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General Summary

Because of the failures of current eradication approaches and the logistical difficulties of implementing them, novel parasite control strategies need to be developed. An interesting aspect of parasitic 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 responses in intermediate hosts, (3) immune responses to helminth infection, and (4) vector epidemiology.

Research Activities

Dissection of blood sucking behavior of mosquitoes
Exploring the molecular mechanism of the blood-sucking behavior of female mosquitoes is a critical step for fighting against vector-borne diseases, such as dengue and malaria, because pathogens are transmitted when mosquitoes are gorging on blood. To dissect the molecular mechanism of blood-sucking behavior, we performed RNA sequencing analysis and compared the expression pattern of genes in dengue and Zika virus-vector mosquito (*Aedes aegypti*) brains before and after blood suction. With this analysis, we noticed that the expression level of transient receptor potential-like (TRPL) was increased immediately after blood taking. With the CRISPR/Cas9 system, we have generated *TRPL* mutant mosquitoes. We found that camphor can directly activate TRPL in cultural cells. Furthermore, mosquitoes did not take camphor via membrane feeding when camphor was mixed with a phagostimulant. With these results, we hypothesize that mosquitoes stop sucking blood when they sense a camphor-like molecule in blood by TRPL, the expression of which is increased during the course of engorgement. We will investigate the blood/camphor taking behavior of *TRPL* mutants and verify our hypothesis.

Dissecting molecular interaction between entomopathogenic fungi and mosquito
Entomopathogenic fungi can invade a wide range of insect hosts in terrestrial and aquatic habitats and cause severe damage to insects. Upon fungal invasion, the innate immune system of insects mounts a response recruiting 2 distinct but complimentary forms, humoral and cellular immunity. Studies of insect-fungi interactions provide valuable insights into the coevolutionary arms race between entomopathogens and their hosts. In this study, to unveil fungi-insect molecular interactions, we performed biochemical characterization of virulence factors from *Beauveria bassiana*, a major entomopathogenic fungus capable of directly penetrating the cuticle of haematophagous insect hosts, the *Anopheles* and *Aedes* mosquitoes. Strains of *B. bassiana* were isolated from wild adult mosquitoes collected in Japan and West Africa, after which their pathogenicity against

laboratory-reared mosquitos (*A. aegypti*) was evaluated. Adult mosquitos infected with the *B. bassiana* 60-2 strain (B60-2) exhibited severe lethality, whereas mosquitos infected with the B9-3-1 strain showed less of an effect on their survival rate. Culture supernatants of B60-2, but not B9-3-1, showed more toxicity toward the adult mosquitoes when grown in the modified medium. To identify factors that can determine the different pathogeneses between these 2 strains of *B. bassiana*, the secreted proteins in the culture supernatant were compared, resulting in 2 proteins in the B60-2 culture supernatant being identified as candidate virulence factors. These results provide more insights into the ecological state of entomopathogenic fungus-mosquito interactions, which might imply an effective management of mosquito-borne diseases.

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

Maggot debridement therapy (MDT) is an effective method for debriding wounds, such as leg ulcers, supporting wound bed preparation. The larvae of the blowfly species *Lucilia sericata* are the most widely used for MDT owing to their preference for feeding on necrotic rather than healthy tissue. New evidence suggests that maggots might contribute to wound healing in other ways, such as reducing biofilms, disinfecting wounds, and stimulating the growth of healthy tissue. In this study, several new strains of *L. sericata* were screened and established toward developing a maggot strain for more efficient MDT. One corpse-borne strain (strain 28) showed outcomes most favorable than did the conventional *L. sericata* strain for MDT in all 3 evaluation points: food consumptions, body weight, and growth rate. Strain 28 then underwent differential gene expression analysis via RNA sequencing. Compared to the genes of the conventional *L. sericata* strain, 1,623 genes of strain 28 showed higher expression and 1,370 genes showed lower expression. These findings indicate that strain 28 and the conventional strain hold unique gene expression patterns, which are likely related to the capacity of debridement and the potency of wound healing of the respective strains. The findings also suggest that new *L. sericata* strains might be developed for more efficient MDT by modifying these candidate genes.

Novel immune-mediated protection against reinfection of a gastrointestinal nematode causing chronic infection in mice

Heligmosomoides polygyrus, a murine gastrointestinal nematode, establishes in the small intestine of mice for more than 2 months. Orally inoculated infective larvae grow up in the muscular layer of the small intestine and emerge to the gut lumen by day 8 postinoculation. Secondary inoculation, performed at 4 weeks after deworming of the initial inoculation, terminates within 2 weeks after the inoculation making this parasite used as a reinfection model. Comparison of the number of larvae in the muscular layer between primary and secondary inoculation suggested an immune-mediated blockade against larval invasion into mucosal tissue. To confirm this possibility, the early phase of infection kinetics was evaluated. Results showed that the larvae first invade the stomach tissue and re-invade in the upper 1/6 part of the small intestine from days 1 to 2 postinoculation. The recovered number of larvae from the stomach between primary and secondary inoculation on day 1 postinoculation did not differ. However, the number of recovered larvae

from the same part of the small intestine differed between the 2 inoculations on day 2 postinoculation; a significant difference was found on day 6 postinoculation. These findings indicate that the first invasion of larvae into the stomach evokes a memory immune response that blocks the following invasion of the larvae into the upper part of the small intestine during days 1 to 2 postinoculation. Although the species specificity of blockade remains to be examined, this novel mucosal defense would open a new aspect of protective immunity.

Publications

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