three can be correct, depending on which subpopulation is being studied, a finding which helps to unravel the inter-relationships between different parasite populations in cattle and in humans.

MacLeod *et al.* used three minisatellite markers showing extensive allelic variation to compare parasites that were human serum resistant (*T. b. rhodesiense*) and sensitive (*T. b. brucei*) taken from humans and/or cattle in Uganda and Zambia. *Trypanosoma brucei rhodesiense* isolates from humans and cattle in Uganda showed minimal genetic variation, just a few closely related genotypes, and appeared to be clonal and to have persisted for several decades. *Trypanosoma brucei rhodesiense* isolates from Zambia had distinct genotypes and appeared to be clonal or epidemic. *Trypanosoma brucei brucei* isolates from cattle in Uganda, however, showed much greater diversity and better evidence of recombination, albeit with clonal expansion of some genotypes. It appears that not many genotypes ever make it into humans. The results confirm that *T. b. rhodesiense* and *T. b. brucei* are, at least in Uganda, distinct populations. But they also suggest that parasites of humans from Uganda are more closely related to parasites from the same region but a different host species, than they are to parasites from a different region but the same host species.

These findings will, it is hoped, be confirmed by extending the study to other *T. brucei* populations. However, the implication is that the infection of humans from local cattle populations does occur and that it is somehow associated with specific genetic changes in the parasite. There are some clues as to the nature of these changes: it has been shown that human serum resistance gene in *T. b. rhodesiense* has a single variant surface glycoprotein expression site[2]. Other work suggests that *T. brucei* can adapt phenotypically to different host species by switching between VSG expression sites[3]. Determining exactly which genotypic and phenotypic changes are involved in the transmission of trypanosomes from cattle to humans will be a vital step forward in the fight against sleeping sickness.

1 MacLeod, A. *et al.* (2000) Minisatellite marker analysis of *Trypanosoma brucei*: reconciliation of clonal, panmictic and epidemic population genetic theories. *Proc. Natl. Acad. Sci. U. S. A.* 97, 13442–13447
2 Van Hong, H. *et al.* (1998) A VSG expression-site associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. *Cell* 95, 839–846
3 Bitter, W. *et al.* (1998) The role of transferrin-receptor variation in the host range of *Trypanosoma brucei*. *Nature* 391, 499–502

Mark E.J. Woolhouse
mark.woolhouse@ed.ac.uk

In Brief

UNEP approves use of DDT for vector control

On 10 December 2000, United Nations Environmental Program delegates in Johannesburg agreed a treaty that allows DDT production for use against vectors of disease, but not for agriculture. Countries using DDT must notify WHO and follow WHO guidelines; developed countries are encouraged to support research into development of alternatives and pay 'agreed incremental costs'. Although 11 other persistent organic pollutants are to be banned by 122 countries, DDT was accepted to be unique. A ban would put an unethical burden on undeveloped countries and cost many lives. Although widespread spraying in agriculture might have harmed wildlife, there is no convincing evidence that DDT is harmful to people, but there is evidence that the incidence of malaria greatly increased when countries in which the disease is endemic stopped using it. DDT is cheap and effective and the small doses that are required for spraying the walls of houses are unlikely to affect the environment.

This sensible outcome is a tribute to the hard work of many malariologists and particularly to the advocacy of the Malaria Foundation International (http://www.malaria.org).

Janice Taverne
j.taverne@ucl.ac.uk

Letters

More panantigens in *Leishmania*

José Requena *et al.*[1] have recently reviewed evolutionary conserved immunogens in *Leishmania* infections and have put forward the concept of panantigens. Here, we present a further example of a panantigen and discuss its relationship with those presented previously.

Earlier work by Le Ray *et al.*[2] identified an immunodominant, visceral leishmaniasis-specific immunogen, antigen 24 (AG24) in *Leishmania infantum*, using precipitation-in-gel techniques. Further work has highlighted the similarity of this antigen with the 20S proteasome of the parasite, based on several pieces of evidence: (1) AG24 is composed of 6–9 proteins in the 21–31 kDa range[3]; (2) a mouse antiserum obtained by genetic immunization with a *L. infantum* 26 kDa proteasome subunit recognized a 26 kDa component of AG24 (B. Couvreur *et al.*, unpublished); (3) a monospecific rabbit antiserum directed to AG24 recognized a purified preparation of proteasome from *L. mexicana* in western blots[4]; and (4) sera from visceral leishmaniasis (VL) patients or from experimentally infected animals recognized the purified proteasome in precipitation-in-gel techniques. In the experiments with sera from VL patients or from experimentally infected animals, the precipitation line formed by the purified proteasome preparation fused with the AG24 precipitation line, indicating full antigenic identity (B. Couvreur *et al.* unpublished). As the proteasome is an evolutionarily conserved, multicomponent complex and as the human proteasome has been shown to be the target of antibodies in several autoimmune conditions, it seems certain that the leishmanial proteasome possesses the main features of panantigens.

So far, the precise antigenic determinants recognized by antibodies elicited against the proteasome of the parasite during VL have not been identified. Clues might come from previous work on purified 20S proteasomes from various eukaryotes[5]. Using rabbit antisera directed to the purified 20S proteasomes, Tanaka *et al.* observed no crossreaction between the intact complexes of species as close as human and rat, in conditions where the integrity of the native structure is preserved (i.e. Ouchterlony's immunodiffusion). However, when the complexes were dissociated and subunit proteins linearized in western blots, crossreactivity was recorded[5]. This

 © 2001 Elsevier Science Ltd. All rights reserved.

indicates that the native proteasome particle can be a very specific antigen and that the determinants responsible for specificity might be due to the conformation of the subunits or even the quaternary structure of the complex as well as to, or instead of, the amino acid sequence of individual subunits.

Features of panantigens proposed to be responsible for their high immunogenicity also apply to the 20S proteasome. The proteasome is abundant (up to 1% of protein in a typical mammalian cell), very stable and particulate in nature. Moreover, its relatively large size (~750 kDa, 12×17 nm), and the repetitive arrangement of subunits (14 related subunits, each present twice) could further facilitate the capture and processing by antigen-presenting cells (APC). However, the observation that recognition of AG24 proteasome is observed only in VL[2] might have implications pertinent to the immunological mechanisms leading to its recognition.

Bernard Couvreur*
Alex Bollen
Laboratory of Applied Genetics, Free University of Brussels, rue des Professeurs Jeener et Brachet 12, B-6041 Gosselies, Belgium.

Jean-Claude Dujardin
Dominique Le Ray
Laboratory for Protozoology, Prince Leopold Institute of Tropical Medicine, 155 Nationalestraat, B-2000 Antwerpen, Belgium.
*e-mail: bernard.couvreur@var.fgov.be

References

1 Requena, J.M. *et al.* (2000) Evolutionary conserved proteins as prominent immunogens during *Leishmania* infections. *Parasitol. Today* 16, 246–250
2 Le Ray, D. *et al.* (1973). Diagnostic immunoélectrophorétique de la leishmaniose viscérale à l'aide d'un extrait antigénique hydrosoluble de *Leishmania infantum*. *Ann. Soc. Belg. Med. Trop.* 53, 31–41
3 Couvreur, B. *et al.* (1997). Molecular characterization of antigen 24, a specific immunodominant antigen family from *Leishmania infantum*. *Parasitology* 115, 611–619
4 Robertson, C. (1999) The proteasome of *Leishmania mexicana*. *Mol. Biochem. Parasitol.* 103, 49–60
5 Tanaka, K. *et al.* (1988) Proteasomes (Multiprotease complexes) as 20S ring-shaped particles in a variety of eukaryotic cells. *J. Biol. Chem.* 263, 16209–16217

More panantigens in *Leishmania*

Response from Requena *et al.*

The proteasome is a multi-subunit, cylinder-shaped structure that constitutes a molecular machine specialized in intracellular protein degradation[1]. Given its functional role, it is not surprising that proteasomes are present in the three phylogenetic domains (Bacteria, Archaea and Eukarya) and that, consequently, the proteasomal subunits are evolutionarily conserved proteins.

Couvreur *et al.* (this issue), report that antigen 24 (AG24), a complex immunogen of *Leishmania infantum* formed by 6–9 different proteins[2], is related to the leishmanial proteasome. The AG24 was identified by its strong antigenicity in both natural and experimental *Leishmania* infections and used as a reference standard for serodiagnosis of visceral leishmaniasis (VL). Couvreur *et al.* indicate that a monospecific rabbit antiserum against the AG24 complex recognizes purified *L. mexicana* proteasomes. The antigenicity of the *Leishmania* proteasome has been confirmed by recent work[3] showing that an *L. donovani* cDNA, with 46% sequence similarity to the proteasome a-type subunit from humans and other eukaryotes, could be isolated using the sera from VL patients. Remarkably, DNA vaccination with an eukaryotic expression vector containing this cDNA generated a delayed development of the cutaneous lesions in *L. major*-infected mice.

However, proteasome components have also been described as antigens during other infectious diseases, for example, those caused by *Schistosoma mansoni* infection[4]. In addition, some proteasome subunits have been found to be primary targets of autoantibodies present in the sera from patients with myositis and systemic lupus erythematosus[5].

Couvreur *et al.* also note that the *Leishmania* proteasome possesses features attributed to several groups of *Leishmania* proteins referred to as panantigens, a concept recently developed by our group[6]. The proteasome does share the following characteristics with panantigens: (1) it is an abundant cellular component; (2) it possesses a high evolutionary conservation; (3) it forms a stable and multi-component structure; (4) it is a prominent immunogen during *Leishmania*-infections; and (5) it is the target for autoantibodies in autoimmune diseases. Therefore, we agree fully with their claim and welcome the proteasome into the framework of *Leishmania* panantigens. It will now be interesting to investigate, in greater detail, the molecular basis that determines the immune behavior of these groups of *Leishmania* antigens.

Jose M. Requena
Carlos Alonso*
Manuel Soto
Centro de Biología Molecular 'Severo Ochoa', Universidad Autónoma de Madrid, E-28049 Madrid, Spain.
*e-mail: calonso@cbm.uam.es

References

1 Voges, D. *et al.* (1999) The 26S proteasome: a molecular machine designed for controlled proteolysis. *Annu. Rev. Biochem.* 68, 1015–1068
2 Couvreur, B. *et al.* (1997) Molecular characterization of antigen 24, a specific immunodominant antigen family from *Leishmania infantum*. *Parasitology* 115, 611–619
3 Christensen, C.B.V. *et al.* (2000) Molecular characterization of a *Leishmania donovani* cDNA clone with similarity to human 20S proteasome α-type subunit. *Biochim. Biophys. Acta* 1500, 77–87
4 Harrop, R. *et al.* (1999) Characterization, cloning and immunogenicity of antigens released by lung-stage larvae of *Schistosoma mansoni*. *Parasitology* 118, 583–594
5 Feist, E. *et al.* (1996) Proteasome alpha-type subunit C9 is a primary target of autoantibodies in sera of patients with myositis and systemic lupus erythematosus. *J. Exp. Med.* 184, 1313–1318
6 Requena, J.M. *et al.* (2000) Evolutionarily conserved proteins as prominent immunogens during *Leishmania* infections. *Parasitol. Today* 16, 246–250

Revisiting amebiasis

Concerning the article by Petri *et al.*[1], we agree strongly that there is an urgent need to revisit the epidemiology of amebiasis with tools that allow us to not only diagnose amebic infection more accurately, but also to differentiate *Entamoeba histolytica* from *Entamoeba dispar*.

However, the article is remiss on several points. First, in a study conducted in Berlin, and recently in Toronto, amebiasis remains an important disease among returning travelers and recent immigrants in large cosmopolitain centers

http://parasites.trends.com 1471-4922/01/$ – see front matter © 2001 Elsevier Science Ltd. All rights reserved.