Department of Tropical Medicine

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

General Summary

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

Research Activities

Malaria and mast cells

Malaria is one of the most serious protozoan diseases. The pathogenesis of malaria is believed to depend on immune responses to *Plasmodium* proliferating in the blood. We have proposed a hypothesis that pericapillary mast cells are a major factor in the pathogenesis of malaria. We examined the molecules activating mast cells. Mast cells have two receptors, namely the IgE receptor in acquired immunity and toll-like receptor (TLR) in innate immunity. For the IgE receptor, anti-*Plasmodium* IgE antibody was detected in mice infected with the parasite, and peroxiredoxin was identified as an antigen for IgE antibody production. Moreover, peroxiredoxin induced tumor necrosis factor from mast cells through TLR4. The stimulation of TLR4 by peroxiredoxin required CD14, MD-2, and MyD88. The direct binding of peroxiredoxin and TLR4 was confirmed by coprecipitation of these molecules. These findings suggest a new concept that TLR4 can induce innate immunity through proteins instead of lipopolysaccharides.

Immune responses against gastrointestinal parasites

Gastrointestinal parasites require energy for active establishment in the gut against intestinal flow and peristaltic motion. We determined the ATP value of individual adult *Nippostrongylus brasiliensis* during the course of immune-mediated expulsion from the small intestine in rats. The ATP values of adult worms taken from the mucosa of the proximal small intestine, the preferred site of adult *N. brasiliensis*, were higher than those of worms from other sites of the small intestine. The reduction of ATP values in worms from unfavorable sites was observed not only at expulsion phase, but also at established phase(s) of the infection suggesting that the energy metabolism of the parasites is independent of the host immune response. When adult worms with low ATP values were surgically implanted into the small intestine of naïve rats, the worms re-established themselves in the recipients and completely restored the ATP values. Short-term *in vitro* culture of adult worms under low oxygen tension resulted in low ATP values in the worms. These results suggest that adult *N. brasiliensis* are dislodged from their preferred site by intact energy metabolism activity.

Protective immunity against re-infection by *Hymenolepis nana* onchospheres develops

within 24 hours after primary infection; however, the mechanism is unknown except for the requirement for CD4-positive cells. Histological observation revealed that when the interval between primary and secondary infections was as short as several days, protection took place on the mucosal surface; secondaily infected onchospheres could not invade host intestinal tissue. When the interval was longer than 3 weeks, the onchospheres could penetrate the mucosal tissue once but were then driven out. Antibodies are not involved in these protection mechanisms. These results suggest a novel protective immune mechanism in the gut.

Effect of artificial gastrointestinal fluids on the excystation and metacystic development of Entamoeba invadens

The effect of artificial gastric fluid containing 0.5% pepsin and 0.6% hydrochloric acid (pH 1.8) on the excystation and metacystic development of *E. invadens* was examined. Excystation was enhanced by pretreatment of cysts with artificial gastric fluid for 30 to 60 minutes at 37°C but not at 26°C. In addition, 0.6% hydrochloric acid had an enhancing effect on excystation comparable to that of artificial gastric fluid. Metacystic development was slightly enhanced by pretreatment with artificial gastric fluid. An artificial intestinal fluid containing 1% pancreatin, 1% sodium bicarbonate, and 5% ox bile (pH 8.0) had a significant toxic effect on cysts. These results suggest that gastric fluid but not intestinal fluid at 37°C enhances excystation in *Entamoeba* infection.

Differences in protein profiles of the isolates of Entamoeba histolytica and Entamoeba dispar by surface-enhanced laser desorption ionization time-of-flight mass spectrometry ProteinChip assays

Surface-enhanced laser desorption ionization time-of-flight mass spectrometry (SELDI-TOF MS) ProteinChip assays were used for protein profiling of different isolates of *E. histolytica* and *E. dispar*. When SELDI-TOF MS spectra of *E. histolytica* strain HM-1: IMSS were compared with those from 4 other laboratory strains grown under the same culture conditions, different peak patterns were observed among these strains independent of their zymodeme types. Similarly, 5 Japanese isolates of *E. histolytica* grown under the same culture conditions revealed different peak patterns among themselves. The spectra of 2 isolates of *E. dispar* showed the presence of peaks specific for *E. dispar* isolates and the absence of peaks common to *E. histolytica* isolates. Thus, the SELDI-TOF MS spectra accurately reflect proteins of *E. histolytica* and *E. dispar* isolates, showing their phenotypic differences and providing a unique means of distinguishing them.

Publications

Furuta T, Kikuchi T, Akira S, Watanabe N, Yoshikawa Y. Roles of the small intestine for induction of toll-like receptor 4-mediated innate resistance in naturally acquired murine toxoplasmosis. *Int Immunol* 2006; **18:** 1655–62.

Furuta T, Kikuchi T, Iwakura Y, Watanabe N. Protective roles of mast cells and mast cell-derived TNF in murine malaria. *J Immunol* 2006; **177:** 3294–302.

Watanabe N, Tomimori Y, Terakawa M, Ishiwata K, Wada A, Muto T, Tanaka T, Maruoka H, Nagahira K, Nakatsuka T, Fukuda Y. Oral administration of chymase inhibitor improves dermatitis in NC/Nga mice. *J Invest Dermatol*

2007; **127:** 971–3.

Makioka A, Kumagai M, Takeuchi T (Keio Univ), Nozaki T (Gunma Univ). Characterization of protein geranylgeranyltransferase I from the enteric protist *Entamoeba histolytica*. *Mol Biochem Parasitol* 2006; **145:** 216–25.

Makioka A, Kumagai M, Kobayashi S[1], Takeuchi T[1] ([1]Keio Univ). Effect of artificial gastrointestinal fluids on the excystation and metacystic development of *Entamoeba invadens*. *Parasitol Res* 2006; **98:** 443–6.

Morimoto M[1], Yamauchi Y[1], Shinkawa S[1], Ishiwata K, Hayashi T[1] ([1]Yamaguchi Univ). Nematode infection in Alymphoplasia (*aly*) mice: worm species-dependent differential effect of defects of the gut-associated lymphatic tissue system. *Scand J Immunol* 2006; **63:** 365–70.

Watanabe N, Kikuchi T, Iwakura Y, Kawazu S, Kano S, Furuta T. Protective roles of mast cell-derived TNF in murine malaria. *Proc 11th Intl Cong Parasitol* 2006; 637–9.

Ishiwata K, Watanabe N. Primed mucins allow attainment and adhesion, but prevent establishment of challenged adult *Nippostrongylus brasiliensis* in the small intestine in mice. *Proc 11th Intl Cong Parasitol* 2006; 453–6.

Reviews

Watanabe N. Parasitic infection and allergy (in Japanese). *Allergology* 2006; **21:** 173–7.