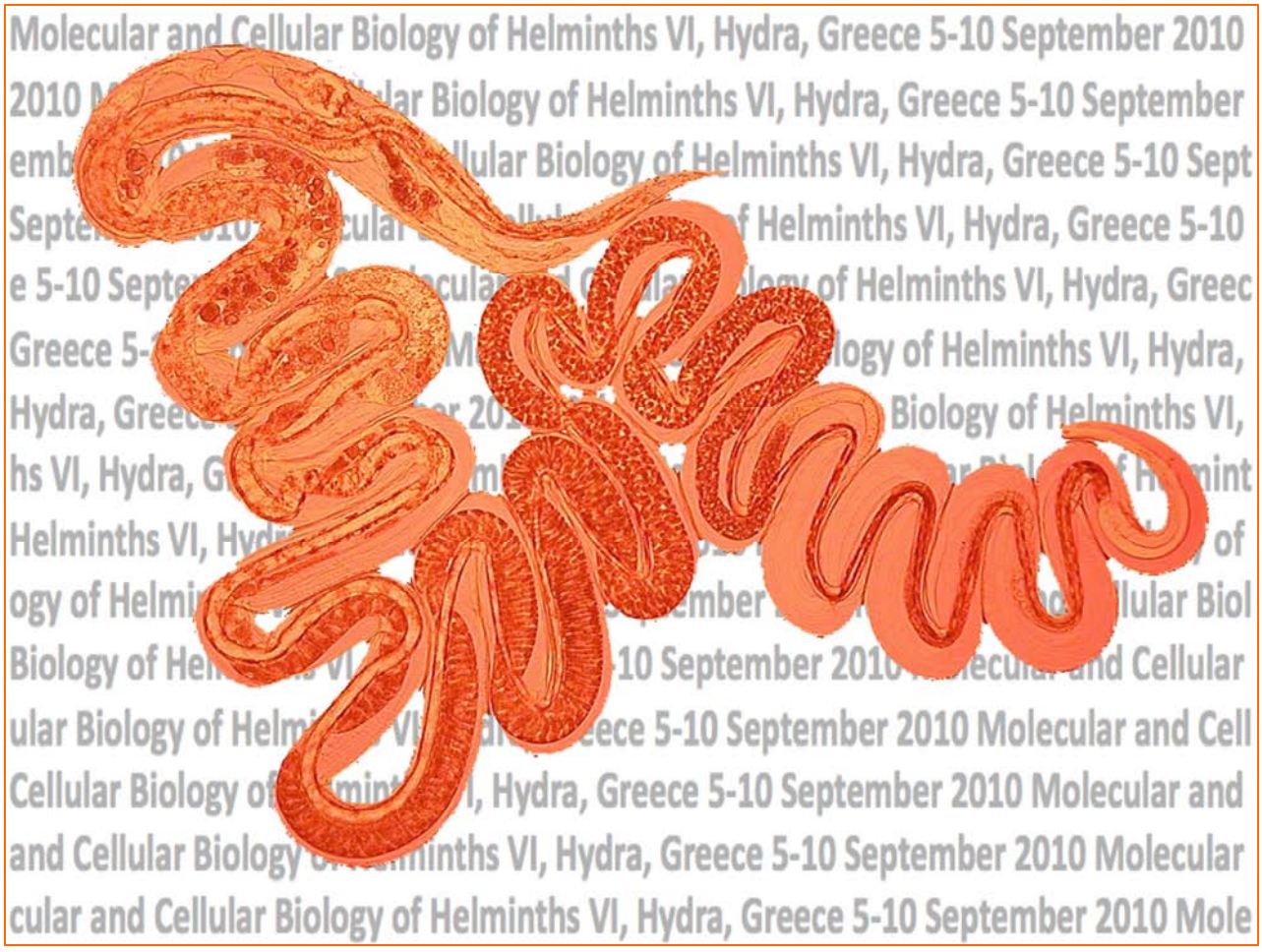
MOLECULAR AND CELLULAR BIOLOGY OF HELMINTH PARASITES VI

BRATSERA HOTEL
HYDRA, GREECE
5-10 SEPTEMBER 2010



This meeting was made possible through the generous support of the

Burroughs Wellcome Fund

MOLECULAR AND CELLULAR BIOLOGY OF HELMINTH PARASITES

I. 6-9 September 1997, Edinburgh, UK
"Parasitic Helminths from Genomes to Vaccines"

II. 8-11 July 1999, Edinburgh UK
"Parasitic Helminths from Genomes to Vaccines II"

III. 14-19 September 2002, Hydra, Greece
"Molecular and Cellular Biology of Helminth Parasites III"
Special Issue of *International Journal of Parasitology* **33 (11):** 1127-1302

IV. 6-11 September 2005, Hydra, Greece
"Molecular and Cellular Biology of Helminth Parasites IV"
Special Issue of *International Journal of Parasitology* **36 (6):** 615-733

V. 12-17 September 2008, Hydra, Greece
"Molecular and Cellular Biology of Helminth Parasites V"

VI. 5-10 September 2010, Hydra, Greece
"Molecular and Cellular Biology of Helminth Parasites VI"

ORGANISERS

Kleoniki Gounaris (Imperial College, UK, 2002-2010), Malcolm Kennedy (University of Glasgow, UK 1997-2002), Mark Blaxter (University of Edinburgh 1997-1999), Ed Pearce (University of Pennsylvania, USA 2005-2008), Murray Selkirk (Imperial College, UK 2002-2010), Rick Maizels (University of Edinburgh 1997-2010)

ADMINISTRATION FOR 2010

Annie Hetherington

COVER IMAGE

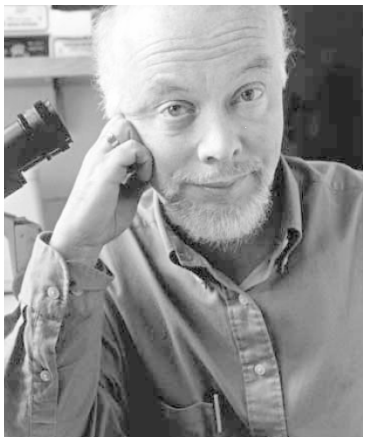
Adult female *Heligmosomoides polygyrus*
(Photograph by Janice Murray, University of Edinburgh)

Programme Summary

Sunday 5 September	Monday 6 September	Tuesday 7 September	Wednesday 8 September	Thursday 9 September
Registration and refreshments from 17:00	**Helminthomics**	**Genes & Development**	**Molecular Interactions**	**Immunity & Modulation**
	09:00-11:00 David Bird Nancy Holroyd Tom Nutman Sergio Verjovski-Almeida	**09:00-10:40** Karl Hoffmann Frederic Landmann Warwick Grant John Hawdon	**09:00-10:40** Robert Greenberg Moniek Meevissen Svenja Beckmann Richard Martin	**09:00-10:40** Nicola Harris James Hewitson Ruth Forman Mark Wilson
	Coffee Break			
	11:30-13:10 Mark Viney Claudia Welz Cecilia Fernández Larry McReynolds Tom Unnasch	**11:10-12:50** Alison Knight Najju Ranjit Katia Cristina Oliveira James Lok Murray Selkirk	**11:10-12:50** Colette Dissous Jaap van Hellemond Jürgen Krücken Jose Lozano Niki Gounaris	**11:10-12:50** Maria Periago Elena Pinelli Sabine Specht Katrin Gentil Soraya Gaze
	Lunch/Free time			
19:30 Welcome **19:45** Keynote Lecture, James McKerrow	**16:30-18:10** Christoph Grevelding Collette Britton Bernd Kalinna Ron Hokke	**16:30-18:10** Adrian Wolstenholme Balachandran Ravindran Sabine Lorenz Klaus Brehm	**16:30-18:10** David Williams Franco Falcone Pamela Knight William Horsnell	**16:30-18:10** William Harnett Wiebke Hartmann Minka Breloer Rick Maizels
21:00 Welcome Reception	**18:15-20:15** **Poster Session 1**	**19:00 Boat Trip to Vlychos Taverna**	**18:15-20:15** **Poster Session 2**	**20:00 Farewell Banquet**

KEYNOTE SPEAKER

James McKerrow
University of California
http://pathology.ucsf.edu/mckerrow//mckerrow.html



Jim McKerrow is Director of the Sandler Center for Drug Development on the Mission Bay Campus of the University of California, San Francisco. His background is unique with eclectic, cross-disciplinary scientific and medical training. Following graduation from Haverford College as a double major in Biology and Chemistry he did his doctoral work at the University of California, San Diego, influenced there by several of the pioneers in the relatively new fields of molecular genetics and recombinant DNA research. Taking a different path than his peers, he followed his PhD training with medical training at SUNY Stony Brook. After medical school McKerrow did postgraduate training in Internal Medicine and elective work in Infectious Disease at Columbia University. He immigrated back to the West Coast to also complete a Pathology residency at the University of California, San Francisco, at which time his mother said, "You've been a student for too long, get a real job." McKerrow finally did postdoctoral work at UCSF in cell biology and biochemistry, and joined the faculty in 1980. He found parasitology and tropical medicine to be an ideal focus for his eclectic background. A major influence in his life was time spent in India and Nepal following his medical residency. There a friend working in a vaccination program introduced him to the challenges of healthcare in developing countries. In 1996 he organized a group of research faculty into the UCSF Tropical Disease Research Unit, the model for the Sandler Center that he directs today. He appears frequently in the media including All Things Considered, NY Times Science Tuesday, the BBC, Channel 5 and 7 News, and Mythbusters on the Discovery Channel. He was the recipient of the Gregor Mendel Medal in 2010.

Dvorak J, Caffrey CR, Grevelding C, Beckman, Engel J, McKerrow JH. Biolistic transformation of *Schistosoma mansoni*: studies with modified reporter-gene constructs containing regulatory regions of protease genes. *Molecular & Biochemical Parasitology* **170**: 37-40, 2010

Abdulla MH, Ruelas DS, Wolff B, Snedecor J, Lim KC, Xu F, Renslo AR, Williams J, McKerrow JH, Caffrey CR. Drug discovery for schistosomiasis: hit and lead compounds identified in a library of known drugs by medium-throughput phenotypic screening. *PLoS Negl Trop Dis* **3**: e478, 2009

Caffrey CR, Rohwer A, Oellien F, Marhöfer RJ, Braschi S, Oliveira G, McKerrow JH, Selzer PM. Comparative chemogenomics strategy to predict potential drug targets in the metazoan pathogen, *Schistosoma mansoni*. *PLOS One* **4**: e4413, 2009

Hansell E, Braschi S, Medzihradszky KF, Sajid M, Debnath M, Ingram J, Lim, K-C, McKerrow JH. Proteomic analysis of skin invasion by blood fluke larvae. *PLoS Negl Trop Dis* **2**: e257, 2008

SCIENTIFIC PROGRAMME

All timings include minimum of 5 minutes for discussion.

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-Sunday 5th September-

17:00-19:30	Registration, Bratsera Hotel
19:30	Introduction: Rick Maizels, University of Edinburgh, UK
19:45	Plenary lecture: James McKerrow, University of California San Francisco, USA. Biochemical insights into schistosome invasion and development.
21:00	Welcome Reception, Bratsera Hotel

Day 1 ~ Monday 6 September ~ HELMINTHOMICS

Session 1
Chair - Murray Selkirk, Imperial College London, UK

9:00	David Bird, North Carolina State University. USA. Evolution of parasitic ability in a nematode: Horizontal gene flow, gene diversification and convergent evolution.
9:30	Nancy Holroyd, Wellcome Trust Sanger Institute, Hinxton, Cambridge UK. Parasitic helminth genomics at WTSI.
10:00	Tom Nutman, National Institutes of Health, Bethesda, USA. The *Loa Loa* genome: Comparative analyses (with *Wuchereria bancrofti* and *Brugia malayi*) with an emphasis on the absence of Wolbachiae in *Loa loa.*
10:30	Sergio Verjovski-Almeida, Universidade de São Paulo, Brazil. High-throughput RNA sequence of *Schistosoma mansoni* adult males and females.

11:00 - 11:30 Coffee Break

Session 2
Chair- Karl Hoffman, Aberystwyth University, UK

11:30	Mark Viney, University of Bristol, UK. Genetic mapping in *Strongyloides ratti*.
11:50	Claudia Welz, University of Veterinary Medicine, Hannover, Germany. Transcriptional changes during the percutaneous migration of *Ancylostoma caninum* larvae.
12:10	Cecilia Fernández, Universidad de la República, Montevideo, Uruguay. Precursors of small RNAs in the *Echinococcus granulosus* transcriptome.
12:30	Larry McReynolds, New England Biolabs, USA. Complexity of small RNAs in *Brugia malayi.*
12:50	Thomas Unnasch, University of South Florida, USA. Analysis of promoter structure and regulated gene expression in *B. malayi.*

13:10 - 16:30 Lunch/Free Time

Session 3
Chair- Klaus Brehm, University of Würzburg, Germany

16:30	Christoph Grevelding, Justus-Liebig-University, Giessen, Germany. Inhibitor and RNAi approaches to investigate signalling molecules in *Schistosoma mansoni*.
17:00	Collette Britton, University of Glasgow, UK. In vitro and in vivo RNAi in *Haemonchus contortus.*
17:20	Bernd Kalinna, University of Melbourne, Australia. Suppression of cathepsin B by RNAi in *Schistosoma.*
17:40	Cornelis H. Hokke, Leiden University Medical Center, Netherlands. Glycomics-driven discoveries in schistosomiasis.

18:15 - 20:00 Poster Session 1
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Day 2 ~ Tuesday 7 September ~ GENES AND DEVELOPMENT

Session 4
Chair - Collette Britton, University of Glasgow, UK

9:00 Karl Hoffmann, Aberystwyth University, UK. Detection of DNA methylation in *Schistosoma mansoni.*
9:30 Frederic Landmann, University of California Santa Cruz, USA. Asymmetric *Wolbachia* segregation during early *Brugia malayi* embryogenesis.
9:50 Warwick Grant, La Trobe University, Melbourne, Australia. Conserved and divergent elements of the *Caenorhabditis elegans* dauer signalling pathways in *Parastrongyloides trichosuri.*
10:10 John Hawdon, George Washington University, USA. Role of *Ancylostoma caninum* transcription factor DAF-16/ FoxO during the rescue of developmental arrested larvae.

10:40 - 11:10 Coffee Break

Session 5
Chair- Warwick Grant, La Trobe University, Australia

11:10 Alison Knight, Australian National University, Canberra, Australia. VHA-19, a nematode-specific protein, is critical for reproduction in *Caenorhabditis elegans* oocytes.
11:30 Najju Ranjit, University of Pennsylvannia, USA. Functional studies of *Ss-daf-2*: an insulin signaling pathway intermediate in *Strongyloides stercoralis.*
11:50 Katia Cristina Oliveira, University of São Paulo, Brazil. Molecular characterization of the TNF-alpha receptor in *Schistosoma mansoni* adult worms.
12:10 James Lok, University of Pennsylvania. USA. Infective third stage larvae of *Strongyloides stercoralis* detect the host chemoattractant urocanic acid as a volatile odorant.
12:30 Murray Selkirk, Imperial College London, UK. Activation of *Nippostrongylus brasiliensis* infective larvae.

12:50 - 16:30 Lunch/Free Time

Session 6
Chair- David Bird, North Carolina State University, USA

16:30 Adrian Wolstenholme, University of Georgia, USA. Using *Caenorhabditis elegans* to study glutamate-gated chloride channels from *Haemonchus contortus.*
17:00 Balachandran Ravindran, Institute of Life Sciences, Bhubaneswar, India. Apoptosis in a pathogenic nematode involves mitochondrial pathway.
17:20 Sabine Lorenz, University of Würzburg, Germany. Host-derived FGF stimulates *Echinococcus multilocularis* development by activation of a biochemically unusual cestode FGF receptor.
17:40 Klaus Brehm, University of Würzburg, Germany. Targetting flatworm signalling cascades for the development of novel anthelminthics.

19:00 ~ Boats depart from Hydra port for Vlychos

19:30 Dinner, Vlychos Taverna

Day 3 ~ Wednesday 8 September ~ MOLECULAR INTERACTIONS

Session 7
Chair-Adrian Wolstenholme, University of Georgia, USA

9:00 Robert Greenberg, University of Pennsylvania, USA. Physiological roles and pharmacological sensitivities of *Schistosoma mansoni* multidrug resistance transporters.
9:30 Moniek Meevissen, Leiden University Medical Center, Netherlands. The *S. mansoni* egg glycoprotein κ5 induces granulomas in a pulmonary mouse model via its GalNAcβ1-4GlcNAc-containing N-glycans.
9:50 Svenja Beckmann, Justus-Liebig-University, Giessen, Germany. Imatinib (Gleevec) causes dramatic effects on morphology, pairing stability, and survival of adult *Schistosoma mansoni* in vitro.
10:10 Richard Martin, Iowa State University, USA. Voltage-activated ion-channel in Clade III nematodes as drug target sites.

10:40 - 11:10 Coffee Break

Session 8
Chair- Billy Harnett, University of Strathclyde, UK

11:10 Colette Dissous, Institut Pasteur Lille, France. *Schistosoma mansoni:* Kinase signalling in mitosis and developmental processes.
11:30 Jaap van Hellemond, Erasmus University Medical Center, Rotterdam, Netherlands. Acetate formation in helminths and other parasites.
11:50 Jürgen Krücken, Freie Universität Berlin, Germany. Nematode P-glycoproteins as mediators of unspecific resistance against macrocyclic lactones and other anthelmintics.
12:10 Jose Lozano, Wageningen University, Netherlands. A nematode venom allergen/ASP-like protein interacts and inhibits an extracellular cathepsin-like cysteine protease required for fungal resistance.
12:30 Niki Gounaris, Imperial College London, UK. Modulation of leukocyte function by nematode secreted nucleotide metabolising enzymes.

12:50 - 16:30 Lunch/Free time

Session 9
Chair- Niki Gounaris, Imperial College London

16:30 David Williams, Rush University Medical Center, Chicago, USA. Characterization of phytochelatin synthase of *Schistosoma mansoni*, a potential target for schistosomiasis drug development.
17:00 Franco Falcone, University of Nottingham, UK. Nuclear Translocation of IPSE/alpha-1.
17:20 Pamela Knight, University of Edinburgh/Roslin Institute, UK. Transcriptomic analysis of early events in ovine abomasal mucosa in response to infection with the nematode *Teladorsagia circumcincta*.
17:40 William Horsnell, University of Cape Town, South Africa. IL-4Rα cellular responses and mechanisms underlying adaptive immunity to *N. brasiliensis.*

18:15 - 20:00 Poster Session 2

Day 4 ~ Thursday 9 September ~ IMMUNITY AND MODULATION

Session 10
Chair- Rick Maizels, University of Edinburgh, UK

9:00 Nicola Harris, École Polytechnique Fédèrale de Lausanne, Switzerland .Antibodies function to promote helminth-Induced basophilia.
9:30 James Hewitson, University of Edinburgh, UK. Immunomics of *Heligmosomoides polygyrus.*
9:50 Ruth Forman, University of Manchester, UK. Functional analysis of the interferon gamma network by susceptibility of mice to chronic *Trichuris muris* infection.
10:10 Mark Wilson, NIMR, Mill Hill, London, UK .Distinct molecular signatures of regulatory T cells following Schistosome and *Leishmania* infection.

10:40 - 11:10 Coffee Break

Session 11
Chair- Nicola Harris, École Polytechnique Fédèrale de Lausanne, Switzerland

11:10 Maria Periago, Centro de Pesquisas René Rachou- FIOCRUZ, Brazil. Update from the Human Hookworm Vaccine Initiative.
11:30 Elena Pinelli, National Institute for Public Health and Environment, Netherlands. Immune response to trickle infection with *Toxocara canis* and its association with experimental allergic airway inflammation.
11:50 Sabine Specht, University Hospital Bonn, Germany. CCL17 controls mast cells to maintain skin integrity for the defense against filarial larval entry.
12:10 Katrin Gentil, University Hospital Bonn, Germany. The role of eosinophils in experimental filarial infection with .
12:30 Soraya Gaze, James Cook University, Queensland, Australia. Using human hookworm to treat celiac disease: immune responses during a clinical trial.

12:50 - 16:30 Lunch/Free time

Session 12
Chair - Tom Nutman, National Institute of Health, USA

16:30 William Harnett, University of Strathclyde, UK. Therapeutic potential of the *Acanthocheilonema viteae* secreted product, ES-62.
17:00 Wiebke Hartmann, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany. Immune response against unrelated antigens is drastically suppressed in *Litomosoides sigmodontis* infected mice.
17:20 Minka Breloer, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany. *Strongyloides ratti* infection induces expansion of regulatory T cells that interfere with nematode-specific immune responses and parasite clearance.
17:40 Rick Maizels, University of Edinburgh, UK. Competing T cell subsets in *Heligmosomoides polygyrus* infection.

20:00 Farewell Dinner at Bratsera Hotel

Friday 10 Sept : Departure

POSTER SESSION 1: MONDAY 6 SEPTEMBER, 18:15-20:00

1	Carmen Aranzamendi, RIVM, Netherlands	Inhibition of Toll-like receptor signalling by *Trichinella spiralis* excretory/secretory products is restricted to TLR4.
2	Young Mee Bae, Seoul National University, Korea	Predominance of IL-10 and TGF-β production from RAW 264.7 cells in response to crude antigen of *Clonorchis sinensis*.
3	Michiel Bexkens Erasmus Medical Center, Netherlands	Schistosomal lyso-phosphatidylserine: a novel Toll Like Receptor 2 ligand.
4	Alexandra Blanchard-Letort, INRA, Nouzilly, France	Transcriptomic approaches to identify genes involved in animal nematode virulence.
5	Raul J. Bobes, Universidad Nacional Autonoma de Mexico	Biological implications of DNA compaction in *Taenia crassiceps cysticerci*.
6	Jana Broadhurst, University of California San Francisco, USA	Regulation of retinoic acid synthesis during *Schistosoma mansoni* infection and its contribution to egg-elicited T cell response.
7	Denice T Y Chan, Imperial College London, UK	Venom allergen homologue / ASP-Like proteins (VALs) in *Nippostrongylus brasiliensis.*
8	Keith Choe, University of Florida, USA	SKN-1 as a potential xenobiotic detoxification and developmental target.
9	Chris Cluxton, Trinity College Dublin, Ireland	Functional characterisation of *Schistosoma mansoni* signalling molecules.
10	Alexis Cogswell, Rush University, USA	Approaches to understanding *Schistosoma mansoni* female worm sexual development.
11	Anna Crisford, University of Southampton, UK	Emodepside is a resistance breaking anthelmintic drug with selective toxicity to the parasite's SLO-1 calcium-activated potassium channel.
12	Julia Fahel,Trinity College Dublin, Ireland	Identification, characterization and functional analysis of immunomodulatory molecules from *Schistosoma mansoni.*
13	Verena Gelmedin, George Washington University, USA	*Ancylostoma* spp: Heat shock response during the transition to parasitism.
14	Warwick Grant,La Trobe University, Australia	Protein biosynthesis and lifespan determination in parasitic nematodes: a bioinformatic analysis.
15	Yvonne Harcus, University of Edinburgh,UK	Proteomic, Transcriptomic and Genomic Analysis of *Heligmosomoides polygyrus.*
16	Jana Janssen, Institute of Parasitology and Tropical Veterinary Medicine, Germany	Bioinformatical analysis and genetic diversity of P-glycoproteins in ivermectin-susceptible and resistant *Parascaris equorum*.
17	Yan Jin, Seoul National University, Korea	Regulation of immune response in mouse dendritic cells by the treatment of *Clonorchis sinensis* crude antigen.
18	Michelle Joyner, University of Southampton, UK	Investigating the effects of compounds with anthelmintic potential: Amidantel, Bay d 9216 and Tribendimidine.
19	Malcolm Kennedy, University of Glasgow, UK	Structural and ligand binding studies of the nematode-specific FAR and nemFABP small lipid binding proteins.
20	Silke Leutner, Justus-Liebig University, Giessen, Germany	A combined study applying microarray and Super-SAGE detects pairing-dependent transcription in *Schistosoma mansoni* males.

POSTER SESSION 1 ~ DAY 1~ MONDAY 6 SEPTEMBER 18:15-20:00

POSTER SESSION 2: WEDNESDAY 8 SEPTEMBER, 18:15-20:00

21	Janice Murray, University of Edinburgh, UK	VAL proteins: Towards a function.
22	Nadege Gouignard, Institut Pasteur Lille, France	Venus kinase receptors of *Schistosoma mansoni.*
23	Natalie Nieuwenhuizen, University of Cape Town, South Africa	Isolation, characterization and cDNA sequencing of *Anisakis pegreffii* haemoglobin.
24	Alasdair Nisbet, Moredun Research Institute, UK	Immunomodulatory molecules from *Teladorsagia circumcincta*.
25	Guilherme Oliviera, FIOCRUZ, Brazil	The phylogenome o*f Schistosoma mansoni:* the evolutionary history of proteins.
26	Larissa Podust, University of California San Francisco, USA	Targeting sterol biosynthesis in helminths for the development of new chemotherapy.
27	Natalia Pouchkina-Stantcheva and Peter Olson, Natural History Museum, UK	Development of RNA interference in *Hymenolepis*.
28	Anna Protasio, Wellcome Trust Sanger Institute, UK	"I've got you under my skin" – Differentially expressed genes between cercariae and skin schistosomula identified using sequencing technologies.
29	Marta C. Romano, CINVESTAV, Mexico	*Taenia solium* and *T. crassiceps* capacity to synthesize and interconvert sex steroid hormones.
30	Lucien Rufener, Novartis Animal Health, Switzerland	Monepantel allosterically activates DEG-3H/DES-2H channels of the gastrointestinal nematode *Haemonchus contortus*.
31	Thomas Schnieder, University of Veterinary Medicine, Hannover, Germany	Genes associated with transition from free-living to parasitic stages of the bovine lungworm *Dictyocaulus viviparus.*
32	Michael Smout, James Cook University, Australia	A granulin-like growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini*, promotes proliferation of host cells.
33	Christina Strube, University of Veterinary Medicine Hannover, Germany	Stage dependent transcriptional changes of daf-12 during the life cycle of *Dictyocaulus viviparus*
34	Francesca Tamarozzi, Liverpool School of Tropical Medicine, UK	Wolbachia drives Type 17 immunity in *Onchocerca volvulus* (river blindness).
35	Mark Taylor, Liverpool School of Tropical Medicine, UK	A-WOL: Anti-*Wolbachia* drug discovery and development for the treatment and control of filariasis.
36	Martha Truscott, Aberystwyth University, UK	The effects of *Schistosoma mansoni* haemozoin on macrophage activation in a changing cytokine milieu.
37	Irene Ajonina Ungitoh, Westfalian Wilhelms University, Germany	Novel approaches to analyse excretory-secretory proteins from *Onchocerca*.
38	Johnny Vlaminck, Ghent University, Belgium	Intestinal immunity against *Ascaris suum* in pigs: The search for targets and effector mechanisms.
39	Saskia de Walick, Erasmus Medical Center, Rotterdam, Netherlands	Targeting of host lipoproteins by the parasitic worm *Schistosoma mansoni*.
40	Hae Joo WI, Seoul National University Colege of Medicine, Korea	Immune suppressive property of thioredoxin peroxidase from *Clonorchis sinensis*.
41	Magdalena Zarowiecki, Wellcome Trust Sanger Institute, UK	The *Hymenolepis microstoma* genome and transcriptome.

POSTER SESSION 2 ~ DAY ~ WEDNESDAY 8 SEPTEMBER 18:15-20:00

Travelling Fellowships

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Through the generous support of the Burroughs Wellcome Fund, the following individuals were awarded travelling fellowships to attend the meeting.

Bernd Kalinna, University of Melbourne

Jana Broadhurst, University of California San Francisco


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ABSTRACTS
FOR ORAL PRESENTATIONS

Evolution of parasitic ability in a nematode:
Horizontal gene flow, gene diversification and convergent evolution.

D.M. BIRD, E.H. SCHOLL, J.P. CROMER, P. DIGENNARO AND C.H. OPPERMAN

DEPARTMENT OF PLANT PATHOLOGY, NC STATE UNIVERSITY, RALEIGH NC, USA

Root-knot nematodes (RKN: *Meloidogyne* spp.) are widely distributed throughout temperate and tropical regions and are responsible for major yield losses on food and fiber crops. They render plants more susceptible to drought stress and are a significant contributing factor to a looming world food crisis. Our recently completed genome sequence of the 54 Mbp diploid RKN, *M. hapla*, provides a research platform to study the genetic and biochemical basis for parasitism. One key feature the *M. hapla* genome is that it encodes a large cadre of genes apparently acquired during evolutionary history from bacteria, perhaps representing the legacy of ancient nematode-bacterial symbioses. These genes, many of which have seemingly obvious roles in plant-parasitism, have been amplified into families with diverse sub-specialties and at least partial redundancy. RKN also encodes sets of genes encoding small secreted proteins with sequence similarity to plant peptide hormones. We have developed bioassays to assess the functional role of these apparent ligand mimics in the host-parasite interaction. Based on initial computational analyses, it appears that these mimics may have evolved by convergent evolution. Like genes acquired by HGT, ligand mimic genes in RKN appear to be expanding and diversifying into families. Collectively, RKN genes with strong inter-kingdom analogues appear to lie at the core of the parasitic armory.

Parasitic Helminth Genomics at the Wellcome Trust Sanger Institute

NANCY HOLROYD, ON BEHALF OF THE PARASITE GENOMICS GROUP

WELLCOME TRUST SANGER INSTITUTE, HINXTON, CAMBRIDGE, UK

Despite their importance globally, both medically and economically, parasitic helminth research has remained relatively untouched by genomics. The Wellcome Trust Sanger Institute is committed to producing reference genomes using *de novo* sequencing approaches for a cross-phyla list of parastic helminths that include hookworms, whipworms, threadworms, Schistosomes, a tapeworm and the filarial parasites. We aim to uncover the genomic basis for differences in the biology of these parasites. The reference genomes are being produced using a mixture of sequencing technologies (Illumina, 454 and limited capillary sequence data). Manual improvement of the assemblies is being performed *in silica* and employing custom capillary sequencing. Several software packages support this process, enabling both large scale global viewing of the genomic and associated data, and in depth manipulation down to the base level. *De novo* sequencing relies heavily upon reliable mapping information. We are using a number of different mapping sources including genetic markers, happy map markers, large insert read pairs and fingerprint maps. We are also developing a new physical mapping approach based on second generation sequencing that could provide a powerful additional tool for *de novo* genome sequencing. Gene prediction is a major challenge in genome analysis. Second generation sequencing technologies are being employed to directly sequence transcriptomes and identify coding regions by alignment to the genome sequence. Comparative sequencing of related strains or species will be undertaken to identify candidate genes or other sequences relating to species-specific differences. An overview of the projects and the approaches being used to produce these reference genomes will be detailed.

The *Loa loa* genome: Comparative analyses (with *Wuchereria bancrofti and Brugia malayi*) with an emphasis on the absence of *Wolbachia in Loa loa*

NUTMAN,T.B., FINK,D.L., RUSS,C., YOUNG,S., ZENG,Q., KOEHRSEN,M., ALVARADO,L., BERLIN,A., BORENSTEIN,D., CHAPMAN,S.B., CHEN,Z., DESJARDINS, ENGELS,R., FREEDMAN,E., GELLESCH,M., GOLDBERG,J., GRIGGS,A.,GUJJA,S., HEILMAN,E.R., HEIMAN,D., HEPBURN,T., HOWARTH,C., JEN,D., LARSON,L., LEWIS,B., MEHTA,T., PARK,D., PEARSON,M., RICHARDS,J., ROBERTS,A., SAIF,S., SHEA,T., SHENOY,N., SISK,P., STOLTE,C., SYKES,S., WALK,T., WHITE,J., YANDAVA,C., HAAS,B., HENN,M.R., NUSBAUM,C. AND BIRREN,B.

NATIONAL INSTITUTES OF HEALTH, BETHESDA, MD, USA

Filarial worms are parasitic nematodes that dwell within the lymphatics and the subcutaneous tissues of up to 170 million people worldwide. Among the 8 filarial infections of humans, those that cause loiasis, onchocerciasis, and lymphatic filariasis are important causes of morbidity. The study of these parasites and the diseases they cause has been greatly hampered by the fact that for most of these organisms, humans are the only definitive host and parasite material must be purified from human blood or tissues. In fact, *B. malayi*, was selected to be the first parasitic nematode to be fully sequenced despite its relatively minor current importance as a human pathogen because it is the only major filarial parasite of humans that can be maintained in small laboratory animals. In late 2009, we generated sequence coverage to single adult worms to characterize polymorphisms from *W. bancrofti*. and *O. volvulus*, and we generated a draft assembly of *Loa loa* from microfilariae, in order to study crucial phenotypic difference among these closely related filarial species. *Loa loa* was targeted for this initial work for two important reasons. First, among the 4 pathogenic filarial parasites, *Loa loa* is the least well studied, though *Loa loa*, is gaining clinical prominence because of serious adverse events following treatment, including death. A second justification for prioritizing *Loa loa* was the fact that unlike any of the other filarial parsites of humans, *Loa loa* does not contain the alpha-proteobacterial endosymbiont, Wolbachia. As currently assembled, the *Loa loa* genome is 86.81 Mb with a GC content of 31%. There are 16330 predicted protein coding genes in the current annotation, ~5000 more than predicted for the Brugia genome. Although a much more thorough analysis will be presented, it is almost certain that *Loa loa* does not contain orthologs of any of genes in the wBm genome related to pathways hypothesized to be missing in *Brugia malayi* (but provided for by wBm) namely those involved in purine biosynthesis, heme biosynthesis, and riboflavin biosynthesis. Further, *Loa loa* does not appear to have any genome-scale insertion of Wolbachia DNA into it's own genome. More extensive comparative analyses will be available in September.

High-throughput RNA Seq of *Schistosoma mansoni* adult males and females

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Schistosoma mansoni is one of the causative agents of schistosomiasis, a major health problem in developing countries. In the past seven years, two high-throughput sequencing projects have contributed a considerable amount of molecular information on the parasite, covering a significant portion of both the transcriptome (Verjovski-Almeida, S. et al., Nature Genetics 35: 148-157, 2003) and the genome (Berriman, M. et al., Nature 460: 352-358, 2009). The most recent estimate of the number of genes is approximately 13,000. However, the fragmented nature of the data is still apparent, as approximately 3,000 genes predicted in the genome have no evidence of transcription in the available EST databases, while approximately 7,000 EST contigs in the public databases do not map to the genome sequence and/or to the predicted genes.
The present work aims at obtaining more extensive transcriptome coverage of the S. mansoni adult stage. The high-throughput pyrosequencing technology has been employed. Using an approach developed in our laboratory, we established a novel method that allows for the construction of directional cDNA libraries ready to be used in the 454 Roche pyrosequencer. Long EST sequences (average 400 bp) both from the 5'- and the 3'-end of messages were obtained. A total of over 1.4 million ESTs were obtained from adult males or females; comparison of these reads with gene predictions from the *S. mansoni* genome project using Blastn indicates that 10,300 (80%) of the 12,845 gene predictions deposited in GenBank have been sampled, evidencing an extensive coverage of adult worms transcriptome. Adequate coverage of the entire predicted gene was obtained for a number of transcripts. In addition, the 5'-most exon of predicted genes was confirmed and/or corrected for many of the transcripts. A thorough analysis of the functional categories of genes expressed in adults is under way. A detailed mapping of the individual EST reads to the genome (with no assembly) is being done to identify alternative splicing isoforms. First results will be presented. Supported by grants from Fundação de Amparo a Pesquisa do Estado de São Paulo and from FINEP, Financiadora de Estudos e Projetos, Brazil.

Genetic mapping in *Strongyloides ratti.*

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Genetics is a very powerful biological tool, which has been used with great success with model species, such as *Caenorhabditis elegans.* For some obvious reasons this approach has been used rather little with parasitic nematodes. The parasitic nematodes *Strongyloides* spp. and *Parastrongyloides* spp. have life-cycles which particularly favour a genetic approach, and thus there are great prospects for genetic approaches with these groups. To this end we constructed a genetic map of *S. ratti*. We did this by identifying 74 'anonymous' genetic markers that differentiated two *S. ratti* isofemale lines. We then followed the inheritance of these markers through a cross and backcross. The resultant genetic map places the markers into three linkage groups (two autosomes and an X chromosome) spanning 90cM. We found no evidence of strong conservation of chromosomal location or extensive synteny between *S. ratti* and *C. elegans*. This work now opens the possibility of mapping important, and interesting, traits of *S. ratti*. Two examples of such traits are parasite survival within a host and free-living developmental route, both of which vary between isofemale lines in this species. Other traits could be mapped too.

Transcriptional changes during the percutaneous migration of *Ancylostoma caninum* larvae

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Besides other ways of infection, the infective larvae of the canine hookworm *Ancylostoma caninum* are able to invade their host percutaneously. An *in vitro* model for the migration of infective larvae through isolated skin has been used in several previous studies. We optimised the *in vitro* system and called it PERL (percutaneous larval migration) chamber-system. Subsequently, the PERL chamber system was used to produce and to collect migrated larvae, i.e., larvae that had migrated completely through the skin. The bulk of larvae completed migration through whole canine skin within 12-16 hours. Total RNA was isolated and transcribed, and selected transcripts were analysed using a real-time PCR setup. To allow for relative quantification, three reference genes were analysed. These genes were the 60S ribosomal acidic protein, β-tubulin, and the cAMP dependent protein kinase A, all of which have been used as reference genes in previous studies on *A. caninum*. Six genes were selected as genes of interest, since these genes have previously been found to be differently transcribed between infective and migrated or serum-stimulated larvae. The selected genes were 1) the previously described metalloprotease *mtp-1*, 2) *daf-12*, which is involved in the regulation of the dauer-like free-living stage, 3) a lysozyme gene, 4) a gene coding for a DNA polymerase, 5) a gene coding for a protein resembling the leukotriene-4-hydrolase, and 6) the precursor of *Ancylostoma* secreted protein 1, *asp-1*. For comparison, the transcription of the respective genes was analysed in serum-stimulated larvae, which are the current model for early parasitic stages of *A. caninum*, and in infective larvae as well as in control populations. Furthermore, skin samples were collected at several time points of the migration experiments to analyse the transcription levels of larvae during skin migration. According to the real-time PCR results, the migrated larvae clearly differ from infective as well as from serum-stimulated larvae. Results and differences between the transcription patterns of the larval populations will be discussed.This work is supported by the German Research Foundation (DFG).

Precursors of small RNAs in the *Echinococcus granulosus* transcriptome

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A transcriptomic survey of *E. granulosus* larval stages carried out several years ago has substantially contributed to the identification of gene families acting at the interface between the parasite and its definitive and intermediate hosts. Although this was a small scale project (10000 ESTs, clustered into 2700 potential gene products [1]), it yielded valuable data because ESTs were generated from full-length enriched libraries (of either oligo-capped or *trans*-spliced cDNAs) prepared with carefully selected parasite materials [2]. Here, we describe an initially overlooked feature of the *E. granulosus* transcriptome: the presence of a diverse set of transcripts containing regions with high similarity to EgBRep, a middle repetitive DNA element, showing structural similarities to mobile elements. We propose that these transcripts may correspond to precursors of piRNAs, a class of small RNAs probably associated with parasite somatic stem cells (neoblasts).

The initial assembly of *E. granulosus* ESTs predicted 2600 polypeptides. Interestingly, the contigs from a majority of the remaining 100 clusters – about 700 ESTs mostly from oligo-capped libraries of all stages - contained fragments showing 92-96% identity with antisense EgBRep. Some of them were among the clusters with higher abundance in the dataset (notably, EGC000310 with 258 ESTs; and EGC003058 with 122 ESTs). When examined in detail, the corresponding contigs were found to be predominantly derived from full-length transcripts of ~900 nt flanked by stretches hitting the EgBRep (150 nt at the 5'-end; 140 nt at the 3'-end). However, in spite of conforming to an overall general structure and of showing a global identity of about 90%, the individual ESTs were extremely diverse. Interestingly, putative orthologues of these transcripts were identified among *E. multilocularis* ESTs, whereas a search against the parasite draft genome retrieved about 100 contigs. Finally, some EgBRep-containing transcripts were also found to be derived from full-length *trans*-spliced *E. granulosus* cDNAs; the latter were shorter (~250 nt) and included the 140 nt 3'-end fragment similar to EgBRep. We propose that these EgBRep-containing cDNAs would derive from piRNA precursors. piRNAs (piwi-interacting RNAs) are a class of stunningly diverse small non-coding RNAs implicated in germ-line development and in maintaining germ-line DNA integrity in metazoa; they have been described as forming an "innate immune system" that discriminates transposons from endogenous genes and selectively silences the former. piRNAs have recently been found to predominate among small RNAs in the neoblasts from the free-living platyhelminth *Schmidtea mediterranea*. Although several aspects of piRNA biogenesis and function remain obscure, the information currently available supports the view that the EgBRep-containing transcripts could be piRNA precursors. Indeed, piRNAs are known to originate from long single-stranded precursors, transcribed by RNA polymerase II; in addition, planarian primary piRNAs have been found to map antisense to transposable elements. We discuss the putative significance of these observations in the context of *Echinococcus* spp. biology.

Small RNA complexity in the nematode *Brugia malayi*

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Brugia malayi is a parasitic nematode that is the cause of lymphatic filariasis. In order to understand the role of small RNAs in this parasite, RNAs from 18 to 30 bases in length were cloned and sequenced using Solexa technology. About 14 million reads matching the *B.malayi* genome were obtained from five libraries, representing three parasitic stages: adult males, adult females and microfilariae (equivalent to the 1st larval stage). Of the 143 miRNAs that we identified, eighty-five were represented by at least 50 reads in one of the libraries. These 85 miRNAs belong to 50 distinct families, of which 32 are conserved in arthropods, vertebrates and helminths. Several differentially expressed miRNAs were identified. For example, the level of mir-71 was 6-fold higher in microfilariae than in either adult males or females. In contrast, members of the miR-36 family appear to be specific to females. A role for these miRNAs in the transition from the blood born L1 stage to adults will be investigated.

Small RNAs from repeated DNA sequences were also identified. The Hha I repeat is a tandem 320 bp sequence that represents about 10% of the *B. malayi* genome. Small RNAs from a 50 bp region of this repeat were found in all three parasitic stages. Treatment of the RNA with tobacco acid pyrophosphatase prior to sequencing increased the number of small RNA reads derived from the Hha I repeat, suggesting that the repeat RNAs contained a 5'-triphosphate moiety and are likely to be primary transcripts. Small RNAs derived from the Pao family of retrotransposons were also sequenced. Small RNA reads were identified from both the coding and non-coding strands of this retrotransposon, suggesting that *B malayi* transposable elements are regulated via the RNAi pathway. Analysis of this data set should provide novel insight into the evolutionary history of small RNA-mediated silencing pathways in nematodes, in addition to how these pathways regulate the parasitic life cycle of *B. malayi*.

Transfection of developmentally competent *B. malayi*.

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Transient transfection of isolated *B. malayi* embryos by biolistic bombardment has proven to be useful in defining promoter structure and function in this human filarial parasite. However, isolated transfected embryos are developmentally incompetent. A method of producing developmentally competent transfected parasites is therefore needed. We have found that L3 parasites can be chemically transfected *in situ* in the peritoneal cavity of a gerbil. The *in situ* chemically transfected parasites are developmentally competent, producing adult parasites with an efficiency similar to that obtained from implanted untreated L3. To refine this system, a reporter plasmid was constructed consisting of the a secreted gaussia luciferase reporter gene (gLUC) sequence flanked by the 5' and 3' untranslated domain of the *B. malayi* HSP70 gene, and containing the first intron derived from the *B. malayi* HSP70 gene inserted into the gLUC ORF. Cultured adult parasites and progeny mf derived from L3 transfected with this construct secreted gLUC into the culture medium. When the transfected mf were mixed with blood, fed to mosquitoes and the resulting L3 collected, the L3 also secreted gLUC into the culture medium. Transfected adults and progeny mf contained transgenic DNA, and the transgenic mRNA produced in these parasites was found to be correctly cis- and trans-spliced. These data suggest that it is possible to produce developmentally competent transfected *B. malayi* and that the transgenic sequences are inherited remain transcriptionally active in all lifecycle stages. Furthermore, the data demonstrate that gLUC may be employed as a selectable marker to identify transfected parasites. These studies open the way to using transgenesis to study all lifecycle stages of *B. malayi*.

Inhibitor and RNAi approaches to investigate signalling molecules in *Schistosoma mansoni*.

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Within the trematodes schistosomes are the only members living dioeciously. A remarkable feature of schistosome biology is the essential influence of the male on the sexual maturation of the female. During a constant pairing contact the male induces mitoses and differentiation processes in the reproductive organs of the female, ovary and vitellarium. By delivering oocytes and vitelline cells both organs contribute likewise to egg production. To understand the molecular basis of gonad differentiation processes in the female, we have started to identify and clone genes coding for signalling proteins with functions in the gonads. Besides elucidating fundamental biological processes, such molecules could serve as potential targets to interfere with the development of this parasite to combat schistosomiasis since its pathology is caused by the eggs.
The signalling molecules of our main interests are cellular tyrosine kinases (CTKs). Among the CTKs isolated and characterised so far are members of the Src and Syk families, which were found to be expressed in the reproductive organs of both genders. Besides CTKs, also the transforming growth-factor β receptor 1 (TGFβ-R1), identified by Pearce et al. and LoVerde et al., was found to be transcriptionally active in the gonads. To analyse the function of these molecules in more detail, we have started to apply a combination of different methods. These included *in vitro* culture studies with adult schistosomes to investigate physiological effects of chemical inhibitors blocking specific enzyme activities and confocal laser scanning microscopy to study the morphological consequences of these experimental approaches. Yeast two/three-hybrid library screenings and interaction studies were done to identify and characterise binding partners of molecules of interest and microarray analyses to get an overview about global gene expression changes upon inhibitor treatment. Finally RNAi was applied to silence specific gene functions and *Xenopus* oocyte transfection approaches combined with GVBD (germinal vesicle breakdown) assays to confirm the repressive effects of specific inhibitors for individual kinases. Results will be presented that demonstrate the potential of combining these methods to functionally characterise schistosome genes. Furthermore, first models of signal transduction cascades will be proposed that are active in the ovary and the vitellarium regulating mitotic activity and differentiation.

In vitro and in vivo RNAi in *Haemonchus contortus*.

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RNA interference (RNAi) has been applied very successfully to *Caenorhabditis elegans* to study gene function, but has proven less effective in parasitic nematode species. We have extended previous analysis of RNAi in the sheep gastrointestinal nematode *Haemonchus contortus* to examine why only some genes seem to be susceptible to RNAi. Target genes were selected based on their expression level in the L3 stage or on their site of expression. Target genes with a high number of ESTs from L3 stage larvae could not be reproducibly silenced by soaking in dsRNA in the presence of lipofectin. However targeting genes putatively expressed in tissues accessible to the environment resulted in consistent RNAi silencing. We obtained specific and reproducible silencing for several genes including the *H. contortus* vaccine candidate *H11.* We are currently testing RNAi knockdown of genes that may produce phenotypes that can be monitored in vitro. In addition, we have examined in vivo effects of RNAi silencing. Pre-treatment of *H. contortus* infective larvae with control dsRNA had no effect on infectivity or viability in sheep, demonstrating the feasibility of in vivo studies. In contrast, pre-exposure of larvae to *H. contortus H11* dsRNA resulted in significant reductions in faecal egg count (FEC) and worm burden. We have increased the number of *H. contortus* target genes reproducibly silenced by RNAi and shown that in vivo studies can be used to examine essential gene function during infection.

Silencing of Cathepsin B1 in schistosomes using vector-based RNAi

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Proteolytic enzymes are attracting increasing interest not only because they are involved in the catabolism of proteins by mammalian cells, but also because of their role in tumour invasion, metastasis and apoptosis. In helminth parasite these enymes have been implicated to facilitate host invasion, moulting and ecdysis, feeding, and evasion of the host immune response. These key functions render them potential targets at which to direct novel anti-parasite chemotherapy and immunotherapy. Schistosomes feed on ingested host blood and use the digested haemoglobin as a major source of amino acids. The haemoglobin digestion pathway is essential for parasite development, growth and reproduction. The potential of intestinal peptidases as targets for chemotherapy of schistosomiasis has driven the molecular study of these enzymes. The entire pathway for haemoglobin degradation consists of at least 8 proteolytic enzymes which break down haemoglobin. Numerous questions remain regarding the exact mechanisms driving the digestive process *in vivo*; the structure of the signalling cascade; the contribution of the various components and degree of redundancy.

We have recently described the use of pseudotyped Moloney Murine Leukaemia Virus to accomplish transformation of *S. mansoni*. Here, we report the design of a retroviral vector expressing a dsRNA hairpin loop, thus coupling a powerful delivery vehicle with a potent RNA interference (RNAi) mechanism to specifically silence expression of *SmCB1*. We observed striking suppression in transcript levels, accompanied by a complete ablation of the cognate enzyme activity in transduced worms which resulted in a lethal phenotype. To our knowledge, this is the first report of this technology – retroviral vector-based RNAi - being successfully employed in any helminth parasite. These findings underscore the potential and power of vector-based RNAi to evaluate potential drug targets and biochemical pathways for novel interventions in schistosomes.

Glycomics-driven discoveries in schistosomiasis

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Schistosomes, parasitic flatworms with a complex life cycle, infect about 200 million people in (sub-)tropical areas worldwide and cause an enormous burden of disease. Glycans and glycoconjugates expressed by schistosomes play a prominent role in the parasite's biology, in particular in the interaction with the human host. A large amount of structural data regarding glycosylation of different schistosome life stages and glycoconjugate subsets has been collected in the past decade, but many significant gaps in our knowledge of the schistosomal glycome remain. While previously schistosome glycoconjugates have been identified as diagnostic targets, we currently focus our research on glycans associated with immunity. On the one hand, we study the structure-function relation of glycans of individual immunomodulatory schistosome egg glycoproteins, while on the other hand we perform global glycomics studies of different developmental stages of the schistosome with the aim of identifying novel glycosylated intervention targets. Detailed mass spectrometry-based glycosylation analysis at the level of individual glycoproteins revealed that some major *S. mansoni* egg glycoproteins such as omega-1 and IPSE/alpha-1 carry similar N-glycans with typical α3-fucosylated core modifications and Galβ1-4(Fucα1-3)GlcNAc (LeX) termini. In contrast, other egg glycoprotein antigens such as kappa-5 contain a completely different subset of glycans with multiple GalNAcβ1-4GlcNAc (LDN) antennae on a fucosylated and xylosylated core. These differential glycan modifications determine each antigen's binding characteristics to lectins of the immune system such as DC-SIGN, MR, MGL and galectins, and the implications of this will be discussed. At a more global level, we have recorded glycoprotein-derived glycan profiles of developmental stages of *S. mansoni*, including cercariae, early/late schistosomula, juvenile/adult worms, eggs and miracidia. These profiles indicated that the expression of many glycans and glycan elements gradually shifts between the different stages, but also some sharp developmental switches were found such as for the expression of xylosylated glycans which is completely absent in the worm stages. A large portion of the antibody response to schistosomes is directed against these different glycan elements. At present it is unclear whether such antibodies may confer any protective mechanisms or not. We have printed hundreds of glycans and glycan fractions purified during the structural investigations on a glycan micro array. Preliminary studies in which we have screened the glycan array with sera from schistosome-infected individuals to determine the serum antibody response profiles in relation to variables such as infection intensity, duration, and treatment will be presented. The generated data and the possibilities for the development of glycan-based intervention methods for schistosomiasis will be discussed.

DNA methylation regulates egg production in the pathogenic blood fluke *Schistosoma mansoni*.

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The blood fluke *Schistosoma mansoni* is a biomedically important human parasite responsible for schistosomiasis, a debilitating and chronic neglected tropical disease of the developing world. Understanding the molecular mechanisms underpinning the complex developmental progression from free-living to parasitic life stages as well as elucidating how schistosomes produce pathogenic eggs will uncover urgently needed anthelmintic targets. Towards this end, we show that the recently characterised genome of this parasite is epigenetically modified, with cytosine methylation detectable in multiple schistosome life stages occupying diverse ecological niches. Moreover, we identify the co-regulation of both a DNA methyltransferase (SmDCMT2) and a methyl binding domain protein (SmMBD) during lifecycle progression, which further implicates the DNA methylation machinery in schistosome developmental pathways. The developmental significance of a methylated genome was investigated by treating sexually-mature adult male and female schistosome pairs with 5-azacytidine (5-Aza), a potent inhibitor of DNA methyltransferase activity. Here, we show that 5-Aza, while not demonstratively lethal, reproducibly inhibits cytosine methylation and significantly reduces the production of phenotypically normal eggs. Collectively these observations demonstrate that DNA methylation is linked to platyhelminth development, contributes to disease transmission and can be effectively targeted as a novel strategy for schistosomiasis control.

Asymmetric *Wolbachia* segregation during early *Brugia malayi* embryogenesis

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Filarial nematodes are the causative agents of human filariasis, affecting over 150 million individuals. The most pathogenic diseases, lymphatic filariasis and onchocerciasis, (river blindness) comprise a major cause of global morbidity in the tropics, with over 1 billion people at risk of these arthropod-transmitted infections. Three filarial nematode species are responsible for lymphatic filariasis: *Wuchereria bancrofti, Brugia malayi* and *Brugia timori,* causing lymphatic pathologies that include hydrocoele and lymphoedema (elephantiasis). These parasitic nematodes rely on alpha-proteobacterial *Wolbachia* endosymbionts for development, viability and fertility. Antibiotic treatments deplete *Wolbachia*, resulting in embryonic arrest and a decrease in microfilarial (larval) production. Human trials with doxycycline or rifampicin provide evidence for long-term sterilization and macrofilaricidal (adulticidal) effects against both lymphatic filariasis and onchocerciasis. *Wolbachia* also play a significant role in the pathogenesis of filarial disease. One feature of the symbiotic relationship left unresolved is the localization and segregation patterns of *Wolbachia* during embryogenesis, which is essential to understand its specific localization in adult somatic tissue and the germline. To address this issue, we developed fixation, immunofluorescent staining and imaging protocols to characterize *Wolbachia* in whole-mount *B. malayi* embryos and adult specimens at the tissue, cellular and sub-cellular levels. We will present the asymmetric and lineage-specific segregation patterns of *Wolbachia* during the initial stages of embryogenesis that resemble that of some *Caenorhabditis elegans* polarity and lineage-specific determinants, and suggest that *Wolbachia* may interact with the counterparts of these determinants in *B. malayi.*

Conserved and divergent elements of the *Caenorhabditis elegans* dauer signaling pathways in *Parastrongyloides trichosuri*

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Dauer formation in *Caenorhabditis elegans* and other free-living nematodes is initiated in response to the accumulation of a cocktail of fatty acid-derived metabolites in the environment (termed “dauer pheromone") in conjunction with other environmental signals, most notably temperature and the availability of food. Under a given set of food and temperature conditions, exceeding a threshold concentration of “pheromone” initiates a signal that is transduced via highly conserved cyclic nucleotide, insulin/IGF and TGF-β pathways. These pathways converge at DAF-12, a nuclear hormone receptor activated by dafachronic acid. It has long been hypothesised that dauer development was an important factor of the evolution of parasitism, and that the infective stage of at least some parasisitic nematodes is derived directly from the dauer of free-living nematodes. Parasites of the genera *Strongyloides* and *Parastrongyloides* have complex life history strategies in which it is proposed that the switch between free-living and parasitic development has evolved directly from the dauer switch and signal transduction pathways in *C. elegans*. We have shown that in *P.trichosuri*, there is conservation of a “pheromone” signal, and that the setting of the threshold at which free-living to parasitic life history switching occurs has a strong genetic component. Furthermore, we have cloned the major components of the insulin/IGF pathway, and have tested their function in transgenic *C. elegans*, to show that some functions of this pathway appear to be well conserved and others are not. Similarly, we show that TGF-β signaling is divergent between *P. trichosuri* and *C. elegans* but that dafachronic acid signaling is likely conserved. Thus, the free-living/parasite switch in *P. trichosuri* is likely to be evolutionarily derived from the dauer switch in free-living nematodes in a broad EvoDevo sense, but the details of the function of the signal transduction pathways clearly differ.

Role of *Ancylostoma caninum* transcription factor DAF-16/ FoxO uring the rescue of developmental arrested larvae

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Almost 800 million people are infected with the hookworms *Ancylostoma duodenale* and *Necator americanus,* causing disabling and persistent disease particularly in poor tropical regions. The infectious hookworm larvae (iL3) are soil-living, non-feeding and developmental arrested. They resume development to the reproductive stage in response to host-specific cues encountered during invasion. The underlying molecular mechanisms of the re-initiation of the developing process are mostly unknown. Similarities between the developmentally arrested stages dauer stage from the non-parasitic *Caenorhabtidis elegans* and the hookworm L3 suggest that similar mechanisms might be involved. Exit from dauer arrest in *C. elegans* is dependant on the inactivation of the FoxO class forkhead transcription factor DAF-16. In the absence of insulin like signaling (ILS), DAF-16 enables the expression of dauer stage-associated genes, including genes involved in the heat shock response and metabolism. ILS starts a cascade of phosphorylation events that culminate in the phosphorylation of DAF-16 on conserved threonine or serine residues by AKT/ protein kinase B. Phosphorylated DAF-16 is escorted from the nucleus, resulting in expression of genes involved in reproductive development, and therefore recovery from dauer arrest. Our evidence suggests a similar role for DAF-16 in hookworms. The mechanism of phosphorylation and nuclear exclusion was investigated using heterologous expression systems, cellular fractionation, and imaging. Further, complementation assays of *C. elegans* dauer defective mutants with wild type and AKT phosphorylation site mutants of hookworm DAF-16 indicate functional orthology in the control of dauer recovery. The data suggest that the hookworm DAF-16 is a pivotal player in the hookworm infectious process and the transition to parasitism during infection.

VHA-19, a nematode-specific protein, is critical for reproduction in *Caenorhabditis elegans* oocytes

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VHA-19 is a *Caenorhabditis elegans* nematode-specific protein of unknown function that has predicted protein homologues in nematode parasites of sheep, humans and plants. Thus, the function of VHA-19 in *C. elegans* is of interest as it may be similar in nematode parasites. Previously we have used RNA interference to show that VHA-19 is essential for *C. elegans* larval development and reproduction. Here, we show that VHA-19 is critical for reproduction in the *C. elegans* oocytes specifically and that VHA-19 is involved in secretion of receptors to the oocyte plasma membrane and probably secretion in the oocyte and early embryo more generally. While VHA-19 does not appear to be required for fertilisation in *C. elegans*, it does appear to have a role in cytokinesis in the early to late embryo, which is consistent with its predicted role in secretion. Interestingly, VHA-19 has also been predicted by bioinformatics analysis to be associated with the vacuolar ATPase complex (v-ATPase), a complex that contributes to pH gradients in the cell and is involved in many crucial processes. The implications of these findings will be discussed.

Functional studies of *Ss-daf-2*: an insulin signaling pathway intermediate in *Strongyloides stercoralis*

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Arrested larval development is a feature of many parasitic nematode life cycles. The infective larval stages of these parasites, the third-stage (L3) in most cases, exist in a state of developmental arrest prior to invading the host. Longevity, sometimes involving life spans measured in years or decades, is also a characteristic of many parasitic nematodes, particularly of their arrested larval stages. A molecular signal transduction pathway, resembling a vertebrate insulin/insulin-like growth factor (IGF) signal pathway, regulates both third-stage larval arrest (dauer development) and lifespan in the free-living nematode *Caenorhabditis elegans.* We hypothesize that *Strongyloides stercoralis* have conserved this signal transduction pathway and use it to regulate infective larval development and lifespan. Such regulatory elements could constitute critically needed leads in the search for new drug and vaccine targets.

We recently identified an insulin-like receptor kinase in *S. stercoralis*, dubbed *Ss-daf-2,* using a PCR homology strategy. Phylogenetic analysis indicates that *Ss-daf-2* is the homolog of *daf-2* in *C. elegans*. Preliminary studies of *Ss-daf-2* expression via semi-quantitative RT-PCR revealed the message in all life stages of *S. stercoralis* assayed so far, with lower levels of expression occurring in both postparasitic and postfree-living L1. Having obtained the full genomic and cDNA sequences of *Ss-daf-2*, we have begun functional studies of this gene. Methods that would allow direct functional study of *S. stercoralis* genes via transgenesis and targeted gene silencing are still under development. As an alternate approach, we studied the functional capabilities of this insulin pathway intermediate from *S. stercoralis* by assessing its ability to complement dauer-regulation in various loss-of-function mutations in *C. elegans* (*Ce*) *daf-2*. The most straightforward example of such an experiment was to ask whether the product of *Ss-daf-2* is capable of complementing dauer regulatory function of the temperature sensitive allele e1370 of *Ce-daf-2.* We reason that if the *S. stercoralis* gene product serves to restore any degree of function in *daf-2* mutants in *C. elegans*, then we can infer capability to perform a similar function in *S. stercoralis*. In addition to the studies involving heterologous genetic complementation, we are using a direct approach, involving characterization of phenotypes resulting from expression of mutant forms of *Ss-daf-2* in *S. stercoralis* itself. We have generated constructs fusing the *Ss-daf-2* promoter, and the *Ss-daf-2* and *gfp* coding sequences to ascertain anatomical expression patterns. The phenotypic effects of mutations in the kinase domain of the molecule are being assessed in variations on the heterologous complementation-rescue experiment and in transgenic *S. stercoralis*. For example, phospho-mimetic mutations at phosphorylation sites within the kinase domain of insulin receptor kinases bring about dominant gain of function in other systems. Under our hypothesis of dauer-like regulation of L3 development by *Ss-daf-2*, these mutant constructs are expected to bring about dauer deficient phenotypes when expressed in *C. elegans* and to suppress formation of infective L3 in transgenic *S. stercoralis*. Supported by NIH grants AI-050688, AI-022662 and RR-02512.

Molecular characterization of the TNF-alpha receptor in *Schistosoma mansoni* adult worms

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Schistosoma mansoni is the major causative agent of schistosomiasis. During the infection process, the parasite takes advantage of many host signals (such as hormones and cytokines) to complete its development in the human body. Tumor necrosis factor-alpha (TNF-alpha) is a human cytokine involved in skin inflammatory responses, and although its effect on the adult parasite's metabolism and egg-laying process has been previously described, a comprehensive assessment of the TNF-alpha pathway and its downstream molecular effects was lacking.

Recently our research group identified a *S. mansoni* homolog gene to TNF-alpha receptor (SmTNFR). This gene encodes a complete receptor sequence composed of 599 amino acids containing four cysteine-rich domains as described for other TNFR members. Real-time RT-PCR experiments revealed that SmTNFR highest expression level is in cercariae, 3.5 times higher than in adult worms. Downstream members of the known human TNF-alpha pathway were identified by *in silico* analysis, revealing a possible TNF-alpha signaling pathway in the parasite. To investigate putative molecular consequences of cytokine treatment on the parasite, two developmental stages (3h-old-schistosomula and adult worms) were treated with human TNF-alpha and changes in the gene expression profiles determined by microarray experiments (Oliveira K. C. *et al*, 2009, PlosNTD 3 (12): e556). In addition to previously described effects, these data suggest that human TNF-alpha can influence parasite development opening a new perspective to understand the molecular cross talk between host and parasite.

Recent experiments aimed at the molecular characterization of SmTNFR in adult worms. In order to identify interaction partners of this receptor we performed a screening with a Yeast Two Hybrid (YTH) library and discovered different binding partners. Additionally, direct YTH interaction studies were done between SmTNFR and SmTRAF (TNF Receptor Associated Factor), a schistosome homolog of a known member of the TNF signaling pathway). Finally *in situ* hybridization experiments were performed to localize SmTNFR and SmTRAF messages in adult worms. First results will be presented.

Infective third-stage larvae of *Strongyloides stercoralis* detect the host chemoattractant urocanic acid as a volatile odorant.

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Soil dwelling parasitic nematodes such as hookworms and threadworms that infect the host by active skin penetration must first perceive the host and orient towards it in order to establish contact. Among the host cues that could mediate this behavior are volatile and aqueous chemical attractants. In this vein, urocanic acid was recently identified as the primary aqueous soluble attractant in canine skin for infective larvae of the threadworm *Strongyloides stercoralis*. As an aqueous soluble molecule, urocanic acid has thus far been presumed to act as a contact attractant. The present study was designed to ascertain whether urocanic acid could act as a volatile attractant.

We adapted a bioassay for attractancy to volatile chemicals in *Caenorhabditis elegans* to determine whether infective larvae (L3i) of *S. stercoralis* would orient toward volatile urocanic acid. *S. stercoralis* L3i migrated toward sources of volatile urocanic acid at rates virtually identical to larvae migrating along gradients of the aqueous compound. The magnitude of the volatile response was dose-dependent with an ED_{50} of 45 mM, approximating the concentration of urocanic acid in mammalian skin. L3i in open plate cultures could orient towards a source of volatile urocanic acid at a distance of 25.5 cm, the longest range tested. Larvae pre-exposed to 200mM urocanic acid for 60 minutes exhibited significantly (two-fold) lower chemotactic indices in the presence of the volatile compound than non-pre-exposed controls. Larvae desensitized by pre-exposure to a high concentration of urocanic acid regained their sensitivity in time-dependent fashion when allowed to recover for periods of up to 60 minutes in the absence of the attractant. Such reversible adaptation or habituation to volatile odorants is a common feature of olfactory responses in a wide range of metazoan organisms. Dauer larvae of *C. elegans* also exhibited marked chemotaxis towards sources of volatile urocanic acid.

We conclude that L3i of *S. stercoralis* perceive urocanic acid as a volatile odorant and could use it as an attractant to mediate host finding over ranges of at least 25 cm. Physiological concentrations of urocanic acid suffice to elicit these behaviors. Observed adaptation to volatile urocanic acid by *S. stercoralis* L3i supports our characterization of this phenomenon as a true olfactory response. That dauer larvae of *C. elegans* exhibit the same behavior suggests that this aspect of host finding in a skin-penetrating parasitic nematode could be derived from olfactory mechanisms by which ancestral free-living nematodes located colonies of bacteria for feeding. Further work is now underway to identify the chemosensory neurons and the associated odorant receptors that mediate the response to volatile urocanic acid in *S. stercoralis* L3i. Supported by NIH AI-022662 and RR-02512.

Activation of *Nippostrongylus brasiliensis* infective larvae is regulated by a pathway distinct from the hookworm *Ancylostoma caninum*

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Developmentally arrested infective larvae of strongylid nematodes are activated to resume growth by host-derived cues encountered during invasion of the mammalian host. Exposure of *Nippostrongylus brasiliensis* infective larvae to elevated temperature (37°C) is sufficient to activate signalling pathways which result in resumption of feeding and protein secretion. This occurs independently of exposure to serum or glutathione, in contrast to the hookworm *Ancylostoma caninum*, and is not initiated by chemical exsheathment. Neither a membrane permeant analogue of cyclic GMP nor muscarinic acetylcholine receptor agonists stimulated feeding at 20°C, and high concentrations of both compounds inhibited temperature-induced activation. LY294002, an inhibitor of phosphatidylinositol 3-kinase, Akt inhibitor IV, an inhibitor of Akt protein kinase, and ketoconazole, an inhibitor of cytochrome P450, all blocked resumption of feeding and protein secretion at 37°C. Serotonin increased the rate of feeding assessed by uptake of radiolabelled bovine serum albumin, but could not initiate feeding independently of elevated temperature. Collectively, the data suggest that the early signalling events for larval activation in *N. brasiliensis* differ substantially from *A. caninum*, but that they may converge at pathways downstream of phosphatidylinositol 3-kinase involving steroid hormone synthesis.

Using *Caenorhabditis elegans* to study glutamate-gated chloride channels from *Haemonchus contortus.*

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It is very difficult to carry out genetic experiments or study gene function in parasitic nematodes, and so for many years there has been interest in using *C. elegans* as an expression system for parasite genes. However, it is clear that the different nematode species differ in their genetic complement, with several gene families proving to vary in size between species, and so we need to know how accurately the results obtained in *C. elegans* might reflect the real role of the parasite gene. We are interested in the ion channels that act as anthelmintic drug targets in parasites. These channels include the glutamate-gated chloride channels and nicotinic acetylcholine receptors; both are multi-subunit proteins encoded by gene families that vary in size between species. Glutamate-gated chloride channels (GluCls) are targets of the macrocyclic lactone anthelmintics. *C. elegans* (DA1316) with mutations in three of their GluCl subunit genes (*avr-14*; *avr-15*; *glc-1*) have high level resistance to ivermectin. Our aim is to assess whether ivermectin sensitivity in this strain can be restored by expressing GluCl subunit cDNA from *Haemonchus contortus*, a parasitic nematode from the same clade as *C. elegans*. We have used particle bombardment to create transgenic *C. elegans* lines, and have measured the rescue using "thrashing" (swimming) assays in ivermectin solutions. As a positive control, we showed that transformation of DA1316 with *Cel-avr-14B* under the control of its own promoter rescued drug sensitivity. DA1316 transformed with *Hco-avr-14B* cDNA behind the *Cel-avr-14* promoter showed a 93% reduction in the thrashing rate (5.5±2.7 thrashes/min) compared to the control triple mutant strain (76.2±6.1 thrashes/min) in $1x10^{-6}$ M ivermectin. Dose - response curves for the effects of ivermectin on the thrashing of these worms were very similar to those obtained with DA1370 (a *avr-15*; *glc-1* double mutant), indicating that the *H. contortus* cDNA completely rescued the *avr-14* component of the drug resistance phenotype of DA1316. DA1316 containing *Hco-avr-14A* cDNA did not show any rescue of ivermectin sensitivity at $1x10^{-6}$ M (70.0±7.5 thrashes/min) and the dose - response curve was almost unchanged. Our data using *C. elegans* support previous *in vitro* experiments indicating that the *Hco-avr-14B* subunit may be important for the anthelmintic effects of ivermectin, and that *Hco-avr-14A* does not form ivermectin-sensitive channels, but differ from our earlier data showing that both *H. contortus* gene products can rescue a behavioural defect in *avr-14* mutant *C. elegans*. We believe that *C. elegans* transgenics expressing anti-parasitic drug targets could be important in future studies of drug resistance mechanisms.

Apoptosis in a pathogenic nematode involves mitochondrial pathway.

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Genetic analysis in free living soil nematode *C. elegans* has been instrumental in elucidation of central cell death machinery in eukaryotes. Regulators of apoptosis such as Bcl-2, Apaf-1 and caspases are evolutionarily conserved from worms to mammals. However mitochondrial pathway of apoptosis involving participation of Cytochrome-c has been demonstrated only in flies and vertebrates and not in nematodes and this has raised issues on the evolutionary conservativeness of mitochondrial pathway of apoptosis. Here, by using confocal microscopy and flow cytometry, we provide first ever experimental evidence for reactive oxygen species (ROS) mediated mitochondrial outer membrane permeabilisation (MOMP), cytosolic release of Cytochrome-c and interaction of Cytochrome-c with CED-4 during apoptosis in a pathogenic filarial nematode *S.digitata.* Significant degree of apoptosis of developing embryo (collected from adult female worms of amicrofilaraemic cattle) could be demonstrated in comparison to embryo of worms collected from microfilaraemic animals. Treatment with lipid peroxidation products resulted in significant induction of apoptosis of developing embryo. These insights offer novel explanations for effector mechanisms of anti-fecundity host immunity operational in helminth infections. Potential to develop apoptosis inducing drugs to block embryogenesis in helminth parasites is another translational outcome of the findings of this study. Molecular docking studies revealed significant interaction between Cytochrome-c of *C.elegans* and the evolutionarily conserved α/β (P-loop)–ATP binding domain of CED-4 suggesting a role for Cytochrome-c during apoptosis in *C.elegans* also.

Host-derived FGF stimulates *Echinococcus multilocularis* development by activation of a biochemically unusual cestode FGF receptor

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Fibroblast growth factors (FGFs) and their cognate receptors (FGFRs) are conserved throughout the metazoan kingdom. FGFRs are receptor tyrosine kinases (RTKs) which activate cytoplasmatic signal transduction pathways such as the mitogen-activated protein kinase (MAPK) cascade, the phosphatidylinositol 3-kinase/Akt, and the phospholipase C-gamma pathway, thereby regulating a multitude of biological functions such as developmental processes, homeostasis and differentiation.
For the model cestode *E. multilocularis*, we herein describe the characterization of a member of the FGFR family, EmFR, which is the first receptor of this type to be identified in a parasitic helminth. The deduced amino acid sequence of EmFR is 41% identical to FGFR4 of *Rattus norvegicus* and to the FGFR1 of the free-living flatworm *Dugesia japonica*. Interestingly, the extracellular domain of EmFR exhibits only one single immunoglobulin-like domain, whereas all other known FGFRs from vertebrates and invertebrates display two or three immunoglobulin-like domains. Deep sequencing transcriptome analyses and RT-PCR confirm that EmFR is expressed in all *Echinococcus* larval stages that are involved in the infection of the intermediate host. Functional studies using the *Xenopus* oocyte expression system clearly indicate that EmFR can sense both acidic and basic FGF of human origin, resulting in an activation of the EmFR tyrosine kinase domain. *In vitro* experiments further demonstrate that mammalian FGF significantly stimulates proliferation and development of *E. multilocularis* metacestode vesicles and primary cells in culture. Furthermore, DNA synthesis and the parasite's Erk-like MAPK cascade module was stimulated in the presence of exogenously added mammalian FGF. By using the small molecule compound BIBF1120 (FGF receptor inhibitor), the activity of EmFR in the Xenopus oocyte system could be effectively blocked. Addition of BIBF1120 to in vitro cultivated *Echinococcus* larval material led to detrimental effects concerning the generation of metacestode vesicles from parasite stem cells, the proliferation and survival of metacestode vesicles, and the de-differentiation of protoscolices towards the metacestode. Taken together, these data demonstrate the presence of a functional FGFR-FGF signaling pathway in *E. multilocularis* that is able to functionally interact with host-derived cytokines and that plays an important role in larval parasite development.

Targeting flatworm signalling cascades for the development of novel anthelminthics.

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Diseases due to parasitic flatworms are prevalent world-wide and often very difficult to treat, particularly in the case of larval cestode infections. In search for new target molecules for anti-parasitic chemotherapy, we concentrate since recent years on factors of the mitogen-activated protein kinase (MAPK) cascades since these evolutionarily conserved enzymes are exceptionally well studied from the biochemical point of view, including the availability of a plethora of small molecule compounds to modify their activities. In the model cestode *Echinococcus multilocularis*, the causative agent of alveolar echinococcosis, we could characterize several members of the MAPK protein family, including Erk1/2-, Erk7-, p38- and JNK-subfamily members, as well as upstream acting MAPK kinases (MAPKKs) and MAPKK kinases. On the basis of transcriptome deep sequencing data and RT-PCR, all these factors are well expressed in the larval stages that are involved in the infection of the intermediate host. Yeast two hybrid and protein-protein interaction studies demonstrated that the parasite's Erk-like MAPK module, which controls stem cell differentiation in response to host-derived cytokines, is formed by the intracellular kinases EmRaf, EmMKK2 and EmMPK1. While inhibitors against the JNK-subfamily did not show detrimental effects on in vitro cultivated *Echinococcus* metacestode vesicles or primary cells, the parasite's Erk-like MAPK module could be effectively blocked in the presence of the MAPKKK- and MAPKK-inhibitors BAY 43-9006 and PD184352. However, although this treatment led to a marked dephosphorylation of EmMPK1 and inhibited parasite growth in vitro, no metacestode vesicle killing was obtained. Interestingly, totipotent parasite stem cells clonally expanded in the presence of these drugs, indicating that BAY 43-9006 and PD184352 modify parasite stem cell differentiation but have only limited activity on fully differentiated *Echinococcus* cells. The most promising novel drug target we identified is the parasite's p38-like MAPK EmMPK2 that displays several marked amino-acid exchanges in its kinase domain, when compared to human orthologs, and behaves like a constitutively overactive member of the MAPK family. The activity of EmMPK2 could be effectively blocked in the presence of the two pyridinyl imidazoles SB202190 and ML3403 in enzymatic assays and treatment of parasite larvae with these substances led to effective killing at concentrations that did not affect cultured mammalian cells. Pyridinyl imidazoles are thus a novel and potent anti-*Echinococcus* compound family and, due to the presence of EmMPK2-like MAPKs in trematodes, could also have a broad spectrum activity against flatworm parasites. Among a small molecule library of 900 related pyridinyl imidazoles, we are currently screening for compounds that are highly specific for the parasite's p38-like MAPK and have limited affinity for mammalian p38-orthologs. In addition to MAPK cascade signalling factors, we recently also characterized components of the parasite's PI3-kinase/Akt-pathway that acts downstream of *Echinococcus* insulin receptors and demonstrated in vitro killing of parasite stem cells in the presence of PI3-kinase inhibitors. Taken together, these results indicate that the intracellular signalling cascades that control flatworm stem cell differentiation and proliferation are well suited as targets for anti-parasitic chemotherapy.

Physiological roles and pharmacological sensitivities of *Schistosoma mansoni* multidrug resistance transporters.

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One potential physiological target for new antischistosomal drugs is the parasite's system for excretion of wastes and xenobiotics. Multidrug resistance (MDR) transporters are members of the ATP-binding cassette (ABC) superfamily of proteins. These transporters are ATP-dependent efflux pumps involved in removal of toxins and xenobiotics from cells, and are also critical in mammals for transport of signaling molecules such as immunomodulators. When overexpressed, they mediate multidrug resistance, a phenomenon in which cells that are resistant to one drug show cross-resistance to a broad range of other agents. MDR transporters are also associated with drug resistance in parasitic helminths, and targeting of them could perhaps provide a strategy for overcoming or attenuating drug resistance. There are several different MDR transporters, including P-glycoprotein (Pgp), multidrug resistance associated proteins (MRPs), and breast cancer resistance protein (BCRP), all of which are represented in the *S. mansoni* genome. SMDR2, the *S. mansoni* homolog of Pgp, is upregulated in worms following exposure to sub-lethal concentrations of praziquantel (PZQ), the current drug of choice against schistosomiasis. Furthermore, SMDR2 is expressed at significantly higher levels in an Egyptian worm isolate with reduced PZQ susceptibility, and both it and SmMRP1, a homolog of MRP1, are expressed at higher levels in PZQ-refractory juvenile worms. PZQ appears to be transported by SMDR2, and also potently inhibits SMDR2 transport of rhodamine, indicating that the drug may be disrupting normal excretory activity in the parasite. RNAi-mediated knockdown indicates that both SMDR2 and SmMRP1 function in *S. mansoni* egg production. We are currently further characterizing the physiological roles and pharmacological sensitivities of these transporters, both *ex vivo* and within the murine host. These studies may provide leads for new drug targets, as well as novel strategies for potentiating the action of PZQ and possibly overcoming PZQ resistance. This work is supported by NIH grant R01 AI073660.

The *S. mansoni* egg glycoprotein κ5 induces granulomas in a pulmonary mouse model via its GalNAcβ1-4GlcNAc-containing N-glycans

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Schistosomes are parasitic helminths that infect over 200 million people in (sub-) tropical areas around the world. The main pathology of schistosomiasis is initiated by a large number of parasite eggs that instead of being excreted, get trapped in various organs such as the liver. Here, egg antigens induce a granulomatous inflammatory response, which eventually leads to fibrosis and severe organ damage.

Previously, granuloma formation has been explored by injection of antigen-coated Sepharose beads as artificial eggs into the caecal vein of mice [1]. Beads that carry SEA (soluble egg antigens) of *S. mansoni* with intact glycans, but not with glycans destroyed by periodate treatment, gave rise to granulomas comparable to those around schistosome eggs in terms of cellular content and expression of adhesion and extracellular matrix components. Interestingly, synthetic model glycoconjugates with terminal Gal(NAc)β1-4GlcNAc (LDN) groups were also able to induce this type of granulomas. In contrast, glycoconjugates with terminal Lewis X, fucosylated LDN or GlcNAc, also abundant glycan elements in SEA, elicited only a monolayer of macrophages.

These data led to the hypothesis that *S. mansoni* egg glycoproteins that carry LDN glycans are capable of inducing granulomas. Using mass spectrometry-based glycosylation analysis, we demonstrate that the major GalNAcβ1-4GlcNAc (LDN)-containing glycoprotein in SEA is kappa-5. Kappa-5 was shown to contain either three or four N-glycosylation sites, occupied mainly with glycans with α1,3/α1,6-linked core fucoses, a β1,2-linked core-xylose and tri-antennary LDN branches. To investigate whether kappa-5 is able to induce granuloma formation via its LDN-branches, we used a pulmonary bead model in mice. In this model, SEA-coated beads gave rise to granulomas, comparable to granulomas elicited by SEA beads in the hepatic model. Beads coated with kappa-5 were able to induce granuloma formation, while specific removal of the LDN moieties by exo-hexosaminidase treatment greatly reduced the capacity of kappa-5 to induce granulomas.

Next, we investigated the recognition of kappa-5 by immune cells via carbohydrate-binding receptors. In an *in vitro* assay, SEA components have been shown to be internalized by dendritic cells via the C-type lectin receptors DC-SIGN, Mannose Receptor (MR) and MGL [2]. Using ELISA and a fluorescent bead adhesion assay, we were able to demonstrate that kappa-5 binds to DC-SIGN but not to MR. Surprisingly, no known DC-SIGN ligands, such as Lewis X or terminal mannoses, are present on kappa-5.

To conclude, our data suggest that kappa-5 plays a role in the induction of periovular granulomas in schistosomiasis and that this property is partly dependent on its terminal LDN structures. Furthermore, we show that kappa-5 is able to bind to DC-SIGN via an unknown glycan structure, possibly (indirectly) affecting its granulomogenetic properties.

Imatinib (Gleevec) causes dramatic effects on morphology, pairing stability and survival of adult *Schistosoma mansoni* in vitro

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The parasitic helminth *Schistosoma mansoni* is the causing agent of schistosomiasis, one of the most prevalent parasitic infections in the world. The only widely used drug to treat and control schistosomiasis is Praziquantel. However, there is evidence from laboratory experiments but also from field studies that point to the development of tolerance or resistance. Furthermore, no vaccine is available yet. Thus, great international efforts are made to search for next-generation anti-schistosomal agents based on new drug targets. Here we present that Imatinib (Gleevec; STI-571), a compound used in human cancer therapy to treat chronic myeloid leukemia or gastrointestinal stromal tumors, dramatically affected the morphology and physiology of adult *S. mansoni in vitro*.

During our experimental approaches to identify and characterize signal transduction molecules of *S. mansoni* with functions during gametogenesis we recently identified the cellular tyrosine kinase SmTK6. Sequence analyses showed its significant similarity to two kinase families, Src and Abl, thus phylogenetically SmTK6 represents an intermediate of both kinase families. Additionally, we identified two Abl-kinase homologs (SmAbl1, SmAbl2) in the *S. mansoni* genome data set, which were cloned. Using *in situ*-hybridization experiments, the transcription of the three kinases (SmTK6, SmAbl1, SmAbl2) was localized in the gonads (ovary, vitellarium, testes), the ootype-surrounding area, the parenchyma, and/or the gastrodermis. This relatively wide expression pattern indicated a variety of physiological functions of these three kinases. Therefore, we hypothesized diverse effects upon inhibiting these Abl kinases and treated adult schistosomes with the Abl kinase-specific inhibitor Imatinib *in vitro*. This 2-phenylaminopyrimidin derivative competitively binds the ATP docking-site within the catalytic domain of Abl kinases, thus preventing subsequent phosphorylation and signaling events. Following schistosome treatment we observed dramatic concentration- and time-dependent effects such as swellings along the whole body, a reduced pairing stability, and most importantly a significantly reduced viability of the worms. By confocal laser scanning microscopy we finally discovered severe morphological changes in the gonads, but also pathological alterations of the gastrodermis that probably caused the death of the parasites. Further functional studies are ongoing to investigate the cellular role of the Abl kinases and the effect of Imatinib in more detail. Beyond that animal experiments are planned to elucidate the potential of Imatinib to serve as an alternative medicine for schistosomiasis.

Voltage-activated ion-channels in Clade III nematodes as drug target sites

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Many existing anthelmintic drugs affect neuromuscular transmission. Pyrantel and ivermectin open ion-channels that are known as ligand gated ion-channels (LGICs). In contrast to our advancing knowledge of nematode LGICs and parasite genomes, little is known about the physiology and pharmacology of nematode parasite voltage-activated ion-channels. This lack of knowledge limits novel drug development and expansion of the use of existing drugs. Our objective is to address this knowledge gap and to provide a solid framework for using and developing nematode voltage-activated ion-channels as anthelmintic drug targets. We have examined the effects of the FMRFamide neuropeptides, AF2, AF3 and PF1 on voltage-activated K currents and voltage-activated Ca current in the Clade III nematode, *A. suum.* We found that: AF2 and AF3 increased the voltage-activated Ca current with little effect on the K currents; PF1 decreased the voltage-activated Ca current and increased a Ca-dependent voltage-activated K current. These observations are consistent with the excitatory effects of AF2 and AF3 and the inhibitory effects of PF1 on muscle contractility. The cyclooctadepsipeptide, emodepside, belongs to a new class of anthelmintic that has been suggested to release PF1 and to mimic the effects of PF1. We also investigated the effects of emodepside on voltage-activated currents in *A. suum* muscle in order to compare the effects of emodepside with PF1. We found that low concentrations of emodepside, 1 µM, increased a Ca-dependent voltage-activated K current, but had no affect the voltage-activated Ca currents. Thus, emodepside mimics some but not all of the effects of PF1. Our observations are consistent with the hypothesis that emodepside has a more direct effect on a Ca-dependent voltage-activated K channel (Slo-1) but not with emodepside producing release of PF1. We are very pleased to acknowledge Dr Achim Harder, Bayer AG, for his continued advice and enthusiasm. NIH AI 047194 supported this work.

Schistosoma mansoni: Kinase signalling in mitosis and differentiation processes.

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Mating and sexual maturation of adult schistosomes lead to the production of numerous fertilized eggs, which are released with the faeces and thus assure disease transmission. However, a large part of these eggs gets trapped in host tissues and is mainly responsible for the pathogenic consequences of schistosomiasis. Therefore, any strategy aiming at a reduction of levels of parasite reproduction and worm fecundity can constitute a valuable approach towards the control of both transmission and pathology of schistosomiasis. During the last few years, evidence has been given that conserved kinase signalling cascades can orchestrate schistosome sexual development, and that components of the TGFβ receptor pathway (LoVerde et al, 2007) and several cytoplasmatic tyrosine kinases (CTKs) of the Src and Syk families play crucial roles in differentiation of reproductive organs and gametogenesis (Beckmann et al, 2010). Polo-like kinases (Plks) are conserved regulators of the cell cycle progression during M-phase, primarily involved in the assembly and dynamics of the mitotic spindle apparatus and in the regulation of the activation of cyclin-dependent protein kinases. Due to their overexpression in a wide range of tumour cells they constitute important targets for anti-cancer therapy (Strebhardt and Ullrich, 2006). We recently identified two Plks in the genome of *S. mansoni*: SmPlk1 and SmSak, homologous respectively to Plk1 and Plk4 in humans. We showed that the anti-cancer drug BI2536 inhibits the kinase activity of SmPlk1 and caused in adult worms of *S. mansoni* profound alterations of the reproductive organs in males and females and reduction of gamete production (Long et al, 2010), suggesting that SmPlk1 can represent a novel therapeutic target against schistosomiasis. SmPlk1 and SmSak have conserved catalytic and regulatory domains and their activation is, as for other members of the Plk family, dependent on their phosphorylation on a specific threonine residue present in their catalytic loop. We have characterized in the parasite *S. mansoni* a Ser/Thr protein kinase, SmSLK (for Ste20-like kinase), with a structure similar to that of mammalian LOK and SLK and *Xenopus* xPlkk kinases (Yan et al, 2007), three members of the SLK family which have the capacity to phosphorylate Plks in vertebrate organisms. Therefore, we have considered the potential of SmSLK for the activation of SmPlks. By expressing schistosome proteins in *Xenopus* oocytes, we have demonstrated that the SmSLK kinase was able to activate SmPlk1 (but not SmSak), but its activity was dependent on its previous cleavage by caspases. According to this, SmSLK might be considered as a novel and unexpected regulator of gametogenesis and fecundity in schistosomes acting as an upstream kinase of SmPlk1.

Acetate formation in helminths and other parasites.

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Formation and excretion of acetate as a metabolic end product of energy metabolism occurs in many helminths and protozoan parasites, such as the parasitic helminths *Fasciola hepatica, Haemonchus contortus* and *Ascaris suum*, and the protozoan parasites, *Giardia lamblia*, *Entamoeba histolytica*, *Trichomonas vaginalis* as well as *Trypanosoma* and *Leishmania* species. In all these parasites acetate is a main end product of their energy metabolism. Acetate formation does not occur in their mammalian hosts, and therefore, acetate production could be a novel target for the development of new anti-parasitic drugs. In parasites, acetate is produced from acetyl-CoA by two different reactions that are catalyzed by either a cytosolic acetyl-CoA synthetase or an organellar acetate:succinate CoA-transferase (ASCT). In parasitic helminths acetate is only produced by the ASCT reaction, which yields succinyl-CoA for ATP formation via succinyl-CoA synthetase (SCS). Based upon our recent work on the ASCTs of *F. hepatica, T. vaginalis* and *Trypanosoma brucei*, we identified the existence of three subfamilies of enzymes within the CoA-transferase family. Enzymes of these three subfamilies catalyze the ASCT reaction in eukaryotes via the same mechanism, but the subfamilies share little sequence homology and differ in kinetic properties. However, the CoA-transferases of the three subfamilies are all present inside ATP-producing organelles of parasites, those of subfamily IA in the mitochondria of trypanosomatids, subfamily IB in the mitochondria of parasitic worms and subfamily IC in hydrogenosome-bearing protozoan parasites. Differences and similarities of the distinct ASCT subfamilies in parasites will be discussed.

Nematode P-glycoproteins as mediators of unspecific resistance against macrocyclic lactones and other anthelmintics

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Frequent and uncontrolled use of anthelmintics has led to multi-resistant nematode strains in particular in small ruminants. However, resistance against anthelmintics is also spreading in parasites of cattle and horses. In addition to direct alterations of anthelmintic target proteins (e.g. SNPs in β-tubulin mediating resistance to benzimidazoles), unspecific resistance mechanisms which increase tolerance towards several drugs simultaneously have to be considered as potent mechanisms that can compromise efficient parasite control. Increased extrusion of drugs by ABC transporters such as P-glycoproteins (Pgps) is an obvious mechanism that might contribute to the development of multi-drug-resistance. Detailed knowledge about the Pgp subfamilies encoded in the genomes of different parasitic nematodes, their substrate specificity and their natural degree of sequence variability is crucial to develop models for risk prediction regarding the spread of drug and multi-drug resistant parasites in different hosts. This is particularly important as frequent prophylactic treatment against one nematode (e.g. *Parascaris*) might rapidly select for drug resistance of co-infecting parasites (e.g. Cyathostominae).

In vitro assays clearly show that Pgp-inhibitors are not only able to increase susceptibility of nematodes towards ivermectin (IVM) but to completely abolish resistance. Furthermore, antibody-based detection reveals higher expression of Pgps in IVM-resistant compared to susceptible populations of *Cooperia oncophora* and *Ostertagia ostertagi*. Noteworthy, the Pgp repertoire of nematodes is far more complex than that of vertebrates. There are e.g. 14 functional Pgps and 9 closely related half-transporters encoded in the genome of *C. elegans*. Though our knowledge about Pgps of parasitic nematodes is currently still fragmentary, there is plenty of evidence that parasites also express a battery of Pgps which might variably contribute to resistance against anthelmintics. Our current cloning and sequencing projects have identified a substantial body of full-length and partial Pgp cDNA sequences from *C. oncophora*, *Parascaris equorum*, and *Cylicocyclus elongatus*. Phylogenetic analysis of these sequences together with data available from *Caenorhabditis*, *Haemonchus*, *Brugia*, *Onchocerca*, and *Pristionchus* reveals that the Pgp subfamilies present in *C. elegans* are conserved. Orthologues for most *C. elegans* Pgps can also be found in parasitic nematodes and there are even Pgp-subfamilies in parasitic nematodes which are absent in *C. elegans*. Moreover, a high number of SNP can be found in different parasite populations and their frequency in susceptible and resistant populations differs significantly. However, since resistant isolates matched with parental susceptible isolates are largely missing, functional analyses are required to define the potential of certain Pgps to contribute to certain resistance phenotypes. We have therefore developed sensitive and reproducible *in vitro* assays to compare dose-response-curves between wild-type and Pgp-deficient *C. elegans* strains. In combination with functional rescue, these methods will be used to compare the ability of individual Pgps from parasitic nematodes to confer resistance to specific classes of anthelmintics and are also suitable to quantify drug selection advantage of different Pgp alleles found in parasite populations.

A nematode venom allergen/ASP-like protein interacts and inhibits an extracellular cathepsin-like cysteine protease required for fungal resistance

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Several animal and plant parasitic nematodes have the capability to remain within the host for a long time. To do so, immunoevasive and immunosuppressive strategies have evolved in parasitic nematodes requiring a range of nematode-secreted proteins. Secretory proteins produced in the esophageal gland cells of plant-parasitic nematodes are believed to include suppressors of plant innate immunity. A venom allergen/ancylostoma secreted-like protein from *Globodera rostochiensis* (designated Gr-VAP1) was identified, by cDNA-amplified fragment length polymorphism (cDNA-AFLP), as being strongly up-regulated in invasive J2s. In situ hybridization microscopy showed specific expression of Gr-VAP1 in the subventral esophageal glands. Gr-VAP1 codes for an open reading frame of 219 amino acids including signal peptide for secretion and a single SCP domain. Temporal expression analysis of Gr-VAP1 in different developmental stages revealed up-regulation in the motile J2s and adult males. Knocking-down Gr-VAP1 expression with RNA interference, significantly reduced the infectivity of the nematodes on host plants. Protein interaction studies using Gr-VAP1 and a tomato root cDNA library, in a yeast-two-hybrid screening, resulted in the identification of various interacting host proteins associated with plant immunity. A pull-down assay confirmed the physical interaction of Gr-vap1 with an extracellular cathepsin-like protease from the host named Rcr3. Enzyme inhibition experiments by protease profiling showed that Gr-vap1 inhibits Rcr3. Others have shown before that Rcr3 conditions disease resistance to fungi in plants. Our results suggest that plant-parasitic nematode venom allergens are required for parasitism and are important modulators of innate immunity by regulating host protease activities.

Modulation of leukocyte function by nematode secreted nucleotide metabolising enzymes

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Nucleotides are released into the extracellular space following cell/tissue damage, mechanical stress, hypoxia or inflammation and regulate a variety of cell responses. They exert their effects by ligating to and activating a number of G-protein-coupled receptors, collectively referred to as purinergic receptors. We have identified a number of nucleotide metabolising enzymes secreted by *Trichinella spiralis* and have shown that they are responsible for altering intracellular signalling pathways resulting from purinergic receptor activation and thus modulating cell function. We have shown that these enzymes can inhibit nucleotide–induced dendritic cell chemotaxis, platelet aggregation, and mucosal mast cell effector functions and have attributed these effects to the action of the secreted 5´-nucleotidase (5´NT). Here we report on the effects of purinergic receptor activation on bone marrow derived macrophages and modulation of these effects by *Trichinella spiralis* secreted proteins. Extracellular nucleotides inhibit NO production and increase arginase activity in macrophages and both these effects are further amplified by the secreted 5´NT due to the generation of adenosine and P1 purinergic receptor activation. However, nucleotides have no effect on cytokine release in the absence of LPS stimulation. Following TLR stimulation, which leads to an increase in IL-6 and TNFα release, P1 receptor activation via adenosine leads to a decrease in LPS-induced IL-6 and TNFα production but a sustained increase in IL-10 release. We also observed that the total secreted glycoproteins from *Trichinella spiralis* act directly on macrophages to modulate effector functions. We showed this to be due to the glycans and identified the lectin with which these glycans interact on macrophages. Adenosine generation antagonises these effects possibly due to cAMP elevation triggered by purinergic receptor activation.

Characterization of phytochelatin synthase of *Schistosoma mansoni*, a potential target for schistosomiasis drug development.

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Currently only one drug, praziquantel, is used for schistosomiasis treatment. However, reliance on a single drug is problematic; there is evidence that drug-resistant isolates of *S. mansoni* and *S. haematobium* have evolved and laboratory selection for praziquantel-resistant worms has been demonstrated. Therefore, there is an urgent need to identify new targets and drugs for schistosomiasis treatment. Ideal drug targets are essential for pathogen survival, unique to the pathogen and drugable. We hypothesized that one such potential target in schistosomes is phytochelatin synthase (PCS). Phytochelatins (PCs) are peptides known to chelate heavy metals in plants, fungi, nematodes and algae. PCs are synthesized enzymatically from the tripeptide glutathione (gamma-Glu-Cys-Gly) by PCS and have the repeating structure of (gamma-Glu-Cys)$_N$-Gly, where N = 2 to 11 repeats. PCS proteins belong to the papain superfamily of cysteine proteases and display conservation of 3D geometry of the catalytic Cys-His-Asp triad. A PCS gene is present in the *S. mansoni* genome. In contrast, there are no PCS genes encoded in the human genome; instead humans use Cys-rich metallothionine proteins to chelate heavy metals. A PCS sequence with ~75% identity is encoded in *S. japonicum* genome. We found that three *S. mansoni* PCS transcripts are produced by alternative splicing from the single PCS gene, potentially encoding three different proteins. The *S. mansoni* PCS predicted proteins are ~50% identical (in the catalytic domain) with PCS proteins from other organisms. Two PCS proteins are thought to be cytoplasmic and one to be mitochondrial. Two predicted PCS proteins contain a complete PCS catalytic domain (approximately amino acids 80-300) are proteins of a total of 591 amino acids (mitochondrial) and 512 amino acids (cytoplasmic). Both of these proteins have long C-terminal extensions (~300 amino acids) with no similarity to PCS proteins from other organisms. Expression of either PCS protein in *Saccharomyces cerevisiae* greatly increased tolerance to Cd^{2+} ion toxicity (50 µM in vector only control to 1,500 µM in yeast expressing PCS). PCS-induced Cd^{2+} ion tolerance was lost upon culture of the yeast with the glutathione biosynthesis inhibitor L-buthionine sulfoximine. Formation of phytochelatins by recombinant PCS in *S. cerevisiae* was analyzed by HPLC-mass spectrometry and PCs were identified containing 2-5 repeat units. *S. mansoni* PCS was found by reverse transcriptase PCR to be expressed in all mammalian stages of worm develop. Together, these findings suggest that SmPCS is a potential candidate for drug development for schistosomiasis. Experiments using reverse genetics (RNA interference) to validate PCS as an essential gene are under way. We are also developing assays suitable for high throughput screening of compound libraries for PCS inhibitors.
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Translocation of the interleukin-4 Inducing principle from *Schistosoma mansoni* eggs (IPSE/alpha1) to mammalian cell nuclei

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Interleukin-4 inducing principle from schistosome eggs (IPSE/alpha-1) is a protein produced exclusively by the egg stage of the trematode *Schistosoma mansoni*. IPSE/alpha-1 is a secretory glycoprotein which activates human basophils in an IgE-dependent, but non antigen-specific mechanism. Sequence analyses revealed a potential nuclear localization signal (NLS) at the C-terminus of IPSE/alpha-1. This sequence ('125-PKRRRTY-131') is both necessary and sufficient for nuclear localization of IPSE or IPSE-EGFP fusion proteins. While transiently expressed EGFP-IPSE/alpha-1 is exclusively nuclear in Huh7 and U-2 OS cell lines, a mutant lacking amino acids 125-131 shows both nuclear and cytoplasmic staining. Moreover, insertion of the IPSE/alpha-1 NLS into a tetra-EGFP construct renders it nuclear, and alanine scanning mutagenesis reveals a requirement for the KRRR residues. Fluorescence microscopy depicts, and Western blotting further confirms, that recombinant unglycosylated IPSE/alpha-1 protein added exogenously is rapidly internalized by CHO cells, and accumulates in their nuclei in a NLS-dependent manner. In contrast, a mutant protein in which the NLS motif was disrupted by triple mutation of the RRR residues to AAA (IPSE/alpha-1 3R mutant) is able to penetrate CHO cells but does not translocate to the nucleus. Furthermore, the uptake of glycosylated IPSE/alpha-1 is confirmed in human primary cells, using monocyte-derived dendritic cells, and found to be a carbohydrate-, calcium- and temperature-dependent process. Live cell imaging shows that IPSE/alpha-1 is not targeted to lysosomes and can be seen in the cytosol. In contrast, basophils do not require the presence of an intact NLS for activation, and we are currently investigating whether IPSE/alpha-1 is taken up by this primary cell type. Taken together, our results suggest that this parasite protein has additional nuclear functions in host cells.

Transcriptomic analysis of early events in ovine abomasal mucosa in response to infection with the nematode *Teladorsagia circumcincta*.

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Parasitic gastroenteritis (PGE), caused principally by the abomasal nematode *Teladorsagia circumcincta*, is the most commonly diagnosed systemic disease of sheep in the U.K. The principal means of control, which depends on the use of anthelmintics, is failing due to the rapid emergence of drug resistance in the target nematodes. Immunity develops slowly on repeated exposure to the parasite, but there is currently no vaccine to the disease. The development of a vaccine is hampered by a lack of knowledge of the host-parasite interaction to incoming larvae. Here we seek to define the earliest molecular events, in both host and parasite, occurring within the first hours of incoming larvae contacting the epithelial surface of the abomasum. The aim is to improve our understanding of the early host/parasite interaction in both immune and naïve animals, as well as in animals where immunity is waning following cessation of exposure to the parasite.

To address the host response, we exploited microarray, qRT-PCR and proteomic techniques to analyse gene expression in abomasal mucosae from naïve, immune and "immune-waning" animals, both in their unchallenged state and 24 hours after oral challenge with *T.circumcincta* larvae. There were a number of significantly differentially expressed transcripts/ proteins between sheep in the three different immune states. The transcripts which are most upregulated in immune animals relative to naïve animals were granulysin, which is normally associated with cytotoxicity, 5-lipoxygenase activating protein (FLAP), a component of the eicosanoid pathway, a number of mast-cell associated transcripts, and galectin 15, (OvGal11), which may alter mucus composition. Interestingly serum albumin was the most significantly down-regulated transcript in immune animals compared to both the naïve and "immune-waning" animals. Proteomic analysis of abomasal extracts confirmed loss of serum albumin protein and appearance of albumin degradation products in immune and immune waning animals. Notably galectin-15 protein was strikingly up-regulated only in the immune animals, similar to the transcritomic findings. Additionally, the loss of a number of chaperone / heat shock proteins was also detected in these sheep. The lack of significant alterations in the levels of mRNA encoding chaperone/ HSPs as assayed in the microarray analysis suggests either degradation (apoptotic or artifactual) or active extracellular transport (e.g. in exosomes). These results will be compared with transcriptomic changes detected in the abomasum 48 hours post-challenge, and with data from a 454 sequence comparison of larvae exposed to naïve and immune host abomasal products. It is intended to use bioinformatics tools to identify key genes and seek correlates between host/parasite gene expression, enabling a better understanding of the early epithelial responses which potentially contribute to rejection and/or establishment of incoming larvae. (Funding; BBSRC (BB/E01867X/1).

IL-4Rα dependent cellular responses and mechanisms underlying adaptive immunity to *N. brasiliensis*

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Disease resolution against primary helminth infections depends on T_H2 polarisation via IL-4Rα expression on non-haematopoietic cells however specific roles for IL-4Rα expression in the rapid protective immunity to re-infection are not understood. Using mouse models unable to express IL-4Rα or with T- or B- cell specific disruption of IL-4Rα expression we examined the efficacy of host immune responses to re-infection against *Nippostrongylus brasiliensis***.** Our results demonstrated protective immunity to *N. brasiliensis* to be mediated via IL-13 signaling through IL-4Rα. Optimal killing of parasites following re-infection requires IL-4Rα expression on T- and B-cells. Both T-cell and B-cell IL-4Rα expression was required for optimal cytokine and antibody responses. Transfer of T- or B-cells from infected animals mediated IL-4Rα dependent protection against primary infection independent of antibody. Analysis of cellular responses revealed roles for T-cell IL-4Rα expression in the induction of effector T-cell populations and novel B-cell functions independent of antibody production. This study demonstrated haematopoetic expression of IL-4Rα is essential for optimal immunity against parasitic nematodes. Furthermore disrupted IL-4Rα expression on T- and B-cells severely impacted on the development of host immunity.

Antibodies function to promote helminth-Induced basophilia

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Basophils have been recently reported to play an important role as antigen presenting cells capable of provoking Th2 immune responses, and as effector cells important for protective immunity against helminth parasites. We now demonstrate an essential role for antibodies in promoting increased basophil production following infection with the murine helminth *Heligmosomoides polygyrus.* Our data shows that helminth-induced basophilia requires the production of isotype-switched antibodies, most likely IgG or IgE. Antibodies promote basophil production in the bone marrow and are therefore essential for helminth-induced increases in blood and tissue basophil numbers. In contrast, Th2 cell differentiation and IL-3 production are not altered by the absence of isotype class switched antibodies. These findings demonstrate that helminth-induced basophilia requires $CD4^+$ T cell-dependent antibody production but occurs independently of $CD4^+$ T cell cytokine production

Immunomics of *Heligmosomoides polygyrus* infection

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The mouse intestinal nematode parasite *Heligmosomoides polygyrus* is a widely used immunological model of chronic helminth infection, and yet its antigenic profile is barely characterised. We are analysing adult parasite excretory/secretory antigens (HES), antibody responses to which are >10-fold stronger than those against a somatic extract of adult worms. Moreover, functional studies have shown the presence of a TGF-β-like molecule in HES which induces Foxp3+ regulatory T cells (Tregs), and that day 28 infection sera (pAb) blocked this effect. We therefore undertook a systematic analysis of both the proteomic composition of HES, and the profile of antibody responses to this antigen. Some 60 distinct components within HES have been identified by matching 2-D SDS-PAGE protein spots to an adult cDNA sequence dataset. Western blot analysis of anti-HES antibodies revealed that even after 28 days' infection, polyclonal antibodies (pAbs) are highly focussed onto just three antigens, all members of the venom allergen-like / activation associated protein (VAL/ASP) family. These 3 antigens, termed Hp-VAL-1, 2 and 6, are among the most abundantly secreted proteins of adult *H. polygyrus*. The pAb response detected by Western blot is predominantly IgM, and is recapitulated by a number of IgM monoclonal antibodies (mAb) generated from infected mice, indicating an immunodominant, common epitope. Hp-VAL-1, 2 and 6 share limited amino acid identity, but contain numerous predicted O-glycosylation sites within an inter-domain linker region, suggesting an antigen O-glycan epitope is present. This epitope was not phosphorylcholine, was not sensitive to PNGaseF digestion, but was destroyed by chemical deglycosylation with TFMS. In contrast to the anti-glycan IgM response, the IgG1 response, predominant in B cells from the draining mesenteric lymph nodes, was largely directed against conformational (protein) epitopes that were not detectable by Western blot. Nevertheless, immuno-precipitation of labelled HES revealed that the IgG1 response was also largely against the VAL proteins, specifically Hp-VAL-1 and 2, as well as against the non-glycosylated but highly secreted VAL-3 and 4. Neither anti-VAL carbohydrate nor anti-VAL protein mAbs were able to block HES induced Treg expansion, and preliminary experiments testing passive immunisation with anti-VAL IgG1 mAb have not indicated accelerated parasite expulsion. In conclusion, adult *H. polygyrus* secrete abundant levels of several VAL homologues, which contain immunodominant glycan targets of IgM and protein targets of IgG, the protective effects of which remain to be determined.

Functional analysis of the interferon gamma (IFNγ) network by susceptibility of mice to chronic *Trichuris muris* infection

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A protective immune response to the gastrointestinal nematode *Trichuris muris* depends on the generation of a T helper type 2 immune response. Dissecting the role of interferon gamma (IFNγ) in the inflammatory response and expulsion of the parasite will provide important understanding of the cellular mechanisms underlying susceptibility to the parasite. In order to determine the influence of IFNγ on a specific cell type we have used a conditional gene targeting approach, utilising a cell specific inactivation of the IFNγ receptor (IFNγR2). We have generated mice, which lack the IFNγR2 on all cells (global knockout), T cells (CD4 Cre), macrophages/neutrophils (LysM Cre) and T cell, macrophages/neutrophils (CD4 Cre x LysM Cre). Mice were infected with a low dose of *T. muris* (20 eggs) which normally drives a T helper type 1 response leading to susceptibility and a chronic infection. Our data demonstrates that establishment of a chronic *T. muris* infection is dependent on IFNγR2 as global deletion of IFNγR2 is sufficient to make a susceptible mouse resistant with expulsion of the parasite. Further work with the cell specific knockout mice has demonstrated that IFNγR2 positive T cells are more important than IFNγR2 positive macrophages. We have demonstrated the deletion of IFNγR2 on T cells or macrophages/neutrophils does not result in resistance to the parasite. However, mice lacking IFNγR2 on T cells did have a lower worm burden than the control mice. The role of IFNγ on T cells and macrophages appears to be non redundant as the double conditional knockout (CD4 Cre x LysM Cre) can expel the parasite. This demonstrates both T cells and macrophages that are responsive to IFNγ are required for the establishment of chronic infection. Conditional knockout mice represent powerful model for dissecting the role of specific cell types in the response to infection. Combining two Cre lines has made it possible for the first time to identify the non-redundant role of two critical cell types.

Distinct molecular signatures of regulatory T cells following *Schistosome* and *Leishmania* infection.

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Foxp3^{+} Regulatory T cells (Tregs) provide an essential immunological counter-balance, preventing hyper-inflammatory disorders such as autoimmunity and allergy and constraining excessive immune responses following infection. Tregs have been studied in a wide range of tissues, immunological environments and diseases and may have suffered from an overly generalized description of their phenotype. We hypothesized that just as effector responses vary significantly, Tregs are shaped by their immunological environment and develop unique characteristics, appropriate to control the specific effector immune response. To test this model we used two important human parasitic infections, the protozoan parasite, *Leishmania major* and the blood fluke, *Schistosoma mansoni*, both of which are influenced by Tregs and are controlled by distinct immunological responses (IFNγ-dominated Th1 responses for *L. major* and IL-4-dominated Th2 responses for *S. mansoni*). Using Foxp3^{gfp} reporter mice, we FACS-purified Tregs from the ear of 9-week *L. major* infected mice (Th1-Treg), the liver of 9-week *S. mansoni* infected mice (Th2-Treg) and from the spleen of naïve mice. Treg-derived RNA was size separated with large (>200bp) RNA profiled using Affymetrix Gene 1.0 ST arrays with the small (<200bp) RNA fraction examined using next generation sequencing (NGS) to create transcriptional signatures of the different Treg populations. Gene expression data revealed significant heterogeneity between the three Treg populations, in alignment with our initial hypothesis. Similarly, NGS data identified unique microRNA (miRNA) profiles. Follow-up *in silico* analyses revealed that several of the miRNAs that are differentially expressed in the infected conditions may contribute significantly toward the observed global gene expression changes. We are currently testing the functional relevance of these miRNAs in Treg populations. Parallel in-vitro and in-vivo models of Th1 or Th2-mediated airway inflammation have been developed to validate candidate miRNA-mRNA interactions. Tregs adopt a unique gene expression profile, which may reflect the nature of the immunological environment and the specific function of Tregs in that environment. The newly discovered unique properties of Tregs identified, may be used to block Treg function and enhance immunity to parasitic infections, or enhance Treg function in hyper-inflammatory conditions, such as autoimmunity and allergy. This work was supported by the Intramural Research Program at the National Institutes of Health, USA.

Update from the Human Hookworm Vaccine Initiative: developing anthelminthic vaccines to combat anemia caused by hookworm infection and intestinal schistosomiasis.

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Iron Deficiency Anemia (IDA) is among the most common disease in the developing world, accounting for 50% of the global burden from all anemia and causing approximately 841,000 deaths and 35 million DALYs lost annually. Most of the disability from IDA occurs in sub-Saharan Africa, Southeast Asia, and the tropical regions of the Americas were co-infections with the hookworm *Necator americanus* and the trematode *Schistosoma mansoni* are common. Here we review our progress in developing recombinant protein vaccines for these two important helminth infections. Previously, our approach to a hookworm vaccine was the development of recombinant proteins from the infective larval stage (L3) of *N. americanus* (e.g., *Na*-ASP-2); this vaccine development path has now been halted due to type 1 hypersensitivity responses upon vaccination with *Na*-ASP-2 in individuals resident in areas with active *N. americanus* transmission. Our new approach to the hookworm vaccine is to focus solely on the identification of the essential components involved in parasite blood feeding and to genetically-engineer these components as recombinant proteins and then either combine them in a single formulation (a chimeric-based vaccine) or co-administer them as separate vaccine formulations. From approximately two dozen proteins involved in the hookworm blood feeding process, two lead candidate antigens have been selected for product and clinical development based on their efficacy in animal trials (hamster and canines): *Na*-GST-1 and *Na*-APR-1. The primary target of our schistosomiasis vaccine development program are the apical membrane proteins from *S. mansoni* (e.g., *Sm*-TSP-2), whose efficacy in a mouse model has been extensively demonstrated and is now in process development for cGMP manufacture, with clinical testing in Brazil scheduled for next year. This presentation will focus on our strategies for the combined product and clinical development of these hookworm and schistosome vaccines, including plans for the scale up of their manufacture, clinical testing in endemic areas of Brazil and Africa, access to these vaccines post licensure, and the potential strategies for incorporating them into existing helminth control programs that would result in sustainable reductions in anemia in developing countries.

Immune response to trickle infection with *Toxocara canis* and its association with experimental allergic airway inflammation

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Toxocara canis is a roundworm of dogs and the causative agent of human toxocariasis. Transmission occurs after ingestion of *Toxocara* eggs present in soil contaminated with dog feces. After ingestion the larvae penetrate the intestinal wall and migrate to different organs including the lungs. Human epidemiological studies suggest that allergic manifestations occur more often in *Toxocara*-seropositive children. We have previously shown using a murine model that a single infection with *T. canis* leads to exacerbation of allergic airway inflammation. Usually, hosts acquired helminth infections by continued exposure to the infective stages. In this study we aim at studying the immune response to parasite acquisition under a more natural frequency of exposure and to determine its effect on allergic airway inflammation.To this purpose BALB/c mice were infected orally with 250 *Toxocara canis* embryonated eggs every three days for two weeks. To study the effect of infection on allergic airway inflammation the mice were first infected and then sensitized and challenged with ovalbumin (OVA).Results indicate that a trickle *Toxocara*-infection alone, leads to increased: pulmonary inflammation, expression of IL-4 and IL-5 in lungs, total IgE and *Toxocara*-specific IgG1 in serum, all of which persisted up to 73 days after infection. In combination with OVA, exacerbation of pulmonary inflammation and eosinophilia was observed. The levels of total and OVA-IgE in serum and the expression of cytokines in lungs increased in the *Toxocara*-infected + OVA treated animals but the differences were not significant.Several factors may influence whether an infection with helminths protects one from or enhances allergic manifestations. *Toxocara canis* is not a natural infection of humans and whether the pathogen infects a definitive or an accidental host may influence the outcome of infection on allergy. Another factor may be the frequency of infection. In this study we show that not only a single (sporadic) infection but also a repetitive chronic *Toxocara* infection results in exacerbation of allergic airway inflammation.

CCL17 controls mast cells to maintain skin integrity for the defense against filarial larval entry

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The first barrier that is encountered by filarial parasites is the host's skin, a site for immediate responses against invading pathogens. However, little is known about mechanisms of early local events in filarial infections. Bone marrow derived-DCs not only upregulate activation markers CD40 and CD80, but also secrete significant amounts of CCL17, a chemokine known to be produced upon microbial challenge. We further observed that mice deficient for the chemokine CCL17 had an up to 4fold higher worm burden compared to heterozygous controls by day 10 of infection with the murine filaria *Litomosoides sigmodontis*. This was associated with greater influx and degranulation of mast cells in the skin, with the consequence of increased vascular permeability and facilitation of larval establishment. This phenotype was reverted by inhibition of mast cell degranulation with disodium cromoglycate. In addition, we showed that CCL17-mediated vascular permeability was dependent on the presence of *Wolbachia* endosymbionts and TLR-2 signaling. Our findings reveal that maintaining mast cell homeostasis by CCL17 represents a mechanism to control filarial larval entry by limiting mast cell dependent vascular permeability of skin vessels.

The role of eosinophils in experimental filarial infection with *Litomosoides sigmodontis*.

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Infections with the filarial nematodes *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* cause lymphatic filariasis mainly in Asia and Africa with a tremendous socio-economic impact in these areas. We were investigating the role of eosinophils in the pathogenesis of this disease using a murine model of filarial infection.Mice were infected with *Litomosoides sigmodontis* via natural infection. Larvae migrate to the pleural cavity that they will reach at around 8-10dpi, mature by 28dpi and start releasing microfilariae by 60dpi. Mice were therefore euthanized at 15, 30 and 60 dpi and immunological and parasitological parameters were examined.To investigate the requirement of eosinophils in pathogenesis of filarial disease we were infecting Balb/c mice, eosinophil deficient ΔdblGATA mice and mice deficient in the eosinophil-attracting chemokine Eotaxin-1. Interestingly, we found that parasite burden at 60dpi was significantly increased in both ΔdblGATA and Eotaxin-1$^{-/-}$ mice, although only ΔdblGATA showed decreased eosinophil migration to the pleural cavity. Lack of Eotaxin-1 was compensated by production of Eotaxin-2 and RANTES in the pleural cavity. Normal eosinophil migration to the pleural cavity together with increased worm burden in Eotaxin-1$^{-/-}$ mice implicated Eotaxin-1 as inflammatory marker in addition to its chemotactic function. Stimulating purified eosinophils with recombinant Eotaxin-1 or Eotaxin-2 led to increased IL-6 production. We could confirm by *in vitro* stimulation with soluble *L. sigmodontis* protein extract that eosinophils from Eotaxin-1$^{-/-}$ mice produce decreased levels of IL-6 in comparison to Balb/c eosinophils. This was reflected by decreased levels of IL-6 in the pleural cavities of ΔdblGATA and Eotaxin-1$^{-/-}$ mice.From this study, we conclude that eosinophil migration and activation are required for parasite killing and demonstrate a specific role for Eotaxin-1 in eosinophil activation rather than migration.

Using human hookworm to treat celiac disease: immune responses during a clinical trial

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Epidemiological and experimental evidence indicates that infection with parasitic helminths leads to suppression of inappropriate immune responses, such as inflammatory gut conditions. Celiac disease (CD) is a complex gluten-induced enteropathy that develops in genetically susceptible individuals: HLA-DQ2 is present in 95% of cases. *Necator americanus* is a uniquely human gastrointestinal nematode parasite that still infects over 500 million people in developing countries and promotes its survival by modulating the immune system. Experimental infection of healthy volunteers with low numbers of *N. americanus* infective third-stage larvae has proven benign, so we recently completed a double-blinded clinical trial, with the goal of treating CD pathology with *N. americanus*. Twenty CD sufferers on a long-term gluten-free diet were recruited, ten of whom were experimentally infected with *N. americanus*, while the remainder received placebo control. At week 20 after infection, oral gluten was administered for 5 days to induce inflammation, with the trial finishing at week 21. Celiac pathology was determined by histology and questionnaire for symptom severity, both of which showed a trend towards decreased pathology in the hookworm-infected group. Immunological parameters were evaluated in the blood and small bowel mucosa before and after gluten challenge. The hookworm infected group showed significantly increased spontaneous production of IL-5 in the mucosa and antigen-specific IL-4, IL-5 and IL-13, indicating a parasite specific TH2 response. Levels of spontaneous mucosal IFN-gamma and IL-17A were higher after gluten intake in the control group than in the hookworm infected group. IFN-gamma production by peripheral blood mononuclear cells after stimulation with a gluten-derived MHC II-restricted peptide (Q65E) was significantly higher in the control group after gluten challenge. In the hookworm-infected group, however, this celiac inflammatory response was again suppressed. Although the mechanism of this suppression is unknown, results from this trial suggest that hookworm infection causes important changes in immune cell types after gluten exposure, both *in vivo* and *in vitro*, and that hookworm-derived products show promise as therapies for a range of inflammatory diseases.

Therapeutic potential of the *Acanthocheilonema viteae* secreted product, ES-62

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ES-62 is the major protein secreted by the rodent filarial nematode, *Acanthocheilonema viteae.* Mediated largely through the unusual post-translational addition of phosphorylcholine (PC) to an N-type glycan, ES-62 has been found to modulate immune cell signal transduction pathways utilising phospholipase D (PLD)/sphingosine kinase (SphK), protein kinase C, (PKC) MAPkinase and NF-B. This results in ES-62 exhibiting a broad range of anti-inflammatory activities including inhibition of mast cell degranulation, reduction in antigen-driven B-cell proliferation and inhibition of macrophage pro-inflammatory cytokine production. By employing knockout mice it has been shown that ES-62 activity is dependent on TLR4 but unlike the classic TLR4 ligand, LPS, ES-62 is active against cells derived from HeJ mice that have a TLR4 point mutation, thereby arguing for a difference in mechanism of action between these two pathogen products. Studies in mast cells indicate that ES-62 forms a complex with TLR4 at the plasma membrane and that this results in sequestration, followed by internalisation and degradation of PKC-, a molecule essential for mast cell degranulation due to its role in facilitating PLD/SphK-mediated Ca^{2+} mobilisation. PKC- sequestration does not occur in response to LPS, again highlighting differences in the consequences of interaction between TLR4 and the two ligands.As ES-62 has anti-inflammatory properties, we hypothesised that it might have potential as a treatment against diseases associated with aberrant inflammation. It was thus tested in a number of mouse models of inflammation and found to offer protection in some of them, specifically in collagen-induced arthritis, ovalbumin-induced airway hyper-responsiveness and oxazolone-induced immediate-type hyper-sensitivity in the skin. ES-62 also blocked development of the kidney damage and arthritis that arise spontaneously in the MRL/lpr mouse. Analysis of immunological parameters indicated that ES-62 activity correlated with inhibition of production of molecules known to be important in promoting inflammation in some of the different disease contexts, e.g., IL-4 in airway-hyper-responsiveness and TNF- and IL-17 in arthritis. Recently we have prepared a library of small PC-based molecules for testing for immunomodulatory activity comparable to ES-62, with the ultimate aim of designing small molecule anti-inflammatory drugs.

Immune response against unrelated antigens is drastically suppressed in *Litomosoides sigmodontis* infected mice

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Human filarial parasites cause long-term chronic infections of considerable morbidity particularly in the tropics. Filariae have developed multiple mechanisms to evade, exploit and suppress the host's immune system leading to impaired immune response against the parasite itself and to concurrent infections and vaccinations. Here, we evaluated immune modulation by filarial nematodes against model antigens in a murine model of helminth infection. Resistant C57BL/6 mice and susceptible BALB/c mice were infected naturally with *Litomosoides sigmodontis* via blood sucking mites. To selectively analyze the capacity of microfilariae (MF) we isolated MF from the blood of infected cotton rats, the natural reservoir of the nematode, and injected them intravenously into C57BL/6 mice. We compared the humoral immune response of infected and naïve mice to thymus dependent (TD) or independent (TI) model antigens to analyze the impact of different laraval stages on the efficiency of vaccinations. Proliferation of adoptively transferred CFSE-labeled T cell receptor transgenic OT-II T cells was compared in naïve and *L. sigmodontis* infected mice.Concurrent *L. sigmodontis*-infection resulted in C57BL/6 and BALB/c mice in a dramatically reduced IgG1, IgG2a/c, IgG2b and IgG3 response against TD antigens. This suppression of TD specific IgG response was mediated by different larval stages: L4, young adults and fertile adults worms. In contrast, injection of microfilariae in C57BL/6 mice selectively suppressed the Th2-associated IgG1 response whereas the proinflammatory IgG2c response was increased. The humoral response against TI antigens however was unchanged in infected and naïve mice suggesting interference with T helper cell function rather than direct suppression of B cells in *L. sigmodontis* infected mice. Supporting this notion adoptive transfer of B cells from naïve mice into *L. sigmodontis* infected B cell deficient hosts resulted in the suppression of Ig response to subsequent TD immunization. B cells purified from infected mice however, mounted normal Ig responses to TD immunization upon transfer into naive B cell deficient mice. To directly analyze T helper cell function we show that infection with *L. sigmodontis* and injection of MF strongly inhibited proliferation of OVA-specific OT-II T cells *in vivo* in an adoptive transfer model. The reduction of total numbers of OT-II T cells in the spleens from *L. sigmodontis* infected mice was associated with the increased induction of Foxp3+ Treg within the transferred OT-II T cells and an enhanced Th2 cytokine response upon OVA-specific *in vitro* stimulation. To analyze the impact of host derived Treg or anti-inflammatory cytokines such as IL-10 we used depletion of regulatory T cells (DEREG) mice or mice that are deficient for IL-10 selectively in T cells. Neither depletion of Treg in the recipient mice nor IL-10 deficiency in CD4+ T cells restored the suppression of OT-II T cell proliferation arguing for a Treg independent suppression of OT-II T cell proliferation in an ongoing infection. Taken together we provide evidence that infection with *L. sigmodontis* strongly interferes with immune response against bystander antigens via modulation of T cell functions and induction of a regulatory phenotype.

Strongyloides ratti infection induces expansion of regulatory T cells that interfere with nematode-specific immune responses and parasite clearance.

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Pathogenic nematodes are large multicellular organisms that often reside for years within their mammalian hosts. In order to escape expulsion by the hosts immune system and to avoid the induction of immune pathology, helminths need to dampen the hosts immune response. To this end helminths utilize regulatory pathways such as the induction of regulatory T cells (T_{reg}) and regulatory receptors, that are intrinsic parts of the mammalian immune system. To study the function of regulatory T cells during nematode infection we employ the "Depletion of T_{reg}" (DEREG) mice. These mice are transgenic for a bacterial artificial chromosome expressing a diphtheria toxin (DT) receptor-enhanced green fluorescent protein fusion-protein under the control of the forkhead box P3 (*foxp3)* gene locus. They allow the specific detection and quantification of $Foxp3^+$ T_{reg} as well as their selective depletion by DT injection.Infection of BALB/c-DEREG mice with the nematode *Strongyloides ratti* induced an early increase in T_{reg} numbers starting at day two post infection. T_{reg} expanded both locally in the mesenteric lymphnodes draining the gut where the parasitic adults reside, but also centrally in the spleen. Application of DT led to a transient deletion of T_{reg} that resulted in dramatically reduced numbers of parasitic female adults in the gut and a reduced larval output in comparison to both: *S. ratti* infected DEREG mice that did not receive DT and wildtype mice that had been treated with DT. The numbers of migrating larvae in head and lung were unchanged, suggesting that the improved host defense in the absence of T_{reg} targeted the parasitic adults. Supporting this notion, we observed an increased concentration of murine mast cell protease-I in the serum upon T_{reg} ablation, indicating increased activation of mast cells that play a central role in the final expulsion of *S. ratti* from the gut. Depletion of T_{reg} increased antigen-specific proliferation in spleen and mesenteric lymphnodes as well as the production of both, Th1 like and Th2 like cytokines to antigen and polyclonal T cell stimulation. Moreover, the absence of T_{reg} during first infection did not interfere with the partial resistance to secondary infection, suggesting that the absence of T_{reg} did not affect generation of protective immune memory.Cytotoxic-T-cell-Antigen-4 (CTLA-4), a central mediator of T cell regulation, is constitutively expressed on T_{reg} but is also upregulated on activated T effector cells. The neutralization of CTLA-4 signaling by application of a blocking antibody *in vivo* again improved resistance of BALB/c mice to *S. ratti* infection. Reduced worm burden in the absence of CTLA-4-signaling was associated with an improved antigen-specific Th2 like cytokine response whereas polyclonal cytokine production was unchanged. Taken together, our results strongly suggest that *S. ratti* induces the expansion of T_{reg} and actively suppresses the T cell mediated immune response and expulsion of parasitic adults.

Competing T cell subsets in *Heligmosomoides polygyrus*

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The intestinal nematode parasite Heligmosomoides polygyrus stimulates and expands multiple subsets of host T cells, with contending influences on the outcome of infection. In susceptible strains of mice (C57BL/6 and BALB/c) there is an activation and expansion of Foxp3+ regulatory T cells (Tregs), including Tregs specific for bystander antigens such as ovalbumin. The development of bystander suppression may explain why Tregs from H. polygyrus infected mice are able to suppress allergen-specific airway inflammation when transferred into uninfected recipient animals. The induction of Foxp3+ Tregs can be reproduced in vitro by exposing naive peripheral non-regulatory T cells to H. polygyrus excretory-secretory (HES) products, and HES stimulates conversion to Foxp3+ Tregs in a very similar manner to host TGF-b, through the TGFb signalling pathway. Tregs generated in vitro by HES are able, on transfer to allergic mice, to suppress airway allergy in the same model used for Tregs from in vivo parasite infections. In resistant strains of mice (eg SJL) the Treg population appears to be less activated (measured by CD103 expression) and a much prompter and stronger Th2 response is mounted. Resistant mice also develop numerous intestinal granulomas around the site of larval infiltration into the gut wall. In addition to a Treg:Th2 antagonism that determines the outcome of infection, we were also interested in the how the inflammatory immune response may counteract the development of protective Th2 immunity. The susceptible phenotype of the C57BL/6 mouse is attenuated or reversed in the absence of certain pro-inflammatory immune genes, including IL-6, IL-23p19 and MyD88; we are therefore now dissecting the role of Th1 and Th17 populations, and of potentially pro-inflammatory signals from commensal microbes, in competing with or inhibiting protective Th2 responses in chronic infection.

Poster Session Abstracts

1. Inhibition of Toll-like receptor signalling by *Trichinella spiralis* excretory/secretory products is restricted to TLR4

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Helminths secrete several molecules that can modulate the host immune response, which has been suggested to be relevant for parasite survival. Modulation of the innate response is an importantprocess in which dendritic cells (DCs) play an important role in the initiation of the adaptive immune response. In this process, activation of TLRs plays a pivotal role. We have previously shown that the excretory/ secretory products of *T. spiralis* (TspES) suppress *E. coli* LPS- but not *N. meningitidis* LPSinduced DC maturation in vitro. The present study aims at identifying the mechanisms involved in the suppressive effect of TspES. Since smooth or S-form LPS (*E. coli*) is known to depend on CD14 for TLR4 activation while rough or R-form LPS (*N. meningitidis*) does not, we suggested that CD14 might be blocked or suppressed by TspES. Results indicate that TspES suppresses CD14 cell surface expression on *E. coli* LPS-pulsed DCs. Compensation of the expression of CD14 by adding CD14 transfected HEK293 cells resulted in partial recovery of DCs maturation. In addition, other LPS were tested to determine whether the effect of TspES on LPS depends exclusively on the S- vs. R-form of this molecule. By comparing different LPS, we have found that TspES suppresses also the R-form of *E. coli* LPS. These results indicate that CD14 is not the only molecule involved in the suppressive effect of TspES. We also tested whether DC maturation by other TLR ligands was affected by TspES however, no changes were observed. Only TLR4 activation was inhibited by these helminth's products. Finally, the effect of TspES on expression of different genes involved in the TLR-signalling pathway was tested and will be presented. The suppressive effect of TspES on DC activation induced by LPS from enterobacteria, as a survival strategy of this parasite will be discussed.

2. Predominance of IL-10 and TGF-β production from RAW 264.7 cells in response to crude antigen of *Clonorchis sisnensis*

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It is well known that parasitic helminths can modulate the host immune responses. *Clonorchis sinensis* (*C. sinensis*) is a liver fluke and the fundamental immunoregulatory mechanism of *C. sinensis* is investigated using murine macrophage RAW 264.7 (RAW) cell line. We found that *C. sinensis* crude antigen (CA) was able to differentiate macrophage RAW cells into dendritic-like cells detected by morphological observation. Along with morphological changes, CA induced the premoninant secretion of anti-inflammatory cytokines such as interleukin (IL) 10 and Tumor Growth Factor (TGF) β. In addition, the levels of proinflammatory cytokines such as IL-4, 5, 6, TNFα and γ-IFN were not significantly changed. In order to understand if morphological changes are accompanied with upregulation of activation markers, cell surface markers involved in antigen recognition, presentation and T cell activation were checked. Flowcytometry showed that the levels of CD40, CD80, CD86, MHC Class II, and TLR4 were unchanged. To clarify the signaling cascade, we focused on MAPK pathways and found that CA exerts it's activation signal via ERK and JNK pathway. Taken together, these data demonstrate that CA from *C. sinensis* might modulate host immune responses via up-regulation of anti-inflammatory cytokines via the regulation of MAPK.

3. Schistosomal lyso-phosphatidylserine: a novel Toll Like Receptor 2 ligand

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Schistosoma mansoni is a parasitic helminth that causes schistosomiasis, a chronic disease in which the immune response of the host is skewed towards a Th-2 type response. Chronic schistosomiasis also leads to down-regulation of inflammatory responses of the host. Induction of this reduced T-cell response is dependent on specific activation of dendritic cells, a process finally resulting in increased interleukin 10 (IL-10) production by regulatory T-cells. Previous experiments have shown that several schistosome-specific phospholipids affect dendritic cells such that mature dendritic cells gain the ability to induce the development of IL-10 producing regulatory T-cells. Since a TLR2-blocking antibody inhibited the induction of regulatory T-cells, schistosomal phospholipids are able to activate dendritic cells by TLR2 activation. Using mass spectrometry, schistosomal lysophosphatidylserine (lyso-PS), a phospholipid with a serine headgroup and only a single fatty acid attached to its glycerol backbone, was identified as a novel class of lipids that can activate TLR2. This activity appears to be a specific property of schistosomal lyso-PS species, because neither commercially available lyso-PS (16:0) nor phosphatidylserine (PS) fractions isolated from the mammalian host were able to activate TLR2. Schistosomes are unable to synthesize phospholipids de novo, but they are able to modify lipids obtained from their host. By these modifications schistosomes form unique PS molecules, in which the attached acyl chains are not only longer but they also contain more unsaturated bonds when compared to those found in their host. In addition, adult schistosomes, and especially the outer surface membranes of the tegument, are enriched with lyso-PS molecules. To further investigate the structure/function relationship of lyso-PS in TLR-2 activation, we have developed a novel method to produce several synthetic lyso-PS molecules with distinct fatty acids attached to the glycerol backbone (e.g. 18:1, 18:3, 20:1, and 24:1). The ability of these synthetic lyso-PS molecules to activate TLR-2 has been investigated in vitro in mammalian cells that were transiently transfected with TLRs. The specific structural properties of the lyso-PS molecules required for TLR-2 activation will be discussed.

4. Transcriptomic approaches to identify genes involved in animal nematode virulence

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Gastro-intestinal nematodes are responsible for great economical losses on ruminants, estimated at 1.5 billon$ per year around the world. This is due to production losses but also to the cost of anthelminthic treatments to control gastro-intestinal nematode populations. To date, the efficiency of the chemical compounds used is dramatically reduced by the apparition and the emergence of resistant population of nematodes. De facto, new strategies to control parasitic diseases related to nematode infections need to be developed. Possible strategies consist in blocking nematode installation in the host. But this implies to better understand the mechanisms involved in penetration and maintenance of the nematodes in the digestive tract of the animals. Indeed, to date little is known about the parasite virulence factors involved in the different parasitic stages of the gastro-intestinal nematodes. Among the different species which colonize the digestive tract of ruminants, *Haemonchus contortus* was the best choice to explore the host-nematode interaction mechanisms at the molecular level, because of the data already available on its genome. We developed a transcriptomic approach (Suppressive Subtractive Hybridization) to highlight the genes differentially expressed between the free infective stage (L3) and the early parasitic stage (L4) of the nematode *H. contortus*. Two different libraries were constructed and 200 random clones were analyzed by dot blot. Among them, 46 clones were selected and sequenced. These EST belong to 9 contigs and the corresponding full length cDNA revealed that 7 of them possess a signal peptide. This suggests the secretion of the corresponding proteins. Homolog search in databases revealed strong similarity with known proteins involved in lipid metabolism, retinol transport as for example or with unknown proteins. RT-PCR analyses confirmed the specific expression or over expression during the parasitic stages. Our work provided interesting clues to go further on the understanding of the host-parasite dialog. Because multiple infections are not rear in ruminants, homologues were search in the sister species *Teladorsagia circumcincta* with the aim to identify common potential target for the development of new treatments. Because of the biological constraints encountered to study the host responses in ruminants, we are also developing an interaction model on mice. Indeed, *Heligmosomoides bakeri* is a sister species of the ruminant nematodes that is adapted to the mice and the immune responses to gastro-intestinal nematodes are well known on this model. However little is known about the parasitic virulence factors on this model. Then, we currently used the data obtain in the lab on cattle nematodes to easier access to the host-parasite molecular dialog. This approach will give a large overview of the molecular diversity of the parasitism genes among the Rhabditina clade of nematodes and will give precious information to choose the best target to elaborate new strategies control of parasitic nematodes

5. Biological implications of DNA compaction in *Taenia crassiceps cysticerci.*

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Taenia crassiceps is a tapeworm parasite that requires, like other cestodes, a predator-prey relationship among its two hosts. In this case, definitive and intermediate hosts are usually foxes and mice. A peculiar trait of *T. crassiceps* is the larval ability to bud, allowing multiplication of the cysts in the peritoneal cavity of mice. This ability has popularized the use of murine *T. crassiceps* as a model for cysticercosis, because it is conveniently maintained under laboratory conditions through simple intraperitoneal passage of cysts from mouse to mouse. Usually, 10 cysticerci are injected into the peritoneal cavity of the recipient mouse using syringes with needles of 0.4 or 0.8 mm in diameter. Infections are left during 2 or 5 months. In order to determine the genome size of *Taenia crassiceps*, cell nuclei from cysts of the ORF strain, were recovered at different post-infection times in mice. After staining with propidium iodide, the nuclear DNA content was estimated through cytofluorometry, using chicken red blood cells as a known standard. Cysts nuclei from 5 months infections showed a DNA content equivalent to a haploid genome size of 175.7±2.3 Mb, whereas the nuclei from 2 months infections had a significantly lower DNA content, equivalent to a genome size of 118.8±1.72 Mb. This lower DNA content was associated to the fragmentation of the cysts tissues during the intraperitoneal mouse to mouse passage of cysts through syringe needles. On the other hand, protein profile was estimated by two dimensional electrophoresis (2-DE). At least 656 protein spots were resolved for late (high DNA content), in contrast to 535 for early infections (low DNA content). Cysticerci recovered from late infections showed a majority of proteins with isoelectric points between 5-7, and molecular masses between 20 and 150 kDa, whereas those from early infections showed less proteins with isoelectric points over 6 and molecular masses over 70 kDa. Cysticerci from early infections showed a lower expression of proteins in the 5-7 isoelectric point region and 15-30 kDa. In both cases, an abundance of proteins appeared between 4-5 isoelectric point and 48-67 kDa. Same results in proteins expression were obtained with cysticerci obtained from 2 months infected mouse with different needless diameter. This genomic changes promoted by laboratory manipulation, illustrates the remarkable plasticity of this parasite that could be involved in natural processes of parasite's adaptation and survival. Further studies will be conducted in order to determine if this lower DNA content is due to differences in staining amongst these two different laboratory manipulations of the cysts or are different adaptations of the parasites. This work was supported by IMPULSA program, *Taenia solium* Genome Project from Universidad Nacional Autónoma de México.

6. Regulation of retinoic acid synthesis during *Schistosoma mansoni* infection and its contribution to egg-elicited T cell responses

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Vitamin A (retinol) plays a critical role in immune function. Upon delivery to tissues, retinol can be oxidized intracellularly to yield retinoic acid (RA), a transcriptionally active metabolite that mediates the effects of vitamin A in the immune system. When secreted by antigen presenting cells, RA signaling in helper T cells promotes Th2 and T_{reg} differentiation. Despite convincing *in vitro* evidence that RA plays a critical role in shaping helper T cell responses, the importance of this signaling pathway in the setting of infection remains obscure. Given the importance of Th2 and T_{reg} cells in regulating egg-induced immune pathology during infection with *Schistosoma mansoni*, we sought to determine whether RA synthesis was increased in this context. We found that the expression of retinal dehydrogenase (Raldh), the rate-limiting enzyme in RA synthesis, was greatly increased in granuloma-afflicted tissues of C57BL/6 mice. Furthermore, immunofluorescence staining demonstrated that Raldh co-localized with CD11b in granulomas, suggesting that alternatively activated macrophages (AAMφ) may be competent for RA synthesis. Induction of classical and alternative activation of bone marrow-derived macrophages *in vitro* clearly confirmed that Raldh expression is a characteristic of AAMφ. To investigate the contribution of RA signaling to T cell responses during *S. mansoni* infection, we analyzed infected, vitamin A deficient mice. In the absence of RA signaling, Th2 responses were significantly diminished in the intestinal immune compartment and liver. Surprisingly, the expression of Foxp3 was strikingly increased during vitamin A deficiency, suggesting the induction of an RA-independent T_{reg} population. Our results suggest that vitamin A is more important for the development of egg-elicited Th2 responses, rather than for the induction of $Foxp3^+$ T_{reg} cells during *S. mansoni* infection.

7. Venom allergen homologue / ASP-Like proteins (VALs) in *Nippostrongylus brasiliensis*

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The venom allergen homologue / ASP-like proteins (VALs) are a major class of nematode secreted proteins in the pathogenesis-related protein (PRP) superfamily. First discovered in the canine hookworm *Ancylostoma caninum*, homologues have since been discovered in most nematodes. In *Nippostrongylus brasiliensis*, eight variants of VALs (*Nb*VAL1-8) have been identified, and they show differential expression across lifecycle stages. All eight variants appear to be secreted by adult worms and at least two seem to be secreted by infective larvae (L3), although we find no evidence that they are involved in penetration of host skin. The *Nb*VALs are immunologically reactive; mice infected with *N. brasiliensis* develop antibodies to VALs typical of a type 2 response including IgE, and 4 out of 10 animals showed immediate-type hypersensitivity reactions in skin tests with *Nb*VAL4 and *Nb*VAL7. Immunisation with *Nb*VAL7, a protein secreted by both infective larvae (L3) and adult worms, yields strong immunological responses but confers no protective effects in terms of worm burden or parasite viability. These data suggest VALs from different nematode species may induce type 1 hypersensitivity reactions, placing constraints on their inclusion in vaccine development.

8. SKN-1 as a potential xenobiotic detoxification and developmental target

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Multidrug resistance is a growing problem in nematodes. In pathogenic organisms and tumor cells, multidrug resistance is mediated by enzymes that detoxify xenobiotics. Transcription factors that control the expression of xenobiotic detoxification genes are promising, but largely unexplored, multidrug resistance targets. The transcription factor SKN-1 activates the expression of multiple xenobiotic detoxification genes in *Caenorhabditis elegans*. SKN-1 is also essential for development of the pharynx and intestine in embryos. Pharmacological compounds that target SKN-1 would provide new tools for studying multidrug resistance and have the potential to inhibit development in embryos and drug resistance in adults. We recently used genome-wide RNAi screening to identify a principal pathway regulating SKN-1. Genetic, molecular, and biochemical data support a model in which the WD40 repeat protein WDR-23 regulates SKN-1 activity by mediating ubiquitylation and degradation of the transcription factor. Importantly, the homologous mammalian transcription factor Nrf2 is regulated by a distinct molecular mechanism. We propose the WDR-23/SKN-1 pathway as a promising target for drugs that inhibit embryonic development, xenobiotic detoxification, and drug resistance in nematodes without affecting mammalian hosts. The small size, simple culturing characteristics, and genetic tractability of *C. elegans* make it an ideal system in which to screen for pharmacological inhibitors of SKN-1. These inhibitors would provide tools for studying the function of SKN-1 in parasitic species. We have developed a robust assay for SKN-1 activity using in vivo fluorescent reporters in *C. elegans* and plan to use it in high-throughput screens of small molecules. This work is funded by NIH grant R21 NS067678-01.

9. Functional characterisation of *Schistosoma mansoni* signalling molecules

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Toll-like receptors (TLRs) function as primary sensors of molecular patterns, both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Ligand binding activates a signalling cascade that results in the translocation of specific downstream transcription factors into the nucleus, including immunity-related factors (NFκB) and stress response-related factors (CREB and AP1). Although originally described in research on *Drosophila melanogaster* the use of *Caenorhabditis elegans* and *Homo sapiens* homologous pathways have provided key information on the role of the pathway in pathogen clearance, stress response and, in some species, embryogenesis. Previously, we hypothesized that investigation of host or self-recognition signalling pathways, functional or redundant, in parasitic helminths would advance understanding of the evolution of these pathways (Fallon et al., 2001). Schistosome parasites are multicellular eukaryotic organisms with a complex life cycle that involves mammalian and snail hosts. This complex life cycle is dependent upon the schistosome's ability to survive in multiple diverse environments, and hence their ability to respond to this environment appropriately. These same pathways are thought to function at the parasite surface and transduce signals not only to the cells associated with the host –parasite interface but to the nuclei of cells throughout the parasite body to regulate gene expression important in differentiation, homeostasis, parasite migration, immune evasion and reproductive development. The publishing of the first draft of the *Schistosoma mansoni* genome in Nature in 2009 has confirmed the possibility of a functional TLR/IL-1R signalling pathway in this helminth species. Our lab is currently working to identify and functionally characterise this pathway, and its members, in terms of both *Schistosoma mansoni* signalling and evolutionary significance. We have identified early divergent, functionally conserved signalling proteins in the TLR/IL-1R signalling pathway in the helminth parasite *Schistosoma mansoni*. The function of these helminth signalling proteins will be presented.

10. Approaches to understanding *Schistosoma mansoni* female worm sexual development

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Schistosoma mansoni, one of the causative agents of Schistosomiasis, is a trematode species that is unique in that both male and female sexes exist. Constant contact between male and female worms is required for the complete development of the female reproductive organs. Female development is a topic of paramount importance as only mature females are able to produce eggs that generate the pathology associated with schistosomiasis and are essential for transmission of the parasite. Currently, little is known about the process of worm pairing and sexual development, partly because schistosomes do not complete their lifecycle *in vitro*. Because we are not able to manipulate the transformation between larval and adult parasites we are not able to directly observe the process of worm pairing. However, analysis of single sex infections in mice have shown that single sex females (SSF) differ from mixed sex females (MSF) in that they do not reach reproductive maturity and are unable to produce eggs. Because of these differences, we expected that the transcriptome of SSF worms is very different from that of MSF. To better understand female development we have examined both MSF-specific and SSF-specific transcriptomes using serial analysis of gene expression, SAGE. We have identified numerous transcripts differentially expressed between MSF and SSF. The proteins characterized in this study are specific to MSF or SSF and have no known function or similarity to genes with known functions in other organisms. The goal of this study is to uncover the functions of these unknown proteins using both whole mount *in situ* hybridization (WISH) and reverse genetics. Using the data generated by SAGE, we cloned transcripts and made RNA probes for WISH analysis. One of the advantages of WISH is that transcripts can be localized within the context of the whole worm. Using this method we localized phenol oxidase (also known as tyrosinase), a known female specific gene that encodes a protein that functions in the formation of the eggshell. This gene has never been localized using WISH, and we show in this study that it is present in the vitelline cells of female worms only. With this method we have thus far shown that two of the unknown MSF transcripts localize to different cell types of the female reproductive tract including the vitelline cells and the ovary. These proteins did not localize to any part of the male worm, a result that is in agreement with the data generated through SAGE. In addition to the uncharacterized MSF and SSF transcripts we localized peroxiredoxin 1, a protein known to combat peroxide radicals in the adult parasite. We found that this protein localizes to the gynecopheral canal in males. Along with localization studies we will also discuss the effects of RNA interference silencing on these transcripts.

11. Emodepside is a resistance breaking anthelmintic drug with selectively toxicity to the parasite's SLO-1 calcium-activated potassium channel.

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The cyclooctadepsipeptide emodepside is a resistance breaking compound, effective against a wide range of gastrointestinal nematodes including *Haemonchus contortus* and *Trichostrongylus colubriformis* in sheep, *Cooperia oncophora* in cattle, *Toxocara cati, Toxascaris leonina, Ancylostoma tubaeforme* and cestodes in cats. In the model genetic animal, the nematode *Caenorhabditis elegans*, emodepside inhibits neuromuscular function and thus impairs feeding, locomotion and egg-laying in dose dependent manner. Mutagenesis screening for worms resistant to the paralytic actions of emodepside identified a calcium – activated potassium channel SLO-1 as a mediator of the paralysing effects of emodepside. A reference allele for *slo-1, js379*, a predicted functional null, is highly resistant to emodepside. Transgenic *slo-1(js379)* animals expressing wild type copy of *slo-1* behind the native *Pslo-1* promoter have the same sensitivity to emodepside as wild type *C. elegans* with IC50 of 20nM. *Slo-1* belongs to a family of highly conserved potassium channels that regulate cell excitability throughout animal phyla. Bioinformatic analysis revealed a human *kcnma1* channel as a closest mammalian homologue to nematode *slo-1*. SLO-1 and KCNMA1 share 55% identity and 69% similarity in primary amino acid sequences. To investigate selective toxicity of emodepside to the parasites we expressed a closest mammalian homologue *kcnma1* in *slo-1(js379)* mutants. Behavioural assays confirmed that *kcnma1* is a functional homologue of *slo-1* and functions when expressed in *C. elegans*. Conversely, transgenic worms carrying the mammalian homologue are not effected by 1□M and only slightly sensitive to 10□M emodepside. This is well above clinically used doses and thus identifies emodepside as a selectively toxic drug to the worm. Furthermore, to elucidate a mode of action of emodepside we characterised effects of known activators of mammalian BK channels, NS1619 and Rottlerin on locomotion of wild type and transgenic *C. elegans*. In behavioural assays *C. elegans* carrying *kcnma1*, but not *slo-1* or wild type were sensitive to NS1619 after 3 hours exposure and exhibited uncoordinated movement. Rottlerin slowed locomotion of all transgenics and wild type worms after 24 hours exposure. *Slo-1(js379)* mutants were not sensitive to any of the drugs tested. Taken together these data are most parsimoniously explained by a mode of action in which emodepside directly interacts with the nematode calcium-activated potassium channel *slo-1* and has very low affinity to its mammalian homologue *kcnma1*. A mode of action of emodepside is being further investigated using electropharyngeogram (EPG) recordings from the pharyngeal muscle of wild type and transgenic *C. elegans*.

12. Identification, characterization and functional analysis of immunomodulatory molecules from *Schistosoma mansoni*

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Helminth infections can suppress or exacerbate unrelated inflammatory responses in humansand various experimental animal models. *Schistosoma mansoni* is a human parasite that is apotent modulator of the immune system, including the potential suppression of allergicresponses. Previously, we have shown that infection with male schistosomes can ameliorateinflammatory diseases in mouse models of anaphylaxis, asthma and inflammatory boweldisease. *S. mansoni* male worms or worm excretory-secretory (WES) molecules werecultured *in vitro* with cells from different reporter mice to characterise the generation ofsuppressive cell populations. A defined B regulatory cell population was generated *ex vivo* by*S. mansoni* worms and WES, with the cells suppressing allergic inflammation *in vivo* via thegeneration of Tregulatory cells *(*Amu *et al* JACI 2010*)*. We have subjected WES to functionalscreening and proteomic analysis to identify immunomodulatory molecules. The male *S.mansoni* WES proteome has a repertoire of molecules associated with known and unknownmechanisms of modulation of immune function. Targets from functional screens werecharacterized and expressed as recombinant protein, using insect cell expression system, forfurther analysis. Schistosome-derived molecules with novel immunomodulatory activity willbe descried.

13. *Ancylostoma* spp: Heat shock response during the transition to parasitism

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The hookworms *Ancylostoma duodenale* and *Necator americanus* infect approximately 800 million people world-wide, leading to severe iron-deficiency anemia in heavy infections. Children, pregnant women and the elderly of rural and poor populations in tropical and subtropical areas are particularly vulnerable. People acquire the infection through exposure to soil contaminated by the developmental arrested infective third larval stage (iL3). During invasion, host derived cues re-initiate developmental pathways in the arrested iL3 that culminate in the reproductive stage and therefore essential for the completion of the life cycle. The molecular events of the transition from the free-living L3 to the parasitic L3 are poorly understood. Negative regulation of the class O forkhead transcription factor (FoxO) DAF-16 by the insulin like signaling (ILS) plays a pivotal role in the exit from the developmentally arrested dauer in the model nematode *C. elegans*. During infection, L3 are exposed to a sudden increase in temperature associated with entry into the homoeothermic host. This temperature increase is required for activation, suggesting a possible role for heat shock in the infectious process. In response to heat shock, organisms synthesize stress proteins and chaperones that stabilize denatured proteins and protect against cellular damage. This heat shock response (HSR) is mediated by the heat shock factor (HSF) 1 transcription factor, and negatively regulated by the HSF binding protein (HSB) 1. HSF-1 is also required for normal development and entry into the arrested dauer stage, and shares several target genes with DAF-16. Given the essential role of HSF-1 and DAF-16 in hookworm infection, we have begun characterizing the role of HSF-1 and the HSR in the hookworm infectious process.

14. Protein biosynthesis and lifespan determination in parasitic nematodes: a bioinformatic analysis.

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Experimental down-regulation of protein biosynthesis has been shown to extend lifespan in several organisms, including *Caenorhabditis elegans*. Microarray and Q-PCR analysis of *Strongyloides ratti* and *Parastrongyloides trichosuri* has shown that genes of the Gene Ontology (GO) protein biosynthesis class showed the greatest degree of transcriptional down-regulation in comparisons between free-living and parasitic life cycles. This is consistent with a down-regulation of protein biosynthesis being a component of the >50-fold extension in lifespan between free-living and parasitic adults in these species. To investigate this correlation further, we have analysed to full complement of parasitic nematode ESTs at dbEST. This comprised >1 million sequences from >20 species. The ESTs were assigned to "pre-parasitic" (outside the host) or "parasitic" (infective larvae, and stages inside the host), and the parasite orthologues of all protein biosynthetic machinery components (ribosomal proteins, initiation, elongation and termination factors etc) extracted by BLAST using the *C. elegans* protein biosynthesis machinery genes as query sequences. These ESTs were then used to conduct a "digital northern" analysis across all parasite species in dbEST. This analysis showed that there is a significant decrease in the relative abundance of transcripts encoding protein biosynthesis machinery in parasitic stages as compared to pre-parasitic stages. This observation is suggests that the down regulation measured directly by microarray and Q-PCR in *S. ratti* and *P. trichosuri* is a general feature of all parasitic nematodes, and may be correlated with the long lifespans of many parasites.

15.Proteomic, Transcriptomic and Genomic Analysis of *Heligmosomoides polygyrus*

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We have undertaken an integrated genomic, transcriptomic and proteomic study of the mouse intestinal nematode *Heligmosomoides polygyrus*, to provide a resource for parasite research and to identify the major secreted products released by the immunomodulatory adult worm. The genomic characterisation is currently underway with approximately 24 million paired-end reads from which a preliminary assembly has 708,000 contigs representing 280 MB. (N50 = 424bp, with 19,000 contigs >1 kb). The transcriptomic dataset is constructed at two levels. For the adult cDNA (taken at 14 days post-infection), we have analyzed both normalized and non-normalized cDNA libraries (280,000 and 170,000 reads respectively), to provide a deep profile of rarer transcripts as well as enabling us to conduct a frequency analysis of the most abundant mRNA products. Cluster analysis of the the non-normalized adult cDNA dataset produced ~2,500 isotigs (combinations of contigs likely to derive from the same gene). Addition of the normalized library resulted in a total of 20,235 isotigs, of which ~6,000 are >1 kb in length, and which in total represent 16.6 MB of sequence. Sequence variation likely to represent polymorphism is very evident. We are now analyzing the non-normalised cDNA from eggs, L3 infective larvae, d3 and d5 larvae (the latter being L4 stage), to provide a snapshot of gene expression patterns through the life cycle. Small RNAs have also been sequenced, and both known miRNAs (eg miR-1 and miR-100) and new potential miRNA species identified. The proteomic dataset has a specific focus on products secreted by the adult worm: over 100 secreted components have been matched to the transcriptomic dataset. While most of the analysis is still in progress, the most striking feature has been the prominence of VAL/ASP family members in the adult worm transcriptome and secretome. Some 20 distinct genes have been identified, of which 10 are known encode secreted proteins, and 4 (named VAL-1 to -4) are both dominant secreted products and targets of immundominant serum antibody responses in murine infection.

16. Bioinformatical analysis and genetic diversity of P-glycoproteins in ivermectin-susceptible and resistant *Parascaris equorum*

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Parascaris equorum is a gastrointestinal nematode parasitizing equine hosts. In foals and yearlings it may cause severe small intestinal diseases. Due to its widespread occurrence, high prevalence and pathogenicity, *P. equorum* has become one of the most important parasites of young horses. The most common antiparasitic compound currently used for treatment is the broad spectrum anthelmintic ivermectin, a macrocyclic lactone (ML). However, due to its frequent and often prophylactic use, it has lost potency and resistant *P. equorum* populations have been reported from several countries. Drug resistance can be evoked by unspecific detoxification mechanisms often involving activity of ABC-transporters such as P-glycoproteins (P-gps) leading to increased xenobiotic elimination. MLs can compete with transport of typical P-gp substrates in nematodes suggesting that they can be detoxified by extrusion. In comparison to vertebrates, nematodes posses a large number of different P-gp genes in their genome (e.g. 14 for *Caenorhabditis elegans)*, however, to date no P-gp sequences have been published for ascarids. To analyze the potential involvement of P-gps in ML resistance in *P. equorum*, it is important to determine P-gp coding sequences. Nested RT-PCR using degenerated primers resulted in amplification of several P-gp cDNA fragments. Full length sequences were obtained by 5' and 3' RACE-PCR for two *P. equorum* P-gps with open reading frames encoding P-gps of 1284 and 1247 amino acids, respectively. Blast analyses of deduced amino acid sequences against nematode sequence databases suggested that the first *P. equorum* P-gp is most closely related to *pgp-3* of *C. elegans* (Accession-No.: NP_509901). The second showed the highest similarity to a P-gp-like cDNA of *Onchocerca volvulus* (Accession No.: AAD49436). Both proteins have a typical P-gp architecture with two similar halves, each containing six transmembrane helices and an intracellular ATP binding site in a nucleotide binding domain (NBD). More detailed phylogenetic analyses revealed that the first protein sequence is more closely related to a putative Pgp-16 of *Cooperia oncophora* (unpublished data) than to the Pgp-3/Pgp-4 subfamily. Apparently, no Pgp-16 is encoded in the genome of *C. elegans*, but there is a Pgp-16 like entry in the *Caenorhabditis briggsae* database (Accession-No.: CBG12969 in WormBase). The second *P. equorum* P-gp sequence described here and also the *O. volvulus* P-gp-like protein are orthologs to *Cel*Pgp-11 and should therefore be designated as *Peq*Pgp-11 and *Ovo*Pgp-11. Currently, real-time RT-PCR is used to compare expression *Peq*Pgp-11 and *Peq*Pgp-16 in ML-sensitive and resistant populations. To identify single nucleotide polymorphisms (SNPs) that might be associated with developing resistance, *Peq*Pgp-11 and *Peq*Pgp-16 sequences were compared between several ML-sensitive and resistant populations by SeqDoC analyses. Several informative SNPs were identified showing different allele frequencies in *P. equorum* populations under investigation. However, further functional studies are required to provide conclusive evidence if different alleles differ with regard to affinity for anthelmintics, in particular to ivermectin. Therefore, it is planned to study transport of anthelimintics after expression of P-gps in yeast cells and to desensitize *C. elegans* to anthelmintics by overexpression of *P. equorum* P-gps. This work is supported by the Deutsche Forschungsgemeinschaft (SA973/ 3-1).

17. Regulation of immune response in mouse dendritic cells by the treatment of *Clonorchis sinensis* crude antigen

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Dendritic cells (DC) are regarded as the most potent antigen presenting cells which are related with innate and adapt immunity. After taking up pathogens, DCs will initiate expression of cell surface markers and the secretion of cytokines. The production of cytokines were analyzed and we found that IL-10 and TGF-β production were significantly enhanced in both bone marrow-derived dendritic cells and mouse dendritic cell line, DC2.4 after treated with *Clornorchis sinensis* crude antigen. However, expression patterns of several activation molecules were not changed. In addition, when the DC2.4 cells were treated with *Paragonimus westermani* crude antigen, the production of IL-10 and TGF-β were increased significantly but no induction was noticed in sparaganum (SP) and Cystcercoid (CY) crude antigen treated groups. We also found that DC2.4 cells treated with *C. sinensis* crude antigen resulted in rapid and significant phosphorylation of the mitogen activated protein kinase, extracellular signal-regulated kinase 1/2. Treatment of DC2.4 cells with an extracellular signal-regulated kinase specific inhibitor, led to inhibit interleukin-10 and TGF-β production following *C. sinensis* crude antigen in DC2.4 cells. Our results suggest that crude antigen from *Clonorchis sinensis* have a role in the anti-inflammation of DC cells by producing IL-10 and TGF-β via activation of extracellular signal-regulated kinase 1/2.

18. Investigating the effects of compounds with anthelmintic potential: Amidantel, Bay d 9216 and Tribendimidine.

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Amidantel (Bay d 8815) and its deacylated derivative (Bay d 9216), have been shown to act as agonists at the nicotinic acetylcholine receptor in electrophysiological studies using muscle strips of the parasitic nematode, *Ascaris suum,* and neurones from the *Helix aspersa* snail. These compounds and a further derivative, tribendimidine, have been found to have anthelmintic activity against a range of noteworthy parasitic nematode infections in vivo, including hookworm and ascariasis. The efficacy of amidantel and its derivatives was comparable to currently available drugs. The model genetic organism *Caenorhabditis elegans* has been employed to determine the effects of amidantel, Bay d 9216, and tribendimidine. The predominant effect of these compounds in wild type *C. elegans* is an inhibition of motility. This effect has been observed in body bend and thrashing assays, inhibition is equivalent to that seen with levamisole. Egg laying behaviour is increased on exposure to each of the compounds, both effects that are associated with disruption of neuromuscular transmission. No effect on pharyngeal pumping or developmental timing was observed with any of the compounds. Exposure of wild type worms from the first larval stage through to adulthood, to tribendimidine or levamisole, did not have any effect on the timing of development. Motility was notably affected at L4 stage and the adult worm length was reduced by more than 60% compared to control worms, it remains to be clarified whether this is a morphological or contractile affect. The kinetic profile of these compounds is currently being explored to reveal any differences between the actions of amidantel, Bay d 9216, tribendimidine or levamisole. Such differences will give clues as to the molecular target(s) of these compounds, In conjunction with investigations using wild type *C. elegans,* a reverse genetic screen using transgenic strains mutated in genes expressing acetylcholine receptor subunits or their associated proteins is being undertaken with a focus on those strains with reported resistance to levamisole. Preliminary results suggest an inhibition of motility in some of the levamisole resistant strains. Susceptibility to any of these compounds in levamisole resistant strains would imply a resistance breaking mode of action.This work is funded by Bayer Healthcare AG, Monheim, Germany

19. Structural and ligand binding studies of the nematode-specific FAR and nemFABP small lipid binding proteins

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Nematodes produce, and in some cases secrete, lipid binding proteins (LBPs) of types that are found in no other group of organisms. Three types of small LBP stand out in particular. First, the nematode polyprotein allergens (NPAs), the structure of a typical member of which is known (the ABA-1 allergen of *Ascaris*). Secondly, the fatty acid and retinol binding proteins (FARs) that are major secretory products of parasitic stages, and occur in seven isoforms in *C. elegans* (Ce-FAR-1 to -7). FAR-1s are of particular interest, having been found as abundant components of the secretions of several parasitic nematodes. A crystal structure of one FAR (Ce-FAR-7) is known, but the amino acid sequences of FAR-1s do not model successfully to this structure. So, FAR-1s and FAR-7s probably differ significantly in structure. The last of the unusual, nematode-specific LBPs are the nemFABPs. These are similar to the β-strand rich fatty acid binding proteins (FABPs) whose 3-D structures are virtually congruent in all other animal phyla, and which are not secreted by the synthesizing cells. But, nemFABPs are unique in being secreted (e.g. into the perivitelline fluid of eggs in both oviparous, such as *Ascaris,* and ovoviviparous species, such as *Brugia and Onchocerca*, and are structurally modified from the protein fold typical of other FABPs. We have obtained protein nuclear magnetic resonance spectra of the FAR-1 of the human hookworm, *Necator americanus,* and a nemFABP of *Ascaris,* that should allow us to solve the 3-D structure of these unusual proteins, and identify the amino acids that interact with lipid ligands, some of which are likely to be unusual. Supported by The Wellcome Trust (UK).

20. A combined study applying microarray and Super-SAGE detects pairing-dependent transcription in *Schistosoma mansoni* males

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In the unique reproduction biology of schistosome parasites male worms hold the key role. Without the constant pairing contact to a male partner no differentiation processes occur in the reproductive organs of the female. The female gonads even dedifferentiate upon separation from the male. Although this phenomenon is long known, the molecular basis is still unknown and a proposed-male factor stimulating female development remains unidentified. Classical experiments demonstrated that pairing-experienced (e-)males are faster to stimulate mitotic activity in females than pairing-unexperienced (u-)males. From this it can be concluded that u-males have to reach a status of competence before they are able to induce developmental processes in the females. First results from our studies applying a non-radioactive method for measuring mitotic activity in adult worms kept *in vitro*, supported this assumption that pairing has an influence also on males. The present study aims at the identification of molecules and molecular networks contributing to the genesis of the male-competence or playing roles in the production of a female-stimulating factor. To this end three biological replicas of (e-)male and (u-)male transcriptomes each were comparatively analyzed by 44k microarrays and Super-SAGE. Genes that showed significant differential transcription between the two male groups in both data-sets were selected for *in silico* analyses. The according transcripts are predicted to code for i.e. transcription factors, surface proteins or signal transduction molecules. Allocation of selected proteins to larger networks will help to identify pathways contributing to the male status of competence. Further studies will include the verification of transcription of genes of selected pathways by real-time PCR as well as *in situ* hybridization. Recent results will be presented.

21. VAL proteins: Towards a function…

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Immune evasion is a general strategy employed by helminths to facilitate their survival with the host, and may be mediated by parasite secreted products. The function of some secreted proteins is suggested by sequence similarity, for example to host cytokines such as MIF and TGF-β. However, among the most prominent secreted proteins are those of the VAL family, which belong to a phylogenetically ancient SCP/TAPS gene superfamily. We have found that both the human filarial parasite *Brugia malayi* and the mouse intestinal nematode *Heligmosomoides polygyrus* secrete these proteins. Originally found in dog hookworm, homologues have since been found in many other species such as *Necator americanus*, *Meloidogyne incognita* and *C. elegans*. The VAL proteins are expressed at high levels and often at critical points in the parasite lifecycle, however as yet no single function has been found. We are examining the functions of VAL proteins using three approaches, testing the hypothesis that they are involved in the down regulation of the host immune system thus achieving a "parasite friendly" environment. Firstly, we have generated monoclonal and polyclonal antibodies, which in the case of *H. polygyrus* bind to the adult cuticle, and are being tested for blocking parasite effects in vivo. Secondly, using an insect cell expression system recombinant VAL proteins from both *B. malayi* and *H. polygyrus* are being used to modulate host immune cell responses *in vitro,* illustrated by higher IL-10 production from murine cells. Finally, transfection by homologous recombination within *Leishmania* parasites will allow examination of the effect of VAL genes in vivo against the template of the well-characterised Th1/Th2 balance in the immune response to *L. major* infection.

22. Venus kinase receptors of *Schistosoma mansoni*

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VKRs are atypical Receptor Tyrosine Kinases (RTK) composed of an extracellular Venus FlyTrap (VFT) module linked through a single transmembrane domain to a tyrosine kinase domain. The association of these two domains was described for the first time in *Schistosoma mansoni* with the characterization of SmRTK-1, later renamed SmVKR-1 (Vicogne et al, 2003; Ahier et al, 2009). Recently, we showed that SmVKR-1 belongs to a novel RTK family that includes diverse members in invertebrates mostly present in insects. Structural and phylogenetic studies performed on VFT and TK sequences demonstrated that VKR sequences form monophyletic groups, the VFT group being close to that of $GABA_B$ receptors, which belong to the family of GPCR-coupled receptors, and the TK one being close to that of insulin receptors, which represent RTKs. In accordance with the fact that both GPCRs and RTKs function as dimers, recombinant VKRs were expressed as dimers at the cell surface, and we could also show that were activated by amino-acids (Ahier et al, 2009). By in silico analyses of the recently published *S. mansoni* genome data set (Berriman et al, 2009) we identified a second *vkr* gene *(Smvkr-2)*, which has a common organization and is similar (30% sequence identity) to *Smvkr1*. *S. mansoni* is the only species so far, in which a second *vkr* gene has been detected. We have demonstrated that SmVKR proteins can activate kinase signalling cascades in *Xenopus* oocytes and the hypothesis that SmVKR1 and SmVKR2 could constitute active heterodimers is currently investigated. Both genes are expressed in all the parasite stages, and are abundantly transcribed in miracidia and sporocysts. In adult worms, results of *in situ* hybridizations indicated a concentration of *Smvkr-1* transcripts in mature oocytes of female schistosomes. The biological function of VKR proteins is still unknown but their presence in larvae and the reproductive organs of adults suggests that they could play a role in development and/or reproduction. As VKR are absent from vertebrate hosts, SmVKRs might represent novel specific targets against schistosomes.

23. Isolation, characterization and cDNA sequencing of *Anisakis pegreffii* haemoglobin

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Anisakis pegreffii is a parasitic marine nematode which can cause gastrointestinal disease and allergic reactions if accidentally consumed. It is closely related to the human roundworm, *Ascaris lumbricoides*, which is known to possess a novel haemoglobin molecule that binds oxygen 25 000 times more tightly than human haemoglobin and is thought to protect the worm against host oxidative defences.Recently we generated a monoclonal antibody (4/E8g) against *A. pegreffii* that was found to bind nematode haemoglobin from several species, including *Ascaris lumbricoides*, *Contracaecum* sp. and *Nippostrongylus brasiliensis*. *A.pegreffii* haemoglobin was identified as a 37kDa protein while haemoglobins of *A.lumbricoides* and *N. brasiliensis* were approximately 40kDa and 37kDa, respectively. Interestingly, haemoglobin of *Contracaecum* appeared to have a higher molecular weight of approximately 50kDa. Degenerate PCR and RACE-PCR were used to obtain the cDNA sequence of *A. pegreffii* haemoglobin. Sequence data showed that *A. pegreffii* haemoglobin displayed the strongest similarity to that of *Pseudoterranova decipiens*. Immunoprecipitation with 4/E8g was used to purify haemoglobin from *A. lumbricoides* and *A. pegreffi* and these proteins were investigated for immunogenicity in mice and humans. *Anisakis* haemoglobin was immunogenic and allergenic in mice, but was not recognized by fish processing workers who were exposed to worm antigens. However, we are planning future studies to evaluate its immunogenicity in patients with proven anisakiasis. Importantly, we found that *Ascaris* haemoglobin was immunogenic in humans. Immunohistochemical staining with 4/E8g showed that haemoglobin from *Anisakis* and *Ascaris* are both somatic and excretory-secretory proteins, which may support the putative role for haemoglobin in detoxification of the environment and/or protection against reactive oxidative intermediates produced by the host. In conclusion, we have generated a monoclonal antibody that can be used to purify haemoglobins from several nematode species for use in immunological and functional studies. *A.pegreffii* and *A.lumbricoides* haemoglobins are both somatic and excretory products and currently we are investigating their role in parasite viability and protection against host defences.

24. Immunomodulatory molecules from *Teladorsagia circumcincta*

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Teladorsagia circumcincta, a parasitic nematode that inhabits the abomasum of small ruminants, primarily causes disease in lambs. This parasite is endemic in temperate regions of the world and is currently the major cause of parasitic gastroenteritis (PGE) in sheep in the UK. Natural immunity against *T. circumcincta* does develop after prolonged exposure however, within an experimental model, the development of protective immunity requires a trickle infection of several thousand infective larvae (L3) repeatedly over several months. The requirement for prolonged exposure and continuous infections to produce protective immunity against *T. circumcincta* suggests that the nematode is able to actively manipulate the host immune response to permit worm establishment over an extended period before protective immunity eventually overcomes this regulatory manipulation. Parasitic nematodes produce a variety of host-immunomodulatory factors on their surfaces or in excretory and secretory (ES) products and these are thought to have a role in worm establishment, survival and immunomodulation. Several studies have indicated that a number of parasitic nematodes produce macrophage migration inhibitory factor (MIF) orthologues and we have also recently demonstrated the presence of a putative immunomodulatory Ca^{2+} dependent apyrase (Tci-APY-1) in the ES material of *T. circumcincta*. We will present data describing the stage-specific expression of the Tci-MIF-1 and Tci-APY-1 molecules, their biochemical and biological activities and, based on these parameters, their implied roles in immunomodulation of the host.

25. The phylogenome of *Schistosoma mansoni*: the evolutionary history of proteins.

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Schistosoma mansoni is a Platyhelminth parasite responsible for human schistosomiasis, a tropical neglected disease that affects 210 million people in 76 countries. The parasite predicted proteome (www.*SchistoDB.net*) contains over 11,000 proteins, which remain vastly experimentally uncharacterized. The reconstruction of the evolutionary histories of all proteins encoded in the genome (phylome) of *S. mansoni* was performed through a pipeline as implemented in *PhylomeDB*. Our results covered the analysis of 8.818 phylogenetic trees of 13.285 *S. mansoni* predicted proteins and their homologs in 16 other organisms, including a plant, fungi, nematodes, arthropods, urochordates, and cephalochordates. Using this phylogeny-based approach, we could transfer functional annotations from Gene Ontology (GO) to 5.587 *S. mansoni* proteins, 956 that were previously annotated as "hypothetical" or "expressed protein", corresponding to proteins not experimentally tested. This approach has provided us with a genome-wide view of *S. mansoni*, indicating which genes were gained, lost, or duplicated in this parasite in respect to the other organisms over evolutionary time, providing insights into the parasitic lifestyle of schistosomes. All sequence alignments, phylogenetic trees, and functional annotation will be made publicly available through *PhylomeDB* website at http://phylomedb.org/ providing a powerful resource for the phylogenomics community.

26. Targeting sterol biosynthesis in helminths for the development of new chemotherapy

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Parasitic filarid worms scavenge host cholesterol and use sterol biosynthesis to produce molting hormones. Sterol biosynthesis has proven to be an exploitable metabolic pathway for the development of drugs to treat chronic fungal and protozoan infections. We therefore explored targets within filarid worm sterol biosynthesis pathways using a known inhibitor of CYP51, posaconazole, as a probe. Posaconazole was cidal for filarid worms in micromolar concentrations. It also had an effect on the "surrogate" free-living nematode, *C. elegans*, resulting in a decrease in fecundity and worm size in progeny. Based on these findings, we propose that the target of posaconazole in filarid worms is an enzyme involved in hormone production for adult worm homeostasis as well as molting and embryogenesis.

27. Development of RNA interference in *Hymenolepis*

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The machinery for RNA interference is known to be present in the genome of the tapeworm *Echinococcus* (K. Brehm, pers. comm.). Recently, effective knock down of a housekeeping gene was shown in the sheep tapeworm *Monezia* (Pierson et al., 2009). Although some classes of genes investigated in the latter study (e.g. neurotransmitters) seemed not to be susceptible to RNAi, it is apparent that the mechanism operates in cestodes. Our study is focused on developing RNAi in the model tapeworm *Hymenolepis microstoma* and aimed at characterization of loss-of-function of *hox* genes phenotypes. In particular we are interested in arbitrating between cause and consequence for genes exhibiting segmental expression patterns such as *abdA* and *post-2*. We will first test the RNAi pathway, demonstrating knock down of a housekeeping gene (e.g. actin-1) using dsRNA delivered by electroporation and/or soaking of cysticercoids (an encysted larval form of the worm) derived from the haemocoel of intermediate host *Tribolium confusum*. Treated and untreated cysticercoids will be cultured *in vitro* allowing us to monitor associated changes both in morphology and gene transcript levels (via qPCR). At present we have successfully cultured larvae from encysted cysticercoids to non-strobilate juvenile worms for aprox. 10 days. We are working towards full development *in vitro* along side with reproduction of the entire worm life cycle (i.e. egg to adult) in culture, as it was first demonstrated in the 1970s.

28. "I've got you under my skin" – Differentially expressed genes between cercariae and skin schistosomula identified in *Schistosoma mansoni* using sequencing technologies.

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Schistosoma spp. are helminth parasites responsible for schistosomiasis, a neglected tropical disease endemic in sub-tropical regions of Africa, Brazil, central America and regions of south east Asia. It causes serious morbidity, mortality and economical losses. Praziquantel is the front line drug for treatment and, although very effective, it does not prevent re-infection and the possible emergence of resistant strains is a constant threat. Draft reference genome sequences for *S. mansoni* and *S. japonicum* have been recently published and will undoubtedly become an essential resource in the search for new drug targets and vaccine candidates for control of schistosomiasis. Previous studies have been successful in the identification of gene structures and in the study of differentially expressed genes throughout the parasite's life cycle. However, little is known about the regulation of gene expression during the transformation of the cercariae into the skin stage of the parasite. There have been recent publications featuring microarray studies that look at this transformation. These studies were performed using microarrays and therefore have drawbacks that are inherent to the technology: 1) custom-made arrays make it difficult to compare results across laboratories; 2) microarrays were designed using mainly ESTs databases and therefore are biased towards the measuring of already identified genes, genes highly expressed and genes expressed in life stages other than the skin stage. In addition, these studies used mechanical methods (disruption of the head-tail junction by sheer force and osmotic shock) to obtain the schistosomula stage. In our study of the skin stage, we make two major improvements. First, we use sequencing technology to quantify gene expression. Second we use skin-transformed schistosomula, rather than mechanically transformed, to more closely resemble physiological conditions. Here, cercariae are allowed to penetrate through a freshly excised layer of mouse skin. We used the current genome assembly to identify new genes expressed in these stages and quantify gene expression using the whole repertoire of predicted gene models. Differential expressed genes between the cercariae and the skin transformed schistosomula and between mechanical and skin transformed schistosomula were identified. This study represents the first high throughput transcriptome analysis done by sequencing in the more natural skin transformed schistosomula.

29. *Taenia solium* and *T.crassiceps* capacity to synthesize and interconvert sex steroids hormones. 17β-hydroxyesteroid dehydrogenase is expressed in the cysticerci.

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The larval (cysticerci) stage of *Taenia solium* (*Ts*) develops in pork meat and causes severe disease in the human nervous system. The worms developed from cysticerci have reproductive units called proglottids that contain testis and ovaries in different stages of development. We have demonstrated the ability of cysticerci to synthesize steroid hormones *in vitro*. Using histochemistry and thin layer chromatography (TLC) we have also recently shown that *Ts* and *T. crassiceps* WFU (*Tc*WFU) taenias and cysticerci have a functional 3β-hydroxysteroid dehydrogenase, a key enzyme in the steroidogenic pathways involved in the synthesis of androgens and estrogens in vertebrates. The 17β-hydroxysteroid dehydrogenases (17β-HSDs) are also key enzymes that participate in the formation and the inactivation of sex steroids, having reductive and oxidative functions. In vertebrates the reductive group synthesizes active androgens and estrogens, the oxidative groups inactivate the steroids. The aim of this work was to study the ability of cysticerci and worms from *Ts* and *Tc*WFU to synthesize androgens in the Δ4 steroidogenic pathway and the capacity to interconvert androgens and estrogens, a function performed by 17β-HSDs. In this regard, the expression of this enzyme has been studied by RT-PCR in *Ts* cysticerci. For this purpose *Ts* and *Tc* WFU worms were grown in the intestine of experimentally infected hamsters. Thirty to sixty days postinfection, the worms were recovered and thoroughly washed in PBS plus antibiotics/antimycotics. The parasites were incubated in DMEM plus antibiotics/antimycotics plus tritiated steroid hormone precursors (Progesterone (P_4), Androstenedione (A_4), Estrone (E_1) and 17β-estradiol (E_2)) for 3h at 37°C. Vials containing DMEM and only the tritiated steroid precursors (blanks) were simultaneously incubated. Blanks and parasite culture media were ether extracted and analyzed by thin layer chromatography (TLC) in a dichloromethane-ethyl acetate (8:2 v/v) system. Data are expressed as percent transformation of the tritiated precursors. For RT-PCR studies total ARN was extracted with TrisReagent from *Ts* cysticerci dissected from pig muscles. RT-PCR was performed by SuperScript™ One-Step RT-PCR with Platinum® Taq using gene specific primers designed from a EST sequence identified in the larval library of the *Taenia solum* Genome Project. TLC results showed that *T. solium* and *T. crassiceps* worms synthesize androgens and estrogens from ^{3}H-P4. In addition they interconvert ^{3}H-E_1 into ^{3}H-17β-E_2 and conversely, an observation which strongly suggests the activity of 17β-HSDs. The expression of a 17β-HSD RNAm was found in *T. solium* cysticerci. The sequence has at least 78% homology with 17β-HSD cDNAs of rats, mice and the *Schiquistoma mansoni.* In summary, the present results show for the first time that cestode worms have the ability to synthesize androgens and estrogens by the Δ4-steroidogenic pathways, and they also have the capacity to inactivate these hormones by converting them to weak molecules, or to activate the weak hormones to provide active sex steroids. Finally we have found the RNAm expression of a 17β-HSD that is probably involved in the sex steroid inter-conversion referred above. Partially financed by CONACyT grant # 69347. The authors acknowledge MVZ José A. Jiménez for technical assistance.

30. Monepantel allosterically activates DEG-3/DES-2 channels of the gastrointestinal nematode *Haemonchus contortus*

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Monepantel is the first drug of a new family of anthelmintics, the amino acetonitrile derivatives (AAD), presently used to treat ruminants infected with gastrointestinal nematodes such as *Haemonchus contortus*. Monepantel shows an excellent tolerability in mammals and is active against multidrug-resistant parasites, indicating that its molecular target is (i) absent or inaccessible in the host and (ii) different from those of the classical anthelmintics. Genetic approaches with mutant nematodes have suggested acetylcholine receptors of the DEG-3 subfamily as the targets of AADs, an enigmatic clade of ligand-gated ion channels that is specific to nematodes and does not occur in mammals. Here we demonstrate direct interaction of monepantel, its major active metabolite monepantel sulfone, and other AADs with potential targets of the DEG-3 subfamily of acetylcholine receptors. *Haemonchus contortus* DEG-3/DES-2 receptors were functionally expressed in *Xenopus* oocytes and found to be preferentially activated by choline, to permeate monovalent cations, and to a smaller extent, calcium ions. While monepantel and monepantel sulfone did not activate the channels by themselves, they substantially enhanced the late currents after activation of the channels with choline, indicating that these AADs are type II positive allosteric modulators of *H. contortus* DEG-3/DES-2 channels. Interestingly, the inactive, R-enantiomer of monepantel inhibited the late currents after stimulation of *H. contortus* DEG-3/DES-2 receptors with choline. In summary, we present the first direct evidence for interaction of AADs with DEG-3 type acetylcholine receptors and discuss these findings in the context of anthelmintic action of AADs.

31. Genes associated with transition from free-living to parasitic stages of the bovine lungworm *Dictyocaulus viviparus*

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Dictyocaulus viviparus, the bovine lungworm is the causative agent of parasitic bronchitis in cattle. Transition from the infective free-living third-stage larvae to the parasitic stage living in the host requires a substantial shift in gene transcription to adapt to the new environment since the parasitic larva has to migrate to his definitive settlement, starts to feed, and is confronted with the host´s immune system. To identify genes involved in this fundamental change of the bovine lungworms way of life Suppression subtractive hybridization using free living L3 and parasitic L5 was performed followed by Differential screening and verification via Virtual Northern blot. Stage-specific upregulated transcripts and respective protein sequences were bioinformatically processed by clustering, BLAST, and gene ontology search, domain/motif search and mapping to biological pathways. From the 400 sequenced clones from the parasitic larvae 372 ESTs were obtained which were clustered into 68 representative ESTs (rESTs) consisting of 30 Contigs and 38 Singletons. Sequence comparison revealed that the majority of the upregulated transcripts including the Top1-contig encoded cathepsins and other cysteine proteases which are known to play important roles in tissue migration, digestion and immune evasion or regulation of the host´s immune response, respectively. Gene ontology terms could be assigned to about the half of the rESTs whereas 17 rESTs containing amongst others the Top3-contig represent novel parasite or even *Dictyocaulus* specific genes. Interestingly, transcripts upregulated in infective L3 compared to parasitic L5 mainly encoded rRNA or RNA modifying proteins, enzymes or the like were not identified. This transcriptional status seems to mirror the standby position of this stage which remains on pasture without feeding or further development only waiting for ingestion by the host.

32. A granulin-like growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini*, promotes proliferation of host cells

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The human liver fluke, *Opisthorchis viverrini*, infects millions of people throughout South-East Asia and is a major cause of cholangiocarcinoma (bile duct cancer). The mechanisms by which the parasite causes cancer are multi-factorial, but one process is the secretion of mitogenic parasite proteins into the bile ducts, driving cell hyperproliferation and creating a tumorigenic environment. Using proteomics, we identified a homologue of human granulin, a growth factor involved in cell proliferation and would healing, in the secretions of the parasite. *Opisthorchis* granulin, termed *Ov*-GRN-1, was expressed in most parasite tissues, particularly the gut and tegument. Furthermore, *Ov*-GRN-1 was detected *in situ* on the surface of biliary epithelial cells of hamsters experimentally infected with *Opisthorchis*. Recombinant *Ov*-GRN-1 stimulated proliferation of human cholangiocarcinoma and non-cancerous bile duct cell lines at nanomolar concentrations and antibodies against *Ov*-GRN-1 inhibited the ability of *Opisthorchis* secretions to induce proliferation of fibroblasts and a human cholangiocarcinoma cell line *in-vitro*, indicating that *Ov*-GRN-1 is the major growth factor in *Opisthorchis* secretions. This is the first report of a secreted growth factor from a parasitic worm that induces proliferation of host cells, and supports a role for this fluke protein in establishment of a tumorigenic environment that may ultimately manifest as cholangiocarcinoma.

33. Stage dependent transcriptional changes of *daf*-12 during the life cycle of *Dictyocaulus viviparus*

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The nuclear steroid hormone receptor *daf*-12 regulates lifespan in the free living soil nematode *Caenorhabditis elegans*. In favourable environment the presence of a yet unidentified hormone specifies reproductive development whereas in unfavourable environment unliganded *daf*-12 drives dauer formation and thus retarded aging. Orthologues of this receptor have yet been identified in *Strongyloides* and *Ancylostoma* spp. As in *C. elegans*, activation of the parasite´s DAF-12 induced recovery from the dauer-like infective L3 stage and initiated development into parasitic individuals by initiating feeding behaviour. We now identified *daf*-12 in the bovine lungworm *Dictyocaulus viviparus*, one of the most important parasites of cattle. This identification is a first step in answering the question whether hypobiosis of the bovine lungworm is regulated in the same manner as dauer formation in *C. elegans* or if there is an independent genetic regulation of this phenomenon. Semiquantitative PCR using cDNA of eggs, L1, L3, male and female L5 and adults, respectively, as well as hypobiotic L5 revealed the strongest *daf*-12 transcription in L3 and adult female worms. This might indicate that the process of hypobiosis is driven by a signal cascade different from the dauer pathway. To further clarify the role supposed for *daf*-12 in hypobiosis, quantitative real time PCR using TaqMan minor groove binder probes will be performed using 17 developmental lungworm stages, namely eggs; L1, L2 and L3 from 9 different dates post shedding; male, female and mixed L5; male and female adults as well as hypobiosis induced L3 and hypobiotic L5. The transcriptional changes to be revealed by this analysis will provide further knowledge about the regulatory role of *daf*-12 in bovine lungworms.

34. *Wolbachia* drives Type 17 immunity in *Onchocerca volvulus* (river blindness).

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The interaction between *Wolbachia* bacterial endosymbionts of filarial nematodes and the host's immune system plays an important role in the immunopathogenesis of filarial diseases. A newly described pro-inflammatory immune pathway, involved in auto-immunity and inflammation, is characterised by the production of interleukin-17 (IL-17). The unusual combination of a nematode plus a bacterium could support the development of such Type-17 immune response. We therefore investigated by immunohistochemistry the presence of IL-17 in *Onchocerca volvulus* nodules depleted or not of *Wolbachia* by doxycycline treatment. *Wolbachia*-containing but not *Wolbachia*-depleted onchocercomas showed an abundant IL-17$^+$ cell infiltrate, commonly adjacent to the worms. Moreover, IL-17 appeared to be released onto the cuticle of the worms. We next investigated Th17 lymphocytes as a cellular source of IL-17 by double-staining for CD4 and IL-17. Th17 cells accounted for 5.8% of CD4$^+$ lymphocytes in placebo-treated nodules, dropping to >0.2% in doxycycline-treated onchocercomas. We then assessed macrophages and neutrophils as possible sources of IL-17. We found that IL-17$^+$ cells were negative for the macrophage marker CD68, but, strikingly, >70% of IL-17-producing cells were neutrophils. The IL-17$^+$ neutrophil infiltrate dramatically decreased in *Wolbachia*-depleted nodules. These results suggest that *Wolbachia* induces a Type-17 response both at the level of innate and adaptive immunity.

35. A-WOL: Anti-*Wolbachia* drug discovery and development for the treatment and control of filariasis

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Anti-biotic therapy of filarial nematodes has emerged as a new strategy for the treatment and potential control of onchocerciasis and lymphatic filariasis. This novel approach targets the *Wolbachia* bacterial endosymbionts, which are essential for nematode development, embryogenesis and survival. A 4-6 week course of doxycycline results in the long-term sterility and ultimate death of the adult filarial parasite. The Anti-Wolbachia Consortium (A-WOL) has been established to optimize current regimes and discover and develop new anti-*Wolbachia* drugs to deliver a therapy compatible with current mass-distribution approaches. A-WOL has established screening approaches from target-based HTS to whole organism cell and nematode screens to exploit focused and diversity based libraries of existing and novel drugs and natural products in the search for anti-wolbachial drugs, which are compatible with the community directed delivery of filarial treatment. A-WOL target-discovery aims to identify both genes essential to the survival of *Wolbachia* and define the processes and pathways, which underpin the symbiotic relationship. Identification and validation of target pathways involved in haem and lipoprotein biosynthesis and other metabolic components of the *Wolbachia*-nematode relationship and their progress through the A-WOL screening strategy will be discussed.

36. The effects of *Schistosoma mansoni* hemozoin on macrophage activation - in a changing cytokine milieu.

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Adult schistosomes detoxify free heme, liberated during haemoglobin digestion, via its crystallization into hemozoin, a mechanism shared with *Plasmodium* parasites. *Schistosoma* hemozoin, regurgitated into the host circulation, accumulates in the liver and is phagocytosed by macrophages in the egg-induced granuloma. As a body of evidence from work with *Plasmodium* hemozoin suggests that it has immunomodulatory capacities, the effects of *S.mansoni* hemozoin on macrophage responses to immune stimuli were investigated. Hemozoin, purified from adult worms, was introduced into macrophage cultures, alongside classically or alternatively activating stimuli. Macrophages were also 'switched' from classical to alternative stimulation, mimicking the Th1 to Th2 shift in immune response observed in schistosome infections concomitant with the appearance of eggs and hemozoin in the liver. Measures of arginine metabolism, real-time PCR gene expression profiling and cytokine secretion were used to assess macrophage activation status. Hemozoin has little or no innate stimulatory effect on macrophages at physiological concentrations but does have a significant synergistic effect with IFNγ, as previously reported using *Plasmodium* hemozoin. Crucially, macrophages 'switched' in the presence of hemozoin fail to develop a normal alternatively activated phenotype. Instead, they display a 'mixed' phenotype in which both Arginase-1 activity and Nitric Oxide production occur. Expression of characteristic alternatively activated transcripts is also perturbed; *Arg1* and *Ym1* are expressed normally but *Fizz1* expression is significantly suppressed, explaining previous observations that macrophages are not a major source of *Fizz1* in the granuloma, and suggesting that *S.mansoni* hemozoin subtly manipulates the immune responsiveness and functional capabilities of macrophages.

37. Novel approaches to analyse excretory-secretory proteins from *Onchocerca*

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Onchocerciasis causes chronic morbidities in humans in many African and Latin American countries and hence is of enormous public health importance. Like other filarial parasites, the human pathogen *O. volvulus* and the closely related bovine parasite *O. ochengi* survive within their mammalian hosts for many years, usually without being seriously affected by the host immune system. Excretory-secretory (ES) products from the life cycle stages of the worms have been implicated in shaping the parasite's environment, which includes the modulation of the host immune response. Identification and characterisation of these ES products is a fundamental prerequisite to study the host-parasite cross talk at the molecular level, i.e. the interactions between host and parasite molecules. In a recently started Cameroonian-German cooperation project we apply several novel approaches to analyse ES proteins from *Onchocerca* species. Already known ES products from *O. volvulus* are re-evaluated e.g. by structural analyses. For a systematic examination, ES products collected from *O. ochengi* are currently analysed by mass spectrometric analysis. Furthermore, we have used a bioinformatic approach to identify proteins that are characterised by putative parasite-specific signal peptides. However, the latter approach is often critical, since *in silico* predictions on signal sequences of filarial proteins are sometimes inaccurate. Thus, we have established a means to confirm functional signal peptides in a nematode environment by employing the model *Caenorhabditis elegans*. Parasite derived proteins that contain a putative N-terminal secretory signal are cloned either in full length or in a truncated form omitting the predicted signal sequence in front of the green fluorescent protein. Expression of these fusion proteins is then carried out in appropriate *C. elegans* mutants under the control of the *C. elegans* hsp-16-41 promoter. Known ES products like the *O. volvulus* glutathione S-transferase 1 and the superoxide dismutase 2 have been tested as a proof of principle. The project is funded by the German Research Foundation (DFG), the Alexander von Humboldt-Foundation and the German Academic Exchange Service (DAAD).

38. Intestinal immunity against *Ascaris suum* in pigs: The search for targets and effector mechanisms.

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Round worms (*Ascaris s*pp.) are universal and very important parasites of the small intestines of humans (*A. lumbricoides*) and pigs (*A. suum*). Both parasite species are closely related to each other, have an identical life cycle and are morphologically and antigenically indistinguishable. Following the oral ingestion of *Ascaris* eggs by the host, L3 larvae hatch from the eggs and then penetrate predominantly the wall of the caecum and proximal colon to undergo a hepatopulmonary migration. After this, the larvae ultimately establish in the small intestine and develop into adulthood. A continued exposure to *Ascaris* for several months induces a sterile immunity in both humans and pigs. This immunity is located at the level of the intestine and specifically targets the incoming larvae, preventing them from penetrating the intestinal wall. This so-called pre-hepatic barrier protects the host against the histopathological damage inflicted to the liver and lungs by the migrating larvae**.** The aim of this research project is to identify the effector components of the immune response necessary to prevent this larval migration and the parasite antigens driving it. Intestinal immunity was induced in pigs by infecting them daily with a trickle infection for 30 weeks. The pigs subsequently received a challenge infection and were euthanized two weeks later. At necropsy, there was a 100% reduction in L4s recovered from the intestine and a 97,2% reduction in white spots on the liver in comparison with naïve control animals that received the same challenge infection. The mucus from the intestine was collected and the antibody secreting cells (ASC) were extracted from the local lymph nodes and put into culture. Antibodies purified from the mucus and the ASC culture supernatant were subsequently used to probe L3 larval extracts, egg hatch fluid and L3 E/S material. This resulted in the identification of a 12 kDa antigen specifically recognized by the IgA and IgG antibodies of immune animals. The antigen appears to be highly glycosylated and further steps are currently being taken to characterize it. In addition, intestinal tissue samples collected from the immune and naïve animals are used for gene transcription profiling and histological analysis in order to elucidate the immune effector mechanisms. The outcome of this project will help in the development of an *Ascaris* vaccine that induces a pre-hepatic barrier.

39. Targeting of host lipoproteins by the parasitic worm *Schistosoma mansoni*

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Schistosomes have developed multiple strategies to evade and resist the immune response of the mammalian host, allowing them to reside for many years in the blood vessels. Much research has focused on immune modulatory proteins excreted by schistosome eggs such as IPSE/alpha-1 or Omega-1, and on the Th2 skewed-immune response typically found in helminth infections.
We here describe a completely different mechanism of immune modulation where host lipoproteins serve as transporters of schistosome antigens. Like many schistosome proteins, these antigens are glycosylated with schistosome specific glycoproteins. As a result of transfer of schistosome antigens to host lipoproteins, circulating antibodies against schistosome antigens will indirectly bind to these lipoproteins. We have demonstrated the presence of IgG on low-density lipoprotein particles from serum from infected individuals, whereas no antibodies were found on lipoproteins of healthy controls. Subsequently, these antibody-opsonised lipoproteins are phagocytosed by immune cells carrying an Fc-receptor. Indeed, accumulation of lipids within several types of blood derived immune cells occurred in infected individuals only. *In vitro*, this lipid accumulation was associated with apoptosis and reduced viability of neutrophils. The consequences of these lipoprotein binding antibodies for immune cells and for the anti-helminth host response will be discussed.

40. Immune suppressive property of Thioredoxin peroxidase from *Clonorchis sinensis*.

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Parasitic helminth infection leads to severe immunopathological complications. Likewise, liver fluke *Clonorchis sinensis* (*C. sinensis*) can cause chronic infection while they reside in the bile duct and lead to cholangiocarcinoma in humans. We investigated the immuno-suppressive properties of live *C. sinensis* worms using a non contact co-culture transwell plate using murine macrophage cell line RAW 264.7 (RAW). We found that direct exposure and indirect exposure of live worms induced morphological changes from round shape to spindle-like shape of cells. Along with morphological change, expression of cell surface markers related with T cell co-stimulation and antigen recognition such as CD86 and Toll-like receptor (TLR) 4 were increased by worm exposure. Interestingly, lipopolysaccharide (LPS) induced nitric oxide (NO) and pro-inflammatory cytokine Interleukin (IL) 6 levels were down-regulated by worm exposure. On the contrary, anti-inflammatory cytokine such as IL-10 and Tumor growth factor (TGF) β secretion was up-regulated. In order to identify candidate molecules that are in charge of anti-inflammatory properties of *C. sinensis*, we first cloned and purified an antioxidant exzyme, Thioredoxin peroxidase (TPX, 2-Cys peroxidase) from *C. sinensis*. This has two cysteine residues and sized at 25kDa. TPX treatment was able to induce morphological changes of RAW cells into dendritic-like shape of cells. Moreover, upregulation of LPS-induced cell surface markers such as CD80, CD40, MHC class II, and TLR4 was down-modulated by TPX co-treatment. LPS-induced NO levels were also down-regulated by TPX co-treatment. Here we report that although several molecules of parasites are involved in LPS induced inflammation, TPX of *C. sinensis* might have a role in regulating LPS-induced immune responses.

41. The *Hymenolepis microstoma* genome and transcriptome

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Hymenolepis spp. have been used as laboratory models for teaching and research since the 1950s and thus much of our basic understanding of tapeworm biology, including their physiology, biochemistry, ultrastructure and development stems from work on these species. Characterization of the *Hymenolepis* genome will bring this classical model into the genomics age and will provide a community resource for research on the molecular basis of cestode biology, particularly for those interested in the adult, strobilate stages of the life cycle. We are using a combination of Solexa and 454 high-throughput sequencing to characterize the genome and transcriptome of a highly inbred strain of *H. microstoma* (Nottingham strain). Based on draft genome data we predict the size to be 118 MB with a G-C content of ~35%, highly similar to the genomes of the hydatid tapeworm (*Echinococcus multilocularis*) and the pork tapeworm (*Taenia solium*). Transcriptome sequencing derives from two different life-stages; mid-metamorphosis larvae and whole adult worms, as well as from several different adult tissues. These transcriptome data, together with those of *Echinococcus,* will facilitate assembly and annotation of the genome. We also have collaborations to characterize the MicroRNA targets and to use chromosomal in situ hybridization to assign scaffolds to individual chromosomes. Initial gene annotation and comparative studies will concentrate on the Hox 'clusters' of parasitic flatworms. Data generation is being underwritten by The Sanger Institute as part of a larger effort to characterize the genomes of helminths and other parasites of medical or research significance (see http://www.sanger.ac.uk/Projects/Helminths/).

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