Department of Tropical Medicine

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General Summary

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

Research Activities

Malaria and mast cells
Malaria is a severe protozoan disease of the tropics. We have studied under our own hypothesis that protection and pathogenesis in malaria depend on pericapillary mast cells through innate and acquired immunity. We have demonstrated that mast cell —derived vascular endothelial growth factor (VEGF) is responsible for protection in murine malaria. Human mast cell lines secreted VEGF upon stimulation with an extract of *Plasmodium falciparum*. Serum levels of VEGF were significantly higher in human patients with malaria than in healthy subjects. We then examined VEGF receptor (VEGFR) in murine malaria. The results indicate that VEGFR2 on vascular endothelial cells, but not VEGFR1 on macrophages, is responsible for protection. Serum levels of soluble VEGFR2 were significantly higher in patients with malaria than in healthy subjects. However, levels of soluble VEGFR1 were similar in patients and healthy subjects. These findings suggest that mast cell-derived VEGF participates in protection and pathogenesis through stimulation of VEGFR2 on vascular endothelial cells in human and murine malaria.

Chemotactic properties of adult Nippostrongylus brasiliensis *to mouse intestinal mucus*
Gastrointestinal nematodes are believed to be affected by intestinal mucus because they reside in mucus that covers and protects the intestinal epithelium. Their expulsion is not associated with killing by the host T cell-mediated immune systems but is likely related to the inhospitality of the mucus. Therefore, the chemotactic properties of mouse intestinal mucus on adult *N. brasiliensis* were examined. Adult worms, placed at the center of agar-coated dishes, were incubated at 37°C under 5% CO_2 with mucus taken from mice harboring worms (day 5-6 after infection; established-phase mucus) or during terminated expulsion (day 9-10 after infection; expelled-phase mucus). When expelled-phase mucus was placed at the center of the dish, most worms moved to the edge of the dish. In contrast, fewer worms moved to the edge when the mucus was placed at the edge of the dish. Thus, adult *N. brasiliensis* showed negative chemotaxis to expelled-phase mucus. The worms also showed negative chemotaxis to the established-phase mucus. Interestingly, the worms moved away from bile, a component of mucus, when it was placed at the center of the dish. These results suggest that worms

dislike intestinal mucus and that chemotaxis to intestinal mucus is not associated with adult *N. brasiliensis* expulsion from the small intestine in mice.

Analysis of actin depolymerizing factor cofilin in Entamoeba

Entamoeba histolytica cysts regain motility with the induction of excystation, with the amoeba passing through a small hole made in the cyst wall. Reactivation of motility and its control by actin cytoskeletal reorganization are necessary processes in excystation. This study investigated an important molecule in actin cytoskeletal reorganization: actin depolymerizing factor cofilin. *Entamoeba invadens* was used as a model for the excystation and development of *E. histolytica.* The cysts formed in an encystation medium were transferred into a trophozoite culture medium to induce excystation. A search of the *E. histolytica* and *E. invadens* genome databases indentified 1 type of cofilin for *E. histolytica* (EhCf) and 3 types for *E. invadens* (EiCf-1, Cf-2, and Cf-3). Levels of messenger RNA, measured with the real-time reverse transcriptase polymerase chain reaction, were higher in all EiCf proteins 5 hours after excystation than before the induction of excystation. Immunofluorescence staining with a rabbit anti-EiCf antibody and a mouse monoclonal anti-actin antibody showed that both cofilin proteins and the actin of trophozoites were localized immediately beneath the cell membrane. In particular, staining in pseudopodia was intense for both cofilin and actin, suggesting they are involved in amoeba motility. Cofilin and actin were also localized around the area immediately below the cell membrane in cysts. These findings demonstrate increased cofilin expression by excystation induction, cofilin colocalization with actin, and a close correlation between cofilin expression and amoeba motility.

Entamoeba transcriptome analysis using full-length complementary DNA

Construction of a complementary (c) DNA library is essential for transcriptome analysis. However, most conventional methods provide only incomplete cDNA lacking the 5′-terminal region. This problem was overcome by the oligo-capping method, which focuses on the cap structure of the 5′-terminal and allows construction of a full-length cDNA library including the mRNA 5′-terminal region. With this method, a full-length cDNA library was developed for *E. histolytica* and *E. invadens* trophozoites and publicized as a Full-Entamoeba database. The database contains information on 1150 and 1238 genes of *E. histolytica* and *E. invadens,* respectively. Comprehensive analysis revealed that the 5′-end untranslated region of *E. histolytica* and *E. invadens* cDNA is significantly shorter than those of other organisms by a mean of 12 and 10 base pairs each. The newly constructed full-length cDNA database complements the *Entamoeba* genome database and is useful for analyzing the transcriptional/translational regulatory mechanisms of amoeba genes.

Publications

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