様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 ２０ 日現在

機関番号：１２６０１
研究種目：若手研究
研究期間：2022～2023
課題番号：２２Ｋ１５４４８

研究課題名（和文）Mechanism of malaria-induced disruption of hematopoietic stem cell niche in the bone marrow

研究課題名（英文）Mechanism of malaria-induced disruption of hematopoietic stem cell niche in the bone marrow

研究代表者
ＬＥＥ　ＭＩＣＨＥＬＬＥ（Lee, Michelle Sue Jann）

東京大学・医科学研究所・特任助教

研究者番号：１０８２１４２３

交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：感染と炎症は、骨髄造血幹細胞（HSC）ニッチの損傷に寄与している可能性がある。本研究では、マラリアがサイトカインCXCL12を著しく高発現する間葉系幹細胞（CAR）細胞の変化を通して骨髄のBリンパ球ニッチを抑制することを見出した。CAR細胞集団の減少にもかかわらず、マラリアはHSPCの増殖を促進する。しかし、マラリアではHSPCの分化能のバランスが崩れる。CAR細胞と骨芽細胞の両方が抑制されることにより、骨髄におけるBリンパ球造血とB細胞の維持が弱まる。マラリア感染時の骨髄における既存の長寿命プラズマ細胞の減少は、体液性免疫の低下に寄与している可能性がある。

研究成果の学術的意義や社会的意義
This study reveals the effect of malaria on immune cell development in the bone marrow. We found that malaria reduced pre-existing long-lived plasma cells in the bone marrow, which raises the concern of impaired antibody responses against other infections after malaria infection.

研究成果の概要（英文）：Infection and inflammation may contribute to the damage of bone marrow hematopoietic stem cell (HSC) niches. In this study, we found that malaria suppresses B lymphocytic niche in the bone marrow through the alteration of CXCL12-abundant reticular (CAR) cells. Despite the decreased of CAR cell population, malaria enhances the expansion of hematopoietic stem and progenitor cells (HSPCs). However, the differentiation potential of HSPCs is imbalance during malaria. The suppression of both CAR cells and osteoblasts dampens B lymphopoiesis and the maintenance of B cells in the bone marrow. The decreased of pre-existing long-lived plasma cells in the bone marrow during malaria infection may contribute to compromised humoral immunity(Lee MSJ et al., 2024, International Immunology).

研究分野：マラリア免疫学

キーワード：Malaria　Bone marrow　Hematopoiesis　B cells

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Bone marrow consists of hematopoietic stem cells (HSC) that give rise to immune cells. The bone marrow mesenchymal stromal compartments play important roles to provide HSC niches for hematopoiesis to make blood cells and immune cells, as well as to provide lymphocytic niche for B cell development and maintenance of long-lived plasma cells to fight diseases (Sugiyama et al., 2018). CXCL12-abundant reticular (CAR) cells surrounding the sinusoids are the major cellular component of perivascular HSC niches, that are in close contact with HSC and B cell progenitors (Nagasawa T, 2015). Stresses from infection and inflammation may alter or damage the HSC niches in the bone marrow (Nombela-Arrieta and Isringhausen, 2017). We previously reported that the retention of the byproduct of *Plasmodium* parasites within the bone marrow causes chronic inflammation-induced osteoporosis in mice (Lee et al., 2017). Other studies showed evidences that malaria causes compromised immunity and rapid declined in antibody responses against previous vaccinations (Banga et al., 2015). Hence, we hypothesize that malaria may affect host immunity through the alteration of HSC niche in the bone marrow.

２．研究の目的

This study aims to evaluate the effects of malaria on hematopoiesis and maintenance of immune cells in the bone marrow. This study further aims to address the mechanism that causes alteration of CAR cells that maintain the HSC and lymphocytic niches in the bone marrow during malaria infection.

３．研究の方法

To examine the effects of malaria on bone marrow HSC and lymphocytic niches, bone marrow cells from *Plasmodium yoelii* Non-Lethal (*Py*NL)-infected mice on day 0 (uninfected), 15 (peak infection) and 30 (recovered) were isolated, and gene expressions were evaluated by quantitative PCR. Using CXCL12-GFP transgenic mouse, histology of femurs was performed to visualize the distribution of CAR cells and the structure of sinusoide vessels in the bone marrow. 3D imaging of thick bone sections cleared by TDE method was performed using light sheet fluorescence microscopy. To examine the effects of malaria on CAR cell populations in the bone marrow, CAR cell populations were examined by flow cytometry method at a time course manner. To evaluate the alteration of CAR cells during malaria infection, CAR cells were sorted by flow cytometry and gene expressions of transcription factors, chemokine, cytokine and fibrotic markers were examined by quantitative PCR. To examine the effects of malaria on subsets of hematopoietic cells in the bone marrow, flow cytometry was performed to examine the populations of HSC, multipotent progenitor cells (MPPs), erythroid progenitors, myeloid cells, granulocytes, T cell subsets, B cell progenitors, short-

lived plasmablasts and long-lived plasma cells in the bone marrow. Antibody-secreting cells were examined using ELISPOT assay. T cell activation and IFNγ production in bone marrow were also examined. The role of IFNγ on CAR cell and B cell development in the bone marrow were also examined using CXCL2-GFP expressing IFNγR knockout mice.

4．研究成果

This study revealed that malaria infection depletes CAR cells in the bone marrow, causing the reduction of CXCL12 chemokine and IL-7 cytokine which are crucial for the retention and development of B cells within the bone marrow. 3D imaging of CAR cell distribution and structure of sinusoid vessels showed vasodilation and CAR cell populations were reduced during acute malaria infection (Figure 1). We also found malaria causes the atypical upregulation of Sca1 expression on CAR cells. However, the upregulation of Sca1 on CAR cells did not affect the gene expressions of CAR cell markers such as *Foxc1*, *Ebf3*, *Kitl*, *Cxcl12* and *Il7*, and did not induce the fibrotic marker *Col1a1*, *Col3a1* and *Col6a3*. Although malaria infection enhances the expansion of hematopoietic stem and progenitor cells in the bone marrow, we found that there was a bias in the differentiation potential of multipotent progenitros (MPPs), which skew towards MPP2 (megakaryocyte / erythrocyte potential) and MPP3 (granulocyte / myeloid potential), while suppressing MPP4 (lymphocyte potential). Further analysis of erythroid development stages confirmed increased erythropoiesis and myelopoiesis, while lymphopoiesis is suppressed including common lymphoid progenitor (CLP), pre-B cells, immature B cells, mature B cells, and plasma cells. We found that malaria induces an increase of activated CD4 T cells with increased IFNγ production in the bone marrow. The increase of CD4 T cells and CD8 NKT cells in the bone marrow are IFNγR-dependent. The upregulation of Sca1 on CAR cells is IFNγ-dependent, however, the depletion of CAR cells and B cell subsets in the bone marrow is IFNγ-independent (Lee et al., 2024).

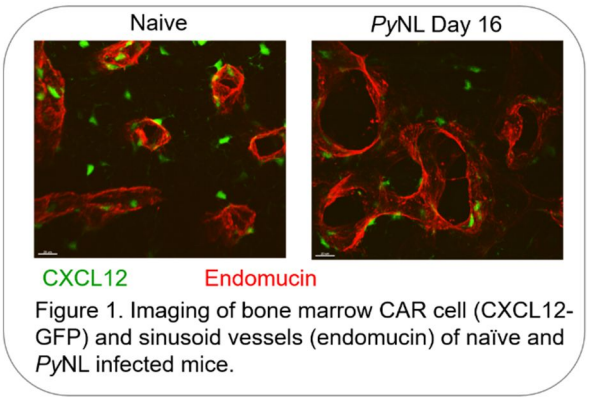

Figure 1. Imaging of bone marrow CAR cell (CXCL12-GFP) and sinusoid vessels (endomucin) of naïve and *Py*NL infected mice.

In this study, we found that CAR cells and most B cell populations in the bone marrow recovered after recovery from malaria infection, despite the accumulation of *Plasmodium* products in the bone marrow after recovery from infection. These findings suggest the need to properly diagnose and treat asymptomatic malaria cases which are common in humans to prevent compromised humoral immune response to vaccinations. This work is published in International Immunology journal (Lee et al., 2024).

＜引用文献＞

1. Banga, S., J.D. Coursen, S. Portugal, T.M. Tran, L. Hancox, A. Ongoiba, B. Traore, O.K. Doumbo, C.-Y. Huang, J.T. Harty, and P.D. Crompton. 2015. *PLoS One*. 10:e0125090.

2. Lee, M.S.J., K. Maruyama, Y. Fujita, A. Konishi, P.M. Lelliott, S. Itagaki, T. Horii, J. wen Lin, S.M. Khan, E. Kuroda, S. Akira, K.J. Ishii, and C. Coban. 2017. *Sci. Immunol.* 2:eaam8093.
3. Lee, M.S.J., J. Matsuo-Dapaah, C. Del Rosario Zorrilla, Y. Omatsu, T. Nagasawa, S. Uemura, A. Iwama, K.J. Ishii, and C. Coban. 2024. *Int. Immunol.* dxae012. doi:10.1093/intimm/dxae012.
4. Nombela-Arrieta, C., and S. Isringhausen. 2017. *Front. Immunol.* 7:1-12.
5. Sugiyama, T., Y. Omatsu, and T. Nagasawa. 2018. *Int. Immunol.* 31:5-11.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　1件）

１．著者名 Lee Michelle Sue Jann、Matsuo Dapaah Julia、Del Rosario Zorrilla Camila、Omatsu Yoshiki、Nagasawa Takashi、Uemura Shun、Iwama Atsushi、Ishii Ken J、Coban Cevayir	４．巻 36
２．論文標題 Acute malaria suppresses the B lymphocytic niche in the bone marrow through the alteration of CXCL12-abundant reticular cells	５．発行年 2024年
３．雑誌名 International Immunology	６．最初と最後の頁 dxae012
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/intimm/dxae012	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

〔学会発表〕　計2件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Michelle Sue Jann Lee
２．発表標題 Effects of malaria on bone marrow hematopoietic stem cell niches
３．学会等名 第15回寄生虫感染免疫研究会
４．発表年 2023年

１．発表者名 Michelle Sue Jann Lee
２．発表標題 Effects of malaria on bone marrow hematopoietic stem cell niches
３．学会等名 第51回日本免疫学会学術集会
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関