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研究課題名（和文）Molecular mechanisms of bone-specific immunopathology during malaria

研究課題名（英文）Molecular mechanisms of bone-specific immunopathology during malaria

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研究成果の概要（和文）：マラリアに感染することによって、免疫が強く活性化され、マラリア原虫の生成物の骨髄への侵入が誘導されることで、骨の恒常性が阻害されることを明らかにしました。本チームは、マウスマラリアモデルを用いて、一度のマラリア感染であっても、マラリア原虫生成物が徐々に骨髄ニッチに蓄積し、骨吸収を行う細胞である破骨細胞によって「食べられる」ことを示しました。本研究によって、マラリア感染者において長期的かつ持続的骨量減少の危険性が明らかとなり、また、マラリア治療においては骨量減少に対する治療もあわせて行う必要があることがわかりました。

研究成果の学術的意義や社会的意義
Our results highlight the risk of bone loss in malaria-infected patients. Bone loss is sustained even after recovery from malaria.Therapies promoting bone health like Vitamin D treatment coupled with anti-malarial treatment may help to improve bone health in malaria-infected individuals.

研究成果の概要（英文）：Malaria has deadly complications; however, the long term pathological consequences of chronic malaria infection are poorly understood. We suspected that there is an association between growth retardation and malaria infection. Therefore, we investigated possible negative impact of malaria infection on bone remodeling and growth. Using mouse models mimicking chronic and self-clearing Plasmodium infection we showed that infection causes significant and long term bone loss in adult mice and growth retardation in young mice. Bone remodeling is completely suppressed during the acute phase of infection, but is highly activated immediately after the clearance of parasites, with increased osteoclastic activity skewing the balance toward bone resorption. Osteoclasts are activated by the key osteclastogenic cytokine RANKL, which was upregulated in osteoblasts through MyD88-dependent inflammation, triggered by the accumulation and long term persistence of parasite products in the bone marrow.

研究分野：Immunoparasitology

キーワード：Bone loss　osteoclast　osteoblast　malaria　　Vitamin D　RANKL　MyD88　Plasmodium

１．　研究開始当初の背景

Malaria is a deadly disease affecting millions each year with its deadly as well as chronic, unforeseen complications. People living in malaria-endemic regions are repeatedly infected and acquire partial immunity over the years. Although this partial immunity prevents them from developing severe complications, however, they remain with a persistent, low parasite density in the blood. There is little known about the long-time complications of this condition in the entire community of endemic areas. We hypothesized that rather than the parasite burden itself, the complications induced by the dysregulated immune responses and the tissue damage done by the parasites and their products can cause chronic and irreversible sufferings.

２．　研究の目的

Recent evidence suggests that there is physical growth retardation in young children in Africa which might be associated with infectious diseases, particularly with high incidence of malaria infection. Furthermore, an increased incidence of porous bone lesions have also been reported in malaria endemic regions, suggesting infection may compromise bone integrity. Despite the importance of bone tissue in health and development, and the known interaction between *Plasmodium* parasites and bone marrow cells whereby parasites circulate, reside and infect, the pathology of malaria in bone is poorly understood. Therefore, we aimed to address the direct effect of malaria infection on bone and bone environment..

３．　研究の方法

We have designed this study to characterize the effects of malaria infection on bone by using various *Plasmodium spp.* (*Py*NL, *Pcc*, *Pb*A, and *Pb*AΔpm4Δbp2 parasites) in mice. Age- and gender-matched littermates of the same strain were used in each experiment. Mice were sacrificed on acute, convalescence and the chronic phases of the infections and bone morphometric analysis were performed. The ethanol fixed femurs were scanned by three-dimensional micro-computed tomography to detect bone loss. For the treatment of bone loss, different doses of alfacalcidol (VitD3 analog) were administered orally.

In vitro bone marrow osteoclasts and calvarial osteoblast cultures were generated and used for various stimulations, and data were generated from RNAs extracted.

４．　研究成果

Several researches have focused on the malaria’s deadly complications; however, the long term pathological consequences of chronic malaria infection are poorly understood. In our studies we suspected from public information that there is an association between growth retardation and malaria infection in endemic areas. Therefore, we investigated possible negative impact of malaria infection on bone remodeling and growth, particularly in the young. Using mouse models mimicking chronic and self-clearing *Plasmodium* infection, we showed in Lee et al. (*Science Immunology, 2017*) that infection causes significant and long term bone loss in adult mice, and growth

retardation in young mice. Bone remodeling is completely suppressed during the acute phase of infection, but is highly activated immediately after the clearance of parasites, with increased osteoclastic activity skewing the balance toward bone resorption. Furthermore, we found that osteoclasts are activated by the key osteclastogenic cytokine, RANKL, which was up-regulated in osteoblasts through MyD88-dependent inflammation, triggered by the accumulation and long term persistence of parasite products in the bone marrow.

The main finding of our study is that, although *Plasmodium* infection is resolved systemically, the bone marrow stays highly active after parasite clearance, evolving into a state of chronic inflammation. This is due to the long term accumulation of *Plasmodium* products in the bone marrow and attributes uniqueness to the malaria-mediated bone loss. Moreover, MyD88, an adaptor molecule for most of the TLRs and IL1-related signals, is involved in malaria-induced bone loss through controlling inflammatory responses induced by *Plasmodium* products. However, TLR9, NLRP3 or IL-1 signaling alone had a minimal role in malaria-induced bone loss, suggesting that as yet unknown *Plasmodium* recognition machinery upstream of MyD88 may be involved. Our future efforts have focused on the delineation of these mechanisms causing malaria-induced bone loss.

We crucially found in our studies a remedy for the bone loss caused by malaria. We found that alfacalcidol, a VitD3 derivative, protects *Plasmodium*-infected mice from bone loss possibly via suppression of *Plasmodium* product-induced inflammatory cytokines. Our findings have been appreciated very well by scientific community as well as public. Our study was chosen as a cover of Science Immunology journal (June 2017 cover, https://immunology.sciencemag.org/content/2/12). Also, Altmetric score of manuscript has reached to 151, revealing the important social effect of this study. The doctoral student Michelle Lee who is the first author of this study won prestigious Ikushi Prize with these findings (The 8th JSPS Ikushi Prize Awardee, https://www.jsps.go.jp/english/e-ikushi-prize/awards_fy2017_01.html).

５．　主な発表論文等
〔雑誌論文〕（計　　件）

1. Lee MSJ, Natsume-Kitatani Y, Temizoz B, Igari Y, Tsukui T, Kobiyama K, Kuroda E, Ise W, Inoue T, Kurosaki T, Mizuguchi K, Akira S, Ishii KJ, Coban C. B cell-intrinsic MyD88 signaling controls IFNγ-mediated early IgG2c class switching in response to a particulate adjuvant. **European J Immunology, 2019**, May 14. doi:10.1002/eji.201848084. PMID: 31087643. *(Peer reviewed)*

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4. Coban C, Lee MSJ, Ishii KJ. Tissue-specific immunopathology during malaria infection. **Nature Reviews Immunology, 2018** doi:10.1038/nri.2017.138. *(Peer reviewed)*

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6. Wing JB, Kitagawa Y, Locci M, Hume H, Tay C, Morita T, Kidani Y, Matsuda K, Inoue T, Kurosaki T, Crotty S, Coban C, Ohkura N, Sakaguchi S. A distinct subpopulation of $CD25^-$ T-follicular regulatory cells localizes in the germinal centers. **Proc Natl Acad Sci U S A. 2017** Jul 11. pii: 201705551. doi: 10.1073/pnas.1705551114. PMID: 28698369. *(Peer reviewed)*

7. Lee MSJ, Maruyama K, Fujita Y, Konishi A, Lelliott PM, Itagaki S, Horii T, Lin JW, Khan SM, Kuroda E, Akira S, Ishii KJ, Coban C*. Plasmodium products persist in the bone marrow and promote chronic bone loss. **Science Immunology, 2017,** June 2; 2 (12), pii: eaam8093. DOI: 10.1126/sciimmunol.aam8093. (Journal Issue Cover) *(Peer reviewed)*

8. Lelliott PM, Coban C. IFN-γ protects hepatocytes against Plasmodium vivax infection via LAP-like degradation of sporozoites. **Proc Natl Acad Sci U S A, 2016**, Jun 10. pii: 201607007. PMID: 2728682. *(Peer reviewed)*

〔学会発表〕（計　　件）

Invited Talks:

1. Coban C. Mysterious interactions between Plasmodium parasites and their host.The 14^{th} NUS-Nagasaki Joint Symposium / 2^{nd} Medical Sciences Cluster Infectious Disease Symposium, May 27-29, 2019, National University of Singapore, SINGAPORE.

2. Coban C. Mysterious interaction of Plasmodium parasites with their host. Special lecture at Hacettepe University Medical School, Department of Pediatrics, May 2, 2019, Ankara, TURKEY.

3. Coban C. Mysterious interaction of Plasmodium parasites with their host. The 4^{th} International Molecular Immunology & Immunogenetics Congress (MIMIC IV 2019), April 27- 30, 2019, Bursa, TURKEY.

4. Coban C. Imaging interactions between Plasmodium parasites and their host. Japan-UK Workshop on Infectious Disease Research supported by AMED and MRC. January 21-22, 2019, TKP Tokyo St. Otemachi Conference Center, Tokyo, JAPAN.

5. Coban C. Immunology of Malaria. XXXVIII. Turkish Microbiology Congress 2018. November 4-8, 2018, Starlight Hotel and Convention Center, Antalya, TURKEY.

6. Coban C. Immunology of host-Plasmodium interactions. The 20th JSI Summer School, August 22-23, 2018, Ibusuki, Kagoshima, JAPAN.

7. Coban C. Learning from mouse models: How to understand and treat tissue specific immunopathology during malaria infection? ICOPA 2018, 14th International Congress of Parasitology Meeting, August 19-24, 2018, EXCO, Daegu, S. KOREA.

8. Coban C. Malaria and Vitamin D. The 4th Japanese Society of Osteoimmunology Meeting, June 24-26, 2018, MICE, Okinawa, JAPAN.

9. Coban C. Host-Plasmodium interactions at tissue level. Immunology at the Forefront. The 9th International Symposium of IFReC. January 26, 2018, ICHO Kaikan, Osaka University, Osaka, JAPAN.

10. Coban C. New insights into the interactions between malaria parasites and the host innate immune response. EMBO Workshop, Modelling infectious diseases in the cell and host. January 22-25, 2018, A-Star, SINGAPORE.

11. Coban C. Host-Plasmodium interactions at tissue level. 13th Tsukuba Primate Center Forum, NIBIOHN at Tsukuba. November 10, 2017, Tsukuba, JAPAN.

12. Coban C. Plasmodium products persist in the bone marrow and promote chronic bone loss. Malaria Gordon Research Conference. July 2-7, 2017, Les Diablerets Conference Center, SWITZERLAND.

13. Coban C and Ozdemir SK. High performance novel sensing of receptor-ligand interactions by whispering gallery mode (WGM) resonators. 2017 Yamada Science Foundation Meeting, May 27th, 2017, Tokyo Conference Center, Tokyo, JAPAN.

14. Coban C. Tissue-specificity during malaria infection. The 13th Nagasaki-Singapore Medical Symposium / Leading Program International Symposium 2017, May 18-19, 2017, Nagasaki University, Nagasaki, JAPAN.

15. Coban C. Tissue-specific Immunopathology during Malaria. Protein Island Matsuyama (PIM) International Symposium 2016. September 16-17, 2016. Ehime University, Matsuyama, JAPAN.

16. Coban C. Tissue-specific Immunopathology during Malaria. The John Curtin School of Medical Research (JCSMR), Australian National University (ANU), August 19, 2016, Canberra, AUSTRALIA.

17. Coban C. Tissue-specific Immunopathology during Malaria. Molecular Immunology & Immunogenetics Congress (MIMIC-III), April 27- 30, 2016, Antalya, TURKEY.

Meeting Presentations (Oral or poster):

1. Aykac K, Lee MSJ, Kahyaoglu P, Erci E, Matsuo-Dapaah J, Abrenica CS, Coban C. Identification of cell-types responsible for MyD88-dependent malaria-induced bone loss *(Poster Presentation, Award Winner for meeting registration and accommodation).* The 4th International Molecular Immunology & Immunogenetics Congress (MIMIC IV 2019), April 27- 30, 2019, Bursa, TURKEY.
2. Lee MSJ, Coban C. Plasmodium products persist in the bone marrow and promote chronic bone loss. (*Poster Presentation*). The 16th Awaji International Forum on Infection and Immunity, September 5-8, 2017, Hyogo, JAPAN.

3. Lee MSJ, Coban C. Plasmodium products persist in the bone marrow and promote chronic bone loss. (*Poster Presentation*). Malaria Gordon Research Conference. July 2-7, 2017, Les Diablerets Conference Center, SWITZERLAND.
4. Lee MSJ, Coban C. Plasmodium products persist in the bone marrow and promote chronic bone loss. (*Poster Presentation*). The 3rd Osteoimmunology Meeting. June 27-29, 2017, ANA Intercontinental Ishigaki, JAPAN.
5. Lee MSJ, Maruyama K, Akira S, Coban C. Plasmodium infection modulates bone homeostasis (*Oral Poster Presentation*). The 6th NIF Winter School on Advanced Immunology, January 22-26, 2017, SINGAPORE.
6. Lee MSJ, Maruyama K, Akira S, Coban C. Plasmodium infection modulates bone homeostasis (3-C-W26-12-O/P-2016A-0145) (*Best Oral Poster Presentation).* The 45th Annual Meeting of Japanese Society for Immunology, December, 18-20th, 2016, Okinawa, JAPAN.
7. Lee MSJ, Maruyama K, Akira S, Coban C. Malaria-induced bone disorder triggered by chronic inflammation in the bone (# 635) (*Poster Presentation*). The 16th International Congress of Immunology 2016 (ICI Melbourne), August 21-26, 2016, Melbourne, AUSTRALIA.

〔図書〕（計　　件）

Lee MSJ, Coban C. Mucosal vaccines for malaria. In the book Mucosal Vaccines: Innovation for Preventing Infectious Diseases, 2e (Edited by Hiroshi Kiyono and David W. Pascual), 2019, in press.

〔産業財産権〕：Not applicable.

〔その他〕ホームページ等

Web site of Malaria Immunology Lab: http://malimm.ifrec.osaka-u.ac.jp/index.html. Cover of Science Immunology journal (June 2017 cover): https://immunology.sciencemag.org/content/2/12). Altmetric score of manuscript has reached to 151, revealing the important social effect of this study. The doctoral student Michelle Lee who is the first author of this study won prestigious Ikushi Prize with these findings (The 8th JSPS Ikushi Prize Awardee, https://www.jsps.go.jp/english/e-ikushi-prize/awards_fy2017_01.html).

６．研究組織

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