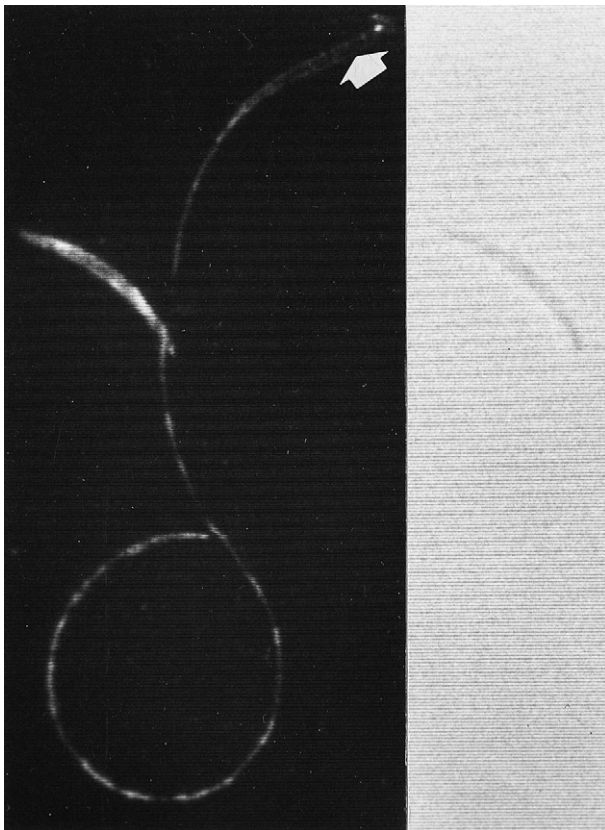


Research letters

Thrombospondin-related adhesive protein (TRAP) of *Plasmodium berghei* and parasite motility

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Thrombospondin-related adhesive protein (TRAP) plays a part in malaria sporozoite recognition and entry into host hepatocytes.^{1,2} Little is known about how this molecule, mainly localised in the parasite micronemes, contributes to sporozoite invasion.³ The presence of conserved adhesive motifs within the amino acid sequence of TRAP suggested that this molecule could interact directly with ligands on the surface of host cells.⁴ We observed that air-dried *Plasmodium berghei* sporozoites, while being processed for immunofluorescence, leave a trail of TRAP immunoreactive material on the microscope slide, suggesting that TRAP was released during parasite movement. We looked for TRAP after inducing sporozoites to glide on microscope slides at 37°C. Trails of TRAP immunoreactive material were observed behind most sporozoites (figure). Gliding sporozoites occasionally detached from microscope slides leaving an immunoreactive footprint on the glass that reveals a uniform distribution of TRAP along the surface of the parasite body (figure arrow). These findings show that in motile



Confocal fluorescence and transmission microphotographs

Sporozoites incubated at 37°C on a microscope slide for 10 min in culture medium. Gliding sporozoites shed bright trails of TRAP, a footprint of TRAP reactive material is left by a detached sporozoite (arrowhead). Magnification 630×, zoom factor 2.6 for image acquisition.

sporozoites TRAP is translocated to the parasite surface and is continuously shed behind in a similar fashion to that described for the circumsporozoite (CS) protein.⁵

We have investigated TRAP function during sporozoite movement with freshly dissected *P. berghei* salivary-gland sporozoites and a polyclonal mouse serum previously shown to specifically recognise TRAP. Examination by direct microscopy of live sporozoites revealed that parasites incubated with TRAP antiserum at 37°C showed a reduction in gliding motility. We carried out a quantitative analysis of this inhibitory effect by immunofluorescence with a biotin-labelled monoclonal antibody directed against the CS protein. We identified motile sporozoites by the presence of an immunoreactive trail of CS protein. This experiment showed that the TRAP antiserum inhibited, in a dose-dependent manner, sporozoite gliding. Sporozoites incubated with TRAP antiserum diluted 1/50 and 1/100 slowed CS-protein gliding trails by 19.5% and 41%, respectively. These percentages were significantly lower than those observed by incubating sporozoites with a control mouse serum raised against the recombinant protein 85A from *Mycobacterium tuberculosis*.

TRAP binding to host ligands would allow sporozoites to interact with biological surfaces thus providing molecular anchors for parasite gliding. These results also shed new light on the mode of action of TRAP antibodies in blocking sporozoite invasion of hepatocytes. TRAP antibodies would primarily impair sporozoite motility and, as a consequence of this, the ability of parasites to invade host cells. The demonstration that TRAP is implicated in sporozoite motility contributes to the understanding of the molecular mechanisms that lead to parasite infection of host cells, and provides the rationale for eliciting TRAP antibodies to block sporozoite invasion of hepatocytes.

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