PARASITOLOGY

Dissecting the role of LPG in *Leishmania* virulence

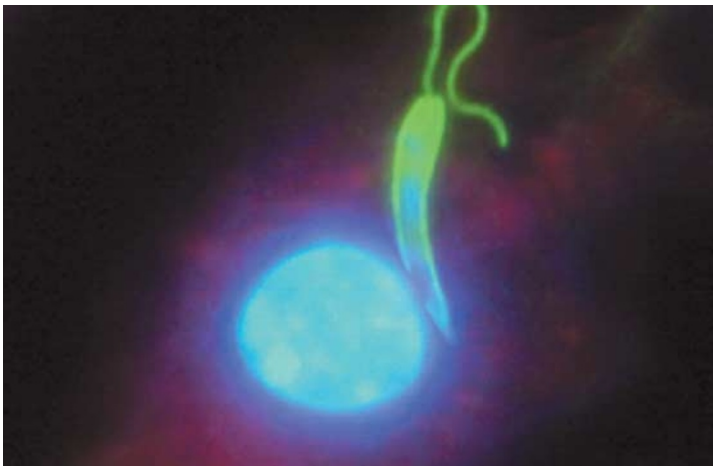

Image showing a *Leishmania major* promastigote entering a murine macrophage *in vitro* kindly provided by S.M. Beverley (Washington University School of Medicine, USA). Blue, DNA (hoechst dye); green, *Leishmania* phosphoglycans (antibody WIC 79.3); red, anti-tubulin (macrophage cell body).

A recent publication from Stephen Beverley's group continues their intriguing dissection of the role of lipophosphoglycan (LPG) in the virulence of the protozoan parasite *Leishmania*.

Leishmania is transmitted into mammalian hosts in its promastigote form and is soon phagocytosed by macrophages. Early *in vitro* assays using purified components indicated that LPG is involved in many of the processes essential for the survival of the parasite within the host, including resisting the innate immune system and entering macrophages. However, the specificity of these initial experiments was always of concern to *Leishmania* researchers as LPG shares its main structural domains with many other *Leishmania* secreted and surface proteins.

To address these concerns, Gerald Späth in Beverley's group used gene targeting to create a null mutant of the *Leishmania major lpg1* gene, which encodes an enzyme essential for LPG biosynthesis. In previous work, they established that *lpg1*$^-$ parasites are specifically defective in LPG and are attenuated for virulence. In this latest paper, Späth *et al.* have used their *lpg1*$^-$ mutants to take a more detailed look at the true biological function of *Leishmania* lipophosphoglycan.

Incubation of purified promastigotes with human serum demonstrated that *lpg1*$^-$ parasites are sensitive to lysis by human serum, and that the serum factor responsible is complement. This confirms that LPG has a role in resisting the innate immune defences in humans. Surprisingly, further results indicated that, in mice, lysis by complement is not an effective defence mechanism against *Leishmania*. The authors therefore comment that for *Leishmania*, and perhaps for other pathogens, inbred mice might not be suitable models to use for analysis of the role of the lytic functions of complement in infection.

Flow cytometry work showed that *lpg1*$^-$ parasites could still be opsonised by complement and enter macrophages; so, contrary to previous work with purified proteins, it would seem that *Leishmania* LPG is not a major adhesin involved in macrophage attachment and entry. However, *lpg1*$^-$ promastigotes did show a significant increase in sensitivity to oxidative stress compared with wild-type parasites, indicating that LPG is important in resisting the effects of intracellular oxidants.

The *in situ* effects of the *lpg1* mutation were studied by using fluorescence microscopy to follow the progress of phagolysosomal fusion within infected macrophages. Unexpectedly, the data indicated that although LPG is involved in the transient inhibition of phagolysosomal fusion soon after *Leishmania* enters the host cells, this inhibition is not essential for *Leishmania* survival within macrophages.

The ability to use genetic techniques such as targeted gene disruption is a relatively recent development for researchers working on protozoan parasites such as *Leishmania*. This new study shows that the careful use of these techniques is extremely valuable and can yield surprising results.

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Stephen Beverley's lab: http://www.microbiology.wustl.edu/dept/fac/beverley.html

Entre 2

lpg1
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=nucleotide&list_uids=7239376&dopt=GenBank

References and links

ORIGINAL RESEARCH PAPER Späth, G.F. *et al.* The role(s) of lipophosphoglycan (LPG) in the establishment of *Leishmania major* infections in mammalian hosts. *Proc. Natl Acad. Sci. USA* **100**, 9536–9541 (2003).

FURTHER READING Beverley, S.M. Protozomics: Trypanosomatid parasite genetics comes of age. *Nature Rev. Genet.* **4**, 11–19 (2002).

WEB SITES

Stephen Beverley's lab: http://www.microbiology.wustl.edu/dept/fac/beverley.html