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

Possible cause of red meat allergy by tick bites

Recent several studies suggest that patients in the eastern United States with red meat allergy have serum immunoglobulin (Ig) E against ticks. The IgE recognizes galactose-α-1,3-galactose (α-Gal), which exists in ticks and in beef and pork, i.e., red meat. Japanese spotted fever, caused by *Rickettsia japonica* and transmitted via the tick *Haemaphysalis longicornis*, is endemic in Shimane Prefecture. Interestingly, the same area has many patients with red meat allergy. Therefore we collaborated with the Department of Dermatology, Shimane University School of Medicine, to examine the association of tick bites with red meat allergy. The carbohydrate α-Gal was detected in the salivary gland protein of *H. longicornis*, maintained for more 30 years in a laboratory, by immunoblotting with an anti-α-Gal antibody. The reactive several bands were approximately 50 kDa, whereas those of beef were 250 kDa or greater. Approximately 50 kDa of salivary gland protein-specific IgE was detected in the sera of 24 of 30 patients with the allergy. These IgE bound several bands of different molecular weight in beef protein. The binding was lost after the blotted membrane was treated with sodium periodate and suggests that the recognition of IgE includes carbohydrates. These results suggest that sensitization to tick salivary gland protein containing α-Gal is also a cause of red meat allergy in Japan. This result implies that tick bites transmit several pathogens and also cause red meat allergy.

Development of novel maggot debridement therapy with genetically modified blowflies

Maggot debridement therapy is a biotherapy technique in which sterile *Lucilia sericata* maggots are applied to ulcers. Although the precise mechanisms by which maggots heal ulcers are still unknown, numerous successful cases, and some less-successful cases, have been reported. Therefore, performing maggot debridement therapy with genetically modified maggots — specifically, maggots that have enhanced feeding and expanded targets — was recognized as a way to establish a novel technique for treating a wider range of cases more quickly. First, the relation between the olfactory mechanisms and feeding activity of

maggots was analyzed. The olfactory perceptions are speculated to differ between field-caught wild *L. sericata* and laboratory strains because of their feeding targets, various vertebrate remains, including the human body and ground beef/pork. Three independent lines were established by crossing field-caught blowflies, both morphologically and genetically identified, in each collected group. One of the olfactory-related genes, odorant receptor coreceptor (*Orco*), which is expressed in all olfactory receptor cells, was determined and analyzed for its sequences in each established fly strain and laboratory strain. All amino acid sequences of *Orco* were conserved and suggested a lack of diversity attributed to flies' feeding targets. As the next step to establish a novel method of maggot debridement therapy, genetically modified *L. sericata* maggots, based on the obtained sequence of *Orco*, are being produced.

Elucidation of molecular basis of tick host detection

Tick-borne diseases represent 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. Mosquitoes are known to have a remarkable ability to locate blood meals using host body emanations, such as CO_2, smell, and heat acting as strong mosquito attractants. Recently, evidence for thermosensitive sensilla on mosquito appendages has been uncovered. The activation of a transient receptor potential, an ion channel involved in various types of sensory reception, including thermoreception, chemoreception, mechanoreception, and photoreception, was reported to be caused by an increase in temperature in mosquitoes from 25°C to 37°C. In contrast, tick forelegs are antennae necessary for recognizing distant hosts with 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 effects of ambient temperature on locomotion in the larval, nymph, and adult stages.

Dissection of the blood-sucking behavior of mosquitoes

Exploring the molecular mechanism of the blood-sucking behavior of female mosquitoes is a critical step in fighting against vector-borne diseases, such as dengue and malaria, because pathogens are transmitted when mosquitoes are gorging on blood. In blood, ATP is known to serve as a phagostimulant. To confirm the nature of phagostimulants, we examined whether other nucleotides can be sucked by mosquitoes. Both AMP and deoxy-AMP served as phagostimulants like ATP, while adenosine and guanosine triphosphate did not. This finding suggests the existence of a chemoreceptor that specifically recognizes adenine nucleotides. To isolate candidate chemoreceptors of ATP, we performed RNA sequencing analyses of mosquito midguts to compare the gene expression pattern of pre- and post-ATP/blood suction. Changes in the expression of several molecules were common between ATP and blood suction, suggesting that these molecules are chemoreceptors of adenine nucleotides in the midgut of mosquitoes.

Revisiting a method for diagnosing toxoplasmosis: Development of the Toxoplasma *Killing Observation test*

Toxoplasma gondii, the most successful protozoan, infects approximately one-third of

persons worldwide. In most people who are infected, except immunocompromised patients and pregnant women, toxoplasmosis is a self-limited disease with mild symptoms or no symptoms. Immunocompromised patients, such as those who have acquired immunodeficiency syndrome, have undergone organ transplantation, or use steroids, are at risk for *Toxoplasma* encephalitis, pneumonitis, and retinitis. Many types of serodiagnosis are widely used to detect toxoplasmosis around the world. However, because *Toxoplasma* immunoglobulins G and M are the only available serodiagnostic agents in Japan, diagnosis is complicated in some cases. The Sabin-Feldman dye test, which was reported in 1948, is used to evaluate the aggregate ability of the tachyzoite-cidal immunoglobulin titer in a patient' serum. This classic serodiagnostic test still has high sensitivity and specificity as a standard diagnosis. A problem with the dye test is the complicated evaluation method, in which stained tachyzoites must be counted through visual recognition. We examined this problem with a tachyzoite expressing green fluorescent protein, which is an alternative marker for evaluating the deactivation of tachyzoites. The new, improved dye test, the *Toxoplasma* Killing Observation test, has the advantages of its objectivity and retention for evaluation and achieves outcomes equivalent to those of the classic Sabin-Feldman dye test.

Publications

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