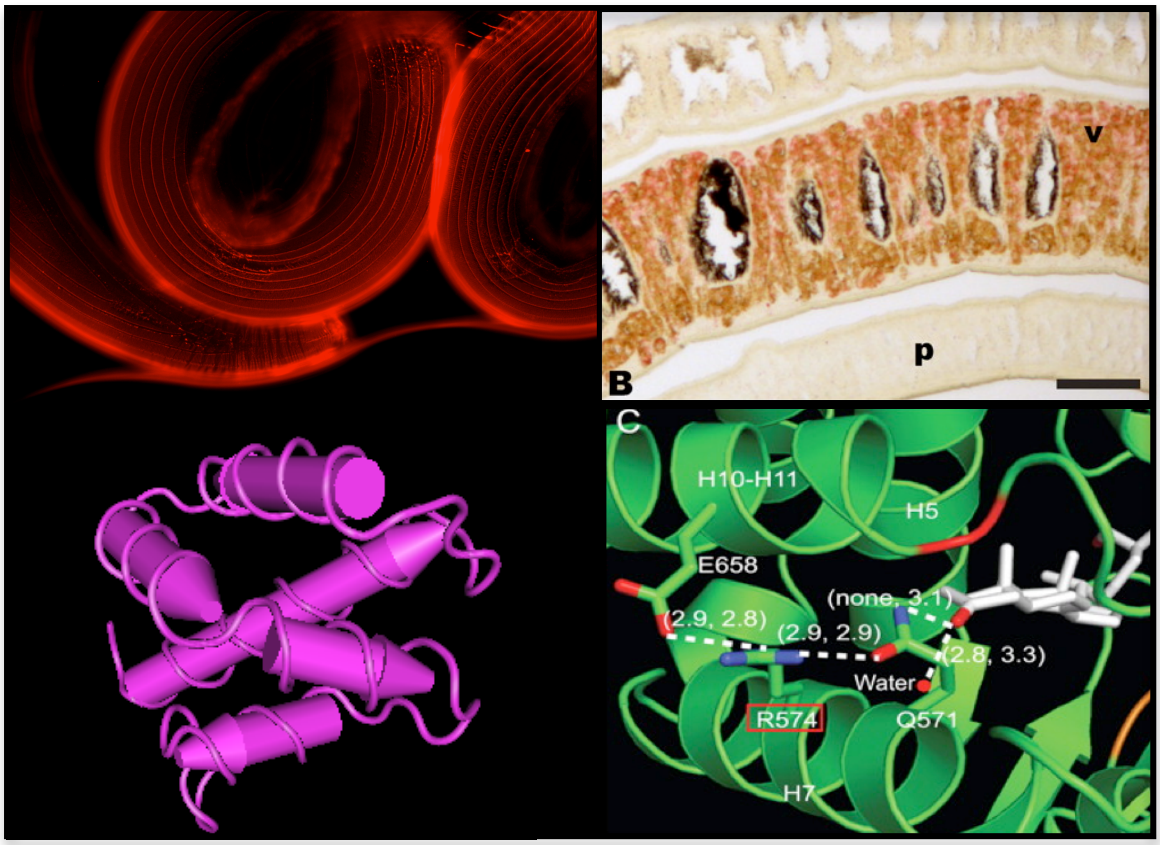
MOLECULAR AND CELLULAR BIOLOGY OF HELMINTH PARASITES VII

BRATSERA HOTEL,
HYDRA, GREECE
2-7 SEPTEMBER 2012



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MOLECULAR AND CELLULAR BIOLOGY OF HELMINTH PARASITES VII

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"
Special Issue of *Experimental Parasitology* (in press)

VII 2-7th September 2012, Hydra, Greece
" Molecular and Cellular Biology of Helminth Parasites VII"

ORGANISERS, 2012

Kleoniki Gounaris (Imperial College, UK)
Rick Maizels (University of Edinburgh, UK)
Ed Pearce (University of Pennsylvania, USA),
Murray Selkirk (Imperial College, UK),

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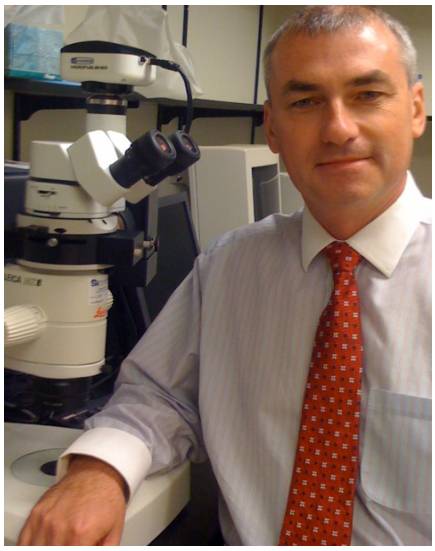
Programme Summary

Sunday 2 September	Monday 3 September	Tuesday 4 September	Wednesday 5 September	Thursday 6 September
Registration and refreshments from 18:00	**Systems**	**Development & Biochemistry**	**Molecular Interactions**	**Immunity**
	08:30-10:10 Paul Sternberg Adrian Streit Mark Viney Peter Geldhof	**08:30-10:10** Raffi Aroian Aaron Maule Gabriel Rinaldi James Lok	**08:30-10:10** Cornelius Hokke Nicole Driessen Alvaro Diaz Tony Page Patrick Skelly	**08:30-10:10** David Artis Alykhan Vira Minka Breloer Katherine Smith
	Coffee Break			
	10:40-12.20 Matt Berriman Nancy Holroyd Raul Bobes Pascal Mäser Neil Young	**10:40-12.20** Eileen Devaney Amy Buck Bernadette Connolly Katerina Artavanis-Tsakonas Robert Greenberg	10:40-12.20 Richard Martin Carolyn Behm John Gilleard Bart Landuyt Phillip Felgner	**10:40-12.20** Judith Allen Laura Layland William Horsnell Clarissa Da Costa Judy Appleton
	Lunch/Free time			
20:15 Welcome **20:30** Keynote Lecture, David Bird	**16:30-18:10** Klaus Brehm Thomas Quack Shelly Michalski Jonathan Stoltzfus Padraic Fallon	**16:30-18:10** Christophe Grevelding Stan Huang Mathieu Vanderstraete Jaap Van Hellemond Jose Lozano	**16:30-18:10** Alex Loukas William Harnett Corinna Schnoeller Balachandran Ravindran	**16:30-18:10** Graham Le Gros Wiebke Hartmann James Hewitson Andrew MacDonald Maria Yazdanbakhsh
21:30 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



David Bird,
North Carolina State University, California, USA

David Bird received a Ph.D. in Biochemistry from the University of Adelaide, Australia, in 1984, and then spent three years researching *C. elegans* developmental genetics with Don Riddle in Columbia, Missouri. Following that training, David joined the faculty of the University of California-Riverside, and in 1995 moved to NC State University. Dr. Bird serves as Director of the NCSU Bioinformatics Research Center, is Director of the university's Genomic Sciences Graduate Program, and holds the title William Neal Reynolds Distinguished Professor. The primary focus of his research group is to understand the mechanisms underlying parasitic interactions between nematodes and plants, and David was a pioneer in framing the key questions in the context of nematode and host development. His group and collaborators have been instrumental in establishing the root-knot nematode, *Meloidogyne hapla*, as the preeminent genetic system to model less-tractable nematode-host interactions, and as a platform for comparative genomics (www.hapla.or)

Raffi V. Aroian
University of California, San Diego

Raffi Aroian received a Ph.D. in Biology from the California Institute of Technology with Paul Sternberg in 1992 using molecular genetics to study the role of epidermal growth factor receptor in the development of the *Caenorhabiditis elegans* vulva. He performed his postdoctoral studies at the University of California, San Francsicso with Bruce Alberts, where he used cell biology and biochemistry to study the role of the actin cytoskeleton in early embryonic development of *C. elegans*. He was appointed to the faculty of the University of California, San Diego in 1997, where he has been since. Initially his laboratory worked on the establishment of embryonic polarity in *C. elegans*. His group now studies mechanism of action of anthelmintics as well as *Bacillus thuringiensis* crystal proteins. His group is exploring how crystal proteins can be used as anthelmintics to cure intestinal roundworm infections and studying structure/function relationships in these proteins. His group is also studying four different models of intestinal roundworms, mostly studying anthelmintics and developing new methodologies for studying anthelmintics. A second major focus of his group is studying innate immune responses to bacterial pore-forming proteins. In addition to his teaching and service at UC San Diego, he also founded a non-profit research organization, Wormfree World Institute (www.wfwins.org), dedicated towards developing new cures and diagnostics for intestinal roundworm infections.

David Artis
University of Pennsylvania, USA

Dr Artis completed his doctoral research training at the University of Manchester, UK focusing on immune regulation of parasitic helminth infections. Following receipt of a Wellcome Trust Prize Traveling Fellowship, he undertook his fellowship training at the University of Pennsylvania where he continued his research training in examining the regulation of immunity and inflammation at mucosal sites. Dr Artis joined the faculty at Penn in 2005 and became an Associate Professor of Microbiology in 2010. Dr Artis is actively involved in teaching of undergraduates, graduates and vet students and plays an active role in the Immunology Graduate Group, the Cell and Molecular Biology Graduate Group and the Institute for Immunology. Administratively, he is Director of the Penn Gnotobiotic Mouse Facility, Chair of Admissions for the Immunology Graduate Group and a member of the Executive Committees of the Institute for Immunology and the Immunology Graduate Group. He has developed a research program focused on dissecting the pathways that regulate innate and adaptive immune cell function at mucosal sites in the context of health and disease. Dr Artis is funded by NIH, CCFA and BWF and has published 43 primary papers and 18 reviews in high profile journals including *Nature*, *Science*, *Nature Medicine*, *Nature Immunology* and *Journal of Experimental Medicine*. He has been the recipient of the Crohns and Colitis Foundation of America Young Investigator Award, the AAI Junior Faculty Award, the Lady Barbara Colyton Prize for Autoimmune Research, and the International Cytokine Society Junior Faculty Award.

Alex Loukas

James Cook University, Australia

Alex obtained his PhD from the University of Queensland in Brisbane, Australia in 1995. His first postdoc was with Rick Maizels at the University of Edinburgh in the UK, and he then moved to George WashingtonUniversity in the US to join the human hookworm vaccine initiative. In 2004 obtained a career development fellowship from the NHMRC and returned to Brisbane to establish the Helminth biology laboratory at Queensland Institute of Medical Research. In 2010 he moved to James Cook University in Cairns as a professorial tropical research leader and founding director of the Centre for Biodiscovery and Molecular Development of Therapeutics. He holds an NHMRC Principal Research Fellowship and has grant funding from the NHMRC, ARC, NIH, Gates Foundation and large pharma. He is also editor-in-chief of the International Journal for Parasitology. Alex's major research interest is the molecular basis of host-parasite interactions, with a particular focus on characterising the functions of proteins secreted by helminths that parasitise humans in developing countries. Research projectsinclude the development of vaccines for human hookworm disease and schistosomiasis, molecular pathogenesis of infection with the carcinogenic liver fluke, characterising the secretomes of parasitic helminths, and the use of human helminths and their secreted proteins as therapies for autoimmune and allergic diseases. He is the author of more than 180 peer-reviewed publications and has received numerous awards for his research.

Paul Sternberg

California Institute of Technology

Paul Sternberg graduated from Hampshire College in 1978 and joined the Biology Department at M.I.T. for his Ph.D., which he received in 1984. His graduate work on the Genetic Control of Nematode Development was under the supervision of H. Robert Horvitz. He then pursued postdoctoral research on yeast mating type with Ira Herskowitz at UCSF, and returned to C. elegans when he joined the Caltech Biology Division faculty in 1987, where he is now Thomas Hunt Morgan Professor of Biology. He is an Investigator with the Howard Hughes Medical Institute, with whom he joined in 1989. He became lead-PI of WormBase in 1999 and started the Caltech branch of WormBase. He served on the board of directors of the Genetics Society of America from 2000-2003. At Caltech, he led the Biology graduate program for several years, helped found the BioEngineering graduate option, and is now director of the Center for Biological Circuit Design, part of the new Information Science and Technology initiative.

SCIENTIFIC PROGRAMME

All timings include minimum of 5 minutes for discussion.

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-Sunday 2nd September-

18:00-20:00 Registration, Bratsera Hotel

20:15 Introduction: **Rick Maizels**, University of Edinburgh, UK

20:30 Plenary lecture: **David Bird**, NC State University,
Genetical genomics of nematode parasitism

21:30 Welcome Reception, Bratsera Hotel

Day 1 ~ Monday 3 September ~ SYSTEMS

Session 1
Chair : Rick Maizels

08:30 **Paul Sternberg**, California Institute of Technology.
Genomic and chemical ecology approaches to nematode communication

09:10 **Adrian Streit**, Max Planck Institute for Developmental Biology, Germany. Genetic and molecular analysis of life history switches in nematodes of the genus *Strongyloides*

09:30 **Mark Viney**, University of Bristol. Natural variation in dauer larva formation and major life-history traits of wild isolates of *Caenorhabditis elegans*

09:50 **Peter Geldhof**, Ghent University, Belgium. An integrated approach to unravel the host-parasite interplay during the hepatopulmonary migration of *Ascaris suum*

10:10 - 10:40 Coffee Break

Session 2
Chair : Christoph Grevelding

10:40 **Matt Berriman**, Wellcome Trust Sanger Institute, Hinxton, UK. Fifty helminth genomes

11:00 **Nancy Holroyd**, Wellcome Trust Sanger Institute, Hinxton, UK Laying solid foundations for future helminth genomic research: producing parasitic helminth reference genomes

11:20 **Raul Bobes**, Institute of Biomedical Research, Universidad Nacional, Mexico.
The *Taenia solium* genome project: completed at last! and future directions

11:40 **Pascal Mäser,** Swiss Tropical and Public Health Institute.
The genome of the heartworm, *Dirofilaria immitis*

12:00 **Neil Young,** The University of Melbourne,
The *Schistosoma haematobium* genome and its implications

12:20 - 16:30 Lunch/Free Time

Session 3
Chair : Kleoniki Gounaris

16:30 **Klaus Brehm,** University of Würzburg, Germany. Characterization of the trans-spliced transcriptome and polycistronic transcription units in *Echinocccus multilocularis*

16:50 **Thomas Quack**, Justus-Liebig-University, Germany. Isolation of gonad-specific RNA, proteins, and cells from *Schistosoma mansoni* adults by a novel organ- and cell-isolation approach

17:10 **Shelly Michalski**, University of Wisconsin Oshkosh.
Transcriptome analysis of *Brugia malayi* using deep sequencing.

17:30 **Jonathan Stoltzfus**, University of Pennsylvania. A transcriptomic analysis of infectious larvae development in the parasitic nematode *Strongyloides stercoralis*

17:50 **Padraic Fallon**, Trinity College Dublin. Heminths and the immunome of today

18:15 - 20:15 Poster Session 1
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Day 2 ~ Tuesday 4 September ~ DEVELOPMENT & BIOCHEMISTRY

Session 4
Chair - Carolyn Behm

08:30 **Raffi Aroian,** University of California San Diego,
Gut worms? *Bacillus thuringiensis* crystal proteins as cures for intestinal roundworms

09:10 **Aaron Maule**, Queen's University Belfast.
Eri-1 expression correlates with RNAi susceptibility in plant parasitic nematodes

09:30 **Gabriel Rinaldi**, George Washington University.
Germline transgenesis and insertional mutagenesis in *Schistosoma mansoni* mediated by murine leukemia virus

09:50 **James Lok,** University of Pennysylvania.
Transposon-mediated chromosomal integration of transgenes in the parasitic nematode *Strongyloides ratti* and establishment of stable transgenic lines

10:10 - 10:40 Coffee Break

Session 5
Chair- Klaus Brehm

10:40 **Eileen Devaney**, University of Glasgow.
The microRNAs of *Caenorhabditis elegans* : could they play a role in drug resistance?

11:00 **Amy Buck**, University of Edinburgh.
Small RNA expression and secretion in vesicular exosomes by *Heligmosomoides polygyrus*

11:20 **Bernadette Connolly**, University of Aberdeen.
Investigating the mechanism and evolution of spliced leader *trans*-splicing in nematodes

11:40 **Katerina Artavanis-Tsakonas**, Imperial College London.
Ubiquitin pathway involvement during *Trichinella spiralis* infection

12:00 **Robert Greenberg**, University of Pennysylvania.
Role of schistosome ABC transporters in parasite physiology and drug susceptibility

12:20 - 16:30 Lunch/Free Time

Session 6
Chair- Judy Appleton

16:30 **Christophe Grevelding**, Justus-Liebig-University Giessen, Germany.
Discovery of platyhelminth-specific alpha/beta integrin families and evidence for beta-integrin function in signaling complexes within the gonads of *Schistosoma mansoni*

16:50 **Stanley Huang**, Washington University School of Medicine.
Fatty acid oxidation Is essential for egg production by the parasitic flatworm *Schistosoma mansoni*

17:10 **Mathieu Vanderstraete,** University Lille Nord de France.
Molecular and functional studies of the *Schistosoma mansoni* Venus kinase receptors SmVKR1 and SmVKR2: potential roles in larval development and oogenesis

17:30 **Jaap Van Hellem,** Erasmus University Medical Center & Harbor Hospital, the Netherlands.
The role of the kinetic properties of lactate dehydrogenase in the metabolic switch of *Schistosoma mansoni* cercariae.

17:50 J**ose Lozano**, Wageningen University, The Netherlands.
A nematode venom allergen/ASP-like protein is required for plant-parasitism and induces a hypersensitive response through an extracellular leucine-rich repeat receptor

19:00 ~ Boat departs from Hydra port for Vlychos

19:30 Dinner, Vlychos Taverna

Day 3 ~ Wednesday 5 September ~ MOLECULAR INTERACTIONS

Session 7
Chair- Judi Allen

08.30 **Cornelius Hokke**, Leiden University Medical Center.
Schistosome glycosylation and the application of glycan arrays for monitoring antibody responses in human schistosomiasis cohorts

08:50 **Nicole Driessen**, Leiden University Medical Center.
Specific glycan elements determine differential binding of individual egg glycoproteins of the human parasite *Schistosoma mansoni* by host C-type lectin receptors

09:10 **Alvaro Diaz**, Universidad de la Republica, Uruguay.
The *Echinococcus granulosus* laminated layer carbohydrates: structure and recognition by host lectin receptors

09:30 **Tony Page**, University of Glasgow.
The nematode astacins: key enzymes in cuticle collagen synthesis and moulting

09:50 **Patrick Skelly**, Tufts University.
Schistosome Surface Enzymes

10:10 - 10:40 Coffee Break

Session 8
Chair- James Lok

10:40 **Richard Martin**, Iowa State University.
Anthelmintic AChR channels from the Clade III nematodes *Brugia malayi* and *Ascaris suum*: the same but different

11:00 **Caroline Behm**, Australian National University.
Investigation and validation of a potential new drug target for nematodes

11:20 **John Gilleard,** University of Calgary.
Population genetic analysis of *Haemonchus contortus* and *Teladorsagia circumcincta* suggests independent benzimidazole resistance mutations frequently arise in the field

11:40 **Bart Landuyt**, KU Leuven.
A unique nematode peptide library for the development of novel anti-helminthic drugs

12:00 **Phillip Felgner,** University of California.
Using genome-wide proteome microarrays to understand the humoral response to infectious diseases

12:20 - 16:30 Lunch/Free time

Session 9
Chair- Graham Le Gros

16:30 **Alex Loukas**, James Cook University.
The therapeutic hookworm - of mice and men, mechanisms and molecules

17:10 **William Harnett,** University of Strathclyde.
Development of small molecule analogues of the *Acanthocheilonema viteae* immunomodulator ES-62 for therapeutic purposes

17:30 **Corinna Schnoeller**, Imperial College London.
The helminth-derived immunomodulator AvCystatin blocks Th2-driven lung eosinophilia in mice infected with respiratory syncytial virus

17:50 **Balachandran Ravindran**, Institute of Life Sciences, Bhubaneswar, India.
A filarial glycoprotein and chitohexose activate macrophages by alternate pathway through TLR4 and block endotoxemia

18:15 - 20:00 Poster Session 2

Day 4 ~ Thursday 6 September ~ IMMUNITY

Session 10
Chair- Eileen Devaney

08:30 **David Artis,** University of Pennsylvania.
Dangerous liaisons: parasites and the immune system

09:10 **Alykhan Vira**, University of Cape Town.
The role of muscarinic acetylcholine receptor M3 in immune response to pathogens using transgenic murine models

09:30 **Minka Breloer**, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany.
Different impact of regulatory T cells on nematode-induced immune evasion in BALB/c and C57BL/6 mice

09:50 **Katherine Smith**, University of Edinburgh.
IL-6 controls natural Treg expansion and Th2-mediated expulsion following chronic helminth infection

10:10 – 10:40 Coffee Break

Session 11
Chair- Alex Loukas

10:40 **Judith Allen**, University of Edinburgh.
Macrophage activation in helminth infection: Where inflammation is anti-inflammatory

11:00 **Laura Layland,** Technical University of Munich, Germany**.**
Schistosoma mansoni triggers Dectin-2 which activates the Nlrp3 inflammasome and alters adaptive immune responses

11:20 **William Horsnell**, University of Cape Town.
Influence of maternal helminth infection on offspring immunity

11:40 **Clarissa Prazeres Da Costa**, Institute of Medical Microbiology, Munich, Germany.
The immune phase of helminth infection during pregnancy determines the allergic immune response in offspring

12:00 **Judy Appleton**, Baker Institute for Animal Health, Cornell University, USA.
Eosinophils regulate immunity and larval growth in mice infected with *Trichinella spiralis*.

12:20 - 16:30 Lunch/Free time

Session 12
Chair - Ed Pearce

16:30 **Graham Le Gros**, Malaghan Institute of Medical Research, Wellington, NZ.
Role of innate cytokines in protective immunity against helminth parasite infections

16:50 **Wiebke Hartmann**, Bernhard Nocht Institute for Tropical Medicine, Germany.
Different developmental stages of a filarial nematode dampen the vaccination efficiency of model antigen immunizations

17:10 **James Hewitson**, University of Edinburgh.
Vaccine-induced sterile immunity to intestinal nematode infection through antibody-dependent mechanisms

17:30 **Andrew MacDonald**, University of Edinburgh.
Epigenetic control of Th2 induction by dendritic cells

17:50 **Maria Yazdanbakhsh,** Leiden University Medical Center
Omega-1 and rapamycin : human dendritic cell induced Th2 responses

20:00 Farewell Dinner at Bratsera Hotel

Friday 7 Sept: Departure

POSTER SESSION 1: MONDAY 3 SEPTEMBER, 18:15-20:15

1	**Kathrin ARNDTS**	University Clinic Bonn	Elevated adaptive immune responses are associated with latent infections of *Wuchereria bancrofti*
2	**Allison BANCROFT**	University of Manchester	Immunological silence and chronic *Trichuris muris* infection
3	**Mariana BONILLA**	Universidad de la República, Montevideo	Thioredoxin-glutathione reductase-dependent redox networks in flatworm parasites
4	**Cinzia CANTACESSI**	The University of Melbourne	SCP/TAPS proteins of parasitic helminths - future prospects
5	**Michelle CASTELLETTO**	University of California at Los Angeles	Host seeking and the evolution of olfactory behavior in parasitic nematodes
6	**Betina CORSICO**	University of Glasgow	Structures of the nematode-specific FAR-1 and nemFABP lipid binding proteins
7	**Matthew DARBY**	University of Cape Town	Protective immunity against *Nippostrongylus brasiliensis* requires IL-13 production and antigen presentation by IL-4Rα responsive B cells
8	**Harriet DICKINSON**	University of Cambridge	Platyhelminth tegumental allergen-like proteins in *Schistosoma haematobium*: from ESTs to human immune responses
9	**Colette DISSOUS**	Institut Pasteur de Lille	SmSak, the second polo-like kinase of *Schistosoma mansoni* acts in tandem with SmPlk1 to regulate cell division
10	**Vanessa DUFOUR**	McGill University	Characterization and distribution of glutamate-gated ion channels in *Schistosoma mansoni*
11	**Jan DVORAK**	Institute of Molecular Genetics, Prague	Trypsin proteases from *Schistosoma mansoni* in host-parasite interactions?
12	**Cecilia FERNÁNDEZ**	Universidad de la República, Montevideo	An expanded and functionally diverse family of Kunitz inhibitors from *Echinococcus granulosus*
13	**Ana Maria FERREIRA**	Universidad de la República, Montevideo	Characterisation of the native lipid moiety of *Echinococcus granulosus* antigen B
14	**Jana HAGEN**	The University of Melbourne	Exploring the role of *S. mansoni* egg proteins in immune regulation using microRNA-adapted shRNA constructs
15	**Steffen HAHNEL**	Justus-Liebig-University, Giessen	Transmembrane receptor signaling in the reproductive organs of the blood fluke *Schistosoma mansoni*
16	**John HAWDON**	George Washington Medical Center	Investigating DAF-16/FOXO target gene regulation during early parasitic hookworm development
17	**Min HU**	Huazhong Agricultural University	Molecular characterization of RIO protein kinase-encoding genes Ss-rio-1 and Ss-rio-3 of *Strongyloides stercoralis*
18	**Marc HÜBNER**	University Hospital Bonn	Chronic *L. sigmodontis* infection improves *E. coli* induced hypothermia and bacterial clearance
19	**Henry MCSORLEY**	University of Edinburgh	Secreted products of the helminth *Heligmosomoides polygyrus* suppress Type 2 immunity and airway allergic inflammation in mice

POSTER SESSION 2: WEDNESDAY 5 SEPTEMBER, 18:15-20:15

20	**Elisabeth JANACEK**	University of Vet Medicine Hannover	Gene transcriptional changes during development from immature to adult bovine lungworms
21	**Adela JILKOVA**	Institute of Organic Chemistry & Biochemistry, Prague	Structural basis for inhibition of the Cathepsin B drug target from the human blood fluke, *Schistosoma mansoni*
22	**Coralie MARTIN**	Museum National d'Histoire Naturelle, Paris	The chemokine CXCL12 is essential for the clearance of the filaria *Litomosoides sigmodontis* in resistant mice
23	**Erin MCCAMMICK**	Queen's University, Belfast	Triclabendazole and its metabolites impact multidrug resistance protein expression in *Fasciola hepatica* newly excysted juveniles
24	**Janice MURRAY**	The University of Edinburgh	Diversification, expression and function of secreted VAL proteins in *Heligmosomoides polygyrus*
25	**Al NISBET**	Moredun Institute, Edinburgh	Control of a parasitic nematode in sheep by vaccination with recombinant antigens
26	**Lise PEETERS**	K.U. Leuven	Comparative study of the cholecystokinin GPCR signaling system in free-living and parasitic nematodes
27	**Anna PROTASIO**	Wellcome Trust Sanger Institute	Transcriptome analysis of the early skin-stage schistosomula of *S. mansoni*
28	**Emma RINGQVIST**	AgResearch, New Zealand	Immuno-modulation of dendritic cell responses by the nematode parasite surface glycolipid CarLA
29	**Irma SCHABUSSOVA**	Medical University, Vienna	*Oesophagostomum dentatum* extract induces regulatory responses and prevents the development of allergy in mice
30	**Susan STASIUK**	University of Calgary	Functional analysis of benzimadazole metabolism and efflux in the model nematode *Caenorhabditis elegans*.
31	**Christina STRUBE**	University of Veterinary Medicine Hannover	Transcriptional profiles of daf-16, daf-12 and daf-9 do not support the hypothesis of *Dictyocaulus viviparus* L3 being equivalent to the *Caenorhabditis elegans*-dauer larva.
32	**Thomas TIMM**	University of Giessen	Phosphocholine in parasitism : biosynthesis and immunological implications of PC-epitopes
33	**Lenka ULRYCHOVA**	Institute of Molecular Genetics, Prague	Recombinant production and characterization of trypsin-like proteases from *Schistosoma mansoni*.
34	**Georg VON SAMSON-HIMMELSTJERNA**	Freie Universität Berlin	Indication for potential interaction of Emodepside and γ-aminobutyric acid (GABA)-gated chloride channel receptor signaling
35	**Rhiannon WHITE**	Imperial College London	Characterisation of the *Trichinella spiralis* deubiquitinating enzyme, TsUCH37, an evolutionarily conserved proteasome interaction partner
36	**Adrian WOLSTENHOLME**	University of Georgia	Novel nicotinic receptors from parasitic nematodes
37	**Qing YE**	Huazhong Agricultural University,China	Identification and molecular characterization of RIO protein kinase-encoding genes Sj-rio-1 and Sj-rio-2 from *Schistosoma japonicum*
38	**Magdalena ZAROWIECKI**	Sanger Institute	Tapeworm genomes " the Good, the Bad and the Well-assembled

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;

Marianna Bonilla *– Universidad de la República, Montevideo, Uruguay*

Alykhan Vira *– University of Cape Town, South Africa*

Qing Ye *- Huazhong Agricultural University, China*

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FUND

ABSTRACTS
FOR ORAL PRESENTATIONS

Genetical genomics of nematode parasitism

DAVID M BIRD[1,2], VALERIE M WILLIAMSON[3], ELIZABETH H SCHOLL[2], PETER M DIGENNARO[2], DAHLIA NIELSEN[1]
[1]NC STATE UNIVERSITY, BIOINFORMATICS RES. CNTR., RALEIGH, NC, 27695-7566, [2]NC STATE UNIVERSITY, PLANT PATHOLOGY, [3]UC-DAVIS, NEMATOLOGY

Host-parasite interactions reflect the co-evolved interaction between two genomes. Given suitable genetic and genomic resources for both partners, it should be possible to directly examine the parasitic interaction in a comprehensive, gene-by-gene manner. We have initiated such a project for the plant-parasitic, root-knot nematode, Meloidogyne hapla, and the plant host, Medicago truncatula. We selected M. hapla because of its agricultural importance, the phenotypic diversity of field isolates, tractable genetics, and small genome. A well-annotated sequence spans >99% of the 54 Mb M. hapla genome (www.hapla.org), and sequence skims of other inbred field isolates have yielded numerous SNPs for mapping. Genetically, M. hapla is a simple diploid (n=16) that reproduces by facultative meiotic parthenogenesis. This reproductive mode permits both out-crossing and inbreeding. Using these resources, we have begun to address the question: “How does the genetic makeup of the pathogen influence host gene expression?” Specifically, we consider the influence of allelic variation at each individual nematode locus on the expression of each and every plant gene. Natural genetic and phenotypic variation in field isolates of M. hapla has been captured in several, highly inbred nematode parental lines, and 140 recombinant inbred progeny lines (RIL) developed as a mapping population. Our broad approach is to perform a cross-species expression quantitative trait locus (eQTL) mapping experiment, followed by regulatory network inference to characterize the cross-talk between organisms. Replicate pools of host plants have been individually infected with the 140 nematode RILs and the combined transcriptomes of each individual determined by Illumina-based RNA-Seq. Deconvoluting the data allows us to determine expression levels of each plant and each pathogen gene, and also to genotype the pathogen line (based on SNPs) in one assay. Initial analyses have yielded an unprecedented insight into M. hapla genome organizati!on, and both nematode and host expression profiles.

Genomic and chemical ecology approaches to nematode communication

PAUL W. STERNBERG[1], JAGAN SRINIVASAN[1], ANDREA CHOE[1], ADLER R. DILLMAN[1], ANUSHA NARAYAN[1], YEA-RA JO[1], HILLEL SCHWARTZ[1], DANIEL LEIGHTON[1], IGOR ANTOSHECHKIN[1]1, FRANK C. SCHROEDER[2], STEPHAN H. VON REUSS[2], TATSUJI CHUMAN[3], AARON DOSSEY[3], RAMADAN AJREDINI[3], ARTHUR S. EDISON[3], L. HEIKKINEN[4], M. LAKSO[4], KELLEY FRACCHIA[5], ALI MORTAZAVI[5], ERICH M. SCHWARZ[1], GARY WANG[4]

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Ascarosides have emerged as small molecule communication signals among nematodes in addition to their structural role in ascarid eggs. We have found that ascarosides not only serve as population density signals for *Caenorhabditis elegan*s dauer formation, but also as mating pheromones and aggregation pheromones. Our recent results indicate that many nematodes produce ascarosides, and many can respond to synthetic ascarosides originally identified from *C. elegans*. Since each species appears to produce a distinct set of asacarosides, and each species responds to a distinct set, we hypothesize that there is an ascaroside code by which many nematodes communicate. We will discuss progress our several current approaches to elucidate mechanisms of ascaroside-mediated communication. In one approach, we are using single cell transcriptional profiling to identify genes expressed in the four male specific CEM neurons, which we find are direct sensors of mating pheromones ascr#3 and as cr#8 based on whole-cell patch clamp electrophysiology. We hope to identify receptors among the genes expressed specifically in these cells by comparing the transcriptomes of multiple sensory neurons. We are also pursuing genomic sequences of species for which we have characterized the production and response to ascarosides with the ultimate goal of identifying and perturbing relevant genes. For example, *Panagrellus redivivus* uses ascr#1 as male attractant, and we have assembled and annotated its genome, with help from the mapping of mixed stage mRNAs and small ncRNAs. We are similarly analyzing genomes from *Steinernema*, *Heterorhabditis*, among other genera.

Genetic and molecular analysis of life history switches in nematodes of the genus *Strongyloides*

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The parasitic females of the nematode genus *Strongyloides* live in the small intestines of their hosts and reproduce parthenogenetically. In addition to producing parasitic offspring (homogonic life cycle), they can also form a facultative free-living sexual generation (heterogonic life cycle). The entire progeny of the free-living generation is female and parasitic. We are further characterizing the male specific chromatin diminution event we identified in *S. papillosus* (Nemetschke et al 2010 Curr Biol 20:1687-1695). We also perform comparative analyses of chromosome numbers and sex chromosomes in different species of *Strongyloides* and we ask if X chromosome dosage compensation exists in *Strongyloides* spp. The switch between the homogonic and heterogonic cycles is likely homologous with the dauer switch in *C. elegans*. Nevertheless, many of the factors involved in the control of these switches are likely to differ. To identify genes that control this switch in *S. ratti* we mutagenized a strain (ED321) that predominantly undergoes the heterogonic life cycle. Analysis of mutants with an elevated proportion of homogonic development is in progress. Additionally we performed transcriptome comparisons between young larvae of ED321 and ED5, which forms very few free-living individuals. We compared three samples: i) ED321 progeny of the parasitic generation (destined to form free-living adults of both sexes); ii) ED5 progeny of the parasitic generation (destined to form parasitic females) and iii) ED321 progeny of the free-living generation (destined to form parasitic females). The number of differentially regulated genes is relatively small (about 370 genes across all comparisons). Interestingly, the two samples that were the progeny of parasitic mothers but bound for different life cycles (i and ii) differed much less from each other than did the two samples that were both destined to form infective larvae but were the progeny of parasitic or free-living parents respectively (ii and iii).

Natural variation in dauer larva formation and major life-history traits of wild isolates of *Caenorhabditis elegans*

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For *Caenorhabditis elegans*, young larvae can choose to arrest their development as dauer larvae in response to a change in food conditions, temperature and signals from other individuals (measured by ascaroside pheromones). The orthodox view is that dauer larva formation is a population response to poor conditions. We have investigated the dauer larva formation phenotypes (as well as reproduction and lifespan) of different wild *C. elegans* isolates. We have found very extensive differences in how these isolates respond to the same environmental signals; no two isolates seem to show similar responses. Specifically, dauer larva formation of these isolates when exposed to (i) two chemically synthesised ascarosides (singly and as a mixture) shows that these same stimuli can have opposite effects on dauer larva formation, and (ii) dauer pheromone produced by different isolates also have different dauer larva induction properties, as has been seen for *Pristionchus*. These results therefore mean that different worm isolates have fundamentally different environmental sensitivities. We have also found that different *C. elegans* isolates differ in the ascarosides that they produce (specifically, the absolute quantities and the relative ratio of two molecules). Together, these observations suggest the hypothesis that dauer larva formation in *C. elegans* may be a kin-signalling system where different genotypes produce an ascaroside signal that only makes sense to related individuals, such that kin groups have a dauer larva formation phenotype that maximises fitness in their environment. These data are not consistent with the idea that dauer larva formation is a species-wide arrest phenomenon. All the *C. elegans* isolates studied do form dauer larvae (so the phenomena is *C. elegans*-wide), but the specific nature of the relationship between the environment and the dauer formation phenotype is genotype-dependent, diverse and, we suspect, rapidly evolving.

An integrated approach to unravel the host-parasite interplay during the hepatopulmonary migration of *Ascaris suum*

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Common roundworms are important intestinal nematodes of humans (*Ascaris lumbricoides*) and pigs *(Ascaris suum*). 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 adult worms. The first aim of this project was to investigate the intestinal immune responses that hosts develop against the penetrating L3 larvae, the so-called pre-hepatic barrier, and the developing L4 larvae, the so-called expulsion reaction. The pre-hepatic barrier develops after a continued exposure to *Ascaris* for several months and prevents the incoming larvae from penetrating the intestinal tissue. Detailed analysis indicated that the development of this pre-hepatic immune barrier coincided with a marked influx of eosinophils in the intestinal tissue. A similar eosinophil influx was also observed during the expulsion of the L4 larvae from the small intestine, which normally takes place around 17 days post infection. These observations indicate that eosinophils act as effector cells in the intestinal immune response against *Ascaris*. Current research is aimed at unravelling the regulatory mechanisms leading to this eosinophil influx. In addition to the host responses, the second aim was to identify the parasite antigens that potentially trigger or modify these immune responses. Analysis of the transcriptomic datasets generated by next generation sequencing approach of infective L3s from eggs, liver- and lung-L3s and intestinal L4s revealed that tightly regulated transcriptional changes appear in the larvae when they migrate from one organ to another. For example, several molecules, such as C-type lectins and mucins, potentially involved in host-parasite interactions, are amongst the most abundant transcripts in the lung-L3 stage. Determining the exact role of these molecules in the host-parasite interplay needs further research.

Fifty helminth genomes

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Within the genomics field, parasitic helminths are still the "poor relatives", despite their medical and economic importance globally. We are interested in studying the diversity of helminth parasites, their complex interactions with their hosts, and wish to uncover the genomic basis for their biological differences. Taking the breadth of the field into consideration, we have developed the Helminth Genomes Initiative in collaboration with others, including The Genome Institute at Washington University and Gene Pool at Edinburgh University. We are now surveying the genomes of more than 50 helminths including many of the most important, in terms of their impact in human, agricultural and veterinary disease, or use as disease models. Our goal is two-fold: to provide rapid draft data from a broad list of more than 50 parasitic helminths and to produce high quality reference genomes from a subset that includes many key human pathogens (or their common models) such as whipworms, threadworms, Schistosomes and tapeworms.

Laying solid foundations for future helminth genomic research: producing parasitic helminth reference genomes

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It is now possible, using second generation sequencing technologies, to generate helminth genomes and functional genomics data sets at substantially reduced cost, with significantly smaller amounts of starting material and at a much faster pace than ever before. With the imminent wide-scale adoption of third-generation sequencing technologies, sequence data will be delivered from even smaller amounts of genomic DNA. However, the challenge will remain to intelligently assemble, interpret and utilise the avalanche of data. We are therefore committed to producing high quality reference helminth genomes for major clades of parasitic helminths. This is being undertaken using a combination of highly adaptive automated and bespoke manual improvement approaches that deal with the continually evolving sequencing technologies. The reference genome sequences will enable scaffolding of the numerous draft genomes that are and will be available, will provide the foundation for functional genomics experiments and will allow sequence variants to be accurately identified in population genomics experiments. An overview of the strategy employed and progress made in three helminth reference genome projects - *Echinococcus multilocularis*, *Strongyloides ratti* and *Trichuris muris* - will be presented, and will include novel approaches to error correction and quality control as well as examples of how the reference sequence has added value to the draft genome sequence of a related species.

The *Taenia solium* genome project: completed at last! and future directions.

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Tapeworms (Platyhelminthes: Cestoda) are obligate endoparasites of vertebrates, with complex life cycles that frequently involve larval. The most important tapeworm for human health is *Taenia solium* whose larval stage is the cause of cysticercosis and neurocysticercosis, a parasite disease known since antiquity in Western Europe, Africa and South East Asia. Recently, the Mexican Consortium for the Genome of *Taenia solium* has completed the assembly and identification of the expressed genes. Genomic DNA sequencing was carried out through a whole genome shotgun strategy. Different amounts of sequence were obtained using different technologies: Illumina (9504 Mb), 454-Flex (2348 Mb), 454-GS20 (1167 Mb) and Sanger (455 Mb). Sanger included paired-end sequencing of 4 libraries with different insert sizes: 1.2, 3, 5, and 9 kb. We produced a final collection of 12,714 predicted protein coding genes for the *T. solium* genome. The sum of gene lengths was 97.6Mb, which is in agreement with the amount of single copy regions in the genome (~90Mb). As expected, *T. solium* fulfills the profile of a strongly dependent organism; a number of biosynthetic pathways are missing, including those for the synthesis of amino acids, fatty acids and sugars, indicating that this parasite obtains most of the basic nutrients from its host. In addition, several immunomodulatory proteins reported in other parasites (*E. multilocularis*, *S. mansoni*, *S. japonicum*, *C. sirensis* and *Brugia malayi*) were also found in *T. solium*, pointing their possible role in specific evasion pathways to survive in an immunologically competent host, i.e. the up-representation of enzymes involved in the alternative activation of macrophages and inhibition of nitric oxide synthesis, as arginase and ureohydrolase. The whole information obtained from this study will direct future studies on parasite strategies of immune evasion, host-specificity, and development of new drugs and vaccine targets, which can help to control the disease.

The genome of the heartworm, Dirofilaria immitis

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The heartworm *Dirofilaria immitis* is an important parasite of dogs and other mammals, including humans. Transmitted by mosquitoes in warmer climatic zones, it is spreading across Southern Europe and the Americas at an alarming pace. There is no vaccine and chemotherapy is prone to complications. To learn more about this elusive parasite, we have sequenced the genomes of *D. immitis* and its endosymbiont *Wolbachia* using Illumina technology. In the 84.2 Mb of nuclear genomic assembly, we predict 10,179 protein coding genes, and 823 genes in the 0.9 Mb *Wolbachia* genome. The *D. immitis* genome harbors neither DNA transposons nor active retrotransposons, and there is extraordinarily little genetic variation between two sequenced isolates from Europe and the USA. Analysis of 990 orthologous genes resolves filarial nematode phylogeny, placing *D. immitis* next to *Onchocerca* species. Differential presence of anabolic pathways such as heme or nucleotide biosynthesis hints at th e intricate relationship between the heartworm and *Wolbachia*. Comparing the *D. immitis* predicted proteome to other nematodes, and to mammalian hosts, identifies families of potential drug targets, immune modulators and vaccine candidates. This genome sequence will support the development of new tools against dirofilariasis and aid efforts to combat the related human filariases.

The *Schistosoma haematobium* genome and its implications

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The intestinal and urinary forms of schistosomiasis are caused by chronic infections with *Schistosoma* species and affect > 200 million people worldwide. Of the three main species that infect people, *Schistosoma haematobium* has come into the spotlight, because of its high prevalence (> 110 million people infected), as a cause of urogenital disease and its link to malignant bladder cancer and HIV/AIDS in endemic regions. In spite of the impact of urogenital schistosomiasis and complications, little is known about *S. haematobium* compared with other schistosomes. Recently, we sequenced the 385 Mb genome of this parasite from the genomic DNA isolated from a single pair of adult worms using an advanced approach, employing Illumina technology, and undertook extensive analyses of the dataset. We are excited about the prospects that this resource provides for fundamental genomic, genetic, evolutionary, biological, ecological and epidemiological studies and for developing new interventions.

Characterization of the trans-spliced transcriptome and polycistronic transcription units in *Echinocccus multilocularis*

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In nematodes, spliced leader (SL) trans-splicing is associated with processing of polycistronic transcription units (PTU) into translatable mRNAs. Although SL trans-splicing is employed by all major lineages of flatworms, extensive information on the trans-spliced transcriptome in these organisms or on flatworm PTU was missing. Using RNA-Seq, PTU have recently been identified in the trematode *Schistosoma mansoni* and herein we provide evidence that this type of gene expression is also employed by cestodes. Based on transcriptome data, we found around 10% of *E. multilocularis* genes processed involving trans-splicing. We also found that around 300 genes form part of a PTU. The majority of *Echinococcus* PTU comprises two cistrons in which the downstream member is always trans-spliced whereas the upstream partner is trans- or conventionally spliced. We also found PTU that comprise three or four cistrons. A large number of *Echinococcus* PTU is also present with similar st ructure in *S. mansoni*. These data indicate that many PTU were already formed early in flatworm evolution and we indeed obtained evidence that PTU are also present in planarians. Within flatworm PTU there is a clear prevalence of genes that encode important cell-cycle regulators, splicing factors and mitochondrial proteins, orthologs of which are also present in nematode PTU, although trans-splicing in flatworms and nematodes evolved independently. In a large number of trans-spliced genes that are not part of a PTU we could identify 5' UTR introns that are both conventionally spliced and trans-spliced. This indicates that the acquisition of a 5' UTR intron is a necessary prerequisite for subsequent trans-splicing of a gene and eventual incorporation into a PTU. On the basis of genome/transcriptome comparisons between cestodes, trematodes and free-living flatworms, we will discuss the evolution of PTU in flatworms as well as a possible association of trans-splicing with gene expression in flatworm neoblasts.

Isolation of gonad-specific RNA, proteins, and cells from *Schistosoma mansoni* adults by a novel organ- and cell-isolation approach

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Schistosomes are blood-dwelling digenean trematodes that mature as adults in the intestinal or urinary veins, predominantly of mammals. Schistosome couples produce eggs of which approximately 40% fail to pass into faeces or urine (species-dependent) to pursue the complex life cycle, but are dispersed by the blood stream into different organs, where they provoke severe inflammation. This parasitic infection is known as schistosomiasis and considered by the WHO as the second most socioeconomically devastating parasitic disease, next only to malaria, with hundreds of millions infected worldwide. As the eggs represent the causative pathogenic agents, the understanding of egg-forming processes, and therefore of the schistosomal reproductive biology in general, is of fundamental interest. To unravel the signal transduction pathways involved herein, a comprehensive analysis of the sub transcriptome and especially sub proteome of the reproductive organs would be of decisive importance. A prerequisite to fulfil this ambitious aim would be the isolation of reproductive organs (ovary, testes, vitellarium) in sufficient amounts and purity. Therefore, a novel method was developed consisting of a combined detergent/protease treatment ("stripping"), which leads to the successive degradation of the tegument and muscle fibres, finally allowing the preparation of gonads in a pure state. During the establishment of this method a presently undescribed morphological structure ("Q-layer") was discovered, located subjacent to the musculature. Here we present microscopic analyses documenting the stripping-procedure, as well as preliminary results on isolated RNA, protein, and cells from gonad tissues. In the future, such gonad-specific cellular material will help to unravel signal transduction cascades involved in e.g. gametogenesis and/or vitellogenesis. Furthermore, the described technique will facilitate the isolation of isogenic cells and germ cells from reproductive organs for attempts to establish desper ately needed schistosomal cell lines. Finally, the described method could probably also be beneficial for research on other trematode parasites like *Fasciola*.

Transcriptome analysis of *Brugia malayi* using deep sequencing

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Using Illumina high-throughput sequencing, we have undertaken a comprehensive genome-wide survey of the developmental transcriptome of the human filarial parasite *Brugia malayi.* Over 100 million paired-end reads were generated from polyA-tailed mRNA from seven life cycle stages: eggs and embryos, immature MF (of less than 3 days of age), mature MF, L3, L4, adult male and adult female. Examining the developmental transcriptome profiles of *B. malayi* revealed major transitions in RNA expression from eggs through larval stages to adults. Using statistical approaches, we identified groups of genes with distinct life stage dependent transcriptional patterns and functional categories overrepresented in each of these groups. Global transcriptional differences were further evaluated between pairs of stages with particular emphasis on (i) MF maturation, (ii) late larval development, (iii) sex differences, and (iv) intrauterine reproductive processes. Overall, our analysis provides a first comprehensive view of the life cycle transcriptome of *B. malayi*, revealing the dynamics of gene expression during parasite development. Recent findings on the use of these deep sequencing data for genome annotation through Wormbase will be presented.

A transcriptomic analysis of infectious larvae development in the parasitic nematode *Strongyloides stercoralis*

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The parasitic nematode *Strongyloides stercoralis* differs from other nematode species that infect humans, in that its life-cycle includes both parasitic and free-living forms. This distinguishing feature can be leveraged to examine factors necessary for parasite development, specifically, mechanisms governing development of infectious third-stage larvae (L3i). Since L3i are constitutively formed in the post-free-living generation, while free-living adults are predominately formed in the post-parasitic generation, pair-wise comparison of the morphologically indistinguishable first-stage larvae from each generation can yield insights into mechanisms regulating L3i formation. Furthermore, comparison of developmentally arrested L3i to L3 larvae that have resumed development in a permissive host can elucidate mechanisms of L3i activation. To investigate the mechanisms regulating developmental arrest and activation of *S. stercoralis* L3i, we undertook a deep-sequencing study of the polyadenylated transcriptome. Using Illumina HiSeq technology, we generated over 2.3 billion paired-end reads for seven developmental stages: post-parasitic L1 and L3 larvae, free-living females, post-free-living L1 larvae, L3i, L3i activated in vivo in a permissive host, and parasitic females. Although these data are also informative for identifying transcripts and splice variants, this study aims to identify specific sets of differentially expressed genes that play a role in regulating L3i development. Informing the analysis is work from the strictly free-living nematode *Caenorhabditis elegans*, in which regulation of similar developmental stages has been extensively studied. Overall, our analysis provides a unique inter- and intra-species examination of a limited number of genes that are associated with free-living and parasitic developmental fates in the medically relevant pathogen *S. stercoralis.*

Heminths and the immunome of today

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In modern societies there is a growing incidence of a range of inflammatory conditions. The reasons for this exacerbation in immune-mediated diseases is both complex and multifactorial. In effect the function of the immune system of today is aberrant. Therefore while the immunome is the product of adaptation during evolution, it is not fit for purpose in modern man. A reductionist view is that parasitic helminths, with chronic infections with low morbidity and as potent immune modulators, exerted specific selection pressure on the immunome of today. Indeed there is evidence that human genes implicated in causing inflammation today may have evolved to function in the context of infection with helminths. A manifestation of this argument is the premise that infection with helminths may suppress unrelated inflammatory states in humans. There is evidence that deliberate administration of helminth parasites to humans may alter inflammatory diseases such as inflammatory bowel disease. However, helminths do cause pathology in man. In this presentation novel immune cells and modulating mechanisms evoked or usurped by heminths will be presented.

Gut worms? *Bacillus thuringiensis* crystal proteins as cures for intestinal roundworms

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Intestinal roundworms (hookworms, whipworms, *Ascaris*, *Strongyloides*) infect somewhere between 1-2 billion people in the world. Intestinal roundworms are considered a top cause of disease burden in children, with infections leading to growth and cognitive stunting, lower future earnings, malnourishment, decreased school attendance, and immune defects. The drugs currently available have significant deficiencies and so new therapeutics are urgently needed. Our group has pioneered work on *Bacillus thuringiensis* (Bt) crystal (Cry) proteins that target roundworms. Here we will discuss the efficacy of Cry5B against intestinal roundworms. We will present recent data comparing the *in vitro* efficacy of Cry5B versus drugs used clinically against parasitic roundworms. We will present new *in vivo* data on the excellent efficacy of Cry5B in clearing intestinal roundworm infections in small animals when delivered orally. We are in process of mutagenizing the entire Cry5B protein, performing alanine-scanning mutagenesis on each amino acid in the toxin domain. This study is revealing many residues that can be mutated in order to improve toxicity against roundworms and will reveal significant new insights into structure/function relationships in Cry proteins. We are also working on novel delivery systems by which Cry proteins could be used to cure intestinal roundworm infections in humans. Our data reveal that Cry proteins have excellent potential to cure human disease via their effects on roundworms.

Eri-1 expression correlates with RNAi susceptibility in plant parasitic nematodes

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In spite of apparent deficiencies in proteins known to be involved in the uptake and spread of double-stranded (ds)RNA, plant parasitic nematodes (PPNs) display an unusual susceptibility to experimental RNA interference (RNAi) by soaking. Most notably, efficient RNAi has been reported for neuronal gene transcripts, setting PPNs apart from *Caenorhabditis elegans* where nerve cells are refractory to systemic RNAi, a situation reversed in transgenic *C. elegans* expressing the transmembrane protein SID-1 in nerve cells. This susceptibility in PPNs has fostered the development of parasite-resistant transgenic plants expressing PPN-specific dsRNAs. In efforts to select the optimal targets for in planta RNAi-based parasite control, we performed tissue-specific screens for RNAi susceptibility in PPNs. We found broad RNAi susceptibility across gene transcripts expressed in nerve, muscle, hypodermis and sub-ventral gland cells. Exceptions included gene transcripts expressed in the dorsal glands and flp-31, a PPN-specific neuropeptide encoding gene expressed in a tail neuron. Although the chemical induction of pharyngeal pumping and the consequent oral uptake of dsRNA facilitated more efficient flp-31 RNAi, this approach did not alter the RNAi susceptibility of dorsal gland genes. Monitoring a PPN orthologue of the siRNAse eri-1, an inhibitor of RNAi in *C. elegans*, revealed an increased expression in response to RNAi induction across all tissue types regardless of RNAi susceptibility. However, eri-1 upregulation was significantly higher during efforts to silence dorsal gland genes. The enhanced upregulation of eri-1 in dorsal gland cells correlates with its RNAi refractory phenotype and could be key to RNAi susceptibility in parasitic nematodes.

Germline transgenesis and insertional mutagenesis in *Schistosoma mansoni* mediated by murine leukemia virus

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Functional studies will facilitate the characterization of the role and essentiality of newly available genome sequences of the major human schistosomes, S*chistosoma mansoni*, *S. japonicum* and *S. haematobium*. To develop transgenesis as a functional approach for these pathogens, we previously demonstrated that pseudotyped murine leukemia virus (MLV) can transduce schistosomes leading to chromosomal integration of reporter transgenes and short hairpin RNA cassettes. Here we investigated vertical transmission of transgenes through intra-snail stages of *S. mansoni*. Although MLV infection of schistosome eggs from mouse livers was efficient in terms of snail infectivity, >10-fold higher transgene copy numbers were seen in cercariae derived from in vitro laid eggs (IVLE). Targeting IVLE with transgenes increased the likelihood of transducing germline cells. High-throughput sequencing of genomic DNA from schistosome populations exposed to MLV revealed widespread and rando m insertion of transgenes throughout the genome, along each of the autosomes, chromosomes 1 to 7, and the sex chromosomes Z and W, confirming the utility of this approach for insertional mutagenesis. After infecting snails with miracidia from eggs transduced by MLV, sequencing of genomic DNA from cercariae released from the snails also revealed the presence of transgenes, demonstrating that transgenes had been transmitted through the asexual developmental cycle, and thereby confirming germline transgenesis. In addition, the germline-transmitted transgene encoding neomycin phosphotransferase rescued cultured schistosomules from toxicity of the antibiotic G418. These findings provide the first report of wide-scale, random insertional mutagenesis of chromosomes and of germline transmission of a transgene in schistosomes. Transgenic lines of schistosomes expressing antibiotic resistance could advance functional genomics for these significant human pathogens.

Transposon-mediated chromosomal integration of transgenes in the parasitic nematode *Strongyloides ratti* and establishment of stable transgenic lines

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Genetic transformation is a potential tool for analyzing gene function and identifying new drug and vaccine targets in parasitic nematodes. We have developed a robust system for transgenesis in *Strongyloides* spp. using gonadal microinjection for gene transfer. Using this system, transgenes are expressed in promoter-regulated fashion in the F1 but are silenced in subsequent generations, presumably because of their location in repetitive episomal arrays. To counteract this silencing, we explored transposon-mediated chromosomal integration of transgenes in *S. ratti.* To this end, we constructed donor vectors encoding green fluorescent protein (GFP) under the control of the Ss-act-2 promoter with flanking inverted terminal repeats specific for the piggyBac transposon. In three experiments, free-living *Strongyloides ratti* females were transformed with one of two donor vectors and a helper plasmid encoding the piggyBac transposase. Pooled results for the three experiments indicate that 5.8% of F1 larvae were GFP-positive. We inoculated rats with 34 F1 GFP-positive infective larvae (L3i), and 1.9% of 6014 F2 individuals resulting from this host passage expressed GFP. We cultured GFP-positive F2 individuals to produce GFP-positive F3 L3i for additional rounds of host and culture passage. GFP expression frequencies in subsequent generations were 15.9% in F3, 98.8% in F4, 76.5% in F5 and 98.68% in F6. The three resulting transgenic lines now have uniform GFP expression among all progeny after at least 10 generations of passage. Chromosomal integration was confirmed by Splinkerette PCR, which revealed the transgene flanked by *S. ratti* genomic sequences corresponding to at least 5 discrete integration sites. BLAST searches of flanking sequences against the *S. ratti* genome mapped the integrations to five contigs: 75336, 74996, 74278, 75034 and 75488, three of which are on Chromosome I. This result provides the basis for two powerful functional genomic tools in *S. ratti*: heritable transgenesis and insertional mutagenesis.

The microRNAs of *Caenorhabditis elegans* : could they play a role in drug resistance?

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Drug resistance in veterinary nematodes represents a major challenge to the food and livestock industry worldwide. However the molecular mechanisms by which resistance arises in parasite populations are poorly understood. We are investigating the hypothesis that resistance is correlated with alterations in microRNA (miRNA) activity. miRNAs are small non-coding RNAs that bind to target mRNAs and regulate their expression. They are important modulators of drug sensitivity in tumour cells and we propose that they may play similar roles in parasitic nematodes. miRNAs could influence the expression of drug transporters or sub-units of various ion channels and thus affect sensitivity to drug. We compared miRNA expression by microarray in ivermectin-resistant and wild type *Caenorhabditis elegans*. The drug resistant worms were selected by continuous growth on increasing concentration of ivermectin. This analysis demonstrated that a single miRNA was over-expressed (x40-fold) in ivermectin-resistant worms compared to wild type worms. These results were confirmed by qRT-PCR. Over-expression of this same miRNA in wild type *C. elegans* conferred resistance to ivermectin. Bioinformatic predictions highlighted multiple possible mRNA targets; worms containing a mutation in one predicted target gene appear to be resistant to ivermectin. Our on-going studies are aimed at defining the role of this microRNA in drug resistance.

Small RNA expression and secretion in vesicular exosomes by *Heligmosomoides polygyrus*

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We have conducted a high-throughput sequencing analyses of small RNAs expressed around the life cycle of the murine nematode parasite *Heligmosomoides polygyrus*. A total of 61.5 million Illumina reads were recovered from duodenal adult worms, eggs and free-living infective L3 larvae. Several hundred mature miRNAs were identified which derive from predicted pre-miRNAs mapped to the *H. polygyrus* genome sequence determined in-house. The most highly expressed miRNAs are conserved in other nematodes and display development-specific expression patterns similar to those reported in *C. elegans*. For example, 6 species of the miR-35 family are restricted to the egg, consistent with their association with embryonic development, while the conserved miR51/58 and -85/81 families are present throughout the life cycle. However, over half of the pre-miRNAs detected from small RNA reads in the adult library appear to have evolved recently and are not conserved in other nematodes. In addition, a specific family of highly abundant pre-miRNAs were identified which appear to be conserved in other nematodes and display enrichment in the pre-miRNA form compared to mature miRNA form, suggesting unusual regulation. We also detect a specific subset of pre-miRNAs and miRNAs in the secretion products of adult *H. polygyrus* based on sequencing, which we have further validated by rt-PCR. These miRNAs appear to be stabilized against degradation by RNases and associate with higher molecular weight structures that pellet upon ultracentrifugation. Analysis of purified miRNA-containing complexes by transmission electron microscopy reveals vesicle-like structures of 50-100 nM. These structures appear similar to exosomes, membrane vesicles of endosomal origin that contain miRNAs and have been shown to play a role in cell-to-cell communication in mammals. We are currently testing the hypothesis that *H. polygyrus* secretes vesicle-bound miRNAs able to modulate RNA stability and/or translation in murine cells.

Investigating the mechanism and evolution of spliced leader trans-splicing in nematodes

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Spliced leader (SL) *trans*-splicing has been identified in a wide range of eukaryotes, but much of our understanding of this process in nematodes has come from studies in *Caenorhabditis elegans* and other Chromadorian nematode species. Based on our own work and that of others spliced leader *trans*-splicing is almost certainly a conserved feature of mRNA processing in all nematodes, although questions remain as to its origin and evolution within the phylum. In addressing this, recent work by our group on the extent and nature of spliced leaders in a variety of non-Chromadorian nematodes is providing valuable insight into the likely nature of SL trans-splicing in the ancestral nematode. In addition, as SL *trans*-splicing is absent from vertebrates or plants, it represents an excellent target for the development of novel, broad-specificity anti-nematode treatments. However, despite the fact that the basic steps in spliced leader *trans*-splicing are known, how these steps are achieved at the molecular level is not understood. To address this we have recently developed a novel sensitive and specific, reporter gene-based screening strategy that allows us to visualise loss of spliced leader *trans*-splicing in vivo. We have validated our assay by showing that it is able to detect sub-lethal defects in this process, and have used this assay to show the involvement of three proteins previously implicated in in vitro experiments. This system is allowing us to carry out genome-wide screens to isolate genes that are involved in SL *trans*-splicing and in parallel to screen small molecules and natural product libraries for products with novel anthelmintic properties

Ubiquitin pathway involvement during *Trichinella spiralis* infection

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Many intracellular pathogens manipulate host cells to their advantage in order to maximize survival and replication. In most cases these manipulations lead to the destruction of host tissue once the pathogen has successfully established an infection. *Trichinella spiralis* is unique in that its life cycle comprises both extra and intracellular stages, the latter of which results not in the destruction of the host cell, but rather in its reprogramming to suit the worm's development. Infected skeletal muscle cells undergo pronounced changes including nuclear enlargement, de-differentiation, collagen capsule formation and angiogenesis. Perhaps most striking is the ability of the worm to cause cell cycle re-entry and subsequent arrest at the G2/M interface in terminally differentiated cells. *T. spiralis* likely achieves these manipulations through the direct secretion of proteins into the host cell. Only a subset of the estimated 150 proteins contained in this secre ted fraction have been characterized and, notably, the elements responsible for the cell-cycle changes have not been identified. The ubiquitin pathway has been highly conserved throughout evolution and there is ample evidence of viral and bacterial pathogens encoding host-targeted proteins able to interfere with this pathway. Eukaryotic parasites possess a Ub-pathway of their own as well as the potential ability to interfere with host Ub. In the case of parasites such as *Trichinella* with intracellular life-cycle stages and secretory abilities, it is possible that Ub-pathway proteins are involved in parasite-derived, host-targeted manipulations. We are investigating the role of *T. spiralis*-derived Ub-pathway components in skeletal muscle cell reprogramming seen during nurse cell development. We are using a combination of functional chemistry, proteomics and and biochemical approaches to probe the worm excretory-secretory fraction, with a particular focus on cell cycle control.

Role of schistosome ABC transporters in parasite physiology and drug susceptibility.

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Schistosomiasis affects hundreds of millions worldwide, yet only a single drug (praziquantel, PZQ) is available in most parts of the world. Though indispensable, PZQ has shortcomings, including an unknown mode of action and lack of activity against juvenile schistosomes. Reliance on a single drug for a disease of this magnitude clearly represents a treacherous situation, particularly when considered in light of the looming threat of drug resistance. Members of the ATP-binding cassette (ABC) superfamily of proteins mediate transport of toxins and xenobiotics from cells and are associated with development of multidrug resistance in mammals and resistance to antiparasitic drugs, including anthelmintics. We hypothesize that schistosome ABC transporters such as SMDR2 and SmMRP1 could provide attractive targets for novel antischistosomal agents that act on their own or as adjuncts to PZQ. In support, we find that SMDR2 and SmMRP1 are upregulated in worms exposed to sub -lethal concentrations of PZQ, and that higher basal expression of these two transporters is associated with reduced susceptibility to PZQ. PZQ also acts as both an inhibitor and a likely substrate of recombinant SMDR2. Disruption of SMDR2 or SmMRP1 expression or function leads to reduced egg production by worms in culture, and administration of Pgp inhibitors to *S. mansoni*-infected mice results in reduced liver egg burden and liver pathology. Most significantly, co-administration of Pgp inhibitors with PZQ potentiates PZQ activity against adult worms, as well as normally PZQ-refractory juvenile schistosomes. We are currently confirming these results and extending them to investigate components of the adaptive signaling pathways activated by schistosomes in response to PZQ. These findings indicate that drug transporters such as SMDR2 and SmMRP1 may be excellent candidate targets for new anthelmintics that act either on their own or that enhance the activity of currently available therapeutics. Supported by NIH grant R01AI073660.

Discovery of platyhelminth-specific alpha/beta integrin families and evidence for beta-integrin function in signaling complexes within the gonads of *Schistosoma mansoni*

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During the last decade convincing evidence has accumulated that signal transduction pathways in *S. mansoni* control proliferation and differentiation of female oocytes and vitellocytes following pairing (1). Among the genes intensively characterized are cellular tyrosine kinases (CTKs) such as SmTK6, SmTK4, and SmTK3, which were found to colocalize in the reproductive organs, presumably acting in a multi-kinase complex (2). Recent results from interaction studies indicated that this complex is controlled by receptor tyrosine kinases (RTK) such as SmVKR1 (3), an atypical RTK (4). Evidence from other biological systems suggested that integrin receptors may be additionally involved in activating such RTK-CTK complexes (5). We have identified and cloned one beta-integrin receptor (Smb-Int1) and four alpha-integrin receptors (Sma-Int1 - Sma-Int4) of *S. mansoni*, analyzed their amino acid sequences, evolutionary classification, tissue-specific transcriptional profiles, and examined the potential of Smb-Int1 to interact with intracellular signaling molecules as well as its function in adults by a novel RNAi approach. Data will be presented demonstrating (i) the discovery of new platyhelminth-specific integrin clades containing branches discriminating orthologs of free-living and parasitic classes, (ii) colocalization of Smb-Int1 with the schistosome CTKs SmTK6, SmTK4, and SmTK3 in the gonads, (iii) interactions of Smb Int1 with these CTKs, and (iv) the usefulness of a combinatory siRNA approach, which provided first evidence for a decisive role of Smb-Int1 during oogenesis. Our findings shed new light on the functions of integrins in schistosomes and the complexity of the processes involved in their reproductive biology, which may be representative for other trematodes as well.

Fatty acid oxidation is essential for egg production by the parasitic flatworm *Schistosoma mansoni*

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Schistosomes are parasitic flatworms that cause the neglected tropical disease schistosomiasis. As adult males and females, they live as closely associated pairs within the portal vasculature. Females are dependent on males to achieve reproductive maturity, and when fecund produce hundreds of eggs each day. Schistosomes have been considered to have an entirely carbohydrate based metabolism. However, we have found that fatty acid β-oxidation (FAO) coupled to oxidative phosphorylation is essential for female schistosomes to make eggs. These processes are linked to a dynamic network of lipid droplets within proliferating and differentiating cells in a specialized reproductive organ, the vitellarium. Our data point to the importance of FAO for the compartmentalized process of egg production in schistosomes. As such, it represents a potential target for inhibiting the transmission of an important human pathogen.

Molecular and functional studies of the *Schistosoma mansoni* Venus kinase receptors SmVKR1 and SmVKR2: potential roles in larval development and oogenesis

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Venus kinase receptors (VKRs) form a new family of receptor tyrosine kinases. Atypically, VKRs contain an extracellular Venus Flytrap (VFT) domain, a ligand-binding domain activated by small molecules such as aminoacids (Ahier et al., 2009). Vkr genes are found in diverse eumetazoan genomes, from cnidarians to echinoderms and are particularly well conserved in protostomian species (Vanderstraete et al., in prep). In the platyhelminth *Schistosoma mansoni*, two VKRs have been described, SmVKR1 and SmVKR2 (Gouignard et al., 2011). Quantitative RT-PCR as well as in situ hybridization indicated a large expression of both genes in larval stages and in female ovaries. RNA interference experiments performed on sporocysts and adult worms further confirmed the implication of SmVKRs in larval development and oogenesis. Using Xenopus laevis oocytes for protein expression, we demonstrated that SmVKR1 could bind and be activated by amino acids, mainly by L-Arginine, whereas SmVK R2 activation was triggered by calcium ions. In order to decipher the downstream signalling pathways of SmVKR1 and SmVKR2, we have started to identify binding partners of these receptors by the screening of an adult worm cDNA library using the yeast two-hybrid system. Our results suggest that both SmVKR1 and SmVKR2 participate in cytoskeleton rearrangement and in developmental mechanisms. Potential substrate/adapters for SmVKR1 have been identified and their function in the activation pathway of the receptor is under investigation.

The role of the kinetic properties of lactate dehydrogenase in the metabolic switch of *Schistosoma mansoni* cercariae.

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The lifecycle of the blood dwelling fluke *Schistosoma mansoni* comprises free-living and parasitic stages that encounter environmental conditions that differ in substrate availability. Free-living stages of *S. mansoni* use as energy source their endogenous glycogen stores, which are aerobically degraded via the Krebs cycle to CO2, at the same time producing ATP via oxidative phosphorylation. When cercariae penetrate the final host and transform into schistosomula, they use external glucose from the host and switch immediately from mitochondrial CO2 production from glycogen, to lactate production from glucose via glycolysis. We postulated that it are the special kinetic properties of the glycolytic enzymes of *S. mansoni* that allow this instantaneous and reversible switch from aerobic metabolism to fermentation, depending on the external glucose supply. Hexokinase, phosphofructokinase and lactate dehydrogenase are key enzymes that regulate the glycolytic flux to lactate. In previous studies we have shown that schistosomal hexokinase is distinct from mammalian hexokinase as it is poorly inhibited by its product glucose-6-phosphate. We now show that schistosomal lactate dehydrogenase (SmLDH) also differs from other eukaryotic LDHs, including the isozymes present in the final host. Purified and recombinantly expressed SmLDH was shown to be inhibited by physiological concentrations of ATP and this inhibition could be abolished by addition of intermediates of glycolysis. In this respect SmLDH bares more resemblance to many prokaryotic LDHs than to eukaryotic ones. Furthermore, recombinantly expressed mutated forms of SmLDH revealed the binding pocket for the glycolytic intermediates. The kinetic differences with LDH of the host, which is mirrored in differences in primary structure of SmLDH, will be discussed as SmLDH is a potentially interesting drug target because anaerobic glycolysis is for all stages in the final host the only pathway available to generate ATP.

A nematode venom allergen/ASP-like protein is required for plant-parasitism and induces a hypersensitive response through an extracellular leucine-rich repeat receptor

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Plants lack the seemingly unlimited receptor diversity of a somatic adaptive immune system as found in vertebrates, and rely on only a relatively small set of innate immune receptors to resist a myriad of pathogens. Here, we show that disease resistant tomato plants use an efficient mechanism to leverage the limited non-self recognition capacity of their innate immune system. We found that the extracellular plant immune receptor protein Cf-2 of the currant tomato (*Solanum pimpinellifolium*) has acquired dual resistance specificity by sensing perturbations in a common virulence target of two independently evolved effectors, one of a fungus and a venom allergen/*Ancylostoma* secreted-like protein from the nematode *Globodera rostochiensis*. The Cf-2 protein, originally identified as a monospecific immune receptor for the leaf fungus *Cladosporium fulvum*, also mediates disease resistance to the root parasitic nematode *Globodera rostochiensis*. The Cf-2-mediated dual resista nce is triggered by effector-induced perturbations of the apoplastic papain-like protease Rcr3pim of *S. pimpinellifolium*. Binding of the venom allergen-like effector protein Gr-VAP1 of *G. rostochiensis* to Rcr3pim perturbs the active site of this cysteine protease. In the absence of the Cf-2 receptor, Rcr3pim increases the susceptibility of tomato plants to *G. rostochiensis*, thus showing its role as a virulence target of plant parasitic nematodes. Furthermore, both nematode infection and transient expression of Gr-VAP1 in tomato plants harboring Cf-2 and Rcr3pim trigger a defense-related programmed cell death in plant cells. Overexpression of parasitic venom-allergen like proteins in host plants, leads to an increased susceptibility to oomycetes, fungi and nematodes, suggesting an important role of plant parasitic venom-allergens in modulating innate immunity. Our data further demonstrate that monitoring host proteins targeted by multiple pathogens broadens the spectrum of disease resistances mediated by single plant immune receptors.

Schistosome glycosylation and the application of glycan arrays for monitoring antibody responses in human schistosomiasis cohorts

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The parasitic blood fluke *Schistosoma mansoni*, like all organisms, expresses a range of complex glycans as part of glycoproteins and glycolipids. The expression and biosynthesis of functional and immunogenic glycan elements is developmentally regulated and cell- and species-specific. In infected humans a large proportion of the antibody response to schistosomes is directed against the numerous glycan epitopes expressed by the different life cycle stages of the parasite. At present it is unclear if (a subset of) such anti-glycan antibodies can confer protection from (re-)infection or not. To address this question we have first unravelled N- and O-glycan profiles of *S. mansoni*, including the invasive larvae, juvenile as well as adult worms, eggs and miracidia. These profiles indicated that the expression of many potentially antigenic glycan motifs gradually shifts between the different stages, but that also glycan motifs exist that are unique for a particular life cycle stage. A library of defined anti-glycan monoclonal antibodies in combination with immune fluorescence microscopy was applied to study the parasite surface expression of glycan motifs during development. Subsequently, we have printed the hundreds of glycans purified during these structural studies on glass slides to create glycan microarrays. The arrays were then screened with sera from cohorts of *S. mansoni*- or *S haematobium*-infected individuals to monitor antibody responses to the different glycan antigens. Overall anti-glycan antibody titers are higher in children than in adults, but response profiles to particular subsets of glycans are correlated to other variables such as infection intensity, treatment or re-infection. The application of the array for the discovery of glycan vaccine targets for schistosomiasis and other helminth infections will also be discussed. (Supported by NWO-CW ECHO grant 700.58.003 and the EU-FP7 project TheSchistoVac 242170)

Specific glycan elements determine differential binding of individual egg glycoproteins of the human parasite *Schistosoma mansoni* by host C-type lectin receptors

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During infection with the blood fluke *Schistosoma mansoni,* the parasite's eggs induce the formation of granulomas and a Th2-type immune response. These processes are at least partly triggered by glycosylated egg antigens interacting with C-type lectin receptors (CLR) on host antigen-presenting cells such as dendritic cells (DC). We studied in detail the binding of two abundant, differently glycosylated egg antigens, kappa-5 and IPSE/α1, by the primary CLR involved: DC-specific ICAM3-grabbing non-integrin (DC-SIGN), macrophage galactose-type lectin (MGL) and mannose receptor (MR). Egg glycoprotein kappa-5 carries tri-antennary N-glycans containing terminal GalNAcβ1-4GlcNAc (LDN) of which <5% is fucosylated (LDN-F), while IPSE/α1 mainly carries di-antennary Galβ1-4(Fucα1-3)GlcNAc (LeX) structures. Glycan array analysis in which glycoconjugates fixed to glass slides were probed with Fc-constructs of each CLR showed strong binding of DC-SIGN-Fc to most fucosyla ted structures including LeX and LDN-F, of MGL-Fc to LDN(-F), and of MR-Fc mainly to mannosylated structures. Representing interactions in the more natural context of glycoproteins secreted/excreted from eggs, native soluble kappa-5 and IPSE/α1 were examined for their binding by cellular CLR on DC. Unexpected on basis of the glycan array data, the major receptor on DC for IPSE/α1 is MR. α-Fucosidase-treated IPSE/α1 analysed for binding to cell lines expressing single CLR, confirmed that cellular MR binds the LeX-fucoses. Interestingly, while the MGL-ligand LDN is the major glycan on kappa-5, all three CLR, DC-SIGN, MGL and MR, contributed to a similar extent in the DC-binding of kappa-5. Exoglycosidase-treatment of kappa-5 revealed that the antenna fucoses of LDN-F are essential for its recognition by DC-SIGN, while LDN is required for MGL-binding. Kappa-5-binding by MR may involve either LDN-F antennae or the fucosylated and xylosylated trimannosyl-chitobiose core. The se data provide a structural basis for the interaction between DC and individual egg glycoproteins and subsequent CLR-dependent immunomodulation.

The *Echinococcus granulosus* laminated layer carbohydrates: structure and recognition by host lectin receptors

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Echinococcus larvae are protected by the extracellular matrix called laminated layer (LL), a unique structure central to the biology of these infections. We previously showed that the *E. granulosus* LL includes abundant nano-deposits of calcium inositol hexakisphosphate. The other major component of the LL is a meshwork of mucins, as now made clear by electrophoretic and lectin-binding studies and by an in-depth glycomic analysis. The LL carbohydrates are indeed based on the conventional cores 1 and 2 of mucin-type O-glycans. The core Gal residue is elongated with a variable number of Gal beta1-3 residues, and this "main chain" can be capped with a single Gal(alpha1-4) residue. The "main chain" can also be ramified with GlcNAc(beta1-6) residues, which can in turn be decorated with the Gal(alpha1-4)Gal(beta1-4) disaccharide. The same disaccharide can decorate the core 2 GlcNAc residue. Hence both the "main chain" branches and core 2 can display the P1 blood group antigen trisaccharide, explaining a reactivity long reported in the LL. The structural motifs summarised apparently account essentially for the total of the LL carbohydrates, and are related to those previously reported in glycans carried by an *E. multilocularis* mucin. Cell-surface lectin receptors are important in decoding self and microorganisms and hence shaping immune responses. Contrary to our expectation, neither the E. granulosus nor the E. multilocularis LL mucins were bound by any member of a panel of 30 recombinant lectin receptors known to be expressed in "systemic" human macrophages. In contrast, both materials were recognised by the mouse Kupffer cell receptor (KCR). This receptor also recognised five synthetic glycans representing the major LL carbohydrate motifs. The KCR is expressed only in rodent liver macrophages. As rodents are possible ancestral intermediate hosts for Echinococcus, and the liver is the major infection organ, the interaction may be evolutionarily shaped.

The Nematode Astacins: key enzymes in cuticle collagen synthesis and moulting

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The nematode cuticle is a collagenous extracellular matrix that is repeatedly shed and re-synthesised throughout post-embryonic development via the moulting cycle. We study the enzymes involved in the synthesis, modification, processing and crosslinking of the cuticle components in the tractable model *Caenorhabditis elegans*. Using a combination of genetics and biochemistry we have identified key astacin zinc metalloproteases that are responsible for cuticle collagen processing, moulting and the maintenance of structural integrity and viability in *C. elegans*. We have identified and the orthologues from *Haemonchus contortus* and *Teladorsagia circumcincta* and we will report on the characterization and chemical disruption of these enzymes in *C. elegans* and these key Trichostrongylid species.

Schistosome surface enzymes

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Intravascular schistosomes are covered by an unusual double-lipid bilayered membrane. We are interested in the molecular composition of this host-interactive structure. A series of enzymes has been identified at this site. We have hypothesized that some of these function to impede host immunological signaling through Danger Associated Molecular Patterns (DAMPs). For example, by employing RNA interference (RNAi), we have shown that tegumental ATPdiphosphohydrolase (SmATPDase) is capable of cleaving the pro-inflammatory DAMP, ATP, from the parasite's external environment. Furthermore, tegumental alkaline phosphatase (SmAP) can cleave exogenous AMP to generate the potent anti-inflammatory mediator, adenosine. In this manner the parasites may generate and maintain a less immunologically hostile external environment. A third enzyme, tegumental phosphodiesterase (SmNPP-5) exhibits a unique distribution; electron microscopy reveals unusual, discrete clusters of SmNPP-5 immunogold staining throughout the tegument. While the precise molecular function of SmNPP-5 is not yet known, we have shown that suppressing SmNPP-5 gene expression, using RNAi in schistosomula, severely impedes the ability of the parasites of establish infection. SmNPP-5 can therefore be considered a virulence factor for schistosomes. Other tegumental enzymes include carbonic anhydrase (SmCA) which likely functions in pH regulation and acetylcholinesterase (SmAChE) which may directly impact host vascular physiology. Our work is designed to help understand how the host interactive surface enzymes of intravascular schistosomes contribute to the global success of these parasites by impacting host immunology and physiology and by controlling the biochemistry of the parasite's local environment.

Anthelmintic AChR channels from the Clade III nematodes *Brugia malayi* and *Ascaris suum*: the same but different

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Why do some cholinergic anthelmintics work against some nematode parasites but not others? There are some effective anthelmintics for treating adult *Ascaris suum*, but drugs for treating adult *Brugia malayi* are very limited. Both parasites are Clade III nematodes and appear to have the same AChR channel subunits (UNC-63, UNC-29, UNC- 38, ACR-8), but they may combine in different ways to produce a range of different muscle AChR channel subtypes. Here we report development of a new preparation and initial observations from adult *B. malayi* muscle Whole-cell voltage clamp recordings from *B. malayi* showed that activation of AChR channels produces a dose-dependent and reversible inward current. The agonist potency series was: acetylcholine> tribendimidine> levamisole> pyrantel> bephenium. The potency series from *A. suum* was tribendimidine> pyrantel> bephenium> levamisole> acetylcholine, suggesting that AChR subtypes present in *B. malayi* are different to that of *A. suum.* Twelve single channel patches from *B. malayi* containing AChRs, were observed using acetylcholine or levamisole as the agonist. Detailed analysis revealed that single-channel conductance values of *B. malayi* were within a narrow range (26.3:28.5 pS) compared to that of *A. suum* (18:53 pS). The wide range of conductances observed in *A. suum* is a result of multiple nAChR subtypes present, while the narrow range in *B. malayi* may be due to the presence of fewer AChR channel subtypes. The subunits that make up the levamisole AChR channel in *A. suum* and *B. malayi* appear very similar, so why is there a difference in conductance ranges? It is interesting to speculate that unlike *A. suum*, *B. malayi* may express only a single subtype of muscle AChR channel due a non-plastic arrangement of their AChR subunits; further experiments are required to test this.

Investigation and validation of a potential new drug target for nematodes

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In a screen for conserved nematode proteins that exhibit highly compromised RNA interference (RNAi) phenotypes in *C. elegans* we identified UDP-galactopyranose mutase (GLF-1; E.C.4.4.99.9) as a potential anti-nematode drug target. RNAi of glf-1 in *C. elegans* confirmed the published phenotypes (Kamath et al. 2003 Nature 421, 231; Novelli et al. 2009 Devel. Biol. 335, 340), including locomotory defects progressing to paralysis, abnormal larval development, and cuticular permeability to the fluorescent DNA-binding dye, Hoechst 33258. RNAi of glf-1 in wild-type *C. elegans* also led to potentiation of the effects of levamisole, ivermectin and mebendazole. We cloned the putative orthologue of glf-1 from Haemonchus contortus and used RNAi of the free-living larval stages of H. contortus (Zawadzki et al. 2012 Parasitol. in press) to show that knockdown of Hc-glf-1 caused abnormal development from L1 to L3, reduced locomotory activity and permeability to Hoechst 33258. To assess the physiological functions of Hc-GLF-1 we tested whether expression of Hc-glf-1 could rescue the functions of glf-1 in *C. elegans* using a "differential RNAi" technology (Britton & Murray 2002 Parasitology 122, 21; Zawadzki et al. 2012 Parasitology in press). We constructed transgenic strains of *C. elegans* that expressed both Hc-glf-1 and *C. elegans* glf-1. When *C. elegans* glf-1 was selectively knocked down by RNAi, the strains expressing Hc-glf-1 did not exhibit the glf-1 RNAi phenotype, indicating that expression of Hc-GLF-1 effectively replaced the critical functions of GLF-1. This work validates GLF-1 as a potential new drug target for parasitic nematodes and shows that a drug targetting nematode GLF-1 could act potentially both as a nematotoxin and a potentiating agent for other anti-nematode drugs. Research funded by Meat and Livestock Australia and Australian Wool Innovation.

Population genetic analysis of *Haemonchus contortus* and *Teladorsagia circumcincta* suggests independent benzimidazole resistance mutations frequently arise in the field

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Anthelmintic drug resistance now seriously compromises the control of parasitic nematodes of livestock and Its emergence in human parasites is of increasing concern. Understanding how resistance mutations arise and spread is an important goal to inform the design of control programs and develop strategies to minimize the spread of resistance. Several Single Nucleotide Polymorphisms leading to amino acid substitutions in the isotype I β-tubulin polypeptide (P167,P198 and P200) are associated with benzimidazole resistance and have been studied in detail in the sheep nematode parasites *Haemonchus contortus* and *Teladorsagia circumcincta*. This provides an excellent system in which to study how anthelmintic resistance mutations originate in the field. We have undertaken a detailed population genetic study of these two parasite species on seven different UK farms. Using panels of microsatellite markers, we have found that T. circumcincta has significantly higher levels of genetic variation but markedly less geographical sub-structuring than H. contortus. We hypothesize that this is due to epidemiological differences (survival of infective stages on the pasture) leading to greater population bottlenecking for *H. contortus*. Sequencing of isotype I β-tubulin alleles across the seven farms again reveals a lower level of genetic diversity for *H. contortus* than *T. circumcincta*. For *H. contortus,* phylogenetic and haplotype network analysis suggests at least 4 independent origins for resistance mutations found across the seven farms sampled. For *T. circumcincta* an even greater number of independent origins are inferred. Our analysis supports a model of multiple independent origins of anthelmintic resistance by recurrent mutation accompanied by translocation and spread. This contrasts with examples of rare mutations followed by simple selective sweeps in insecticide and antimalarial resistance. These results have major implications for the application of population genomic approaches to identify areas of parasitic nematode genomes under selection and loci contributing to anthelmintic resistance.

A unique nematode peptide library for the development of novel anti-helminthic drugs

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Over the past 25 years, a unique peptidomics technology platform was developed in our institute that enables the identification of endogenous neuropeptide hormones from different species, including nematodes. This peptidomics approach combines a unique peptide extraction method with multi-dimensional liquid chromatography separation, high resolution tandem mass spectrometry and dedicated, in-house developed bioinformatic algorithms for peptide identification. Over the past decade, more than 250 novel endogenous neuropeptide hormones have been identified in *C. elegans* alone with this peptidomics approach. For more than 10 of these peptides we have already been able to elucidate the physiological function and identified the cognate G-protein coupled receptor. This unique peptide library is currently also successfully being used to find ligands for receptor targets in parasitic nematodes. Knowledge on the natural ligands of target receptors offers the opportunity to specifically engineer peptide mimetics or small molecule drugs that bind to the relevant domain of the receptor, but that have better pharmacological properties. We therefore believe that our peptide library forms a strong basis for future anti-helminthic drug development.

Using genome-wide proteome microarrays to understand the humoral response to infectious diseases

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We developed an approach to construct and probe protein microarrays on a genome-wide scale from more than 25 medically important infectious microorganisms including, *P. falciparum*, *P. vivax*, *S. mansoni, S. japonicum, B. melitensis, M. tuberculosis*, 13 other bacteria and 15 viruses. We made more than 36,000 plasmids, printed the encoded proteins on 25,000 microarrays and probed them with 12,000 serum specimens determining disease associated antibody profiles. The individual proteins printed on these chips capture antibodies present in serum from infected individuals and the amount of captured antibody can be quantified using fluorescent secondary antibody. In this way a comprehensive profile of Abs that result after infection can be determined that is characteristic of the type of infection and the stage of disease. The broad goals of this research activity are to develop a more detailed understanding of how the immune system responds to medically important infectious microorganisms by quantifying the immune response to infection against thousands of antigens on an antigen specific, patient specific, and population specific basis, and to identify serodiagnostic and subunit vaccine antigens. The approach permits assessing the repertoire of Abs generated in response to infections or vaccinations from large collections of individual patient sera while consuming a small amount of each sample (<2µl), and can be used to perform large-scale sero-epidemiological, longitudinal and sero-surveillance analyses not possible with other technologies. Studies completed so far indicate that we are only at the beginning of our understanding of the humoral response to infections and they highlight the need and opportunity to continue to develop and apply genome-wide approaches to understand infectious disease. Here we will describe progress on projects where we produced and screened a schistosome tegument membrane array and a hookworm entire secretome array.

The therapeutic hookworm - of mice and men, mechanisms and molecules

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Parasitic helminths have received much attention of late due to their utility as novel therapies for human inflammatory diseases. Of these helminths, we consider the hookworm, *Necator americanus*, to be the most appropriate for the following reasons: (1) good safety profile in low dose experimental infections/clinical trials; (2) long-lived enteric infection; (3) strong immuno-epidemiological associations between infection and reduced prevalence of inflammatory diseases. To further address the therapeutic potential of hookworms, we utilised a combination of live hookworm therapy in human trials and administration of excretory/secretory (ES) products in murine models of inflammatory diseases. We assessed the systemic and mucosal responses of humans to experimental *N. americanus* infection and showed that inflammatory Th17 responses are suppressed in the gut, possibly via mechanisms that include the induction of IL-22 and suppression of IL-23. Using mouse models of colitis, we showed that administration of ES products alone (in the absence of a live helminth infection) induces the same general immune phenotype as that observed in human hookworm infections, and protects against inflammation. Protective ES moieties were of protein origin, so we utilised various chromatographic separation and proteomic techniques to characterise the ES proteome and identify the proteins of interest. Two ES proteins have been isolated and produced in recombinant form in yeast, both of which protect against colitis and airway obstruction in mouse models. We are now exploring the mechanisms by which these recombinant proteins suppress inflammation, and have established a pathway for their pre-clinical development through partnership with large pharma.

Development of small molecule analogues of the *Acanthocheilonema viteae* immunomodulator ES-62 for therapeutic purposes

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ES-62 is a major secreted immunomodulator of the rodent filarial nematode *Acanthocheilonema viteae*. As a consequence of its anti-inflammatory properties, ES-62 can significantly reduce disease development in mouse models of asthma, cardiovascular disease, arthritis and lupus. These findings are consistent with the hypothesis that parasitic worm infection can protect humans from developing diseases associated with aberrant inflammation and raise the possibility that ES-62 may have therapeutic potential. However ES-62 is an immunogenic glycoprotein and is thus not suitable for use as a drug. Nevertheless, its anti-inflammatory activity is dependent on a post-translational modification, phosphorylcholine (PC) attachment, thus raising the possibility of designing small molecule analogues (SMAs) based around PC. Approaching 100 SMAs have been synthesized and tested for immunomodulatory activity in vitro, mainly by determining effects on cytokine production by macrophages exposed to PAMPs and mast cells activated via FcεRI. Two SMAs : S3 and S5, which were found to inhibit the inflammatory responses of the two cell-types, were selected for testing in vivo and, as with ES-62, were found to reduce disease development in both the collagen-induced arthritis (CIA) and ovalbumin-induced airway hypersensitivity models. The essentially identical results obtained between ES-62 and the SMAs allied to their all sharing a similar structural feature naturally led us to assume the same mechanism of action. However, further analyses with S5 showed that unlike for ES-62, the in vitro effects on macrophages are retained in $TLR4^{-/-}$ mice, and its protective effects in CIA are not obviously reliant on targeting the IL-17 inflammatory axis. More recently, S5 has been subjected to metabolomics and microarray analysis and consistent with its anti-inflammatory properties, a notable finding from macrophage microarray data was that the gene showing most down-regulation (x9) was IL-1B. S5 and S3 are now being subjected to further development towards producing novel, effective anti-inflammatory therapeutics.

The helminth-derived immunomodulator AvCystatin blocks Th2-driven lung eosinophilia in mice infected with respiratory syncytial virus

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Respiratory syncytial virus (RSV) is the major world-wide cause of viral bronchiolitis, the commonest cause of acute lower respiratory tract illness in infancy. Pathology seems to be mainly dependent on excessive immune reactivity, and no effective drugs are available to treat infection. Parasitic helminths have evolved potent modulators of immune responses in order to persist in their hosts, thereby potentially blocking other host immune reactions as well. For example, the immunomodulator AvCystatin, a cysteine proteinase inhibitor derived from *Acanthocheilonema viteae*, has successfully been used to attenuate murine allergic airway inflammation. We therefore investigated the effect of recombinant AvCystatin in RSV-induced eosinophilic airway inflammation. Mice were sensitized with vaccinia virus expressing the RSV G-protein (vvG) and challenged with RSV, causing a Th2-mediated enhanced inflammation. Treatment of mice with AvCystatin completely ablated eosinophil influx into the airway and reduced excessive weight loss. Th2 cytokine (IL-4, IL-13) and chemokine (RANTES, Eotaxin) production, linked to vvG RSV pathology, was also diminished in the airways. Th1-related cytokines were partly modulated, with a reduction of IFN-γ but no change in TNFα levels. In keeping with ameliorated pathology, we found that AvCystatin treatment increased the numbers of CD4+ IL-10+ Tr1 cells in lungs and airways of RSV challenged animals. These findings extend previous reports that AvCystatin modulates macrophage responses to an adaptive response as a mechanism of protection. Remarkably, the effects of AvCystatin were specific to reduction of airway immunopathology, as viral load and persistence were not enhanced, which supports the feasibility of using AvCystatin as a potential drug in virus-related airway inflammation.

A filarial glycoprotein and chitohexose activate macrophages by alternate pathway through TLR4 and block Endotoxemia

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We report here that a filarial glycoprotein binds to murine macrophages and human monocytes thro TLR4 and activates them through alternate pathway and in the process inhibits LPS mediated classical activation which leads to inflammation associated with endotoxemia. The active component of the nematode glycoprotein mediating alternate activation of macrophages was found to be a carbohydrate residue, Chitohexaose. Murine macrophages and human monocytes upregulated Arginase 1 and released high levels of IL-10 when incubated with chitohexaose and features similar to alternately activated macrophages. However, Macrophages of C3H/HeJ mice which are non-responsive to LPS due to a mutation in TLR4 gene failed to get activated by Chitohexaose suggesting that a functional TLR4 is critical for alternate activation of macrophages also. Chitohexaose inhibited LPS induced production of inflammatory molecules TNF-α, IL-1β and IL-6 by murine macropahges in vitro and in vivo. A dministration of Chitohexaose completely protected mice against endotoxemia when challenged with a lethal dose of LPS. Furthermore, Chitohexaose was found to reverse LPS induced endotoxemia in mice even 6/24/48 hrs after its onset. Monocytes of subjects with active filarial infection displayed characteristic alternate activation markers and were refractory to LPS mediated inflammatory activation suggesting an interesting possibility of subjects with filarial infections being less prone to develop of endotoxemia. These observations that innate activation of alternate pathway of macrophages by chtx through TLR4 has offered novel opportunities to cell biologists to study two mutually exclusive activation pathways of macrophages being mediated through a single receptor.

Dangerous Liaisons: parasites and the immune system

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Employing diverse models of microbial colonization, pathogen infection and chronic inflammation, research in the Artis lab is examining how mammalian host genetics and signals derived from commensal microbial communities influence innate and adaptive immune responses in the skin, lung and intestine. Intestinal epithelial cells (IECs) were recently shown to play a critical role in maintaining the balance of tolerance, immunity and inflammation at barrier surfaces including the gastrointestinal tract. Based on these findings, there are three major research areas in the lab. First, we are employing inducible deletion or overexpression of genes in IECs to interrogate how they regulate the functions of intestinal myeloid and lymphocyte lineages. The long-term goals of these studies are to improve oral vaccination against enteric infections and prevent chronic inflammation associated with diseases including food allergy and inflammatory bowel disease. Second, we are employing gnotobiotic mice to examine the influence of commensal microbial communities on intestinal and peripheral immune cell development and function. Our findings indicate that commensal microbes have a major regulatory influence on CD4+ T cell and granulocyte function associated with susceptibility to multiple inflammatory diseases. To determine if the immune system reciprocally regulates the acquisition and/or composition of commensal microbial communities, we are undertaking high-throughput pyrosequencing analyses of bacterial communities in murine models of health and disease. Third, we are investigating how IECs regulate allergen- or helminth-induced type 2 inflammation at mucosal sites. Secretion of IEC-derived cytokines including IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) appear to be important early events in influencing dendritic cell and CD4+ T cell responses required these responses. Our recent studies suggest that IECs also govern extramedullary hematopoiesis that can influence the development of T_H2 cytokine responses. It is hoped that the results of these studies will advance understanding the pathophysiology of multiple mucosal inflammatory diseases, including asthma, allergy and inflammatory bowel disease and provide a framework to test the therapeutic potential of manipulating IEC responses in these disease states.

The role of muscarinic acetylcholine receptor M3 in immune response to pathogens using transgenic murine models

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Muscarinic receptors are G protein coupled receptors that bind acetylcholine, initiating an intracellular signalling cascade. There are 5 subtypes of muscarinic receptors (i.e M1-M5), first identified in the central nervous system but subsequently discovered on other non-neuronal cells. M3 muscarinic receptors (M3R) in particular are expressed on immune cells such as T cells, macrophages and dendritic cells where their function remains unknown. In our study, we aim to elucidate the role of M3 receptors on immune cells, using a mouse strain lacking the M3 receptor (M3R-/-) and a nematode parasite *Nippostrongylus brasiliesis.* The absence of M3R significantly delayed parasite expulsion during a primary infection with 2-fold higher parasite burden and severely impaired clearance in the lung after a secondary infection together with significant reduction in TH2 cytokine production in intestine and lungs. There was an increase in M3 mRNA expression in CD4 T cells after infection and addition of muscarinic receptor specific agonists in vitro significantly increased TH2 cytokine production. Furthermore, adoptive transfer of naive wildtype CD4 T cells into M3R-/- mice abrogated the increased parasite burden observed. Also, adoptive transfer of CD4 T cells from infected M3R-/- into naive WT mice did not provide protection against subsequent infection with *N. brasiliensis* whereas transfer of corresponding WT CD4 T cells resulted in 3 fold decrease in parasite burden. Therefore, we can conclude that M3R on CD4 T cells is required for its effective development which is subsequently important for mounting protective immune responses and TH2 cytokine production against *N. brasiliensis*. This study highlights a novel previously unknown function of muscarinic receptors on immune cells and adds to existing literature on acetylcholine receptor regulation of immune functioning. Furthermore, the existence of approved M3R selective drugs such as SpirivaÂ ® makes this receptor an attractive target to improve protective immune responses during disease.

Different impact of regulatory T cells on nematode-induced immune evasion in BALB/c and C57BL/6 mice

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To avoid expulsion by their host's immune response, pathogenic nematodes exploit regulatory pathways that are intrinsic parts of the mammalian immune system. Foxp3+ regulatory T cells (Treg) represent one central mediator of immunological homeostasis. We have shown recently that *Strongyloides ratti* infection induced a rapid increase in Treg numbers. Using BALB/c "Depletion of Treg mice" (DEREG) we demonstrated that these nematode-induced Treg interfered with the successful immune response since transient depletion of Treg led to increased cytokine production, accelerated mast cell degranulation and improved expulsion of *Strongyloides* from the small intestine. Here we compare the impact of nematode-induced Treg on host defense in different mouse strains. Although Treg were induced with comparable kinetics in BALB/c and C57BL/6 mice, Treg depletion did not change worm burden in C57BL/6-DEREG mice, whereas worm expulsion was reproducibly improved in BALB/c-DEREG mice. The efficiency of Foxp3+/CD4+ Treg depletion in lymphatic organs and in small intestine was comparable in both strains. We also observed similar increases in polyclonal IgE serum concentrations and IFN-gamma as well as IL-13 production upon Treg depletion. Production of *Strongyloides* antigen-specific IL-9, in contrast, was enhanced selectively in Treg-depleted BALB/c-DEREG but not in Treg-depleted C57BL/6-DEREG mice. Likewise, accelerated mast cell degranulation as a consequence of Treg depletion was observed in BALB/c-DEREG mice only. Taken together our findings suggest that control of IL-9 and mast cell-mediated nematode expulsion was differentially regulated in BALB/c and C57BL/6 mice. While Treg appear to be dominant regulators in BALB/c mice, other regulatory elements, that remain to be identified, may play a more central role in C57BL/6 mice.

IL-6 controls natural Treg expansion and Th2-mediated expulsion following chronic helminth infection

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Interleukin-6 plays a vital role in establishing the balance between pro- and anti-inflammatory responses following infection by determining naïve T cell polarisation towards the Th17 or Treg phenotype. In order to clarify how this cytokine impacts upon inflammation and regulation during Th2 differentiation, we tested the role of IL-6 in the acute inflammatory response, which expels the Th2 cell-inducing gastrointestinal helminth *Nippostrogylus brasiliensis*, and in the Treg/Th2-stimulating environment of chronic *Heligmosomoides polygyrus* helminth infection. In IL-6-deficient BALB/c mice, IL-17 responses to soluble antigen was found to be IL-6-dependent but in mice undergoing live infection with either parasite species, a significant IL-6-independent CD4+IL-17+ T cell response was engendered. Adult worm expulsion and the Th2 response were unaffected in IL-6-deficient mice following acute *N. brasiliensis* infection; however, these mice showed a much greater capacity to clear chronic *H.polygyrus* infection. Enhanced expulsion of *H. polygyrus* in IL-6 deficient mice was not recapitulated by IL-17 neutralisation, but was associated with reduced levels of Foxp3+Helios+ regulatory T cell expansion and an enhanced Th2 response early in infection including parasite-specific IL-4, IL-10 production and serum IgE antibodies. Similar enhancement of Th2 and reduction of Foxp3+Helios+ Tregs was observed in wild-type mice receiving anti-IL-6 monoclonal antibody. To establish if Treg levels influenced the outcome of infection in this model, IL-6 deficient mice infected with *H.polygyrus* were administered anti-IL-2:IL-2 antibody complexes. Administration resulted in the expansion of natural and induced regulatory T cells, a reduced granulamatous response and enhanced persistence of adult worms in vivo. This study reveals an integral role for IL-6 in mediating resistance to helminth infection by determining the balance between natural Treg and Th2 effectors in vivo.

Macrophage activation in Helminth infection: Where inflammation is anti-inflammatory

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Macrophages activated by the Th2 cytokines IL-4 and IL-13 are found in high numbers at the site of helminth infection. We have demonstrated that macrophage accumulation in this setting does not require recruitment of blood monocytes but can result from local expansion of the resident F4/80hi population. Proliferative expansion of macrophages occurs in a broad range of helminth-infected tissues. The canonical Th2 cytokine IL-4, which is responsible for alternative activation and suppression of pro-inflammatory cytokines, also directly drives local macrophage proliferation. Thus, the anti-inflammatory nature of the Th2 response goes beyond the release of downregulatory molecules and is an intrinsic part of the process itself. Although both proliferation and alternative activation are IL-4 induced, they are independently regulated and likely reflect very different functional pathways and evolutionary origins.

***Schistosoma mansoni* triggers Dectin-2 which activates the Nlrp3 inflammasome and alters adaptive immune responses**

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The propensity of helminths, such as schistosomes, to immunomodulate the host's immune system is an essential aspect of their survival. Previous research has demonstrated how soluble schistosomal egg antigen (SEA), which is a complex mixture of highly glycosylated proteins and lipids, can dampen TLR-signalling during innate immune responses. We could show that the suppressive effect by SEA on TLR-signalling is simultaneously coupled to the activation of the Nlrp3 inflammasome and thus IL-1 beta production. Since IL-1 beta has been shown to be an essential orchestrator against several pathogens we studied the in vivo consequences of *Schistosoma mansoni* infection in mice deficient in the central inflammasome adapter ASC and Nlrp3 molecule. These mice failed to induce local IL-1 beta levels in the liver and showed decreased immunopathology. Interestingly, with the exception of IL-13, helminth-specific Th1, Th2 and Th17 responses were down-regulated. Further in vitro studies also elucidated that the SEA-mediated Nlrp3 inflammasome activation depends upon a (glyco-)protein component within the SEA, which binds to the Dectin-2/FcR gamma (Fc receptor gamma chain) complex and activates the Syk kinase signaling pathway to induce mitochondrial reactive oxygen species and potassium efflux. Interestingly, our current research indicates that S. mansoni infection in Dectin-2 deficient mice also results in dampened immunopathology and skewed antigen-specific responses. Our future studies focus on identifying the SEA component(s) that trigger the C-type lectin receptor Dectin-2 to activate the Nlrp3 inflammasome and thus play a role in inducing the two distinct Th phases during Schistosomiasis. These observations highlight the complexity of S.mansoni-mediated immunomodulation at the interface of immunity and glycobiology and represents an evolutionary evolved example of host-parasite interaction.

Influence of maternal helminth infection on offspring immunity

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Maternally derived immunity is accepted as being an important source of protection to children against potentially lethal infections, such as non-typhoidal salmonella (NTS)(MacLennan et al., 2008). How unrelated maternal infections (e.g. helminths) may influence this protection is poorly understood, but recognised as a research field which needs expansion (Labeaud et al., 2009). Identification and understanding of maternal immune mediators and responses which influence childhood ability to control infection and/or launch strong protective responses to vaccination is a relatively poorly understood. To study this experimentally we infected adult female mice with the parasitic nematode *Nippostrongylus brasiliensis* and then cleared the infection with Ivermectin prior to mating. Offspring were then infected intra-peritonially with *Salmonella*. Effects on offspring ability to launch key B and T cell response were then examined and their influence on any changes in host ability to control infection established. We present data here showing how maternal exposure to N. brasiliensis alters offspring B and T cell responses. This altered immunity is related to significant impairment of offspring ability to control a *Salmonella* infection.

The immune phase of helminth infection during pregnancy determines the allergic immune response in offspring

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In strong contrast to third world countries, Western populations have shown a consistent rise in allergic diseases over the last century. Epidemiological studies and in vivo animal models revealed that due to host immunomodulation, infection with the parasitic helminth *Schistosoma mansoni*, may contribute to a lower prevalence of asthma, mainly by the direct or indirect induction of IL-10 producing B and T cells. Whether maternal helminth-induced immunomodulation affects also the offsprings' immune response and its underlying mechanisms have not been fully investigated up to now. We therefore analyzed the development of OVA-induced allergic airway inflammation in offspring from *S. mansoni* infected dams, which were mated in different stages of infection. Offspring from acutely infected dams, which show a strong Th1 response during pregnancy, were completely protected against allergic airway inflammation. Protection was also seen in offspring from long-term infecte d dams mated in the stage of infection, which is dominated by regulatory T-cell responses. In strong contrast, allergic airway disease was aggravated in offspring from chronically infected dams in which Th2 cytokines were prominent during pregnancy. Mechanistically, we could exclude germline-epigenetic regulation by performing in vitro fertilization of eggs from *S. mansoni* infected mice, carried out by naive foster mice. Furthermore, microarray and RT-PCR analysis revealed a strong downregulation of the vitamin-D-receptor expression and Hsd3b1 only in the placentas of acutely infected dams. Since a lack in vitamin D receptor has been shown to contribute to an inflammatory placenta environment we analyzed heterozygous offspring of acutely infected IFNγ knockout dams. Interestingly, these offspring were no longer protected against allergic airway inflammation. Overall, these data suggest a strong correlation between the immune status of the mum during *S. mansoni* infection and the response against allergic airway inflammation in offspring due to immunological changes in the placenta environment.

Eosinophils regulate immunity and larval growth in mice infected with *Trichinella spiralis*

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Our recent findings challenge the paradigm that eosinophils function exclusively as effector cells that attack parasitic worms. *Trichinella spiralis* initiates the chronic phase of infection when newborn, first-stage larvae infect skeletal muscle cells. We have discovered that eosinophils contribute to the ensuing myositis while simultaneously protecting larvae against immune-mediated destruction. Specifically, we find that larvae are killed in large numbers in two strains of mice in which the eosinophil lineage has been ablated and that parasite survival improves when eosinophils are restored to such mice. Larval clearance is dependent upon nitric oxide. In addition, larval growth is inhibited in the absence of eosinophils and this delayed growth prolongs the period during which larvae are susceptible to the effects of nitric oxide. Cell transfer experiments show that eosinophils are influential at the initiation of the immune response that eventually promotes Th2 cell recruitment to sites of infection. Currently, we are investigating STAT6 signaling as a key pathway in regulating parasite growth and glucose metabolism in infected mice. Our findings begin to define the interplay between metabolism and the immune system that occurs at a site of nematode infection in which the eosinophil functions as a pivotal regulator of immunity.

Role of innate cytokines in protective immunity against helminth parasites infections

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Establishing protective immunity to helminth nematodes through vaccination is currently a major global health objective. Of particular note is human Hookworm which is estimated to infect up to 1 billion people worldwide and poses significant economic and health issues on the poorest countries. Surprisingly, the immune cells, cytokines, effector responses and vaccination strategies that could be used to provide immunity against infections such as hookworm remain largely undefined. We use green fluorescent protein/interleukin-4 (IL-4), reporter mice, cytokine gene deficient mice and truncated infection studies with *N. brasilienisis* and *H. polygyrus* to identify the role different tissues, immune cells and molecules play in mediating immunity to invading helminth parasites. Significantly, we find that the lung is an important site for priming immune protection against repeated helminth infection with IL-4 cytokine mediated immune pathways being key. Surprisingly, the investigation of the pro-allergic innate cytokine IL-25 failed to reveal a significant protective role for this cytokine, while innate inflammatory responses mediated by IL-3, IL-5 and immunoglobulin failed to effect outcomes. The role of IL-4 producing CD4 T cell and basophil recirculation and memory cell development will be discussed with respect to helminth infection induced immunity.

Different developmental stages of a filarial nematode dampen the vaccination efficiency of model antigen immunizations

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One third of the human population is infected with helminths. To promote their longevity and to limit pathology helminths have developed several strategies to suppress the immune response of their host. This immune suppression might act on unrelated third party antigens and interfere with vaccine efficiency. Here, we analyze the impact of concurrent infection with Litomosoides sigmodontis on the immune response to unrelated antigens in mice. Although resistant C57BL/6 mice clear the infection within 50 days, humoral response to thymus dependent (TD) but not to thymus independent model antigens was suppressed in infected mice. As these data indicate an interference of helminth infection with T cell help we directly compared the function of T cell receptor transgenic T cells in infected and noninfected C57BL/6 mice. Proliferation of OVA-specific OT-II T cells was diminished after adoptive transfer in L. sigmodontis infected C57BL/6 mice. In the absence of host-deriv ed B and T cells this suppression was abolished while host-derived regulatory T cells were dispensable for the transduction of helminth-mediated suppression. In contrast, L. sigmodontis-induced, host-derived IL-10 mediated Foxp3 induction in transferred OT-II T cells and contributed to the observed T cell hypoproliferation in infected mice. To analyze the impact of different developmental stages of L. sigmodontis on vaccination efficiency we employed the fully susceptible BALB/c mice. L4 (day 14 post infection), young adults (day 30 p.i.) and mature adults with circulating microfilariae (MF) (day 60 p.i.) induced a generalized suppression of Th1-associated IgG2 and Th2-associated IgG1 antibody response to TD antigens. The magnitude of suppression increased with duration of infection. Strikingly, injection of isolated MF in the absence of parasitic adults led to increased Th1-associated IgG2 response while Th2-associated IgG1 response to model antigens was not altered. These findings strongly suggest that adult worms overrule the proinflammatory capacity of MF.

Vaccine-induced sterile immunity to intestinal nematode infection through antibody-dependent mechanisms

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The excretory/secretory (ES) products of helminth parasites contain molecules capable of inhibiting potentially protective host immune responses. Neutralisation of these putative immune modulators by immunisation may reverse parasite immunosuppression and promote host resistance. To investigate this, mice were vaccinated with adult ES material from the chronic intestinal nematode *Heligmosomoides polygyrus bakeri* (HES), which resulted in long-lasting sterile immunity. In contrast, immunisation with a somatic extract of adult parasites did not induce worm killing. Protection was evident early in infection, with an almost complete absence of egg production, followed by parasite expulsion within two weeks. Immunisation with L4 ES, which shares many proteins with adult HES, also induced sterile immunity, but HES vaccination was unable to promote the clearance of established luminal adult worms. HES vaccination failed to induce protection in B cell deficient C57BL/6 μMT mice, despite robust Th2 responses and alternatively activated macrophage differentiation and similar results were observed in MD4 B cell-sufficient animals with a fixed irrelevant BCR. In contrast, vaccine-induced immunity remained intact after macrophage or mast cell depletion, as well as in eosinophil-deficient IL-5-/- mice. Passive immunisation of naïve C57BL/6 mice with polyclonal IgG from vaccinated mice resulted in significant immunity. Critically, no protection was observed in C57BL/6 IL-4Rα-/- mice despite anti-parasite IgG1 responses comparable to wild-type, indicating that a effector cell type requiring IL-4Rα signalling is required to act in an antibody-dependent manner for the expression of rapid sterilising immunity.

Epigenetic control of Th2 induction by dendritic cells

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Dendritic cells (DCs) play a critical role in Th2 priming during helminth infection, yet the mechanism by which they direct Th2 polarization is poorly understood. Since Th2 inducing DCs display minimal transcriptional activation, we investigated whether Th2 priming by DCs is dependent on epigenetic regulation of gene transcription via methyl-CpG binding domain protein-2 (MBD2), which links CpG methylation to repressive chromatin structure. We found that global $MBD2^{-/-}$ mice mount an impaired Th2 response following injection of eggs from the parasitic helminth *Schistosoma mansoni*. Then, to determine the impact of restriction of MBD2 deficiency to DCs alone, we generated mice with conditional deletion of MBD2 in CD11c+ cells ($CD11c^{cre}MBD2^{fl/fl}$). These animals displayed significantly impaired Th2 development in response to egg challenge, illustrating that MBD2 has a central role in controlling Th2 priming by DCs. To further address this possibility, we generated bone marrow derived DCs from global $MBD2^{-/-}$ mice. $MBD2^{-/-}$ DCs activated with soluble egg antigen (SEA) from *S. mansoni* were less able to prime OT-II T cell proliferation and Th2 polarisation *in vitro*. Furthermore, SEA pulsed $MBD2^{-/-}$ DCs transferred into naïve mice exhibited severely impaired Th2 induction ability compared to WT DCs. These data demonstrate that epigenetic regulation of DCs, via the action of MBD2, can be critical for Th2 induction and development both *in vitro* and *in vivo*. Ongoing work is investigating which genes are targeted by MBD2, with the aim being identification of specific mechanisms employed by DCs that are fundamental for Th2 promotion.

Omega-1 and Rapamycin : human dendritic cell induced Th2 responses

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Blocking mammalian target of rapamycin (mTOR) in dendritic cells (DCs) has been shown to promote T cell tolerance. A recent study has shown that rapamycin-conditioned DCs induce Th2 responses in an in vitro model of murine cells. We have shown that rapamycin conditions human DCs such that upon co culture with naïve T cells, a strong Th2 response characterized by increased levels of IL-4 and decreased IFN-γ production develops. Torin1, another inhibitor of mTOR signaling, induces similar but less potent Th2 skewing. To explore the possible involvement of the mTOR pathway in the induction of Th2 responses by soluble egg antigen (SEA) of *Schistosoma mansoni* or by omega-1, a single molecule within SEA, able to induce strong Th2 responses, DCs were stimulated with LPS in the presence of these helminth antigens or rapamycin for 6 or 48 hours and the phosphorylation of P70S6K, a downstream target of mTOR complex 1, was assessed by western blot. The mTOR pathway is rapidly activated upon stimulation with LPS, as shown by an increased P70S6K-Thr421-on-P70S6K ratio after 6 hours, an effect which is much reduced at 48h. While rapamycin strongly inhibits mTOR activity at both time points, there was no apparent effect of either SEA or omega-1 on the pathway. Similar results were found when mTOR-Ser2448 and S6-Ser235/236 were analyzed. These observations suggest that parasite antigens, in contrast to rapamycin, condition DCs to skew Th2 without apparent modulation of the mTOR pathway. This is further supported by the observation that co-stimulation with SEA and rapamycin promotes a stronger Th2 response than stimulation with either of the two alone.

Poster Session Abstracts

1. Elevated adaptive immune responses are associated with latent infections of *Wuchereria bancrofti*

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In order to guarantee the fulfillment of their complex lifecycle, adult filarial nematodes release millions of microfilariae (MF), which are taken up by mosquito vectors. The current strategy to eliminate lymphatic filariasis as a public health problem focuses upon interrupting this transmission through annual mass drug therapy (MDA). It remains unclear however, how many rounds of MDA are required to achieve low enough levels of MF to cease transmission. Interestingly, with the development of further diagnostic tools a relatively neglected cohort of asymptomatic (non-lymphedema) amicrofilaremic (latent) individuals has become apparent. Indeed, epidemiological studies have suggested that there are equal numbers of patent (MF+) and latent individuals. Since the latter represent a roadblock for transmission, we studied differences in immune responses of infected asymptomatic male individuals (n=159) presenting either patent (n=92 MF+) or latent (n=67 MF-) manifestations of *Wuchereria bancrofti*. These individuals were selected on the basis of MF, circulating filarial antigen in sera and detectable worm nests. Immunological profiles of either Th1/Th17, Th2, regulatory or innate responses were determined after stimulation of freshly isolated PBMCs with either filarial-specific extract or bystander stimuli. In addition, levels of total and filarial-specific antibodies, both IgG subclasses and IgE, were ascertained from plasma. Results from these individuals were compared with those from 22 healthy volunteers from the same endemic area. Interestingly, we observed that in contrast to MF+ patients, latently-infected individuals had lower numbers of worm nests and increased adaptive immune responses including antigen-specific IL-5. These data highlight the immunosuppressive status of MF+ individuals, regardless of age or clinical hydrocele and reveal immunological profiles associated with latency and immune-mediated suppression of parasite transmission.

2. Immunological silence and chronic *Trichuris muris* infection

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The immune response to *Trichuris muris* infection is well characterised with Th1 responses and IFN-γ known to be important in susceptibility. During chronic infection, the host immune system is in intimate contact with both the worm and its excretory secretory, ES, products. The major component of ES is a 43 kDa molecule whose function is currently unknown. qPCR data for the gene revealed the greatest increase in expression to occur at the immunomodulatory, L3 stage. The murine immune response to native, purified 43, P43, recombinant, baculovirus derived 43, B43 and ES from which the 43 kDa had been removed (termed flow through, FT) was compared. Surprisingly, serum responses from *T. muris* infected resistant and susceptible mice showed that there was little antibody recognition of either P43 or B43 whereas there was a strong antibody response to the FT. In vitro re-stimulation assays using resistant BALB/c mice also confirmed that there was little immune recognition of P43 and B43 and that Th2 cytokine production was due to ES proteins in the FT. In susceptible strains of mouse however, cellular responses to both B43 and P43 revealed a significant IFN-γ, IL-6, TNF-α and IL-17 response to *T. muris* infection. Exposure of bone marrow derived dendritic cells, revealed a significant increase in CD11c+, MHC II+, CD86 + cells with both ES and FT whereas P43 and B43 exposure resulted in a negligible increase in these cells.

3. Thioredoxin-glutathione reductase-dependent redox networks in flatworm parasites

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In most organisms the reducing equivalents required for key cellular processes are supplied by thiol-based redox enzymatic systems: the thioredoxin (Trx) and/or the glutathione (GSSG, oxidized form; GSH, reduced form) systems. Flatworm parasites share unique and simplified thiol-based redox systems. In these organisms the reduction of GSSG and Trx is entirely dependent on the selenoprotein Thioredoxin-Glutathione Reductase (TGR), a fusion of a glutaredoxin (Grx) domain to thioredoxin reductase (TR) domains. During the past few years our group has significantly contributed to understanding TGR functions and mechanism. In particular, we have shown that in *Echinococcus granulosus*' TGR the TR activity only requires the TR domains, while GSSG reduction and deglutathionylation (glutathione-protein mixed disulfide reduction) require both TR and Grx domains. Furthermore, we found that the TR domain and EgTrxs provide alternative routes for GSSG reduction and deglutathionylation. The recent survey of *Echinococcus* spp. genomes reveals that these organisms encode a diverse group of monodomain Grxs and Trxs. In addition to the two previously characterized cytosolic and mitochondrial Trxs three cytosolic Trxs have been found. Four monodomain Grxs have been identified: two of them resemble the Grx domain of TGR while the other two have CGFS active sites typical of monothiolic Grxs, normally implicated in iron-sulfur cluster assembly and transport. Our recent work has therefore been aimed at expanding our knowledge *E. granulosus'* TGR-based redox network. After confirming the expression of these genes we have cloned the wild-type and mutant forms of these proteins in order to study their functions and to identify their protein targets. Finally, TGR has been validated as a key drug target for schistosomiasis. Our recent work has shown that this concept can be extended to other trematodes and to cestodes, reinforcing the concept that interference with this key pathway leads to flatworm parasite death.

4. SCP/TAPS proteins of parasitic helminths - future prospects

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Proteins belonging to the SCP/TAPS family have been described for various parasitic helminths, including nematodes (e.g. the hookworms *Ancylostoma caninum* and Necator americanus) and trematodes (e.g. *Fasciola hepatica, Clonorchis sinensis, Opisthorchis viverrini* and *Schistosoma* spp.). Despite the key roles that SCP/TAPS proteins have been proposed to play in a number of fundamental biological processes, such as host-pathogen interactions and the modulation of the host immune response, there is a paucity of information on their genetic relationships, structures and functions. Our recent focus has been to (i) conduct a large-scale analysis of SCP/TAPS from a range parasitic nematodes and trematodes of both human and veterinary health importance by mining of currently available genomic and/or transcriptomic datasets; (ii) undertake a comprehensive, systematic phylogenetic analysis of full-length amino acid sequence data; (iii) predict secondary structures of represen tative protein sequences for each phylogenetic clade; and (iv) investigate differential transcription of genes encoding SCP/TAPS proteins selected species (*O. viverrini* and *F. hepatica*) to provide a framework for future structural and function explorations of members of this protein family.

5. Host seeking and the evolution of olfactory behavior in parasitic nematodes

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How do skin-penetrating parasitic nematodes locate hosts? We are addressing this question using the entomopathogenic nematodes (EPNs) *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* and the mammalian-parasitic nematodes *Nippostrongylus brasiliensis* and *Strongyloides ratti*. We are examining the host-seeking behaviors of parasitic infective juveniles (IJs) in response to the universal host cue carbon dioxide (CO2) as well as a large panel of host-derived odorants. We find that EPN IJs are strongly attracted to CO2. However, the requirement for CO2 as a host-seeking cue varies for different EPNs and different hosts: CO2 is required for attraction to some but not all hosts, and for the host-seeking behavior of some but not all EPNs. By contrast, mammalian-parasitic IJs are repelled by CO2 alone, suggesting they rely on host-specific odors for host location. Both EPNs and mammalian-parasitic nematodes respond to a wide range of chemically diverse odorants. EPNs respond strongly to a number of insect-derived odorants, while mammalian-parasitic nematodes respond strongly to a number of human skin odorants. Overall, parasite odor response profiles reflect host range: species with similar host preferences have similar odor response profiles despite their phylogenetic distance. This suggests a critical role for olfaction in niche partitioning and the evolution of host range among parasitic nematodes. We also find that host-seeking behavior changes dramatically as IJs age, perhaps reflecting the increased urgency of host location for older IJs. We are now investigating the neural basis of host seeking. We have shown that the BAG sensory neurons are required for CO2 attraction by IJs, and we are now identifying the sensory neurons that mediate responses to host-specific odorants. We will then perform a functional analysis of these neurons using calcium imaging. These experiments will provide insight into how parasitic nervous systems have evolved to enable parasite-specific behaviors.

6. *Stuctures of the nematode-specific FAR-1 and nemFABP 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. First, the nematode polyprotein allergens (NPAs), the structure of a typical member of which is now history (the ABA-1 allergen of *Ascaris*). Secondly, the fatty acid and retinol binding proteins (FARs). Seven FAR isoforms are known from *C. elegans*. FAR-1s are of particular interest, having been found as abundant components of the secretions of several plant- and animal-parasitic species. 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. We present the protein nuclear magnetic resonance (NMR) structure of a FAR that was identified form EST surveys from the hookworm *Necator americanus.* The remaining unusual, nematode-specific LBPs are the nemFABPs. These are similar to the cytoplasmic 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 and ovoviviparous species, such as *Ascaris*, *Brugia* and *Onchocerca*), and have unusual structural modifications. We will present protein nuclear magnetic resonance and x-ray crystallographic structures of a nemFABP (As-p18 found in the perivitelline fluid of *Ascaris* eggs) that indeed exhibits novel structural modifications. These modifications were not predicted in previous model building, despite the relative simplicity of the modeling task. The As-p18-expressing clone was provided by Rick Komuniecki, Toledo, USA. Supported by The Wellcome Trust (UK).

7. Protective immunity against *Nippostrongylus brasiliensis* requires IL-13 production and antigen presentation by IL-4Rα responsive B cells

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B cell function in protective TH2 immunity against *N. brasiliensis* infection was investigated in this study. Protection against secondary infection depended on IL-4Rα and IL-13, but not IL-4 or antigen-specific IgG1 production. Re-infection of B cell-specific IL-4Rα-/- mice resulted in increased worm burdens compared to control mice. Impaired protective immunity was related to reduced lymphocyte IL-13 production and B cell MHCII and CD86 surface expression. Adoptive transfer into naive BALB/c mice of *N. brasiliensis* primed IL-4Ra-responsive B cells, but not IL-4Ra or IL-13 deficient B cells, conferred protection against primary *N. brasiliensis* infection. Protection was also B cell MHC class II dependent as naive mice receiving *N. brasiliensis* primed wild-type but not MHCII-/- or BALB/b B cells were protected against primary infection. This B cell driven protection was associated with cognate interactions driving CD4+ T cell co-ordination of immunity. The rapid generation of protective ability by B cells suggested a non-BCR mediated mechanism of antigen processing such as Toll Like Receptors. Transfer of CD4+ T cells co-cultured with N. brasiliensis antigen pulsed Myd88-/- B cells failed to confer protection. These data suggest TLR dependent antigen presenting IL-4Rα-responsive B cells producing IL-13 contribute significantly to CD4+ T cell-mediated protective immunity against *N. brasiliensis* infection.

8. Platyhelminth tegumental allergen-like proteins in the *Schistosoma haematobium*: from ESTs to human immune responses

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The tegumental allergen-like proteins (TALs) are a family of platyhelminth proteins that have two EF-hand, and a dynein-like chain motif architecture. The TALs are most studied in *Schistosoma mansoni*, where human IgE against SmTAL1 (Sm22.6) is correlated with resistance to infection. Significantly, the SmTALs are developmentally expressed in *S. mansoni.* Evidence shows that the pattern of developmental expression for each SmTAL is linked with the human immune responses to it. The TALs clearly make an interesting target for further study in other parasitic platyhelminths. I generated a Hidden Markov model of the TAL structure and searched for matching sequences in numerous organisms. The TALs appear to be restricted to the platyhelminths, but also appear in free-living and parasitic platyhelminths. I have found TALs in *S. haematobium, S. japonicum, S. bovis, Echinococcus multilocularis , Chlonorchis sinensis, Taenia asiatica, Fasciola hepatica, F. gigantica, Opisthorchis viverrini* and *Schmidtea mediterranea*. The phylogeny of this protein family can be modelled, and it raises interesting questions concerning the evolution of the tegument- the interface between the organism and host (or outside world if non-parasitic). The developmental expression and calcium-binding properties and of selected TALs can be studied experimentally. *S. haematobium* is the most prevalent schistosome species that infects man, but until recently the paucity of genomic information for this species meant that the molecular immunology of *S. haematobium* infection was not much studied. Now using an EST database, I have predicted and produced recombinant ShTALs, and used them in an ELISA assay with human sera from an *S. haematobium*-endemic region of Mali. The human immune responses to the ShTALs differ according to their developmental expression. This work increases the understanding of a protein family present in many platyhelminths, and sheds light of the human immune responses against an antigen from a very important human pathogen.

9. SmSak, the second polo-like kinase of S*chistosoma mansoni* acts in tandem with SmPlk1 to regulate cell division

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Polo-like kinases (Plks) control many events throughout the cell cycle. In vertebrates, five genes coding for Plk1-5 were identified, and those encoding Plk1 and Plk4 (or Sak) are essential for embryonic growth and viability. In the *S. mansoni* genome (Berriman et al, 2009), only two genes for SmPlk1 and SmSak (Dissous et al, 2011) were detected. The identification of proteins that control female reproductive development in *S.mansoni* represents a valuable alternative strategy to find novel therapeutical targets against schistosomiasis. We have previously identified SmPlk1, a protein homologous to other Plk1 members with conserved functions in mitotic processes. SmPlk1 was shown to play a key role during G2/M transition and in meiosis resumption when expressed in stage VI *Xenopus* oocytes. The SmPlk1 gene is abundantly transcribed in parasite gonads and the dramatic alteration of schistosome gonads caused by the kinase inhibitor specific for Plk1, BI2536, suggested that SmPlk1 played a crucial role in gametogenesis and parasite reproduction (Long et al, 2010). We have recently characterized SmSak, the second Plk of *S. mansoni*. By using protein expression in *Xenopus* oocytes, we have demonstrated its major function in centriole duplication, a role conserved with other Plk4 proteins. Overexpression of SmSak in oocytes cannot, in contrast to that of SmPlk1, overcome the lack of endogenous Plk1, indicating that different functions must be attributed to SmPlk1 and SmSak in the oocyte maturation process. Surprisingly, the co-expression of both proteins induced spontaneously oocyte maturation. Molecular interaction between SmSak and SmPlk1 was confirmed by co-immunoprecipitation and the specificities of this interaction are currently investigated. Also, the respective implication of SmPlk1 and SmSak kinase activities in the process of maturation observed is under analysis. Such a direct interaction and co-activation between Plk1 and Plk4 kinases is described here for the first time

10. Characterization and distribution of glutamate-gated ion channels in *Schistosoma mansoni*

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Cys-loop ligand-gated ion channels (LGIC) are instrumental for nervous system modulation, both in vertebrates and invertebrates. While LGICs are widely described in nematodes and insects, very little is known about these channels in flatworms, including in *Schistosoma mansoni*. We have previously cloned and characterized 3 glutamate-gated ion channel (GluCl) subunits from *S. mansoni*; these are the first GluCl subunits from flatworms to be successfully cloned and expressed. Bioinformatics and electrophysiology have revealed that not only are the SmGluCl subunits evolutionary unique, but their pharmacological and biophysical properties are also distinct from their counterparts in other invertebrates. In addition, confocal laser microscopy analyses show that these SmGluCl subunits are distributed throughout the central and peripheral nervous systems of *S. mansoni*. Further work is in progress in order to provide a detailed description of SmGluCl distribution in males, females, cercaria and somules. We have also initiated RNAi-based functional studies to assess the roles played by the GluCls in schistosomes. This project has uncovered a completely new aspect of neuronal modulation in flatworms, and brings attention to very appealing new anthelmintic targets which could be used to address the urgent need for new chemotherapeutic options for schistosomiasis.

11. Trypsin proteases from *Schistosoma mansoni* in host-parasite interactions?

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Schistosomes survive many years in the vasculature of mammalian hosts and are known for their ability to actively modulate host physiology. Their molecules involved in host parasite interactions are crucial for successful invasion, maturing and reproduction of the parasite. Schistosomes manipulate vasculature system where they reside and lay eggs by secreting unknown molecules capable to drive massive vasodilatations and produce anticoagulative factors. Our research is currently focused on *Schistosoma mansoni* trypsin-like proteases which may play active role in these processes. There were already indications that *S. mansoni* trypsin-like proteolytic activities influence coagulation and vasodilation but the information is generally very scarce. So far, we were able to identify in *S. mansoni* genome database three trypsin proteases with modulatory potential. Our deduction is based on remarkable similarities in amino acid code with their known vertebrate counterparts involved in vascular regulatory networks. Their closest sequence hits appear among trypsin catalytic domains of vertebrate plasminogens, kallikreins and tryptase-like proteases. Our expression profiling by qRT-PCR confirmed expression in stages residing in mammalian host and imply distinct roles in particular life stages such as schistosomula, adults and mature eggs. Therefore we suppose that these trypsin proteases are indeed involved in the modulatory processes in the host vasculature. Screens of *S. mansoni* trypsin-like proteases by active site probes, synthetic peptide libraries, antibody detection, RNAi knockdown and recombinant productions are currently in the process.

12. An expanded and functionally diverse family of Kunitz inhibitors from *Echinococcus granulosus*

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We have described a multigene family of secreted monodomain Kunitz proteins from the cestode *Echinococcus granulosus* (EgKU-1 : EgKU-8), and provided evidence that they may act at the parasite-dog interface. Kunitz inhibitors are metazoan high affinity serine protease inhibitors; in addition, some of them, until now identified only in Kunitz families from animal venoms, block cation channels. Our recent studies indicate that the *E. granulosus* family contains protease inhibitors as well as channel blockers. The close paralogs EgKU-3 and EgKU-8 behaved as typical Kunitz inhibitors of chymotrypsin and trypsin, respectively (Ki 10-11 M). In turn, EgKU-7 inhibited trypsin with similar Ki; however, it showed an unusual biphasic inhibition pattern: the inhibited steady-state rate of trypsin increased by a factor of 2-3 after 2 hours in the presence of EgKU-7, suggesting that it is hydrolysed by the enzyme. In addition, using whole cell patch-clamp of neurons, we demonstrated that the close paralogs EgKU-1 and EgKU-4, which do not inhibit proteases, block voltage-dependent potassium channels (Kv; IC50 10-7 M); and also acid-sensing ion channels (ASIC; IC50 10-9 M), an activity as yet not described for Kunitz proteins. An analysis of the organisation of EgKUs in the assembled *E. granulosus* genome revealed that the genes encoding functionally diverse proteins are located in different regions, and that the family is considerably larger than originally described. In fact, three supercontigs group the genes encoding: i) the pairs of protease inhibitors EgKU-3/EgKU-8 and EgKU-6/EgKU-7; ii) the channel blockers EgKU-1/EgKU-4; iii) EgKU-2 and EgKU-5, which show some protease inhibitory activity and are considerably divergent from the rest. Interestingly, the first two contigs appear to contain numerous genes encoding additional monodomain Kunitz proteins. Our results highlight an interesting similarity between animal toxins and parasite secretions in the evolutionary mechanisms underlying the functional diversification of expanded Kunitz families.

13. Characterisation of the native lipid moeity 0f *Echinococcus granulosus* Antigen B

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Antigen B (EgAgB) is the most abundant and immunogenic antigen produced by the larval stage of *Echinococcus granulosus*. It is a lipoprotein, whose structure and function have not been completely elucidated. EgAgB apolipoprotein components have been well characterised; they share homology with a group of hydrophobic ligand binding proteins (HLBPs) present exclusively in cestode organisms, and consist of different isoforms of 8-kDa proteins encoded by a polymorphic multigene family comprising five subfamilies (EgAgB1 to EgAgB5). In vitro studies have shown that EgAgB apolipoproteins are capable of binding fatty acids. However, the identity of the native lipid components of EgAgB remains unknown. The present work aimed at characterising the lipid ligands bound to EgAgB in vivo. EgAgB was purified to homogeneity from hydatid cyst fluid and its lipid fraction was extracted using chloroform methanol mixtures. This fraction constituted 40-50% of EgAgB total mass. HPTLC analysis revealed that the native lipid moiety of EgAgB consists of a variety of neutral (mainly triacylglycerides, sterols and sterol esters) and polar (mainly phosphatidylcholine) lipids. GLC analysis showed that 16:0, 18:0 and 18:1(n-9) are the most abundant fatty acids in EgAgB. Furthermore, size exclusion chromatography coupled to light scattering demonstrated that EgAgB comprises a population of particles heterogeneous in size, with an average molecular mass of 229 kDa. The native lipids bound to EgAgB obtained from different *E. granulosus* strains will be compared. Our results provide the first direct evidence of the nature of the hydrophobic ligands bound to EgAgB in vivo and indicate that the structure and composition of EgAgB lipoprotein particles are more complex than previously thought, resembling high density plasma lipoproteins. Results will be discussed considering what is known on lipid metabolism in cestodes, and taken into account the *Echinococcus* spp. genomic information regarding both lipid metabolism and the EgAgB gene family.

14. Exploring the role of *S. mansoni* egg proteins in immune regulation using microRNA-adapted shRNA constructs

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One of the most intriguing aspects of schistosome biology is the interaction of the parasites with the immune system. The immune response underlies the ability of the host to survive while infected, and to eventually develop resistance or immunity to subsequent infection. However, it is also instrumental in the development of disease due to its role orchestrating granuloma formation around tissue-trapped parasite eggs. While schistosomes have clearly evolved mechanisms for evading host immune responses, their normal development is, paradoxically, also dependent upon the presence of a normal immune system. Schistosome eggs are remarkable for their ability to induce strong and highly polarized Th2, Treg and Th17 responses in susceptible mouse strains. IL-4 made by Th2 cells plays the central role in allowing infected hosts to survive acute infection. However, Th2 cells also make IL-13, which stimulates myofibroblasts to make collagen, leading to excessive fibrosis associated with hepatic circum:ova granuloma formation. The identity of the molecules that are instrumental in inducing the Th2 response, Treg and Th17 responses, are largely unknown, especially in vivo. We have designed microRNA-adapted shRNA constructs targeting the secreted egg antigens SmOmega-1, SmIL-4-inducing principle (SmIPSE)/alpha-1 and SmKappa-5 to permit studies on their role in granuloma formation. Schistosome eggs were transduced with lentiviral vectors and in vitro analysis revealed that for example the construct targeting SmOmega-1 supressed its expression by 90%. We are currently injecting transduced eggs into mice to perform immunological studies of egg-induced immune responses after knock-down of all 3 antigens in vivo, and to determine their impact on granuloma formation in lung and liver tissues.

15. Transmembrane receptor signaling in the reproductive organs of the blood fluke *Schistosoma mansoni*

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Schistosomes are blood parasites causing schistosomiasis, an infectious disease of global importance. Due to its tremendous influence on human and animal health many efforts are made to control this parasite. With regard to the fact that the eggs are the pathogenic agents of schistosomiasis, among others the unique reproductive biology of the parasite has come into focus of research (Beckmann et al. (2010) Parasitology 137, 497-520). Prerequisite for female maturation and egg production is a constant pairing contact between both genders. Studies at the molecular level led to the identification of different signaling molecules as key players in gonad development. Although cellular tyrosine kinases (CTKs) were among the signaling proteins discovered to be important for gonad differentiation, our knowledge about signaling pathways controlling this essential step of schistosome development is still incomplete. Especially the role of transmembrane receptors and their ligands as activators of gonad-specific signaling has not been extensively studied yet. In the present study we have elucidated the expression profile of selected transmembrane receptors such as GPCRs, RTKs and a transmembrane mucin (Beckmann et al. (2011) J Biol Chem 286, 42325-42336) in the reproductive organs of adult *Schistosoma mansoni* using different approaches like in situ hybridization and tissue-specific RT-PCRs with cDNA from testes and ovaries, which were obtained by a novel organ isolation method. Furthermore, we performed first interaction studies to identify potential downstream binding partners of candidate receptor molecules. Finally, RNA interference experiments and inhibitor studies were done in our in vitro culture system, providing first hints of biological functions of candidate receptors in adult schistosomes. In summary, our results allow a more detailed view into signaling processes involved in the regulation of gonad development and differentiation.

16. Investigating DAF-16/FOXO target gene regulation during early parasitic hookworm development

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The developmentally arrested third stage larva (L3) of hookworms resumes development in response to host-specific signals during infection. Recovery from the analogous arrested dauer stage of *Caenorhabditis elegans* requires insulin/insulin growth factor (IIS) signaling to negatively regulate the forkhead transcription factor DAF-16/FoxO. IIS and DAF-16 are conserved in hookworms, and are postulated to mediate the resumption of development during infection. However, the precise role of DAF-16 in hookworm infection and its downstream effectors are unknown. We used molecular and bioinformatic techniques to identify a group of hookworm DAF-16 target genes. The DNA binding domain of *Ancylostoma caninum* DAF-16 was used to select genomic fragments containing DAF-16 binding elements (DBE) by in vitro genomic selection. The transcript contigs linked to these genomic fragments were identified in silico and defined as candidate direct gene targets of DAF-16. We focused on on e DBE-containing fragment, 2.23, located in the 3' UTR of the small nuclear ribonucleo-protein-3 (Ac-rnr-3), for further analysis. We used promoter-less and minimal promoter luciferase reporter vectors to investigate the effect of fragment 2.23 on DAF-16-driven expression in vitro. Fragment 2.23 showed both promoter and enhancer activity that was depended on the intact DBE, but was independent of its position relative to the reporter gene. We also examined the gene structure and developmental stage-specific expression patterns expression of snr-3 and the neighboring lipid phosphate phosphatase gene by real-time PCR. Our results suggest that hookworm DAF-16 is involved in diverse biological processes throughout hookworm development, and that the fragment 223 DBE might function as a transcriptional enhancer. Further investigation of this and other potential target genes will provide insights into the molecular basis by which hookworm DAF-16 regulates its downstream gene network in hookworm infection.

17. Molecular characterization of RIO protein kinase-encoding genes Ss-rio-1 and Ss-rio-3 of *Strongylodies stercoralis*

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RIO protein kinase is a group of three atypical protein kinases involving in ribosome biogenesis, cell cycle progression and development of organisms. In spite of their functional importance, there is still a paucity of information on these molecules for most parasitic nematodes. In the present study, two RIO protein encoding genes Ss-rio-1 and Ss-rio-3 were characterized from parasitic nematode *Strongyloides stercoralis*. The full length cDNA of Ss-rio-1 is 1820 bp, including a 369 bp 5' UTR, 17 bp 3' UTR and a 1434 bp ORF encoding a protein of 477 amino acids containing RIO1 signature motif. The full length cDNA of Ss-rio-3 is 1736 bp, including a 179 bp 5' UTR, 36 bp 3' UTR and a 1521 bp ORF encoding a protein of 506 amino acids containing RIO3 signature motif. Analysis of gDNA sequences of both genes found that there were two introns in Ss-rio-1 and no intron in Ss-rio-3. The promoter regions of both genes were also isolated with 3128 bp and 486 bp in l ength for Ss-rio-1 and Ss-rio-3, respectively. Each promoter region contains the conserved promoter elements including CAAT box, GATA box, GC box, E-box (CANNTG) and TATA box. RT-PCR analysis on Ss-rio-3 showed this gene was transcribed at all developmental stages including post-parasitic L1, infective L3, free-living adults, post free-living L1 and parasitic females. In order to assess the kinase acitivity, a 55KD recombinant Ss-RIO1 was expressed in E. coli and the subsequent in vitro kinase assay determined its kinase activity. The findings from the present study provide basis for further investigations on elucidating the functions of these molecules in the biological processes of parasitic nematodes.

18. Chronic *L. sigmodontis* infection improves *E. coli* induced hypothermia and bacterial clearance

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Helminths modulate the immune system of their hosts and induce a regulatory, anti-inflammatory milieu that enables not only parasite survival within the host, but also benefits the host. Thus, helminth infections have been shown to improve or prevent autoimmunity in animal and human studies. In this study we investigated the impact of chronic infections with the filarial nematode *Litomosoides sigmodontis* on a systemic inflammation caused by intraperitoneal injection of *E. coli* in mice. Chronic *L. sigmodontis* infection significantly improved *E. coli* induced hypothermia compared to uninfected controls. This correlated with increased levels of the anti-inflammatory cytokine IL-10 in *L. sigmodontis* infected mice, whereas pro-inflammatory cytokines like IL-6, TNF-α, and IFN-γ were not altered 6 hours post *E. coli* injection in *L. sigmodontis* infected mice compared to *E. coli* challenged uninfected controls. Similarly, macrophage activation, as was measured by CD80 and CD86 expression, was not affected in *L. sigmodontis* infected mice compared to uninfected controls after *E. coli* challenge. Interestingly, *L. sigmodontis* infected mice had an improved bacterial clearance from the peritoneal cavity. Cell transfer experiments and phagocytosis assays showed that the improved bacterial clearance in *L. sigmodontis* infected mice was neither due to the increased total number of peritoneal cells in infected mice nor an improved phagocytic activity. Using eosinophil-deficient ΔdblGATA mice and TLR2 gene-deficient mice we were able to demonstrate that the observed protective effect of *L. sigmodontis* infection against an *E. coli* challenge is dependent on eosinophils and TLR2 signaling. This study demonstrates that chronic *L. sigmodontis* infection improves *E. coli* induced hypothermia and clearance of injected bacteria. Helminth-induced eosinophilia and signaling of endosymbiotic *Wolbachia* bacteria via TLR2 are two potential mechanisms that facilitate clearance of injected bacteria in *L. sigmodontis* infected mice and will be the focus of future research.

19. Secreted products of the helminth *Heligmosomoides polygyrus* suppress Type 2 immunity and airway allergic inflammation in mice

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Allergic asthma is less prevalent in countries with parasitic helminth infections, and data from experimental models indicate that parasites such as *Heligmosomoides polygyrus* can protect mice from airway allergy. To characterise the mechanisms and mediators responsible for the suppression of allergy, we tested *H. polygyrus* excretory-secretory (HES) products for their ability to impair allergic inflammation. When HES was added to sensitising doses of ovalbumin, profound suppression of the subsequent airway allergic response was seen, with fewer eosinophils infiltrating the lung, and lower levels of TH2 cytokines and innate type 2 response factors such as Ym-1, RELM-α and RELM-β. The suppressive effect was retained in MyD88 x TRIF double-deficient mice, and is therefore not dependent on TLR ligands. Suppression of allergic sensitization was heat-stable, could not be replicated by administration of mammalian TGF-β, and was unaffected by TGF-βR signalling blockade. Furthermore, the protection against allergy was not accompanied by increased proportions of $Foxp3^+$ Tregs or IL-10, and depletion of $Foxp3^+$ Tregs using a Foxp3-DTR mouse did not lead to abrogation of suppression. Thus in this model, HES acts through a Treg-independent mechanism. HES also has therapeutic potential as administration only at airway challenge of previously fully-sensitized animals suppressed recruitment of innate effector cells, including eosinophils and alternatively activated macrophages. In contrast to suppression at sensitisation, heat-treated HES administered at challenge failed to suppress allergic immune responses. Thus HES contains independent heat-stable and heat-labile immunomodulatory factors that act at different phases of the allergic response in vivo.

20. Gene transcriptional changes during development from immature to adult bovine lungworms

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The bovine lungworm *Dictyocaulus viviparus* is of major importance in grazing cattle in temperate areas. The infection leads to parasitic bronchitis which is accompanied by substantial economic losses. To investigate gene transcriptional changes from the immature, preadult stage, which is also referred to as L5, to adult reproducing worms a subtractive hybridization technique was used. Stage-specific upregulated transcripts and respective protein sequences were bioinformatically analysed by clustering and BLAST identification followed by gene ontology search, domain/motif search and mapping to biological pathways. From the preadult L5 population 300 ESTs were sequenced. These clustered into 96 representative ESTs (rESTs) consisting of 29 contigs and 67 singletons. Most conceptually translated peptides were SCP/TAPS "family" members also known as pathogenesis-related protein (PRP) superfamily (18 rESTs, 39.3% of total ESTs). The next most frequent stage-associated transcripts were H-gal-GP orthologues (galectins and metalloproteases, 6 rESTs, 6.6 % of ESTs) followed by transcripts involved in growth developmental processes, i.e. proteins necessary for cuticle and muscle synthesis (5rESTs, 4.0% of ESTs). Sequencing of 600 ESTs from adult lungworms resulted in 96 rESTs as well. These were represented by 60 contigs and 36 singletons. As expected, the majority of differentially transcribed genes encoded indispensible sex-specific proteins, namely the yolk protein vitellogenin (10 rESTS, 6.8% of ESTs) and proteins containing the major sperm domain (5 rESTS, 7.8% of ESTs). Compared to L5, adult worms have only few stage-associated SCP/TAPS proteins (3 rESTs, 2.2% of ESTs) reflecting the shift in gene transcription towards reproduction.

21. Structural basis for inhibition of the Cathepsin B drug target from the human blood fluke, *Schistosoma mansoni*

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Schistosomiasis is caused by parasitic blood flukes of the genus *Schistosoma* and afflicts over 200 million people worldwide. *Schistosoma mansoni* cathepsin B1 (SmCB1) is a gut-associated peptidase that digests host blood proteins as a source of nutrients. It is under investigation as a drug target. We report crystal structures of SmCB1 complexed with the peptidomimetic vinyl sulfone inhibitors, K11017 and K11777, at resolutions of 1.8 Ã… and 2.5 Ã…, respectively. Also, we calculated the interactions of the subsites of the active-site cleft with a series of vinyl sulfone derivatives by quantum chemistry. Combined, the structural data and inhibition profiling provide insight into the specificity of SmCB1 inhibition. Finally, we demonstrate that the severity of phenotypes induced in the parasite by vinyl sulfone inhibitors correlates with inhibition of SmCB1 activity, thus confirming SmCB1 as a valuable drug target. The present data provide a footing for the continued rational design of anti-schistosomal peptidase inhibitors targeting SmCB1. (This work is supported by grant 203/09/1585 from GACR, grant LH12023 from MSMT, research project Z40550506, the Academy of Science of the Czech Republic, and the Sandler Center for Drug Discovery).

22. The chemokine CXCL12 Is essential for the clearance of the filaria *Litomosoides sigmodontis* in resistant mice

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Filariae are a leading cause of infections which are responsible for blindness, serious dermatological and vascular lesions. It is caused by nematodes that can live for years in the human body. It is estimated that about 180 million people are currently infected with these parasites. Infectious larvae are transmitted through the bite of hematophagous vector. *Litomosoides sigmodontis* is a well established model of filariasis in mouse, the vector being a mite. The progress of the infection depends on the host genotype. We give evidence for a critical role of the chemokine CXCL12 and its receptor CXCR4 in the control of the filarial infection. We comparatively analyzed subcutaneous infection with *L. sigmodontis* in two inbred mouse strains, with different filarial outcomes: one slow clearing strain (BALB/c) and one fast clearing strain (C57BL/6). The delay in parasite clearance observed in BALB/c mice was associated with a lower peak of cell influx in the pleural cavity, and lesser secretion of inflammatory chemokines. Remarkably, rapid parasite clearance was associated with *L. sigmodontis*-specific CXCL12-cell response in C57BL/6 mice. Interfering with CXCL12/CXCR4 interaction increases the survival of *L. sigmodontis* and restrains the influx of cells in the pleural cavity while, conversely, it delays development of the filarial worms. Thus, the CXCL12/CXCR4 axis needs to be considered as key players in the development of *L. sigmodontis* infection.

23. Triclabendazole and its metabolites impact multidrug resistance protein expression in *Fasciola hepatica* newly excysted juveniles

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Due to its efficacy against adult and juvenile liver fluke, triclabendazole (TCBZ) is the drug of choice for the treatment of fasciolosis in both human and veterinary infections. The heavy reliance on TCBZ therapy for liver fluke control coupled with accumulating reports of TCBZ resistance highlight the need to enhance the effective life-span of current drugs, and to identify novel control options. In this study we consider the involvement of multidrug resistance proteins (MDR/MRP) from the ABC transporter family in the susceptibility of F. hepatica to TCBZ and its common metabolites. Previous work on *Schistosoma mansoni* implicates the elevated transcription of two MDR proteins (SmMDR2 and SmMRP1) in the reduced susceptibility of schistosomes to praziquantel. Here we identify orthologues of both of these genes in *F. hepatica* newly excysted juveniles (NEJs) and demonstrate a profound, concentration-dependent modulation of FhMRP1 transcript levels following exposure to TCBZ or its therapeutically active sulfoxide metabolite (TCBZ.SO) in vitro. We have successfully triggered transcript knockdown of both FhMRP1 and FhMDR2 in NEJs using RNA interference (RNAi) methodology, facilitating examination of any resultant alteration in the phenotypic responses of FhMRP1- and FhMDR2-silenced worms to TCBZ and TCBZ.SO.

24. Diversification, expression and function of secreted VAL proteins in *Heligmosomoides polygyrus*

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Parasites employ diverse strategies to ensure their survival within the host, in particular secreting mutiple "immune evasion molecules". In our analysis of the mouse parasite *Heligmosomoides polygyrus*, we have integrated proteomic information on the secretions of this parasite with high-throughput transcriptomics of each stage of the parasite life cycle. In both proteomic and transcriptomics, the Venom allergen/ secreted protein-like (VAL) gene family in particular stands out as being abundantly represented. VAL proteins are found in a wide range of nematode species including *Ancylostoma caninum* in which they were first discovered, and belong to the phylogenetically broad protein SCP/TAPS superfamily. Earlier work indicated that VAL products from some species can elicit IL-10 production from host antigen-presenting cells. Within *H. polygyrus* we have identified 25 different VAL proteins secreted by the adult parasite alone, sharing a single or double conserved cysteine-rich domain structure, with as little as 25% sequence similarity between domains. Comparison with related nematode species indicates an extraordinary species-specific expansion and diversification of VAL protein members has occurred. In addition, transcriptomic analysis identifies differential expression, with some family members (eg VAL-2) being highly adult-specific, while expression of others is maintained around the life cycle. Monoclonal antibodies specific to individual VAL proteins show surface reactivity to the adult worm, some but not all of which can be attributed to glycan side chains on the major VAL products. As the disulphide-bonded domain structure has proven unfavourable to bacterial expression, we have now produced soluble recombinant *H. polygyrus* VAL proteins from both single and double domain gene family members in Hi5 insect cells transfected with pMIBV5HIS plasmids. This system permits isolation of purified recombinant protein free of bacterial contaminants that we are now using to test immunological responses of dendritic cells and macrophages in vitro, and in models of immune pathology in vivo.

25. Control of a parasitic nematode in sheep by vaccination with recombinant antigens

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Teladorsagia circumcincta is the major cause of parasitic gastroenteritis in small ruminants in temperate regions. As sheep can acquire a protective immune response against *T. circumcincta* in natural and experimental circumstances, vaccination is a possible alternative for control. We developed a strategy to identify putative host protective antigens by studying local IgA responses directed at proteins specific to post-infective larvae. Antigens were also selected on the basis of their potential immunomodulatory role at the host/parasite interface. Recombinant versions of five immunogenic molecules were combined with recombinant versions of three proteins that have potential immunoregulatory activities and were administered to sheep as a single vaccine formulation and the animals subsequently subjected to a trickle challenge infection regime with T. circumcincta infective larvae. The trial was performed twice. In both trials, vaccinated sheep had significantly lower mean faecal egg counts (FEC) over the period of the experiment, with an overall mean FEC reduction of 72% (Trial 1) and 58% (Trial 2). During the peak egg shedding periods vaccinated sheep shed 92% and 73% fewer eggs than control sheep in Trials 1 and 2 respectively. At post mortem, vaccinated sheep had 75% (Trial 1) and 57% (Trial 2) lower nematode burdens in the abomasum than those in the control group. These levels of protection are higher than observed in any other system using a recombinant vaccine against a parasitic nematode in the definitive host.

26. Comparative study of the cholecystokinin GPCR signaling system in free-living and parasitic nematodes

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The recently identified CCK neuropeptide signaling system in *C. elegans* constitutes not only the structural, but also the functional homologue of the CCK/gastrin signaling system in mammals, as well as the sulfakinin signaling system in arthropods. Both receptors (ckr-2) and its ligands (nlp-12) are conserved very well in nematodes and have been linked to an important role in the management of energy supplies and fat storage. In addition, food-consumption tests in *C. elegans* revealed a positive effect of the nematode CK signaling on satiety, which is in agreement with their function in vertebrates and arthropods. We identified a NLP-12-like neuropeptide precursor in *Strongyloides ratti* (a gastro-intestinal parasite of rats) and in *Parastrongyloides trichosuri* (a gastro-intestinal parasite of Australian possums). We found the C-terminal motif RPLQFamide of NLP-12 to be conserved among free-living and parasitic nematodes. Thereafter we did a SAR analysis on the native *C. elegans* peptides (DYRPLQFamide and DGYRPLQFamide, NLP-12a and :b respectively). Based on systemic amino acid replacements the Arg-, Leu-, and Phe- residues appear to be critical for high affinity receptor binding. A N-terminal truncated series revealed the essential pharmacophore in *C. elegans* NLP-12b to be the pentapeptide RPLQF-amide. The N-terminus (DY or DGY motif) seems to be irrelevant for receptor activation, however, it is more conserved among other phyla than the C-terminus (RPLQF-amide motif). Therefore it might be nematode-specific and could be used as a starting point in the design of non-peptide mimetics.

27. Transcriptome analysis of the early skin-stage schistosomula of *S. mansoni*

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During the process of infection of the human host, *S. mansoni* parasites change from the cercariae free-living stage into a parasitic life form, the schistosomula. In natural conditions, this transition is triggered by the penetration of the skin barrier during which the cercarial head invades the host while the cercarial tail is lost. The time the parasite resides in the skin of the mammalian host is known to be the most vulnerable stage of the parasite's intra-mammalian life and therefore a good target for the design of intervention strategies. Our work describes the use of massive parallel sequencing known as RNA-seq to investigate genes that are differentially expressed during the cercariae to schistosomula transformation at 3 hours and 24 hours post-transformation. We could confirm the previously reported state of cell cycle arrest at the transcriptional level. However, the up regulation of a significant number of transcription factors, many of them related to neural tissue development, and genes involved in cell signalling (such as GPCRs) suggest this is not a systemic halt but a developmentally regulated process. Reduced expression of key players in protein synthesis suggest that this process might be regulated post transcriptionally but before translation is initiated. Taken altogether, we hypothesised that during the first 24 hours in the mammalian host, parasites suppress their growth but nevertheless prepare themselves at the transcriptional level for a swift transition that would lead to progress to later stages of development and adult worms.

28. Immuno-modulation of dendritic cell responses by the nematode parasite surface glycolipid CarLA

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Gastro-intestinal nematodes (GIN) impose an important problem within the farming industry all over the world and is the largest reason for economic loss coupled to infections in the top three sheep farming countries. The parasites are controlled almost exclusively by the use of anthelmintics, with consequential multiple administrations per animal per year, explaining the strong selective pressure towards GIN resistant strains. The wide spread occurrence of single and multiple resistance to even fairly recently introduced drugs, points to the importance of investigating new ways to control infections. An alternative control approach could be to boost the animal's resistance or resilience to GIN. We are currently investigating one path of how GIN are able to down-regulate the immune response of its host to establish the infection. Some years back, researchers at AgResearch, identified a glycolipid present on the surface of all studied ruminant GIN species. The molecule is shed during the iL3 to L4 moult after infection, and can be induced in vitro during dendritic cell (DC) co-cultures. Antibodies to the molecule were shown to correlate to protection from further infections, but purified CarLA failed to provoke an antibody response. Further studies with CarLA showed that DCs in vitro internalize the molecule but show a limited response. However, co-administration of CarLA and LPS or OVA showed an alteration in surface markers and cytokine production compared to LPS/OVA alone which suggest that CarLA has immune-modulatory properties. Importantly, from a transcriptome microarray study of stimulated DCs with and without CarLA, there were common transcript alterations due to CarLA irrespectively of the co-stimulus used. Several of these genes indicated alterations specific to viral infections, even when stimulated with non-viral products. Here, we present the data of the interesting virus-related genes and the specific interaction of viruses and CarLA during in vitro stimulation of DC.

29. *Oesophagostomum dentatum* extract induces regulatory responses and prevents the development of allergy in mice

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Helminth parasites exert widespread immunomodulatory effects on both the innate and adaptive immune system of the host. Epidemiological and experimental data clearly indicate that infections with certain helminth parasites reduce the development of allergic diseases. Here we sought to investigate whether extract derived from swine nematode parasite *Oesophagostomum denatatum* can prevent the development of allergy in mice. *O. dentatum* extract induced dose:dependent IL-10 and TGF- β secretion by bone marrow-derived dendritic cells. Immunisation of BALB/c mice with *O. dentatum* extracts led to production of antibodies in serum and markedly increased levels of Th2-specific and regulatory cytokines (IL-4, IL-5, IL-10, and TGF-β) in re-stimulated splenocytes and mesenteric lymph node (MLN) cells. Application of *O. dentatum* extract simultaneously with sensitization to major birch pollen allergen Bet v 1 prevented the development of birch pollen-induced airway inflammation, as demonstrated by attenuation of bronchoalveolar lavages (BAL) eosinophil influx, IL-4 and IL-5 levels in lung and lung lymph node cell cultures, peribronchial inflammatory infiltrate, and mucus secretion. Furthermore, these mice had significantly reduced Bet v 1-specific antibody titres in serum (IgG1 and IgG2a) as well as serum IgE-dependent basophil degranulation. We suggest that the suppression was mediated by interf erence with T helper cells function rather than by direct suppression of B cells, as the responses to a thymus-independent model antigen remains unchanged in *O. dentatum*-treated mice. Reduced secretion of Th2-related cytokines by birch pollen-re-stimulated splenocytes and MLN cells was observed in *O. dentatum* extract-treated mice in comparison to sensitized controls. Interestingly, heat inactivation of *O. dentatum* extract did not abolish its capacity to prevent the allergy development. *O. dentatum*-derived molecules showed strong immunomodulating capacity leading to the prevention of allergy in a murine disease model. This observation may lead to the development of new vaccines for prevention/treatment in patients with allergy.

30. Functional analysis of benzimadazole metabolism and efflux in the model nematode *Caenorhabditis elegans*

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There is a need to understand the mechanisms of anthelmintic resistance, to develop approaches that optimize drug potency and to develop new synergists and drugs. An understanding of the way in which anthelmintic drugs are modified, metabolized and excreted from nematodes is central to these objectives. Metabolism of pesticides in insects is known to have a major effect on drug potency and has a major role in insecticide resistance. However, these processes have barely been studied as potential mechanisms of anthelmintic resistance or as factors to consider in anthelmintic drug design and modification. However, nematode genomes contain all the key enzyme families known to be involved in these processes in vertebrates and insects. We have shown that the benzimidazoles: albendazole, mebendazole, fenbendazole and thiabendazole are all metabolized in the model organism *Caenorhabditis elegans* and the resulting metabolites are excreted from the organism. In the case of albendazole, this metabolism involves the addition of a glucose moiety to the parent compound, in complete contrast to metabolism in mammals which involves sulphoxidation and sulphonation. In an attempt to identify the enzymes involved in this novel drug biotransformation reaction that occurs in nematodes, we have used Affimetrix array expression profiling to characterize transcriptomic response of worms to drug exposure. We have identified several cytochrome P450 and transferase genes that are dramatically up-regulated in response to albendazole exposure (cyp-35C1, cyp-35A2, cyp-35A5, ugt-16, ugt-63, and gst-5). We have found these genes are similarly induced by the other members of the benzimidazole drug class but not by several other anthelmintic drug classes. We are currently examining the effects of over-expression and gene knockout/ knockdown (by deletion and RNAi) of these (and other) candidate genes on both the production of drug metabolites and the phenotypic effects of drug exposure on worms.

31. Transcriptional profiles of daf-16, daf-12 and daf-9 do not support the hypothesis of *Dictyocaulus viviparus* L3 being equivalent to the *Caenorhabditis elegans* dauer larva

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In the free living soil nematode *Caenorhabditis elegans*, steroid hormones control development and longevity. These hormones are bile acid-like substances, called dafachronic acids, and are produced by DAF-9. In unfavourable environments, the DAF-16 FOXO transcription factor signalling and DAF-9 dafachronic acid production is missing. Consequently, the DAF-12 nuclear steroid hormone receptor remains unliganded what promotes *C. elegans* dauer formation and thus longevity. In favourable environmental conditions this cholesterol signalling pathway drives short life span, i.e. reproductive development. The developmentally arrested infective L3 (iL3) of parasitic nematodes is suggested to be the equivalent to the *C. elegans* dauer larva and as in this free-living nematode, dafachronic acids induce recovery of *Strongyloides* and hookworm iL3 in parasitism-activated larvae. We have identified daf-12, daf-9 and daf-16 in the bovine lungworm *Dictyocaulus viviparus*, a parasite of major importance in cattle farming. Quantitative real time PCR using 17 developmental stages covering the whole parasite's life cycle showed elevated daf-12 and daf-16 transcription rates in free-living larval stages followed by a marked decrease after transition to a parasitic stage inside the host. The same is true for daf-9 with the exception that the parasitic L4 stage revealed the second highest transcription rate amongst all stages. If one assumes that the transcribed daf-genes are also expressed and functionally active, then there is no shortage of dafachronic acids in all free-living larval stages of the bovine lungworm and consequently no entry into a *C. elegans* dauer-like stage. We hypothesize that the nuclear hormone receptor signalling pathway is conserved among nematodes but fulfils functions in a different biological context. To prove the assumption that there is no dauer equivalent in *D. viviparus*, further experiments are needed to show whether or not the free-living lungworm larvae produce a considerable amount of dafachronic acids.

32. Phosphocholine in parasitism : Biosynthesis and immunological implications of PC-epitopes

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In multicellular parasites (e.g. nematodes and protozoa) proteins and glycolipids have been found to be decorated with PC. Phosphocholine (PC) as a small haptenic molecule present on antigens of parasites can provoke various effects on immune cells leading to immunomodulation of the host's immune system. This immunomodulation allows long-term persistence but also prevents severe pathology due to down-regulation of cellular immune responses. Additionally, PC plays an important role for development and fertility of the parasites. RNAi experiments targeting the glycosphingolipid-biosynthesis and choline-metabolism in nematodes resulted to a dramatic reduction of the offspring production. Furthermore, inhibitor studies with chemical inhibitors sustained the importance of PC-modification for nematode development. PC-bearing components have been found to interfere with key proliferative signalling pathways in B- and T cells, development of dendritic cells and macrophages and mast cell degranulation. These effects contribute to the observed low antibody and cytokine levels and impairment of lymphocyte proliferation. To fully understand the mechanisms of immunomodulation the detailed knowledge of the biosynthesis of the PC-epitopes, their molecular structure and biological function has to be elucidated. The implication of parasite-specific transferases in the biosynthesis of the PC-epitopes and the sensitivity of parasites towards disruption of the choline metabolism offers new perspectives for the development of anti-parasitic drugs and therapies. Furthermore, the immunomodulation provoked by PC preventing inflammatory reactions may be useful in the treatment of inflammatory diseases.

33. Recombinant production and characterization of trypsin-like proteases from *Schistosoma mansoni*

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Schistosomiasis caused by trematode parasites from genus *Schistosoma* is ranked second among parasitic diseases with more than 200 million people infected worldwide. The main goal of our work is to characterize serine proteases of *Schistosoma mansoni* which have potential to participate in host-parasite interactions. Apart of one already partially characterized serine protease SmSP1, we were able to identify other trypsine-like proteases with significant similarities to vertebrate regulatory proteases involved in processes such as coagulation, vasodilatation and inflammation. We suppose that these enzymes could be essential for survival in the vasculature of definitive mammalian hosts including human. We used *E. coli* expression systems in order to produce N- or C- histidine tagged recombinant *S. mansoni* proteolytic enzymes. Recombinant proteins were produced in large quantities in inclusion bodies and purified using affinity chromatography, and subsequently submitte d to several refolding techniques to obtain active proteases. Furthermore, expressions using alternative expression systems are under progress. Recombinant active trypsin-like proteases are characterized using synthetic peptidyl substrates and active site probes. Concurrently, by use of novel sensitive synthetic peptide library we are trying to uncover proteolytic activities of secreted proteases by different life stages of *S. mansoni* infecting definitive mammalian hosts. This includes far less abundant trypsin-like proteases. First results will be presented and the implications of these findings will be discussed.

34. Indication for potential interaction of Emodepside and γ-aminobutyric acid (GABA)-gated chloride channel receptor signaling

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The semi-synthetic cyclooctadepsipeptide emodepside shows a broad spectrum of activity against all major parasitic nematode species including ascarids such as *Toxocara canis*. The anthelmintic cyclooctadepsipeptides (CODP) exhibit a neurotoxic nematocidal effect which was found to be unaltered also against nematode isolates which otherwise are resistant to benzimidazoles, tetrahydropyrimidines or macrocyclic lactones. This indicated that the CODPs act through a novel pathway. Following initial findings indicating that the emodepside parent compound PF1022A might bind to ionotropic GABAA receptors, it was then found that PF1022A and emodepside specifically bind and activate a G-protein coupled mechanism involving a latrophilin-like receptor named lat-1 and found to be expressed mainly in the pharynx. However, using *Caenorhabditis elegans* lat-1 knock out worms it was found that emodepside does still inhibit their locomotion, so that it became clear that the CODP act through at least one further pathway. The voltage-gated and calcium-activated potassium channel SLO-1 was identified as an additional putative emodepside receptor. Functional recombinant expression of parasitic nematode SLO-1 in *C. elegans* SLO-1 knock outs (being significantly reduced in emodepside sensitivity) led to a complete rescue of the emodepside sensitive phenotype. Within the present study a similar approach has been successfully used to study any potential involvement of GABA receptors in the emodepside mode of action. Indeed, it was found that *C. elegans* lacking the unc-49 GABA receptor at the neuromuscular junction show a slightly, but significantly reduced sensitivity to emodepside in both motility and developmental assays. Transformation of unc-49 deficient worms with a plasmid containing *T. canis* unc-49B cDNA under control of the *C. elegans* unc-49 promoter fully rescued the uncoordinated phenotype and restored emodepside sensitivity. These results together with the previous reports of PF1022A binding to emodepside suggest that the GABA signaling pathway is also involved in the mode of action of CODP.

35. Characterisation of the *Trichinella spiralis* deubiquitinating enzyme, TsUCH37, an evolutionarily conserved proteasome interaction partner

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Proteins of the ubiquitin (Ub) /proteasome pathway are highly conserved throughout evolution, and play an important role in cell cycle control and gene transcription, providing interesting and novel therapeutic targets for various diseases. Like all eukaryotic organisms, parasites possess a Ub/proteasome system as well. In order to therapeutically target this pathway in parasites, pathogen Ub/proteasome system components must first be characterised. We are working with *Trichinella spiralis*, an intracellular parasitic nematode capable of surviving for decades in a muscle tissue-derived nurse cell complex within a mammalian host. Although trichinellosis is not usually a fatal disease, zoonotic transmission represents a significant agricultural problem that could benefit from more effective therapeutics. Using derivatized probes based on human Ub, we were able to immuno-precipitate and identify by mass spectrometry 16 different putative *T. spiralis* Ub-associated enzymes from crude lysate of infective larvae. The electrophilic nature and architecture of the probes confer the majority of their specificity to deubiquitinating enzymes (DUBs). Our experiment identified 12 different putative T. spiralis DUBs. We cloned the predicted 930 bp ORF for the putative Ub C-terminal hydrolase 37 homologue, TsUCH37. Using Ub-AMC assays we were able to confirm that this enzyme is active and has Ub-specificity. Through co-precipitation and mass spectrometry we were able to demonstrate that TsUCH37 is proteasome-associated. This shows evidence that the function of this protein as a proteasome interaction partner has been evolutionarily conserved. Inhibition assays showed that members of this enzyme family are important for *T. spiralis* survival and that the use of inhibitor compounds may help elucidate their role in infection.

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Nicotinic acetylcholine receptors are the targets of important old and new anthelmintics, including levamisole, pyrantel, monepantel and tribendimidine. Most of these act at the neuromuscular junction, causing paralysis and some of their receptors have recently been reconstituted in vitro. However, nematodes possess a large number of nicotinic receptor genes, which are likely to play previously uncharacterised roles in behavioral control and may be exploitable as novel drug targets. We have identified a novel gene, acr-26, which is present in many animal parasitic nematodes, but thus far not free-living or plant-parasitic species. In *Ascaris suum*, the subunit protein is expressed on the surface of muscle cells isolated from the head, but not from the middle of the worm. The sequence of ACR-26 is clearly distinct from other nematode nicotinic subunits, and is most closely related to two uncharacterized subunits found in mollusks. Characteristic sequence motifs suggested the possibility that the subunit forms a homomeric acetylcholine-sensitive receptor. When expressed in Xenopus oocytes, ACR-26 from *Ascaris suum* formed channels that were acutely sensitive to acetylcholine, less sensitive to nicotine and rather insensitive to levamisole, properties that clearly distinguished them from the receptors expressed at the neuro-muscular junction of the body-wall muscle. Further pharmacological characterization was limited by the unreliability of expression in vitro. We used RT-PCR and deep sequencing to study the distribution of all the *Ascaris suum* subunits within the parasite. Nicotinic receptors are known to be expressed in the nematode pharynx, and several subunit mRNAs were detected there, including acr-21, for which the pharynx was the only identifiable site of expression. Other subunits were detected in oviduct and gut preparations, as well as in head and body-wall muscle.

37. Identification and molecular characterization of RIO protein kinase-encoding genes Sj-rio-1 and Sj-rio-2 from *Schistosoma japonicum*

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RIO protein kinases, a group of atypical protein kinase were reported to play important roles in cell-cycle progression, 20S pre-rRNA processing and development of many organisms. In spite of their significance, there is no information on these molecules in parasitic trematodes. In the present study, Two RIO protein encoding genes Sj-rio-1 and Sj-rio-2 were characterized from *Schistosoma japonicum*. The full length cDNAs of Sj-rio-1 and Sj-rio-2 were 1412 bp and 2093 bp, respectively. Sequence analyses identified the conserved RIO1 and RIO2 protein kinase motifs from the ORFs of Sj-rio-1 and Sj-rio-2, respectively. Northern blot hybridization determined only one transcript for either Sj-rio-1 or Sj-rio-2. Phylogenetic analysis based on predicted amino acid sequences of each gene from 14 other animal species revealed that Sj-rio-1 and Sj-rio-2 were respectively grouped with their orthologues of *Schistosoma mansoni* with strong bootstrap support (100%). Real-time PCR analyses revealed that Sj-rio-1 and Sj-rio-2 were transcribed in larval and adult stages such as cercariae, skin-stage schistosomula, lung-stage schistosomula, and liver-stage worms. Highest transcription levels were detected in female worms 42-49 days post infection. In vitro culture pairing/separation studies and subsequent real-time PCR analyses showed that Sj-rio-1 and Sj-rio-2 were up-regulated in females following separation from males for 3 days. However, remating of uncoupled females with males did not reduce the transcript levels of both genes to the original level. RNA interference revealed that 1μg/ml and 5μg/ml dsRNA could induce nearly 90% suppression of Sj-rio-1 in 42 days old female worms, while 25μg/ml dsRNA showed less suppression effects. Moreover, recombinant Sj-RIO1 and Sj-RIO2 were expressed in *E. coli* in order to examine the RIO kinase activities and localize the expression in the tissue of this parasite by immunolocalization. The findings here prov ide a first basis for elucidating molecular processes during the reproductive development in *S. japonicum.*

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38. Tapeworm genomes: the Good, the Bad and the Well-assembled

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The Sanger institute is conducting three tapeworm genome projects; *Echinococcus multilocularis* (the fox tapeworm), *E. granulosus* (the hydatid tapeworm) and *Hymenolepis microstoma* (the rodent tapeworm). Here, I will describe the characteristics and quality of the different genome projects; *E. multilocularis* (114 Mb) is the best assembled, with more than half the genome in chromosome-size scaffolds, *E. granulosus* is similar, and the complete *H. microstoma* (140 Mb) genome is in fewer than 1000 scaffolds. I will high-light our most important findings in these tapeworm genomes, including the finding of large-scale inverted repeats in the genome structure, micro-introns and micro-exons in the gene-structures, general gene-content, results of comparative transcriptomics across different life-stages, developmental pathways, metabolic pathways, their use of secreted proteins and protease inhibitors for interacting with their hosts, novel domain architectures in the transcription and splicing machinery, and the presence of drug targets in the gene-repertoire. The genomes and transcriptomes of these worms offer many novel insights to the biology of tapeworms, and these genomes have the potential to greatly increase the ease and speed with which new scientific discoveries in tapeworms can be made.

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Front Cover Illustrations

(Authors in **bold** are attending the Conference)

Top Left : Binding to the surface cuticle of adult *Heligmosomoides polygyrus* by monoclonal antibody to parasite Glycan A [from **Hewitson JP,** Filbey KJ, Grainger JR, Dowle AA, Pearson M, **Murray J,** Harcus Y, **Maizels RM** (2011) *Heligmosomoides polygyrus* elicits a dominant nonprotective antibody response directed at restricted glycan and peptide epitopes. *J Immunol* 187:4764-4777]

Top Right : *In situ* staining for mRNA encoding *Schistosoma mansoni* SM-DLG (Disc-large homologue) in the ovary and vitellarium of adult worm [from Beckmann S, **Hahnel S**, Cailliau K, **Vanderstraete M**, Browaeys E, **Dissous C, Grevelding CG** (2011) Characterization of the Src/Abl hybrid kinase SmTK6 of *Schistosoma mansoni. J Biol Chem* 286:42325-42336]

Bottom Left : Structure of *Ancylostoma ceylanicum* Saposin-like protein SAP-1 [from Willis C, Wang CK, Osman A, Simon A, Pickering D, Mulvenna J, Riboldi-Tunicliffe A, Jones MK, **Loukas A**, Hofmann A (2011) Insights into the membrane interactions of the saposin-like proteins Na-SLP-1 and Ac-SLP-1 from human and dog hookworm. *PLoS ONE* 6:e25369]

Bottom Right : Model of ligand binding site of *daf-12*-homologue of *A. ceylanicum* [from Zhi X, Zhou XE, Melcher K, Motola DL, Gelmedin V, **Hawdon J,** Kliewer SA, Mangelsdorf DJ, Xu HE (2012) Structural conservation of ligand binding reveals a bile acid-like signaling pathway in nematodes. *J Biol Chem* 287:4894-4903]