Department of Tropical Medicine

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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 tropical diseases. We have proposed a hypothesis that pericapillary mast cells are a major factor in the pathogenesis of malaria. We have demonstrated that mast cells release a large amount of tumor necrosis factor, resulting in protection from *Plasmodium* in innate and acquired immunity. Recent studies have shown that mast cells produce vascular endothelial growth factor (VEGF), which activates macrophages and induces adhesion molecules in blood vessels. We have examined the effects of VEGF in murine malaria. VEGF levels in the serum increased after *Plasmodium berghei* infection. Parasitemia was decreased by administration of VEGF to infected mice. On the other hand, treatment with an anti-VEGF antibody increased parasitemia. These findings suggest a protective role of VEGF in malaria. In the next experiment, mast-cell-deficient mice were given injections of cultured mast cells from normal mice and were then infected. These mice had higher protective activity and VEGF levels than did untreated control mice. These results indicate that VEGF from mast cells participates in the protection against malaria and that mast cells are competent for immune protection.

Expulsion mechanisms of gastrointestinal parasites

Interleukin (IL)-4 and IL-13, both of which are derived from T helper type 2 cells, are involved in the expulsion of gastrointestinal nematodes. Experiments in mice have shown that the signal operates through non-bone marrow-derived cells in the small intestine, suggesting that the direct effector cells are not immune cells but cells of other types, such as epithelial cells and smooth muscle cells. Both cytokines are involved in the water/ion regulation of epithelial cells and the contraction of intestinal smooth muscle cells. These functions are also regulated by nerve systems. The selective α2-adrenoceptor agonist domitor inhibits sodium and water secretion into the lumen and the contraction of smooth muscle cells. The expulsion of adult *Nippostrongylus brasiliensis* from the small intestine in mice was suppressed by treatment with domitor. The expulsion was restored following treatment with a selective α2-adrenoceptor antagonist. In fact, treatment with domitor inhibited mucus secretion and peristaltic motion of the small intestine in infected mice. Adult worms in domitor-treated mice

were driven from the mucosa to the lumen and stayed there without being expelled. These findings indicate that the suppression of worm expulsion by domitor was due to inhibition of peristaltic motion. These results also suggest that responses of the nervous system are also involved in immune-mediated expulsion of adult *N. brasiliensis* in mice. Protection against secondary infection by eggs of *Vampirolepis nana* develops within several days after primary infection in mice. We attempted to identify the type of cell involved in this protection by using monoclonal antibodies to lymphocyte antigens. When an anti-CD3 antibody was administrated 1 day before primary or secondary infection, the protection against secondary infection was inhibited. However, administration of an anti-CD4 antibody 1 day before primary infection prevented protection, but treatment 1 day before secondary infection allowed protection. Administration of an anti-CD8 antibody had no effect. These results suggest that the protection depends on $CD4^+$ lymphocytes for the induction phase and $CD4^-CD8^-$ lymphocytes for the effector phase and indicate the participation of a novel subset of lymphocytes for mucosal immune responses.

Analysis of serine proteases, which mediate the excystation and metacytic development of Entamoeba

The functions of cysteine proteases involved in the pathogenicity and differentiation of *Entamoeba histolytica* have been demonstrated, but little is known about the functions of serine proteases. This study examined the involvement of serine proteases in amoebic excystation and metacystic development using *Entamoeba invadens* as the model of *E. histolytica*. Four serine-protease inhibitors — phenylmethylsulphonyl fluoride (PMSF), aminoethylbenzenesulphonyl fluoride, tosylphenylalanylchloromethyl ketone and dichloroisoproterenol — given at different concentrations decreased the number of metacystic amoebae in a dose-dependent manner but did not affect the survival of cysts. PMSF also inhibited the development of metacystic amoebae. PMSF effectively inhibited serine protease activity in cystic lysates. These data demonstrate the involvement of serine proteases in amoebic excystation and development. *E. invadens* has each two types of enzymes of serine protease family member S28 and S9 in the genome databases. The real-time reverse transcriptase polymerase chain reaction revealed that the mRNA expression levels of these serine proteases 5 hours after induction of excystation were higher than those before induction, in which an increase in expression of one type of the S9 enzyme was most significant. These results indicate that serine proteases mediate the excystation and metacystic development of *Entamoeba* and that serine protease mRNA levels in amoeba cysts increase after induction of excystation, especially that of the one type of S9.

Protein analysis of amoeba isolates with a liquid chromatography/tandem mass spectrometry system

Comprehensive analysis of proteins from amoeba isolates was performed with a liquid chromatography-electrospray ionization/tandem mass spectrometry system. Ten strains of cultured *E. histolytica* (including 5 domestic isolates) and 2 strains of a nonpathogenic amoeba, *Entamoeba dispar,* were used. Detected proteins were searched for with

Mascot Search, and the amount of protein was estimated from the exponentially modified protein abundance index (emPAI) for comparison. Actophorin and pyruvate phosphate dikinase, which were detected and identified in all isolates, showed a difference in the emPAI values according to the different protein expression patterns among strains. Alcohol dehydrogenase 3 and coactosin were detected and identified in all *E. histolytica* isolates but not in 2 *E. dispar* strains. In contrast, 40S ribosomal protein S18 and peroxiredoxin were detected and identified in the 2 *E. dispar* strains but not in the *E. histolytica* isolates. These results suggest that the present system is effective for detecting and identifying proteins of amoeba isolates with their expression levels and for distinguishing between *E. histolytica* and *E. dispar*.

Publications

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Reviews and Books

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