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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

Mutual interaction by the mixed infection of 2 species of gastrointestinal nematode
Human gastrointestinal (GI) nematodes usually make chronic and mixed infections. Host immunity to the parasites evokes a response of helper T type 2 cells (Th2) followed by changing the environment of the intestinal tract dynamically, but the responses fail to drive them away from the gut. Murine experiments have shown that the worm expulsion depends largely on interleukin 13/signal transducer and activator of transcription (STAT) 6 signaling. *Nippostrongylus brasiliensis*, a murine GI nematode, penetrates subcutaneously, migrates the lung, reaches the small intestine by day 3 postinfection, and is expelled interleukin 13/STAT6–dependently by day 10 postinfection. Orally infected *Heligmosomoides polygyrus*, another murine GI nematode, grows in the submucosa of the small intestine, returns into the lumen on day 8 postinfection, and also develops a Th2 response. It survives in the tract for more than 2 months. Here, to investigate mutual interaction of 2 different kinds of GI nematode, *H. polygyrus* infection, regarded as a human infection model, was combined with *N. brasiliensis* infection. We examined whether *N. brasiliensis* could extend its parasitic period when *N. brasiliensis* was infected at the time *H. polygyrus* established well in the gut, and we also examined whether *H. polygyrus* was expelled with *N. brasiliensis* when *H. polygyrus* appeared back to the lumen at the time *N. brasiliensis* was expelled. Results suggested that (1) the parasite infected ahead expended its parasitic period with following infection of the other species, (2) consecutively infected parasite underwent influence of the host immunity led by the preceding infection parasite, and (3) the egg production ability of female worms was easily affected by the host immunity apart from worm expulsion. Although single infection experiments have raised an idea that an expulsion mechanism is different depending on the parasite species, this experiment system reflecting a phenomenon of the natural world will provide a fresh perspective in host immune response–mediated mutual interaction between the parasites living together.

Ejection of pathogen-invaded cell maintains midgut wall of malaria vector mosquito

The midgut of disease vectors contributes as a primary and most important physical barrier against pathogens. The overview of antipathogen responses has been unclear, and 2 conflicting models of mosquito midgut cells and *Plasmodium* ookinete invasion have remain unsettled. One model proposed the existence of distinct midgut epithelial cells without microvilli, called Ross cells, into which ookinete preferentially invades; the other proposed midgut wall consists of indistinguishable epithelial cells that change morphologically similar to Ross cells when invaded by ookinete. To reveal the conformation of midgut cells before and after invasion by ookinete, the midgut of mosquitoes (*Anopheles stephensi*) infected with rodent malaria parasite (*Plasmoidum berghei*) was analyzed precisely with confocal microscope for its function as a barrier. The infected midgut wall contained extruding cells, which had no microvilli, with its adjacent cells of both sides contacting in the basal part. Ookinetes were often observed inside or adjacent to extruding cells, indicating that invaded cells were extruded while ookinete migrated to neighboring cells. Meanwhile, the midgut from mosquitoes that had ingested noninfected blood showed a midgut wall with indistinguishable microvillar columnar cells. Our observations, supporting the latter model, demonstrated that mosquitoes positively eliminate parasites by ejecting parasite-invaded cells, whereas parasites that have invaded midgut cells migrate from cell to cell to escape from mosquito's elimination mechanisms. This competition between mosquitoes and parasites may explain the natural balance of vector tolerance and the number of parasites.

Evidence of vertical transmission of severe fever with thrombocytopenia syndrome virus in field-collected ticks

Tick-borne diseases represent major public health issues worldwide. Severe fever with thrombocytopenia syndrome (SFTS) virus is a newly identified *Phlebovirus* genus in the *Bunyaviridae* family causing acute hemorrhagic fevers in parts of East Asia, including China, Korea, and Japan. The SFTS virus has been detected and isolated from diverse species of ticks in the endemic areas. We collected ticks from 15 point localities (over an area of 10 km^2), on the island of Kyushu, Japan, in April and October 2013. In addition, the localities are known to have an abundance of ticks and a recent history of human cases of Japanese spotted fever. A total of 1,168 questing ticks were collected with flagging vegetation (with a white flannel cloth of 170 $\times$ 70 cm). The SFTS virus-specific RNA was detected with the reverse-transcription polymerase chain reaction (RT-PCR) in complementary DNA generated from the RNA of individual ticks. The SFTS virus-specific RNA was detected in 4 species: *Haemaphysalis formosensis*, *Haemaphysalis longicornis*, *Haemaphysalis flava*, and *Haemaphysalis hystricis*. The SFTS virus-specific RNA was not detected with RT-PCR in any field-collected *Ambryomma testudinarium*. Of the ticks collected in April, most (n = 794) were nymphs (SFTS virus positivity = 9.8%), 73 (8.2%) were adults, and only 10 (0%) were larvae. In contrast, in October we collected 8 (0%) nymphs, 38 (0%) adults, and 245 (7.3%) larvae. Interestingly, SFTS virus-specific RNA was also detected in larvae. Larvae may become infected with the SFTS virus via vertical transmission.

Genetic dissection of intermediate host and tapeworm interaction

The dwarf tapeworm, *Hymenolepis nana*, which belongs to the order *Cyclophyllidea*, is the most common cestode of humans. Its intermediate hosts are arthropods, in particular, beetles. Once the intermediate host ingests tapeworm eggs, oncospheres immediately hatch and pass through the insect's gut wall. Cysticercoids develop within the hemocoel, where they survive without loss of infectivity until the intermediate host is ingested by a definitive host. To examine the interaction between the tapeworm and the intermediate host, we employed a reverse genetic approach with the red flour beetle, *Tribolium castaneum*, in which a robust systemic RNA interference (RNAi) response is observed, as a model system to explore host responses to tapeworm infection. Adult knock-down phenotypes in *T. castaneum* were induced by injection of double-stranded RNA (dsRNA) into late instar larvae. We performed RNAi screening targeting several gene transcripts of the Toll and the immune deficiency pathways, which are major signaling pathways of the humoral immune response in insects. Reduction of Toll pathway function, which was induced by RNAi-mediated silencing of myeloid differentiation primary response 88 (*MyD88), Dif1,* and *Dif2*, in addition to Janus kinase/STAT and c-Jun N-terminal kinase components, increased the burden of cysticercoids. On the other hand, RNAi-mediated knockdown of immune deficiency pathway components, Death related ced-3/Nedd2-like caspase (*Dredd*) and immune deficiency (*imd*), had no significant effect on the cysticercoid load. Our findings suggest a pivotal role of specific pathways, such as the Toll signaling pathway, in regulating resistance to tapeworm infection.

The role of gut in Plasmodium-transmitting vector mosquito

Vector-borne diseases rely upon organisms, named vectors, such as mosquitoes, ticks, and sandflies, that have an active role in the transmission of a pathogen from one host to the other. A critical stage in pathogen transmission occurs in the vector midgut, when the pathogens ingested with blood first makes contact with the gut epithelial surface. To understand the response mechanisms within the midgut environment, including those influenced by resident microbiota against pathogens, we focus on both midgut bacteria species and the vector-pathogen interaction that confers diversity to the vector's competency for pathogen transmission. *Serratia marcescens* isolated from either laboratory-reared mosquitoes or wild populations in Burkina Faso shows great phenotypic variation in its cellular and structural features. Importantly, this variation is directly correlated with its ability to inhibit *Plasmodium* development within the mosquito midgut. Furthermore, this anti-*Plasmodium* function conferred by *S. marcescens* requires increased expression of the flagellum biosynthetic pathway that is modulated by the motility master regulatory operon, flhDC. These findings point to new strategies for controlling malaria through genetic manipulation of midgut bacteria within the mosquito.

Publications

Teshima T, Onoe H, Aonuma H, Kuribayashi-Shigetomi K, Kamiya K, Tonooka T, Kanuka H, Takeuchi S. Magnetically responsive microflaps reveal cell membrane boundaries from multiple angles. *Adv Mater*. 2014; **26:** 2850-6.