Department of Tropical Medicine

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General Summary

There is a great need to develop novel strategies for controlling parasites because of the failures of current eradication approaches and the logistical difficulties of implementing them. An interesting aspect of parasitic diseases is that the arthropod vectors that transmit the pathogens can mount immune responses that kill a large proportion of the infecting parasites. Our group is pursuing research that covers 4 topics: 1) modification of mosquito vectorial capacity, 2) vector–parasite interactions, 3) immune responses to helminth infection, and 4) genomics of protozoan parasites.

Research Activities

Analysis of mucosal immunity to gastrointestinal nematode infection

The intestinal tract possesses an immune system that regulates elimination and tolerance to various kinds of foreign substances, such as foods and microorganisms, coming from the mouth. The uniqueness of the intestinal tract's natural and acquired immunity, differing from systemic immunity, is being revealed. Interestingly, most types of human intestinal parasitosis are chronic infections that readily recur. The mucosal immune system has difficulty eliminating intestinal parasites as pathogens. In a mouse model, *Nippostrongylus brasiliensis* infection terminates within 2 weeks and leads to protective immunity against reinfection which is dependent on the Th2 immune response. Dendritic cells (DCs) and T cells in mesenteric lymph nodes were both activated 1 day after nematode establishment in the small intestine. The Th2 response, estimated by interleukin 4 production in the mesenteric lymph nodes, was detected at least 3 days after establishment, and the worms were expelled by 7 days after establishment. Interestingly, DCs decreased their MHC class II expression on cell surfaces after the transient activation. Antigen-presenting ability to specific T cells, followed by clonal proliferation, of DCs 5 days after establishment was half that of DCs 1 day after establishment. CD4-positive DCs, which are supposed to present foreign antigens to T cells, disappeared at the same time. These findings are under evaluation by comparing immune responses to *Heligmosomoides polygyrus* infection whose establishment continues more than one month in the mouse small intestine.

Transcriptome analysis of Entamoeba using an ultrafast sequencer

We have been performing transcriptome analysis of *Entamoeba histolytica* and *Entamoeba invadens*. *E. invadens* is a parasitic amoeba of reptiles which has been used as an alternative model to the encystation of *E. histolytica*, because *E. invadens* has a morphology and life cycle similar to those of *E. histolytica* and can be easily induced to

undergo encystation in an in vitro axenic liquid culture; in contrast, *E. histolytica* rarely forms cysts in vitro. At the outset, we tried to clarify whether the 5' untranslated region (5'UTR) of messenger RNA is as short as indicated by the analysis of a small number of genes. Through cooperative research, sequencing and bioinformatic analysis with various technologies were performed. We have been following sequences of the 2 species: 1) full-length complementary (c) DNA sequences without deletion of the 5' ends with the oligocapping method; 2) large quantities of tag sequences of 35 nucleotides starting from the transcription start site (TSS) obtained with TSS-Seq, a method that combines the advantages of oligocapping method and next-generation sequencers; and 3) RNA shotgun sequence assembly with RNA-Seq. These sequences were mapped on the genome sequences of *E. histolytica* and *E. invadens*. Previously, we published a database for full-length cDNAs of *E. histolytica* and *E. invadens* (http://fullent.hgc.jp/). Now we have integrated high-throughput data with our full-length cDNA database (http://fullent.genome.ad.jp). Integrated tools help users to visualize, search, and download the data. We then confirmed that the 5'UTRs of the genes of *E. histolytica* and *E.invadens* are uniformly short (about 10 nt on average). It became clear with TSS-seq that TSSs are not always fixed but can show variations. RNA-Seq demonstrated the alternative splicing between trophozoites and cysts in some genes of *E. invadens*. More than 500 cDNA sequences were mapped on intergenic regions of *E. histolytica* and *E. invadens*. Analyses of protein families (Pfam database) and RNA families (Rfam database) showed that 29 cDNA sequences were coding sequences of new genes that had not been predicted, 4 were of transfer RNA genes, and 1 was of a 5S ribosomal RNA gene. The other sequences are probably those of noncoding RNA, although structural analysis will be necessary for confirmation.

Amino acid-related host nutrition dynamics during malaria infection

Malaria parasites, which disable and kill more than 1 million people every year repeat division and multiplication in erythrocytes, taking in nutrition from an immediate environment. Free amino acids in the blood plasma might play an important role in the establishment of blood-borne parasites, because the biosynthetic pathways for most amino acids are absent in parasites, which rely on exogenous amino acids for most of their growth requirements. We demonstrated that plasma aminograms, which show the free amino-acid pattern of the blood plasma, change markedly as parasitemia and mortality increase, resulting in infection-dependent nutrition dynamics that belong to different clusters. Moreover, comparison of preinfection plasma aminograms between BALB/c, C57BL6/J, and C3H/HeN mice, which have different sensitivities to malaria, showed that the concentrations of a subset of amino acids increase in proportion to malaria sensitivity. Because amino acid metabolic pathways are constructed as complicated nutritional networks, multivariate interference of amino acids might define the infectious disease process. Our results suggest that plasma aminograms strongly correlate with malaria parasite infection.

Intraspecific diversity of midgut commensal bacteria in Anopheles mosquitoes defines Plasmodium transmission capacity

A critical stage in malaria transmission occurs in the *Anopheles* mosquito midgut, when the malaria parasite, *Plasmodium*, ingested with blood, first makes contact with the gut epithelial surface. To develop novel strategies for controlling malaria, an understanding of the response mechanisms within the midgut environment, including those influenced by resident microbiota against *Plasmodium*, is needed. Here we focus on a midgut bacteria species' intraspecific variation that confers diversity to the mosquito's competency for malaria 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 the ability of *S. marcescens* 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, which is modulated by the motility master regulatory operon, *flhDC*. These findings point to new strategies for controlling paratransgenic malaria through genetic manipulation of midgut bacteria within the mosquito.

Odor-based contagious transmission of pathogen by fly

The housefly and flies in general are considered to be mechanical vectors of many kinds of pathogens, whereas the mosquito serves as the biological vector for those pathogens. Mechanical vectors simply convey pathogens and are not essential for their development or life cycle. To clarify the molecular mechanisms of transmission by fly, we first established a model system for transmission using *Drosophila melanogaster*. Green fluorescent protein-labeled *Escherichia coli* located on the center of an agar-plate was freely ingested by *Drosophila*. Substances excreted in the feces are easily observed as small fluorescent spots on the surface of agar, showing that flies directly feed on *E. coli* and disseminate it by excretion. Flies without antennae, which contain a large set of olfactory receptors, and flies deficient for *Or83b*, which encodes a broadly expressed odorant receptor, showed impaired dissemination of bacteria. Whereas wild-type flies showed behavioral responses to attractive odors released from growing *E. coli*, the *Or83b*-deficient mutants failed to respond to these odors. Volatile compounds emitted from culture supernatant of *E. coli* were trapped and identified with gas chromatography-mass spectrometry. The predominant compound produced by *E. coli* was indole, along with lesser amounts of alcohols. We also showed that LUSH, the *Drosophila* orthologue of indole-binding protein, is required for transmission of *E. coli* as excreted droplets. Given that *Drosophila* LUSH is also known as a component to activate pheromone-sensitive neurons, we suggest that the pheromone-mediating system also promotes feeding behavior in the presence of indole from pathogens, contributing to the transmission of infectious diseases, such as food poisoning.

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