# Department of Tropical Medicine

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## 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 infection*
We have performed studies to test our hypothesis that mast cells and basophils play roles in immune protection and regulation. Recently, the expression of Dectin-1, a molecule related to innate immunity, was reported on mast cells. Then, the protective role of Dectin-1 in *Plasmodium berghei* infection was examined in mice. Deletion of Dectin-1 on mast cells resulted in decreased protection, suggesting that Dectin-1 on mast cells has a protective role. In dengue virus infection in Vietnam, the sera of patients with hemorrhergic fever or shock syndrome exhibited elevated levels of vascular endothelial growth factor, which is a cytokine from mast cells, and higher amounts of chymase and tryptase, which are mast cell-specific enzymes. These results indicate that mast cell activation is involved in the pathogenesis of dengue virus infection. In tick infection in mice, basophils infiltrated at the skin site of infection. Moreover, mast cells and antibodies helped protect against ticks.

*B7-DC and interleukin 4 on antigen-presenting cells in the T helper 2 immune response*
The caliber and magnitude of T-cell responses are regulated by costimulatory molecules following the engagement of T-cell receptors and MHC molecules. The costimulatory molecule B7-DC has the highest homology with B7-H1 in the B7 family, and both molecules bind an immunoregulatory molecule, programmed death 1 (PD-1). Previous studies have shown that B7-DC stimulates the proliferation of T cells and the generation of cytotoxic T lymphocytes, actions that contrast sharply with the inhibitory role of B7-H1. We used an intestinal nematode, *Nippostrongylus brasiliensis*, to induce strong T helper (Th) 2 responses and to evaluate B7-DC function under Th2-polarizing conditions *in vivo*. By either blocking B7-DC expression during *N. brasiliensis* infection or by examining *N. brasiliensis*-infected B7-DC knockout mice, we observed enhanced eosinophilia, the overproduction of serum IgE, and increased Th2 cytokine production along with decreased production of Th1 cytokines, particularly interferon-$\gamma$, indicating that B7-D inhibits Th2 responses. Our results further demonstrate that the inhibition of Th2 responses by B7-DC occurs independently of PD-1 but conceivably acts through an as yet unknown alternative receptor. These results suggest that B7-DC plays an important role in bolstering a robust Th1 response, even under a strong

Th2-polarizing environment induced by *N. brasiliensis* infection. In contrast, flow cytometry and *in vitro* analysis showed that interleukin 4, a representative Th2 cytokine, reduced the antigen-presenting activity of mesenteric lymph node cells. Although several issues remain unresolved, these results suggest that regulation of signals from B7-DC and interleukin 4 ameliorate Th2-associated diseases, such as allergy and hay fever.

*Expression analyses of Entamoeba chitinases in excystation and encystation*

*Entamoeba histolytica* forms chitin-walled cysts in the infected host whose chitin must be disrupted when the amoeba excysts for infection. Chitinase, an enzyme for chitin degradation, is involved in both encystation and excystation. As *E. histolytica* does not encyst in axenic culture, we used *Entamoeba invadens*, a reptilian amoeba, as a model of *E. histolytica* because *E. invadens* can easily encyst and excyst in culture, and studied the messenger RNA expression of chitinases. We retrieved 4 chitinase homologs named EiChit1, 2, 3, and 4 from an *E. invadens* genome database. In trophozoites, Chit2 and 3 were expressed at very low levels. During encystation, the expression of all 4 chitinases increased markedly in the early phase and then decreased in the later phase. In cysts, a large amount of Chit1 and a small amount of Chit4 were expressed. During excystation, as assayed 5 hours after induction, the expression of Chit1 and 4 decreased, and that of Chit2 and 3 increased. These data demonstrate the dynamics of chitinase expression in the differentiation of *Entameoba*.

*Transcription start site and expression analyses of Entamoeba genes using an ultrafast sequencer*

Transcription start site (TSS) sequencing, which is a combination of the oligo-capping method with an ultrafast sequencer, has recently been developed. This method allows us to simultaneously analyze an extensive number of TSSs and, by counting the tag numbers, the level of gene expression. Here we used TSS sequencing to investigate the TSSs of *E. invadens* trophozoites and the change in expression of genes after the induction of encystation. More than 90% of 3534 genes had a 5'-end untranslated region less than 30 base pairs in length, which was similar to data from the 'Full-Entamoeba' database previously constructed by us. As for expression in induced cysts, a chitin-binding lectin was most abundant, and chitinases were expressed at high levels, although many hypothetical proteins were also included among the top 30. Thus, the TSS sequencing was useful for simultaneous analyses of *Entamoeba* TSSs and gene expression.

*Resistance against malaria in thalassemia*

Persons with thalassemia have resistance to malaria. The mechanism of this resistance was examined in the thalassemia mouse but in vein. We focused on human fetal hemoglobin, which persists for the entire life in humans but not in mice. The human fetal hemoglobin gene was introduced to mice to simulate human thalassemia. This thalassemia model mouse had lower parasitemia and survives longer after infection. In addition, this mouse produced much larger amounts of fetal hemoglobin. These results

indicate that resistance to malaria in thalassemia depends on human fetal hemoglobin and not on just thalassemia.

## Publications

***Makioka A, Kumagai M, Kobayashi S[1], Takeuchi T[1] ([1]Keio Univ).*** Involvement of serine proteases in the excystation and metacystic development of *Entamoeba invadens*. *Parasitol Res* 2009; **105:** 977–87.

***Tetsutani K, Ishiwata K, Ishida H, Tu L, Torii M, Hamano S, Himeno K, Hisaeda H.*** Concurrent infection with *Heligmosomoides polygylus* suppresses anti-*Plasmodium yoelii* protection partially by induction of CD4+CD25+Foxp3+ Treg in mice. *Eur J Immunol* 2009; **39:** 2822.

***Ikeshima-Kataoka H, Wada A, Ishiwata K, Watanabe N, Saito S.*** Cloning and expression of cDNA for interleukin 4 from the MSKR inbred strain of *Mus musculus molossinus*. *In Vivo* 2009; **23:** 277.

***Tetsutani K[1], Ishiwata K, Ishida H[1], Tu L[1], Torii M[2], Hamano S[1,3], Himeno K[1], Hisaeda H[1] ([1]Kyushu Univ, [2]Ehime Univ, [3]Nagasaki Univ).*** Concurrent infection with *Heligmosomoides polygylus* suppresses anti-*Plasmodium yoelii* protection partially by induction of $CD4^+$ $CD25^+$ Foxp $3^+$ Treg in mice. *Eur J Immunol* 2009; **39:** 2822–30.