Department of Tropical Medicine

Hirotaka Kanuka, *Professor*　　Kenji Ishiwata, *Professor*

General Summary

Novel parasite control strategies must be developed because of the failures of current eradication approaches and the logistical difficulties to implement them. One 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 response in intermediate host, (3) immune responses to helminth infection, and (4) vector epidemiology.

Research Activities

Introduction of Sparganum proliferum *and its infectivity in laboratory mice*

Sparganum proliferum is a larval form of cestode for which the adult stage has not been identified and, thus, is called an orphan. The sparganum multiply in the human body in the form of branching buds, like the roots of wasabi. The first case in patients was reported by Professor Ijima at the University of Tokyo in 1905. Since then, only 21 cases have been reported worldwide, and many cases had lethal outcomes. Seven cases were from Japan; the estimated infection areas are Tokyo, 3 cases; Kyoto, 2 cases; and Kumamoto, 2 cases. The most recent case was reported from Toranomon Hospital (Aoshima M et al., Nihon Kyoubu-Shikkan-Gakkai Azsshi, 1989). Although the lifecycle and infection route are completely unclear, the pathophysiology of proliferative spargaonosis is summarized as follows. In the parenchyma organs, including bones, the sparganum randomly grow and divide by branching or budding and sometimes metastasize. Current antihelmintic drugs are ineffective. From the National Museum of Nature and Science, Japan, we received the sparganum, which was the only one in the world that has been maintained and isolated from patients. We confirmed the following points in sharing the maintenance of the precious species. (1) Approximately 2 months after transplantation into the abdominal cavity of a mouse, the number of individuals increased by proliferating and dividing in the free state in the abdominal cavity. (2) Occasional lesions were observed in the peritoneum, diaphragm, liver, and lungs. (3) Histologically, no worms were observed in the lesion, but cell infiltration, consisting mainly of round cells, was found around the substance considered to be the sparganum component. (4) The sparganum did not grow or divide even, if it had been maintained in the culture medium for about 3 months, but the number of individuals increased if transplanted into the abdominal cavity of mice. (5) When a sparganum was divided into 2 pieces and the fragment was transplanted into the abdominal cavity of the mouse, it grew. Employing with both culture and animal experiment systems, we are planning to elucidate the mechanism of growth and division of this sparganum and develop therapeutic agents.

Development of a novel method for xenomonitoring to detect virus-derived DNA in mosquitoes by loop-mediated isothermal amplification

Increasing incidence of mosquito-borne diseases, including Dengue fever and Zika virus disease, accelerates the demand for real-time and accurate surveillance data on pathogen-infected mosquitoes. Surveillance of pathogen-infected mosquitoes, namely xenomonitoring, peculiarly achieved success in the program to eliminate lymphatic filariasis. Meanwhile, xenomonitoring for virus-infected mosquitoes has been stagnated. This stagnation is due to most of the viruses inducing mosquito-borne diseases being RNA viruses, which makes infected mosquitoes difficult to store in endemic areas. In 2016, the novel finding that virus-derived DNA (vDNA) is generated in cultured cells and mosquitoes infected with Dengue virus or Chikungunya virus was reported (Goic et al., Nat Commun, 2016). In this study, taking advantages of DNA being stable in dried mosquito samples, we aimed to develop a new xenomonitoring method to detect vDNA instead of virus genomic RNA by loop-mediated isothermal amplification (LAMP), which was named vDNA-LAMP. With an optimized primer set, LAMP was used to detect vDNA in mosquito-derived culture cells, C6/36 cells, and a mosquito host, *Aedes aegypti*, infected with either Dengue or Zika virus. After the LAMP reaction, the target sequence of vDNA was successfully amplified and detected. Moreover, vDNA-LAMP was applied to the field trial of xenomonitoring with wild mosquito samples collected in Burkina Faso, where Dengue fever is endemic. Mosquitoes collected in each household was pooled, and of which DNA was purified and provided for vDNA-LAMP targeting Dengue virus 2. As a result, vDNA of Dengue virus 2 was detected in mosquitoes collected in 4.8% of households. Together, application of vDNA-LAMP to identify virus-infected mosquitoes provided a potential as a new procedure for xenomonitoring.

Loop-mediated isothermal amplification applied to severe fever with thrombocytopenia syndrome virus vDNA detection in ticks

Severe fever with thrombocytopenia syndrome virus (SFTSV) is a newly identified phlebovirus causing acute hemorrhagic fever in East Asia, China, Korea, and Japan. The SFTSV has infected more than 8,000 people and has an infection fatality rate of 6.4% to 20.9%. The virus lifecycle and natural mechanisms of sustained transmission remain to be fully clarified, but transmission via ticks is considered the most plausible route. Although SFTSV infections cause human disease, infections of arthropod vectors are basically nonpathogenic and persist throughout the life of ticks. Recent studies have revealed that DNA forms of arboviral RNA genomes play a significant role in viral persistence in *Drosophila* and *Aedes* species. It is possible that SFTSV DNA forms are also generated in ticks. We conducted a study to detect the appearance of SFTSV DNA forms following infection of wild ticks.

For epidemiological analysis, we collected ticks from 20 locations on Kyushu, Japan, in 2018 and 2019. These sites were selected for the survey on the basis of the number of human cases of SFTS identified in these regions. Ticks were collected by flagging vegetation (using a white flannel cloth of 170 × 70 cm). Of the 379 tick pools subjected to vDNA-LAMP to detect SFTSV vDNA, 11 were positive. Because vDNA is more stable than RNA, this stability might also lead to new methods to study the epidemiology of

SFTSV. These results provided evidence of SFTSV vDNA synthesis in wild ticks, which might have implications for the effective management of tick-borne diseases by xeno-monitoring using vDNA.

Dissection of blood sucking behavior of mosquitoes

Exploring the molecular mechanism of blood sucking behavior of female mosquitoes is a critical step to fight against vector-borne diseases, such as dengue and malaria, because pathogens are transmitted when mosquitoes are gorging on blood. The ATP in erythrocytes of host blood enhances the blood sucking of mosquitoes. Furthermore, mosquitoes reportedly do not exhibit blood sucking when only plasma is presented. From our experiment, we discovered that the ratio of full-engorged mosquitoes was significantly reduced when a mixture of ATP and plasma was presented to mosquitoes compared to when only ATP was presented, suggesting an inhibitory factor for blood sucking in plasma of host blood. This inhibitory effect was observed when supernatant of boiled plasma was used, indicating that the inhibitory factor might be a nonprotein component. We also fractioned this supernatant of boiled plasma with reverse-phase high-performance liquid chromatography and examined the inhibitory effect by presenting each fraction to mosquitoes. Only one hydrophilic fraction exhibited the inhibitory activity. Although many components are still included in this hydrophilic fraction and further investigation is required to identify the factor, we revealed the existence of a negative factor for blood sucking in host blood. Mosquitoes seem to perceive both positive and negative factors when they suck blood and decide whether to continue or to stop blood sucking in a context-dependent manner.

Publications

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