

Department of Microbiology and Immunology

Division of Malaria Immunology

マラリア免疫学分野

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Summary of Activity (Less than 70 words)

Our laboratory investigates how pathogens interact with the host immune system. Initially specializing in malaria immunology, we have expanded our research to include respiratory viral diseases and neglected parasitic infections like leishmaniasis. Our goal is to deepen understanding of immune responses against these pathogens to enhance vaccine and drug development, with potential applications beyond infectious diseases.

1. Elucidation of host-pathogen interactions

MyD88 in osteoclast and osteoblast lineages differentially controls bone remodeling in homeostasis and malaria. Chronic bone loss is an under-recognized complication of malaria, the underlying mechanism of which remains incompletely understood. We have previously shown that persistent accumulation of *Plasmodium* products in the bone marrow leads to chronic inflammation in osteoblast (OB) and osteoclast (OC) precursors causing bone loss through MyD88, an adaptor molecule for diverse inflammatory signals (Lee *et al.*, *Science Immunology*, 2017). However, the specific contribution of MyD88 signaling in OB or OC precursors in malaria-induced bone loss remains elusive. To assess the direct cell-intrinsic role of MyD88 signaling in adult bone metabolism under physiological and infection conditions, we used the Lox-Cre system to specifically deplete MyD88 in the OB or OC lineages. Mice lacking MyD88 primarily in the maturing OBs showed a comparable decrease in trabecular bone density by microcomputed tomography to that of controls after *Plasmodium yoelii* non-lethal infection. In contrast, mice lacking MyD88 in OC precursors showed significantly less trabecular bone loss than controls, suggesting that malaria-mediated

inflammatory mediators are primarily controlled by MyD88 in the OC lineage. Surprisingly, however, depletion of MyD88 in OB, but not in OC, precursors resulted in reduced bone mass with decreased bone formation rates in the trabecular areas of femurs under physiological conditions. Notably, insulin-like growth factor-1, a key molecule for OB differentiation, was significantly lower locally and systemically when MyD88 was depleted in OBs. Our data demonstrated an indispensable intrinsic role for MyD88 signaling in OB differentiation and bone formation, while MyD88 signaling in OC lineages played a partial role in controlling malaria-induced inflammatory mediators and following bone pathology. These findings may lead to the identification of novel targets for specific intervention of bone pathologies, particularly in malaria-endemic regions (Alshaweesh *et al.*, *International Immunology*, 2024. *This study was chosen as Featured Article*).

Acute malaria suppresses the B lymphocytic niche in the bone marrow through the alteration of CXCL12-abundant reticular cells. Bone marrow is a dynamic organ composed of stem cells that constantly receive signals from stromal cells and other hematopoietic cells in the niches of the bone marrow to maintain hematopoiesis and generate immune cells.

Perturbation of the bone marrow microenvironment by infection and inflammation affects hematopoiesis and may affect immune cell development. Little is known about the effect of malaria on the bone marrow stromal cells that govern the hematopoietic stem cell (HSC) niche. In this study, we demonstrated that the mesenchymal stromal CXCL12-abundant reticular (CAR) cell population is reduced during acute malaria infection. The reduction of CXCL12 and interleukin-7 signals in the bone marrow impairs the lymphopoietic niche, leading to the depletion of common lymphoid progenitors, B cell progenitors, and mature B cells, including plasma cells in the bone marrow. We found that interferon- γ (IFN γ) is responsible for the upregulation of Sca1 on CAR cells, yet the decline in CAR cell and B cell populations in the bone marrow is IFN γ -independent. In contrast to the decline in B cell populations, HSCs and multipotent progenitors increased with the expansion of myelopoiesis and erythropoiesis, indicating a bias in the differentiation of multipotent progenitors during malaria infection. Our findings suggest that malaria may affect host immunity by modulating the bone marrow niche (Lee et al., *International Immunology*, 2024. *This study was chosen as Featured Article*).

Diagnostic challenges in cutaneous leishmaniasis due to atypical *Leishmania infantum*. Leishmaniasis, a parasitic infection affecting both humans and animals, is increasingly spreading across Mediterranean and European regions, largely driven by human migration and environmental changes. In countries like Türkiye and across Europe, which have seen large influxes of migrants, the incidence of cutaneous leishmaniasis (CL) is rising, with cases now appearing in cities where the disease was previously undocumented. In these previously non-endemic areas, physicians unfamiliar with the characteristic lesions may misdiagnose CL, particularly in cases with only cutaneous manifestations. This study aimed to evaluate the impact of re-emerging CL on the routine diagnostic practices of pathologists in Türkiye, by retrospectively reviewing cases. We conducted a retrospective analysis of CL cases diagnosed between 2013 and 2022 at a single pathology center in Türkiye, covering multiple provinces. Twelve cases of CL were identified and analyzed based on clinical presentation, pre-diagnosis, histopathological findings, and molecular diagnostics. DNA extraction and PCR were performed on paraffin-embedded tissue samples to identify the *Leishmania* species involved. Out of the twelve CL cases reviewed, seven exhibited morphological findings strongly suggestive of CL (MFSS of CL), warranting further microbiological evaluation. All patients presented with non-healing skin lesions characterized by central ulceration, crater-like formations, or papulonodular lesions. Notably, CL was included in the clinical pre-diagnosis in only 58.3% of cases, while it was not considered in the remaining 41.7% of cases. Clinicians initially pre-diagnosed skin

tumors in six cases (50%), four of which led to wide surgical excision. Histopathological examination in all cases revealed chronic or mixed (acute/chronic) inflammation, predominantly rich in histiocytes. To further investigate the role of *Leishmania* species in the pre-diagnosis, DNA extraction and PCR were performed on paraffin-embedded tissue samples, identifying *L. infantum* as the causative agent in 10 cases and *L. major* in two cases. Notably, *L. infantum* was the causative agent in all five cases initially misdiagnosed as skin tumors, which were also associated with a granulomatous type of chronic inflammation (Eke-men et al., *Frontiers in Medicine*, 2024).

Histone H3.3 variant plays a critical role on zygote-to-oocyst development in malaria parasites. The *Plasmodium* life cycle involves differentiation into multiple morphologically distinct forms, a process regulated by developmental stage-specific gene expression. Histone proteins are involved in epigenetic regulation in eukaryotes, and the histone variant H3.3 plays a key role in the regulation of gene expression and maintenance of genomic integrity during embryonic development in mice. However, the function of H3.3 through multiple developmental stages in *Plasmodium* remains unknown. To examine the function of H3.3, h3.3-deficient mutants (Δ h3.3) were generated in *P. berghei*. The deletion of h3.3 was not lethal in blood stage parasites, although it had a minor effect of the growth rate in blood stage; however, the in vitro ookinete conversion rate was significantly reduced, and the production of the degenerated form was increased. Regarding the mosquito stage development of Δ h3.3, oocysts number was significantly reduced, and no sporozoite production was observed. The h3.3 gene complemented mutant have normal development in mosquito stage producing mature oocysts and salivary glands contained sporozoites, and interestingly, the majority of H3.3 protein was detected in female gametocytes. However, Δ h3.3 male and female gametocyte production levels were comparable to the wild-type levels. Transcriptome analysis of Δ h3.3 male and female gametocytes revealed the upregulation of several male-specific genes in female gametocytes, suggesting that H3.3 functions as a transcription repressor of male-specific genes to maintain sexual identity in female gametocytes. This study provides new insights into the molecular biology of histone variants H3.3 which plays a critical role on zygote-to-oocyst development in primitive unicellular eukaryotes (Tateishi et al., *Parasitology International*, 2024).

2. Adjuvant discovery and development platform

5,6-dimethylxanthenone-4-acetic acid (DMXAA), a partial STING agonist, competes for human STING activation. Stimulator of interferon genes (STING) is one of the key molecules at the intersection of various cytosolic nucleic acid-sensing pathways,

including cyclic GMP-AMP synthase (cGAS), DEAD-box helicase family, and interferon gamma inducible protein. DMXAA is a mouse-selective stimulator of interferon gene (STING) agonist exerting STING-dependent anti-tumor activity. Although DMXAA cannot fully activate human STING, DMXAA reached phase III in lung cancer clinical trials. How DMXAA is effective against human lung cancer is completely unknown. Here, we show that DMXAA is a partial STING agonist interfering with agonistic STING activation, which may explain its partial anti-tumor effect observed in humans, as STING was reported to be pro-tumorigenic for lung cancer cells with low antigenicity. Furthermore, we developed a DMXAA derivative—3-hydroxy-5-(4-hydroxybenzyl)-4-methyl-9H-xanthen-9-one (HHMX)—that can potentially antagonize STING-mediated immune responses both in humans and mice. Notably, HHMX suppressed aberrant responses induced by STING gain-of-function mutations causing STING-associated vasculopathy with onset in infancy (SAVI) in *in vitro* experiments. Furthermore, HHMX treatment suppressed aberrant STING pathway activity in peripheral blood mononuclear cells from SAVI patients. Lastly, HHMX showed a potent therapeutic effect in SAVI mouse model by mitigating disease progression. Thus, HHMX offers therapeutic potential for STING-associated autoinflammatory diseases (*Temizoz et al., Frontiers Immunology, 2024*).

3. Infection and beyond

Our previous research demonstrated that Lipocalin 2 (LCN2), also known as siderocalin or neutrophil

gelatinase-associated lipocalin (NGAL), enhances innate and adaptive immune responses in malaria by modulating iron metabolism (*Zhao et al., Cell Host Microbe, 2012*). Interestingly, LCN2 expression is also elevated in cancer, highlighting its broader role beyond infection. In tumorigenesis, alongside somatic mutations, stroma-associated immunity significantly influences tumor progression. Tumor cells create a supportive microenvironment by releasing mediators, attracting monocytes and leukocytes, and disrupting iron balance through excessive consumption, potentially upregulating LCN2 as an intracellular iron transporter. Recently, we investigated the expression of LCN2 and the immune checkpoint molecule programmed cell death ligand-1 (PD-L1) in breast cancers across molecular subtypes. This retrospective analysis of 89 primary breast cancer cases revealed that LCN2 expression correlates with poor prognostic factors, including high histological grade, elevated Ki-67 proliferation index, and ER/PR negativity. Elevated LCN2 and PD-L1 expressions were significantly associated with triple-negative and HER2-positive breast cancers. These findings demonstrate the prognostic potential of LCN2 and its relevance in immune modulation within the tumor microenvironment. Furthermore, this research suggests the potential for immunotherapeutic applications of LCN2, advancing breast cancer management (*Ekemen et al., Breast Cancer: Targets and Therapy, 2024*). By bridging infection and cancer research, our work demonstrates the versatile roles of LCN2 in regulating immunity and iron metabolism, offering insights into its therapeutic potential in diverse pathological contexts.

Publications

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