Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Cell Biology 84 (2005) 7–13
European Journal of Cell Biology
www.elsevier.de/ejcb

MEETING REPORT

Parasites, flies and men — 21st Meeting of the German Society of Parasitology in Würzburg

Alicia Ponte-Sucre[a,b], Heidrun Moll[a,*]

[a]*Institute for Molecular Biology of Infectious Diseases, University of Würzburg, Röntgenring 11, D-97070 Würzburg, Germany*
[b]*Instituto de Medicina Experimental, Universidad Central de Venezuela, Caracas, Venezuela*

The German Society of Parasitology (Deutsche Gesellschaft für Parasitologie) was founded in 1960 and its 21st biannual meeting took place in Würzburg, Germany, from March 17 to 20, 2004. Whereas interim meetings that are being held every other year focus on specific topics of parasitology, such as the symposia on "Life in Vacuoles" in 2003 and on "Immunomodulation by Parasites" in 2001, the general biannual meetings cover a wide range of topics. This year's meeting at the University of Würzburg was organised by Klaus Brehm and Matthias Frosch (both at the Institute of Hygiene) and Heidrun Moll (Institute for Molecular Biology of Infectious Diseases). It was attended by more than 500 scientists from 16 countries who presented 181 research projects dealing with the topics defence mechanisms and immunology, genomics and proteomics, epidemiology, cell biology and biochemistry, chemotherapy and vaccines, parasite classification and morphology, vectors, intermediate hosts, and veterinary parasitology. In addition, six plenary lectures highlighted the subjects of comparative nematode genomics, cell biology, immunology, and parasite eradication programmes.

Molecular studies on nematodes

The comparative analysis of genes and genomes in nematodes was reviewed by Mark Blaxter (University of Edinburgh). Four out of five animals on this planet are nematodes, most of them marine nematodes, demonstrating the remarkable success of this diverse phylum. They exist as free-living organisms and as parasites that can adapt to challenging environmental conditions and different hosts. The analysis of gene function is important for understanding the molecular basis of parasitism and will contribute to the development of novel therapeutic and preventive strategies. Comparison of the model organism *Caenorhabditis elegans* with other nematodes, such as *C. briggsae* and *Brugia malayi*, has helped to reveal gene function and regulation and demonstrated that each nematode genome is a mosaic of conserved features and evolutionary novelties. Some of the homeobox (Hox) genes, which are clustered in the genomes of flies and vertebrates and are involved in patterning their anterior–posterior axis (Fig. 1), are missing in *C. elegans*. Interestingly, there is a staged loss of Hox genes in the nematodes *C. elegans*, *C. briggsae*, *B. malayi* and *Trichinella spiralis*. The evolution of Hox genes may be indicative of the move to lineal developmental mechanisms in nematodes.

Intestinal helminths are a major cause of morbidity and mortality in equines worldwide. The Cyathostominae are the most common group of these parasites that infect horses and can be extremely pathogenic, with clinical symptoms ranging from chronic weight loss to colic, diarrhoea and death. Resistance to antiparasitic drugs is common, and there is considerable research effort to develop improved tools for the control and diagnosis of this disease. The Cyathostominae include 51 species, and individual horses tend to harbour about 10 common in addition to some rarer species. Jane Hodgkinson (University of Liverpool) reported that by the use of oligoprobes designed from intergenic spacer region sequences, a specific and sensitive PCR-ELISA was developed for the high-throughput species identification of fourth-stage larvae (L4) in the diarrhoeic faeces of cyathostomin-infected horses.

*Corresponding author.
E-mail address: heidrun.moll@mail.uni-wuerzburg.de (H. Moll).

0171-9335/$ - see front matter
doi:10.1016/j.ejcb.2004.10.003

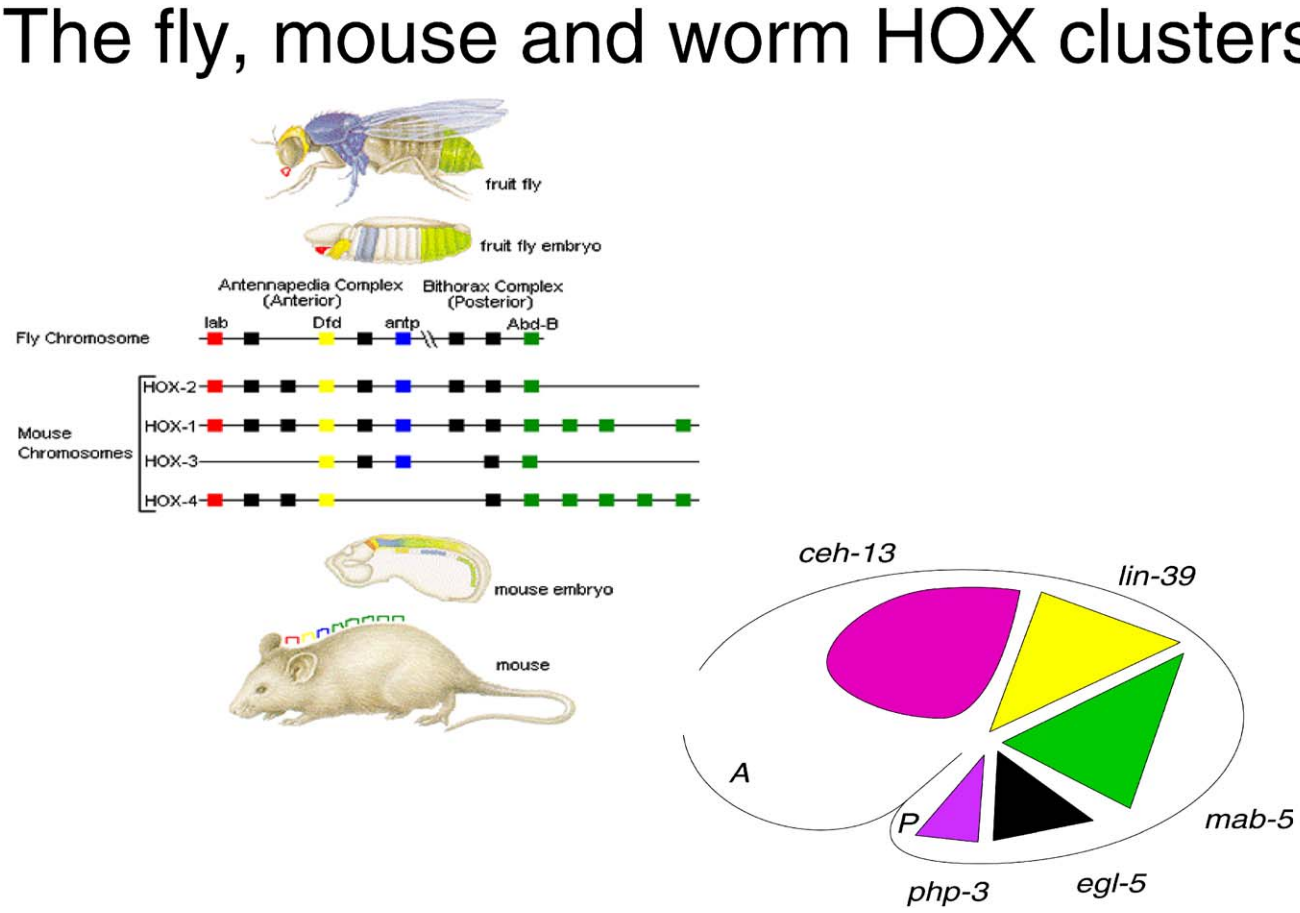

Fig. 1. Hox genes are involved in anterior–posterior patterning of many animals. In mice and flies, the Hox genes are arranged in a cluster, and the order of the genes in the cluster mirrors the domains of expression of the genes along the body axis. In *C. elegans*, there are fewer Hox genes than in other model organisms, and while their expression along the anterior–posterior axis corresponds to that seen in other animals, their genomic organisation is very different. *C. elegans* has lost Hox genes during evolution, and some of these can be found in other species of parasitic nematodes. *C. elegans* ceh-13, labial/Hox1 gene; lin-39, EFF-1 fusogen repressor, mab-5, antennapedia-class homeobox genes homologue; egl-5, abdominal-B homologue; php-3, abdominal-B orthologue (figure provided by M. Blaxter).

Signal transduction pathways in helminth and protozoan parasites

Female schistosomes depend on signals from the male for egg production. Christoph Grevelding (University of Düsseldorf) presented a study on the involvement of protein tyrosine kinases in this process. Using inhibitors that selectively block different classes of tyrosine kinases (Src or Syk), it was found that inhibitors of Src gender-specifically reduced the mitotic activity in females, whereas Syk-specific inhibitors did not affect mitoses in males or females. These results correlated with evidence obtained from the characterisation of the tissue-specific activity of Src-like (TK3/TK5) or Syk-like (TK4) molecules in both genders, suggesting that Src kinases are involved in mitogenic processes during egg production in female schistosomes.

Several talks dealt with the role of mitogen-activated protein kinases (MAPK), evolutionary conserved molecules that regulate the differentiation, proliferation, motility and stress response of all eukaryotic cells. Oxidative stress has been implicated in the defence against trematodes by their intermediate host snails, and Fanny Edele (University of Tübingen) described the activation by phosphorylation of various MAPK (ERK, JNK and p38 MAPK) by oxidative stress in the hemocytes of the schistosome intermediate host snail *Lymnaea stagnalis*. In the protozoan parasite *Leishmania mexicana*, which exists in a flagellated promastigote form and an amastigote form with a rudimentary flagellum, a protein kinase is involved in flagellar length control. Martin Wiese (Bernhard Nocht Institute for Tropical Medicine, Hamburg) reported the identification of a MAPK kinase homologue, designated LmxMKK, that is required for the regulation of

flagellar assembly and cell shape. LmxMKK is exclusively expressed in the promastigote stage and is likely to be regulated by post-translational mechanisms such as phosphorylation. It is the first protein kinase known to be involved in organellar assembly. These observations make *Leishmania* an attractive model for the study of flagellar morphogenesis and function.

Survival strategies of the malarial parasite *Plasmodium falciparum*

Plasmodium falciparum, the causative agent of the most severe form of malaria, causes around one million deaths every year. Upon invasion of erythrocytes, *P. falciparum* modifies its host cell and induces the expression of parasite-derived proteins on it surface. One of these molecules is PfEMP-1, a variant adhesin that is responsible for the retention of infected erythrocytes in microvascular beds. This process of adhesion correlates with severe disease, for example parasite accumulation in the brain and cerebral malaria. More than a dozen of endothelial receptors have been shown to bind PfEMP-1 on infected erythrocytes, including intercellular adhesion molecule-1 (ICAM-1) and CD36, which appear to be responsible for the majority of adhesion seen in isolates from children with malaria and work synergistically in mediating adhesion. Alister Craig (Liverpool School of Tropical Medicine) reviewed the major role of ICAM-1 in mediating *P. falciparum* adhesion to endothelial cells under flow conditions. In the absence of ICAM-1, binding in flow assays was severely impaired. Mutagenesis of the N-terminal domain of ICAM-1, which is involved in parasite adhesion, demonstrated that different parasite variants bind to a similar region of the ICAM-1 molecule. However, there are subtle differences in the contact residues used by the different parasite lines that correlate with their adhesive phenotype and may affect pathogenesis. The definition of binding determinants may facilitate the design of anti-adhesive agents for therapeutic interventions (see also the review article by Chakravorty and Craig (2005) in this issue of the European Journal of Cell Biology).

PfEMP-1 is encoded by the *var* gene family, and switching between members of this gene family is associated with changes in antigenicity and adherence functions. Recently, the *stevor* and *rif* multicopy gene families have been identified in *P. falciparum*. Its members have the potential to be involved in antigenic variation, cytoadherence and pathogenesis by analogy with the *var* multigene family. Jude Przyborski (University of Heidelberg) described the identification of signal sequences that are responsible for trafficking of STEVOR to the parasitophorous vacuole, the erythrocyte cytoplasm and to the Maurer's clefts, membranous structures that have been implicated in transport of parasite proteins to the surface of red blood cells. STEVOR may shield essential parasite proteins from immune attack and thus may contribute to the survival of the parasites in the host.

Cysteine proteases form another group of molecules that are known to play an essential role in the survival of intraerythrocytic *P. falciparum*. They are involved in the degradation of hemoglobin, the cleavage of host cell proteins and the release of parasites from red blood cells. Christoph Gelhaus (University of Kiel) reported the use of a covalent cysteine protease inhibitor to localise and purify potential target proteins of the compound. It was shown that some inhibitor-reactive proteins are transported to the erythrocyte cytoplasm across the membrane of the parasitophorous vacuole, which protects the parasite from potentially harmful substances in the host cytosol and is involved in the bidirectional transport of nutrients and metabolites. Using cysteine protease inhibitors, a protocol for isolation of the parasitophorous vacuole membrane was established that enables the analysis of the protein repertoire of this organelle. The development of a proteomic approach towards the characterisation of parasitophorous vacuoles in *P. falciparum*-infected erythrocytes was reported by Julius Nyalwidhe (University of Marburg). It utilises non-permeant biotin derivatives that are introduced into infected erythrocytes after their permeabilisation with the pore-forming protein streptolysin O. The derivatives gain access to the vacuolar lumen and selectively label proteins in this compartment which can be isolated by affinity chromatography, separated by two-dimensional gel electrophoresis and analysed by MALDI-TOF mass spectrometry to identify novel molecules.

New targets for antimalarial chemotherapy and vaccination

Glutathione S-transferases (GST) occur abundantly in most forms of life and catalyse the conjugation of glutathione with a wide variety of hydrophobic compounds, generally resulting in the intracellular detoxification of substances. In contrast to many other organisms, *P. falciparum* contains only one GST isoenzyme (PfGST). The enzyme has been shown to bind parasitotoxic hemin and thus represents a promising target for antimalarial drug development. Katja Becker (University of Gießen) presented the recently resolved crystal structure of PfGST at a resolution of 1.9 Å (Fig. 2). The protein represents a novel GST isoform that cannot be assigned to any of the described GST classes. In comparison to other GST, in particular

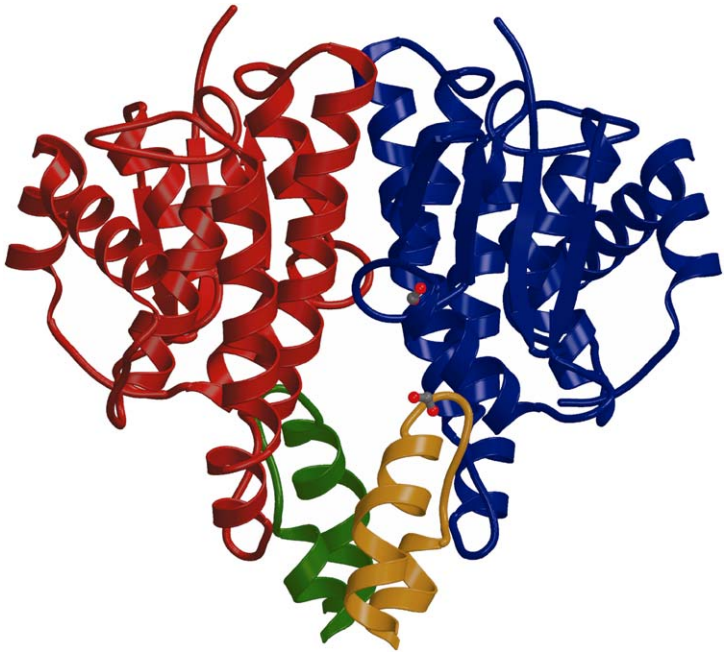

Fig. 2. Assembly of the PfGST homodimer. The two-fold crystallographic axis is located between the two monomers A (blue) and A′ (red), and points approximately vertical in the drawing plane. This dimer AA′ interacts with the crystallographically independent dimer BB′ (gold, green). The formate molecules, shown in ball-and-stick indicate the glutathione-binding site. The figure was drawn using the programmes molscript and raster3d (Kraulis, 1991; Merritt and Bacon, 1997) (figure provided by K. Becker and K. Fritz-Wolf).

the human isoform, PfGST possesses a shorter C-terminal section resulting in a more solvent-accessible substrate-binding site. A future aim of the studies is the identification of a structure-derived inhibitor for rational drug design.

Malaria parasites are transmitted by *Anopheles* mosquitoes which release the sporozoite stage into the skin. Sporozoites are carried through the blood to the liver, where they invade hepatocytes and develop into exoerythrocytic forms (EEF). Salivary gland sporozoites and EEF are suitable targets for immuno- and chemoprophylaxis because they precede the development of the pathogenic blood stages. Ann-Kristin Müller (University of Heidelberg) reported the identification of a set of transcripts that are specifically upregulated in infective salivary gland sporozoites. One of the genes, UIS4, encodes a protein that is transferred to the parasitophorous vacuole membrane, where it is continuously present throughout the liver stage development. The generation of deletion mutants revealed that UIS4 expression is required for complete liver stage development in vitro and the establishment of blood stage infection in vivo. Notably, UIS4 deletion mutants induce complete protection against a subsequent challenge with wild-type parasites.

In another presentation dealing with the search for genes encoding molecules that are essential for the parasite life cycle, and thus represent prospective vaccine candidates, Gabriele Pradel (Cornell University, New York) described the identification of three sexual stage-specific genes in *P. falciparum*, named *PfCCp1* through *PfCCp3*. They encode proteins that can be detected only in gametocytes (Fig. 3) and are striking for multidomain architectures composed of animal- and bacterial-like putative adhesive domains. Orthologues are present in the genome sequences of *Cryptosporidium parvum* and *Theileria annulata*, indicating an evolutionary conserved function. Disruption of the *PfCCp2* or *PfCCp3* gene loci via homologous recombination leads to a developmental blockage in the transition of *P. falciparum* from mosquito midgut to salivary gland sporozoites. Thus, the proteins encoded by the *PfCCp* gene family represent candidates for subunits of a transmission-blocking vaccine.

Pathogenesis of human sleeping sickness

African trypanosomes, the cause of human sleeping sickness, evade the host's immune attack by covering their entire surface with a dense coat of about five million copies of a single antigen termed variant surface glycoprotein (VSG) that is periodically changed. This mechanism of antigenic variation allows a part of the parasite population to avoid killing mediated by serum

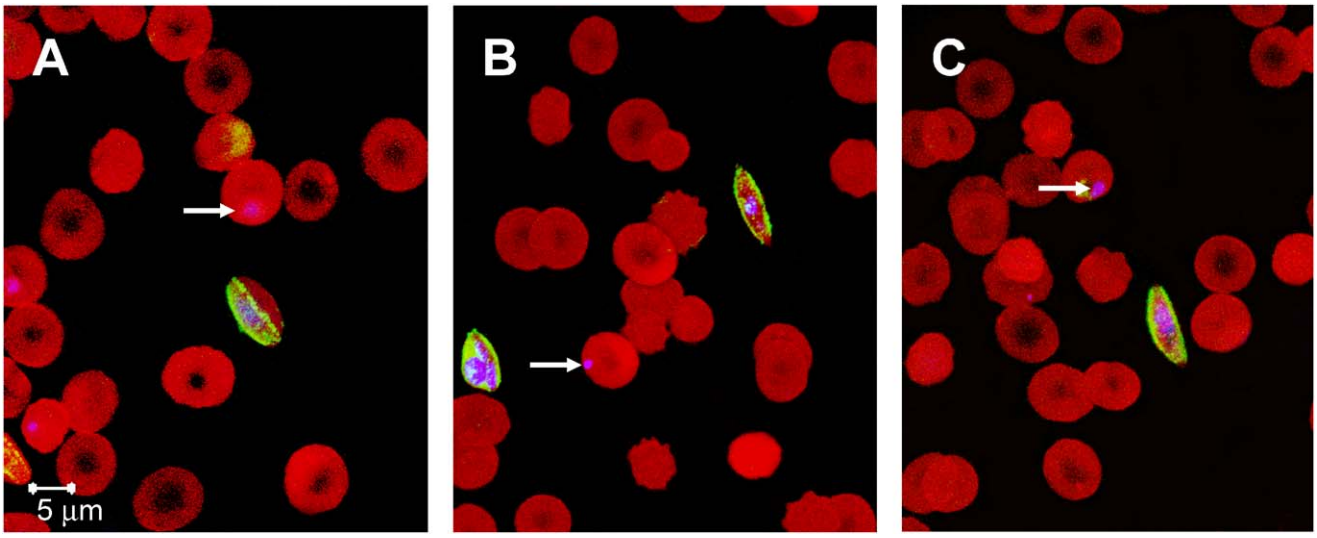

Fig. 3. Indirect immunofluorescence analysis using mouse sera against PfCCp1 (A), PfCCp2 (B) and PfCCp3 (C) revealed a punctate pattern associated with the parasite surface in mature *P. falciparum* gametocytes (green Alexa Fluor 488), whereas asexual stage parasites were not labelled. Erythrocytes were counterstained with Evans Blue (red). Arrows indicate asexual parasites visualised by TOTO-3 nuclear stain (blue) (figure provided by G. Pradel).

antibodies and to repopulate the host. Etienne Pays (University of Brussels) reviewed the mechanisms underlying adaptation of African trypanosomes to man. Resistance of *Trypanosoma brucei rhodesiense* to lysis by normal human serum is conferred by a gene that encodes a truncated form of VSG and is termed serum resistance-associated protein (SRA). Expression of the SRA gene is necessary and sufficient to confer resistance to human serum. The product of SRA is an atypical VSG devoid of surface loops, the part of the molecule that is exposed to the host immune system on the surface of trypanosomes. It was shown that SRA is a lysosomal protein and that the N-terminal α-helical region of SRA is responsible for resistance to human serum. The trypanolytic factor in human serum was identified as apoL-I, a human apolipoprotein associated with high-density lipoprotein. Confocal microscopy demonstrated that apoL-I is internalised through the endocytic pathway and meets SRA in the lysosome. SRA neutralises the lytic activity of apoL-I, presumably through coiled-coil protein–protein interaction.

Current therapy of human sleeping sickness is limited to a few drugs that suffer from unacceptable toxicity and increasing treatment failures. Therefore, it is important to identify drug targets and develop lead compounds that interact with these targets. Thorsten Irsch (University of Heidelberg) reported on the cloning of a gene encoding a glyoxalase II from *T. brucei*. The glyoxalases II of trypanosomatid organisms lack three basic residues that are responsible for binding the glutathione moiety of a substrate analogue in the structure of the human enzyme. Dietmar Steverding (University of Bristol) presented a study showing that different DNA topoisomerase inhibitors, including drugs currently used as anti-cancer agents, exhibit trypanocidal activities and may be used for drug development.

Immune evasion strategies of parasites

An effective immune reaction to the diverse types of microbial pathogens that enter the human body relies on the co-ordinated action of components of the innate and the adaptive immune system. On the other hand, infectious agents evolved a multitude of strategies to interfere with the development of host immunity at several levels. Dendritic cells are specialised antigen-presenting cells that play a key role in connecting innate and adaptive immune responses and, thus, the modulation of their functions provide means to facilitate pathogen survival. The interaction of *P. falciparum* with dendritic cells was summarised by Britta Urban (University of Oxford). Erythrocytes infected with *P. falciparum* adhere to dendritic cells and profoundly reduce their maturation. This is associated with a change in the cytokine profile of these dendritic cells, as hallmarked by reduced interleukin (IL)-12 and enhanced IL-10 production, and a decrease in their ability to stimulate antigen-specific T cell responses. Moreover, a proportion of these T cells is anergic to restimulation and expresses the markers CD4 and CD25, suggesting that the dendritic cells modulated by *P. falciparum*-infected erythrocytes induce functionally unresponsive T cells with characteristics of regulatory T cells. Interestingly, the receptors on

the dendritic cell surface binding infected erythrocytes were shown to be CD36 and CD51, molecules that are also involved in the recognition of apoptotic cells. Thus, the malaria parasite-infected erythrocyte may mimic some of the adhesive ligands of apoptotic cells, which also inhibit the maturation and functional activities of dendritic cells. This effect may have an impact on the disease, as the percentage of mature dendritic cells expressing HLA-DR was found to be significantly reduced in children with acute *P. falciparum* malaria.

The cytokine production of dendritic cells can also be impaired by *Echinococcus multilocularis*, the cause of alveolar echinococcosis which results in progressive destruction of the liver parenchyma and can lead to hepatic failure. Georg Härter (University of Ulm) reported the decrease of IL-12 and IL-10 expression by dendritic cells stimulated with *E. multilocularis* antigen and the downregulation of the chemokine receptor CCR7. The isolation and characterisation of a secretory component of *E. multilocularis* potentially involved in suppressing the cellular immune response was described by Mirjam Walker (University of Bern). Immunofluorescence studies demonstrated that the parasite antigen, termed Em492, is present on the laminated layer (LL) and in the periphery of developing brood capsules (BC) (Fig. 4). Whereas EM492 is supposed to be a carbohydrate structure, a glycoprotein of the hog roundworm *Ascaris suum* was also shown to cause marked suppression of T cell reactivity in vitro and to inhibit the heterologous immune response against a filarial infection in vivo, as reported by Richard Lucius (Humboldt University Berlin).

Major histocompatibility complex (MHC) class II expression by antigen-presenting cells is critical for immune responses mediated by $CD4^+$ T cells. The obligate intracellular parasite *Toxoplasma gondii* is able to establish persistent infections in immunocompetent hosts, and Christine Lang (University of Göttingen) presented data showing that *T. gondii* downregulates activation-induced MHC class II expression in macrophages that involves decreased transcript levels of the class II transactivator CIITA, suggesting a mechanism which may contribute to immune evasion and long-term persistence of the parasite.

The importance of a functional immune response in supporting chemotherapy was addressed by Bruno Gottstein (University of Bern). Using immunocompetent and immunodeficient mouse strains, it was demonstrated that toltrazuril treatment of experimental *Neospora caninum* infection required the support of T cell immunity to be effective.

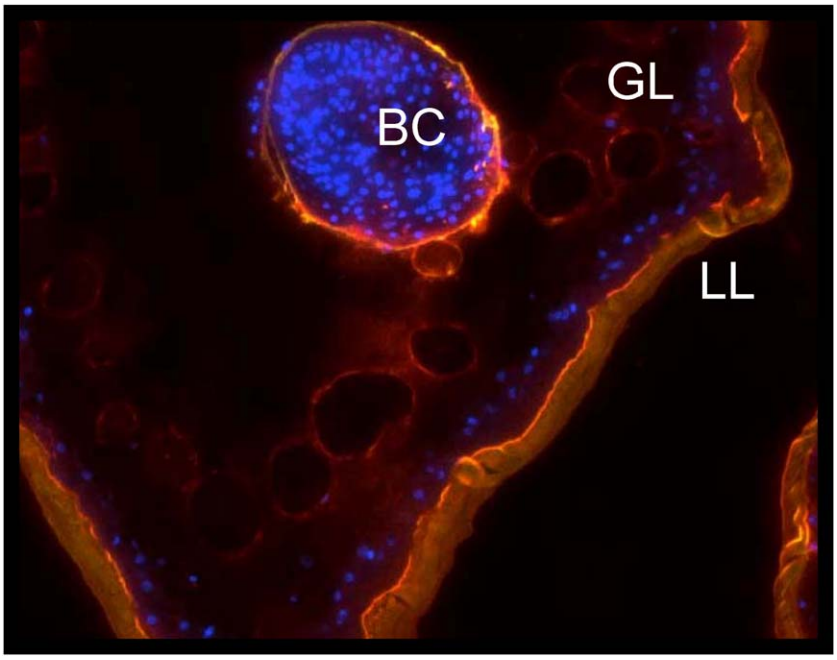

Fig. 4. Section through an in vitro cultured *E. multilocularis* metacestode embedded in LR-White resin. The section was labelled with anti-Em492 antibodies recognising a putative immunomodulatory secretory fraction of the parasite (green) and with an antiserum generated against *E. multilocularis* metacestode (red). Areas of overlapping labelling appear yellowish/orange. Parasite nuclei were stained with Hoechst 22358 (blue). Note that Em492 is concentrated at the outer and acellular LL of the parasite, and is absent from the germinal layer (GL). In addition, Em492 is localised at the periphery of the BC harbouring developing protoscolices (figure provided by M. Walker and A. Hemphill).

Parasite eradication programmes

The challenging task of disease eradication was reviewed by Nevio Zagaria (World Health Organisation, Geneva). The classic eradication programme was the smallpox eradication programme, which reached its goal in 1977. To date, no parasitic disease has been eradicated, but dracunculiasis is a promising candidate for this achievement.

Dracunculiasis, also known as guinea worm disease, is caused by the large female of the nematode *Dracunculus medinensis*. The worm secretes a toxin that causes local inflammation and eventual formation of an ulcer, most frequently on the lower limbs. The time-honoured treatment involves winding the worm gradually on a stick until it is totally extracted and is frequently associated with secondary bacterial infection. Dracunculiasis is rarely fatal but it has a significant socioeconomic impact because of the substantial temporary disability suffered by the patient. There is little evidence of acquired immunity and the same individual can be reinfected many times. The disease is endemic across the Sahel belt of Africa from Mauritania to Ethiopia, having been eliminated from Asia and some African countries. Animals can be infected but do not act as reservoirs of human infection. Dracunculiasis is caught by ingesting infective larvae in drinking water obtained from ponds or open wells.

In 1991, the World Health Assembly declared "its commitment to the goal of eradicating dracunculiasis". The strategy includes safe water supply, health education, case management and vector control. The programme has made significant progress and guinea worm transmission has been certified as having ceased in India, Pakistan, Iran, Senegal and Yemen. The situation continuously improves in Africa, apart from Sudan, and three other African countries, where no cases of guinea worm transmission have been recorded for periods varying from 5 to 9 years, are in pre-certification status. The outcome of this major effort has been a remarkable 98% reduction in the number of cases, from an estimated 3.3 million worldwide in 1986 to only 75,223 cases reported in 2000, all in Africa. Towards the end of his presentation, Nevio Zagaria raised the question whether guinea worm disease is "a neglected disease or one of the diseases occurring in neglected populations" and emphasised that health is a human right.

References

Chakravorty, S.J., Craig, A., 2005. The role of ICAM-1 in *Plasmodium falciparum* cytoadherence. Eur. J. Cell Biol. 84.

Kraulis, P.J., 1991. Molscript: a program to produce both detailed and schematic plots of protein structures. J. Appl. Crystallogr. 24, 946–950.

Merritt, E.A., Bacon, D.J., 1997. Raster3D: photorealistic molecular graphics. Methods Enzymol. 277, 505–524.