別紙２

Results report

１．Title of Research and Development : Role of TLRs in patients with severe complicated malaria due to *Plasmodium vivax* and the development of diagnostic method for predicting the severity

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４．Results of Research and Development:

Severe cases of malaria are mainly caused by human malaria parasite, *Plasmodium falciparum*, leading to death if not promptly treated. Although *P. vivax* has long been considered as a benign infection, there has been an increase in the reported cases of severe malaria due to *P. vivax* in recent few years. However, the pathogenesis in severe vivax malaria is not fully understood. To investigate pathogenesis in severe vivax malaria, the clinical data related to the vivax malaria were collected at Postgraduate Institute of Medical Education & Research (PGIMER), Chandigarh, India. The informed consent was taken from patients before taking the clinical history and blood samples. Clinical data were analyzed in line with WHO criteria for severe malaria. Analysis of data obtained from the 90 *P. vivax* malaria patients revealed that 48% of patients showed the same clinical manifestation as severe malaria. The clinical manifestations observed in severe vivax malaria are severe anemia, thrombocytopenia, renal failure, jaundice, respiratory distress, and hypoglycemia to multi-organ dysfunction. Especially, thrombocytopenia was found in 75% of severe vivax malaria patients, suggesting that thrombocytopenia may be associated with increasing severity in vivax malaria.

The severity in vivax malaria patients may be attributed to the various factors, such as parasitic, host and environmental factors. In this study, we focused on the host factors responsible for the causation of severe complicated vivax malaria. We first investigated the various toll like receptors (TLRs), such as TLR4 and TLR9, in 90 subjects with *P. vivax* malaria. As results, we found that the nucleotide change was observed in all the targeted toll like receptor genes with varying frequency. In the available sequences, mutations in the TLR4 and in the TLR 9 were observed in 19 of 80 subjects and in 8 of 35 subjects, respectively. These findings suggest that the mutations in toll like receptors might be associated with pathogenesis of severe vivax malaria.

To investigate pathogenesis in severe vivax malaria, we tried to establish attenuated rodent malaria parasites and examined key factors determining the severity of malaria. *Plasmodium* parasites are unable to synthesize purine rings de novo. Thus, they rely on the host for a supply of purine nucleosides and then synthesize purine nucleotides through a purine salvage pathway. We demonstrated that the growth and virulence of *P. berghei* (*Pb*) ANKA, a high virulent-rodent malaria parasite, were suppressed by purine restriction. In mice infected with purine restricted parasites, progression of thrombocytopenia was delayed compared with mice infected with intact parasite. Subsequent purine restriction resulted in a substantial reduction in ATP levels relative to intact parasites, suggesting that the ATP levels in parasites might be useful for predicting the disease severity during malaria. Moreover, the attenuated parasites were readily cleared by wild-type mice, but not by TLR2/4/9-deficient mice and γδT cell-deficient mice. These results suggest that polymorphism or mutation of immune factors may be associated with the severity of malaria due to *P. vivax*.

Further analyses of clinical cases with severe vivax malaria (Indian side) and mouse malaria models (Japanese side) would contribute to understanding the pathogenesis and developing a diagnostic method for predicting the severity due to *P. vivax*.