

PARASITE BIOLOGY

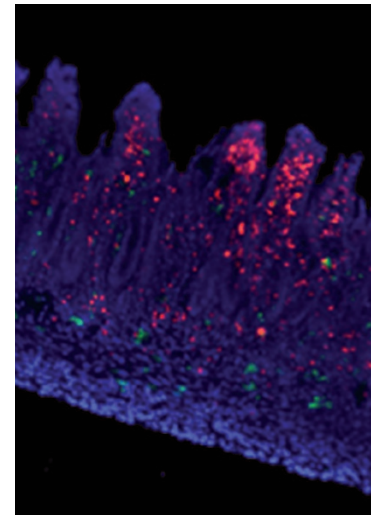
The stowaway traveller

Toxoplasma gondii is transmitted through contaminated water and food to establish infection in the small intestine of its mammalian host before spreading and forming cysts in various tissues. Little is known about the first stages of infection, and it is unclear which cells are the initial targets and how the infection then amplifies in the intestine. A new study now sheds light on these questions using two-photon microscopy in a mouse model of *T. gondii* infection.

Previously, it had been shown that *T. gondii* enters migrating leukocytes and uses them to leave the intestinal tract and spread between tissues. The current study set out to test whether this mode of host cell-mediated dissemination also plays a part in the intrainstestinal spread of *T. gondii* following oral infection of mice. Fluorescence microscopy of RFP-expressing *T. gondii* revealed that mostly individual, isolated parasites were present in infected villi early after inoculation, and foci containing multiple parasites, frequently at the top of neighbouring villi, could be detected reliably only at later time points. This suggests that there is local parasite replication at the site of entry, resulting in parasite release back into the lumen and subsequent luminal spread. Using lysozyme M–GFP reporter mice, in which both neutrophils and intestinal

macrophages express high levels of GFP, it was possible to show that these immune cells accumulated near the parasite foci in the intestinal tissue and lumen. Further analysis by flow cytometry showed that 10% of intestinal neutrophils contained parasites and that neutrophils made up 50% of all parasite-containing immune cells in the small intestine. To determine whether *T. gondii* had actively invaded neutrophils or had been phagocytosed, isolated neutrophils were stained for the parasite dense granule protein GRA6, which is a component of the parasitophorous vacuole formed by the parasite during invasion. The majority of parasite-containing neutrophils showed a GRA6 staining pattern typical of intact, invasive parasites. In time-lapse two-photon microscopy images of explanted intestinal tissue, parasite-invaded neutrophils exhibited high motility inside the tissue and were observed migrating across the intestinal epithelium and in the luminal mucous layer. Finally, purified invaded neutrophils could transmit *T. gondii* to cultured human fibroblasts and to naive mice.

Taken together, these results suggest that *T. gondii* uses neutrophils to spread through the intestinal lumen, and this unusual mode of transport might allow an initially small number of parasites to efficiently establish multiple foci. Other pathogens,



Immunofluorescence image of parasite foci (red) and neutrophils (green) in the small intestine of orally infected mice. Image courtesy of E. A. Robey, University of California, Berkeley, USA.

such as *Salmonella* and *Listeria* spp., also use retrograde release into the intestinal lumen to amplify locally, but whether immune cells are involved in these cases remains to be determined.

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ORIGINAL RESEARCH PAPER Coombes, J. L. et al. Motile invaded neutrophils in the small intestine of *Toxoplasma gondii*-infected mice reveal a potential mechanism for parasite spread. *Proc. Natl Acad. Sci. USA* 6 May 2013 (doi:10.1073/pnas.1220272110)

FURTHER READING Hunter, C. A. & Sibley L. D. Modulation of innate immunity by *Toxoplasma gondii* virulence effectors. *Nature Rev. Microbiol.* 10, 766–778 (2012)