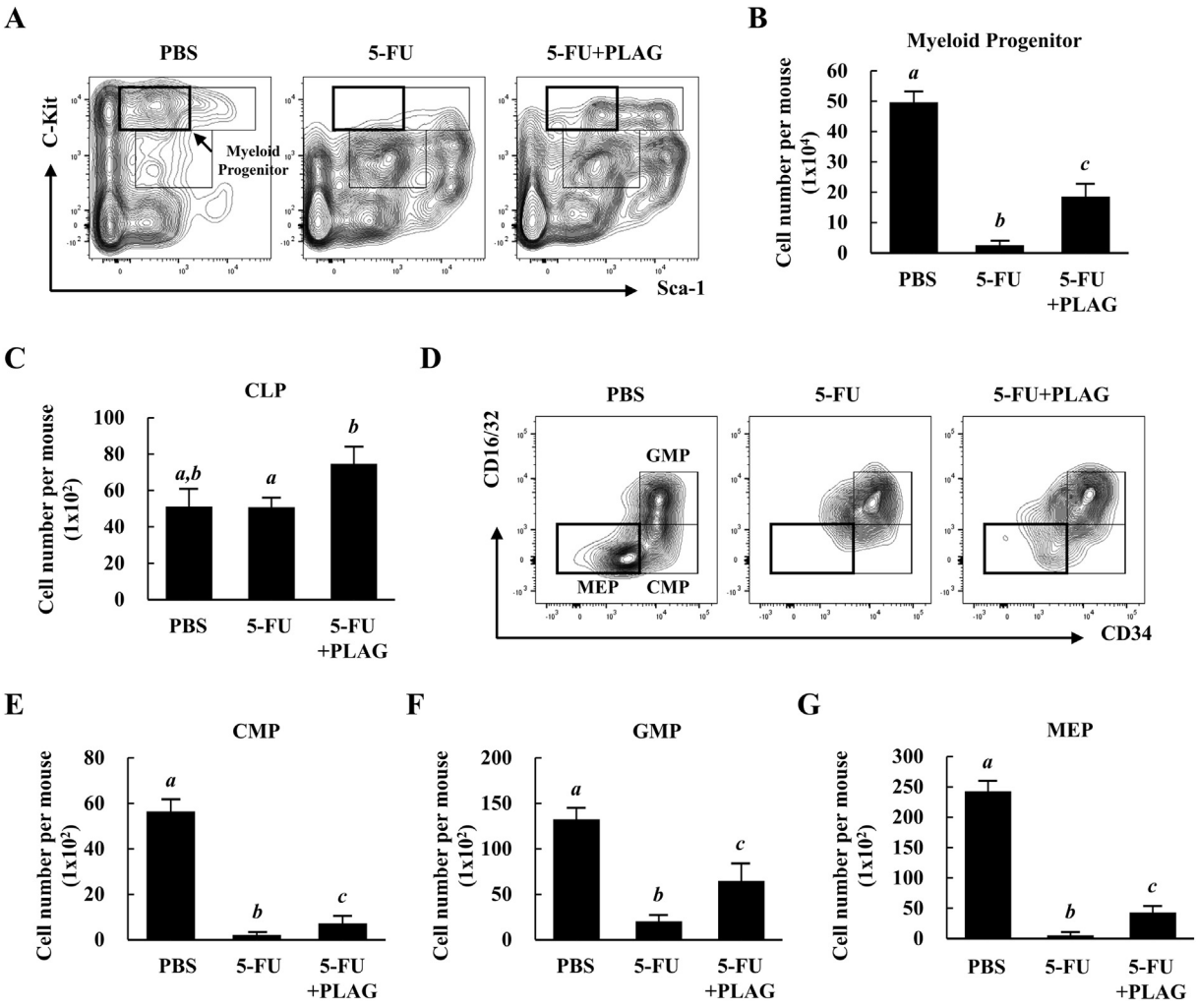

Fig. 3. 5-FU-induced MEP loss is decreased by PLAG treatment (n = 5 per group).
Mice were treated as described in Fig. 2A, and bone marrow-derived cells were analyzed for myeloid progenitor (Lin^- $Sca1^+$ $c\text{-}Kit^+$), CLP (Lin^- $Sca1^{low}$ $c\text{-}Kit^{low}$ $CD127^+$), CMP (Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD34^{low}$ $CD16/32^{low}$), GMP (Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD34^+$ $CD16/32^+$), and MEP (Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD34^-$ $CD16/32^-$) lineages (as described in Table 1) (A and D). Absolute numbers of hematopoietic progenitor subsets were represented as bar graphs (B, C, E, F and G). Numbers represent cells retrieved from tibias and femurs of each mouse. Data represent one experiment performed in triplicate. Each group contained five mice. Results are presented as mean ± s.e.m. Statistical analysis was performed by one-way ANOVA, followed by Duncan's *post hoc* test. Different letters (*a*, *b*, *c*) show values having significant difference at $p < 0.05$. The same letter represents groups that are not statistically significant.

Table 3
Hematological indices following 5-FU and PLAG treatment (n = 5 per group).

Frequency of total BM cells per mouse	PBS	5-FU	5-FU + PLAG
CLP (%)	0.11 ± 0.02 *a*	0.32 ± 0.03 *b*	0.27 ± 0.04 *b*
Myeloid Progenitor (%)	1.05 ± 0.03 *a*	0.17 ± 0.09 *b*	0.68 ± 0.17 *c*
CMP (%)	0.12 ± 0.01 *a*	0.01 ± 0.01 *b*	0.03 ± 0.01 *b*
GMP (%)	0.28 ± 0.01 *a*	0.13 ± 0.05 *b*	0.24 ± 0.09 *a*
MEP (%)	0.51 ± 0.02 *a*	0.04 ± 0.03 *b*	0.16 ± 0.04 *c*

Mice (n = 5 per group) were injected with 150 mg/kg 5-FU on day 0 and were daily administrated with 250 mg/kg PLAG as described in Fig. 2A. After 7 days, the frequency of hematopoietic lineage-negative cells (Lin^-) and progenitor populations was analyzed using specific markers as described in Table 1. Data represent one experiment performed in triplicate. Results are presented as mean ± s.e.m. Each group contained five mice. Statistical analysis was performed by one-way ANOVA, followed by Duncan's *post hoc* test. Different letters (*a*, *b*, *c*) show values having significant difference at $p < 0.05$. The same letter represents groups that are not statistically significant.

4. Discussion

We previously reported that gemcitabine-induced platelet loss in blood was significantly blocked by PLAG treatment in a mouse model of neutropenia [21]. In this study, we further confirmed this with other findings that PLAG treatment increased a cell population and the absolute cell counts of MEP in mice model of 5-FU-induced thrombocytopenia (Fig. 3G). The 5-FU-induced decreased circulating platelet numbers were also elevated in PLAG-treated mice (Fig. 4A and B). PLAG has the potential to stimulation of hematopoietic stem cells, especially megakaryocytes (Fig. 1). Because MEP differentiation was specifically increased during PLAG administration, the circulating platelets were also naturally affected as a result. In Yang HO, et al. study, PLAG treatment powerfully enhanced the colony forming ability of Lin^- $Sca1^+$ cells and stimulated the proliferation of megakaryocyte than 50 ng/ml of IL-11 treatment [19]. Collectively, these data indicate that PLAG may relieve thrombocytopenia via effects that facilitate the alleviation of chemotherapy-induced bone marrow cell loss; however, the molecular function of PLAG in the differentiation signaling pathway toward MEP remains unknown.

Platelets maintain homeostasis in the coagulation system and play a critical role in inflammation. These cells directly bind to pathogens via surface molecules, such as lectin, integrin, Toll-like receptor, and Fc receptors [24]. Activated platelets interact with leukocytes to modulate the immune response [25]. Thrombocytopenia patients have a relatively high incidence of sepsis, and mice with thrombocytopenia

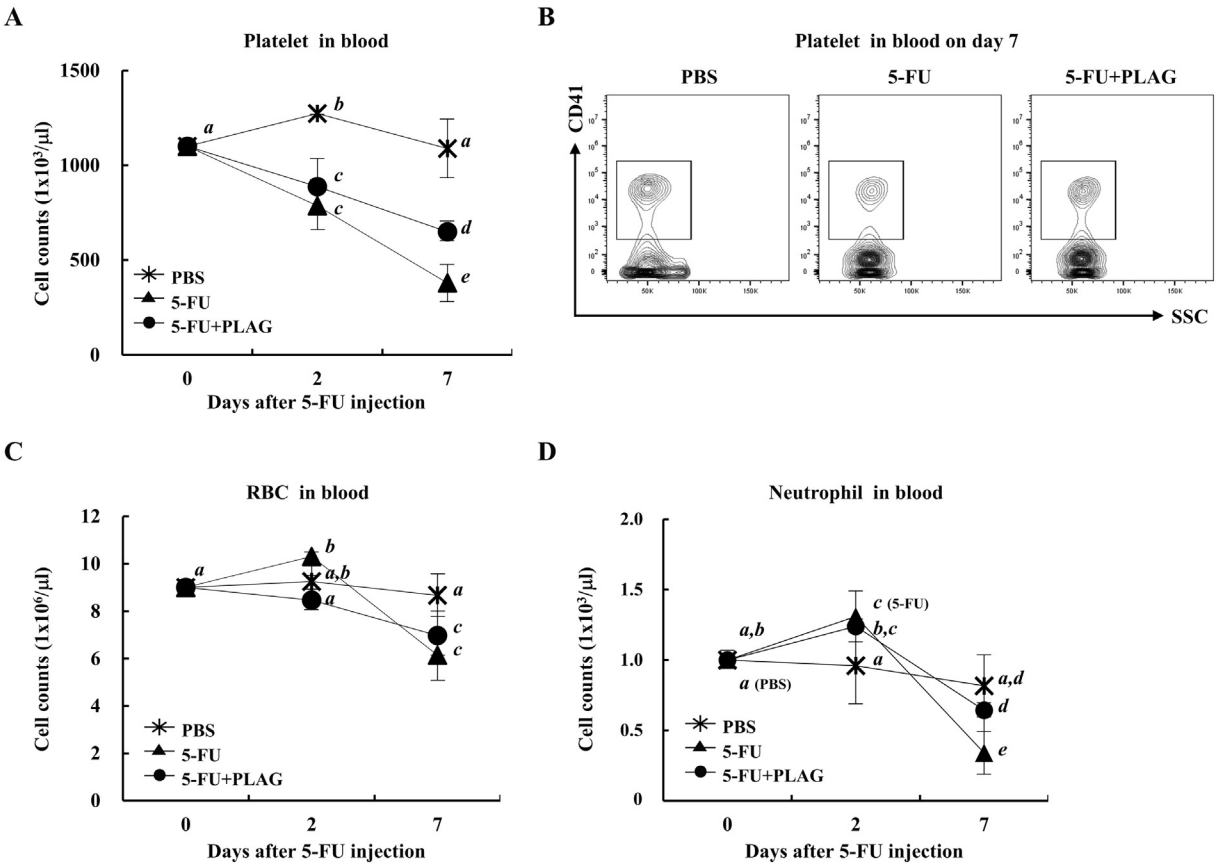

Fig. 4. 5-FU-induced thrombocytopenia is decreased by PLAG treatment (n = 5 per group).
The groups of mice were treated as described in Fig. 2A, and the circulating platelet, erythrocyte, and neutrophil in blood were counted (A, C, and D). X, control; ▲, 150 mg/kg 5-FU-treated group; ●, 250 mg/kg PLAG and 150 mg/kg 5-FU-treated group. Data represent one experiment performed in triplicate. Results are presented as mean ± s.e.m. Each group contained five mice. Statistical analysis was performed by one-way ANOVA, followed by Duncan's *post hoc* test. Different letters (*a*, *b*, *c*, *d*, *e*) show values having significant difference at $p < 0.05$. The same letter represents groups that are not statistically significant. Flow cytometry analysis was performed using rat anti-mouse CD41 antibodies for platelet detection in whole blood cells on day 7 (B). Data represent one experiment performed in triplicate.

frequently exhibit massive hemorrhage due to inflammation [26,27]. Because tumor-related inflammation can affect host metabolism and their cells may undergo apoptosis in a series, the number of circulating platelet is considered important in cancer patients [28].

Chemotherapy complications include thrombocytopenia, bone marrow suppression, neutropenia, oral mucositis and cachexia [29,30]. Bone marrow is the manufacturer and a supply center for red and white blood cells that support bodies of many animals including human. Therefore, bone marrow suppression leads to an increase in the risk of infection and leukemia. PLAG noticeably reduced 5-FU-induced bone marrow suppression (Fig. 2B). This induced the significant difference of the absolute cell numbers between 5-FU and PLAG/5-FU treated groups in all myeloid progenitor subsets (Fig. 3D–G). Besides, we previously reported that PLAG augments the therapeutic effect of pegfilgrastim on chemotherapy drug-induced neutropenia through regulation of neutrophil extravasation [21]. PLAG is considered to have an anti-inflammatory role in chemotherapy-induced oral mucositis, and, subsequently, accelerates the healing of ulceration and reduces inflammation [22]. The representative inflammatory cytokine IL-6 was increased by 5-FU/scratching, but were significantly reduced by PLAG administration. Moreover, several *in vivo* tests showed that PLAG administration does not induce side effects or toxicity [31], and a Phase 1 clinical trial by the Food and Drug Administration (FDA) was performed to assess the safety, tolerability, and pharmacokinetics of single-dose and multiple-dose oral administration of PLAG (EC-18) in healthy adult male volunteers (ClinicalTrials.gov Identifier: NCT02532712). Therefore, based on our findings, PLAG may successfully associated with anticancer drugs and augments their therapeutic effect on various side effects caused by chemotherapy.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2017.10.005.

Author contributions

JWK and HRL designed the project. HRL, NNY, and JSJ were performed *in vivo* assay and bone marrow cell sampling. Flow cytometry assay was carried out by HRL and NNY. KYS and SYY were analyzed the experiment data and reviewed the manuscript. HRL collected data and wrote the paper. SJC was edited the manuscript and modified data. JWK was supervised the study and reviewed the manuscript.

Conflict of interests

The authors have declared no conflicting interests.

Acknowledgments

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