

Department of Tropical Medicine

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General Summary

There is a great need to develop novel parasite control strategies because of the failures of current eradication approaches and the logistical difficulties to implement them. One interesting aspect of these diseases is that the vector arthropods that transmit the pathogens can mount immune responses against the infection that will kill a large proportion of parasites. Our group is pursuing research that covers 4 topics: (1) vector-parasite interactions, (2) infection response in intermediate host, (3) immune responses to helminth infection, and (4) vector epidemiology.

Research Activities

Intestinal mucus barrier against re-infection of a gastrointestinal nematode

Heligmosomoides polygyrus (Hp), one of the murine gastrointestinal nematodes, infection has been employed as a re-infection model of parasite immunology. The swallowed larvae of infective stage once penetrate mucosa into muscularis where they grow up, then appear back to the lumen of the small intestine in mice. Although Hp persist to reside on the mucosa for over two months in initial infection, re-infected Hp after deworming is expelled from the small intestine soon after establishment on the mucosa. Many immunological studies focused on the protective immune response against Hp in the lumen of the gut, however, the number of re-infected Hp just after establishment on the mucosa is lower than those of initially infected Hp suggesting that acquired protective immune response to Hp also includes penetration blockade of the larvae at the mucosa surface. Here we confirmed the blockade and examined related mechanisms. Infective larvae were inoculated 4 weeks after deworming of initial infection, then the growing larvae in the muscularis of the small intestine were counted under the dissecting microscope on day 6 after the challenge infection. Number of larvae in the immune mice was lower than that of naïve mice. The statistical significant difference in the total numbers of larvae was derived from the difference in upper part 1/6 of the small intestine where initially infected larvae prefer to penetrate and grow up. The blockade persisted for 10 weeks after deworming, and depended on Th2 cells and Fcε receptor. Results suggest that acquired protective immunity against Hp consists of two mechanisms; 1) accelerated expulsion of adult worms from the gut lumen, and 2) blockade of larval penetration into gut mucosa. The latter newly found-barrier mechanism would be valuable for the mucosal vaccine strategy.

*Toward the establishment of more suitable strain of *Lucilia sericata* for maggot debridement therapy*

Maggot debridement therapy (MDT) is one of biotherapy that involves the application of

green bottle fly, *Lucilia sericata*, larvae (Maggot) to the necrotic wounds. Maggot application to the wound promotes removal of necrotic tissues, disinfection, and healing. Toward the establishment of more suitable strain of fly for MDT, a measure to evaluate the ability of maggot for debridement has been developed. Maggots fed on nonliving human tissue, removed during surgical debridement, or mixture of ground beef and pork were compared for their growth by weighing. Each maggots group showed respective rise curves, indicating maggot actually eats nonliving human tissues. Our result suggests the necessity of evaluating maggot for their debridement ability prior to the application to MDT. Controlling maggot pupation by reducing ecdysteroids was also examined, which could lead to the extension of days between each treatment. Maggots injected with Ecdysteroid-22-oxidase (E22O), an ecdysteroid-inactivating enzyme, showed extended larval period without onset of pupation, thus suggesting potency of improved medical maggots applicable on wound for longer period. Our evaluation and improvement of medical maggots potentially amend therapeutic effect and patient outcome with MDT.

Elucidation of molecular basis of tick host detection

Tick-borne diseases present major public health issues worldwide. Blood-sucking insects dedicate many of their sensory abilities to detect and follow the physical and chemical signals emitted by their hosts. In general, it is known that mosquitoes are remarkable for their ability to locate blood meal using host body emanations such as CO₂, smell, and heat acting as strong mosquito attractants. Recently, evidence for thermosensitive sensilla on mosquito appendages has been uncovered. It was reported that the activation of a transient receptor potential, via ion channels involved in various types of sensory reception, including thermo-, chemo-, mechano-, and photoreception, is caused by an increase in temperature from 25°C to 37°C in mosquitoes. On the contrary, tick forelegs are known as antennae necessary for the recognition of distant hosts using the Haller's organ, a sensory structure containing sensilla on the dorsal surface of the leg. To understand the molecular processes by which ticks sense external thermal signals, we investigated the impact of ambient temperature on larval, nymph, and adult stage locomotion. To verify a previously unknown role for the tick Haller's organ during host recognition, we used an automated device that was able to quantify selected host-detecting behaviors (Etho Vision XT, Sophia Scientific). *Haemaphysalis longicornis* strongly reacted to a stimulus of carbon dioxide, but *Amblyomma testudarium* did not react. This data suggests that response behaviors to inducing stimuli, such as heat or carbon dioxide, are different depending on the host. We will investigate the participation of TRPA1 molecules in host exploratory behavior by the behavior analysis system.

Dissection of blood sucking behavior of mosquitoes

Exploring the molecular mechanism of blood sucking behavior of female mosquitoes is one of the critical steps to fight against vector-borne disease such as dengue and malaria, since pathogens are transmitted when mosquitoes are gorging on blood. It has been known that ATP in blood serves as a phagostimulant. To identify the candidate chemoreceptor that perceives ATP in mosquitoes, we focused on gustatory receptors (Grs) of *Drosophila melanogaster* from following two reasons: 1) sequence of P2X receptor that per-

ceives ATP in broad animals is missing in mosquitoes and *Drosophila*, and 2) nonvolatile compounds are generally perceived by Grs in *Drosophila*. Two-choice behavior assay showed *Drosophila* also show feeding preference to ATP and not to adenosine. Each 116 RNAi strain against 53 Grs was specifically expressed in neurons and feeding preference was examined by two-choice behavior assay. Among 53 Grs, 17 Grs seem to contribute to perceive ATP and 7 Grs to adenosine. Some of these Grs are conserved in mosquitoes, suggesting that they can be the strong candidates of ATP/adenosine receptors in mosquitoes. Thus, we revealed that *Drosophila* shows the same feeding preference to ATP as mosquitoes, and Grs seem to intermedate this chemoreception.

Seroepidemiology and risk assessment of Toxoplasma gondii infection in HIV/AIDS patients

In HIV-infected patients, AIDS develops with decreased CD4 positive lymphocytes. Toxoplasma encephalitis is one of the AIDS indicator diseases that its risk increases when CD4 positive lymphocyte becomes 100/ μ l or less. Majority of cases are caused by reactivation of bradizoites in brains, which forms latent infections. However, there is no adequate assessment of toxoplasma seroprevalences and its risk factors among Japanese HIV-infected patients. We collected serums from 400 HIV-infected patients who visited our hospital outpatient clinic and conducted serological evaluation of *T. gondii* specific-antibody levels by anti-toxoplasma IgG (ELISA), with confirmation by Sabin-Feldman Dye Test. As a result, 33 cases (8.3%) of patients were *T. gondii* IgG antibody positive, and every case was Sabin-Feldman Dye test positive. The obtained prevalence of seropositivity was equivalent to the previous survey that was conducted in pregnant women in Japan; there was no correlation with HIV infection. Also, a correlation between seropositivity and with a history of cat rearing was found, rather than having a habit of consuming rare meat from the questionnaire survey to the participants. Consumption of rare bivalve, which is reported to be a probable risk factor in other studies, was not correlated with toxoplasma infection in our study.

Publications

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