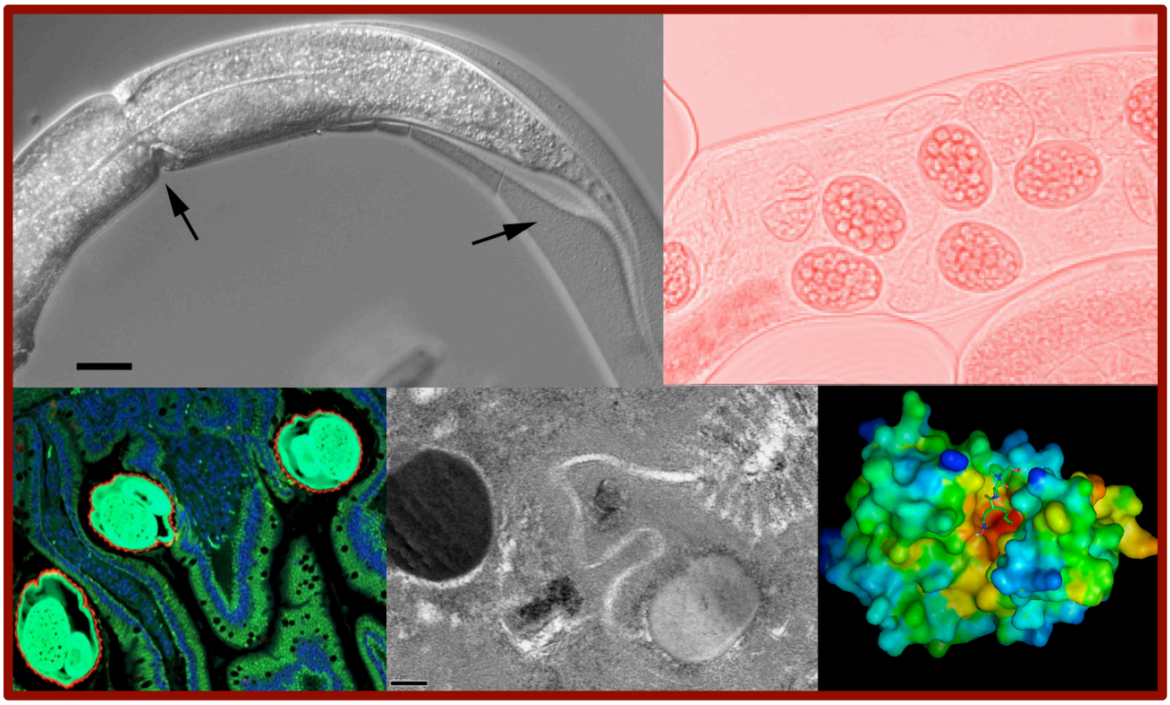
Molecular and Cellular Biology of Helminths VIII



1st - 6th September 2014

Bratsera Hotel, Hydra, Greece

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MOLECULAR AND CELLULAR BIOLOGY OF HELMINTH PARASITES

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

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

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

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

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

VI. **5-10 September 2010, Hydra, Greece**
'Molecular and Cellular Biology of Helminth Parasites VI'
Special Issue of *Experimental Parasitology* **132** (1) : 1-102

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

Dates of MCBHP-IX Meeting: 1-6 September 2015

ORGANISERS, 2014

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Rick Maizels (University of Edinburgh, UK)
Ed Pearce (Washington University at St Louis, USA)
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Cover photos, clockwise from top left, *Moulting defects in C. elegans nas-36 mutant (Tony Page), H. polygyrus eggs in adult female (Janice Murray); C. elegans DPY-31 protein structure with astacin inhibitor modeled to active site (Tony Page); intestinal section of H. polygyrus (Gillian Coakley); H. polygyrus adult sections in mouse duodenum (Janice Murray).*

	Monday 1 September	Tuesday 2 September	Wednesday 3 September	Thursday 4 September	Friday 5 September	Saturday 6 September
	ARRIVE	Developmental Biology	Helminth Genomes	Tissue Interactions	Mechanisms of Immunity	DEPART
09:00		James Collins	Makedonka Mitreva	Richard Grencis	Bill Gause	
09:20		Uriel Koziol				
09:40		Peter Olson	Steve Paterson	Michael Hsieh	Katie Smith	
10:00		Tania Rozario	Matt Berriman	Mirjam Mebius	Bill Horsnell	
10:20		Frederic Landmann		Chris Johnston	Minka Breloer	
		10:40-11:00 Coffee break				
		RNAs in Helminths	Pathways for Drug Development	Molecular Interactions	Immune Cell Interactions	
11:00		Collete Britton	Stephen Doyle	Sasi Bennaru	Miguel Stadecker	
11:20		Larry McReynolds	Christoph Grevelding	Jaap van Hellemond	Matthew Darby	
11:40		Bernadette Connolly	Sophie Parker-Manuel	Alvaro Diaz	Tiffany Bouchery	
12:00		Ana Protasio	Gillian Stepek	Cecilia Casaravilla	Corinna Schnoeller	
12:20		Peter Sarkies	Paula Ribeiro	Katerina Artavanis-Tsakonas	Ed Pearce	
		12:40-4:30 Afternoon break				
		Transgenesis and Plasticity	Targetting Cholinergic Signalling	Innate Immunity	Immunomodulation	
4:30	Registration Opens at Bratsera Hotel	James Lok	Robin Beech	Padraic Fallon	Nicola Harris	
4:50		Emitt Jolly	Claude Charvet	Judy Appleton		
5:10		David Bird	Richard Martin	Christian Schwartz	Henry McSorley	
5:30		Poster Pitches, 18x2 minutes	Ronald Kaminsky	Poster Pitches, 18x2 minutes	Bill Harnett	
5:50					Cathryn Nagler	
		6:10 Break/End of Sessions				
6:30	Refreshments	Poster Session 1		Poster Session 2		
7:30	Keynote Lecture: Phil Newmark		Boat Trip and Dinner at Vlychos Taverna		Farewell Dinner at Bratsera Hotel	
8:30	Wellcome Dinner Bratsera Hotel	End of Session		End of Session		

NOTES

Monday 1 September

Chair: **Ed Pearce,** *Washington University at St Louis*	
19:30	Keynote Lecture: **Phil Newmark**, *University of Illinois at Urbana-Champaign* Germ cell development and regeneration in planarians: implications for understanding parasitic flatworms
21:00	**Welcome Reception, Bratsera Hotel**

Tuesday 2 September

09:00 - 10:40 Session 1: Developmental Biology

Chair: **Murray Selkirk**, *Imperial College London*		
09:00	**James Collins,** *University of Illinois at Urbana-Champaign*	Adult somatic stem cells rapidly renew the schistosome host-parasite interface
09:20	**Uriel Koziol,** *University of Würzburg*	The unique stem cell system of the immortal larva of *Echinococcus multilocularis*
09:40	**Peter Olson,** *The Natural History Museum*	How to make a tapeworm: identifying developmental signals and switches through transcriptomics and spatial gene expression
10:00	**Tania Rozario,** *Wellcome Trust Sanger Institute*	Dividing somatic cells with potential stem-cell functions are distributed throughout the entire adult rat intestinal tapeworm, *Hymenolepis diminuta*.
10:20	**Frederic Landmann,** *CNRS*	Asymmetrically inherited *Wolbachia* endosymbionts influence the host embryonic polarity in *Brugia malayi*.

11:10 – 12:50 Session 2: RNAs in Helminths

Chair : **Christoph Grevelding,** Justus-Liebig-University Giessen, Germany		
11:10	**Collete Britton,** *University of Glasgow*	microRNAs – identifying roles in parasitic nematode development and host-parasite interactions
11:30	**Larry McReynolds,** *New England Biolabs*	Identification and regulation of embryonic miRNAs in *Brugia malayi*
11:50	**Bernadette Connolly,** *University of Aberdeen*	Spliced-leader trans-splicing and operons: conserved features of nematode genomes and possible targets for anthelminthic drugs.
12:10	**Ana Protasio,** *Wellcome Trust Sanger Institute*	Non-coding RNAs in the intra-mammalian development of *Schistosoma mansoni.*
12:30	**Peter Sarkies,** *Wellcome Trust/Cancer Research UK Gurdon Institute*	Ancient and novel small RNA pathways compensate for the loss of piRNAs in multiple independent nematode lineages

16:30 - 18:10 Session 3: Transgenesis and Plasticity

Chair : **Judy Appleton**, *Cornell University*		
16:30	**James Lok,** *University of Pennsylvania*	Developmental "reprogramming" of larval *Strongyloides stercoralis* by manipulation of steroid-nuclear hormone receptor signaling.
16:50	**Emitt Jolly,** *Case Western Reserve University*	Schistosome transgenesis and genetic analysis
17:10	**David Bird,** *North Carolina State University*	The basis for agronomic flexibility in a plant parasitic nematode
17:30	**Pitches for Poster Session 1**	

Tuesday 2 September

17:30-18:10 (2 min poster presentations, 1 slide each)

Chair: **Niki Gounaris**, *Imperial College London*			
1	**Nor Aziz**	University of Bristol	Pattern of incidence of *Angiostrongylus vasorum* in urban, suburban, and rural slugs revealed by real time PCR
2	**Hayley Bennett**	Wellcome Trust Sanger Institute	An unusual case of human cerebral sparganosis, and whole genome sequencing reaches further into the phylum Cestoda
3	**Gillian Coakley**	University of Edinburgh	Secreted exosomes from *Heligmosomoides polygyrus* modulate cellular responses of the murine host
4	**Katarzyna Donskow**	University of Warsaw	Oral therapy for colitis using L4 nematode antigen fractions
5	**Caroline Durrant**	Wellcome Trust Sanger Institute	A genome on the verge of extinction
6	**Jan Dvorak**	Institute of Molecular Genetics, Prague	A global profiling of *Schistosoma mansoni* secreted proteases into mammalian host.
7	**Cecilia Fernández**	Universidad de la República, Montevideo	Terminal repeat retrotransposons in miniature (TRIMs) are highly expressed in *Echinococcus spp*.
8	**Warwick Grant**	La Trobe University	Population genetics, transmission zones and ivermectin response of *Onchocerca volvulus* in West Africa.
9	**Marthe Heylen**	University of Antwerp	*Schistosoma mansoni* soluble egg antigens reduce the severity of experimental colitis in mice by affecting colonic T cell responses.
10	**Nancy Holroyd**	Wellcome Trust Sanger Institute	A broad survey of parasitic helminth genomes
11	**Vicky Hunt**	University of Bristol	The molecular basis of parasitism in the nematode *Strongyloides ratti*
12	**Shin Kang**	Pusan National University	Parasitic nematode-induced CD4+Foxp3+T cell is able to ameliorate allergic airway inflammation
13	**Malcolm Kennedy**	University of Glasgow	The FAR proteins of nematodes – family diversity, structural diversity, binding site diversity
14	**Roz Laing**	University of Glasgow	Ivermectin resistance in UK field populations of *Haemonchus contortus*
15	**Erin Logan**	University of Cape Town	The effect of early-life helminth exposure on children's responses to childhood vaccines.
16	**Ben Wen Li**	Washington University School of Medicine	Localization of nicotinic acetylcholine receptors (nAChRs) transcripts in adult *Brugia malayi* indicates their potential involvement in reproduction of filarial nematodes
17	**Zhigang Lu**	Justus-Liebig-University Giessen	Isolation and characterization of vitelline cells from *Schistosoma mansoni*
18	**Aya Masuda**	Scotland's Rural College/University of Edinburgh	Nutritional regulation of resistance to nematodes in mammals

18:30-20:30 Poster Session 1 and Drinks, Bratsera Hotel Courtyard

Wednesday 3 September

09:00 - 10:40 Session 4: Helminth Genomes

Chair: **Richard Grencis,** *University of Manchester*		
09:00	**Makedonka Mitreva,** *Washington University at St Louis*	Beyond the helminth genomes
09:40	**Steve Paterson**, *University of Liverpool*	A draft genome sequence for *Fasciola hepatica* reveals extensive gene duplication and polymorphism
10:00	**Matt Berriman,** *Wellcome Trust Sanger Institute*	Large scale comparative genomics of helminths

11:10 – 12:50 Session 5: Pathways for Drug Development

Chair: **Collete Britton,** *University of Glasgow*		
11:10	**Stephen Doyle**, *Latrobe University*	Population genomic approaches toward understanding anthelmintic resistance in *Onchocerca volvulus*
11:30	**Christoph Grevelding,** Justus-Liebig-University Giessen, Germany	Imatinib, a potential lead compound against schistosomes and other platyhelminths? Lessons from in vitro and in vivo studies.
11:50	**Sophie Parker-Manuel,** Justus Liebig University, Giessen	Potassium channel activity has a role in schistosome muscle function and egg production, as shown by the characterisation of SmKK7 and SmERG.
12:10	**Gillian Stepek,** *University of Glasgow*	Identification of novel inhibitors of the nematode astacin metalloprotease, DPY-31, that has an essential role in cuticle formation of free-living and parasitic nematodes
12:30	**Paula Ribeiro,** *McGill University*	Functional genomics of acetylcholine receptors in *Schistosoma mansoni*

16:30 – 18:10 Session 6: Targetting Cholinergic Signalling

Chair: **Nicola Harris,** *Ecole Polytechnique Federale de Lausanne*		
16:30	**Robin Beech,** McGill University	Ligand-gated ion-channel evolution and functional diversity of the Trichostrongylid levamisole receptor
16:50	**Claude Charvet,** French National Institute for Agricultural Research	Functional investigation of nematode parasite specific acetylcholine receptors
17:10	**Richard Martin,** *Iowa State University*	Nicotinic anthelmintics and diversity of nAChRs in the Clade III nematode, *Brugia malayi*
17:30	**Ronald Kaminsky,** *Novartis Centre de Recherche Santé Animale*	Discovery and commercialization of the new Anthelmintic monepantel

Thursday 4 September

09:00 - 10:40 Session 7: Tissue Interactions

Chair: **Bill Gause,** *Rutgers, The State University of New Jersey*		
09:00	**Richard Grencis,** *University of Manchester*	Chronic Infection by *Trichuris muris*: modulating the microflora
09:40	**Michael Hsieh,** *Stanford University*	*Schistosoma haematobium* eggs induce urothelial abnormalities through p53- and IL-4 receptor-α-dependent pathways
10:00	**Mirjam Mebius,** *Erasmus University Medical Center*	Interactions between schistosomes and the host haemostatic system
10:20	**Chris Johnston,** *University of Edinburgh*	A role for helminths in achieving immunological tolerance

11:10- 12:50 Session 8: Molecular Interactions

Chair: **Ed Pearce,** *Washington University at St Louis*		
11:10	**Sasi Bennaru,** *National Institutes of Health*	Loa loa secretome: Comparative proteomic analyses of urine, plasma and in vitro microfilaria-derived excretory secretory products towards biomarker for high burden
11:30	**Jaap van Hellemond,** *Erasmus University Medical Center*	The tegument membranes of adult *Schistosoma mansoni* have a specific and unusual phospholipid composition
11:50	**Alvaro Diaz,** *Universidad de la República, Uruguay*	Inhibition of the PI3K/Akt pathway in dendritic cells by particles from the *Echinococcus granulosus* laminated layer
12:10	**Cecilia Casaravilla,** *Universidad de la República, Uruguay*	Inflammasome activation by particles from the *Echinococcus granulosus* laminated layer
12:30	**Katerina Artavanis-Tsakonas** *Imperial College London*	Targeting of host ubiquitin pathway by *Trichinella spiralis*

16:30 – 8:10 Session 9 : Innate Immunity

Chair: **Miguel Stadecker,** *Tufts University School of Medicine*		
16:30	**Padraic Fallon,** *Trinity College Dublin*	Elicitation and functions of innate lymphoid type 2 cells in helminth infections.
16:50	**Judy Appleton,** *Cornell University*	Versatility of eosinophils in nematode infection
17:10	**Christian Schwartz,** *University Clinic Erlangen*	The role of basophils during immune responses against helminths
17:30	**Pitches for Poster Session 2**	

Thursday 4 September

17:30-18:10 (2 min poster presentations, 1 slide each)

Chair: **Niki Gounaris**, *Imperial College London*			
19	**Marina Mourao**	Fundação Oswaldo Cruz-FIOCRUZ	Mitogen-activated protein kinases are involved in the reproductive development and survival of *Schistosoma mansoni*
20	**Janice Murray**	University of Edinburgh	Excretory/secretory products from *Heligmosomoides polygyrus*: the VAL proteins
21	**Katherina Oeser**	University Clinics Erlangen	Role of T cell-derived IL-4 and IL-13 during infections with *Nippostrongylus brasiliensis*.
22	**Tony Page**	University of Glasgow	Nematode moulting enzymes as potential drug targets
23	**Mi Park**	Pusan National University	CCR7-dependent immune regulation of *Trichinella spiralis* Infection
24	**Najuu Ranjit**	QIMR Berghofer Medical Research Institute	Utilising protein inhibitors and transgenic vectors to identify genes in the insulin-signaling pathway, which are essential for development and survival in *Schistosoma* spp.
25	**Lukus Roberts**	Imperial College London	Acetylcholine: a co-stimulator for pulmonary immune responses against *Nippostrongylus brasiliensis*?
26	**Fabio Simbari**	University of Edinburgh	Compartive biochemical analysis of nematode and mammalian exosomes.
27	**Danielle Smyth**	University of Edinburgh	*Heligmosomoides polygyrus* excretory/secretory products can protect against T cell-mediate colitis through continuous delivery in vivo.
28	**Adrian Streit**	Max Planck Institute for Developmental Biology	Different *Strongyloides stercoralis* populations in humans and dogs in rural Combodi.
29	**Rebekah Stuart**	Aberystwyth University	Towards revealing Cytochrome P450 contribution to the detoxification capacity of Liver Fluke
30	**Thomas Tzelos**	Moredun Research Institute	The development of RNA interference (RNAi) in the parasitic nematode *Teladorsagia circumcincta* as a method for screening vaccine candidates
31	**Lenka Ukrychova**	Institute of Molecular Genetics, Prague	Characterization of *Schistosoma mansoni* trypsin-like serine protease SmSP2: a new chapter of *Schistosoma* degradome
32	**Rachel Vaux**	Imperial College London	The development of a *Trypanosoma musculi*-based in vivo heterologous expression system to investigate parasitic nematode gene function
33	**Mark Viney**	University of Bristol	The role of SCP/TAPS genes in the parasitic life of *Strongyloides ratti.*
34	**Rhiannon White**	Imperial College London	The individual effects of novel *Trichinella spiralis* proteins on host cells.
35	**Ruud Wilbers**	Wageningen University	Expression of Schistosome-derived Omega-1 with diantennary glycans carrying Lewis X motifs in *Nicotiana benthamiana* plants
36	**Hak Sun Yu**	Pusan National University	Intestinal helminth infection improve intestinal homeostasis via alteration of intestinal bacteria population

Friday 5 September

09:00 - 10:40 Session 10: Mechanisms of Immunity

Chair: **Warwick Grant,** La Trobe University		
09:00	**Bill Gause,** *Rutgers, The State University of New Jersey*	Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion.
09:40	**Katie Smith,** *Cardiff Institute of Infection and Immunity*	Efficient chronic parasite expulsion is co-ordinated by IL-25R expression and IL-4Rα signalling within the innate immune system
10:00	**Bill Horsnell,** *University of Cape Town*	Contribution of Surfactant Protein D to host immunity to *Nippostrongylus brasiliensis* infection
10:20	**Minka Breloer,** *Bernhard Nocht Institute for Tropical Medicine*	Concerted action of adaptive immunity and mucosal mast cells is required for final elimination of *Strongyloides ratti* from the small intestine

11:10 – 12:50 Session 11: Immune Cell Interactions

Chair: **Niki Gounaris**, *Imperial College London*		
11:10	**Miguel Stadecker,** *Tufts University School of Medicine*	A novel role of innate immunity in orchestrating pathogenic Th17 cell responses in schistosomiasis
11:30	**Matthew Darby,** *University of Cape Town*	The influence of maternal *Nippostrongylus brasiliensis* infection on offspring immunity
11:50	**Tiffany Bouchery,** *Malaghan Institute of Medical Research*	ILCs and CD4 T cells co-operate to maintain AAM activation in *Nippostrongylus brasiliensis* lungs
12:10	**Corinna Schnoeller,** *Imperial College London*	Non-neuronal cholinergic signalling in immunity to infection: a role for M3 muscarinic receptors.
12:30	**Ed Pearce,** *Washington University at St Louis*	The metabolic regulation of alternative macrophage activation in immunity to helminthes

16:30 – 18:10 Session 12: Immunomodulation

Chair : **Rick Maizels**, *University of Edinburgh*		
16:30	**Nicola Harris,** *Ecole Polytechnique Federale de Lausanne*	Antibodies trap migrating helminth larvae and promote timely tissue repair
16:50	**Henry McSorley,** *University of Edinburgh*	Blockade of early innate allergic responses by *Heligmosomoides polygyrus* excretory/secretory products
17:10	**Bill Harnett,** *University of Strathclyde*	A small molecule analogue of the *Acanthocheilonema viteae* immunomodulator ES-62 inhibits inflammation in concert with activation of the Nrf2-dependent anti-oxidant response
17:30	**Cathryn Nagler,** *University of Chicago*	Commensal bacteria protect against food allergen sensitization.
20:00	**Farewell Banquet, Bratsera Hotel**	

ABSTRACTS

KEYNOTE LECTURE

Germ cell development and regeneration in planarians: implications for understanding parasitic flatworms

BO WANG, JIM COLLINS, HARINI IYER, AMIR SABERI, AND **PHILLIP NEWMARK**

HOWARD HUGHES MEDICAL INSTITUTE, DEPARTMENT OF CELL AND DEVELOPMENTAL BIOLOGY, UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Planarians are free-living flatworms with amazing regenerative abilities. Their regenerative prowess is based upon a population of adult stem cells, called neoblasts,that serve as the source of new tissue during regeneration and tissue homeostasis. Using the functional genomics tools available for studying planarians, we have been investigating how these stem cells give rise to the germ cell lineage and how reproductive system development and regeneration are controlled systemically. This talk will discuss how our work on planarian germ cell development led us to study the biology of schistosomes, parasitic flatworms with great significance for global health. We have shown that, like planarians, schistosomes have neoblast-like stem cells in the adult stage of the life cycle, providing one potential explanation for their longevity. Extending this work to the intramolluscan stage of the schistosome life cycle, we find that the so-called germinal cells in the sporocysts resemble neoblasts morphologically and express similar genes that are required for germinal cell proliferation and maintenance. We have used single-cell RNA sequencing to characterize the heterogeneity of the germinal cell population, and have identified co-regulated gene clusters that define apparently distinct cell lineages. With these molecular markers, we can follow these lineages in space and time throughout the parasite's development in both snail and mammalian hosts. Thus, applying the lessons learned from studying planarians has provided new insights into the biology of their parasitic cousins.

Adult somatic stem cells rapidly renew the schistosome host-parasite interface

JAMES COLLINS

HOWARD HUGHES MEDICAL INSTITUTE AND DEPARTMENT OF CELL AND DEVELOPMENTAL BIOLOGY, UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN, URBANA, IL 61801, USA

Schistosomiasis is among the most prevalent human parasitic diseases, affecting more than 200 million people worldwide. The etiological agents of this disease are trematode flatworms (*Schistosoma*) that live and lay eggs within the vasculature of the host. These eggs lodge in host tissues, causing inflammatory responses that are the primary cause of morbidity. Because these parasites can live and lay eggs within human hosts for decades, elucidating the mechanisms that promote their longevity is of fundamental importance. Although adult pluripotent stem cells, called neoblasts, drive long-term homeostatic tissue maintenance in long-lived free-living flatworms (e.g., planarians), little is known about whether similar cell types exist in the schistosome. Here, we describe a population of neoblast-like cells in *Schistosoma mansoni*. These cells resemble planarian neoblasts morphologically and share their ability to proliferate and differentiate into derivatives of multiple germ layers. Capitalizing on available genomic resources and RNAseq-based gene expression profiling, we find that these schistosome neoblast-like cells express a fibroblast growth factor receptor ortholog that is required for stem cell maintenance. To further characterize the cellular roles for neoblasts in adult schistosomes, we examined the transcriptional profile of parasites 2-3 weeks following neoblast ablation. These studies, coupled with EdU pulse-chase experiments, indicate that a large fraction of differentiating neoblasts are destined to become cells that integrate into a syncytial epidermal structure called the tegument. Since this structure serves as the interface between the parasite and its host, understanding this neoblast-tegument relationship may address the unresolved question of how schistosomes evade the host immune system. We expect that future studies deciphering the function of these neoblast-like cells will have important implications for understanding the biology of these devastating parasites.

The unique stem cell system of the immortal larva of *Echinococcus multilocularis*

URIEL KOZIOL[1], CECILIA FERNÁNDEZ[2], KLAUS BREHM[1]

[1] *INSTITUTE FOR HYGIENE AND MICROBIOLOGY, UNIVERSITY OF WÜRZBURG, GERMANY.* [2]*CÁTEDRA DE INMUNOLOGÍA, FACULTAD DE QUÍMICA, UNIVERSIDAD DE LA REPÚBLICA, URUGUAY*

From classical studies, it is assumed that in cestodes undifferentiated stem cells (so-called “germinative cells”) are the only source of cell proliferation, similarly to the neoblasts of free-living flatworms. However, nothing is known about the properties of germinative cells regarding their heterogeneity and gene expression patterns. In this work, we investigated the germinative cells of the metacestode larva of the cestode *Echinococcus multilocularis*. This larva grows continuously like a mass of vesicles, infiltrating the tissues of the intermediate host and generating protoscoleces by asexual budding. We demonstrate that only the germinative cells proliferate by morphological criteria and by developing for the first time molecular markers of differentiated cells in *E. multilocularis*, including markers for previously undescribed nerve cells in the larval vesicles. The germinative cells are heterogeneous at the molecular level, since only specific sub-populations express homologs of the post-transcriptional regulators *nanos* and *argonaute*, suggesting lineages of germinative cells with different potencies. Experiments of recovery after partial germinative cell depletion indicate extensive self-renewal capabilities for individual germinative cells. In spite of the similarity in morphology and function between the *E. multilocularis* germinative cells and the neoblasts of other flatworms, important differences are observed in their gene expression patterns. Furthermore, cestodes and trematodes lack orthologs of *piwi* and *vasa*, classical germ-line markers in many animals with key functions in the somatic neoblasts of free-living flatworms. The lack of *piwi* is particularly striking: although this gene family has a conserved role in the control of transposable elements, very few of these elements are present in cestode genomes, suggesting efficient alternative mechanisms for their control. Finally, we show that a novel family of non-autonomous retrotransposons has escaped repression and is massively expressed in the germinative cells.

How to make a tapeworm: identifying developmental signals and switches through transcriptomics and spatial gene expression

PETER D OLSON, M. ZAROWIECKI AND MATT BERRIMAN

WELLCOME TRUST SANGER INSTITUTE, WT GENOME CAMPUS

Rodent-hosted tapeworms of the genus *Hymenolepis* have been widely employed as laboratory models since the 1950s, but were barely characterised on a genetic level before the recent publication of four tapeworm genomes in 2013. An even more recent assembly of the *H. microstoma* genome, full gene annotation and multiple transcriptome profiles now make this and other tapeworm species among the best characterised of any animal model system. We're using these data to identify up-regulated signalling molecules and transcription factors (TF) associated with different aspects of tapeworm development, including metamorphosis, strobilation, maturation and senescence, and using in situ hybridisation (ISH) to identify more precisely their spatial expression patterns. The profile of up-regulated developmental genes during larval metamorphosis is most similar to that of strobilation in the adult worm and includes fewer factors than seen during maturation of the reproductive organs, with the ovaries found to express the vast majority of TF. The latter is dominated by conserved homeobox, zinc finger and forkhead box TF, but also includes novel zinc finger TF lacking homologs in other animals. In contrast, signalling molecules associated with the Wnt, Notch and TGFB pathways are up-regulated during formation of the body (i.e. larval metamorphosis and adult strobilation). Stem-cell related factors (e.g. Pumilio, Dicer, P53) are expressed ubiquitously, but there is some evidence that separate paralogs of these factors are partitioned between the soma (ie. neobloasts) and germ-line. Whole-mount ISH reveals spatial patterns corresponding to body regions, cells or organs. Whereas no demarcation between 'neck' and strobila can be identified morphologically, expression patterns show a discrete zone of proliferation well-defined by developmental genes. Similarly, organ and cell-specific factors have been identified that can be used to mark and sort cell types for more focused transcriptomic profiling in the future.

Dividing somatic cells with potential stem-cell functions are distributed throughout the entire adult rat intestinal tapeworm, *Hymenolepis diminuta*.

TANIA ROZARIO AND PHILLIP A. NEWMARK

DEPARTMENT OF CELL AND DEVELOPMENTAL BIOLOGY, HOWARD HUGHES MEDICAL INSTITUTE, UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN, ILLINOIS, USA

The remarkable growth potential of tapeworms has been attributed to neoblast-like stem cells resident in the germinative region (GR) directly posterior to the base of the suckers. It has been long accepted that the GR contains the only dividing somatic cells in the adult tapeworm based on observations that tritiated thymidine uptake was confined to the parenchyma of the GR (Bolla and Roberts, J. Parasitol, 1971). Using *H. diminuta*, we find that somatic cell divisions are distributed throughout the length of the adult worm and not confined to the GR. We successfully labeled dividing cells using two methods: 1) uptake of the thymidine analog F-ara EdU to label cells in S-phase and 2) immunostaining using an anti-phosphohistone H3 antibody to label mitotic cells. We confirm that the dividing cells do not co-localize with various markers of differentiated cell types and thus likely represent stem cells or committed progenitors. Furthermore, we amputated 6-day old worms to remove the scolex and all or most of the GR and cultured the posterior amputees in vitro to observe their capacity for growth. The posterior amputees increased in length five-fold and successfully specified proglottids with mature reproductive structures. This strongly suggests that the dividing cells outside of the GR retain the potential to fuel growth and strobilation. We are currently pursuing various amputation and regeneration assays to determine whether the dividing cells throughout the length of the adult possess the same growth potential. In summary, *H. diminuta* (and possibly other tapeworms) have greater regenerative potential than has been previously appreciated.

Asymmetrically inherited Wolbachia endosymbionts influence the host embryonic polarity in *Brugia malayi*

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While bacterial symbionts influence a variety of host cellular responses throughout development, there are no documented instances in which symbionts influence early embryogenesis. Here we demonstrate that Wolbachia, an obligate endosymbiont of the parasitic filarial nematodes is required for proper Anterior-Posterior polarity establishment in the filarial nematode *B. malayi*. Characterization of pre- and post-fertilization events in *B. malayi* reveals that unlike *C. elegans*, the centrosomes are maternally derived and produce a cortical-based microtubule organizing center prior to fertilization. We establish that *Wolbachia* rely on these cortical microtubules and dynein to concentrate at the posterior cortex. *Wolbachia* also rely on PAR-1 and PAR-3 polarity cues for normal concentration at the posterior cortex. Finally, we demonstrate that *Wolbachia* depletion results in distinct anterior-posterior polarity defects. These results provide a striking example of endosymbiont-host co-evolution operating on the core initial developmental event of axis determination.

microRNAs – identifying roles in parasitic nematode development and host-parasite interactions

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microRNAs play essential roles in regulating cell proliferation, differentiation and organ development. *C. elegans* miRNAs *lin-4* and *let-7* are absolutely essential for correct developmental progression from early to mid larval stages and from L4 to adult worms. miRNAs regulate gene expression post-transcriptionally and bind with partial complementarity, often but not exclusively, to the 3'UTR of their target mRNAs, leading to mRNA degradation and/or translational inhibition. By deep sequencing and bioinformatic approaches we previously identified miRNAs in *Haemonchus contortus* and *Brugia pahangi*. While some miRNAs are conserved in a range of nematodes, most are species-specific, based on current data. This suggests rapid evolution of miRNA sequences for adaptation to different environments. By microarray analysis we have identified sets of miRNAs significantly up- or down-regulated at specific developmental transitions and are currently focussing on those that change in expression level between pre-and post-infective L3 stages, to identify potential roles in larval arrest and development. We have also identified miRNAs enriched in *Haemonchus* gut tissue and in ES products. *Hco-mir-5352* is of particular interest as it is expressed only in parasitic stages of gastrointestinal nematodes, can be detected in adult ES products and from target prediction analysis may regulate host T cell activation. Using target prediction programs, 3'UTR datasets, reporter gene assays, and cross-linking immunoprecipitation we are investigating pathways regulated by specific miRNAs. miRNA inhibitors and mimics have potential to alter miRNA activity, thus aiding functional analysis as well as providing a potential novel approach to parasite control.

Identification and regulation of embryonic miRNAs in *Brugia malayi*

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MicroRNAs play a central role in the development of nematodes. To study the expression and regulation of miRNAs in the filarial parasite, *Brugia malayi,* we have used both deep sequencing of miRNAs and *in vivo* reporter constructs to measure their activity. Sequencing of small RNAs from microfilaria and embryos identified miR-71 as the most abundant miRNA. Studies in *C. elegans* suggest that this miRNA may have a central role in the stress response in dauer and adult parasites. MiR-71 has been shown to extend life span in adult parasites through the DAR-16/FOXO pathway (Boulias & Horvitz 2012 Cell Metab 15:439-450). To measure the activity of endogenous miR-71 in *Brugia malayi*, reporter constructs were made using a luciferase gene with a target sequence complementary to miR-71 in the 3' UTR. These reporters were used to transiently transfect embryos of *B. malayi.* Reporters that contained sequences complementary to the miR-71 target had an 80% reduction in luciferase activity when compared to embryos transfected with the parental plasmid. The use of transfected reporters can be used to assay for mRNA targets of endogenous miRNAs. We have developed a simple, sensitive, two step ligation based method for miRNA detection. The SplintR ligase [Chlorella virus DNA ligase] can very efficiently ligate two adjacent DNA oligonucleotides that are hybridized to a miRNA splint. The enzyme is much more efficient for this reaction than T4 DNA ligase (Lohman et al 2014 Nucl Acid Res 42:1831). The ligated oligos can then be amplified and measured by qPCR. We have detected sub-femtogram amounts of miRNA using this protocol. The method can also detect single base mismatches in miRNAs when the DNA oligonucleotides are designed to have the ligation junction at the mismatch. We believe that ligation based RNA detection method should be of general use for a variety of RNA studies.

Spliced-leader trans-splicing and operons: conserved features of nematode genomes and possible targets for anthelminthic drugs

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The organisation of genes into operons, clusters of genes that are co-transcribed to produce polycistronic pre-mRNAs, is a trait found in a wide-range of eukaryotic groups, although their distribution is sporadic and it is unlikely that they represent an ancestral eukaryotic trait. Operons are present in *Caenorhabditis elegans* and other members of the Chromadorea, one of the three main nematode taxa. Their distribution in the other two nematode taxa is not known. We will show data identifying the first putative operons in *Trichinella spiralis* and Trichuris muris, two members of the Dorylaimia. Consistent with the mechanism of polycistronic RNA resolution in other nematodes, the mRNAs produced by genes downstream of the first gene in the operons are trans-spliced to spliced leader RNAs, and we can detect polycistronic RNAs derived from these operons. Importantly, a putative intercistronic region from one of these potential operons confers polycistronic processing activity when expressed as part of a chimeric operon in *C. elegans*. We find that the orthologues of genes located in operons in the Dorylaimia are more likely to also be in operons in the Chromadorea, consistent with models of operon evolution, and have identified putative operons conserved between the two taxa. Our data suggest that operons and SL trans-splicing are common features of all nematodes and predate the radiation of the nematode phylum. It is striking that, at least to date, all eukaryotes in which operons usage is widespread also undergo SL trans-splicing, suggesting that the resolution of polycistronic RNA is dependent upon SL trans-splicing. Importantly, SL trans-splicing is absent from vertebrates, raising the possibility that this process could be a target for the development of anthelminthic drugs. To this end we have developed and tested an assay to identify such drugs/small molecule inhibitors and will present results of the initial screen.

Non-coding RNAs in the intra-mammalian development of *Schistosoma mansoni*.

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Schistosomes are parasitic helminths and the causative agent of schistosomiasis, also called bilharzia, a disease affecting circa 200 million people in Africa, south East Asia and south America – some of the most underprivileged regions of the world. *Schistosoma mansoni* is the preferred species kept in the laboratory due to its ease of propagation both in the snails and the rodent models. Its genome, transcriptome and at a certain extent the proteome of the intra-mammalian stages of these parasites have been studied. However, the role of non-coding RNAs in the development of this parasite has been left unknown. Non-coding RNAs (ncRNAs) include all RNA species that are not translated into a protein product. These include the well-known ribosomal rRNAs, transfer-RNAs, small nucelolar RNAs among others. Other ncRNAs include microRNAs and long intergenic non-coding RNAs (lincRNAs). miRNAs are ~21nt long RNA molecules specially processed to undertake key roles in regulating the availability of messenger RNAs (mRNAs). A number of them have been described in schistosomes, yet there is nothing known about their potential targets. LincRNAs are commonly >200 nt and lack coding potential. Some play roles in gene regulation, often mediated by promoting sequence-specific chromatin assemblies. They are somehow conserved in closely related species, which makes the in silico identification a challenge. We used strand-specific RNAseq and microRNA-seq libraries from several time points of mechanically transformed schistosomula (0, 3, 6, 12, 24, 48 and 72 hours old larvae) to identify both miRNAs and lincRNAs. Using both in silico and experimental methods we found novel and possibly stage specific miRNAs as well as lincRNAs in the intra-mammalian early stages of *Schistosoma* development. The potential role of miRNAs and lincRNAs in parasite development is discussed. This study represents the first of its kind for a parasitic helminth.

Ancient and novel small RNA pathways compensate for the loss of piRNAs in multiple independent nematode lineages

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Small RNAs act at the front line of defense against transposons across the entire eukaryotic kingdom. Within animals, the Piwi-interacting small RNAs (piRNAs), which associate with Piwi proteins and target transposons for silencing, are widely conserved and are essential for fertility in fruit flies, mammals, zebrafish and the nematode *C. elegans*. However, other small RNA pathways appear restricted to specific phyla. To date the evolutionary relationship between different small RNA pathways targeting transposons remains mysterious. To address this question we sequenced small RNAs from multiple evolutionarily distant nematode species, producing the first comprehensive analysis of how small RNA pathways evolve within a single phylum. Surprisingly, piRNAs are absent in all independent nematode lineages with the exception of the clade containing *C. elegans,* raising the question of how transposons can be controlled in the absence of piRNAs. We found that there are at least two evolutionarily distinct pathways in nematodes that compensate for the absence of piRNAs. Both pathways involve RNA-dependent RNA polymerase (RdRP). Whilst one pathway is unique to nematodes, a second, more ancient pathway is an RNA-directed DNA methylation pathway, hitherto unknown in animals, which bears striking similarity to transposon-control pathways in fungi and plants. Our results highlight the rapid, context-dependent evolution of the small RNA world and suggest that transposon defence by piRNAs in animals may have replaced an ancient RNA-dependent RNA polymerase pathway ancestral to all eukaryotes.

Developmental "reprogramming" of larval *Strongyloides stercoralis* by manipulation of steroid-nuclear hormone receptor signaling.

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The infective third-stage larva (iL3) is common to the life cycles of many parasitic nematodes. iL3 are morphologically similar to dauer larvae of the free-living nematode *Caenorhabditis elegans*. Dauer arrest in C. elegans is governed in part by the DAF-12 nuclear hormone receptor and its dafachronic acid (steroid) ligands. DAF-12 signaling is conserved in the parasitic genera *Strongyloides* and *Ancylostoma* and probably others. *Strongyloides* are opportune subjects for study in this area because they can switch between development to iL3 or to free-living females. In *S. stercoralis*, progeny of free-living females develop exclusively to iL3. Exogenous Δ7 dafachronic acid (Δ7DA) can substitute for host-like cues (serum and elevated temperature) to stimulate resumption of development by cultured iL3 of S. stercoralis and Ancylostoma caninum. Moreover, exogenous Δ7DA stimulates formation of advanced second-generation free-living juveniles of *S. stercoralis* and even reproductively competent, second-generation free-living females of *S. papillosus*. Presently we defined the Δ7DA dose requirements for stimulating second-generation free-living development by *S. stercoralis* and explored the kinetics of this process to characterize it as gradual or as a discrete "triggering" event. Δ7DA stimulates second-generation free-living development by *S. stercoralis* with an IC50 of approximately 250 nM. In kinetic experiments, worms were treated with Δ7DA at concentrations bracketing the IC50 for increasing intervals, then removed from the compound and their subsequent development assessed. No advanced free-living larvae or adults appeared in cultures treated with Δ7DA for 24 hours. By contrast, 48 hours' exposure induced second-generation free-living development at 63% the frequency seen in continuously exposed controls. Thus, Δ7DA can trigger a second generation of free-living individuals in *S. stercoralis* within 48 hours of egg deposition, an interval in which second-stage larvae (L2) appear. The first morphological indicator of this triggering was significant enlargement of the genital primordium in DA-treated L2 compared to controls.

Schistosome transgenesis and genetic analysis

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Although the use of gene overexpression, gene knockouts or knockdowns are straightforward genetic tools applied in many model systems, gene misexpression and genetic manipulation of schistosome genes in vivo has been exceptionally challenging, and plasmid based transfection inducing gene expression is limited. We recently reported the use of polyethyleneimine (PEI) as a simple and effective method for schistosome transfection and gene expression. Now, we use schistosome plasmid transgenesis to define, compare and contrast gene expression profiles from endogenous and nonendogenous promoters in the schistosomula stage of schistosomes in schistosomes- important to misexpress (underexpress or overexpress) gene product levels. In addition, we overexpress schistosome genes in vivo using a strong promoter, and show plasmid-based misregulation of genes in schistosomes producing a clear and distinct phenotype, death. These data focus on the schistosomula stage, but foreshadow strong potential for genetic characterization of schistosome molecular pathways, and potential for use in overexpression screens and drug resistance studies in schistosomes using plasmid-based gene expression.

The basis for agronomic flexibility in a plant parasitic nematode

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Plant-parasitic nematodes reduce global agricultural production by ~15%, contributing to malnutrition and loss of income; root-knot nematodes (RKN: *Meloidogyne* spp.) are the most culpable. Field isolates of RKN exhibit substantial diversity of pathogenicity traits, ranging from net egg production, to the ability to break host resistance. On the assumption that phenotypic diversity reflects genetic variation between individuals, we established inbred lines of *M. hapla* (VW8, VW9, LM) that capture much of the diversity seen in the field. Recombinant inbred lines were developed from crosses of these strains, and genes conditioning various traits were mapped using a panel of 28,000 SNPs. These markers were constrained to coding regions so that genotypes could be deduced from RNA-Seq data. Using our *M. hapla* tool-kit (a whole genome sequence for each of the three wild isolates, 2 billion mapped ESTs, and a robust linkage map), we ask "how does genetic diversity in the pathogen influence host gene expression?" We developed a revolutionary cross-species, expression-QTL mapping approach to reveal: a) which nematode loci are responsible for the changes in host expression, and b) what those changes are. Our approach provides expression quantification for all host and all parasite genes, whilst simultaneously providing information on DNA sequence variation within the nematode genome. Based on analysis of 98 RILs from the LM X VW9 cross, we have identified 127 host-plant genes whose expression levels are influenced by allelic variation at one or more, tightly defined parasite loci. The eQTL map also revealed broad loci in the M. hapla genome, including a locus that spans ~120 kb and encodes 12 genes that mimic a key family of plant-peptide hormones. We have solved the structure of these peptides, and present a model for their central role in the parasitic interaction.

Beyond the helminth genomes

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In the last decade there has been a constant increase in the number of helminth genomes published (and in progress) which is a trend that correlates to ever-improving molecular techniques for obtaining biological material, performing DNA extraction and library construction, and predominantly to ever-evolving sequencing chemistry and instrumentation, as well as to decreased sequencing costs. These helminth genomes provide an invaluable resource that facilitates the development of postgenomic tools used to investigate the immunobiology of helminth diseases and accelerate discoveries essential for control, prevention and diagnostics of helminth infections. Such post-genomics tools (functional genomics, proteomics, interactomics etc) provide the basis for a multi-omics approach and prove information essential for developing novel diagnostics, vaccines and anthelmintics. Elucidating the complexity of the helminth proteome (structure and function), evolutionary insights gained from comparative analysis, and subsequent laboratory studies to confirm the predictions will generate exceedingly greater advances in helminth research than the decoding of the genome. Specific post-genomic application we are working on will be presented, including i) bioinformatics and cheminformatics approaches, along with laboratory screening of nematodes spanning the phylum Nematoda, that have led us to identify and characterize targets with broad-control potential, ii) a systems biology approach including immunoaffinity chromatography and mass spectrometry to efficiently identify candidate diagnostic antigens, and iii) deep-sequencing approaches to routinely characterize clinical isolates and through variant studies explain varied pathogenicity and responsiveness to treatment.

A draft genome sequence for *Fasciola hepatica* reveals extensive gene duplication and polymorphism

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A draft genome sequence is presented for *Fasciola hepatica*, which is a digenean parasite of livestock that causes significant disease and loss of productivity. The assembled genome size was c. 1.3 Gb with a scaffold N50 of 206 Kb and gene prediction was assisted by RNAseq data from different developmental stages. This assembly was used to discover and elucidate gene families previously implicated as potential vaccine candidates or as underlying anthelmintic resistance. Major differences in transcriptional profiles through development were seen among different members of these gene families, especially for cathepsins and legumains, supporting their role in infection of vertebrate hosts. Despite the ability of *Fasciola* to self-fertilise and expand clonally within its intermediate snail host, substantial levels of polymorphism were observed across the genome, including in vaccine candidates and potential drug resistance loci. These patterns of duplication, differential expression and polymorphism suggest that the genetic diversity and evolutionary potential of *Fasciola* should be considered in the design of sustainable drug or vaccine treatments.

Large scale comparative genomics of helminths

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The availability of parasite genome sequences has changed the face of parasitology. However, due to the economics of producing larger-sized genomes, and the difficulty in obtaining material, some key areas of parasitology have lagged behind, particularly parasitic helminths. To address the need for genomic resources in helminths, we have also produced high quality reference genomes for some key human pathogen species (or their model equivalents) including as whipworms, threadworms, Schistosomes and tapeworms. Alongside these reference sequences, more than 50 other species have been sequenced and assembled. Using an automated pipeline, 1.6 million genes have been predicted. From these genes, more than 100k gene families have been predicted using a tree-based approach. From these data, major lineage specific differences in gene content can be identified, as well as gene families that have undergone extreme changes across the phyla.

Population genomic approaches toward understanding anthelmintic resistance in *Onchocerca volvulus*

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Investigation of anthelmintic resistance genetics typically employs a "reverse genetics" approach of guessing what might be a plausible mechanism (target site insensitivity; drug metabolism/efflux) and searching for evidence of selection at the gene(s) encoding components of the mechanism. This adaptationist approach relies on the validity of two assumptions that are rarely, if ever, tested: (1) that differences in allele frequencies at candidate loci arise due to directional selection acting on a resistance-conferring allele that is rare in susceptible populations, and (2) that resistance is mediated by a single gene with high heritability and will therefore behave as a Mendelian trait. An alternative approach, made possible by genome-scale sequencing, is to simply scan the parasite genome for evidence of genetic differentiation between susceptible and resistant worms. The advantage of this approach is that it makes no assumptions about mechanism, mode of inheritance or selection. The only assumption is that resistance has a heritable (genetic) component that will manifest as genetic difference(s) between resistant and susceptible worms. We have undertaken a genome scanning approach in *Onchocerca volvulus* populations that show differing response to ivermectin using a combination of population genomic approaches, including pooled genome resequencing and RAD(restriction associated DNA)seq on individual worms. We have uncovered multiple genomic regions that differentiate susceptible and resistant worms, an observation that is most consistent with a polygenic mode of resistance. Furthermore, these regions are often population specific, suggesting local and independent acquisition of resistance. Lastly, population dynamic modeling of these data suggest that genetic drift rather than directional selection is the most likely mechanism by which resistance has arisen in these populations. Given the growing accessibility and reduced cost of next generation sequencing technologies, we propose that population genomic approaches such as those applied here should begin to redefine how drug resistance is approached and understood.

Imatinib, a potential lead compound against schistosomes and other platyhelminths? Lessons from *in vitro* and *in vivo* studies.

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Schistosomiasis is one of the most prevalent parasitic diseases worldwide. No vaccine is available, and there is only one drug to control the disease, namely praziquantel (PZQ). Evidence from laboratory and field studies has indicated the potential of schistosomes to develop PZQ resistance. This motivates the search for alternative treatment options. We discovered that the Abl-kinase inhibitor imatinib (Gleevec/Glivec from Novartis; STI-571), which is used in human cancer therapy, dramatically decreases gametogenesis, egg production and survival of adult *S. mansoni in vitro*. Recent *in vitro* studies with other platyhelminths also indicate imatinib's lethality suggesting that the target proteins are conserved which is supported by genome data. As molecular targets of imatinib in *S. mansoni* we identified the Abl kinase orthologs SmAbl1/SmAbl2 by inhibitor competition assays in *Xenopus* oocytes. Microarray analyses and qRT-PCR experiments based on RNA from inhibitor-treated couples demonstrated a wide influence on a variety of physiological processes. Because the *in vitro* lethality of imatinib could not be replicated in mouse and hamster models of schistosome infection, we considered the possibility of host factors interfering with the compound's efficacy. Therefore, we tested the influence of alpha-1 acid glycoprotein (AGP) and serum albumin (SA), two major blood components, in *in vitro* experiments. Both proteins decrease, in a dose dependent manner, imatinib's efficacy against larval and adult *S. mansoni*. AGP exerted the stronger effect. This negative effect, however, was partially reversed by erythromycin a competitor of AGP. From these results, we suggest the routine inclusion of AGP and SA in *in vitro* experiments to evaluate the potential efficacy of presumptive candidate compounds. Furthermore, as rodents produce much higher amounts of circulating AGP relative to humans, we conclude that rodents are unsuitable infection models for such candidate compounds.

Potassium channel activity has a role in schistosome muscle function and egg production, as shown by the characterisation of SmKK7 and SmERG.

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SmKK7 was first identified in the material released by cercariae during mechanical transformation. Localisation studies revealed the presence of SmKK7 transcripts in peripheral nerve cells in larvae and adult worms. The protein is distributed throughout an extensive nerve net in cercariae, schistosomula and adult worms. In cercariae the protein is present in nerve endings at the very anterior. In adults, the KK7-containing network extends into dorsal tubercles, and is prominent in the suckers. Nerve endings were documented in these areas using electron microscopy. SmKK7 was named for its homology to BmKK7, a potassium channel blocker in scorpion venom. The specific channels affected by BmKK7 are from the ERG family of inward-rectifying voltage-gated potassium channels. Human ERGs (hERGs) maintain electrical stability of the heart. Drosophila with mutations in their ERG channels are hyper-excitable. Schistosomes possess potassium channels with homology to ERGs. To investigate the function of these channels in schistosomes, paired ex-vivo adults were treated with dofetilide, an ERG channel inhibitor. Treated worms exhibited dramatic 'corkscrew' motion 30 minutes after addition of the inhibitor. Eggs laid by dofetilide-treated worms were deformed in comparison with those laid by control worms. Inhibition of SmERG channels led to aberrant regulation of muscle activity, thus affecting the physical production of eggs. RNAi was successfully carried out to knockdown both SmKK7 and SmERG in coupled adult worms. Whilst no difference was observed in the number of eggs laid per female in either treatment, the SmKK7-knockdown worms laid deformed eggs. 'Corkscrew' motion was observed in SmERG-knockdown worms corroborating the results from the inhibitor studies. Morphological analysis of RNAi and inhibitor-treated worms, including confocal microscopy, will be presented. The importance of SmERGs and SmKK7 in schistosome physiology, and their suitability as drug targets will be discussed.

Identification of novel inhibitors of the nematode astacin metalloprotease, DPY-31, that has an essential role in cuticle formation of free-living and parasitic nematodes

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Parasitic nematodes cause chronic, debilitating infections in both livestock and humans worldwide, and have developed multiple resistance to the anthelmintics currently available. The protective cuticle of these parasites has a key role in nematode survival, and its synthesis is a complex multi-step process, involving numerous enzymes, including astacin metalloproteases. Nematode AStacin (NAS) metalloproteases are crucial to the development of the free-living nematode, *Caenorhabditis elegans*, with specific roles in hatching, moulting and cuticle synthesis. DPY-31 (NAS-35) has a crucial role in proper cuticle formation of *C. elegans*, and bioinformatic analyses indicated DPY-31 homologues in nematode species throughout the five nematode phylogenetic clades. Functional conservation was shown between the enzyme in *C. elegans* and its parasitic homologues from *Haemonchus contortus*, *Teladorsagia circumcincta* and *Brugia malayi* when the parasitic enzymes fully rescued the *C. elegans dpy-31* mutant. Specific groups of compounds have previously been found to be potent inhibitors of procollagen C-proteinases. DPY-31 is a homologue of vertebrate BMP-1, a procollagen C-proteinase; thus, these same specific compound groups may also be potent inhibitors of DPY-31. Various compounds, unknown for their inhibition activity, were identified through *in silico* modelling to the active-site of *C. elegans* DPY-31, and a large number of these contained one of the functional groups present in the specific groups of compounds previously found to inhibit procollagen C-proteinases; these functional groups bind to the zinc in the active-site of DPY-31. These compounds were screened against *C. elegans* L4 and in specific colorimetric enzyme activity assays against recombinant DPY-31 from *H. contortus*, *T. circumcincta* and *B. malayi*. The most potent compounds had greater inhibition activity, and caused stronger body morphology defects, than a known broad-spectrum metalloprotease inhibitor and a known astacin metalloprotease-specific inhibitor. Therefore, these novel inhibitors of DPY-31 may be promising as future anti-nematode drugs.

Functional genomics of acetylcholine receptors in *Schistosoma mansoni*

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Acetylcholine (ACh) receptors play an important role in the control of motor activity in schistosomes and therefore are regarded as potential targets for drug discovery. The *Schistosoma mansoni* genome encodes at least 10 predicted subunits of ACh-gated channels, which are distantly related to nicotinic ACh receptors (nAChRs) from other species. To identify receptors involved in motor control we performed an RNAi phenotypic screen in cultured schistosomula and adult worms. These studies identified several subunits that produced either hypoactive or strongly hyperactive RNAi phenotypes, suggesting the existence of multiple cholinergic channels that either stimulate or inhibit movement of the worm. Bioinformatics analyses revealed that the subunits belong to two separate clades within the nAChR tree, one clade that includes "classical" cation-selective channels and the other is a novel clade of predicted ACh-gated chloride channels. One of these subunits was expressed in mammalian cells and shown to form a functional cholinergic chloride channel, using a novel fluorescence-based iodide flux assay. To further elucidate the mode of action, we performed immunolocalization studies with subunit-specific antibodies. The results show predominantly neuronal expression for all the subunits tested, suggesting these receptors influence movement indirectly by modulating neuronal input to the musculature. Besides ACh-gated channels, *S. mansoni* also has one predicted metabotropic ACh receptor, which is a member of the GPCR superfamily (SmGAR). Interestingly this receptor appears to be developmentally expressed and is strongly upregulated in early stage schistosomula. Functional expression studies in yeast showed that SmGAR is a functional acetylcholine receptor that displays a high level of constitutive activity and unusual pharmacology. Furthermore, RNAi suppression of SmGAR led to a reduction in motility of larval schistosomula. These results strengthen our belief that schistosome cholinergic receptors are attractive therapeutic targets due to their important role in motor control and their apparent divergence from host receptors

Ligand-gated ion-channel evolution and functional diversity of the Trichostrongylid levamisole receptor

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Levamisole is widely used to cure parasitic nematode infections, where it binds to and activates a class of acetylcholine receptors (L-AChRs) expressed at neuromuscular junctions. The L-AChR of the model nematode *Caenorhabditis elegans* contains subunits encoded by unc-38, unc-63, lev-8, lev-1 and unc-29. In contrast, *Haemonchus contortus* possesses four copies of unc-29 and the L-AChR contains in addition, unc-38, unc-63 and acr-8. UNC-29.1, UNC-29.3 and UNC-29.4, but not UNC-29.2 produce functional channels in Xenopus oocytes when combined with H. contortus subunits, with similar affinity for ACh but differing response to LEV and other agonists and antagonists. All four, including UNC-29.2, are functional when expressed with the remaining subunits from *C. elegans* in *Xenopus*. This is confirmed in vivo by rescue of a *C. elegans* unc-29 KO by transfection of each unc-29 copy. This suggests co-adaptation between UNC-29.2 and the other H. contortus subunits is responsible for the lack of a receptor, rather than changes limited to UNC-29.2. Specific UNC-29 copy antibodies allow us to localize the novel L-AChR targets in *H. contortus* in vivo and assist in the search for in vivo partners for UNC-29.2 and a potentially new class of receptor. The UNC-29 copies of *H. contortus* are functionally divergent and produce receptors wtih distinct pharmacology. Comparison of the different subunits and the unusual properties of UNC-29.2 provides an experimental platform to investigate the details of ligind binding, gating and the evolution of subunit assembly. Taken as a whole, this work establishes, critically, that evolutionary differences between nematode species must be taken into account when using *C. elegans* as a model and that parasite receptors must be examined directly.

Functional investigation of nematode parasite specific acetylcholine receptors

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Acetylcholine receptors of parasitic nematodes (AChRs) represent major targets of widely used anthelmintics. AChRs are ion channels located at the neuromuscular and neuronal junctions involved in fast synaptic neurotransmission. They are made of 5 identical subunits (homopentamers) or 5 different subunits (heteropentamers) depending on the presence or absence of two adjacent cysteine residues involved in the acetylcholine binding site. There is a large repertoire of genes encoding for AChR suhunits in nematodes (25 genes in the model-nematode *C. elegans),* and the aim of this present project is to investigate the AChR diversity in parasitic nematode to identify and characterize novel drug targets. Based on genomic data analyses we have identified two AChR subunits named ACR-26 and ACR-27 that appear to be specifically present in parasitic nematodes of mammalian and absent in free living nematodes. We show that these two subunits are shared by the clade V Trichostrongylid species as well as the clade III ascarid and filarid species. The full-length cDNA of Hco-ACR-26 and Hco-ACR-27 were cloned in the strongyle *Haemonchus contortus*, a nematode parasite of great economic importance for the livestock industries. Immunolocalization and qRT-PCR analysis of these subunits show differences in the expression pattern in the adult worms and larval stages. We report that these two subunits are able to form a functional AChR when expressed into *Xenopus laevis* oocytes. The detailed pharmacological characterization of this new AChR revealed some unexpected properties of interest that may be affected by the stoichiometric arrangement of the subunits. Our results improve the understanding of AChR diversity in parasitic nematodes and provide a solid basis for the development of novel anthelmintic compounds specific for mammalian parasites.

Nicotinic anthelmintics and diversity of nAChRs in the Clade III nematode, *Brugia malayi*

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Lymphatic filariasis (elephantiasis) is caused by the filarial nematode, *Brugia malayi,* which is grouped in Clade III with *Ascaris suum*, because of molecular similarities. Treatment of adult *Brugia* infections relies on a small number of anthelmintic drugs that are limited in efficacy. Recently, novel cholinergic anthelmintics, like tribendimidine and derquantel, have been found to be effective against *Ascaris suum* and have increased interest in pharmacology of parasite nicotinic receptors (nAChRs). We are exploring receptor selectivities of nicotinic anthelmintics and diversities of Clade III nAChRs in *Brugia malayi*. Our aim is to synergize pharmacological research efforts made on Clade III parasites. Cytoplasm from single muscle cells of Brugia malayi was collected for PCR using patch micropipettes. The cells expressed putative levamisole receptor subunits (Bma-unc-63, Bma -unc-29, Bma -unc-38 & Bma-acr-8) suggesting that cholinergic anthelmintics may be active on *Brugia* muscle. Whole-cell patch-clamp recordings were made from muscle cells at 35o C that revealed bursts of spontaneous excitatory post-synaptic currents (EPSCs) due to input from motor neurons. Perfusion with acetylcholine, tribendimidine, levamisole, pyrantel or bephenium produced non-desensitizing dose-dependent inward currents due activation of nAChRs. Interestingly, as the inward currents increased, the amplitude of the EPSCs decreased, because the release of acetylcholine from motor neurons could no longer open already open receptor channels. Application of derquantel revealed that it was also a potent nicotinic receptor antagonist of *Brugia malayi* nAChRs, suggesting a therapeutic significance. Interestingly, ivermectin produced a slow outward current, and also inhibited the acetylcholine current responses suggesting that, in addition to its effect as a GluCl positive allosteric modulator, it had a negative allosteric modulator effect on nAChRs of *Brugia*. We are currently looking for additional nAChR subtypes in *Brugia* and *Ascaris* that may be useful for combination therapies.

Discovery and commercialization of the new anthelmintic monepantel

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Until recently, only three broad-spectrum classes of anthelmintics for the control of gastrointestinal nematodes of livestock were available: the benzimidazoles (BZs), the imidazothiazoles (IMZ) and the macrocyclic lactones (MLs). Resistance of gastrointestinal nematodes to all three drug classes has severely threatened successful control in livestock in many parts of the world. In 2008, Novartis Animal Health reported the discovery of the Amino-Acetonitrile Derivatives (AADs) as a potential new class of broad-spectrum anthelmintics for livestock. In 2009, monepantel was introduced into the market as Zolvix® and is now commercially available in all major sheep producing countries. The objective of the presentation is to introduce the AADs, their discovery, their safety and efficacy profiles, the selection of monepantel as the first candidate for commercial use and, finally, the investigation on and elucidation of the mode of action of this active ingredient.

Chronic Infection by *Trichuris muris*: modulating the microflora

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The mouse whipworm *Trichuris muris* naturally exists as a chronic low-level infection. In the laboratory such low level infection is associated with a modulated Th1 immune response via interleukin (IL) -10 and IL-22. The parasite occupies the epithelium and lumen of the caecum and proximal colon, major sites of intestinal microflora. We now show that long-term infection is associated with marked dysbiosis although the mechanisms underlying this are unknown. Following anthelmintic treatment the microbial populations slowly return towards those observed in uninfected animals, suggesting direct parasite or anti-parasite host response influences. We also observed that chronic infection is associated with the production of intestinal lipocalin-2, an IL-22 regulated host anti-microbial molecule that sequesters iron as part of the intestinal innate defence system. Moreover, we speculate that the major secreted parasite derived protein contributes to modulation of the dysbiosis via its capacity to avidly bind divalent cations including iron, reflecting a complex interplay between parasite, microflora and host immunity during chronic intestinal helminth infection.

***Schistosoma haematobium* eggs induce urothelial abnormalities through p53- and IL-4 receptor-α-dependent pathways**

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The urothelium responds vigorously to *Schistosoma haematobium* infection(urogenital schistosomiasis). Defining the pathways underpinning these responses will help us understand how urogenital schistosomiasis-associated egg expulsion, hematuria, and bladder cancer develop. p53 is of particular interest, given its known role in carcinogenesis. The phenotype of mice featuring tamoxifen-inducible cre activity in cells expressing uroplakin-3a, a urothelial-specific gene(Upk3a-GCE mice), was confirmed by crossing them with TdTomato-floxed-EGFP mice and administering tamoxifen to their progeny. Expectedly, these progeny switched from TdTomato to EGFP expression in their urothelium. We next crossed Upk3a-GCE mice to p53-floxed mice. These progeny were given tamoxifen or vehicle to render them urothelial p53-haploinsufficient or –intact. Then, we injected *S. haematobium* eggs or vehicle into the bladder walls of these mice. Three months later, male p53-intact, egg-injected mice exhibited similar histological changes as their p53-haploinsufficient counterparts, while female p53-intact, egg-injected mice featured no urothelial ulceration and their p53-haploinsufficient counterparts often had significant ulceration. These histological changes led us to examine how urothelial cell cycle status is molded by egg-induced immune responses, specifically IL-4 receptor-α(IL4Rα). We injected eggs or vehicle into the bladder walls of wild type(wt), IL4Rα$^{-/-}$, and myeloid-associated IL4Rα-deficient(IL4RαLysMCre) mice. Three weeks later, single cell suspensions of bladder urothelia were prepared and stained with DAPI and anti-CD45 and EpCAM antibodies. Urothelial cell cycle status was analyzed via DAPI staining of CD45^{-} EpCAM^{+} cells. Relative to vehicle controls, egg-injected wt mice demonstrated more and fewer urothelial cells in S and G2/M phase. Although egg-injected, IL4RαLysMCre mice featured similar urothelial responses as wt counterparts, egg-injected IL4Rα$^{-/-}$ mice exhibited fewer urothelial cells in S phase(Fig.5). Thus, IL4Rα signaling through non-myeloid cells seems to affect urothelial cell cycle status; this effect may target DNA synthesis, a crucial process in carcinogenesis. These pathways result in histologically apparent urothelial lesions, including p53-dependent ulceration in females.

Interactions between schistosomes and the host haemostatic system

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Adult schistosomes reside in the human bloodstream for years and adult females release around 300 eggs into the circulation daily. Both the outer surface of the eggshell and the outer surface of the adult worm form a direct site of interaction between the parasite and its host. Egg excretion is an essential step in the life-cycle of schistosomes, but mechanisms involved in this process are largely unknown. Research from our lab shows direct binding to the *Schistosoma mansoni* eggshell of von Willebrand factor and other plasma proteins involved in blood clotting. Using deletion-mutants, we demonstrated that it is the A1 domain of von Willebrand factor that binds to the eggshell. These results suggest that binding of plasma proteins to the eggshell promotes binding to the endothelium, initiating the passage of the egg through the blood-vessel wall to be excreted in the end. From adult schistosomes it is known that they affect the immune system of the host, but they also interfere with haemostasis. The adult worm is a potential activator of blood coagulation. The worms seriously alter blood flow, and therefore endothelial function, leading to hypercoagulability. However, schistosomiasis patients do not show an increased risk of thrombus formation, which indicates that schistosomes interfere with the haemostatic system of the host. Research from our lab on the mechanisms employed by *S. mansoni* to interfere with the haemostatic system show that *S. mansoni* indeed contains components that inhibit platelet activation (primary hemostasis), and also components that induce fibrin formation (secondary hemostasis). In addition, *S. mansoni* is also able to stimulate activation of the fibrinolytic system. This could explain the hyperfibrinolytic state observed in schistosomiasis patients. In short, schistosomes interfere with primary and secondary coagulation pathways, which may explain the absence of thrombotic complications in schistosomiasis patients.

A role for helminths in achieving immunological tolerance

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Helminth worms currently infect more than one quarter of the world's population and their success as parasites owes much to their active immunomodulation of the host immune response. The resulting effects of suppressed allergy and autoimmunity have been widely discussed; however we have also noted a literature recording extended transplant tolerance in helminth-infected hosts. Accordingly, we hypothesised that helminth infection reduces the immune response to allograft transplantation and may offer a therapeutically tractable approach. To test this hypothesis, C57BL/6 mice were implanted with a subcutaneous minipump delivering a continuous infusion of secreted products from the model mouse intestinal parasite, *Heligmosomoides polygyrus*. Simultaneously, fully allogeneic skin grafts were performed from BALBc donors. Seven days later, lymphocytes were isolated from allograft draining lymph nodes and analysed by flow cytometry. Flow cytometric analysis reveals a 41.7% increase in the mean percentage of $CD4^+CD25^+Foxp3^+$ regulatory T cells (of total $CD4^+$ cells) in treated vs. untreated mice ($p=0.0085$). Treatment with parasite products also increased mean expression of the regulatory cell surface receptor PD-1, specifically in the effector CD4+ T cell population, by 62.2% ($p=0.03$). In conclusion, our results demonstrate that helminth-derived products can powerfully induce regulatory immunological mechanisms in the presence of a fully-allogeneic transplant. This was achieved with physiological concentrations, similar to those experienced by millions of (largely asymptomatic) patients with chronic helminth infection. Identification of the specific mechanisms involved in suppression of allograft rejection by helminth parasites may lead towards development of safe and effective novel therapeutic strategies.

Loa loa secretome: Comparative proteomic analyses of urine, plasma and in vitro microfilaria-derived excretory secretory products towards biomarker for high burden

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Among parasitic helminths, *Loa loa* presents a challenge for the mass drug administration programs in areas co-endemic for *W. bancrofti* and *O. volvulus* because of the severe adverse events (SAE's) in cases of very high microfilaraemia. To identify microfilarial-derived *Loa*-specific biomarker(s) in service of the development of a rapid diagnostic immunoassay-based tool we characterized the excretory/secretory proteome of *Loa loa*-mf as well as the *Loa*-specific proteins found in urine and plasma of *Loa*-infected and -uninfected individuals. *Loa loa* microfilariae (~20 x 106) were purified from the blood of *Loa*-infected patients cultured in vitro and the excretory-secretory (ES) products characterized by mass spectrometry. A total of 1273 proteins were identified of which over 200 proteins were identified by having at least 2 unique peptides. Similar to *B. malayi* microfilarial ES, among the most abundant proteins identified were the endochitinase, cyclophilins, and a phosphatidyl ethanolamine binding protein. In addition several hypothetical proteins unique to *Loa loa* were identified. To further identify if any of these ES proteins were present in body fluids, proteomic analyses of urine and plasma of *Loa*-infected individuals (depleted of the top 12 to 20 human abundant proteins) resulted in the identification of 18 (from urine) and 29 (from plasma) *Loa*-proteins found only in *Loa*-infected individuals that were identified by having at least 2 unique peptides. Antibodies to *Loa loa* mf ES and immunogenic peptides from 4 of these mf- and *Loa*-specific proteins have been raised and immunoassays have been developed in hopes of having a point of care diagnostic tool.

The tegument membranes of adult _Schistosoma mansoni_ have a specific and unusual phospholipid composition

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The complex outer-surface structure of adult schistosomes (the tegument) is essential for survival of the parasite and consists of a syncytium of fused cells covered by at least two closely-apposed lipid bilayers. The membranes of the tegument form the interactive surface with the host, and therefore, the components of the tegument membranes are likely to be involved in the complex interaction between the parasite and its host. Proteome analysis by multiple laboratories demonstrated that the tegument membranes comprise a specific set of proteins, correlating with the specific function of these outer-surface membranes. Although schistosomes are also known to contain various specific lipids, such as lyso-phospholipids and long chain fatty acids with desaturations at unusual locations, the location of these lipids within the adult schistosomes is so far not known. We now identified the lipid composition of the tegument membranes of adult *S. mansoni* worms. The species composition was analysed of all four major phospholipid classes, including the lyso-phospholipid species. Thereby we showed that (1) the tegument comprises many schistosome-specific and tegument-specific phospholipids, (2) the species composition of most phospholipid classes in the tegument differs dramatically from that of total worms as well as from that of host blood cells, and (3) the tegument membranes are especially enriched in lyso-phosphatidylserine and lyso-phosphatidylethanolamine. Since some of these lyso-phopholipids are known to affect host immune cells, we also examined excretion of these tegument-specific phospholipid species *in vitro* and *in vivo*. In conclusion, the tegument membranes are not only unique in structure and protein composition and they contain many unusual phospholipids.

Inhibition of the PI3K/Akt pathway in dendritic cells by particles from the *Echinococcus granulosus* laminated layer

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Echinococcus larvae are protected by a unique mucin-based extracellular matrix called the laminated layer (LL). The shedding of LL particles is necessary for larval growth. We previously observed that such particles (pLL) induce unconventional maturation phenotypes in mouse bone marrow-derived and peritoneal dendritic cells (DCs). We are now exploring the signalling basis of these changes. Treatment of DCs with pLL did not activate central components of the NFkB or MAP kinases pathways, or inhibit them upon TLR stimulation. However, pLL inhibited the activation of the central effector of the PI3K pathway, Akt, upon stimulation with LPS and under basal culture conditions. pLL treatment mimicked the effects of the Akt inhibitor triciribine in terms of co-stimulatory molecules: it caused CD86 but not CD40 up-regulation, and when added together with LPS, potentiated CD86 while inhibiting CD40 up-regulation. Further, when added together, pLL and the inhibitor appeared redundant in causing these changes. In contrast, the PI3K inhibitor wortmannin abrogated the effects of pLL, possibly reflecting a need for particle phagocytosis. Reduction of disulphide bridges in pLL, previously observed to decrease drastically the material's capacity to condition DCs, also negated its capacity to inhibit Akt activation, suggesting the participation of certain cysteine-containing apomucin sequences. Data using gene-deficient DCs suggest that the PIP_3 phosphatase SHIP is necessary for part of the effects of pLL. The PI3K/Akt pathway is necessary for alternative activation (of macrophages) in response to IL-4. In DCs *in vitro*, pLL inhibited Akt phosphorylation induced by IL-4, and alternative activation. As a co-stimulus with LPS, IL-4 potentiates IL-12p70 while inhibiting IL-10 production by BMDCs. pLL counteracted both effects of IL-4 in the presence of LPS, and concomitantly inhibited Akt phosphorylation induced by the combined stimuli. Thus, the parasite-derived material has distinct immune modulatory effects on mammalian DCs.

Inflammasome activation by particles from the *Echinococcus granulosus* laminated layer

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Larval *Echinococcus granulosus* grows in internal organs as fluid-filled structures (hydatids). Hydatids, which elicit little inflammation in spite of their massive size, expose a mucin-based extracellular matrix termed the laminated layer (LL). For the parasite to grow, particles must be shed from the external strata of the LL. We have observed that a preparation of LL particles (pLL) induces an unconventional maturation phenotype in BMDC, which includes the selective up-regulation of CD86 and, after TLR stimulation, inhibition of CD40 expression and potentiation of IL-10 production. Similar changes are observed in peritoneal DCs after pLL injection in mice. In the present work, we report that pLL also induces the release of IL-1β and IL-18 in BMDCs. This activity was much potentiated after LPS priming, and was inhibited by the caspase-1 inhibitor Z-YVAD-FMK. It was also inhibited by extracellular KCl, suggesting participation of the NLRP3 inflammasome. Inhibition of caspase-1 had no effect on the unconventional maturation phenotype induced by pLL. However, as previously observed for induction of the mentioned phenotype, the reduction of disulphide bonds in pLL strongly diminished its capacity to elicit IL-1b. This suggests that non-glycosylated, cysteine-containing termini of LL mucin backbones are necessary for both inflammasome-independent and inflammasome-dependent signals in DCs. Upon intraperitoneal injection, pLL elicited peritoneal IL-1b, which was much potentiated by co-injection of LPS. Thus with respect to inflammasome activation, pLL behaves similarly to alum and other particulate adjuvants. However, when assayed in BMDCs, alum did not enhance IL-10 production. Also, according to preliminary results, pLL did not act as an adjuvant in vivo. Then, we are intrigued by the possibility that certain inflammasome-independent cellular changes elicited by pLL in DCs reflect an adaptation to minimise any adjuvant capacity of shed LL materials, which would be inherent to their particulate nature.

Targeting of the host ubiquitin pathway by *Trichinella spiralis*

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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 such as helminths possess a Ub-pathway of their own as well as the potential ability to interfere with host Ub. In the case of *Trichinella*, with intracellular life- cycle stages and secretory abilities, it is possible that parasite-derived Ub- pathway proteins are involved in the manipulation of the host during 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 force terminally differentiated cells to undergo cell cycle re-entry and subsequent arrest at the G2/M interface, processes usually dependent on a functional ubiquitin pathway. Indeed, we have confirmed that the ES/secreted proteins of *T. spiralis* muscle larvae demonstrate both E2 ubiquitin conjugating and E3 ubiquitin ligase activity. Proteomic analysis attributes the E2 activity to the secretion of TsUbE2L3, a worm-derived enzyme with homology to mammalian UbE2L3. Human UbE2L3 is known to play a role in controlling the G1/S phase of the cell cycle and known to target the tumour suppressor protein p53 for proteasomal degradation via ubiquitination. Through biochemical analysis we have shown that the worm-derived enzyme is also able to manipulate levels of host p53. These findings demonstrate that parasites are able to interfere with host ubiquitin machinery, and provide insight into one of the many mechanisms .*T spiralis* uses to regulate the host cell cycle to its advantage.

Elicitation and functions of innate lymphoid type 2 cells in helminth infections.

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The use of helminth in mouse models was instrumental in the first discovery of new innate lymphoid cells (ILCs) that initiate type 2 responses. These group 2 ILC (ILC2) cells have now been shown to functions beyond that described by helminthes. Indeed ILC2 are implicated in the genesis of inflammation cascades that are intrinsic to a number of major human diseases including skin inflammation (atopic dermatitis), pulmonary fibrosis, lung inflammation (idiopathic pulmonary fibrosis and asthma) and obesity. Furthermore, helminth studies facilitated the analysis of the relative roles of key cytokines, interleukin (IL)-25, IL-33 and thymic stromal lymphopoietin (TSLP) as well as transcription factors, such as retinoid-related orphan receptor (ROR)a, in innate and adaptive type 2 responses. In this presentation the parasite and immunological factors that stimulate ILC2 expansion will be described and the functional roles of ILC2 in helminth infections will be presented.

Versatility of eosinophils in nematode infection

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Eosinophilia is a feature of the host immune response that distinguishes parasitic worms from other pathogens, yet defining the function of eosinophils in worm infection has been challenging. Eosinophils protect larval stage *Trichinella spiralis* in skeletal muscles of mice by inhibiting local production of nitric oxide and supporting larval growth. IL-10 limits NO-mediated toxicity; however, the relationship of eosinophils to IL-10 has not been defined. Here we report that by producing IL-10 at the initiation of infection, eosinophils expand IL-10^+ myeloid dendritic cells and IL-10^+ $CD4^+$ T lymphocytes that protect larvae. In contrast, published findings show clearly that eosinophils adhere to and kill *T. spiralis* larvae in the presence of antibodies *in vitro*. By challenging previously infected eosinophil-ablated mice, we obtained evidence that eosinophils do protect the host in the context of secondary infection. The results document a versatility of function for eosinophils that reflects the immune contexts in which they operate.

The role of basophils during immune responses against helminths

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Basophils are potent IL-4 producing cells and are associated with T helper 2 (Th2) cell-polarized immune responses found during allergic inflammation and parasitic infections. The role of basophils in the initiation of Th2 immune responses and their role during the effector phase is still highly debated. We established a new mouse strain (Mcpt8Cre) where basophils are specifically and constitutively deleted. To gain new insights into the role of basophils in protective immunity against helminths we infected these mice with the gastrointestinal nematodes *Nippostrongylus brasiliensis* and *Heligmosomoides polygyrus,* and the trematode *Schistosoma mansoni*. Moreover, we generated mixed bone-marrow chimeric mice in which only basophils are deficient for activating Fc receptors or IL-4/IL-13 to decipher the mechanism behind basophil-mediated worm expulsion. Differentiation of Th2 cells in draining lymph nodes and their accumulation in tissues appeared to be basophil independent in these infection models. During infection with *S. mansoni* we could not observe any differences in egg counts, number and size of granulomas, collagen expression, eosinophilia and Th2 cell cytokine production. Basophils were also not required for eosinophilia and worm expulsion during primary infections with *N. brasiliensis* or *H. polygyrus*. However, basophils were required for efficient worm expulsion in a secondary infection. By using mixed bone marrow chimeras we were able to show that basophil-derived IL-4/IL-13 as well as antibody-mediated activation of basophils was required for an optimal immune response against *N. brasiliensis* and *H. polygyrus*. These results demonstrate the importance of basophils during the memory type 2 immune response against certain helminths, while they appear dispensable for Th2 induction and antibody production. Basophils therefore constitute a critical component for protective immunity against reinfection with helminths which is probably triggered by helminth-specific antibodies generated during the primary infection.

Neutrophils prime a long-lived effector macrophage phenotype that mediates accelerated helminth expulsion.

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The innate immune cells mediating macroparasite clearance remain largely undefined. We examined their role in acquired resistance to the parasitic nematode, Nippostrongylus brasiliensis (Nb) hypothesizing that they may mediate the markedly accelerated CD4+ cell-independent worm clearance occurring after secondary inoculation. After secondary inoculation, parasitic larvae in the lung are surrounded by macrophages and showed reduced ATP, indicating impaired metabolism. As late as one month after Nb inoculation, lung macrophages, transferred to naïve recipients, accelerated parasite clearance. Primed macrophages adhered to larvae in vitro and triggered increased mortality through CD11b-dependent mechanisms. Neutrophil depletion impaired the recall response and depletion of neutrophils in primed mice abrogated the protective effects of transferred macrophages in recipient mice and inhibited their binding to L3. Global transcriptome analyses of sort-purified lung neutrophils from Nb inoculated mice revealed a markedly different expression pattern from lung neutrophils isolated from LPS inculated mice, indicating that neutrophils, like macrophages, develop a distinct alternatively activated phenotype (N2) after helminth infection. These data thus indicate that differentially activated neutrophils in the context of a type 2 immune response prime a long-lived effector macrophage phenotype that binds metazoan parasites and directly mediates rapid worm damage and clearance.

Efficient chronic parasite expulsion is co-ordinated by IL-25R expression and IL-4Rα signalling within the innate immune system

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Interleukin-25 is reported to play a vital role in the generation of type-2 responses and in controlling immunity to acute parasite infection. However, it is not yet clear how this cytokine contributes to type-2 inflammation and parasite expulsion following chronic helminth infection. Using IL-25R-deficient BALB/c mice infected with *Heligmosomoides polygyrus*, we find that Th2 responses and adult worm burdens were equivalent to wild-type BALB/c mice at the peak of Th2 inflammation. However, by day 28 post-infection, adult worm expulsion was significantly delayed in IL-25R-deficient mice. Injection of rIL-25 at the peak, but not the initiation, of Th2 inflammation was able to enhance parasite expulsion in more susceptible C57BL/6 mouse strains. Construction of bone-marrow chimeras demonstrated that the IL-25 responsive cell was of a hematopoietic lineage and use of immune-deficient RAG-/- mice, provided with an exogenous source of IL-4, demonstrated that the IL-25 responsive cell was innate and that it required IL-4Rα signalling in order to effectively mediate helminth clearance. This work generates the novel and fascinating hypothesis that IL-25R signalling may be redundant during the early phases of chronic helminth infection and that it is most effective in driving parasite expulsion subsequent to initial IL-4Rα signaling.

Contribution of Surfactant Protein D to host immunity to *Nippostrongylus brasiliensis* infection

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Immunity to nematode infections requires epithelial cell driven production of cytokines and chemokines including IL-33 and TSLP. These drive disease resolving TH2 immunity by inducing effector responses such as goblet cell mucus secretion. Pulmonary alveolar type II epithelial (ATII) cells are an important source of IL-33, but also secrete large quantities of the collectin Surfactant Proteins (SPs). SPs have important antigen recognition functions and appear to be positively regulated by TH2 cytokines. Any role for SPs in controlling nematode infections is unknown. We found that pulmonary concentrations of SP-D were upregulated following *Nippostrongylus brasiliensis* (Nb) infection, this was dependent on host TH2 responsiveness. Manipulation of pulmonary SP-D levels enhanced host control of Nb infection. This related to SP-D preferentially binding lung stage L4 Nb and enhanced innate cell responses associated with control of Nb infection. These included increased pulmonary ILC2 populations and alternative activation of alveolar macrophages (alvM). Macrophages are a major target of ATII secreted SP-D. We found that treatment of alvM with SP-D ex vivo then transfer into a naïve host conferred protection against Nb infection. Therefore SP-D, in part at least, contributes to host protective immunity against Nb by enhancing both binding the parasite and driving alternative activation of alvM. These findings represent the first identification of SP-D as an important contributor to host protective immunity to Nb infection.

Concerted action of adaptive immunity and mucosal mast cells is required for final elimination of *Strongyloides ratti* from the small intestine

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Immunological functions of mast cells (mc) during helminth infection remain poorly understood. Mice that lack mc due to mutations in the receptor tyrosine kinase kit have been employed as standard models to study mc function, despite the multiple additional immunological and non-immunological alterations caused by dysfunctional kit. Here we use recently generated kit-independent mc-deficient mouse strains to re-evaluate the role of mc during infection with the nematode *Strongyloides ratti.* Numbers of tissue migrating larvae were alike in mc-competent and mc-deficient littermates but parasite burdens in the small intestine were significantly increased in all mc-deficient mouse strains analysed. Mice that selectively lack connective tissue mc (ctmc, Dudeck 2011) terminated infection by day 40, i.e. with kinetics comparable to wildtype littermates, despite their initially increased parasite burden. In sharp contrast, mice that lack ctmc and mucosal mc (mmc, Feyerabend 2011) continued to release low amounts of *S. ratti* DNA for more than 100 days, carrying living and fertile female adults in the small intestine at these late time points. *S. ratti*-specific cytokine and antibody production was comparable in mc-competent and mc-deficient mice. Moreover, improved eradication of tissue migrating larvae was induced by vaccination also in the absence of mc. Thus our collective data suggest that mc do not function as initiators of adaptive immune responses to *S. ratti.* While both, ctmc and mmc contribute to parasite control during early intestinal infection, specifically mmc represent central and indispensible effector cells in the final eradication of *S. ratti* from the small intestine. Mc-competent $Rag1^{-/-}$ mice controlled *S. ratti* numbers during early infection (day 6) but were unable to expulse *S. ratti*, thus suggesting that final eradication was dependent on mmc and adaptive immunity. The molecular mechanism of this immune-mediated mc activation and regulation during early and late stages of infection is under current investigation.

A novel role of innate immunity in orchestrating pathogenic Th17 cell responses in schistosomiasis

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In murine schistosomiasis, immunopathology and cytokine production in response to parasite eggs is uneven and strain dependent. CBA mice develop severe egg-induced hepatic granulomatous inflammation associated with prominent T helper 17 (Th17) cell responses driven by dendritic cell (DC)-derived IL-1β and IL-23. Such Th17 cells fail to develop in low-pathology BL/6 mice, and the reasons for these strain-specific differences in antigen presenting cell (APC) reactivity to eggs remain unclear. We now show by gene profiling that CBA DCs display a markedly higher expression of C-type lectin receptors (CLRs). In particular, expression of CD209a, a murine homologue of human DC-specific ICAM-3-grabbing non-integrin (DC-SIGN), was >18-fold higher in CBA than BL/6 DC. Higher CD209a expression was observed in CBA splenic and granuloma APC subpopulations, but only DCs induced Th17 cell differentiation in response to schistosome eggs. Gene silencing in CBA DCs, and over-expression in BL/6 DCs, demonstrated that CD209a is essential for egg-induced DC IL-1β and IL-23 production which is associated with ERK1/2 MAP kinase activation necessary for subsequent Th17 cell development. These findings reveal a novel genetically-determined innate parasite-sensing mechanism leading to the development of Th17 cells that mediate severe immunopathology in a helminthic disease.

The influence of maternal *Nippostrongylus brasiliensis* infection on offspring immunity

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In early life the immature immune system has a reduced ability to control infection. This susceptibility is offset by transfer of protective immune components from the mother. Helminth infections are widespread and can have a long lasting influence on host immunity. Children of mothers exposed to helminth infections may display T cell sensitization to endemic helminth infections and associations have been made between maternal helminth infection and altered immune responses to childhood diseases and vaccinations. Helminth infections induce a highly polarized Th2 response. We hypothesize that a memory-type Th2 response may be transferred to offspring in utero and through breast milk in the form of lymphocytes, antibodies and cytokines, imprinting on early offspring immune development. Our study has showed that, in mice, maternal infection with the helminth *N. brasiliensis* inherently alters offspring immunity, increasing T cell and B cell population development and proliferation. This was especially evident in the early germinal centre formation in the spleens of pups born to infected mothers. Pups born to N. brasiliensis exposed mothers had increased populations of both CD4+ cells and CD8+ cells related to altered thymic populations, as well as higher sub-populations of central memory and effector CD4+ cells compared to pups born to naive mothers. Unexpectedly this early cell induction is not disease specific. Our initial data suggests that these early activated cells are able to respond to several Th1 or Th2 infections, by increased levels of IFNg or IL-13 production respectively. *N. brasiliensis* infected mothers transfer protective passive immunity against *N. brasiliensis* to their offspring. The *N. brasiliensis* associated maternal protection could be transferred by nursing alone; naïve pups nursed by a previously infected mother also had reduced parasite burdens. Together, these data indicate that maternal exposure to a helminth infection offers some protection against infant infections

ILCs and CD4 T cells co-operate to maintain AAM activation in *Nippostrongylus brasiliensis* lungs

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Establishing sterilizing immunity to helminth nematodes through vaccination is currently a major global health objective. Amongst the helminths infecting humans, hookworms are currently infect an estimated 1 billion people, and are considered to be the leading cause of anaemia worldwide. To date, the gut immune response has been considered as the principal source of protection against geohelminths but data is emerging that other tissue sites including skin and lung could also be important. To date, little information is available concerning the specific components of the immune response that confer resistance or immunity to hookworms, principally due to the absence of an adequate model. We use the closely-related rodent parasite *Nippostrongylus brasiliensis* to model the early stages of hookworm infection that may confer subsequent immunity. Using gene deficient mice, truncated infection studies and fluorescent labelling of the worms, we show that the lung is the major protection site against *Nippostrongylus brasiliensis* infection. Furthermore, we have identified a novel developmental defect in the worms occurring during the moult 3 process which is strongly dependent on STAT6-mediated immune pathways and that is associated with the acquisition of protective immunity in the lung. We further show that this protection is mediated by alternative activation of interstitial macrophages, themselves maintained by CD4 T cells and ILC2s. The implications of these findings in the development of a vaccine against hookworm will be discussed.

Non-neuronal cholinergic signalling in immunity to infection: a role for M3 muscarinic receptors.

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Previous studies have shown that smooth muscle cell IL-4Ra–deficient mice exhibit impaired immunity to the parasitic nematode *Nippostrongylus brasiliensis*, and that this is accompanied by reduced expression of the M3 muscarinic acetylcholine receptor (M3R) in the intestine. We show here that the M3R itself plays an important role in immunity to both *N. brasiliensis* (primary and secondary infection) and *Salmonella typhimurium*, as $M3R^{-/-}$ mice were impaired in their ability to resolve infection with either pathogen. CD4+ T cell activation and cytokine production were reduced in $M3R^{-/-}$ mice, which produced less IL-13 in response to *N. brasiliensis* and less IFN-γ in response to *S. typhimurium*. Ex vivo lymphocyte stimulation of cells from intact BALB/c mice infected with *N. brasiliensis* and *S. typhimurium* with muscarinic agonists enhanced production of IL-13 and IFN-γ respectively, and levels of M3R mRNA were upregulated on CD4+ T cells during infection with either pathogen. We also investigated non-cholinergic signalling in innate cells and could measure a moderate but significant enhancement of IL-13 in innate lymphoid cells after stimulation with muscarinic agonists. Finally, we investigated which cells have the capacity to synthesise the agonist acetylcholine *in vivo* in choline acetyltransferase (ChAT) reporter mice, which indicated both adaptive and innate immune cells as possible sources. Interestingly, many parasitic nematodes which colonise mucosal surfaces secrete acetylcholinesterases (AChEs). The function of these enzymes has been postulated to suppress cholinergic signalling which promotes smooth muscle contraction and exocrine gland secretion, but our data also support a potential immunomodulatory role for AChE in suppressing T cell cytokine production.

The metabolic regulation of alternative macrophage activation in immunity to helminths

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Macrophages are crucial for immunity and can adopt different activation states depending on context. Interferon-γ (IFNγ) in combination with TLR agonists promotes M1 (or classical) activation, whereas the cytokines IL-4 and IL-13 promote M2 (or alternative) activation. Whereas M1 macrophages are inflammatory and are important for immunity to microbial pathogens, M2 macrophages are generally considered to play a more anti-inflammatory role and to be important for immunity to parasitic helminths. We are interested in the metabolic differences between M1 macrophages, which rely on aerobic glycolysis, and M2 macrophages, which utilize fatty acid oxidation (FAO) to fuel mitochondrial oxidative phosphorylation, and whether or not these metabolic states can be manipulated to promote or inhibit cell function to change disease outcome. The presentation will focus on our recent findings on the role of regulated lipolysis in the generation of fatty acids for FAO in M2 macrophages.

Antibodies trap migrating helminth larvae and promote timely tissue repair

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Infections with intestinal helminths severely impact on human and veterinary health, particularly through the damage that these large parasites inflict when migrating through host tissues. We have recently reported an important role for antibodies in the rapid trapping of tissue migrating larvae of the murine parasite *Heligmosomoides polygyrus* (*Hp*) (1). Trapping was mediated by antibody-activated macrophages, and the upregulation of Arginase-1 (Arg1) activity within these cells and resulted in impaired development of adult worms (Esser-von Bieren, J. PLoS Pathog. 2013;9(11):e1003771.). Further investigations identified CD11b as the major complement receptor mediating macrophage adherence to the larval surface, whilst activation of the macrophage to express Arg1 required the activating IgG receptor FcgRI (CD64), which was largely bound by IgG2a. We additionally observed that antibodies acted to promote intestinal wound healing by enhancing the production of the CXCR2 ligands, CXCL2 and CXCL3, and promoting myofibroblast migration. CXCL2 and CXCL3 production by macrophages was activated by antibodies together with helminth larvae, whilst myofibroblasts could produce these chemokines directly in response to helminth larvae through a process dependent on Dectin-2 and FcR γ-chain. Collectively our findings suggest that host immunity can act to both reduce adult worm burdens and to promote timely tissue repair through the inhibition of larval motility and the induction of host chemokines critical to wound contraction.

Blockade of early innate allergic responses by *Heligmosomoides polygyrus* excretory/secretory products

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Heligmosomoides polygyrus is a natural nematode parasite of mice, known to suppress pathology in a number of mouse models of asthma, colitis and autoimmunity. *H. polygyrus* excretory/secretory products (HES) can replicate many suppressive effects of infection. Administration of allergenic extracts of the clinically relevant fungus *Alternaria alternata* induces a rapid T cell-independent, but IL-33- and type 2 Innate Lymphoid Cell (ILC2)-dependent eosinophilia, and can also act as a TH2 adjuvant by priming a classical allergic airway response to OVA protein. When HES was coadministered with *Alternaria*, suppression of eosinophilia, ILC2 and type 2 cytokine responses was seen; both at the early (<48h) T cell independent phase, and later during T-cell dependent airway inflammation in response to OVA challenge. Importantly, HES suppressed release of IL-33 to the bronchoalveolar spaces at very early timepoints (1h) post-*Alternaria* treatment, which was central to suppression in this model. *Alternaria*-induced IL-33 release, and suppression by HES, could be modelled in vitro using cultures of naive lung cells. IL-33 release from intracellular stores is induced by *Alternaria*- or freeze-thaw-induced necrosis, and while IL-33 release is suppressed in both treatments, necrosis is unaffected. HES can not directly degrade IL-33, and therefore suppression operates by activation of host cell-intrinsic pathways. We are presently investigating the mechanism of IL-33 suppression by HES, focussing on caspase/inflammasome activation, purinergic signalling and induction/suppression of other IL-1 family members. Furthermore, using size and charge fractionation of HES we have isolated a pool of molecules with suppressive activity. We are currently attempting to identify and express the active molecule(s) within HES, with a view to developing these molecules as therapeutics for human disease.

A small molecule analogue of the *Acanthocheilonema viteae* immunomodulator ES-62 inhibits inflammation in concert with activation of the Nrf2-dependent anti-oxidant response

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ES-62 is the major secreted product of the rodent filarial nematode *Acanthocheilonema viteae*. The molecule possesses many anti-inflammatory properties, which afford protection in several mouse models of allergic and autoimmune disease. As the protective effects are due to covalently attached phosphorylcholine (PC) moieties and ES-62 itself is not suitable as a therapy for such conditions, we have constructed a library of more drug-like compounds – PC-based ES-62 Small Molecule Analogues (SMAs), and investigated them for similar anti-inflammatory activity. SMA 12b has been found to mirror ES-62 in protecting mice against collagen-induced arthritis (CIA), ovalbumin-induced airway hypersensitivity, oxazolone-induced contact sensitivity and the kidney damage that develops spontaneously in MRL/lpr mice. SMA 12b's protective effects against CIA are shared with another SMA, 11a. However, whereas ES-62 and SMA 11a activity in this model correlates with targeting of pathological Th17/Th1 responses, this is not the case with SMA 12b. For this reason 12b, was subjected to further analysis. Focusing on macrophages, microarray analysis revealed that SMA12b down-regulated a number of genes associated with pro-inflammatory cytokine responses (e.g., IL-1β) and cell migration and recruitment (e.g., CXCL10) but upregulated genes that play an inhibitory role in inflammation (e.g., CD200R1). Ingenuity Pathway Analysis (IPA) of Transcription Factors (TFs) predicted inhibition of NF-κB signaling and this was confirmed in functional assays. IPA analysis also suggested activation of Nrf2, a key regulator of the anti-oxidant response and in support of this, a large group of genes involved in protection against oxidative stress (e.g., in synthesis of glutathione) and that are controlled by this TF, were upregulated. Nrf2 and NF-κB have been reported to counter-regulate gene induction and thus, we are currently further dissecting how Nrf2 impacts on SMA12b's immunomodulatory activity.

Commensal bacteria protect against food allergen sensitization.

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The rapidly increasing prevalence of autoimmune and allergic disease shows no sign of abating. To understand what factors might be driving this increase, we have turned to the trillions of bacteria that populate the gastrointestinal tract, known collectively as the microbiota. Twenty-first century environmental interventions, including widespread antibiotic use, consumption of a high fat/low fiber Western diet, elimination of previously common enteropathogens (including helminthic parasites), reduced exposure to infectious disease, Caesarean birth and formula feeding have perturbed mutually beneficial interactions established with the commensal microbiota over millions of years of co-evolution. Recent work has linked commensal dysbiosis to an expanding range of complex immune-mediated diseases, including diabetes, inflammatory bowel disease, arthritis and allergic asthma. Whether changes in the composition of the intestinal microbiota also play a role in regulating non-responsiveness to the other major luminal constituent - food – has been poorly understood. Murine models developed in our laboratory demonstrate that sensitization to a food allergen is enhanced in mice that have been treated by neonatal antibiotic administration (Abx) or are devoid of commensal microbes (germ free). By selectively colonizing germ free mice, we have shown that the allergy-protective capacity is contained within the Clostridia, a class of anaerobic spore-forming Firmicutes that reside in close proximity to the colonic epithelium. Moreover, reintroduction of a Clostridia-containing microbiota to Abx-treated mice blocks sensitization to a food allergen. Microarray analysis of intestinal epithelial cells isolated from gnotobiotic mice helped to identify a novel innate mechanism by which Clostridia protect against sensitization to dietary antigens. Defects in intestinal permeability have been implicated in aberrant allergic responses to food, but the mechanisms governing uptake of dietary antigen have not been clear. We find that Clostridia colonization induces the production of the barrier protective cytokine IL-22 by both innate lymphoid cells and T cells in the colonic lamina propria. IL-22 acts to reduce uptake of orally administered dietary antigen into the systemic circulation, thereby protecting against sensitization. Our data therefore suggest that maintenance of tolerance to dietary antigen requires the induction of both antigen specific regulatory T cells and a bacteria-induced barrier protective response. These findings will inform the development of novel approaches to prevent or treat sensitization to food based on interventions that modulate the composition of the gut microbiota.

NOTES

POSTER SESSION 1

ABSTRACTS

1. Pattern of incidence of *Angiostrongylus vasorum* in urban, suburban, and rural slugs revealed by real time PCR

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Angiostrongylus vasorum (Nematoda; Metastrongyloidea) is a parasite of the heart and pulmonary system of domestic dogs (*Canis lupus familiaris*) and red foxes (*Vulpes vulpes*). Infection is acquired by intentional and accidental ingestion of gastropods containing *A. vasorum* larvae (Moeremans et al., 2011). Information on which gastropod species can act as intermediate hosts, and the distribution of infected individuals is lacking. Such information is needed to evaluate the role played by slug populations as intermediate hosts of infection and the probability of transmission of this and many other endoparasites to domestic dogs and other canid species. To address this knowledge gap, 877 slugs from suburban (Underhill park, Cwmdokin Park and West Cross), urban (Woodlands Tce, Sainsbury's CP and Landore) and rural (Penrice, Gelli hir and Pilton Green) were collected between October and November 2012. Slugs were identified morphologically and by sequencing of 16S mitochondrial ribosomal DNA (rDNA) (Rowson et al., in press). 180 slugs were extracted for amplification of the second internal transcribed spacer (ITS-2) of r(DNA) using a Real Time PCR assay (Jefferies et al., 2009). We identified the occurrence of *A. vasorum* in terrestrial slugs from different areas (urban suburban, and rural) in Swansea, United Kingdom. We predicted that differences in the prevalence of *A. vasorum* in infected slug species should differ along an urban-rural gradient, reflecting variation in patterns of intermediate host community use by the parasite. The preliminary results suggest that there was a statistically higher prevalence of *A. vasorum* in suburban areas compared to rural and urban areas ($p\ 0.026$). Infection was more common in suburban than in rural slugs ($p = 0.003$). Among 43 slugs that tested positive for *A. vasorum*, the common species were Arion spp. of which 65% were *A. rufus* (n=28), 19% were *Limax maculatus* (n=8) and 16% *A. flagellus* (n=7). Future research will focus on determining how slug species utilisation by *A. vasorum* varies between sites. This will facilitate studies into the adaptive value of a broad intermediate host range in varying conditions of host availability and environmental variation.

2. An unusual case of human cerebral sparganosis, and whole genome sequencing reaches further into the phylum Cestoda

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A patient presented in the East of England with a range of neurological symptoms and a migrating lesion was observed in their brain. A formalin-fixed paraffin-embedded biopsy sample from the patient was morphologically identified as a larval tapeworm of the order Pseudophyllidea. We used PCR to refine the diagnosis to a species level, identifying the worm as *Spirometra erinaceieuropaei*. Previously molecular information regarding *Spirometra erinaceieuropaei* has been limited, as for all species belonging to this order of tapeworm. Short paired-end sequencing libraries were generated from 12.5 ng gDNA. The genome was assembled using Velvet, and scaffolded using SSPACE. Output from gene prediction software, cross-species comparative algorithms and BLAST alignments with known cDNAs were integrated by MAKER to build evidence-informed gene models. We used the genome sequence to re-visit the outcomes of drugs used in the treatment of the patient and predict how this parasite could have responded to alternatives. Using a targeted search of the genome we also considered whether putative drug targets for more common tapeworm infections are applicable to these infections. We ran genome-wide comparisons of *Spirometra erinaceieuropaei* with other cestodes, including *Echinococcus multilocularis* and *Taenia solium*. We identified expanded gene families, many of which are likely involved in host-parasite interaction. In complement to this analysis we also BLAST searched the specialist MEROPS database to identify and class sets of proteases and protease inhibitors. We observed a large number of leucyl aminopeptidases, serine endopeptidases, and Kuntiz-type protease inhibitors. These observations possibly reflect the wide range of hosts in which this species is found. In summary we have built a de novo genome assembly from a clinical specimen, and ran the first comparative analysis for a member of this tapeworm order. We hope that the genome will be useful to determine the most appropriate treatment strategies for future cases.

3. Secreted exosomes from *heligmosomoides polygyrus* modulate cellular responses of the murine host

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Exosomes are nanovesicles providing a mode of communication amongst eukaryotic cells through transfer of proteins and RNAs. Recent indications suggest that pathogen derived exosomes, such as those discovered in Leishmania donovani, are able to modulate the onset of a host inflammatory immune response, thus promoting parasite survival. Here, we examine secreted vesicles from the murine gastrointestinal nematode *Heligmosomoides polygyrus*, and their potential role in host-helminth interactions. Transmission electron microscopy reveals vesicle-like structures of 50- 100 nM in the secretory product recovered by ultracentrifugation, and potential evidence of multi-vesicular bodies in the worm intestine. An intestinal origin is supported by proteomic data which show enrichment of worm intestinal proteins in the exosomes. Microarray analysis of exosome-treated small epithelial cells reveals significantly reduced expression of a number of genes, including those involved in the regulation of signaling and the immune response, such as Dual Specificity Phosphatase 1 (DUSP1). Furthermore, we found that exosomes significantly reduce expression of classical activation markers, as well as inflammatory cytokine production in the macrophage cell line RAW 264.7. Finally, in vivo studies using a model of lung inflammation, indicate that exosomes modulate some cellular components of this response, shown by a reduction in type-2 innate lymphoid cells from lung tissue and bronchoalveolar lavage (BAL) fluid eosinophils. This work suggests that exosomes secreted by parasitic nematodes could mediate cross-phylum communication and may help to suppress the host inflammatory response.

4. **Oral therapy for colitis using L4 nematode antigen fractions.**

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The important aspect of helminth therapy (HT) and helminth-derived product therapy (HDPT) research is the characterization of the key molecules responsible for immunomodulation. These could be used as drugs to control inflammation and autoimmune diseases. Our recent study indicated that in live nematode therapy of intestinal autoimmune diseases, such as colitis, the changes in the small intestinal milieu promote intestinal nematode larval adaptation and improve worm growth. The plasticity of the nematodes proteome is a consequence of evolutionary adaptation. Adaptation of the parasite is beneficial for the host because it inhibits inflammatory disease but increases parasite survival. The complex signaling pathways that nematodes activate to regulate the host immune system can be investigated with using fractions of the antigen. Our preliminary study on L4 *Heligmosomoides polygyrus* established that parasite somatic antigens and some of the L4 fractions inhibit colitis symptoms in absence of live infection. In this study, mice with DSS- induced colitis received L4 somatic extract and fractions orally. The small dose of L4 somatic antigen administered orally, significantly suppresses infiltration of a heterogeneous population of inflammatory cells into the colon from 3 days post treatment. A lower amount of the antigen in the fraction or a more complex protein mixture in it may explain the effect. Therefore the fractions might be extremely useful in the development of intervention strategies for inflammatory reactions and precise detection of the mechanism induced by parasite during autoimmune diseases.

5. Genome on the Verge of Extinction

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Dracunculus medinensis (Guinea worm) is a nematode that causes the disease dracunculiasis. This was once a major parasitic infection, and widespread across tropical Africa and Asia. However, infection is entirely preventable, through provision of clean water and behaviour change, which has led to a drop in cases from around 3.5 million cases per annum in the 1980s to fewer than 150 cases in 2013. The disease is now eradicated in all but four countries. The aim is that dracunculiasis will be the second human disease to be eradicated, the first parasitic disease and the first disease to be eradicated without the use of a vaccine or drug. However, dracunculiasis was believed to be extinct for a decade in Chad until new cases emerged in 2010. The pattern of the current outbreak in Chad does not cluster by village or water source as expected and a large number of dogs were also infected. It appears that dogs are now the main host in Chad, with human cases being sporadic and incidental, transmitted by a common paratenic host. Building on de novo genome assemblies for *D. medinensis* and the related *D. insignis*, we describe genome-wide patterns of diversity in *D. medinensis* samples from Chad and from other parts of the range. We are aiming to understand both the current diversity of the population in endemic countries and the diversity of populations before the eradication campaign, and to more formally understand the epidemiology of canine and human cases in Chad.

6. A global profiling of *Schistosoma mansoni* secreted proteases into mammalian host.

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Schistosoma mansoni genome contains more than 300 putative genes coding proteases from 61 protease families. We focused on proteases excreted/secreted into the host environment by in vitro cultivated life stages: newly transformed schistosomula (NTS), adults and eggs. Due to high complexity of samples it is difficult to deconvolute particular activities. Therefore we employed new method for multiplex substrate-profiling by mass spectrometry (MSP-MS) followed by liquid chromatography-tandem mass spectrometry. This method utilizes mixtures of 124 unique synthetic peptides in the length of 14 residues with 1 612 potential cleavage sites. Advantages of this approach are in fast direct cleavage assays with specificity and kinetic profiles for any exo- and endo- peptidase detected by mass spectrometry while multiple proteolytic profiles can be resolved by class-specific protease inhibitors. Taken together this approach represents powerful tool for robust analysis of proteolytic activities (degradome) secreted by different *schistosoma* life stages and has potential to determine particular proteases by their cleavage signature. Most of cleavages detected were unique for each S. mansoni life stage. Judged by cleavage patterns, only a few secreted proteases are shared by NTS, adults and eggs indicating different survival strategies. We detected as well high levels of unknown activities originating from metallo-proteases and serine proteases found particularly in the eggs. We were able to detect surprisingly high number of proteolytic activities secreted by S. mansoni eggs (represented by more than 300 unique cleavages). Some cleavage patterns are unique and specific for particular stages thus suitable for specific probes development with diagnostic potential. The number of cleavages together with the level of proteolytic activity found in egg samples is surprising and suggesting importance of secreted proteases for egg pathogenesis.

7. Terminal repeat retrotransposons in miniature (TRIMs) are highly expressed in *Echinococcus* spp.

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We previously described that a set of long non-coding RNAs, with partial direct repeats in the 5' and 3' regions, are the most highly expressed transcripts in *E. granulosus* larval stages (metacestodes and protoscoleces) and that presumed orthologs of these abundant transcripts are also expressed by *E. multilocularis* metacetodes (Parkinson et al (2012) PLoS NTD 6(11), e53401). The availability of *Echinococcus* spp. genomes has allowed us to find out that the loci corresponding to these transcripts have all the hallmarks of TRIMs, a class of short (<1000 bp) non-autonomous LTR retrotransposons, which are ubiquitous in plants but have only recently been described in metazoans. Indeed, "Eg/EmTRIMs" include: complete terminal LTRs with 5'TG and 3'CA (indispensable for integration); a polypurine tract adjacent to the 3'LTR (for second strand switching during reverse transcription); and direct repeats of 4 or 5 bp external to the LTRs (that are generated by the integration process, target site duplication). In addition, mapping of the ESTs indicated transcriptional promoter and terminator/polyadenylation activities from the 5' and 3' LTRs, respectively. Solo-LTRs (single LTRs), probably arising by unequal homologous recombination of a TRIM, were also identified and found to initiate transcription of either non-coding RNAs or downstream coding sequences. Interestingly, the presence of these transposable elements appears to be a trait of the Taeniidae because TRIMs homologous to the ones in Echinococcus were also found to be transcribed in *Taenia solium* but not in *Hymenolepis microstoma* or *Schistosoma* spp. We propose that the expression of Eg/EmTRIMs is a major feature of the biology of Echinococcus stem cells. In particular, it could be related to the mechanism whereby the taeniids maintain genome integrity without a canonical piRNA pathway.

8. Population genetics, transmission zones and ivermectin response of *Onchocerca volvulus* in West Africa.

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The population structure of, and gene flow within and between, helminth populations are largely unexplored aspects of parasite biology but are critical to a full understanding of parasite population dynamics, drug responses and epidemiology. Malaria control campaigns have made extensive use of population genetic data, but few if any examples of inclusion of population genetic parameters in helminth control campaigns exist. This is particularly true of filarial parasites, where the gene flow within and between populations are a potentially powerful tool with which parasite transmission zones could be defined more accurately. We have explored the population structure of *Onchocerca volvulus* in West Africa, using a combination of whole genome sequencing, whole genome RADseq, random nuclear SNPs, mitochondrial re-sequencing and partial re-sequencing of onchocerca *Wolbachia* (wOv). The O. volvulus mitochondrial and nuclear genomes are very variable, and *O. volvulus* in West Africa is highly subdivided genetically over several spatial scales that do not align well with simple notions of savannah and forest "strains" or ivermectin treatment. The wOv genome, in contrast, is >10-fold less variable than either the nuclear or mitochondrial genomes to the extent that we have so far been unable to assay sufficient genetic wOv variation to determine the genetic structure the *Wolbachia* population. Surprisingly, we have shown that single worms may harbor >1 *Wolbachia* genome sequence, and that while there is little wOv sequence variation there is extensive wOv genome copy number variation across West Africa. We are currently working to select genetic markers suitable for a low-tech LAMP assay able to define parasite transmission zones using skin snip or vector surveillance clinical samples in the field. In addition, a preliminary analysis of ivermectin response population genetics suggests that genetic drift rather than selection is the primary determinant of differences in *O. volvulus* ivermectin responses.

9. ***Schistosoma mansoni*** **soluble egg antigens reduce the severity of experimental colitis in mice by affecting colonic T cell responses.**

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Helminth-derived molecules are being identified at an increasing tempo as a potential new therapeutic approach for immune-mediated diseases. We investigated the therapeutic potential and the underlying immunological mechanisms of *Schistosoma mansoni* soluble egg antigens (*Sm*SEA) in a mouse model of chronic colitis. Colitis was induced in immunocompromised SCID mice by the adoptive transfer of $CD4^+CD25^-CD62L^+$ T cells. Two weeks post-transfer, *Sm*SEA treatments were started (study 1: 1 x 20 µg *Sm*SEA/week (i.p.); study 2: 2 x 20 µg *Sm*SEA/week (i.p.)). From the start of the treatment (week 2), clinical outcome (bodyweight, stool consistency, mobility, pilo-erection) and colonic inflammation were assessed at different time points by clinical disease score and colonoscopy, respectively. At the end of the studies (study 1: week 6; study 2: week 4), colons were macroscopically examined and lamina propria mononuclear cells (LPMC) were isolated from the colons and prepared for flow cytometric (FCM) T cell characterization. Administration of *Sm*SEA improvemed all the inflammatory parameters studied (body weight, clinical disease score, colonoscopic score and macroscopic inflammation score). In the 6-week study this beneficial effect on inflammation however diminished in time and the FCM T cell characterization of LPMC revealed no immunological effects of the *Sm*SEA treatment on the T cell response. However, in study 2 the mice were sacrificed at the moment a beneficial effect of SmSEA was shown on the inflammatory parameters (i.e., 4 weeks) and then the administration of *Sm*SEA significantly downregulated the number of IL-17A producing T cells (Th17 cells) and significantly upregulated the number of IL-4 producing T cells (Th2 cells) in the colon LPMC. Administration of *Sm*SEA reduced the severity of colitis in the adoptive transfer mouse model characterized by an increased Th2 response and a suppressed Th17 response in the colon at the time of maximal anti-inflammatory effect.

10. A broad survey of parasitic helminth genomes.

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The Helminth Genomes Initiative is a collaboration between the Sanger Institute, The Genome Institute at Washington University and Gene Pool at Edinburgh University. The aim is to survey the genomes from many of the most important parasitic worms and uncover the genomic basis for major differences in their biology. The data will be mined for diagnostic markers, putative targets for intervention or new evolutionary insights. To date, more than 50 helminth genomes have been sequenced, some of which have large, repetitive genomes (above 1 Gbase), presenting a major challenge for sequence assembly. This problem can be compounded by polymorphism in the sample sequenced, and/or contamination of the sample by DNA from host or commensal species. To overcome these challenges, and be able to process the huge quantity of data, we have established several bioinformatics pipelines and workflows for genome assembly, gene prediction and functional annotation of the millions of genes the genomes contain. The draft assemblies have undergone extensive quality control, including the use of a custom-built pipeline based on phylogenetic analyses to identify and discard contaminant sequences, and use of tools such as CEGMA and REAPR to analyse assembly completeness and likely error rates, respectively. The genomes are available via a newly launched website, WormBase-ParaSite (parasite.wormbase.org), which will enable phylogenic querying of orthologs and paralogs and the distribution of specific genes and gene families to be viewed across multiple species and sub-clades.

11. The molecular basis of parasitism in the nematode *Strongyloides ratti*

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The *Strongyloides* lifecycle includes a parasitic female-only stage, which inhabits the small intestine of its host, and a facultative, dioecious free-living adult generation. These adult life-cycle stages are genetically identical, so that comparing parasitic and free-living stages offers an almost unique opportunity to discover the molecular adaptations required to be a successful parasitic nematode. We have used quantitative mass spectrometry and RNAseq analyses to compare the proteome and transcriptome of parasitic and free-living females of S. ratti, a parasite of rats. We find that 15% of genes are differentially expressed between these two life stages. Many of the genes with upregulated expression in the parasitic stage are physically clustered in the genome. Clusters comprise 2-18 adjacent genes, mostly from the same gene families and therefore with likely similar functions. Approximately 20% of the genes in these clusters code for astacins of the zinc metalloproteases family. The largest clusters are mainly CAP domain-containing genes. These gene families are therefore likely to be key to parasitism in *Strongyloides* and possibly other parasitic nematodes. We further compared RNAseq data for parasitic and free-living females of two closely related species – S. stercoralis, a parasite of humans, and *S. venezuelensis*, a non *S. ratti*–sister species parasite of rats. Together with comparisons of other parasitic nematode species, we have identified genes and proteins important for parasitism that are (i) unique to *S. ratti*, *S. stercoralis* and *S. venezuelensis* (ii) unique to the *Strongyloides* genus, and (iii) common across many parasitic nematodes.

12. Parasitic nematode-induced $CD4^+Foxp3^+$T cell is able to ameliorate allergic airway inflammation

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Recently, many studies have been reported about down-regulation effects on immunologic diseases by parasite infection. In previous study, we found that allergic airway inflammation was ameliorated and $CD4^+CD25^+Foxp3^+$T (T_{reg}) cells were recruited by *Trichinella spiralis* infection. In order to know the function of T_{reg} cell which was induced by parasite, we compared the effects of T_{reg} cells which were obtained from *T. spiralis* infected mice and uninfected mice on experimental allergic airway inflammation. After 4 weeks *T. spiralis* infection, we isolated Foxp3-GFP fusion protein expressed cell (Foxp3 e GFP cell) from Foxp3-GFP tagging transgenic mice using cell sorter. We injected only one time Foxp3 e GFP cell, isolated from *T. spiralis* infected [Inf(+) $Foxp3^+$] mice or uninfected [Inf(-) $Foxp3^+$] mice, to mice via tail vein before [preventive effect]/or after [therapeutical effect] the induction of allergic airway inflammation using OVA-Alum sensitization and challenge. Severe allergic airway inflammation was induced after OVA-alum sensitization and challenge, they showed immune cell recruitment around airway, epithelial cell proliferation, goblet cell hyperplasia, mucin production, MUC2 and MUC5 gene expression in lung. However, these phenomenons were significantly reduced in the Inf(+) $Foxp3^+$ mice. Concentration of Th2 related cytokines IL-4, IL-5, and IL-13 in bronchial alveolar lavage fluid and level of OVA specific IgE and IgG1 in serum of Inf(+) $Foxp3^+$ mice were significantly reduced than those of control mice. Although some ameliorated phenomenon of allergic airway inflammation was observed Inf(-)$Foxp3^+$ mice, most of them was less distinct than those of Inf(+)$Foxp3^+$ mice. We found that $CD4^+CD25^+Foxp3^+$T cell population significantly increased in lung draining lymph node of Inf(+) $Foxp3^+$ injected mice. These data identify Foxp3+ T cells induced with *T. spiralis* as capable of regulating air way allergic responses.

13. The FAR proteins of nematodes – family diversity, structural diversity, binding site diversity

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The fatty acid and retinol-binding proteins (FARs) of nematodes differ significantly from proteins with similar ligand binding properties found in other groups of organisms. Each species possesses several isoforms (eight in *Caenorhabditis elegans*), and genome sequencing ventures continue to reveal unanticipated diversity within the FAR family in parasitic species. The various FARs exhibit similarities in their lipid-binding characteristics, but both subtle and radical differences are increasingly discernible by fluorescence-based spectrophotometric methods. Here we illustrate such differences for FARs from *Necator americanus* and *Onchocerca volvulus*. We present the nuclear magnetic resonance (NMR) and X-ray crystallographic structures of a member of a new family of FARs from *N. americanus,* Na-FAR-1, illustrating the differences in molecular structure between *apo* and *holo* forms. We describe Na-FAR-1's distribution within the parasite, and show how its structure differs from that of the only other FAR structure known (from *C. elegans*). We further describe how its lipid binding site is more complex than previously suspected of FARs, and show how NMR can be used to observe progressive changes in structure with increasing ligand loading.

14. ROZ LAING [1]; AXEL MARTINELLI [2]; KIRSTY MAITLAND [1]; LENKA LECOVA [3]; CHARLOTTE BURGESS [4]; ANDREW REZANSOFF [5]; LIBBY REDMAN [5]; PHIL SKUCE [4]; JAMES COTTON [2]; JOHN GILLEARD [5]; ANDREW TAIT [1]; EILEEN DEVANEY [1];

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Anthelmintic resistance is a major threat to the UK sheep industry and is an emerging concern for parasite control in other species. The mechanisms underlying resistance in parasitic nematodes are not fully understood and current recommendations for sustainable parasite control are based largely on theory. We are investigating genetic changes associated with ivermectin resistance (IVM-R) in the sheep parasite *Haemonchus contortus*, using both candidate gene and whole genome approaches, to improve our understanding of how resistance arises and spreads. SNPs in numerous candidate genes have been associated with IVM-R, but the relevance of these mutations to resistance in the field remains unclear. We examined UK field populations of *H. contortus*, differing in ivermectin treatment history, for evidence of selection at candidate gene loci (glc-5, avr-14 and lgc-37) using capillary sequencing and RFLP, combined with microsatellite marker analysis. High levels of polymorphism were identified at all loci and a degree of population sub-structuring was apparent. These factors can confound candidate gene analysis and may underlie the plethora of genes associated with IVM-R to date. Our second, global approach is to use RAD-seq to genotype individual worms from UK farm populations with differing anthelmintic regimes to identify markers associated with ivermectin selection. We are also genotyping H. contortus larvae from IVM-susceptible and IVM-resistant laboratory isolates and two IVM-resistant backcrosses. This approach does not rely on prior assumptions as to the mechanism of IVM-R and provides genome-wide coverage of markers, which will be used to identify regions of the genome under selection.

15. The effect of early-life helminth exposure on children's responses to childhood vaccines

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Helminth infections are common in sub-Saharan Africa and are able to influence immune responses to unrelated infections and vaccines, although their precise effect on vaccine responses remains unclear. We are investigating possible associations between early-life helminth exposure on children's immune responses to vaccination. Serum samples from child study participants from Worcester, Cape Town were analysed via antibody ELISA for the presence of anti-*A. lumbricoides*, anti-*T. trichiura* and anti-measles vaccine antibodies. Anti-helminth IgG4 was detected in serum against *A. lumbricoides* and *T. trichiura* antigens, indicating helminth exposure. A significant positive association was observed between both helminth IgG4 antibody titres and measles vaccine IgG responses (*A. lumbricoides* IgG4 vs measles IgG **p = 0.002; *T. trichiura* IgG4 vs measles IgG ***p < 0.0001). These preliminary findings suggest that early-life helminth exposure increase measles vaccine IgG responses. The study will be broadened through the investigation of immune responses to tetanus toxoid and *H. influenzae* (Hib) vaccines in relation to helminth immune responses, as well as through comparison with serum samples from a child cohort located in Nyanza Province, Kenya (high helminth infection prevalence) tested for reactivity to the above-mentioned antigens.

16. Localization of nicotinic acetylcholine receptors (nAChRs) transcripts in adult *Brugia malayi* indicates their potential involvement in reproduction of filarial nematodes

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We have recently reported that high level expression of avr-14 (a glutamate-gated chloride channel gene and a target of ivermectin) in reproductive tissues of *Brugia malayi* may explain the sterilizing effect of ivermectin on filarial worms. We now report results of parallel localization studies for nicotinic acetylcholine receptors (nAChRs) which are the targets of several anthelmintic drugs such as levamisole and pyrantel. The recent introduction of novel nicotinic anthelmintics underlines the importance of nAChR as anthelmintic targets. The levamisole nAChRs type in *C. elegans* is composed of five subunits (Cel-unc-29, Cel-unc-38, Cel-unc-63, Cel-lev-1 and Cel-lev-8). Nine nAChRsubunit genes (nAChRs) are present in the B. malayi genome including orthologues of Cel-unc-29, Cel-unc-38, and Cel-unc-63. We have studied expression of these genes by qRT-PCR in B. malayi adult females, males, and microfilariae (Mf). Six of eight nAChRs genes studied exhibited differential expression across these stages. Four were more highly expressed in males, one in females, and one in Mf. We performed in situ hybridization with cRNA probes to localize expression for five *B. malayi* nAChRs genes in adult worms. Most of them had similar expression patterns with strong signals in developing embryos and uterus wall in females, in spermatogonia and vas deferens in males, and in and lateral chords in both males and females. For example, Bm1_35890 (an orthologue of cel-unc-29 which encodes a subunit of a levamisole-sensitive nAChR), had high expression signals by in situ in both male and female worms. In females, strong expression signals were detected in the ovary, developing embryos, and lateral chords, with moderate expression in the uterus wall adjacent to stretched Mf. Expression signals were weak in stretched Mf in the uterus. Expression signals in males were strong in spermatogonia and in the wall of vas deferens.

17. Isolation and characterization of vitelline cells from *Schistosoma mansoni*

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The parasitic trematode *Schistosoma mansoni* is different from other trematode species because it lives dioeciously. The female worms need to be constantly paired with male partners in order to reach maturation and to obtain reproductive capabilities. An important indicator of maturation is the appearance of a massive, highly proliferative organ: the vitellarium. This is composed of many lobes containing different cells. During maturation vitelline cells undergo four developmental stages, and cells at each stage have different intracellular compositions (Erasmus 1975). It is very intriguing to understand how the tissue and cells become mature upon pairing. Based on a novel approach for isolating testes and ovaries from adult *Schistosoma mansoni* (Hahnel et al, 2013), we were also able to isolate pure vitelline cells from mature females. Cytological analyses using specific stains demonstrated among others the viability of these cells. RNA and protein of good quality were extracted and used for further analyses. RT-PCR was performed to detect the transcription of genes related to 1) stem cell characteristics (eg *vlg3, nanos2*), 2) eggshell formation (eg *tyrosinase*, *p14*), and 3) calcium signaling (eg *ORAI-1*, *hippocalcin*). Some of the transcripts were also validated by *in situ* hybridization. FACS analysis was performed to separate vitelline cells at different stages of development by size, granularity and auto-fluorescence. First results indicated that it was possible to obtain defined subpopulations of vitelline cells. Finally, it has been shown that triglycerides stored in the vitellarium are an important energy source for egg production (Huang et al, 2012). Having access to isolated organs and even cells, in which lipids can be stained, will allow more detailed studies in the future to analyze the importance of lipids for female development and egg biosynthesis.

18. Nutritional regulation of resistance to nematodes in mammals

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Breakdown of immunity to gastrointestinal nematode infection during parturition is believed to have nutritional basis. Previous studies have shown up-regulation of pro-inflammatory pathways and down-regulation of genes related to Type 2 immunity in animals offered a Low Protein (L) diet compared to those offered a High Protein (H) diet. It is hypothesised that dietary protein has ability to modulate immune response, especially shifting the Type 1/Type 2 dichotomy against nematode infection. To investigate this hypothesis, a well-established *Nippostrongylus brasiliensis* re-infected lactating rat model was used. During the second half of gestation, rats were offered diets with either 6 (L-) or 21 (H-) % protein, and during lactation either 10 (-L) or 30 (-H) % protein, creating a 2 x 2 factorial experiment. Samples were collected at parturition, day 3 and day 9 post infection. Gestation diet affected foetal growth significantly, where H- dams showed heavier pup weight (p<0.05) at parturition. Pup growth was larger in L-H and H-H group compared to other two. Spleen weight, which is indicative of immunological response, was significantly greater in H- fed animals at parturition (p<0.05). Spleen weight also showed similar tendency to pup growth where L-H and H-H group were heavier than other two groups. The number of worm eggs in the colon content did not differ between groups, however, worm burden tended to be lower in L-H and H-H groups (p=0.086). Analysis of serum samples to determine IgE and IgG levels, gut histology for effector cells and expression analysis of genes associated with regulation of Type 1/Type 2 is currently ongoing. This study is expected to shed light into the molecular interaction between nutrient supplementation and immunity to nematodes which will lead to the development of novel diet-based therapeutics strategies for parasite control.

POSTER SESSION 2

ABSTRACTS

19. Mitogen-activated protein kinases are involved in the reproductive development and survival of *Schistosoma mansoni*

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Although the roles of Mitogen-activated protein kinases (MAPKs) are unclear in schistosomes, protein kinases are increasingly approved as targets for drug development with a rising number of eukaryotic Protein Kinase (ePK) inhibitors under development. In other organisms, MAPKs connect cell-surface receptors to regulatory targets within cells and influence a number of tissue-specific biological activities such as cell survival, differentiation and proliferation. Here, we employed RNA interference (RNAi) to elucidate the functional roles of six *S. mansoni* genes involved in MAPK signaling pathway. First, the ePKs were identified in the predicted proteomes of *S. mansoni*, *S. japonicum* and *S. haematobium* by HMM searches, the genes were classified, annotated and selected regarding their putative essential function in the parasite. Key proteins, such as SmRas, SmERK1, SmERK2, SmJNK, SmCaMK2 and Smp38 were chosen for experimental validation. Gene silencing by RNAi and pharmacological inhibition were used to elucidate the functional role of MAPK signaling pathway proteins in *S. mansoni*. Mice were injected with post-infective larvae (schistosomula) subsequent to RNAi and the development of adult worms observed. The data demonstrate that SmJNK contributes to the transformation and survival of the parasites whereas SmERK and Smp38 seems to be involved in egg production as infected mice had significantly lower egg burdens and female worms that had underdeveloped ovaries. Additionally, as the Smp38 treated worms exhibited tegumental damage. We also observed that Smp38 is involved in the activation of detoxification enzymes. Furthermore, it was shown that the c-fos transcription factor was overexpressed in parasites consequent on RNAi of SmERK1, SmJNK and SmCaMK2 confirming the role of c-fos in gene regulation in this parasite's MAPK signaling cascade. Our results help characterize the importance of MAPK pathway in the normal development and survival of the schistosome parasite and suggest some of these enzymes as useful drug targets against schistosomiasis.

20. Excretory/ secretory products from *Heligmosomoides polygyrus*: the VAL proteins

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The intestinal parasite *Heligmosomoides polygyrus* maintains itself in mice for many months, imposing a broad immunosuppressive effect. Interference with host immunity is believed to be mediated by the release of a potent cocktail of molecules termed excretory/ secretory products (HES). These have been studied at both transcriptomic and proteomic levels identifying a spectrum of molecules with potential immunomodulatory function. The most predominant are a set of >25 VAL proteins (**V**enom allergen, ***A****ncylostoma* secreted protein **L**ike), belonging to a larger family of CAP (Cysteine-rich/Antigen 5/Pathogenesis-related) proteins also termed SCP (sperm coat protein) classified in Pfam00188. These proteins are prominent across the Nematode phylum from plant parasites to the free-living *C.elegans,* and are also represented as extracellular proteins in insect venom, mammalian sperm coats and even in the leaves of infected tomato plants. First described from the dog hookworm *Ancylostoma caninum*, the VAL proteins are often expressed at critical points in the parasite's lifecycle such as during the transition to parasitism. Given the prevalence of these molecules, their structural variety and the extent to which they are expressed we hypothesise that they are intimately involved in manipulation of host/parasite interactions. To examine the biological function of VAL molecules, we have used insect cell systems to express soluble recombinant proteins of Hp-VAL-1 and -4, representing respectively a double- and single-domain form as found throughout the CAP superfamily. Labelled VAL proteins have been used in confocal and whole-mount immunofluorescence microscopy to demonstrate binding of these and other HES proteins to distinct gut epithelial cell types, in particular Paneth and Goblet cells, indicating that these parasite products target specific ligands expressed within the epithelial layer of their murine host.

21. Role of T cell-derived IL-4 and IL-13 during infections with *Nippostrongylus brasiliensis*.

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Parasitic worm infections provoke type 2 immune responses characterized by elevated immunoglobulin E (IgE)-levels and increased numbers of eosinophils, mast cells, basophils and T helper type 2 cells (Th2). The cytokines IL-4 and IL-13 play a crucial role in type 2 immunity and they can be produced by T cells but also by cells of the innate immune system. To further analyze the in vivo functions of IL-4 and IL-13, mice which lack the expression of these cytokines only in T cells (4-13Tko) or in all cells (4-13ko) are characterized during infections with the gastrointestinal helminth *Nippostrongylus brasiliensis*. In BALB/c wild-type mice N. brasiliensis is expelled in less than two weeks after the infection. Here we demonstrate that both 4-13Tko and 4-13ko mice mounted impaired Th2 responses, which was for example reflected in low IgE levels and decreased numbers of alternatively activated macrophages. Furthermore, the mobilization of eosinophils and basophils was decreased in 4-13Tko and 4-13ko mice. But only a complete lack of IL-4 and/or IL-13 led to impaired activation of intestinal epithelial cells and goblet cells which resulted in defective worm expulsion. Thus, innate IL-4/IL-13-producing cells appear to play a critical role to control the primary infection with *N. brasiliensis.*

22. Nematode moulting enzymes as potential drug targets

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The nematode cuticle is a collagenous extracellular matrix that is synthesised repeatedly during development via the moulting process. The enzymology of cuticle collagen biosynthesis and more importantly the enzymology of cuticle ecdysis and moulting represent potential novel targets for nematode control. Using the *Caenorhabditis elegans* model system we have used forward and reverse genetics approached to identify and characterize key moulting enzymes and have focused on the pro-collagen C-peptidase astacin metalloproteases. Mutation of the enzyme-encoding gene DPY-31 reveals an essential developmental role for this enzyme in *C. elegans*, having a temperature sensitive lethal phenotype at 20°C with severe Dumpy survivors at 15°C. We have identified the orthologues of this gene in the parasitic nematodes *Brugia malayi, Teladersagia circumcinta* and *Haemonchus contortus*, that are able to complement the mutant phenotypes in *Ce-dpy-31*. Using in silico-modelling we have screened available chemical libraries and have tested several hundred potential inhibitors using in vivo and in vitro assays in *C. elegans* and *H. contortus*. The screening process will be described and the main finding of this study will be presented.

23. CCR7-dependent immune regulation of *Trichinella spiralis* Infection

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The chemokine receptor CCR7 and its ligands, CCL19 and CCL21, are known to be critical for a number of essential immunological processes throughout the development of an immune response [especially T-cell homeostasis and regulatory T-cell (T_{reg}) function]. In previous our study, we demonstrated that *Trichinella spiralis* infection could ameliorate artificially induced inflammation in mouse model and elicit T_{reg} cell recruitment. In order to know the role of CCR7 in immune response to *T. spiralis* infection, we investigated CCR7 and their ligand expression level during *T. spiralis* infection and evaluated T cell differentiation by *T. spiralis* excretory-secreted (ES) proteins treated bone marrow derived dendrite cells (BMDCs) with or without anti-CCR7 antibody. The expression of CCL19, CCL21, and CCR7 were significant increased in *T. spiralis* infected splenic DCs and muscle over the time course. However, the expression of these genes was not increased in intestine. In addition, we developed BMDCs in the presence or absence of ES protein and analyzed several surface markers of DCs using flow cytometry. The results showed that ES proteins stimulated activation marker of DCs: the expression of MHCII, CD40, CD80, CD86 and CCR7 was higher than in DCs cultured in medium alone. Secondly, we evaluated T cell population after ES protein treated BMDC and naive T cell with anti-CCR7 antibody. After treatment anti-CCR7, the number of IL-4-secreting $CD4^+$ T cells (Th2 cells) and $CD4^+CD25^-Foxp3^+$ T cells (T_{reg}) were significantly decreased. The Th2 and Treg related cytokine level was also decreased in anti-CCR7 antibody treated group. These results showed that CCR7 activation of DCs might be important in Th2 and T_{reg} cell activation in *T. spiralis* infection.

24. Utilising protein inhibitors and transgenic vectors to identify genes in the insulin-signaling pathway, which are essential for development and survival in *Schistosoma spp*.

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The insulin-signaling pathway has been shown to be a good target for drug development in many parasites such as *Leishmania*, *Trypanosomes* and *Trichomonas*. *Schistosoma spp*. utilise human insulin for energy and development; thus targeting molecules within this pathway could lead to a better understanding of which genes are essential for parasite development and survival. Using a panel of protein inhibitors, we have identified specific molecules in the pathway which we think are crucial for parasite survival. The results indicate that inhibitors, which target the mTOR protein were most effective. Death was prevalent in somules and adults at concentrations higher than 50µM, and at lower concentrations caused granule formation, vacuoles in the cell body, abnormality in the tegument and reduced motility. Development of reproductive organs was also affected, suggesting that targeting this pathway could reduce fecundity. In comparison, inhibitors which target the PI3K and Akt proteins did not have much effect at lower concentrations and only at higher concentrations were we able to detect signs of internal structural damage and reduced motility. These results indicate that targeting proteins that are further downstream of the insulin-signaling pathway could be more effective. Unlike protozoan parasites, for which techniques for genetic manipulation are well advanced, schistosomes and other helminths lag substantially. With the recent availability of MLV vector pLNHX, we are now able to construct vectors targeting our specific gene of interest in order, to perform gain- or loss-of function studies in different developmental stages of schistosomes. Vector constructs that target the insulin receptor, specifically the kinase domain as well as other molecules in the pathway particularly PI3K, Akt and mTOR, are of main interest and we are currently looking to see whether knocking down or performing mutagenesis on these genes will have any significant effect on the development and survival of schistosome parasites.

25. Acetylcholine: a co-stimulator for pulmonary immune responses against *Nippostrongylus brasiliensis?*

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A number of nematode parasites secrete acetylcholinesterases (sAChE) within the host. These enzymes break down acetylcholine (ACh), hypothesised to play an important role as a co-stimulator or mediator of immune responses. With this in mind, we sought to characterise cells which are involved in immunity to nematode infection and capable of producing ACh, in order to ascertain whether these cells are potential targets for helminth-derived sAChE. Using the *Nippostrongylus brasiliensis* model of nematode infection, and a choline acetyltransferase (ChAT)-eGFP reporter mouse strain, we were able to demonstrate that a number of hematopoietic cells in the lung have the capacity to synthesise ACh, and up-regulate this during infection, with interesting differences in the early, acute inflammatory phase and later chronic phase of tissue repair. Among these, type-2 innate lymphoid cells (ILC2) and effector/memory CD4+ Th2 cells displayed the most striking differences in expression of ChAT. Mass spectrometry was used to quantify ACh release from sorted cells, while RT-PCR was used to further characterise expression of cholinergic receptors and other features of ACh producers. Given the emerging role of ILC2 cells in allergy and respiratory disease such as asthma and COPD, and the current use of anti-cholinergic drugs to treat a number of these pathologies, exploring the production of ACh by immune cells and its downstream signalling effects may not only reveal new information about host-parasite interactions, but may also provide new targets for treatment of respiratory disorders.

26. Compartive biochemical analysis of nematode and mammalian exosomes.

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Exosomes are bioactive nanovesicles (50-100nm diameter) secreted by many cell types as a form of cell-to-cell communication. They play diverse functions in biological processes including host-pathogen interactions, antigen presentation and immune cell signalling. Viruses such as HIV have also evolved to exploit this pathway to facilitate their spread. Recently, exosomes have also shown promise as therapeutic tools for the delivery of small molecules and siRNAs. Different parasites have been shown to secrete exosome-like vesicles (*Echinostoma caproni*, *Fasciola Hepatica, Leishmania*). We have identified exosomes in the secretion products of the mouse parasite nematode *Heligmosomoides polygyrus*. These vesicles pellet upon ultracentrifugation and as shown by Transmission Electron Microscopy are round in shape with a diameter of ~100nm. In order to better investigate the nature of these vesicles, we performed a comprehensive mass-spec analysis of their lipid content, compared to the rest of the secretion products (supernatant) and the adult worm. In parallel we carried out similar studies with host cell exosomes derived from small intestinal epithelial cells (MODE-K cells), a cell type in close proximity to the parasite *in vivo*. Our results show that the *H. polygyrus* vesicles are enriched in sphingolipids (Ceramide and Sphingomyelin) compared to the rest of *H. polygyrus* secretion products or the adult worms. Similarly, MODE-K exosomes present higher levels of these sphingolipids' species compared to the cells extracts. The global phospholipids analysis shows comparable composition for both types of exosomes but reveals some differences in specific phospholipids' species. Confocal microscopy together with FACS analysis showed that both parasite and host vesicles are actively taken-up by mouse epithelial cells. Dynamic light scattering studies suggest differences in the charge of the exosomes from *H. polygyrus* compared to mouse. All together these results suggest that *H. polygyrus* secreted exosomes display similar but distinct lipid composition compared to mammalian exosomes as well as differences in their size and charge. We speculate that these differences might mediate specificity in their uptake or functional properties within their environment and could underpin how *H. polygyrus* has evolved to manipulate its host.

27*. H. polygyrus* excretory/secretory products can protect against T cell-mediate colitis through continuous delivery in vivo.

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Inflammatory bowel diseases (IBD) are becoming increasingly prevalent in affluent societies, but remain at low incidence in most tropical countries with higher rates of parasite infection. The possibility that parasites may inhibit the development of IBD has been raised in recent studies in both humans and mice showing successful suppression of IBD pathology with helminth parasites. In order to translate the anti-inflammatory effect seen with live parasite infection into a more pharmaceutical pipleline, we tested the capability of parasite secreted products, specifically excretory/secretory antigens (HES) from the gut dwelling helminth *Heligmosomoides polygyrus*, in suppressing colitis. We first studied two models of IBD induced by disruptive chemical treatment of the intestinal tract with dextran sodium sulfate (DSS) and trinitrobenzene sulfonic acid (TNBS). These acute, rapid-onset pathologies could not be overcome by HES, delivered through intraperitoneal injection, even though there was some evidence of protection, seen by reduced weight loss in mice receiving HES. To assess a more slow-onset model of IBD, we transferred naïve/effector T cells (sorted as CD4+CD25-FoxP3- cells from GFP-Foxp3 reporter mice) into RAG-deficient recipients. In this setting, colitis develops over 3-5 weeks. We also modified the administration of HES by implantation of i.p. mini-pumps capable of slow delivery of molecules osmotically over a two or four week time period. In this model, we now found that small concentrations of HES continuously released over the disease incubation period prevented the characteristic weight loss and reduced the histological damage seen in the untreated mice. Further studies are under way to evaluate if a therapeutic schedule of HES administration (given after the onset of mild colititic symptoms) and to testing of defined HES immunomodulators expressed as soluble recombinant proteins in mammalian expression systems.

28. Different *Strongyloides stercoralis* populations in humans and dogs in rural Combodia.

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In the scientific literature generally all *Strongyloides* sp. nematodes found in humans or dogs are considered *S. stercoralis*. Recently, the analysis of mitochondrial sequences from a relatively small number of *S. stercoralis* suggested at least some degree of host specificity. To determine if *S. stercoralis* in humans and in dogs interbreed and if they are transferred between the two hosts, we isolated individual *Strongyloides* sp. worms from villagers in Cambodia and from their animals. First, we compared the nuclear small ribosomal subunit rDNA (*SSU*) sequences. While all *Strongyloides* from pigs and chickens were clearly distinct from the ones derived from humans, the worms present in humans and dogs were much more closely related. Nevertheless we found six different SSU genotypes, five of which appeared to be host specific. Three occurred only in dogs, two only in humans. The sixth genotype (genotype 1) was the by far most frequent one in humans but was also found at a very low frequency in dogs. Next we analyzed the sequences of the mitochondrial, only maternally inherited, gene *cox1*. With respect to *cox1*, all worms isolated from people grouped together. The same was the case for worms from dogs except for the ones with the *SSU* genotype 1. These worms clearly grouped with their human derived counterparts. Therefore, humans and dogs carry mostly different populations of *S. stercoralis*. Nevertheless, zoonotic transmission of *S. stercoralis* from dogs to humans remains possible. Strikingly, we never found hybrids between different *SSU* genotypes although they co-occurred in the same host individuals. Either *S. stercoralis* in our sampling area reproduce non-sexually or the different *SSU* genotypes interbreed only within the genotype and represent different species. To resolve this we are currently analyzing individual females and their progeny in order to determine if within *SSU* genotype crossing does occur.

29. Towards revealing Cytochrome P450 contribution to the detoxification capacity of Liver Fluke

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Fasciolosis of livestock is a global threat to food security and is now an increasing food borne risk to humans. At present there are no commercial vaccines to underpin control programmes. Therefore, reported treatment failures and resistance to Triclabendazole (TCBZ) is of particular concern as TCBZ is the only anthelmintic with activity against both mature and pathogenic immature fluke. To secure future anthelmintic control of fasciolosis, uncovering the parasites full detoxification capacity must be of paramount importance. Currently, there is a limited understanding of the Phase I anthelmintic detoxification capacity in Platyhelminthes, with reductionist biochemistry failing to directly detect Cytochrome P450 (CYP450) expression in adult parasitic worms. We are testing the hypothesis that liver fluke has a broad based Phase I and Phase II capacity to detoxify anthelmintics despite reductionist biochemistry predicting limited detoxification potential and the reliance on members of the abundant glutathione transferase (GST) superfamily to simply passively bind toxins. The newly available genomic and transcriptomic databases of liver fluke provide a new tool to systematically reveal the parasite's detoxification capacity (the detoxome). Genome and transcriptome mining has identified two liver fluke CYP450s, termed FhCYP450-1A1 and a potentially novel FhCYP450-2 in *Fasciola hepatica* and *F. gigantica*. The detection of Phase I P450 transcript expression in adult liver fluke starts to readdress the previously perceived importance of Phase II level detoxification in parasitic flatworms. Delineating the role of CYP450s via functional genomics will provide a pipeline to support the design of new anthelmintics and also potentially provide new biomarker approaches to measure anthelmintic resistance.

30. The development of RNA interference (RNAi) in the parasitic nematode *Teladorsagia circumcincta* as a method for screening vaccine candidates

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Teladorsagia circumcincta is a major cause of ovine parasitic gastroenteritis in temperate climatic regions and the developed resistance to the major anthelmintic drug classes challenges the future control of the parasite. Vaccination is a potential alternative control method since sheep are able to develop protective immunity. Although potential vaccine candidates have been revealed, the increasing gene datasets suggest that vaccine-target selection may be aided by screening methods such as RNA interference (RNAi). RNAi is a reverse genetic mechanism that causes highly specific gene silencing. In this study we tried to develop an RNAi platform for *T. circumcincta* that could be used to screen potential vaccine targets. Four vaccine candidates were selected for knock-down which included: two members of the Activation-associated Secreted Proteins (ASPs); a Macrophage migration Inhibitory Factor-like (*Tci-mif-1*) and a Surface Associated Antigen gene (*Tci-saa-1*). The results have shown a successful knock-down for the ASP but not for the latter two targets after 1 hour of soaking in gene-specific double stranded RNA (dsRNA). These results illustrate both the inconsistency and the target specificity of RNAi in *T. circumcincta.* Similar inconsistencies have been observed in the past with other parasitic nematodes. Moreover, inconsistencies were observed within the ASP targets with the silencing effect not being reproducible after four subsequent successful experiments. A number of parameters that might affect the efficacy of RNAi were examined and it was found that the storage period of the larvae is an important factor for the consistency of the RNAi results. Larvae stored for less than a month at 4°C were consistently susceptible to RNAi whilst larvae stored for more than a month were not susceptible to RNAi.

31. Characterization of *Schistosoma mansoni* trypsin-like serine protease SmSP2: a new chapter of *schistosoma* degradome

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Proteolytic activities are integral part of many physiological processes and in schistosomes are crucial for parasite successful invasion, survival and reproduction. In *Schistosoma mansoni*, besides of relatively well characterized enzymes there are groups of proteases which are neglected such as trypsin-like serine proteases from family S1 of clan PA. In mammals are these proteases responsible for vital processes such as blood coagulation, fybrinolysis, imunity, digestion and apoptosis. Schistosome proteases S1 share high similarities with human host although their genome contains significantly less genes coding proteases with features typical for S1 family. Here we are focused on characterization trypsin-like protease from *S. mansoni* called SmSP2. SmSP2 is according to our data highly expressed in life-stages residing in definitive host (schistosomula, adult worms). We employed RNA in situ hybridization in order to localize the expression and presence of the gene in the parasites. Several modifications of SmSP2 were recombinantly expressed in *Escherichia coli* and *Pichia pastoris* expression systems. Recombinants of SmSP2 were subsequently purified via affinity chromatography, and submitted to several refolding techniques to obtain active proteases when necessary. Active recombinants were characterized using synthetic peptidyl-substrates. Finally, the substrate activity screening assay was utilized to identify the most suitable substrates and inhibitors which help us to determine enzymatic specificity. First results will be presented and the implications of these findings will be discussed.

32. The development of a *Trypanosoma musculi*-based in vivo heterologous expression system to investigate parasitic nematode gene function

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Much is still not known about the function of many parasitic nematode genes, particularly in relation to their immunomodulatory behaviour. One of the reasons for this is the lack of suitable techniques for genetic manipulation, such as RNA interference and transgenesis. A gain of function approach, through the use of a heterologous expression system, may be more effective. We are developing a system using the natural protozoan parasite of mice, *Trypanosoma musculi*, as an in vivo vehicle for the heterologous expression of genes from parasitic nematodes. We have successfully propagated *T. musculi* axenically in vitro, and made constructs to incorporate exogenous genes into the parasite genome and target proteins for secretion. *T. musculi* was successfully genetically modified to express detectable amounts of green fluorescent protein when a construct was incorporated into the ribosomal RNA gene array. Constructs have been made to express and secrete proteins from parasitic nematodes which are known or suspected to be immunomodulatory in order to test this system. Proteins currently under investigation are acetylcholinesterase B from *Nippostrongylus brasiliensis*, which is thought to neutralise cholinergic immune signalling, 5'-nucleotidase from *Trichinella spiralis*, thought to disrupt purinergic signalling, MIF1 from *Brugia malayi*, which synergises with IL-4 to produce alternatively activated macrophages, and NIF from *Ancylostoma caninum*, which binds to CD11b on leukocytes, preventing extravasation and activation. Data will be presented on relevant transgenic trypanosomes which are being used to validate the system.

33. The role of SCP/TAPS genes in the parasitic life of *Strongyloides ratti*.

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Strongyloides spp. are successful parasites and infect a wide variety of vertebrate hosts. We have used a combined proteomic and transcriptomic approach to discover genes and gene products that are likely to underlie the parasitic lifestyle in the rat parasite *S. ratti*. Specifically, we compared the proteome and transcriptome of genetically identical parasitic females and free-living females of *S. ratti*. Among the genes and gene products uniquely present (or present in significantly greater quantities) in the *S. ratti* parasitic female are many SCP/TAPS genes / gene products. SCP/TAPS proteins act as calcium chelators in a broad range of signalling processes. They are characterized by the presence of a single or double CAP domain. Of the 88 SCP/TAPS genes found in *S. ratti,* 65 are upregulated in parasitic females by a mean 288 (± 50 SE) fold change; none are upregulated in free-living females. Of these 65, 64 contain a single CAP domain, and 32 contain a signal peptide domain suggesting they're secreted. The majority of these genes are physically adjacent on chromosome II, in clusters of up to 18 genes. Phylogenetic analysis of all upregulated CAP-domain containing genes showed genes within clusters were more closely related to each other than they were to genes in other clusters, suggesting clusters have resulted from tandem gene duplication. These data together with recent reports of SCP/TAPS gene upregulation in other parasitic nematodes is growing evidence to suggest that these genes have a key role in nematode parasitism.

34. The individual effects of novel *Trichinella spiralis* proteins on host muscle cells

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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 it's 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, cell cycle re-entry and arrest, collagen capsule formation and angiogenesis. Although *T. spiralis* proteins secreted directly into the muscle cell are thought to be responsible for these changes, to date only a subset of these proteins have been characterized. We have performed a detailed proteomic analysis of the secreted proteins of *T. spiralis* muscle larvae. Of the 340 proteins identified, many functions can be predicted based on identity to orthologous proteins in other organisms, however several others appear to be *T. spiralis*-specific. We are interested in characterizing these novel proteins in order to understand what function they play in nurse cell development and how they individually manipulate host cell homeostasis. We have selected a panel of proteins based on their abundance in the ES and are employing proteomic and biochemical analyses to investigate the function of each when individually expressed in mammalian skeletal muscle cells.

35. Expression of Schistosome-derived Omega-1 with diantennary glycans carrying Lewis X motifs in *Nicotiana benthamiana* plants

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Omega-1 is a T2 ribonuclease (RNase) secreted by *Schistosoma mansoni* eggs and is a key factor for the induction of Th2 cell differentiation. Induction of Th2 responses by omega-1 is dependent on its RNase activity as well as the *N*-glycan mediated internalisation by antigen presenting cells. Omega-1 carries two core-difucosylated diantennary *N*-glycans containing terminal Lewis X motifs. Lewis X is known to be an immunomodulatory glycan due to its interaction with DC-SIGN. However, the main receptor required for omega-1 induced Th2 polarisation was shown to be the mannose receptor. The exact role of *N*-glycans on the immunomodulatory properties of helminth secreted glycoproteins remains to be elucidated. As the purification of a single glycoprotein from Schistosome egg extracts is relatively inefficient, a platform is required that enables high expression of these helminth glycoproteins with their native *N*-glycan structures. Here we show that Schistosome-derived omega-1 can be efficiently produced in *Nicotiana benthamiana* plants by means of agroinfiltration. Omega-1 was purified from the intercellular space (apoplast) of *Nicotiana benthamiana* leafs and was shown to have RNase activity. Omega-1 produced in wild-type plants carried diantennary *N*-glycans containing typical plant β1,2-xylose and core α1,3-fucose, but lacked terminal GlcNAc's. By the controlled co-expression of two glycosyltransferases, omega-1 could be engineered to carry terminal Lewis X motifs. Furthermore, both wild-type omega-1 and omega-1 carrying Lewis X motifs were able to induce Th2 cell polarisation. All-in-all our results demonstrate that plants are a promising platform for the expression of helminth glycoproteins carrying engineered *N*-glycans, which opens up a new field of research.

36. Intestinal helminth infection improve intestinal homeostasis via alteration of intestinal bacteria population

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The microbiota in the human, including bacteria, parasites, fungi, and viruses, have co-evolved with the host in a symbiotic relationship. A complex interplay between the host immune system and the microbiota is necessary for gut homeostasis. However, the mechanism by which parasites affect gut homeostasis remains poorly understood. It was recently reported that mice infected with the parasitic helminth, *Trichinella spiralis*, show ameliorated colonic inflammation in a murine model of inflammatory bowel disease. We propose a mechanism that *T. spiralis* infection maintains gut homeostasis, which in turn helps modulate host immunity. To begin to address this hypothesis, we quantified the effect of *T. spiralis* infection on the population of intestinal bacteria in the absence of inflammation, using wild-type C57BL/6 mice. It was observed that there was a significant shift in the population of bacterial phylum in the *T.spiralis* infected mice. Members of the bacterial phylum, Verrcomicobia, significantly increased and Firmicutes phylum decreased in the feces of infected mice. We quantified the effect of *T. spiralis* treatment on the population of intestinal bacteria in DSS-induced gut inflammation and when *T. spiralis* was removed by treatent with the anti-helminthic drug, Flubendazole (20μg/g), after 21 to 25 days post *T. spiralis* infection. In *T. spiralis* infected mice was seen ameliorated colonic inflammation and increased anti-inflammatory cytokine and cells, such as IL-10 and regulatory T cells. But groups with *T. spiralis* removed or non-infected were not ameliorated. Most of entire bacteria number and alteration of intestinal bacteria population were reduced in *T. spiralis* removed and non-infected groups. However, *T. spiralis* infected mouse were not reduced in the entire bacteria number and elevated in the population of intestinal bacteria. These data suggested that *T. spiralis* infection might influence to intestinal homeostasis.

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