



Research article

Hematopoietic progenitor cells specifically induce a unique immune response in dental pulp under conditions of systemic inflammation

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ABSTRACT

Teeth are exposed to various stimuli, including bacterial, thermal, and physical stimuli. Therefore, immune cells present in the normal dental pulp and the immune response to these stimuli have been studied. However, the relationship between systemic inflammation, such as that induced by viral infection, and changes occurring in dental pulp is not well known. This study aimed to investigate the immunological and hematological responses to systemic inflammation in dental pulp. Poly(I:C), a toll-like receptor 3 agonist, was injected into mice every two days to simulate a systemic inflammatory state in which type I interferon (IFN-I) was produced. The untreated normal state was defined as a steady state, and the states of acute and chronic inflammation were defined according to the period of administration. Changes in the abundance and dynamics of hematopoietic and immune cells in dental pulp, bone marrow and peripheral blood were quantitatively investigated in the steady state and under conditions of inflammation induced by IFN-I. We found that dental pulp in the steady state contained only a few hematopoietic cells, but a greater variety of immune cells than previously reported. B cells were also found in the steady state. An increase in multipotent progenitor cell levels was observed in the dental pulp during both acute and chronic inflammation. The increased multipotent progenitor cells in the dental pulp during acute inflammation tended to differentiate into the myeloid lineage. On the other hand, there was an influx of B cells into the dental pulp during chronic inflammation. These results revealed that a unique immune response is induced in the dental pulp by systemic inflammation, which would lead to a significant change in the perspective of dentists on the utility of dental pulp in the management of systemic diseases.

1. Introduction

Dental pulp has recently attracted attention as a source of stem cells due to the discovery of mesenchymal stem cells (MSCs) in dental pulp, and its clinical applications have been investigated [1, 2]. Many studies have been conducted using human dental pulp because it is relatively easy to obtain compared to that of animals. However, it is difficult to examine dental pulp under various conditions in humans. The major cell types that make up dental pulp include fibroblasts, odontoblasts, endothelial cells, Schwann cells, MSCs, and immune cells. An important role of dental pulp is to protect the teeth by inducing an appropriate immune

response to various stimuli and antigens. Macrophages, dendritic cells, and T cells are known to be present in the dental pulp at a steady state. The presence of natural killer (NK) cells, NK T cells, and small amounts of B cells has also been suggested [3]; however, it is considered that macrophages, dendritic cells, and T cells are mainly responsible for immune surveillance in the steady state and during early inflammation. Once pathogen invasion occurs, the innate immune response is initiated, and leukocytes are mobilized from the circulatory system to the site of infection. As inflammation progresses, the number of B cells increases in dental pulp [4, 5, 6, 7, 8]. Previous studies using human teeth have shown that dental pulp contains few hematopoietic stem and progenitor

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cells (HSPCs) that supply immune cells [9]. However, it has also been reported that cells derived from hematopoietic stem cells (HSCs) are present in the dental pulp, periodontal ligament, and alveolar bone, and that hematopoietic cells differentiate into dental tissue cells [10]. This raises a new possibility for the existence of HSPCs in dental pulp. In addition, some cell populations in dental pulp are important components of the hematopoietic niche in the bone marrow [11, 12, 13]. Therefore, we hypothesized that mouse dental pulp has the same unique proportion and distribution of hematopoietic and immune cells as bone marrow.

The bone marrow niche maintains HSCs, which are important sources of blood cells [14]. Hence, the changes that occur in the bone marrow during inflammation have been well investigated by administering lipopolysaccharide, polyinosinic:polycytidylic acid [poly(I:C)], and interleukin-1 β , or by knocking out the receptors for inflammatory

cytokines. As a result, it was found that in response to peripheral inflammation and infection, the proliferation of HSPCs in bone marrow increased, and cells with a biased differentiation tendency were produced to supply the cells necessary for the immune response [15]. In oral cavity, when systemic immunity is weakened due to infection or systemic disease, oral mucosal symptoms such as aphthous stomatitis, oral candidiasis, and xerostomia are likely to occur [16, 17]. However, the changes in dental pulp in the presence of systemic infection are unknown.

This study aimed to investigate the changes that occur in the dental pulp during systemic inflammation induced by type I interferon (IFN-1). This research will lead to a better understanding of the pathophysiology of inflammation in dental pulp and provide new insights for the advancement of pulp immunology as a platform for basic research on immunity and hematopoiesis in dental pulp.

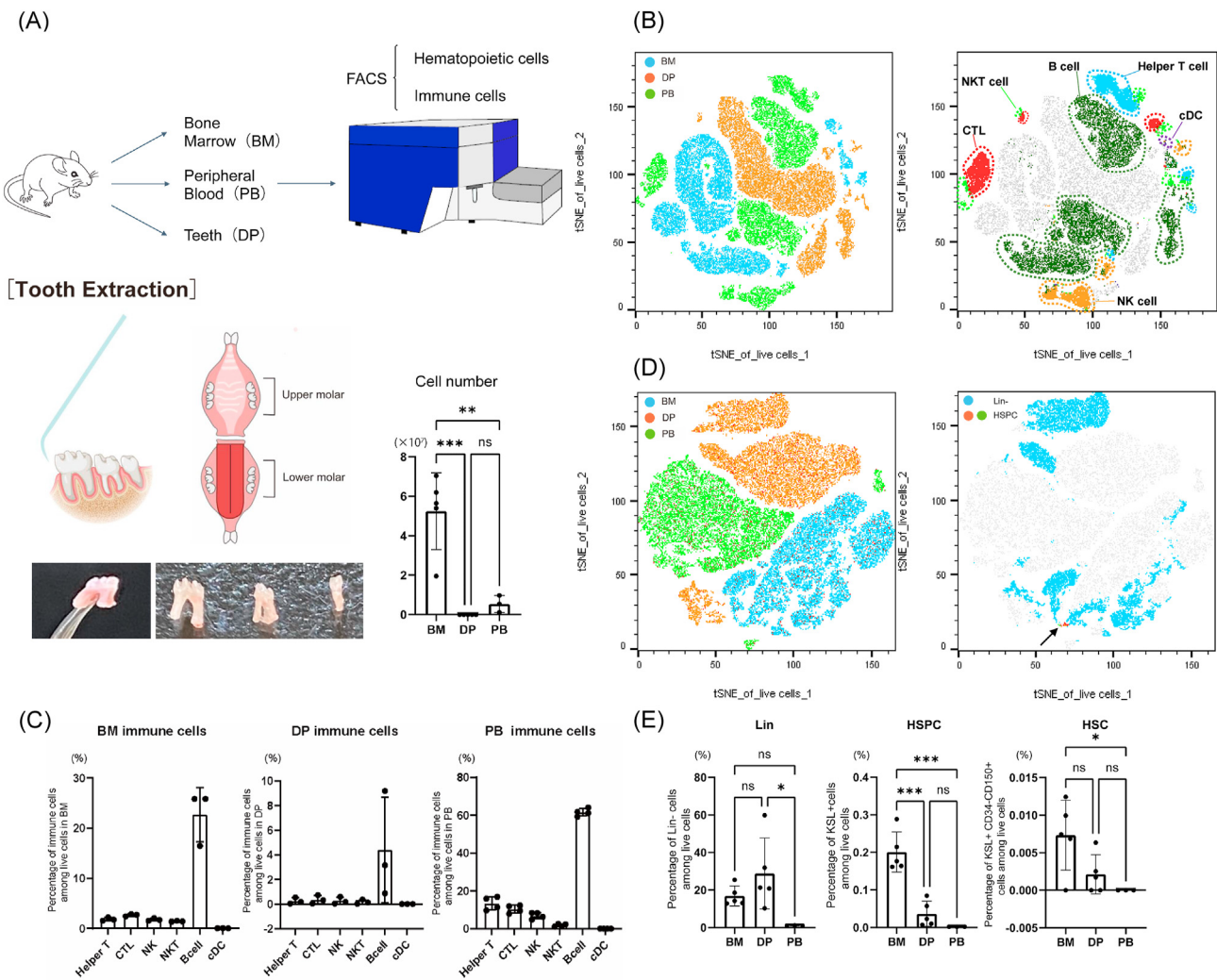


Figure 1. Various immune cells and hematopoietic cells exist in dental pulp. (A) Schematic diagram of the experiment and the method of tooth extraction. Tooth extraction was performed after carefully irrigating the surface of the tooth with PBS so as not to contaminate it with blood and other tissues. The graph shows the number of live bone marrow cells (BM), molar cells (DP), and peripheral blood cells (PB). Bars represent the mean \pm SEM. Significance was calculated using one-way analysis of variance. **denotes $P < 0.01$, ***denotes $P < 0.001$, and n.s. denotes not significant. (3–10 mice/group) This figure was created using BioRender.com. (B) The percentage of each immune cell present in bone marrow, dental pulp, and peripheral blood was examined via flow cytometric analysis. These data were subjected to t-SNE analysis. The figure on the left shows the total number of live cells among the three cell types: bone marrow (blue), dental pulp (orange), and peripheral blood (green). The figure on the right shows the distribution of immune cells in bone marrow, dental pulp, and peripheral blood. (C) Percentage of immune cells in bone marrow (BM), dental pulp (DP), and peripheral blood (PB) in the steady state. Bars represent the mean \pm SEM. (4–6 mice/group, $n = 10$). (D) The percentages of Lin-cells, hematopoietic progenitor and stem cells (HSPCs), and hematopoietic stem cells (HSCs) in bone marrow, dental pulp, and peripheral blood were examined via flow cytometric analysis. The figure on the left shows the total number of live cells among the three cell types: bone marrow (blue), dental pulp (orange), and peripheral blood (green). In the figure on the right, the blue area indicates Lin-cells and the arrow indicates HSPCs and HSCs in BM, DP, and PB. (E) Percentage of HSPCs in BM, DP, and PB in the steady state. Bars represent the mean \pm SEM. Significance was calculated using one-way analysis of variance. * $P < 0.05$, *** denotes $P < 0.001$, **** denotes $P < 0.0001$, and n.s. denotes not significant. (3–10 mice/group, $n = 13$).

2. Materials and methods

2.1. Mice

All experiments were conducted using female C57BL/6-CD45.2 mice strains, aged 7–9 weeks, which were purchased from Japan SLC, Inc. (Shizuoka, Japan). All mice were kept under specific-pathogen-free conditions with free access to food and water. Two to six mice were housed per cage in a temperature- and humidity-controlled room (temperature, 22.0 ± 2.0 °C; humidity, $55.0 \pm 15\%$; 12-hour light/dark cycle). All animal protocols were approved by the Animal Care and Use Committee of the Institute of Medical Science, University of Tokyo (approval no. PA19-03), and this study complied with the Animal Research: Reporting of In Vivo Experiments Guideline version 2.0. The upper and lower molars, pelvis, femur, tibia, and blood were collected from the mice and used in the experiment. Tooth extractions were performed using sharp dental explorers. The teeth were then irrigated with phosphate-buffered saline (PBS) to remove all

traces of blood, and the periodontal ligament was excised to the greatest extent possible. Peripheral blood was collected from the infraorbital vessels. The mice were randomly allocated to the poly(I:C)-treated and non-treated (control) groups. Age- and sex-matched mice underwent intraperitoneal injection with 10 $\mu\text{g/g}$ body mass of poly(I:C) (Tocris Bioscience, Canada) in PBS at 2-day intervals for up to 3 or 7 days.

2.2. Flow cytometry

Mouse bone marrow cells were flushed and isolated from the tibia, femur, and pelvis. Dental pulp cells were collected by crushing the upper and lower molars, carefully irrigating the pulp cavity, and crushing the pieces with PBS. Blood cells were collected by lysing blood, as described above. Hematopoietic cells, bone marrow cells, dental pulp cells, and peripheral blood cells were stained with a lineage antibody cocktail (biotinylated CD4, CD8, CD11b, CD45R, TER119, LY-6G, and CD127) before being stained with fluorescein isothiocyanate (FITC)-conjugated

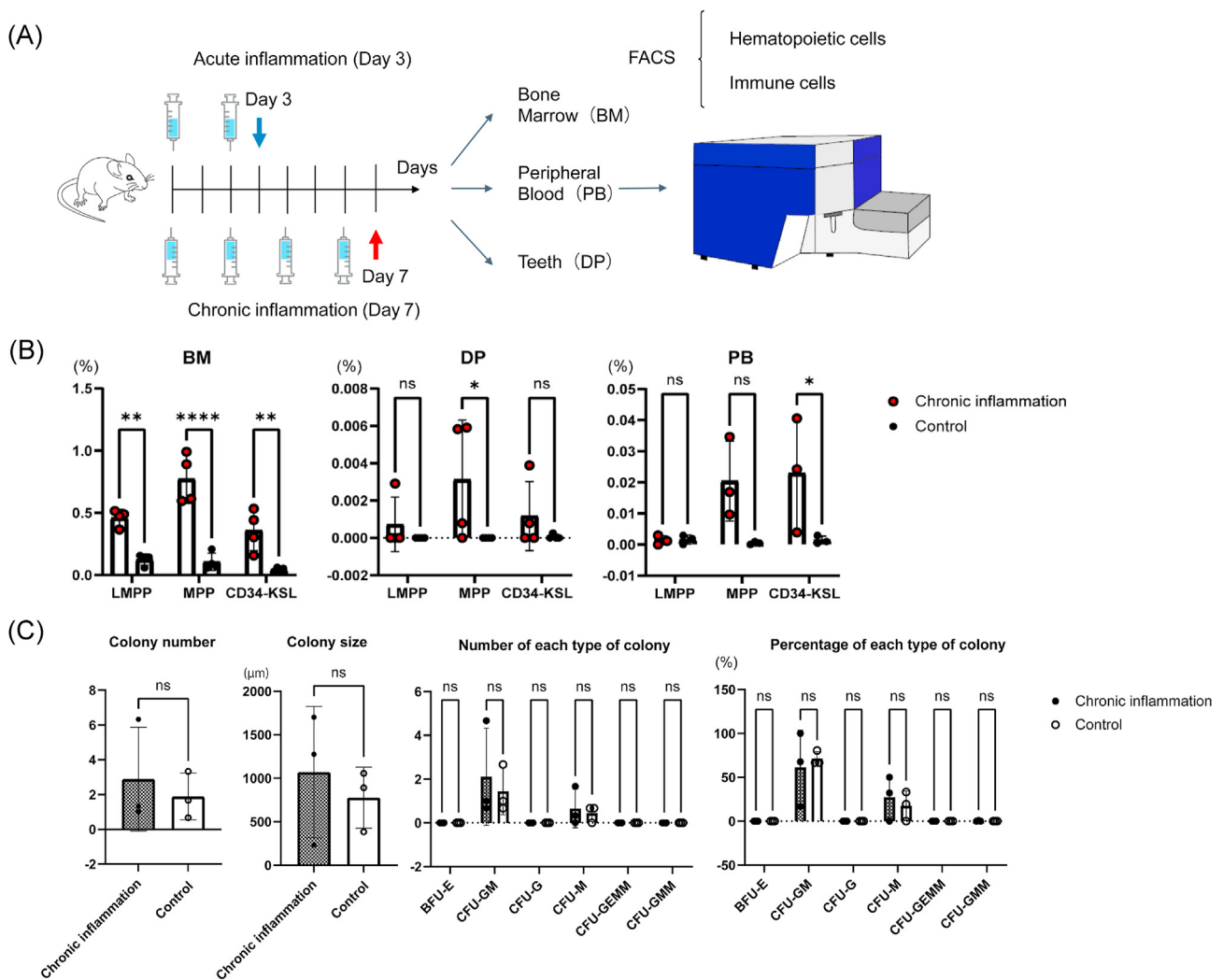


Figure 2. MPPs induced by chronic IFN- I exposure do not show active proliferation or differentiation in dental pulp. (A) Experimental design and time-course for acute and chronic poly(I:C) treatment. (B) Percentages of CD34-KSLs, multipotent progenitor cells (MPPs), and lymphoid-primed multipotent progenitor cells (LMPPs) in BM, DP, and PB of poly(I:C)-treated mice and non-treated mice at 24 h after chronic poly(I:C) treatment. Bars represent the mean \pm SEM. Significance was calculated using two-way analysis of variance. * denotes $P < 0.05$, ** denotes $P < 0.01$, **** denotes $P < 0.0001$, and n.s. denotes not significant. (6–8 mice/group, $n = 16$). (C) CFU assay using dental pulp cells of chronic poly(I:C)-treated and non-treated mice. The left two graphs show the number and size of colonies on day 12. The right two graphs show the number and percentage of burst-forming unit-erythroid (BFU-E); colony-forming unit-granulocyte, macrophage (CFU-GM); colony-forming unit-granulocyte, macrophage (CFU-G); colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM); and colony-forming unit-granulocyte, macrophage, megakaryocyte (CFU-GMM). Bars represent the mean \pm SEM. Significance was calculated using the unpaired t -test and two-way analysis of variance. * $P < 0.05$, *** denotes $P < 0.001$, **** denotes $P < 0.0001$, and n.s. denotes not significant. ($n = 18$).

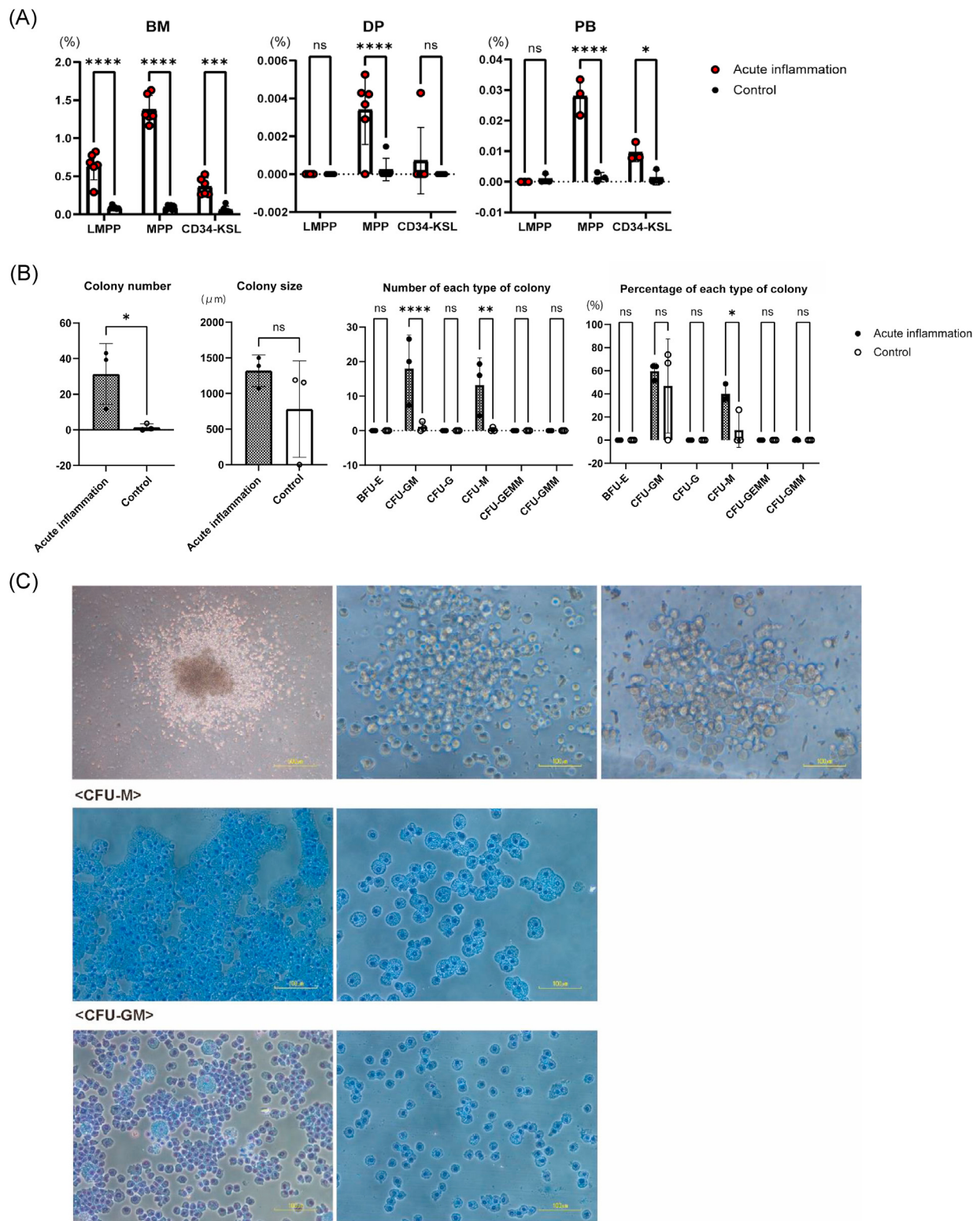


Figure 3. Acute IFN-I exposure induces myeloid progenitor cells in dental pulp. (A) Percentages of CD34-KSL, MPPs, and LMPPs in BM, DP, and PB of poly(I:C)-treated mice and non-treated mice at 24 h after acute poly(I:C) treatment. Bars represent the mean \pm SEM. Significance was calculated using two-way analysis of variance. * denotes $P < 0.05$, *** denotes $P < 0.001$, **** denotes $P < 0.0001$, and n.s. denotes not significant (6–12 mice/group, $n = 20$). (B) CFU assay using dental pulp cells of acute poly(I:C)-treated and non-treated mice. The left two graphs show the number and size of colonies on day 12. The right two graphs show the number and percentage of each type of colony. Bars represent the mean \pm SEM. Significance was calculated using the unpaired *t*-test and two-way analysis of variance. * denotes $P < 0.05$, ** denotes $P < 0.01$, *** denotes $P < 0.0001$, and n.s. denotes not significant ($n = 12$). (C) Representative image of a colony (left) ($\times 4$), (middle, right) ($\times 20$) and representative image of chromatic figures of CFU-M ($\times 20$) and CFU-GM ($\times 20$). The cells were round and their sizes varied according to the cell type and the degree of maturation.

anti-CD34, APC-conjugated anti-c-Kit, PE-conjugated anti-Sca-1 antibodies, and streptavidin-APC/eFluor 780 for 60 min. Where indicated, the cells were also stained with PE/Cyanine7-conjugated anti-CD150, PE/Cyanine7-conjugated anti-CD16/32, and Brilliant Violet 421-conjugated anti-CD135 antibodies. Immune cells were analyzed by staining bone marrow cells, dental pulp cells, and peripheral blood cells with

APC-eFluor780-conjugated anti-CD3e, PE/Cyanine7-conjugated anti-CD4, APC-conjugated anti-CD8, Brilliant Violet 510-conjugated anti-CD11b, PE-conjugated anti-CD11c, eFluor450-conjugated anti-CD45R, and FITC-conjugated anti-CD49b antibodies. T-distributed stochastic neighbor embedding (t-SNE) analysis was performed using FlowJo version 10.7.1 (BD Biosciences, NJ, USA).

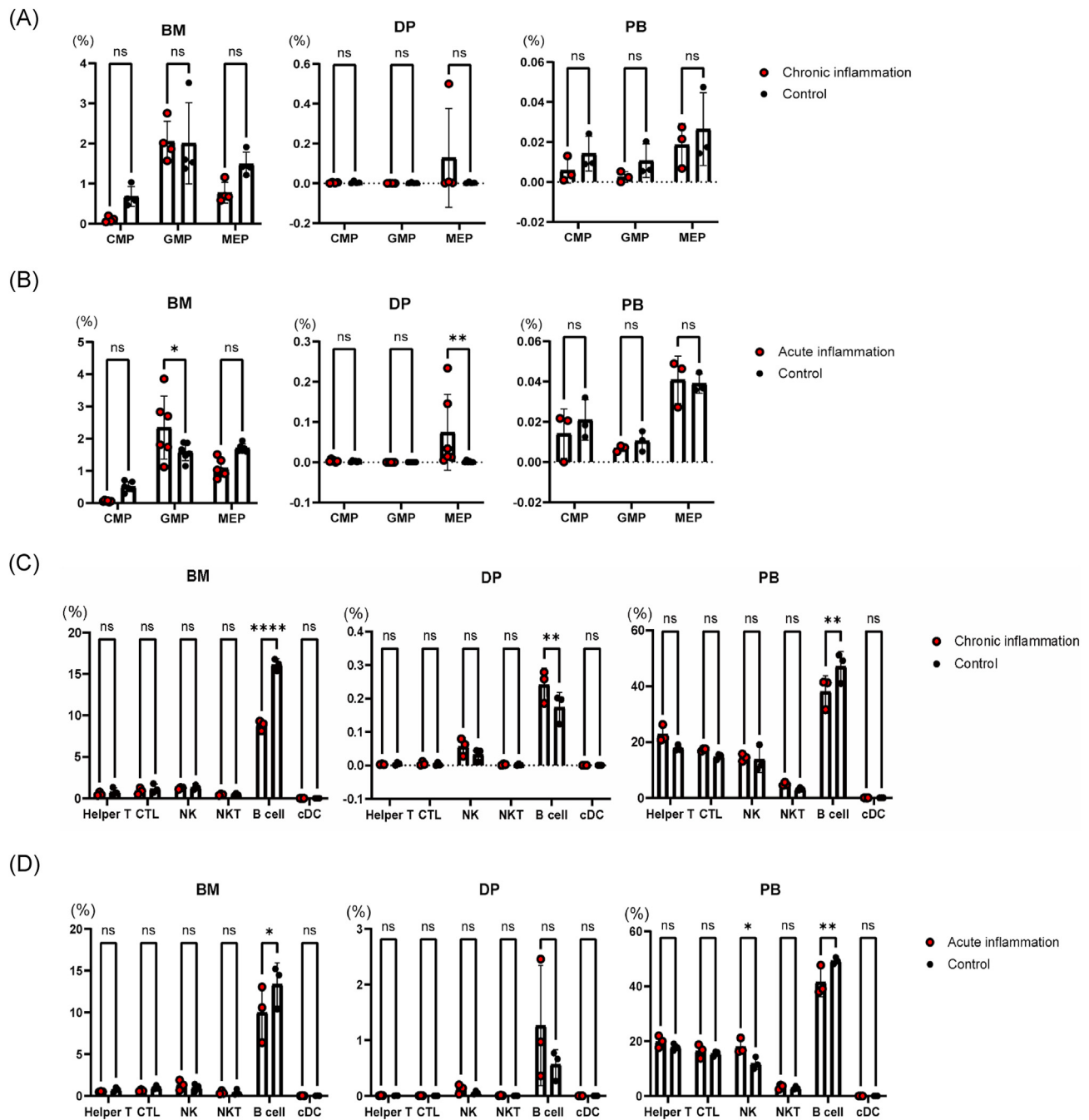


Figure 4. Inflow of B cells in pulp during chronic systemic inflammation. (A) Percentages of common myeloid progenitor cells (CMPs), granulocyte-monocyte progenitor cells (GMPs), and megakaryocyte-erythroid progenitor cells (MEPs) in BM, DP, and PB of poly(I:C)-treated mice and non-treated mice 24 h after chronic poly(I:C) treatment. Bars represent the mean \pm SEM. Significance was calculated using two-way analysis of variance. n.s. denotes not significant (6–8 mice/group). (B) Percentages of CMPs, GMPs, and MEPs in BM, DP, and PB of poly(I:C)-treated mice and non-treated mice at 24 h after acute poly(I:C) treatment. Bars represent the mean \pm SEM. Significance was calculated using two-way analysis of variance. * denotes $P < 0.05$, ** denotes $P < 0.01$ and n.s. denotes not significant (6–12 mice/group). (C) Percentage of immune cells in BM, DP, and PB of poly(I:C)-treated mice and non-treated mice at 24 h after chronic poly(I:C) treatment. Bars represent the mean \pm SEM. Significance was calculated using two-way analysis of variance. ** denotes $P < 0.01$, and n.s. denotes not significant (6 mice/group, $n = 12$). (D) Percentage of immune cells in BM, DP, and PB of poly(I:C)-treated mice and non-treated mice at 24 h after acute poly(I:C) treatment. Bars represent the mean \pm SEM. Significance was calculated using two-way analysis of variance. * denotes $P < 0.05$, ** denotes $P < 0.01$, and n.s. denotes not significant (6 mice/group, $n = 12$).

2.3. Colony-forming unit assay

Dental pulp cells were obtained from the molars of untreated mice and mice treated acutely or chronically with poly(I:C). A total of 2.8×10^4 pulp cells suspended in 300 μ L of Iscove's modified Dulbecco's medium supplemented with 2% fetal bovine serum was vigorously mixed with 4.1 ml of MethoCult (StemCell Technologies, Inc.; cat #03434) medium containing penicillin-streptomycin (final concentration 100 units/mL, 100 μ g/mL). Cells in MethoCult medium were plated in triplicate with 7×10^3 in sterile 35 mm Petri dishes. The dishes were incubated at 37 °C in an atmosphere containing 5% carbon dioxide. Colonies made up of at least 30 cells were scored using an inverted microscope on day 12.

2.4. Statistical analysis

Three or more biological replicates were used in each experiment, including the statistical analyses. The statistical analyses were performed as indicated in the figures using GraphPad Prism version 9.1.2 (GraphPad Software Inc., CA, USA). Analysis items with $P < 0.05$ were considered statistically significant.

3. Results

3.1. A unique proportion of hematopoietic and immune cells is present in dental pulp in the steady state

To accurately analyze the proportion of hematopoietic and immune cells in the dental pulp in the steady state, the cell population obtained by crushing the extracted teeth of mice and irrigating the pulp cavity was used as dental pulp cells. For comparison with dental pulp cells, bone marrow cells obtained from the pelvis, femur, and tibia, and peripheral blood cells were used to ensure that dental pulp cells could be successfully harvested without contaminating these cells. In this way, the ratio of immune cells and hematopoietic cells in dental pulp was analyzed, although the number of pulp cells in mice was much smaller than that obtained from the bone marrow and peripheral blood (Figure 1A). In this study, we focused on T cells (CD3+CD4+/
 CD3+CD8+), NK cells (CD3-CD49b++), NK T cells (CD3+CD49b+), dendritic cells (CD45R/B220-CD11b + CD11c++), and B cells (CD3-CD45R/B220+). The presence of these cells was examined by flow cytometry after which t-SNE analysis was performed. As a result of clustering immune cells in the bone marrow, dental pulp, and peripheral blood, most cells in the dental pulp were non-hematological cells; however, T cells, NK cells, NK T cells, and B cells were present in the dental pulp in the steady state (Figure 1B). Quantitative analysis of immune cells in each tissue showed a high ratio of B cells among the immune cells present in dental pulp, bone marrow, and peripheral blood (Figure 1C). To examine the hematopoietic cells present in the pulp, flow cytometric analysis followed by t-SNE analysis was performed in the steady state. However, clustering HSPCs in the bone marrow, dental pulp, and peripheral blood revealed that dental pulp contained very few HSPCs (Figure 1D). Quantitative comparisons showed that the presence of these hematopoietic cells in the dental pulp was not comparable to that in the bone marrow (Figure 1E). These results indicate that there is a greater variety of immune cells in the dental pulp in the steady state than previously reported. We also found that only a small number of hematopoietic cells was present in the dental pulp.

3.2. Multipotent progenitor cells with low proliferative capacity flow into dental pulp during chronic systemic inflammation

Poly(I:C) was injected into mice every 2 days to induce systemic inflammation, based on the report published by Pietras et al. [18]. The untreated normal group was defined as existing in a steady state. The group assessed on day 3 was considered as the acute IFN-I exposure group and defined as existing in a state of systemic acute inflammation. Similarly, the group assessed on day 7 was considered as the chronic IFN-I exposure group and defined as existing in a state of chronic inflammation. Changes in the proportion of hematopoietic cells in the dental pulp under these conditions were examined via flow cytometric analysis (Figure 2A). The results of the present study also showed significant increases in the levels of CD34-c-Kit + Sca-1+Lin-cells (CD34-KSLs), multipotent progenitor cells (MPPs), and lymphoid-primed MPPs (LMPPs) in the bone marrow in the presence of chronic

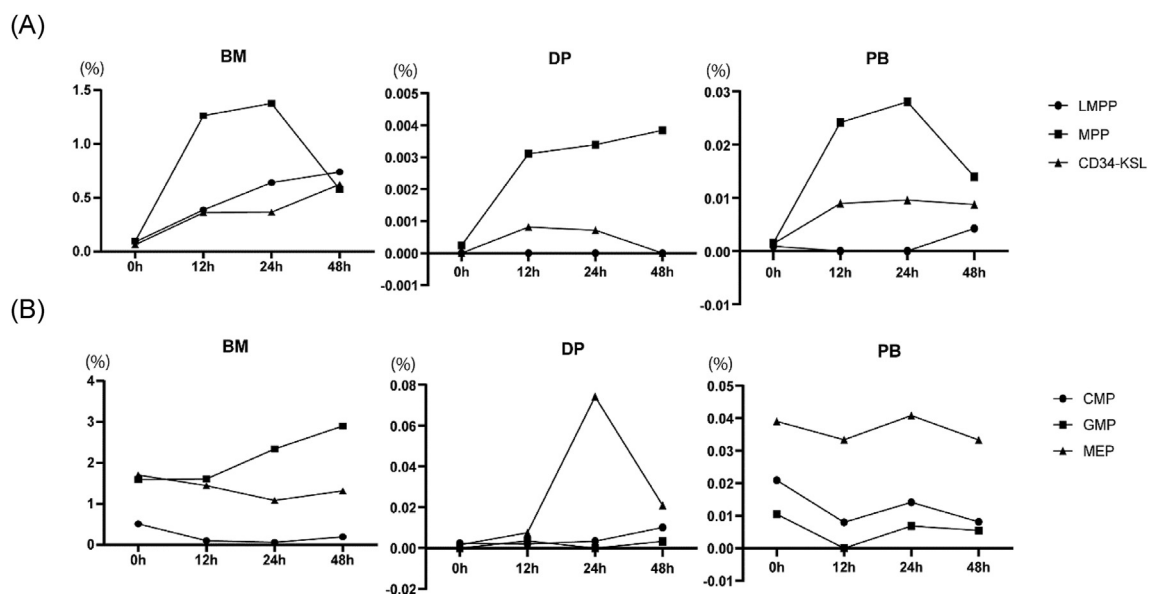


Figure 5. Dynamics of the percentage of hematopoietic stem and progenitor cells in the dental pulp under conditions of acute inflammation. (A) Dynamics of CD34-KSLs, multipotent progenitor cells (MPPs), and lymphoid-primed multipotent progenitor cells (LMPPs) in the BM, DP, and PB after acute IFN exposure (6–12 mice/time point). The values at 0 h indicate the non-inflammatory state. The values from 12 h to 48 h indicate the progress after the completion of administration. (B) Dynamics of common myeloid progenitor cells (CMPs), granulocyte-monocyte progenitor cells (GMPs), and megakaryocyte-erythroid progenitor cells (MEPs) in the BM, DP, and PB after acute IFN exposure (6–12 mice/time point). The values at 0 h indicate the non-inflammatory state. The values from 12 h to 48 h indicate the progress after the completion of administration.

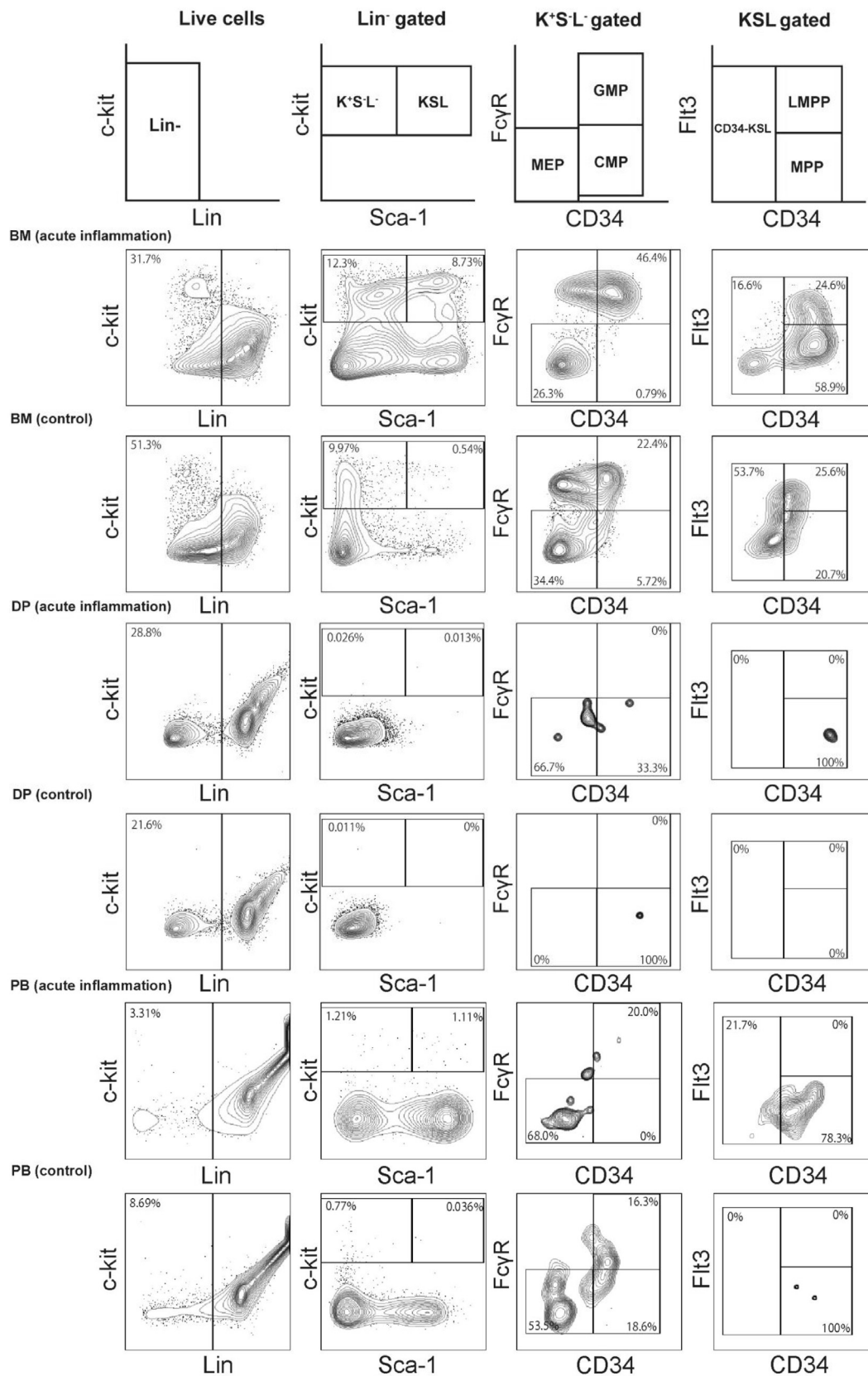


Figure 6. Representative flow cytometric data of hematopoietic stem and progenitor cell populations in the BM, DP, and PB of mice treated with poly(I:C) for 3 days (acute inflammation). Flow cytometric analysis was performed using BM, DP, and PB cells of poly(I:C)-treated mice (acute inflammation) and non-treated mice (control) to evaluate CD34-KSL and phenotypically defined MPPs, LMPPs, and myeloid progenitors (CMPs, GMPs, and MEPS).

inflammation. In peripheral blood, only CD34-KSL levels were significantly increased. In contrast, in the dental pulp, there was a significant increase in MPP levels ($P < 0.05$) (Figure 2B). A colony-forming unit (CFU) assay of dental pulp cells in the presence of chronic inflammation revealed that most colonies were composed of macrophages or a mixture of neutrophils and macrophages. However, the number of colonies was as low as that observed in the steady state, indicating that there were few MPPs in the activated state (Figure 2C).

3.3. The level of MPPs with a tendency toward myeloid differentiation increases in dental pulp in the presence of acute systemic inflammation

In acutely inflamed bone marrow, there were significant increases ($P < 0.05$) in the levels of CD34-KSLs, MPPs, and LMPPs, confirming that the response associated with inflammation occurred in the bone marrow, as previously reported [18]. In contrast, only CD34-KSL and MPP levels were significantly increased in peripheral blood, and only MPP levels were significantly increased in dental pulp ($P < 0.05$) (Figure 3A). Thus, the CFU assay of acutely inflamed dental pulp cells revealed a significant increase in the number of colonies ($P < 0.05$), unlike the results obtained among cells in the presence of chronic inflammation (Figure 3B). In addition, there was a significant increase in colonies consisting only of macrophages and mixed colonies consisting primarily of neutrophils and macrophages ($P < 0.05$) (Figure 3B and C). The results of the present study also showed that both acute and chronic inflammation caused HSPC outflow from the bone marrow to the peripheral blood and increased MPP levels in dental pulp. Hematopoietic progenitor cells observed in dental pulp during acute inflammation were found to be actively proliferating and capable of differentiating into myeloid cells.

3.4. B cell influx into the dental pulp during chronic systemic inflammation

Since MPP levels increase in dental pulp during systemic inflammation, we predicted that the levels of common myeloid progenitor cells (CMPs), granulocyte-monocyte progenitor cells (GMPs), and megakaryocyte-erythroid progenitor cells (MEPs) differentiated from MPPs would also increase. Changes in the levels of these cells before and after inflammation were quantitatively examined via flow cytometric analysis. No significant increases in the proportions of CMPs, GMPs, and MEPs were found in the bone marrow, peripheral blood, or dental pulp

during chronic inflammation (Figure 4A). In contrast, when cells obtained from each tissue were examined in the same way in the presence of acute inflammation, only GMP levels were significantly increased in the bone marrow. In dental pulp, there were no significant increases in CMPs or GMPs, contrary to the results of the CFU assay (Figure 4B). Furthermore, the changes in the proportion of immune cells associated with inflammation were examined via flow cytometry. In this study, there was also a significant downward trend in B cell levels in the bone marrow in the presence of chronic inflammation. The proportion of B cells in the peripheral blood also showed a significant decreasing trend. On the other hand, in dental pulp, there was a significantly higher influx of B cells ($P < 0.05$) (Figure 4C). Similarly, decreased B cell levels were observed in the bone marrow during acute inflammation. However, in dental pulp, there was only a slight increase in the level of each immune cell (Figure 4D).

4. Discussion

In the present study, we confirmed that systemic inflammation induces a unique immune response in dental pulp. The choice of teeth affects the results obtained when using mouse dental pulp for research because the characteristics of mouse incisors and molars differ. Mouse incisors are characterized by lifelong growth, which is made possible by MSCs located at the root tips. Thus, the foramen of the incisor root is enlarged, and the boundary between the alveolar bone and dental pulp is unclear, which makes it difficult to avoid contamination of bone marrow cells during pulp cell collection [12, 19]. Therefore, in the present study, mouse molars, whose structure is close to that of human teeth, were used to reflect the environment of human dental pulp. Teeth are surrounded by gingiva and alveolar bone, which can easily lead to contamination of the bone marrow and periodontal tissue during specimen collection. Mucosal and bone fragments attached to the extracted tooth were carefully removed, and the blood was washed off to minimize contamination.

We expected that dental pulp would have a microenvironment similar to that of bone marrow; however, this was not proven in the present study. Although periodontal tissues such as gingiva and jawbone are rich in HSPCs [20, 21], only very small amounts of HSPCs were found in the dental pulp. Previous reports have suggested that hematopoietic cells differentiate into fibroblasts, adipocytes, osteochondrogenic cells, and stromal cells [22, 23, 24, 25]. Normally, these cells differentiate from MSCs; however, a fraction of cells are known to derive from

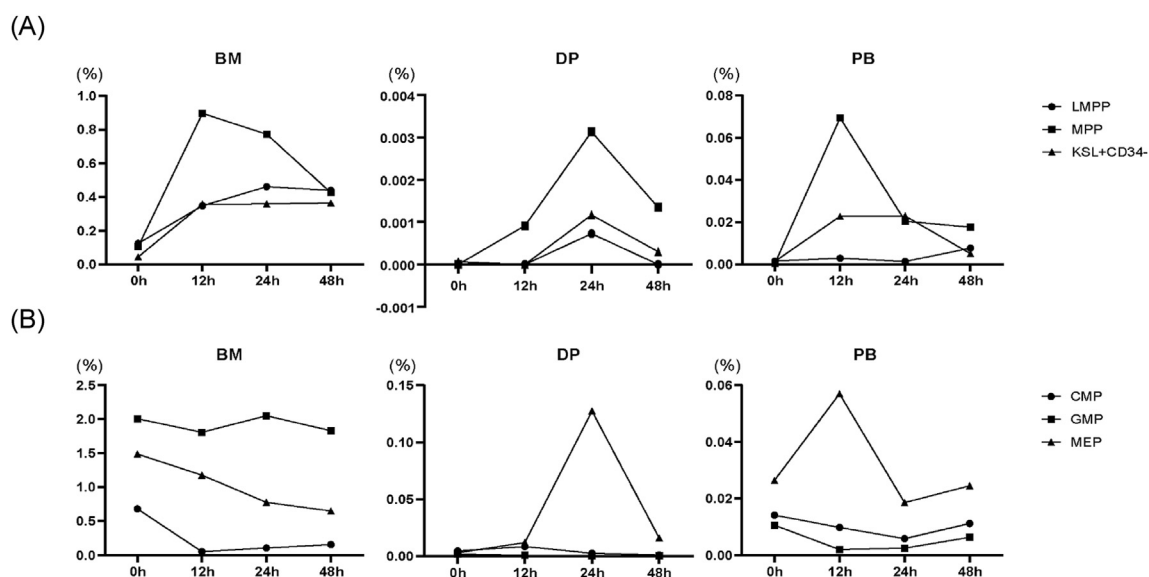


Figure 7. Dynamics of the percentage of hematopoietic stem and progenitor cells in the dental pulp under conditions of chronic inflammation. (A) Dynamics of CD34-KSLs, MPPs, and LMPPs in BM, DP, and PB after chronic IFN exposure. The values at 0 h indicate a non-inflammatory state (6–8 mice/time point). The values from 12 h to 48 h indicate the progress after the completion of administration. (B) Dynamics of CMPs, GMPs, and MEPs in BM, DP, and PB after chronic IFN exposure (6–8 mice/time point). The values at 0 h indicate the non-inflammatory state. The values from 12 h to 48 h indicate the progress after the completion of administration.

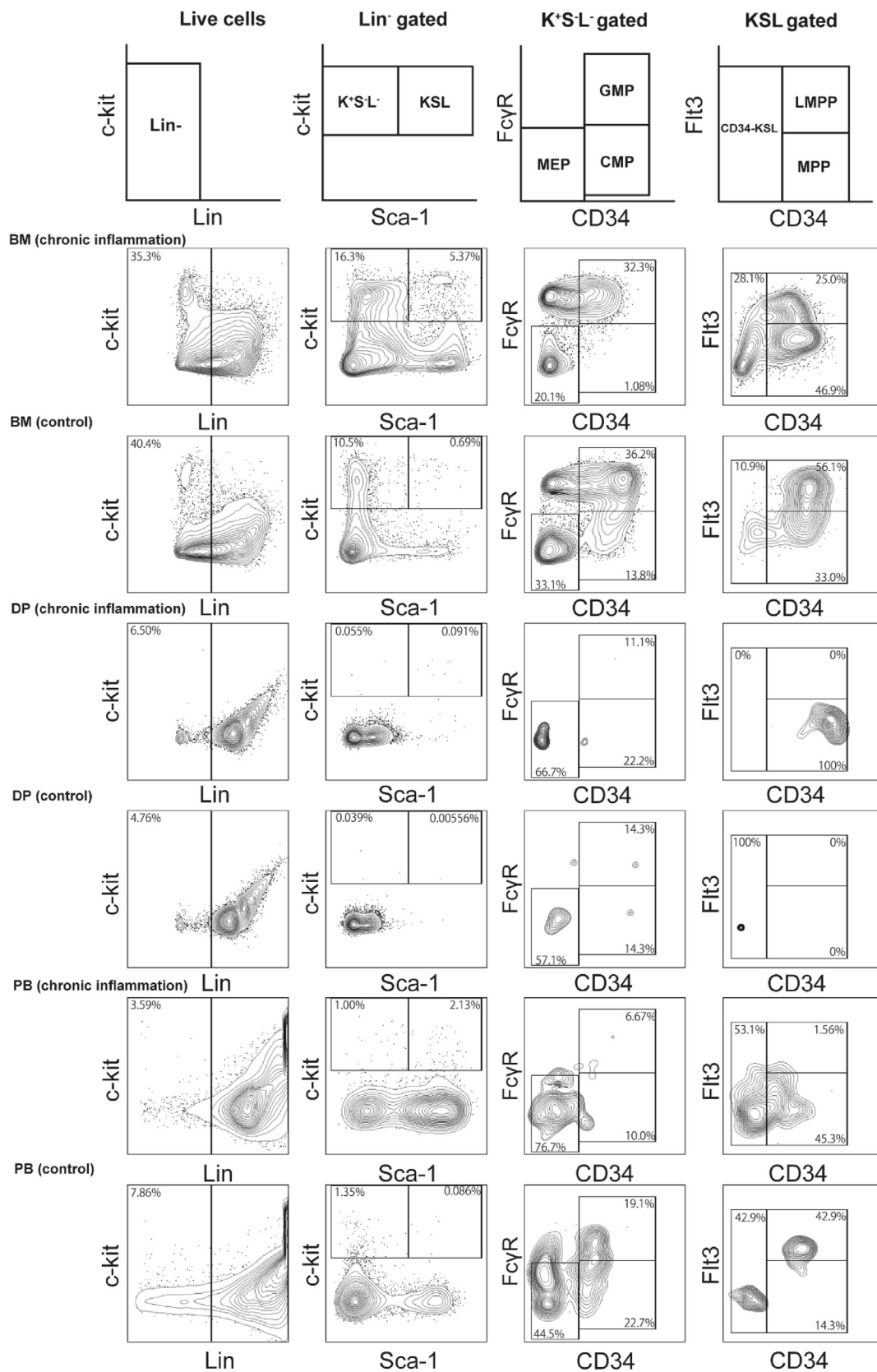


Figure 8. Representative flow cytometric data of hematopoietic stem and progenitor cell populations in the BM, DP, and PB of mice treated with poly(I:C) for 7 days (chronic inflammation). Flow cytometric analysis was performed using BM, DP, and PB cells of poly(I:C)-treated mice (chronic inflammation) and non-treated mice (control) to evaluate CD34-KSL and phenotypically defined MPPs, LMPPs, and myeloid progenitors (CMPs, GMPs, and MEPS).

hematopoietic cells. Some studies have suggested that monocyte/macrophage progenitors differentiate into adipocytes [23]; however, most studies did not identify the type of hematopoietic cells that can differentiate directly into cells of other lineages. Mehrotra et al. also reported that HSCs can differentiate into cells in pulp and 6–24% hematopoietic-derived cells were detected when cultured pulp cells were analyzed [10]. However, this indicates that a smaller number of hematopoietic-derived cells was present before culture and that the number of hematopoietic cells could also have been smaller. Therefore, our results could be consistent with their results and previous studies.

The oral cavity is invaded by many pathogens such as bacteria and viruses, and various external stimuli such as heat and biting forces are generated therein. Therefore, multiple receptors are expressed in dental pulp such that they can respond appropriately to all external pathogens. Toll-like receptor 2 (TLR2) and TLR4 respond to bacteria, and TLR3, TLR7, TLR8, and TLR9 respond to viruses [5, 26, 27]. Hepatitis and human immunodeficiency viruses can be detected in the dental pulp of affected patients. However, no studies have examined in detail the actual changes in the dental pulp [28, 29, 30]. It is well known that extramedullary hematopoiesis occurs outside the bone marrow under conditions of stress, inflammation, infection, and injury, as HSPCs gather in local tissues to replenish immune cells. Extramedullary hematopoiesis has been shown to occur in various tissues such as the skin, joints, bladder, peritoneum, pleura, testis, adrenal gland, and gastrointestinal tract [31]. The results of the present study indicate that extramedullary hematopoietic-like immunoreactivity can occur in the dental pulp in response to viral exposure, even without direct local infection.

Although we expected that the levels of progenitors such as CMPs and GMPs would also be increased, we detected no increase in the levels of these cells in the dental pulp. This finding resembles the response to IFN- γ -induced inflammation in the bone marrow. HSCs in the bone marrow of mice treated with poly(I:C) are known to rapidly enter the cell cycle via direct IFN- γ receptor (IFNAR) signaling and activation of signal transducer and activator of transcription 1 and AKT [32]. Consequently, during acute IFN- γ exposure, the mechanism that keeps cells in quiescence is temporarily suppressed, and transient HSC proliferation occurs in vivo. IFN- γ exposure also induces the reactivation of Sca-1 expression in myeloid progenitor cells, CMPs, GMPs, and MEPs [33]. In fact, the results of the present study also showed a tendency toward a decrease in the CMP fraction and a significant increase in the KSL fraction in bone marrow during acute inflammation (Figures 5 and 6). In dental pulp, there was an increasing trend in the expression of Sca-1, as well as in bone marrow, which could explain why CMP, GMP, and MEP levels were low, and MPP levels were significantly increased in the dental pulp (Figure 6). Despite these results, the CFU assay of pulp cells in the presence of acute inflammation showed an increase in the number of colonies of macrophages and neutrophils, which are descendants of CMPs and GMPs. This showed the possibility that a more differentiated population than GMPs, or a myeloid-biased cell population other than CMPs and GMPs, specifically entered or showed increased levels in dental pulp. In the classical model currently proposed, HSCs gradually lose their stem cell characteristics and differentiate into MPPs, which then differentiate into mature blood cells in each lineage. Although this is widely accepted, the heterogeneity of the HSPC population suggests that there are other differentiation pathways that do not fit the classical model [34, 35, 36]. One such pathway is the myeloid bypass pathway, in which myeloid-restricted repopulating progenitor cells contained in HSCs differentiate directly into myeloid blood cells, rather than undergo a stepwise differentiation process, as in the classical model. The MPP fraction also contains progenitor cells restricted to the myeloid lineage [37]. Additional studies are required to verify the presence of these cell populations in the dental pulp and validate the various possibilities.

In addition, there was a significant increase in B cell levels in the dental pulp during chronic inflammation, whereas the level of T cells, which are typically observed in acquired immunity, did not increase. Moreover, there was no significant increase in LMPP levels in dental

pulp, and the changes in each cellular component over time in bone marrow and peripheral blood under conditions of chronic inflammation did not match the cellular trends in dental pulp (Figures 7 and 8). The levels of granulocytes and B cells decrease in the bone marrow as a result of widespread and sustained IFNAR-dependent apoptosis. The IFNAR-dependent increase in serum levels of proinflammatory cytokines leads to a rapid decrease in lymphoid cell levels in the peripheral blood [18]. These IFNAR-dependent responses may be responsible for the differences in the cell types, proportions, and dynamics observed in the bone marrow, dental pulp, and peripheral blood. These results indicate the existence of a system that specifically induces B cells in the dental pulp during chronic systemic inflammation.

5. Conclusion

The present study offers a new perspective to previous reports by focusing on hematopoietic cells in dental pulp, which have been missed due to their low numbers. Dental pulp may have a specific immune response to systemic inflammation, suggesting the existence of a specific environment and immune system in the dental pulp. Clinically, this is a significant result that is expected to change the perspective of dentists because it revealed the relationship between dental pulp and systemic inflammation, which had not been previously studied. This study will help further our understanding of pulp immunology and provide new insights into dental pulp regenerative medicine.

Declarations

Author contribution statement

Julia Osaki: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Satoshi Yamazaki: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Atsuhiko Hikita and Kazuto Hoshi: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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References

- [1] J. Liu, F. Yu, Y. Sun, B. Jiang, W. Zhang, J. Yang, G.T. Xu, A. Liang, S. Liu, Concise reviews: characteristics and potential applications of human dental tissue-derived mesenchymal stem cells, *Stem Cell.* 33 (3) (2015) 627–638.
- [2] Y. Yamada, S. Nakamura-Yamada, R. Konoki, S. Baba, Promising advances in clinical trials of dental tissue-derived cell-based regenerative medicine, *Stem Cell Res. Ther.* 11 (1) (2020) 175.
- [3] N. Kawashima, I. Wongyaofa, N. Suzuki, H.N. Kawanishi, H. Suda, NK and NKT cells in the rat dental pulp tissues, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 102 (4) (2006) 558–563.

- [4] J.C. Farges, B. Alliot-Licht, E. Renard, M. Ducret, A. Gaudin, A.J. Smith, P.R. Cooper, Dental pulp defence and repair mechanisms in dental caries, *Mediat. Inflamm.* 2015 (2015) 230251.
- [5] H. Yumoto, K. Hirao, Y. Hosokawa, H. Kuramoto, D. Takegawa, T. Nakanishi, T. Matsuo, The roles of odontoblasts in dental pulp innate immunity, *Jpn. Dent. Sci. Rev.* 54 (3) (2018) 105–117.
- [6] M. Jontell, M.N. Gunraj, G. Bergenholtz, Immunocompetent cells in the normal dental pulp, *J. Dent. Res.* 66 (6) (1987) 1149–1153.
- [7] C.L. Hahn, W.A. Falkler Jr., M.A. Siegel, A study of T and B cells in pulpal pathosis, *J. Endod.* 15 (1) (1989) 20–26.
- [8] T. Izumi, I. Kobayashi, K. Okamura, H. Sakai, Immunohistochemical study on the immunocompetent cells of the pulp in human non-carious and carious teeth, *Arch. Oral Biol.* 40 (7) (1995) 609–614.
- [9] S. Aydin, F. Şahin, Stem cells derived from dental tissues, *Adv. Exp. Med. Biol.* 1144 (2019) 123–132.
- [10] K.R. Wilson, I.H. Kang, U. Baliga, Y. Xiong, S. Chatterjee, E. Moore, B. Parthiban, K. Thyagarajan, J.L. Borke, S. Mehrotra, K.L. Kirkwood, A.C. LaRue, M. Ogawa, M. Mehrotra, Hematopoietic stem cells as a novel source of dental tissue cells, *Sci. Rep.* 8 (1) (2018) 8026.
- [11] P. Pagella, L. de Vargas Roditi, B. Stadlinger, A.E. Moor, T.A. Mitsiadis, A single-cell atlas of human teeth, *iScience* 24 (5) (2021) 102405.
- [12] J. Krivanek, R.A. Soldatov, M.E. Kastriti, T. Chontorotzea, A.N. Herdina, J. Petersen, B. Szarowska, M. Landova, V.K. Matejova, L.I. Holla, U. Kuchler, I.V. Zdrilic, A. Vijaykumar, A. Balic, P. Marangoni, O.D. Klein, V.C.M. Neves, V. Yianni, P.T. Sharpe, T. Harkany, B.D. Metscher, M. Bajénoff, M. Mina, K. Fried, P.V. Kharchenko, I. Adameyko, Dental cell type atlas reveals stem and differentiated cell types in mouse and human teeth, *Nat. Commun.* 11 (1) (2020) 4816.
- [13] S. Pinho, P.S. Frenette, Haematopoietic stem cell activity and interactions with the niche, *Nat. Rev. Mol. Cell Biol.* 20 (5) (2019) 303–320.
- [14] S.J. Morrison, D.T. Scadden, The bone marrow niche for haematopoietic stem cells, *Nature* 505 (7483) (2014) 327–334.
- [15] T. Chavakis, I. Mitroulis, G. Hajishengallis, Hematopoietic progenitor cells as integrative hubs for adaptation to and fine-tuning of inflammation, *Nat. Immunol.* 20 (7) (2019) 802–811.
- [16] S.G. Fitzpatrick, D.M. Cohen, A.N. Clark, Ulcerated lesions of the oral mucosa: clinical and histologic review, *Head Neck Pathol.* 13 (1) (2019) 91–102.
- [17] S.R. Porter, V. Mercadante, S. Fedele, Oral manifestations of systemic disease, *Br. Dent. J.* 223 (9) (2017) 683–691.
- [18] E.M. Pietras, R. Lakshminarasimhan, J.M. Techner, S. Fong, J. Flach, M. Binnewies, E. Passegué, Re-entry into quiescence protects hematopoietic stem cells from the killing effect of chronic exposure to type I interferons, *J. Exp. Med.* 211 (2) (2014) 245–262.
- [19] Z. An, M. Sabalic, R.F. Bloomquist, T.E. Fowler, T. Streelman, P.T. Sharpe, A quiescent cell population replenishes mesenchymal stem cells to drive accelerated growth in mouse incisors, *Nat. Commun.* 9 (1) (2018) 378.
- [20] S. Krishnan, K. Wemyss, I.E. Prise, F.A. McClure, C. O'Boyle, H.M. Bridgeman, T.N. Shaw, J.R. Grainger, J.E. Konkel, Hematopoietic stem and progenitor cells are present in healthy gingiva tissue, *J. Exp. Med.* 218 (4) (2021).
- [21] N. Jiang, M. Chen, G. Yang, L. Xiang, L. He, T.K. Hei, G. Chotkowski, D.P. Tarnow, M. Finkel, L. Ding, Y. Zhou, J.J. Mao, Hematopoietic stem cells in neural-crest derived bone marrow, *Sci. Rep.* 6 (2016) 36411.
- [22] M. Ogawa, A.C. LaRue, C.J. Drake, Hematopoietic origin of fibroblasts/myofibroblasts: its pathophysiologic implications, *Blood* 108 (9) (2006) 2893–2896.
- [23] Y. Sera, A.C. LaRue, O. Moussa, M. Mehrotra, J.D. Duncan, C.R. Williams, E. Nishimoto, B.A. Schulte, P.M. Watson, D.K. Watson, M. Ogawa, Hematopoietic stem cell origin of adipocytes, *Exp. Hematol.* 37 (9) (2009) 1108–1120, 1120.e1-1120.
- [24] K.M. Gavin, T.M. Sullivan, J.K. Maltzahn, J.T. Rahkola, A.S. Acosta, W.M. Kohrt, S.M. Majka, D.J. Klemm, Hematopoietic stem cells produce intermediate lineage adipocyte progenitors that simultaneously express both myeloid and mesenchymal lineage markers in adipose tissue, *Adipocyte* 10 (1) (2021) 394–407.
- [25] M. Mehrotra, C.R. Williams, M. Ogawa, A.C. LaRue, Hematopoietic stem cells give rise to osteo-chondrogenic cells, *Blood Cells Mol. Dis.* 50 (1) (2013) 41–49.
- [26] M.J. Staquet, F. Carrouel, J.F. Keller, C. Baudouin, P. Msika, F. Bleicher, T.A. Kufer, J.C. Farges, Pattern-recognition receptors in pulp defense, *Adv. Dent. Res.* 23 (3) (2011) 296–301.
- [27] V. Pääkkönen, P. Rusanen, J. Hagström, L. Tjäderhane, Mature human odontoblasts express virus-recognizing toll-like receptors, *Int. Endod. J.* 47 (10) (2014) 934–941.
- [28] M. Glick, M. Trope, M.E. Pliskin, Detection of HIV in the dental pulp of a patient with AIDS, *J. Am. Dent. Assoc.* 119 (5) (1989) 649–650.
- [29] S. Hernández Viguera, M. Donoso Zúñiga, E. Jané-Salas, L. Salazar Navarrete, J.J. Segura-Egea, E. Velasco-Ortega, J. López-López, Viruses in pulp and periapical inflammation: a review, *Odontology* 104 (2) (2016) 184–191.
- [30] L.G. Siravenha, L.Q. Siravenha, L.D. Madeira, A.B. Oliveira-Filho, L.F. Machado, R.N. Martins Feitosa, A.C. Vallinoto, M.O. Ishak, R. Ishak, Detection of HCV persistent infections in the dental pulp: a novel approach for the detection of past and ancient infections, *PLoS One* 11 (10) (2016), e0165272.
- [31] J.L. Granick, S.I. Simon, D.L. Borjesson, Hematopoietic stem and progenitor cells as effectors in innate immunity, *Bone Marrow. Res.* 2012 (2012) 165107.
- [32] M.A. Essers, S. Offner, W.E. Blanco-Bose, Z. Waibler, U. Kalinke, M.A. Duchosal, A. Trumpp, IFN α activates dormant haematopoietic stem cells in vivo, *Nature* 458 (7240) (2009) 904–908.
- [33] E.M. Pietras, C. Mirantes-Barbeito, S. Fong, D. Loeffler, L.V. Kovtonyuk, S. Zhang, R. Lakshminarasimhan, C.P. Chin, J.M. Techner, B. Will, C. Nerlov, U. Steidl, M.G. Manz, T. Schroeder, E. Passegué, Chronic interleukin-1 exposure drives haematopoietic stem cells towards precocious myeloid differentiation at the expense of self-renewal, *Nat. Cell Biol.* 18 (6) (2016) 607–618.
- [34] E.M. Pietras, D. Reynaud, Y.A. Kang, D. Carlin, F.J. Calero-Nieto, A.D. Leavitt, J.M. Stuart, B. Göttgens, E. Passegué, Functionally distinct subsets of lineage-biased multipotent progenitors control blood production in normal and regenerative conditions, *Cell Stem Cell* 17 (1) (2015) 35–46.
- [35] F.K. Hamey, S. Nestorowa, S.J. Kinston, D.G. Kent, N.K. Wilson, B. Göttgens, Reconstructing blood stem cell regulatory network models from single-cell molecular profiles, *Proc. Natl. Acad. Sci. U. S. A.* 114 (23) (2017) 5822–5829.
- [36] E. Laurenti, B. Göttgens, From haematopoietic stem cells to complex differentiation landscapes, *Nature* 553 (7689) (2018) 418–426.
- [37] R. Yamamoto, Y. Morita, J. Oechara, S. Hamaoka, M. Onodera, K.L. Rudolph, H. Ema, H. Nakauchi, Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells, *Cell* 154 (5) (2013) 1112–1126.