Department of Tropical Medicine

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General Summary

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

Research Activities

Alterations of mucins in mice infected with gastrointestinal helminths
Mucus, of which a major constituent is high molecular weight glycoproteins called mucins, is a primary line of defense for the gastrointestinal mucous membrane. Previously we suggested the possibility that interleukin 13–mediated sialylation of mucins is associated with expulsion of adult *Nippostrongylus brasiliensis* in mice. Here we verified the universality of the above association in other gastrointestinal helminths dwelling in the small intestine of mice: *Heligmosomoides polygyrus* (a nematode) and *Vampirolepis nana* (a cestode). The amount of mucins increased 300% in the jejunum, the habitat of *N. brasiliensis*, at the time of worm expulsion, and the mucins reacted with an antibody (HCM31) against sialo sugar chains. Primary infection with *H. polygyrus* results in chronic infection, whereas secondary infection, performed 4 weeks after deworming the primary infection, terminates within 2 weeks. In *H. polygyrus* infection, the amount of mucins increased 300% to 500% in the jejunum, the major habitat of *H. polygyrus*, and the mucins reacted with HCM31 regardless of the number of times of infection. In *V. nana* infection, the amount of mucins increased in the ileum, the habitat of adult *V. nana*, at the time of worm expulsion, and the mucins reacted with an antibody (PGM34) against the sulfo sugar chain. These results suggest that nematode infection induces sialomucins and that cestode infection induces sulfomucins, although these changes do not appear to be consistently involved in worm expulsion. Further study is needed to confirm our findings.

Transcriptome analysis of Entamoeba with an ultrafast sequencer
We have been performing transcriptome analysis of *Entamoeba histolytica* and *Entamoeba invadens*, which are parasitic amoebas of humans and reptiles, respectively, and have similar morphology and life cycle. *E. invadens* can be easily induced to undergo encystation in an *in vitro* axenic liquid culture and has been used as an alternative model

for the encystation of *E. histolytica*. Using oligo-capping methods (full-length complementary DNA sequence and transcription initiation site [TSS] sequences), we have determined the TSSs of the messenger (m) RNA precisely in large quantities, although TSSs were distributed in clusters and not individually determined. The 5′ untranslated regions of mRNA of these *Entamoeba* species are comprehensively short (12.38 nt and 8.15 nt on average, in 37% and 25% of total predicted genes, respectively). Here we searched the base preference and the conserved motifs, which are position-specific to TSSs. Among the clusters of TSSs, purines (guanine or adenine) were predominant (purine:pyrimidine ratio = 9:1). More than 70% of nucleotides at the most frequent TSS (at the position 0) in a TSS cluster were adenines, and more than 80% of nucleotides at the position −1 were pyrimidines. We found and characterized 5 motifs: AACT and TAT(A/T)(T/A)AA were position-specific to TSSs; AACCCT and AGGGTT were complementary to each other; and many genes with the GGAA motif were specifically expressed during encystation of *E. invadens*.

A simple procedure for permanently stained preparations of E. histolytica and Giardia intestinalis in stool samples using BD SurePath™

BD SurePath™ (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) is new liquid-based cytology system in which negatively charged cells are "smeared" on a positively charged precoated glass slide. By modifying several steps of the BD SurePath™ system, which ordinarily used with Papanicolaou staining, we can easily make trichrome-stained permanent preparations of cysts of *E. histolytica* and *G. intestinalis*. First, stool samples were suspended in the basic solution for Kohn's stain and left to fix for more than 10 minutes. Then the sample was placed in a chamber settled on the precoated (positively charged) glass slide. After the chamber was removed, the slide was immersed in 70% ethanol and stained with trichrome stain. As a result, stool samples were thinly smeared on the slides. Internal structures, such as the nucleus and karyosome, stained clearly. With conventional direct methods, smearing samples thinly and uniformly is difficult; as a result, the morphology of protozoa are difficult to see in the thick part of the smear, and staining is often irregular. In addition, making smears is time-consuming, stools cannot be smeared several days after being passed, and smears of watery stool easily become detached. We were able to overcome these drawbacks by using the BD SurePath™ system.

Molecular interplay between malaria liver stage parasites and the host

Malaria remains a major global health burden, infecting more than 300 million people and resulting in approximately 1 million deaths each year. This grim situation has led to the search for novel control and intervention strategies, in particular, the development of a malaria vaccine. However, these efforts have met with limited success because of the antigenic complexity of the parasite and the differential expression of proteins. Therefore, the next generation of malaria vaccine might exploit a variety of methods for activating an immune response. To better understand the molecular interplay between malaria parasites and hosts for future vaccine development, we have focused on malaria liver stage development, especially the parasitophorous vacuole membrane (PVM), which

is the foremost parasite membrane in the battle against the host. We have found that liver stage parasite PVMs were surrounded by the host cell autophagy marker (LC3) in early liver stage development (< 24 hours after infection); however, most parasites had survived without digestion. Possibly the malaria parasite can control the host cell autophagy response that accompanies digestion. These findings reveal a novel parasite-host interaction and suggest that malaria liver stage parasites possess escape/control/hijack molecules against host digestion for survival inside the host cell.

Differential dynamics of host amino acids in plasma and liver during infection of malaria parasite Plasmodium yoelii

Although malaria is the most significant human parasitic disease, our understanding of the energy metabolism of the principle pathogen, *Plasmodium falciparum*, remains incomplete. Amino acids have long been known to be essential nutrients, and much of the current knowledge of *Plasmodium* energy metabolism is based on early biochemical work, performed with basic analytical techniques, almost exclusively on human plasma with considerable interindividual variability. To further characterize the fate of amino acid metabolism in the malaria parasite, multivariate analysis using statistical modeling of amino-acid concentrations (aminogram) of the plasma and liver were performed in hosts infected with the rodent malaria parasite, *P. yoelii*. Comprehensive and statistical aminogram analysis revealed that *P. yoelii* infection caused marked changes in plasma and liver aminograms and altered the intracorrelation and intercorrelation of amino acid concentrations in the plasma and liver. These findings of the interactions between nutrition and *Plasmodium* infection may provide insight into the protective mechanisms and lead to nutrient-based interventions as low-cost and effective adjuncts to current methods of malaria prevention and treatment.

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 focused 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 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 para-transgenic malaria through genetic manipulation of midgut bacteria within the mosquito.

Odor-based mechanical transmission of bacteria by fly feces

The housefly and flies in general are mechanical vectors of many types of pathogen, whereas mosquitos are biological vectors 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-coated plate was freely ingested by *Drosophila*. Substances excreted in the feces are easily observed as small spots with fluorescence on the surface of agar, showing that flies directly feed on *E. coli* and disseminate them by excretion. Flies without antennae that contain a large set of olfactory receptors or are deficient for *Or83b*, which encodes a broadly expressed odorant receptor, showed impaired dissemination of bacteria. While wild-type flies showed behavioral responses to attractive odors released from growing *E. coli*, the *ORCO* (*Or83b*) 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, which was accompanied by lesser amounts of alcohols. We also showed that LUSH, the *Drosophila* orthologue of an indole-binding protein, is required for transmission of *E. coli* as excreted droplets. Given that *Drosophila* LUSH also activates pheromone-sensitive neurons, we therefore 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.

Publications

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