様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Distinct roles of IL-27 produced by macrophages and dendritic cells in shaping the immune response against Plasmodium parasites

研究課題名（英文）Distinct roles of IL-27 produced by macrophages and dendritic cells in shaping the immune response against Plasmodium parasites

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研究成果の概要（和文）：樹状細胞（DC）とマクロファージは、血液期マラリアにおけるマラリア原虫感染と疾患の重症度を制限するための効果的な適応免疫応答の誘導において重要な細胞です。これらの細胞は、適応免疫の誘導と拡大を制御するインターロイキン-27（IL-27）などのサイトカインを産生します。私たちは、血液期マラリア感染におけるDCとマクロファージによって産生されるIL-27の役割を調査しました。私たちの結果は、DCによって産生されるIL-27が初期の寄生虫血症の増加に関与し、抗原特異的反応の発達を優先的に抑制するのに対し、マクロファージによって産生されるIL-27は疾患の進行を防ぐことを示唆しました。

研究成果の学術的意義や社会的意義
本研究によって、マクロファージにより産生されるIL-27がマラリアの進行を阻止する一方で、樹状細胞により産生されるIL-27がマラリアに対する抗原特異的免疫応答を制御していることを示した。これらの発見は、マラリア原虫感染に対する炎症性免疫応答と抗炎症性免疫応答のバランスを維持する宿主免疫機構の理解に役立つと考えられます。

研究成果の概要（英文）：Malaria is one of the main global burdens, estimating over 200 million cases and 400 thousand deaths occurring annually. Decades of research to develop a vaccine against malaria have yet to establish a highly efficacious vaccine. Therefore, understanding the immune response against malaria is essential.
Dendritic cells (DCs) and macrophages are critical cell types in innate immunity and in the induction of effective adaptive immune responses to limit Plasmodium parasite infection and disease severity during blood-stage malaria. These cells produce cytokines including interleukin-27 (IL-27), which regulate the induction and expansion of adaptive immunity. We investigated the role of IL-27 produced by DC and macrophages in blood-stage malaria infection. Our results suggested that IL-27 produced by DCs involved initial increase of parasitemia and preferentially suppressed development of antigen specific response, while IL-27 produced by macrophages prevent disease progression.

研究分野：Malaria

キーワード：Plasmodium　Interleukin-27　myeloid cells

様　式　Ｃ－１９、Ｆ－１９－１（共通）

１．研究開始当初の背景

Malaria remains one of the most significant global health challenges, with an over 200 million cases and 400 thousand deaths occurring annually. Despite substantial progress in reducing the incidence and mortality rates over the past two decades, malaria continues to pose a severe threat, particularly to children under five and pregnant women. Therefore, unrevealing immune response against malaria is crucial to develop better treatment options.

Interleukin-27 (IL-27) is a heterodimeric cytokine of IL-12 family, which is composed of two subunits: Epstein-Barr virus-induced gene 3 (EBI3) and p28. It signals through a receptor complex consisting of WSX-1 (also known as IL-27Rα) and glycoprotein 130 (gp130). It is primarily produced by antigen-presenting cells, such as dendritic cells and macrophages, in response to microbial stimuli. IL-27 is a critical cytokine in the immune response to malaria, with both protective and pathogenic roles. Its ability to regulate inflammation and influence T cell differentiation makes it a key player in the host's defense against Plasmodium parasites. However, its dual nature necessitates a nuanced approach in considering IL-27-targeted therapies for malaria. We addressed the roles of cytokines derived from innate immune cells in determining disease outcome and shaping adaptive immune response and provide critical information for the development of prophylactic and therapeutic strategies.

２．研究の目的

1. To determine the differential roles of IL-27 produced by DCs and macrophages in the immune modulation during Pcc infection.

2. To determine functional necessity of IL-27 produced by DCs and macrophages for disease control and development of immune memory against *Plasmodium* infection.

３．研究の方法

We generated and used mice lacking IL-27 in DCs (IL27p28$^{fl/fl}$CD11c-Cre) and macrophages (IL27p28^{flfl}LysM-Cre) with C57BL/6 background and IL27p28$^{fl/fl}$ mice as control. *Plasmodium chabaudi chabaudi* used as an infection model. p28$^{fl/fl}$CD11c-Cre, p28$^{fl/fl}$LysM-Cre and control mice were infected with Pcc, and spleen cells analyzed for the activation phenotypes by flow cytometry. Intracellular cytokine production of spleen cells examined upon in vitro stimulation by flow cytometry and cytokine productions will be measured by sandwich ELISA after 48 hours of in vitro stimulation.

４．研究成果

Our data showed IL-27 in the spleen during blood-stage of malaria infection was increased in association with parasitemia, and main cellular sources for this cytokine were innate cells (Fig 1).

DCs are the major antigen-presenting cells for the activation of adaptive immunity, and macrophages are crucial effector cells to control parasitemia. We hypothesized that IL-27 produced by these cells have differential roles in the regulation of immune responses during *Plasmodium* infection. We generated mice lacking IL-27 in DCs or in macrophages and compared the response of these mice to the infection with Pcc. Mice lacking IL-27 in DCs showed delay in the clearance of parasitemia than that in control mice during

the acute phase of the infection (Fig 2).

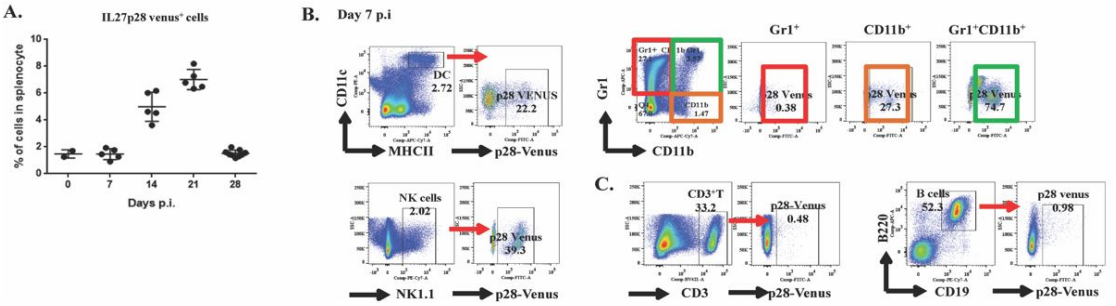

Fig 1. p28-Venus expressing reporter mice were infected with $5x10^4$ iRBC Pcc by i.p. injection. p28-Venus expression in the spleen cells were analyzed every week after infection. (A) The frequency of p28-Venus expressing cells in the spleen. Cells were gated on live cells. (B) Representative dot plots of p28-Venus expressing innate cells in the spleen at day 7 post infection. Cells were gated on $CD3^-$ $CD19^-$ to exclude T and B cells. DC: $CD11c^+MHCII^{hi}$; Macrophage: $Gr1^-CD11b^+$; Monocyte/Neutrophil: $Gr1^+$/ $Gr1^+C11b^+$; NK: $NK1.1^+$ (C) Representative dot plots of p28-Venus expression in $CD3^+$ T cells and mature B cells at day 7 post infection.

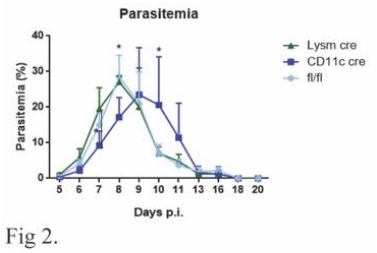

Fig 2.

We further examined antigen specific response development in early and late phase of *Plasmodium* infection. The frequencies of effector $CD4^+$ T and $CD8^+$ T cells were higher in both knockout mice compared to control group in acute phase of infection. However, the frequencies of antigen experienced activated $CD4^+$ T cells ($CD11a^{hi}CD49d^{hi}$) were comparable. There was no difference in IFN-γ producing $CD4^+$ T cells which plays pivotal role in limiting infection, but tissue IFN-γ level was much higher in mice lacking IL-27 in DCs (Fig 3).

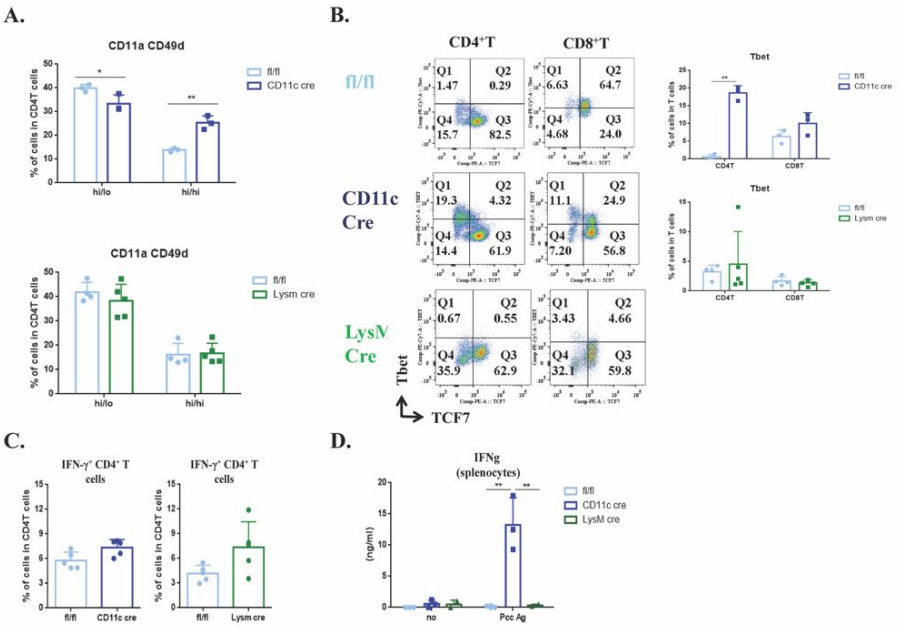

Fig 3. CD11c-Cre, LysM-Cre mice and control $IL27p28^{fl/fl}$ mice were infected $5x10^4$ iRBC Pcc by i.p. injection and spleen cells were analyzed at day 6 p.i.
(A) The frequencies of effector $CD4^+$ and $CD8^+$ T cells populations. (B) Representative dotplots of antigen experienced activated ($CD11a^{hi}CD49d^{hi}$) $CD4^+$ T cells shown. (C) The frequencies of IFN-γ producing $CD4^+$ T cells in the spleen at day 6 p.i. are shown. (D) **)** The spleens were collected at day 6 post infection and the concentration of IFN-γ in the spleen lysate was determined by ELISA.

At chronic phase those antigen experienced cells were higher in mice lacking IL-27 in DCs than those in control mice (Fig 4). Transcription factor T-bet expression Th1 cells are critical in memory formation by

producing IFN-γ, which enhances the long-term immunity and facilitates the rapid response upon re-exposure to intracellular pathogens. We analyzed T-bet expressing $CD4^+$ T and $CD8^+$ T cell populations. The frequencies of T-bet expressing $CD4^+$ T cells were significantly higher in mice lacking IL-27 in DCs than those in control mice, which did not observe in mice lacking IL-27 in macrophages. IFN-γ producing $CD4^+$ T cells were comparable between knockout group and control group. However, splenocytes from mice lacking IL-27 in DCs produced much higher IFN-γ in response to *Plasmodium* antigen.

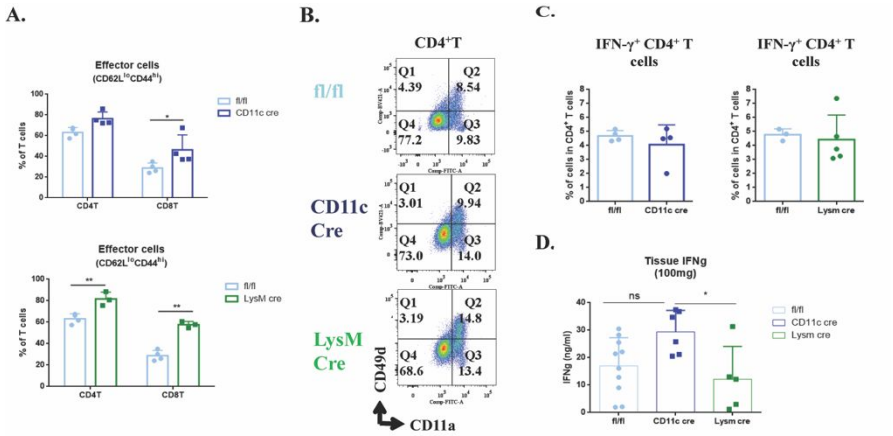

Fig 4. CD11c-Cre, LysM-Cre mice and control $IL27p28^{fl/fl}$ mice were infected $5x10^4$ iRBC Pcc by i.p. injection and spleen cells were analyzed at day 35 p.i.
(A) The frequencies of antigen experienced activated ($CD11a^{hi}CD49d^{hi}$) $CD4^+$ T cells and activated ($CD11a^{hi}CD49d^{lo}$) $CD4^+$ T cells are shown. (B) Profiles of transcriptional factors are shown. Dot plots are representative results and the bar graphs show the frequency of $Tbet^+$ T cells. (C) The frequencies of IFN-γ producing $CD4^+$ T cells in the spleen at day 35 p.i. are shown (D) The spleens were collected at day 35 p.i.. $3x10^5$ splenocytes were stimulated with Pcc crude antigen for 48 hours. Supernatants were collected and the concentration of IFNg was determined by ELISA.

The result showed difference antigen specific response, we evaluated generation of *Plasmodium* specific antibodies and germinal center cells. The strength and functionality of germinal centers are vital for producing high-quality, diverse, and long-lasting antibody responses. We found that frequencies of $CD4^+$ T follicular cells were much higher in mice lacking IL-27 in DCs. Also, $GL7^+$ germinal center B cells frequencies were higher in mice lacking IL-27 in DCs compared to control mice. Following with these results, we found significantly higher amount of *Plasmodium* specific serum antibodies only in mice lacking IL-27 in DCs.

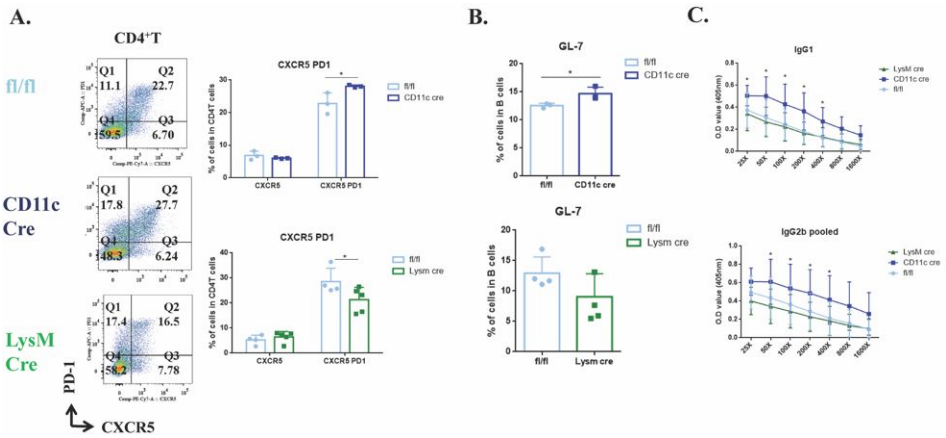

Fig 5. $IL27p28^{fl/fl}$CD11c-Cre, $IL27p28^{fl/fl}$LysM-Cre and control $IL27p28^{fl/fl}$ mice were infected with $5x10^4$ iRBC Pcc by i.p. injection. **(A)** The frequencies of T follicular $CD4^+$ T cells at day 35 p.i. are shown. **(B)** The bar graphs show $GL\text{-}7^+$ B cells in total B mature ($B220^+CD19^+$) cells. **(C)** Pcc crude antigen specific antibodies are examined in the serum collected at day 35 p.i.

Discussion: The immune response to *Plasmodium* infection is complex and involves a coordinated interaction among various cell types, including dendritic cells (DCs), macrophages, monocytes, and natural killer (NK) cells. These cells are key producers of interleukin-27 (IL-27), a cytokine that plays multifaceted roles during the course of the infection. Understanding the distinct contributions of IL-27 from different immune cells can provide valuable insights into the dynamics of host-pathogen interactions and the regulation of immune responses.

During the acute phase of Pcc infection, IL-27 production by DCs appears to be crucial in modulating the early stages of parasitemia. IL-27 from DCs contributes to the initial increase in parasite load, which might seem counterintuitive as an immune response generally aims to control and reduce infection. However, this increase could be a strategic immune regulation where IL-27 suppresses the development of an overly aggressive antigen-specific response. This suppression might prevent excessive inflammation and tissue damage, ensuring that the host can sustain the initial phase of infection without severe pathology.

On the other hand, macrophages produce IL-27 with a protective role that becomes evident as the infection progresses. IL-27 from macrophages prevents disease progression, suggesting its involvement in the resolution phase of the infection. This protective role might be attributed to the cytokine's ability to modulate the immune response, enhancing pathogen clearance while simultaneously preventing immunopathology. By regulating inflammation and promoting effective immune responses, IL-27 produced by macrophages ensures that the host can clear the infection and recover.

The differential roles of IL-27 in DCs and macrophages underscore the complexity of immune regulation during *Plasmodium* infection. In DCs, IL-27 might act to balance the immune response, preventing early hyper-activation that could be detrimental to the host. This regulatory function is crucial during the acute phase when the immune system needs to be tightly controlled to avoid collateral damage. Conversely, in macrophages, IL-27 facilitates a more direct protective role, aiding in the containment and eventual elimination of the parasite, thus preventing chronic infection and associated complications.

Moreover, IL-27's ability to suppress antigen-specific responses when produced by DCs may have significant implications for the development of adaptive immunity. This suppression could delay the activation of T cells and other adaptive immune components, allowing the innate immune response to dominate initially. Once the acute phase is managed, the adaptive immune system can be activated more effectively and in a controlled manner, ensuring a robust and long-lasting immune memory.

In conclusion, IL-27 plays dual roles in the immune response against *Plasmodium* infection, with its effects varying depending on the producing cell type. In DCs, IL-27 modulates early parasitemia and suppresses excessive antigen-specific responses, while in macrophages, it prevents disease progression and aids in pathogen clearance. These findings highlight the importance of cell-specific cytokine production in shaping the immune response and provide potential targets for therapeutic interventions in malaria and other infectious diseases. Understanding these mechanisms further can aid in the development of strategies to manipulate IL-27 pathways, potentially improving outcomes in malaria and other similar infections.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　0件）

１．発表者名 Ganchimeg Bayarsaikhan, Shin-Ichi Inoue, Kazumi Kimura, Hiroki Yoshida, Masahiro Yamamoto and Katsuyuki Yui
２．発表標題 Distinct roles of IL-27 produced by innate cells in shaping the immune response against Plasmodium parasite
３．学会等名 The 51st Annual Meeting of the Japanese Society for Immunology
４．発表年 2022年

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２．発表標題 Interleukin-27 produced by dendritic cells involved in antigen specific response during chronic stage of plasmodium infection
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２．発表標題 Myeloid derived interleukin-27 involvement in immune response to malaria
３．学会等名 The 92nd Annual Meeting of the Japanese Society for Parasitology
４．発表年 2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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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