Journal Club

Rationale for malaria anti-toxin therapy

Glycosylphosphatidylinositols (GPIs) are a distinct class of glycolipids that are present ubiquitously in eukaryotic cells, where they perform a variety of biological processes. GPIs are particularly abundant in protozoa, where they are found as free lipids and attached to proteins. For the asexual blood stages of *Plasmodium falciparum*, several functionally important parasite proteins, including merozoite surface protein 1 (MSP1), are anchored to the erythrocyte membrane through GPI moieties. Because the enzyme specificity of *Plasmodium* GPI biosynthesis differs significantly from that of the mammalian host, it has been speculated that parasite GPIs might provide novel targets for antimalarial drugs or vaccines.

The pathology of malaria infection has long been considered to be the result of the release of toxins of parasite origin. Tumour necrosis factor-α (TNF-α), which is induced by such malarial toxins, was subsequently identified as a major host mediator of disease. Consequently, its production and that of other pyrogenic cytokines is commonly regarded as a surrogate marker for the initiation of malaria pathological processes. The GPI of *P. falciparum* has emerged only recently as a candidate toxin, based on its ability to elicit proinflammatory cytokines from macrophages and endothelial cells by activating NFκB transcription factors and on its ability to cause clinical effects in experimental animals that are akin to acute malaria infection (e.g. fever, hypoglycaemia, dyserythropoiesis and vascular damage in the brain). As *P. falciparum*-infected erythrocytes are sequestered in specific organs, localized elevated toxic responses to the parasite's GPIs can disturb vital physiological functions and cause severe illness and, in the event of cerebral pathology, even death. Antagonists of GPI-mediated signalling and a monoclonal antibody against *P. falciparum* GPIs can each block the induction of toxicity *in vitro*, indicating the feasibility of GPI-based immunotherapy.

Naik *et al.*[1] postulate, because *P. falciparum* GPIs are pathogenicity factors, that semi-immune adults living in regions of malaria endemicity should possess GPI-specific protective immunity. This hypothesis was tested by analysing the anti-GPI antibody response in sera from a large cohort of individuals from western Kenya. Their findings demonstrate for the first time that people in malaria-endemic regions elicit a potent GPI-specific immunoglobulin (Ig) G response, and that this increases in an age-dependent (and possibly exposure-dependent) manner. Thus, although adults and older children have high antibody titres, children susceptible to acute *P. falciparum* infection either have low levels of short-lived antibodies or none at all. Absence of a persistent anti-GPI antibody response was associated with malaria-specific anaemia and fever. Naik *et al.*[1] speculate that antibodies to *P. falciparum* GPIs are involved in protection against clinical infection. This speculation is based on the discovery of a direct correlation between serum anti-GPI IgG and resistance to malaria pathogenesis; a causal link now needs to be established in a suitable experimental model, between the possession of antibodies to parasite GPIs and prevention of pathogenesis. Understanding the factors associated with resistance to clinical infection, so-called anti-disease immunity, might lead to alternative approaches for malaria control. In this regard, GPIs appear to offer a genuine opportunity.

1 Naik, R.S. *et al.* (2000) Glycosylphosphatidylinositol anchors of *Plasmodium falciparum*: molecular characterization and naturally elicited antibody response that may provide immunity to malaria pathogenesis. *J. Exp. Med.* 192, 1563–1575

Andrew Taylor-Robinson
bgyawtr@leeds.ac.uk

One million insects – a lot of parasites?

The medical and economic importance of trypanosomatids that infect vertebrates (e.g. *Trypanosoma brucei*, *T. congolense*, *T. cruzi* and *Leishmania* spp.) and plants (*Phytomonas* spp.), together with their unusual cellular and antigenic characteristics, has resulted in the preferential investigation of these taxa by researchers, whereas trypanosomatids that infect insects have largely been overlooked. More than 300 trypanosomatids that infect insects are described in the literature[1], but many are, at best, synonyms; only 30, from a limited range of hosts and locations, have been studied in the laboratory. Such a situation is obviously inadequate for meaningful phylogenetic studies of trypanosomatids that infect insects and, as illustrated by recent studies of the *Trypanosoma* genus[2,3], inclusion of a wide range of outgroup taxa is also essential for broader evolutionary analyses. The work by Merzlyak and colleagues therefore represents a breakthrough, being the first molecular and phylogenetic study to include sufficient trypanosomatids stocks from insects to allow meaningful analysis of these, until now, poorly studied taxa.

By comparing parasite morphology in the original host, with morphology after time in culture, the authors[4] are able to show how four out of ten insect trypanosomatid isolates significantly altered their predominant morphotype from that observed at the time their of original isolation; interestingly, such isolates also form a monophyletic cluster in the subsequent phylogenetic analyses. Given the importance to date of in-host morphology in the taxonomy of trypanosomatids of insects, such a finding has major implications. Moreover, phylogenetic analysis of small subunit ribosomal RNA-based sequences indicates that at least four of the described genera containing trypanosomatids of insects are polyphyletic (*Blastocrithidia*, *Leptomonas*, *Herpetomonas* and *Crithidia*), again questioning the utility of morphology-based taxonomy for classifying insect trypanosomatids. Finally, phylogenetic analysis of a broad selection of trypanosomatids divides the taxa into two distinct clades – trypanosomes and

nontrypanosome trypanosomatids (e.g. *Leishmania* spp., *Phytomonas* spp. and trypanosomatids of insects) – providing evidence of an early divergence from the monoxenous parasite *Blastocrithidia triatoma*. Such a discovery obviously raises questions relating to the existence and phylogenetic position of other monoxenous parasites of familiar disease vectors (e.g. tsetse and sandflies) and the significance of parallel parasite populations in vectors.

Overall, although this paper[4] perhaps raises more questions than it answers, it is an essential first step towards our understanding of an evidently complex and diverse group of parasites. Moreover, experience suggests that information on such parasites might well prove vital for our understanding of the true evolutionary history of the more familiar trypanosomatid genera.

1 Podlipaev, S.A. (1990) Catalogue of world fauna of Trypanosomatidae (Protozoa). *Proc. Zool. Inst. USSR Acad. Sci.* 217, 1–177
2 Stevens, J.R. *et al.* (1999) The ancient and divergent origins of the human pathogenic trypanosomes, *Trypanosoma brucei* and *T. cruzi*. *Parasitology* 118, 107–116
3 Wright, A-D.G. *et al.* (1999) Phylogenetic position of the kinetoplastids, *Cryptobia bullocki*, *Cryptobia catostomi* and *Cryptobia salmositica* and monophyly of the genus *Trypanosoma* inferred from small subunit ribosomal RNA sequences. *Mol. Biochem. Parasitol.* 99, 69–76
4 Merzlyak, E. *et al.* (2001) Diversity and phylogeny of insect trypanosomatids based on small subunit rRNA genes: polyphyly of *Leptomonas* and *Blastocrithidia*. *J. Eukaryot. Microbiol.* 48, 161–171

Jamie R. Stevens
j.r.stevens@exeter.ac.uk

In Brief

Current Opinion in anti-parasitic drugs

The December 2000 edition of *Current Opinion in Infectious Diseases* (Vol. 13) provides an up-to-date review of the current pharmaceutical and moral issues facing scientists and clinicians working with trypanosomes, *Plasmodium falciparum* and schistosomes. In accordance with the usual *Current Opinion* format, the authors (Michael Barrat; Cecelia Sanchez and Micheal Lanzer; and Donato Cioli, respectively) also provide critical comment on key papers relevant to their fields. In addition, the journal features a comprehensive bibliography (drawn from the top 100 journals in parasitology) of the past year's most important articles. According to the journal's website (www.co-infectiousdiseases.com), it is by provision of these services that *Current Opinion in Infectious Diseases* 'distills the massive amount of primary literature into reliable, concise and thoughtful analyses written by respected opinion leaders'. *SHK*

Vaccine in a patch

Even if we did fully understand the biology and pathology of all the world's infectious diseases, how we might safely and cheaply administer a vaccine to combat these is another matter. Gregory Glen *et al.* (Transcutaneous immunization: a human vaccine delivery strategy using a patch. *Nat. Med.* 6, 1403–1406, 2000) propose that transcutaneous immunization (topical vaccine delivery with the use of a skin patch) is a safe and effective means by which this goal might be achieved.

Glen *et al.* report the safe application of a patch containing *Escherichia coli*-derived enterotoxin to humans, resulting in robust enterotoxin-specific serum antibody responses, as well as some antigen-specific mucosal immunity.

The beauty of this vaccination method is its simplicity: naturally occurring skin hydration that follows patch application allows for non-invasive, non-inflammatory delivery of antigens to underlying antigen presenting cells. Current methods rely on epidermal penetration by injection or gene gun. *SHK*

Setting the stage for *Leishmania*

When a host is infected with a parasite, a symbiosis ensues that functions as a new complex organism; the infected host. The co-existence of these organisms requires integration of both physiological complexes. It has been shown that the immune system plays an important role in the fate of *Leishmania* in the host. White blood cells produce a cytokine context that either allows survival of the parasite or its elimination. This is dependent on the type of T-cell response activated upon infection. A T-helper 1 (Th1) response (characterized by the production of IFN-γ and IL-12) that leads to parasite killing owing to the activation of infected macrophages by IFN-γ. By contrast, a Th2 response (characterized by the production of IL- 4) allows survival of the parasite, probably because of downregulation of IFN-γ production by IL-10. Results from a mouse model indicated that salivary gland lysates of *Plebotomus papatasi*, the mosquito vector that transmits *Leishmania major*, provokes a Th2 response, which allows parasite survival (M.L. Mbow. *et al., J. Immunol.* 161, 5571–5577, 1998). Recently, it was shown that prior exposure of mice to bites of uninfected sandflies induced strong Th1 responsiveness with IFN-γ production, and associated resistance to *Leishmania* infection. These results suggest an alternative means of immunization against *Leishmania* infection (S. Kamhawi *et al., Science* 290, 1351–1354, 2000) and might have implications to the epidemiology of the disease. *TS*

Host IgG and IL-10 are virulence factors in Leishmania infections

Leishmania amastigotes exploit the anti-inflammatory effects of host IgG to evade destruction by macrophages, according to a recently published paper (M.M. Kane and D.M. Mosser, The role of IL-10 in promoting disease progression in *Leishmaniasis*. *J. Immunol.* 166, 1141–1147, 2001). Host IgG bound to the surface of *Leishmania* amastigotes allows them to ligate Fcγ receptors on macrophages. This has the effect of inducing the macrophages to produce high amounts of IL-10 (a cytokine with anti-inflammatory effects) at the expense of pro-inflammatory cytokines such as IL-12 and TNFα. As a consequence, the macrophages are prevented from becoming fully activated and thus they do not become efficient parasite killers.

Further evidence of an important role for IL-10 in promoting amastigote survival was found in IL-10 gene-knockout mice, which show a remarkably improved ability to control *Leishmania* infection compared to normal mice. *SHK*

This month's In Brief was compiled by Sarah Hudson Keenihan (keenihan@namru2.org) and Theo Schetters (theo.schetters@intervet.com)

 1471-4922/01/$ – see front matter