Department of Tropical Medicine

Naohiro Watanabe, *Professor*
Kenji Ishiwata, *Associate Professor*
Asao Makioka, *Associate Professor*
Masahiro Kumagai, *Assistant Professor*

General Summary

Our research is concerned with mast cells and basophils in infection, immune responses to helminth infection, and the growth and differentiation of *Entamoeba*.

Research Activities

Mast cells and basophils in parasitic infections
Mast cells and basophils are known to induce allergic reactions. We have studied under our own hypothesis that these cells play protective roles against parasites. We have demonstrated that mast cell-derived tumor necrosis factor (TNF) and vascular endothelial growth factor (VEGF) are responsible for protection in malaria. Serum levels of VEGF after *Plasmodium berghei* infection were lower in TNF-deficient mice than in wild-type mice, indicating that the secretion of VEGF depends on TNF in murine malaria. In addition, VEGF was likely to be involved in the pathogenesis of malaria through heme oxyganase 1. The effect of basophils was examined with new basophil-deficient mice in which differentiation of basophils could be blocked at any time through a signal from diphtheria toxin receptor by gene manipulation. No difference in protection against primary infection with tick *Haemahysalis longicornis* was found between basophil-deficient mice and wild-type mice. When basophil-deficiency was induced at the time of secondary infection with *Haemahysalis longicornis*, protective activity was significantly reduced, suggesting that basophils play a protective role against secondary infection with ticks.

Exhaustive analysis of glycosylation alterations of intestinal mucus in mice infected with an intestinal nematode, Nippostrongylus brasiliensis
The mucus that covers mucosal epithelium is home to intestinal parasites and serves as a platform for host-parasite interactions, including establishment and expulsion. The main component of mucus is mucin, although many factors, derived from immune cells or epithelium, are dissolved in mucus. Previously, we suggested that interleukin (IL) 13, a type 2 cytokine, up-regulates message level of sialyltransferases and the number of sialomucin-positive goblet cells through IL-4 receptor/Stat6 signal pathway. We herein exhaustively examined immune-mediated glycosylation alterations of intestinal mucus in mice infected with an intestinal nematode, *Nippostrongylus brasiliensis*. Mucus was washed out of the small intestine on day 9 after infection and analyzed by means of lectin array analysis or mass spectrometry; day 9 is when worms are expelled from the gut. In lectin array analysis, the binding affinity of the mucus to 45 known lectins was measured. Twelve lectins showed greater than a 2-fold increase, whereas 3 lectins showed a

decrease of less than 50% when compared with that of the uninfected mucus. After hydrazine degradation treatment, isolated sugar chains were subjected to matrix-assisted laser desorption ionization-time of flight mass spectometry and analyzed with the GlycoMod software tool (http://web.expasy.org/glycomod/). Consistent results of both lectin array and mass spectrometry analyses were increased expression of deoxyhexose (fucose) and conjugated polysaccharides composed of deoxyhexose, hexosamine, and N-acetylneuraminic acid (sialic acid) in day-9 mucus. These results support our previous observations and suggest that host immune responses induce glycosylation alterations of the intestinal mucus to serve as a physical barrier to the enforce physiological condition of the intestine to drive out intestine-dwelling parasites.

The mechanism of encystation and excystation in Entamoeba

We examined changes in gene expression of actin and actin depolymerization factor cofilin of *Entamoeba invadens* during differentiation by real-time reverse transcriptase-polymerase chain reaction. First, we identified 3 cofilins (Cfl-1, 2, 3). Actins and Cfl-2 were expressed in trophozoites and were decreased during encystation. During excystation, actins and 3 cofilins were expressed. Contrary to our expectations, we had previously found that cytochalasin D promoted excystation. Accordingly, we found that the expression of Cfl-1 and Cfl-3 was markedly increased in the presence of cytochalasin D. Thus, we demonstrated that Cfl-1 and Cfl-3 are expressed only during excystation and that the enhancement of excystation by cytochalasin D is associated with the increased expression of Cfl-1 and Cfl-3.

Transcriptome analysis of Entamoeba with an ultrafast sequencer

Data from several genes has shown that the 5′ untranslated region (5′ UTR) of the messenger RNA of *Entamoeba histolytica* is extremely short. However, comprehensive analysis was difficult because the 5′ end of complementary DNA tends to be easily missing. Using transcription start site (TSS) sequencing method, which is a combination of the oligocapping method and ultrafast sequencing, we determined an extensive number of short sequences beginning with the TSS from trophozoites of *E. histolytica* and *Entamoeba invadens*. As a result, short 5′ UTRs (around 10 base pairs) of these *Entamoeba* species have been comprehensively demonstrated. We also sequenced the RNAs of both amoebas and discovered several new genes that had not been predicted from the genome sequences. The exon-intron structure and alternative splicing were also discovered. Promoter sequences are now under investigation. The sequencing of TSSs and RNAs allows us to analyze the level of gene expression by counting tag numbers. Future research will attempt to identify genes related to encystation by comparing levels of gene expression between the trophozoites and cysts of *E. invadens.*

Resistance against malaria in thalasemia

Malaria is a major killer in tropical area. In particular, malaria caused by *Plasmodium falciparum* is lethal and demands research to prevent or cure the disease. However, *P. falciparum* infects only humans and some nonhuman primates. Therefore, performing research in animals is difficult. To overcome this problem, we are developing a mouse

model that is able to survive with human red blood cells (RBCs) rather than mouse RBCs. To eradicate mouse RBCs, hematopoietic stem cells are taken from embryonic lethal thalassemia model mice, which cannot produce RBCs, and transplanted to lethally irradiated severe combined immunodeficiency mice. If this model is successfully developed, it may facilitate malaria research and provide benefits to many patients.

Publications

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