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Abstract of Thesis/Dissertation

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Title : Investigating cross-species immunity and disease modulation in *Babesia* co-infections

(バベシア共感染における種間免疫と疾患調節の研究)

Abstract

Babesiosis and malaria, both significant parasitic diseases, pose considerable challenges to global health. Keeping in view the significance of these diseases, two studies were performed to investigate the co-infections with *Babesia* and *Plasmodium* parasites in murine models.

The first study was focused on two *Babesia* species of different pathogenicity because babesiosis causes high morbidity and mortality in immunocompromised individuals. An earlier study suggested that lethal *Babesia rodhaini* infection in murine can be evaded by *Babesia microti* primary infection via activated macrophage-based immune response during the chronic stage of infection. However, whether the same immune dynamics occur during acute *B. microti* co-infection is not known. Hence, the BALB/c mouse model was used to investigate the host immunity during simultaneous acute disease caused by two *Babesia* species of different pathogenicity. Results showed that *B. microti* primary infection attenuated parasitemia and conferred immunity in challenge-infected mice as early as day 4 post-primary infection. Likewise, acute *Babesia* co-infection undermined the splenic immune response, characterized by a significant decrease in splenic B and T cells leading to a reduction in antibody levels and a decline in humoral immunity. Interestingly, increased

macrophage and natural killer splenic cell populations were observed, depicting their subtle role in the protection. Pro-inflammatory cytokines (i.e. IFN-γ, TNF-α) were downregulated, while the anti-inflammatory cytokine IL-10 was upregulated in mouse sera during the acute phase of *Babesia* co-infection. Herein, the major cytokines implicated in the lethality caused by *B. rodhaini* infection were IFN- γ and IL-10. Surprisingly, significant differences in the levels of serum IFN- γ and IL-10 between co-infected survival groups (day 4 and 6 challenge) indicated that even a two-day delay in challenge infection was crucial for the resulting pathology. Additionally, oxidative stress in the form of reactive oxygen species contributed to the severity of pathology during acute babesiosis. Histopathological examination of the spleen showed that the erosion of the marginal zone was more pronounced during *B. rodhaini* infection, while the loss of cellularity of the marginal zone was less evident during co-infection. Future research warrants investigation of the roles of various immune cell subtypes in the mechanism involved in the protection of *Babesia* co-infected hosts.

Malaria remains one of the most significant health issues worldwide, accounting for 2.6% of the total global disease burden, and efforts to eliminate this threat continue. The key focus is to develop an efficient and long-term immunity to this disease via vaccination or therapeutic approach, and innovative strategies would enable us to achieve this target. Previously, using a mouse co-infection disease model, cross-protection was illustrated between *Babesia microti* and *Plasmodium chabaudi*. Hence, the second study was planned to elucidate the impact of acute *B. microti* Peabody mjr and *Plasmodium berghei* ANKA co-infection on the consequence of complicated malaria in the C57BL/6J mouse model of malaria. Furthermore, immune response and pathological features were analyzed, and the course of the disease was compared among experimental groups. This study established that acute *B. microti* infection activated immunity which was otherwise suppressed by *P. berghei*. The immunosuppressive tissue microenvironment was counteracted as evidenced by the enhanced immune cell population in co-infected mice, in contrast to *P. berghei*-infected control mice. Parasite sequestration in the brain, liver, lung, and spleen of co-infected mice was significantly decreased and tissue injury was ameliorated. Meanwhile, the serum levels of IFN-γ, TNF-α, and IL-12p70 were reduced while the secretion of IL-10 was promoted in co-infected mice. Eventually, co-infected mice showed an extended rate of survival. Hereby, the principal cytokines associated with the severity of malaria by *P. berghei* infection were TNF-α, IFN-γ, and IL-12p70. Moreover, it was evident from our flow

cytometry results that innate immunity is crucial and macrophages are at the frontline of immunity against *P. berghei* infection.

In conclusion, the integrated study provides comprehensive insights into the intricate host immune responses during co-infection with *Babesia* and *Plasmodium*. These findings not only enhance our understanding of the complexities of parasitic diseases but also present potential avenues for innovative therapeutic strategies against both babesiosis and malaria. Further investigations are recommended to unveil the specific mechanisms underlying *Babesia*-mediated suppression of malaria and the nuanced roles of immune cell subtypes in co-infected hosts, paving the way for the development of *Babesia*-based therapies against malaria.