International Vaccine Design Center

Division of Infection Immunology (Human Immune-Profiling Team)

ヒト免疫プロファイリング系・感染免疫学分野

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As part of the International Vaccine Design Center at IMSUT, our team investigate how pathogens interact with human immune system. Initially specializing in malaria immunology, we have expanded our research to include emerging infectious diseases like dengue and COVID-19, respiratory viral diseases, and neglected parasitic infections such as leishmaniasis. By elucidating the virulence factors of these pathogens, our goal is to advance vaccine and drug development.

1. Elucidation of host-pathogen interactions

Chronic bone loss is an under-recognized complication of malaria, with mechanisms still not fully understood. Persistent accumulation of *Plasmodium* products in the bone marrow triggers chronic inflammation in osteoblast (OB) and osteoclast (OC) precursors via MyD88, an adaptor molecule for inflammatory signals (Lee et al., *Science Immunology*, 2017). Following our previous studies, we recently investigated the cell-intrinsic role of MyD88 in bone metabolism under physiological and malaria-infected conditions using Lox-Cre-based depletion of MyD88 in OB or OC lineages. We found that mice lacking MyD88 in maturing OBs exhibited significant trabecular bone loss during *Plasmodium yoelii* infection, comparable to controls. In contrast, mice with MyD88 deficiency in OC precursors showed less bone loss during malaria, indicating that inflammatory mediators are predominantly regulated by MyD88 in the OC lineage. However, under normal conditions, MyD88 depletion in OBs led to reduced bone mass and formation rates due to lower systemic and local levels of insulin-like growth factor-1, crucial for OB differentiation. These results demonstrate MyD88's dual role: as indispensable for OB differentiation and bone formation under normal conditions and as a partial mediator of malaria-induced inflammatory bone pathology in the OC lineage. These findings could inform therapeutic strategies for bone pathologies, especially in malaria-endemic regions and beyond in rheumatological diseases ***(Alshaweesh et al., International Immunology, 2024)***.

Bone marrow (BM) is essential for hematopoiesis and immune cell generation, governed by signals from stromal and hematopoietic cells. Acute malaria alters the BM microenvironment, particularly the CXCL12-abundant reticular (CAR) cell population critical for hematopoietic stem cell (HSC) niches. We recently identified a significant reduction in CXCL12 and interleukin-7 signals during acute malaria, leading to the depletion of common lymphoid progenitors, B cell progenitors, and mature B cells, including plasma cells. Particularly, IFNγ upregulated Sca1 ex-

pression on CAR cells but was not responsible for the CAR cell and B cell population decline. A simultaneous increase in HSCs and multipotent progenitors, along with myelopoiesis and erythropoiesis, indicated a bias in multipotent progenitor differentiation during infection.

These findings emhasize malaria's capacity to modulate host immunity by disrupting the BM lymphopoietic niche, with implications for host-pathogen interactions and immune recovery ***(Lee et al., International Immunology, 2024).***

Leishmaniasis, driven by human migration and environmental changes, is increasingly reported in non-endemic regions like Türkiye and Europe. Rising cases of cutaneous leishmaniasis (CL), particularly those caused by atypical *Leishmania infantum*, present diagnostic challenges. A retrospective study of 12 CL cases in Türkiye (2013–2022) revealed that only 58.3% of cases included CL in the initial clinical pre-diagnosis, while 41.7% were misdiagnosed, often as skin tumors. Misdiagnosed cases led to invasive procedures such as wide surgical excision. Histopathological examinations revealed chronic or mixed inflammation rich in histiocytes. Molecular diagnostics identified *L. infantum* in 10 cases and *L. major* in two. These findings show the need for increased clinical awareness and molecular diagnostics to prevent misdiagnosis and unnecessary interventions, especially in re-emerging non-endemic regions ***(Ekemen et al., Frontiers in Medicine, 2024).***

Taken together, these studies collectively advance our understanding of the intersection between infectious diseases and host pathophysiology, from malaria-induced bone remodeling and immune niche disruption to diagnostic challenges posed by re-emerging cutaneous leishmaniasis.

2. Adjuvant discovery and development platform

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 potently 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.

4. Infections and associated risk factors

Dr. Sakuntabhai's group together with Pasteur Network recently evaluated risk factors for the Crimean-Congo haemorrhagic fever (CCHF) outbreak happened in 2022 in Northern Senegal. CCHF is a severe zoonotic arboviral disease that occurs widely in Eastern and Western Europe, Asia and Africa. The disease

is becoming of growing public health importance in Senegal. However, analysis of tick infestation, CCHF virus (CCHFV) circulation extent and risk factors during ongoing outbreak are scarce. A thorough outbreak investigation was carried out during a CCHF outbreak in Podor (Northern Senegal) in August 2022. Ticks and blood samples were collected from animals (cattle, goats and sheep) randomly selected from confirmed CCHF human cases houses, neighbourhoods and surrounding villages. Blood samples were tested for CCHFV antibodies using a commercial enzyme-linked immunosorbent assay (ELISA) test. Tick samples were screened for CCHFV RNA by RT-PCR. Overall, tick infestation rate (TIR) and CCHFV seroprevalence of livestock were 52.12% (95% confidence interval (CI): 45.54%-58.64%) and 43.28% (95% CI: 36.33%-50.44%), respectively. The TIRs were 87.7% in cattle, 57.6% in sheep and 20.0% in goats. These rates were significantly associated with location, host species and tick control ($p < 0.001$) but not with animal age and sex ($p > 0.7$). CCHFV seroprevalence was 80.4% (95% CI: 67.57%-89.77%) in cattle, 35.4% (95% CI: 25.00%-47.01%) in sheep and 21.2% (95% CI: 12.11%-33.02%) in goats. Age, sex, location, animal host and presence of ticks were significantly associated to the presence of antibodies. The 950 ticks collected included among other species, Hyalomma impeltatum (48.84%) and H. rufipes (10.21%). Five pools of Hyalomma ssp. were found CCHFV RT-PCR positive. These infected ticks included 0.86% (4/464) of H. impeltatum collected on cattle and sheep and 1.03% (1/97) of H. rufipes collected on a sheep. This is possibly the first report on the extend of tick infestation and CCHFV infection in livestock during an outbreak in Senegal. The results highlight the risk of human infections and the importance of strengthening vector, animal and human surveillance as well as tick control measures in this area to prevent CCHF infections in humans ***(Ngom et al., Zoonoses Public Health, 2024).***

Publications

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