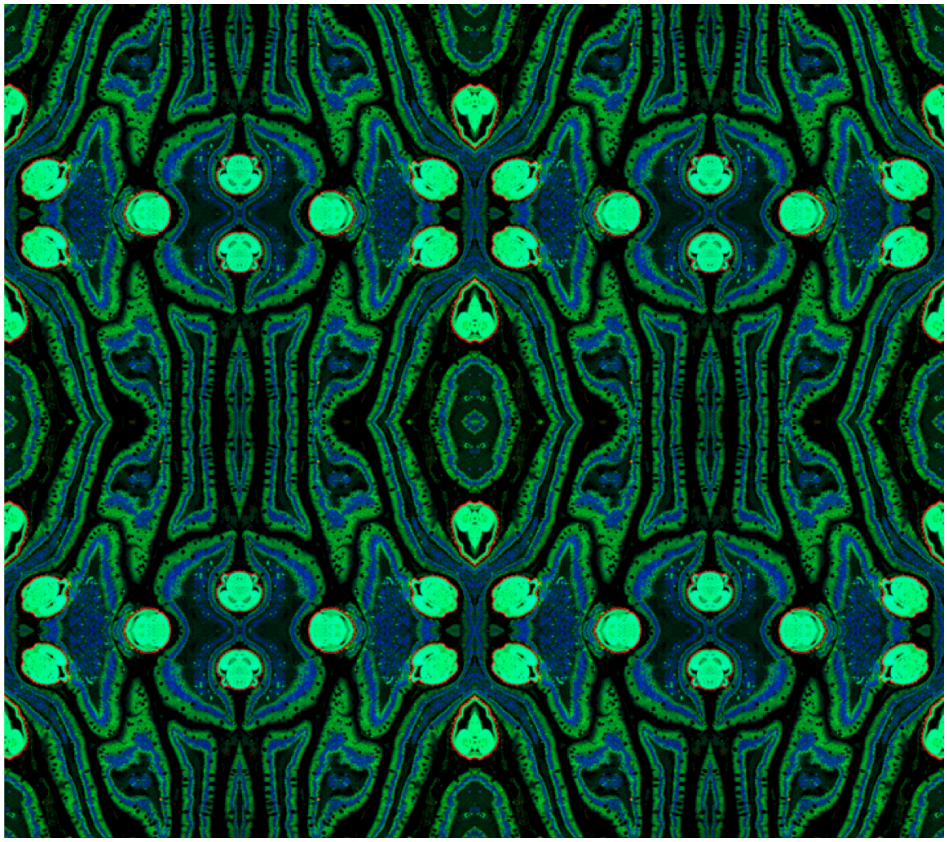
Molecular and Cellular Biology of Helminths XI



3 - 8 September 2017
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'

VIII **1-6 September 2014, Hydra Greece**
'Molecular and Cellular Biology of Helminth Parasites VIII'

IX **31 August – 5 September 2015, Hydra Greece**
'Molecular and Cellular Biology of Helminth Parasites IX'

X **31 August – 5 September 2016, Hydra Greece**
'Molecular and Cellular Biology of Helminth Parasites X'

Dates of MCBHP-XII Meeting: 2-7 September 2018

ORGANISERS, 2017
Richard E Davis (University of Colorado, School of Medicine, USA)
Kleoniki Gounaris (Imperial College, UK)
Rick Maizels (University of Glasgow, UK)
Murray Selkirk (Imperial College, UK)

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Cover photo : **A kaleidoscope view of *Heligmosomoides polygyrus* embedded in murine duodenum, compiled by Janice Murray and Danielle Smyth of Edinburgh and Glasgow Universities**

	Sunday 3 September	Monday 4 September	Tuesday 5 September	Wednesday 6 September	Thursday 7 September	Friday 8 September
	ARRIVE	**Session 1 Hosts, Parasites, Symbionts**	**Session 4 Immunity in Model Organisms**	**Session 7 Development and Gene Expression**	**Session 10 Chemotherapy**	DEPART
09:00		Astra Bryant	**Emily Troemel**	**Guofeng Cheng**	**Tim Geary**	
09:20		Alexandra Grote				
09:40		Alvaro Diaz	Michael Povelones	Dick Davis	John Chan	
10:00		Mike Doenhoff	Michael Blouin	Ronaldo de Carvalho A.	Eileen Devaney	
10:20		Alex Loukas	Euan Allan	Michaela Herz	Guillaume Sallé	
10:40-11:10	**Coffee break**					
		Session 2 Genomics and Engineering	**Session 5 Metabolism and Drug Development**	**Session 8 Immunology II**	**Session 11 Immune Modulators II**	
11:10		**Wormbase Workshop**	Andy Fraser	Maria Yazdanbakhsh	Henry McSorley	
11:30		Kevin Howe	Joseph Turner	Julio Furlong-Sliva	Danielle Smyth	
11:50		Krystyna Cwiklinski	Thomas Spangenberg	Nicolas Pionnier	Taylor Smallwood	
12:10		Jodie Chandler	Philip LoVerde	Ana Gonzalez-Hernandez	Terry Spithill	
12:30		Kim Van Noort	Helmut Haas	Yoanne Mouwenda	Anna Kildemoes	
12:50-4:30	**Afternoon break**					
		Session 3 Immunology I	**Session 6 Host-Parasite Interactions**	**Session 9 Immune Modulators I**	**Session 12 Co-Infection + Vaccines**	
4:30	**Registration Opens at Bratsera Hotel**	**Judith Allen**	**Banchob Sripa**	**Oluwatoyin Asojo**	**Harriet Mpairwe**	
4:50						
5:10		Maria Duque-Correa	Jan Dvorak	Fumi Varyani	Minka Breloer	
5:30		**Poster Pitches,**	Cecilia Fernandez	**Poster Pitches**	John Grainger	
5:50		**15 x 2 minutes**	Ruud Wilbers	**16 x 2 minutes**	Kara Filbey	
6:30	**Pre-lecture drinks**	**Poster Session 1**	*End of Session*	**Poster Session 2**	*End of Session*	
7:30	**Keynote Lecture: Klaus Brehm**		**Vlychos Taverna Dinner (Boat leaves 7:00 PM)**		**Bratsera Farewell Dinner (8:30 PM)**	
8:30	**Welcome Reception**	*End of Session*		*End of Session*		

NOTES

Sunday 3 September

Chair: **Rick Maizels**, University of Glasgow, UK	
19:30	Keynote Lecture: **Klaus Brehm**, University of Würzburg, Germany
20:30	**Welcome Reception and Dinner, Bratsera Hotel**

Monday 4 September

09:00 - 10:40 Session 1: Hosts, Parasites and Symbionts

Chair: **Murray Selkirk**, Imperial College London, UK		
09:00	**Astra Bryant** University of California Los Angeles, USA	Some like it hot: temperature-driven host seeking in human-parasitic nematodes
09:20	**Alexandra Grote** New York University, USA	Searching for metabolic choke points to disrupt the B*rugia malayi* / *Wolbachia* symbiosis
09:40	**Alvaro DIaz** Universidad de la República, Uruguay	Dendritic cells respond to particles from the *Echinococcus granulosu*s laminated layer by a mechanism akin to "membrane affinity triggered signaling"
10:00	**Mike Doenhoff** University of Nottingham, UK	Antigenic cross-reactivity between *Schistosoma mansoni* and allergens: the hygiene hypothesis based on cross-reactive carbohydrate determinants (CCDs)
10:20	**Alex Loukas** James Cook University, Australia	Liver fluke secretions drive wound repair and cancer

11:10 – 12:50 Session 2: Genomics, Gene Expression and Engineering

Chair : **Dick Davis,** University of Colorado, USA		
11:10	**Kevin Howe** EMBL European Bioinformatics Institute, UK	WormBase ParaSite in 2017 and beyond
11:50	**Krystyna Cwiklinski** Queen's University Belfast, UK	A study of *Fasciola hepatica* virulence and invasion using omics tools
12:10	**Jodie Chandler** Malaghan Institute of Medical Research, New Zealand	Molecular analysis of genes induced by blood feeding in *N. brasiliensis*
12:30	**Kim Van Noort** Wageningen University, Netherlands	Characterization of *Schistosoma mansoni* fucosyltransferases for glyco-engineering of 'native' helminth N-glycan structures *in planta*

16:30 - 18:10 Session 3: Immunology I

Chair : **Minka Breloer,** Bernhard Nocht Institute for Tropical Medicine, Germany		
16:30	**Judith Allen** University of Manchester, UK	Local amplifiers of type 2 immunity during helminth infection
17:10	**Maria Duque-Correa** Sanger Institute, UK	Interleukin 10 signaling, but not IL-22 or IL-28, promotes intestinal colonization resistance to opportunistic pathogens and controls immunopathology during whipworm infection
17:30	**Pitches for Poster Session 1**	Posters 1 - 15

<u>Monday 4 September</u> - 17:30-18:00 (2 min poster presentations, 1 slide each)

Chair: **Niki Gounaris**, Imperial College London, UK			
1	**Panat Anuracpreeda**	Mahidol University, Thailand	Novel rapid diagnostic test kits for tropical fasciolosis by *Fasciola gigantica*
2	**Jimmy Borloo**	Ghent University, Belgium	Structural insights in the activation-associated secreted proteins of the cattle parasites *Ostertagia ostertagi* and *Cooperia oncophora* – implications for vaccine development
3	**Clare Collett**	Aberystwyth University, UK	Towards the penside detection of triclabendazole efficacy against liver fluke parasites of livestock
4	**Stephen Cross**	Liverpool School of Tropical Medicine, UK	Tetracycline antibiotics modify both filarial Th2 inflammation and inflammatory-associated lymphatic remodelling in pre-clinical lymphatic filariasis pathology models
5	**Yesid Cuesta Astroz**	Centro de Pesquisas Rene Rachou , Brazil	Helminth secretomes reflect different lifestyles and parasitized hosts
6	**David Curran**	The Hospital for Sick Children, Toronto, Canada	Using metabolic networks to probe the symbiosis between *Wolbachia* and filarial nematodes
7	**Mohammad Davami**	Jahrom University of Medical Sciences, Iran	Current status of cystic echinococcosis in Iran
8	**Chelsea Davis**	Aberystwyth University, UK	Optimising liver fluke (*Fasciola hepatica*) extracellular vesicle purification to understand their role within parasite drug exposure
9	**Samantha Del Borrello**	University of Toronto, Canada	Using simulated anaerobiosis in *C. elegans* as a platform for anthelmintic drug discovery
10	**Claire Drurey**	University of Glasgow, UK	Manipulating the Host: Investigating Targets of *H. polygyrus* Excretory-Secretory Products
11	**Orla Drysdale**	Queen's University Belfast, UK	Upsetting the protease/anti-protease balance could be a novel vaccine strategy against *Fasciola hepatica* infection
12	**Hala Zahreddine Fahs**	New York University Abu Dhabi	High throughput chemical genomics in *C. elegans* to screen for novel anthelmintics and their targets
13	**Pavla Fajtová**	Institute of Organic Chemistry and Biochemistry, Czech Rep	Structural basis for inhibition of the SmCB1 drug target from the blood fluke *Schistosoma mansoni*
14	**Tom Gasan**	Aberystwyth University, UK	Characterisation of *Schistosoma mansoni* Larval Extracellular Vesicle protein-1 (SmLEV-1) an immunogenic, schistosome-specific, protein exhibiting developmentally regulated alternative splicing.
15	**Jana Hagen**	Imperial College London, UK	Lentiviral delivery of artificial miRNAs to *Nippostrongylus brasiliensis* infective larvae

18:30-20:30 Poster Session 1 and Drinks

Tuesday 5 September

09:00 - 10:40 Session 4: Immunity - Model Organisms and Vectors

Chair: **Eileen Devaney**, University of Glasgow, UK		
09:00	**Emily Troemel** University of California San Diego, USA	Microsporidia infection in *C. elegans*: how an obligate intracellular parasite makes itself at home
09:40	**Michael Povelones** University of Pennsylvania, USA	Molecular dissection of mosquito resistance to heartworm infection
10:00	**Michael Blouin** Oregon State University, USA	Association mapping of genes in the snail, *Biomphalaria glabrata*, that confer resistance to *Schistosoma manson*i
10:20	**Euan Allan** Oregon State University, USA	Schistosome infectivity in *Biomphalaria glabrata* is dependent on the Guadeloupe Resistance Complex , and is partially explained by the expression of a single novel protein, Grctm6

11:10 – 12:50 Session 5: Metabolism and Drug Development

Chair: **Tim Geary**, McGill University, Canada		
11:10	**Andy Fraser** University of Toronto, Canada	Establishing *C.elegans* as a tractable system for genetic and drug screens to find modulators of malate dismutation
11:30	**Joseph Turner** Liverpool School of Tropical Medicine, UK	Development of murine models of loiasis and their applications in filarial drug development, diagnostics and immunopathology research
11:50	**Thomas Spangenberg** Merck Global Health Institute, Switzerland	Evaluation of the pharmacokinetic-pharmacodynamic relationship of Praziquantel in the S*chistosoma mansoni* mouse model: possible clinical implications
12:10	**Philip LoVerde** University of Texas Health Science Center, USA	Why does Oxamniquine kill *Schistosoma mansoni* but not *S. haematobium* or *S. japonicum* and can we synthesize a derivative that kills all 3 species of schistosomes?
12:30	**Helmut Haas** helminGuard, Germany	Engaging worms for health - drug discovery on live schistosomes

16:30 – 18:10 Session 6: Host-Parasite Interactions

Chair: **Klaus Brehm**, University of Würzburg, Germany		
16:30	**Banchob Sripa** Khon Kaen University, Thailand	Recent advances in pathogenesis of the carcinogenic liver fluke *Opisthorchis viverrini* and bile duct cancer
17:10	**Jan Dvorak** Institute of Organic Chemistry and Biochemistry, Czech Rep	Secreted serine protease from *Schistosoma mansoni* with hemostatic role.
17:30	**Cecilia Fernández** Universidad de la República, Uruguay	Functional diversity of secreted cestode Kunitz proteins: inhibition of serine peptidases and blockade of cation channels
17:50	**Ruud Wilbers** Waageningen University, Netherlands	Apoplastic venom allergen-like proteins of plant parasitic nematodes modulate the activation of plant innate immunity by cell surface receptors

Wednesday 6 September

09:00 - 10:40 Session 7: Development and Gene Expression

Chair: **Emily Troemel**, University of California San Diego, USA		
09:00	**Guofeng Cheng** Shanghai Veterinary Research Institute, China	Schistosome exosomal miRNAs: potentially important mediators of host-pathogen interactions
09:40	**Dick Davis** University of Colorado, USA	Programmed DNA elimination in nematodes
10:00	**Ronaldo De Carvalho Augusto** Université de Perpignan, France	Chromatin profiling of single larvae of *Schistosoma mansoni*: how to get hold of the stem cells
10:20	**Michaela Herz** University of Würzburg, Germany	Genome wide expression profiling of the *Echinococcus multilocularis* stem cell system

11:10- 12:50 Session 8: Immunology II

Chair: **Judi Allen**, University of Manchester, UK		
11:10	**Maria Yazdanbakhsh** Leiden University Medical Center, Netherlands	Treatment of helminth infections is associated with increased insulin resistance in infected subjects- a study in Indonesia
11:30	**Julio Furlong-Silva** Liverpool School of Tropical Medicine, UK	Lymphatic remodelling is promoted by the initial anti-filarial Th2 response to *Brugia malayi* infection
11:50	**Nicolas Pionnier** Liverpool School of Tropical Medicine, UK	Natural Killer cells are associated with innate control of filarial nematode infection
12:10	**Ana González-Hernández** Ghent University, Belgium	Comparative analysis of the immune response induced by native and recombinant versions of the ASP-based vaccine against gastrointestinal nematodes
12:30	**Yoanne Mouwenda** Leiden University Medical Center, Netherlands	Cellular immune response profiling after the co-administered hookworm vaccine candidates Na-GST-1 and Na-APR-M74 in healthy hookworm-exposed adults in Gabon

16:30 – 18:10 Session 9: Immunomodulators I

Chair: **Terry Spithill,** La Trobe University, Australia		
16:30	**Oluwatoyin Asojo** Baylor College of Medicine, USA	Structural studies of worm proteins
17:10	**Fumi Varyani** University of Glasgow, UK	Role of Macrophage Migration Inhibitory Factor in Immunity to helminths
17:30	**Pitches for Poster Session 2**	Posters 16 - 31

Wednesday 6 September

17:30-18:00 **(2 min poster presentations, 1 slide each)**

Chair: **Niki Gounaris,** Imperial College London			
16	**Margot Lautens**	University of Toronto, Canada	The search for novel anthelmintic targets: Characterizing alternative metabolic pathways in *Caenorhabditis elegans*
17	**Emilie Lefoulon**	New England Biolabs, USA	DNA sequencing within "Russian nested dolls": Large fragment targeted enrichment capture of *Wolbachia* genomes from filarial nematodes
18	**Stephan Loeser**	University of Glasgow, UK	First insights into helminth-induced activation of tuft cells at mucosal barrier surfaces
19	**Amy Marriott**	Liverpool School of Tropical Medicine, UK	Long term in vitro culture of adult *Brugia malayi* parasites to evaluate drug response and host-parasite interactions
20	**Abdolali Moshfe**	Yasuj University of Medical Sciences, Iran	Evaluation of clinical status, diagnosis and treatment of suspected fascioliasis patients and differentiation of *Fasciola* Species by PCR-RFLP
21	**Mayowa Musah-Eroje**	University of Nottingham, UK	The role of a parasite protein termed ''*Fasciola hepatica* transfroming growth factor-like molecule (FhTLM)'' on T cell activation and immuno-suppression.
22	**Ebube Odoya**	Baylor College of Medicine, USA	Chemokines and cytokines Levels in healthy adults and children co-infected with multiple intestinal parasites in Bayelsa state, Nigeria
23	**Babbett Oesterreich**	EUROIMMUN AG, Germany	Use of antigens from *Strongyloides papillosus* instead of *S. ratti* increases ELISA specificity for human strongyloidiasis
24	**Olufunke Oluwatoba**	University of Ibadan, Nigeria	impact of human gene, Tumor Necrotic Factor Super Family 13B, and environmental risk factors on the prevalence and intensity of *Ascaris lumbricoides* infection in Igbo-Ora, Nigeria.
25	**Madeleine Eunice Ongwe**	Leiden University Medical Center, Netherlands	Urinary metabolite investigation in response to hookworm vaccine administrated to healthy adults in Gabon.
26	**Faye Rodgers**	Wellcome Trust Sanger Institute, UK	New whipworm genomes and annotations
27	**Roger Schneiter**	University of Fribourg, Switzerland	The pathogen-related yeast protein Pry1, a member of the CAP protein superfamily, is a fatty acid-binding protein
28	**Bahador Shahriari**	Shiraz University of Medical Sciences, Iran	Genetic diversity of antigen B1 from sheep, cattle and human isolates of *Echinococcus granulosus* in south of Iran
29	**Oluyomi Sowemimo**	University of Lagos, Nigeria	Seroepidemiological study and associated risk factors of *Toxocara canis* infection among preschool children in Osun State, Nigeria

30	**Anfal Yousef**	Liverpool School of Tropical Medicine, UK	The role of autophagy in anti-*Wolbachia* antibiotic therapy
31	**Mathilde Chayé**	Leiden University Medical Center, Netherlands	Identification of schistosome molecules that drive splenic regulatory B cell development

18:30-20:30 Poster Session 1 and Drinks

Thursday 7 September

09:00 - 10:40 Session 10: Chemotherapy and Population Genetics

Chair: **Phil LoVerde**, University of Texas Health Science Center, USA		
09:00	**Tim Geary** McGill University, Canada	The pharmacology of ivermectin as an antifilarial
09:40	**John Chan** University of Minnesota, USA	High throughput screening of a schistosome serotonin receptor identifies potent anthelmintic lead compounds
10:00	**Eileen Devaney** University of Glasgow, UK	Genomics of sex, drugs, and recombination in the gastrointestinal nematode, *Haemonchus contortus*
10:20	**Guillaume Sallé** INRA, University of Tours, France	Global diversity and population genetic structure of the sheep parasite *Haemonchus contortus*

11:10 – 12:50 Session 11: Immunomodulators II

Chair: **Alex Loukas**, James Cook University, Australia		
11:10	**Henry McSorley** University of Edinburgh, UK	A helminth-derived inhibitor of IL-33
11:30	**Danielle Smyth** University of Glasgow, UK	Parasite cytokines : a structurally distinct immunosuppressive TGF-β mimic from an intestinal helminth that potently induces murine and human regulatory T cells
11:50	**Taylor Smallwood** University of Queensland, Australia	The synthesis and characterisation of helminth bioactive peptide as potential therapeutic treatments for autoimmune diseases
12:10	**Terry Spithill** La Trobe University, Australia	Ex vivo immunoproteomic analysis of *Fasciola hepatica* tegumental antigens identifies multiple exosome proteins recognized by antibody from resistant ITT sheep
12:30	**Anna Kildemoes** University of Copenhagen, Denmark	Interaction between commensal gut microbiota and *Schistosoma mansoni* infection in C57BL/6 and type-1 diabetes NOD mouse models

16:30 – 18:10 Session 12: Co-infection and Vaccination

Chair: **Maria Yazdanbakhsh**, Leiden University Medical Center, Netherlands		
16:30	**Harriet Mpairwe** MRC Uganda Virus Research Unit, Uganda	Helminth infections, deworming and allergic conditions in a Ugandan population
17:10	**Minka Breloer** Bernhard Nocht Institute for Tropical Medicine, Germany	*Litomosoides sigmodontis* infection interferes with vaccine induced protection against influenza virus infection
17:30	**John Grainger** University of Manchester, UK	Compartmentalised immune responses provide protection against type 1 pathogens challenge during helminth infection
17:50	**Kara Filbey** Malaghan Institute of Medical Research, New Zealand	Novel immune mechanisms involved in protection in a coinfection model with two distinct helminth parasites

20:30	**Farewell Banquet, Bratsera Hotel**

ABSTRACTS

KEYNOTE LECTURE

Gutting a tapeworm – molecular and cell biological expeditions into Echinococcus multilocularis

KLAUS BREHM

INSTITUTE OF HYGIENE AND MICROBIOLOGY, UNIVERSITY OF WÜRZBURG, GERMANY

Alveolar echinococcosis, caused by the metacestode stage of the fox-tapeworm *Echinococcus multilocularis*, is one of the most dangerous and lethal helminth infections. The disease is characterized by infiltrative, cancer-like growth of the *Echinococcus metacestode* within host organs. For a tapeworm, this is quite unusual behavior since cestode oncospheres typically develop into head-like structures (scolices) without involving an additional, asexually proliferating parasite stage. Since several years we are interested in the peculiar biology of the *Echinococus* metacestode and developed in vitro cultivation systems for parasite vesicles and primary cells by which the infection of the intermediate host can be mimicked under laboratory conditions. We also carried out a whole genome sequencing project for *E. multilocularis* and are currently studying gene function by transcriptome approaches and RNA-interference. Importantly, we established that *E. multilocularis* contains totipotent, somatic stem cells (germinative cells) with homologies to planarian neoblasts and that the cancer-like proliferation of the metacestode is crucially driven by the germinative cells. They are the only mitotically active cells in the parasite and give rise to all differentiated cells. Interestingly, the germinative cells also appear to be resistant towards benzimidazoles, the current drugs against echinococcosis, which explains why chemotherapy against the disease is so ineffective. By studying evolutionarily conserved signaling pathways we demonstrated extensive host-parasite cross-communication involving hormones and cytokines like insulin and TGF-beta. Recently, we showed that the *Echinococcus* metacestode is made up of posteriorized tissue and that the parasite is modulating its anterior-posterior axis (involving *wnt*-signaling) to achieve asexual proliferation before numerous protoscoleces are produced. Our current expeditions make use of these data on parasite stem cells, signaling pathways, and the genome, to develop novel chemotherapeutic measures against echinococcosis.

Some like it hot: temperature-driven host seeking in human-parasitic nematodes

ASTRA BRYANT, ELISSA HALLEM

MICROBIOLOGY, IMMUNOLOGY AND MOLECULAR GENETICS DEPARTMENT, UNIVERSITY OF CALIFORNIA, LOS ANGELES, USA

Skin-penetrating parasitic nematodes infect approximately one billion people worldwide, and are a major source of neglected tropical disease. These parasitic worms are infective exclusively during a soil-dwelling larval stage. The mechanisms by which infective larvae actively locate a suitable host for infection may represent a previously unexplored target for therapeutic intervention. Previous work has shown that the infective larvae of parasitic nematodes respond to several host-emitted sensory cues, including heat. However, our understanding of how strongly infective larvae rely on thermosensation to drive parasitic behavior is incomplete. We investigated the role of heat in host-seeking behaviors of multiple parasitic nematode species, including the human threadworm, *Strongyloides stercoralis*. Using a custom-built behavioral setup, we quantified the movement of infective larvae within precisely controlled temperature gradients. We found that *S. stercoralis* is strongly attracted to heat, with a preferred temperature at least 5°C warmer than human body temperature. In addition, we found that the skin-penetrating rat parasite *Strongyloides ratti* and the passively ingested mouse parasite *Heligmosomoides polygyrus* are also attracted to heat, although less robustly than *S. stercoralis.* The elevated temperature preference of *S. stercoralis* infective larvae likely generates temperature-driven movements that will not asymptote as worms approach a host, a specialization that may be critical for tracking ephemeral thermal targets such as mobile humans. We also tested whether infective larvae synergize multiple sensory modalities during host seeking. *S. stercoralis* attraction to host odors is highly dependent on the local thermal environment, implying a hierarchical coding of sensory information during host seeking. Our findings suggest that parasitic nematodes use temperature as a potent sensory cue to locate hosts for infection. We are currently testing the temperature preferences of other parasitic nematode species, including a human hookworm, *Ancylostoma ceylanicum.* In addition, we are investigating the cellular and circuit adaptations underlying this behavioral specialization.

Searching for metabolic choke points to disrupt the *Brugia malayi* / *Wolbachia* symbiosis

ALEXANDRA GROTE[1], TAO DING[1], DENIS VORONIN[2], SWAPNA SESHADRI[3], DAVE CURRAN[3], SARA LUSTIGMAN[2], JOHN PARKINSON[3], ELODIE GHEDIN[1]

[1]DEPARTMENT OF BIOLOGY, CENTER FOR GENOMICS & SYSTEMS BIOLOGY, NEW YORK UNIVERSITY, [2]MOLECULAR PARASITOLOGY, NEW YORK BLOOD CENTER, [3]MOLECULAR GENETICS, UNIV.TORONTO

Filarial nematodes represent one of the leading causes of disability in the developing world. Many filarial worm species, including *Brugia malayi*, one of the causative agents of lymphatic filariasis, have an obligate endosymbiotic relationship with the alpha-proteobacteria *Wolbachia*. To better understand the molecular interplay between these two organisms, we profiled the transcriptomes of *B. malayi* and *Wolbachia* across the life cycle of the parasite using dual RNA-seq. With these data, we built a co-expression network for the two organisms using weighted gene correlation network analysis (WGCNA). WGCNA is a well-established method by which expression data and trait data are integrated to identify co-expressed pathways. This allowed us to pinpoint functional pathways involved in this essential symbiotic relationship provided by the co-expression of nematode and bacterial genes. For example, during female worm development we find that *Wolbachia* upregulate genes involved in ATP production and purine biosynthesis, as well as genes involved in the oxidative stress response. We have also identified co-expressed pathways required for molting of the worm from L3 to L4, the molt which marks the establishment of infection in the human host. In parallel efforts, these data are also being used to characterize the endosymbiotic relationship at the metabolic level using Flux Balance Analysis, identifying choke points that could be exploited for therapy. By creating a draft metabolic network for *B. malayi* and *Wolbachia*, and using *in silico* knockouts, we will determine the necessary pathways for growth and virulence and determine how these pathways are influenced by the presence of *Wolbachia*.

Dendritic cells respond to particles from the *Echinococcus granulosus* laminated layer by a mechanism akin to "membrane affinity triggered signaling" but with distinctive features

ÁLVARO PITTINI[1], CECILIA CASARAVILLA[1], YAMILA MARTÍNEZ[1], DOMINIK RÜCKERL[2], JUDITH E. ALLEN[2], **ALVARO DÍAZ[1]**

[1] CÁTEDRA DE INMUNOLOGÍA, FACULTADES DE QUÍMICA Y CIENCIAS, UNIVERSIDAD DE LA REPÚBLICA, URUGUAY. [2] FACULTY OF BIOLOGY, MEDICINE AND HEALTH, SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF MANCHESTER, UK

The tissue-dwelling larva of the cestode *Echinococcus granulosus* protects itself with a mucin-based acellular coat called the laminated layer (LL). Particles from this structure need to be shed to allow parasite growth. We have previously determined that mouse bone marrow-derived GMCSF dendritic cells (BMDCs) respond to a preparation of LL mucin particles (pLL) with an unconventional maturation program that includes upregulation of CD80/CD86 but not CD40, and become defective in their capacity to upregulate CD40 in response to TLR agonists. In addition, in TLR agonist-primed BMDCs, pLL activates the NLRP3 inflammasome. Several particulate activators of the NLRP3 inflammasome, including alum, are proposed to act on DCs via "membrane affinity triggered signaling" (MATS). MATS is based on the interaction of particles with plasma membrane lipid polar headgroups, causing lipid raft clustering and receptor-independent activation of the Syk kinase. Here we report that the effects of pLL in BMDCs fulfil conditions associated with MATS, namely: (i) dependence on Syk, (ii) dependence on class I PI3K and on the actin cytoskeleton, even in the absence of phagocytosis, (iii) apparent lack of requirement for molecular-level agonistic motifs in the material. Paradoxically given the PI3K requirement, the interaction with pLL inhibits the activation of Akt, an effect not caused by alum. The inhibition is reflected in diminished phosphorylation of the direct Akt target GSK3 and decreased activity of mTORC1 in terms of p70S6K phosphorylation. The GSK3 hypophosphorylation, known to be associated with enhanced kinase activity, is responsible for the observed defect in CD40 upregulation. We speculate that the MATS mechanism as activated by pLL may have distinctive features that lead to the effects on Akt, GSK3 and CD40. Although triggering MATS in DCs may be an inevitable property of hydrophilic particles, the capacity to inhibit Akt activation may represent a specific adaptation by the parasite.

Antigenic cross-reactivity between *Schistosoma mansoni* and allergens: towards a possible alternative explanation for the hygiene hypothesis based on cross-reactive carbohydrate determinants (CCDs).

MICHAEL J. DOENHOFF[1], JOSEPH E. IGETEI[1], MARWA EL-FAHAM[1], FATOU GAI[1,2], FRANCO H. FALCONE[2], GABRIELE SCHRAMM[3]

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Allergies and other disorders of the immune system have recently increased markedly, particularly in economically advanced countries. The 'hygiene hypothesis' ascribes this to reduced exposure to microbial and parasitic infections and consistent with this, some people with helminth infections are protected against allergic disorders. It has been known for some time that glycoproteins of invertebrates and plants are antigenically cross-reactive due to their carrying carbohydrate epitopes in common (cross-reactive carbohydrate determinants – CCDs). In preliminary experiments rabbit IgG antibodies raised against *Schistosoma mansoni* egg antigens reacted in Western immunoblots with a wide range of molecules in different plants and invertebrates known to be causes of allergy. We have investigated this antigenic cross-reactivity with respect to: (i) determining whether the rabbit anti-*S. mansoni* antibodies reacted with known allergens in the plant and invertebrate extracts; and (ii) determining which *S. mansoni* egg antigens may have induced the allergen cross-reactive antibodies. Allergen molecules which have been found to be reactive in immunoblots with rabbit anti-*S. mansoni* antibodies include: *Hev b 7* in rubber latex, *Ara h 1* in peanut, 5 different known allergens in Timothy grass and birch tree pollens, *Der f 15* from *Dermatophagoides farinae* (house dust mite), a *Per a 3* homologue from cockroach and bee venom phospholipase A. The rabbit IgG antibodies reactive with the above-mentioned allergens were purified by elution from immunoblotted allergen using low pH buffer. These acid-eluted antibodies reacted with 3 immunodominant *S. mansoni* egg antigens IPSE/alpha-1, omega-1 and kappa-5 (though the reactivity with IPSE/alpha-1 may be due to its non-immunological complexing with immunoglobulins). If the results are substantiated with similar observations using sera from schistosome-infected humans, they may offer an explanation for the hygiene hypothesis in terms of schistosome-induced IgG anti-CCD antibodies 'blocking' the reactivity of allergenic IgE antibodies.

Liver fluke secretions drive wound repair and cancer

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The liver flukes *Opisthorchis viverrini* and *Clonorchis sinensis* are considered group 1 carcinogens by the International Agency for Research on Cancer. The only other helminth parasite to have earned this status is *Schistosoma haematobium* due to its association with squamous cell carcinoma of the bladder. The mechanisms by which *O. viverrini* induces cancer are multi-factorial, but we have shown an important role for excretory/secretory (ES) products in this pathogenic process. Both soluble fluke proteins and secreted extracellular vesicles (EVs) can promote proliferation of and cytokine secretion by human cholangiocytes, drive angiogenic processes and accelerate wound repair in vitro and in vivo. Using high-resolution 3D-SIM microscopy and proteomic analyses we showed that liver fluke EVs are internalised by human cell lines and that EV proteins can be detected in bile fluid from infected humans and hamsters. Importantly, antibodies against tetraspanin proteins on the EV surface can block vesicle uptake by host cells, providing a unique target for development of vaccines against parasitic helminths.

WormBase ParaSite in 2017 and beyond

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WormBase ParaSite (http://parasite.wormbase.org) was established 3 years ago as a comprehensive effort to integrate, organise and present data for all nematode and platyhelminth genomes. We currently include genome data for over 100 species, adding value by way of systematic and consistent functional annotation (e.g. protein domains and Gene Ontology terms), gene expression analysis (e.g. alignment of life-stage specific RNASeq sets), and comparative analysis (e.g. orthologues and paralogues). We provide several ways of exploring the data, including genome and gene summary pages, text search, sequence search, a query wizard, bulk downloads, a choice of genome browsers, and a programmatic interface. We will present the core features of WormBase ParaSite, with particular attention paid to recent improvements to functionality. We will also present our plans for the next major phase of development of the project, including enriched suite of tools for exploring gene expression, genomic variation and druggability. We are particularly interested in use-cases and suggestions from the community to help us prioritise future work.

A study of *Fasciola hepatica* virulence and invasion using omics tools

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Infection of the mammalian host with the trematode parasite *Fasciola hepatica* involves parasite activation followed by penetration and migration through the intestinal wall to the liver. As the parasite migrates it is continually developing and growing whilst encountering different host molecules, tissues and micro-environments. Here we report the interrogation of transcriptome and proteome data to investigate the first 24 hours of *F. hepatica* infection, focussing on the infective stage, the metacercariae, and the invasive migratory stage, the newly excysted juveniles (NEJ). Gene transcription analysis within the metacercariae has revealed that metabolic genes are 'switched on' contrary to the view of these stages being dormant. Specifically, the genes associated with aerobic energy metabolism pathways show comparable levels of transcription between the metacercariae and NEJ 1 hour post-excystment. Analysis of the NEJs during the first 24 hours has revealed remarkable differential gene expression. Associated with the up-regulation of gene transcription by the 24hr NEJ is the development of neoblast-like cells that are stimulated and regulated by temperature increases. Secretome analysis shows that cathepsin cysteine proteases play an important role during the early NEJ stages, with the cathepsin L3 and cathepsin B3 proteases present within the top 10 secreted NEJ proteins that represent 70% of the total protein secreted. Localization studies reveal that both FhCL3 and FhCB1/2/3 are present within the gut of the NEJ, consistent with these proteins being secreted. Specifically, FhCB1/2/3 was observed within the newly excysted parasites, compared with FhCL3 which is observed after 1 hour post-excystment, indicating that these proteins are important at different stages of the infection/invasion process. This study provides information on how the parasite prepares for infection and the changes that it must undergo once within the mammalian host, which can be exploited for control strategies to target this early stage of infection.

Molecular analysis of genes induced by blood feeding in *N. brasiliensis*

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The nematode *N. brasiliensis* is a soil dwelling parasites of rodents that has many of the life-cycle characteristics seen in human hookworm infection. After hatching, early larval forms go through a number of moults to reach the iL3 stage that is able to penetrate the skin of its rodent host. It then adopts a physiology and form that enables it not only to survive within its mammalian host but also to mature through further moults to the adult L5 form that can reproduce. Adults mate and females then produce eggs that are dispersed via the gastrointestinal tract back into the environment. We have found that some aspects of the changes that accompany life within its mammalian host can be modelled *in vitro* simply by incubating iL3 stage worms, obtained through standard faeces/charcoal culture, at 37˚C in the presence of blood or haemoglobin (Hb). Indeed, blood-fed iL3 increase in size, remodel their buccal cavity and deposit iron-containing pigment in their gut much as worms do during maturation in the mouse lung. We used RNAseq analysis of worms exposed to elevated temperature and blood/Hb *in vitro* to identify genes that are differentially regulated between the different stages and that could potentially be important during the corresponding developmental transitions. We will present data on these genes, including a subset that is potentially critical for worm survival in a mammalian host and could represent important new targets for vaccine design against human hookworm.

Characterization of *Schistosoma mansoni* fucosyltransferases for glyco-engineering of 'native' helminth N-glycan structures in planta

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Secretory glycoproteins of parasitic helminths are in the spotlight as biopharmaceuticals because of their strong immunomodulatory properties. Clinical trials with live parasites and mouse model studies have shown the potential of helminths and their excretory/secretory (ES) proteins to dampen allergic reactions and autoimmune disorders. Moreover, glycan-dependent mechanisms of action have been shown to be involved in several cases. To further develop helminth-derived ES glycoproteins as biopharmaceuticals, a large-scale expression system is required for the production of recombinant glycoproteins with defined and tailored glycosylation. The trematode *Schistosoma mansoni* produces highly fucosylated N-glycan structures on its glycoproteins, which cannot be synthesized in current production systems. Thereto, co-expression of specific fucosyltransferases in the expression host are required to introduce helminth-like N-glycan modifications. In the GeneDB database 20 different *S. mansoni* fucosyltransferase genes for N-glycosylation can be found. These fucosyltransferases are divided into two groups based on the type of linkage that is formed upon fucose transfer (α1,3 or α1,6). To date one α1,3 fucosyltransferase was characterized *in vitro* using glycan acceptors and was shown to synthesize Lewis X. Since *in vitro* and *in vivo* characterization may differ, characterization in a biological setting, using the Golgi-system, can be more relevant. Thereto, we examined the function of ten selected *S. mansoni* fucosyltransferases by transient co-expression with model proteins in *Nicotiana benthamiana* plants. With this method we have identified *S. mansoni* fucosyltransferases that fucosylate LDN, synthesize Lewis X or are involved in core fucosylation. These functionally characterized fucosyltransferases can immediately be applied to synthesize desired helminth-like N-glycan structures on recombinant glycoproteins in the plant. Therefore characterization of *S. mansoni* fucosyltransferases, other glycosyltransferases and combinations of different glycosyltransferases expands our glyco-engineering toolbox and offers perspectives for large scale production of glycoproteins with functional helminth N-glycan structures in plants.

Local amplifiers of type 2 immunity during helminth infection

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Macrophages activated via the IL-4 receptor alpha (IL-4Rα) are central effectors of the type 2 immune response that controls helminth infection. However, our understanding of how IL-4Rα signals are regulated within the tissue environment remains limited. We found that secreted soluble defense collagens SP-A and C1q are tissue-specific factors that enhance type 2 mediated macrophage activation and proliferation. These proteins act through the atypical myosin, Myo18a, whose surface expression on tissue macrophage is positively regulated by IL-4, revealing an amplification loop for type 2 immune responses in the local tissue. Critically, the local dynamics of macrophage proliferation, activation and recruitment differ between strains of mice that are resistant and susceptible to nematode infection.

Interleukin 10 signaling, but not IL-22 or IL-28, promotes intestinal colonization resistance to opportunistic pathogens and controls immunopathology during whipworm infection

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Whipworms (*Trichuris trichiura*) are soil-transmitted helminths that dwell in the epithelium of the cecum and proximal colon of their hosts, causing the human disease, trichuriasis. Trichuriasis is characterized by colitis attributed to the inflammatory response elicited by the parasite while tunneling through intestinal epithelial cells (IECs). While it is known that IL-10 is critical to prevent morbidity and mortality upon infection of mice with *T. muris* (a mouse model of *T. trichiura* infection in humans), the specific contribution of the members of the IL-10 family of receptors on the regulation of the host responses to whipworm infection remains unclear. Here, we carefully dissected the role of IL-10rα, IL-10rβ, IL-22rα and IL-28rα in the resistance of mice to *T. muris* infections. Our findings demonstrate that while IL-22rα and IL-28rα are dispensable in the host response to whipworms, IL-10 signaling through IL-10rα and IL-10rβ is essential to control cecal pathology, worm expulsion and survival during *T. muris* infections. Interestingly, we found that deficiency of IL-10rα and IL-10rβ results in dysbiosis of the cecal microbiota with an expansion of Enterococcaceae and Enterobacteriaceae, families described as opportunistic bacteria. Moreover, the break of the epithelial barrier after whipworm infection of IL-10rα and IL-10rβ deficient mice, allows the translocation of these opportunistic pathogens to the liver causing organ failure and lethal disease. Importantly, bone marrow chimera experiments indicate that signaling through IL-10rα and IL-10rβ in hematopoietic cells, but not IECs, is crucial to control worm expulsion and immunopathology. On-going experiments focus on understanding the role of IL-10rα in the regulation of macrophage and IECs responses that promote the expulsion of whipworms and mucosal homeostasis. Our findings emphasize the pivotal function of IL-10 signaling in promoting intestinal colonization resistance to opportunistic pathogens and maintaining the intestinal epithelial barrier thus preventing immunopathology during whipworms infections.

Microsporidia infection in *C. elegans*: how an obligate intracellular parasite makes itself at home

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Little is known about natural pathogens of *C. elegans*. We have identified and characterized a natural intracellular pathogen of *C. elegans* that we are calling *Nematocida parisii*, or nematode-killer from Paris (we found this pathogen in a worm isolated from a compost pit near Paris). This work was published in Troemel et al 2008, PLoS Biology. *N. parisii* defines a new genus and species of microsporidia, which comprise a large phylum of intracellular parasites that are most closely related to fungi. Microsporidia are increasingly appreciated to be a serious medical problem, as 15 different species have now been shown to infect humans, most commonly causing intestinal infections. Microsporidia are on the NIH "priority pathogens" list. We have exploited our *C. elegans*/microsporidia model to investigate how microsporidia escape out of host intestinal cells. By tracking when animals are infectious to others and examining different structures within infected intestinal cells, we have found that a cytoskeletal structure called the terminal web is restructured by microsporidia, probably as part of a strategy to escape from host cells. It appears that previously identified pathways important for defense against bacterial infection, like the p38 MAPK pathway, are not important for defense against microsporidia. We are interested in determining which defense pathways *C. elegans* uses to fight off microsporidian infection, with the goal of understanding how the innate immune system controls this medically and agriculturally relevant but poorly understood class of pathogens.

Molecular dissection of mosquito resistance to heartworm infection

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Mosquitoes are essential for the life cycle of the heartworm *Dirofilaria immitis*. Importantly, not all mosquitoes support *D. immitis* infection and identification of factors that restrict infection will likely reveal important molecular interactions that can be targeted to block *D. immitis* infection in mosquitoes. To identify such factors, we have undertaken a comparative analysis of *D. immitis* susceptible and refractory strains of *Aedes aegypti*. While ingested microfilariae migrate from the blood meal to the Malpighian tubules and develop into transmission-stage infective L3 larvae in susceptible mosquitoes, in the refractory strain, microfilariae arrest in Malpighian tubules. Previous genetic crosses between the strains have shown that refractoriness is dominant and exhibits simple Mendelian inheritance. To identify candidate genes responsible for the refractory phenotype, we have performed transcriptional RNA-seq profiling of Malpighian tubules from both strains isolated either prior to blood feeding or 1, 2, and 3 days post feeding on blood containing *D. immitis* microfilariae. We analyzed the RNA-seq data for genes with significantly different expression levels between the strains. Amongst the differences, we found a striking increase in immune gene expression in response to infection in the refractory strain at day 2 and 3 post infection. We also detected heartworm transcripts, which increased in abundance over the time course in susceptible mosquitoes. We have begun screening our candidates by RNAi to determine if preventing their expression can reverse the reverse the refectory phenotype. These studies will identify candidate genes that can be introduced into susceptible mosquito populations via transgenesis to block transmission. New strategies targeting *D. immitis* in its vector has the potential to prevent heartworm transmission to refugia where significant populations of parasites are maintained and are beyond the reach of current control measures.

Association mapping of genes in the snail, *Biomphalaria glabrata*, that confer resistance to *Schistosoma mansoni*

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We have found three regions in the genome of *Biomphalaria glabrata* in which allelic variation influences resistance to infection by *Schistosoma mansoni*. The approaches we used include genome-wide association studies (GWAS) on outbred snails, GWAS on multiple inbred lines derived from the same outbred population, and outlier locus analysis on replicate lines selected to have high resistance. Candidate genes are currently undergoing functional testing. We will also describe a novel approach we used to create a high density linkage map of the *B. glabrata* genome. The current genome assembly is still quite fragmented (over 300,000 scaffolds, most about the size of a gene), making it not that useful for association mapping (i.e. one often cannot know what candidate genes surround a significant marker). Our new map joined 74% of the non-repetitive, gene-containing fraction of the genome into 18 linkage groups that correspond to the 18 chromosomes. This resource greatly advances our ability to do association mapping in *B. glabrata*.

Schistosome infectivity in *Biomphalaria glabrata* is dependent on the Guadeloupe Resistance Complex, and is partially explained by the expression of a single novel protein, Grctm6.

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Schistosomiasis is currently the most devastating helminth disease. Despite relatively effective chemotherapeutic treatments some recent resistance to these treatments has been found, there is no vaccine alternative, and reinfection is common. Understanding and controlling the natural intermediate snail hosts of schistosome parasites is vital to the suppression of this disease, and is currently believed to be the most effective measure for eliminating schistosomiasis from any given region. The Guadeloupe Resistance Complex (GRC), a recently identified genomic region, strongly influences the resistance of Caribbean *Biomphalaria glabrata* snails to infection by *Schistosoma mansoni.* The GRC region contains novel genes which have structural similarity to known pathogen recognition proteins, and there are three distinct haplotypes in the GRC region. Two of these haplotypes are equally susceptible to infection and one is highly resistant to infection. Here we histologically characterise the early clearance of schistosomes from the three distinct haplotypes of the GRC, and show that resistance can be adoptively transferred to susceptible snails using resistant hemolymph. Additionally, we elucidate the structure and role of one of the novel genes in the GRC, *grctm6*. We characterised the tissue expression of Grctm6 in a population of Caribbean snails using custom antibodies, and performed a siRNA knockdown this protein *in vivo*. We show that Grctm6 is not only expressed in *B. glabrata* hemolymph, but that it also has a role in modulating the number of *S. mansoni* cercariae released by infected snails, making it a possible target for the biological control of schistosomiasis.

Establishing *C.elegans* as a tractable system for genetic and drug screens to find modulators of malate dismutation

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Many parasitic helminths occupy anaerobic niches in their hosts. To survive, they cannot use aerobic respiration to produce ATP, but must use alternative pathways. Many parasites (e.g. *Ascaris* and hookwarms) rely on malate dismutation (MD) to generate ATP in their host and this requires an unusual electron carrier, Rhodoquinone (RQ). MD and the pathway of RQ synthesis provides an excellent target for anthelmintics since vertebrate hosts do not use MD and do not make RQ. However no such commercial drugs exist and the enzymes required for RQ synthesis are not known. *C.elegans* is known to make RQ and to be able to use MD. Our goal is to establish conditions in which *C.elegans* uses MD and then conduct genetic screens to identify the enzymes required for RQ synthesis and do drug screens for compounds that interfere with either MD or RQ synthesis. So far, we established an image-based assay that can measure precisely the level of movement in a population of worms. We found that while treating worms with cyanide (KCN) alone results in paralysis through inhibition of the Electron Transport Chain (ETC), when worms are treated with both KCN and a glycolysis inhibitor they are initially paralysed but then unexpectedly recover complete movement. They are thus able to recover by making ATP in a manner than uses neither aerobic metabolism NOR anaerobic glycolysis — one clear possibility is they now use MD. We find that the recovery requires the hypoxia response and complex I activity (as expected for MD). Furthermore, we examined a time course of gene expression across recovery and identify major changes in expression of key metabolic genes consistent with using MD. Finally, we used metabolomics to profile metabolite levels across recovery. We present these results here as well as results of a screen for mutants that cannot recover.

Development of murine models of loiasis and their applications in filarial drug development, diagnostics and immunopathology research

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Both the deployment of current microfilaricides and the development of new macrofilaricides to eliminate onchocerciasis in Africa requires consideration of loiasis co-endemicity. This is because rapid killing of *Loa loa* microfilariae (mf) following treatment in patients with *Loa* hypermicrofilariaemias is linked to the development of severe inflammatory adverse reactions. Study of loiasis at the preclinical level is currently hampered by lack of appropriate small animal models. Here we describe the development of novel mouse models of loiasis and demonstrate their applicability as in vivo microfilaricide drug screens, to identify circulating biomarkers or to study inflammatory responses associated with microfilaricidal treatment. BALB/c WT or SCID were perfused with *Loa* mf. CB.17 SCID, NOD.SCID, NOD.SCID IL-2gc$^{-/-}$ (NSG), BALB/c RAG$^{-/-}$ or RAG$^{-/-}$ IL-2gc$^{-/-}$ (RAGG) strains were infected with *Loa* L3 and evaluated at 3-6 months post-inoculation. Adults were surgically implanted in NSG or RAG$^{-/-}$ mice and evaluated +1 month post-implantation. To evaluate drug responsiveness, microfilariaemic mice were treated with ivermectin (IVM). SCID mice consistently yielded microfilaraemias (~10% of initial inoculates) +7 days post perfusion whereas WT mice yielded significantly lower recoveries. Compound immunodeficient mice (NSG or RAGG) yielded an average recovery of male and fecund female worms of >10% of the initial inoculate at +5-6 months, with microfilaraemias evident. The majority of *Loa* adults (60%) survived one-month post-implant into recipient mice and produced circulating mf. Sera of all mice with patent *Loa* infections tested positive by filarial test strip developed to detect *Wuchereria bancrofti* circulating antigen. IVM induced a rapid decline (>90%) in circulating mf in all mouse strains and efficacy was bolstered in vaccinated WT mice. Multiple circulating pro-inflammatory mediators were identified post-IVM treatments in WT mice when assessed by luminex multiplex analysis. Thus, validated mouse models of loiasis have been established with potential for both translational and basic biological research applications.

Evaluation of the Pharmacokinetic-Pharmacodynamic Relationship of Praziquantel in the *Schistosoma mansoni* Mouse Model: Possible Clinical Implications

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After more than 40 years of use, Praziquantel (PZQ) still remains the drug of choice for the treatment of intestinal and urogenital schistosomiasis. Its anti-parasitic activity resides essentially in the (*R*)-enantiomer. Hitherto neither the molecular target nor the pharmacokinetic-pharmacodynamic relationship have been elucidated. Here we investigated the efficacy and pharmacokinetics of PZQ in the *Schistosoma mansoni* mouse model to determine the key factors that drive its efficacy. Dose-response studies with racemic PZQ with or without addition of an irreversible pan-cytochrome P450 (CYP) inhibitor, 1-aminobenzotriazole (ABT), were performed. In addition, efficacy of PZQ in the presence of the CYP inducer, dexamethasone (DEX), was determined. Plasma samples were obtained by tail vein bleeding at 4 time points. The (*R*)-PZQ levels were determined using a LC-MS/MS method. Non-compartmental pharmacokinetic analysis was performed using PKsolver. In addition, *in vitro* experiments in a host-mimicking environment were conducted. We found that the use of ABT increased (*R*)-PZQ plasma exposures in the systemic circulation by ~10-20 fold but were not predictive of efficacy. The use of DEX decreased plasma exposures of (*R*)-PZQ in the systemic circulation by ~10 fold without reducing efficacy. We extrapolated the (*R*)-PZQ concentrations in mouse portal vein / mesenteric veins from the systemic exposures and found that a free exposure of (*R*)-PZQ of ~ 20 µM*h in the portal vein was needed to obtain a worm burden reduction >60%. This was corroborated by *in vitro* experiments where an exposure to free (*R*)-PZQ of ~ 25 µM for 1 h was required to effectively reduce the viability of *S. mansoni* adult worms.

Why does Oxamniquine kill *Schistosoma mansoni* but not *S. haematobium* or *S. japonicum* and can we synthesize a derivative that kills all 3 species of schistosomes?

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The major species of *Schistosoma* affecting humans are *S. mansoni, S. haematobium,* and *S. japonicum.* There is currently only one method of treatment (monotherapy), the drug Praziquantel. Constant selection pressure through mass chemotherapy - this year will see the administration of over 250 million doses - has yielded evidence of resistance to PZQ. Previous treatment of *S. mansoni* included the use of oxamniquine (OXA), a prodrug that is enzymatically activated in *S. mansoni* but is ineffective against *S. haematobium* and *S. japonicum*. The OXA activating enzyme was identified by our laboratories as being a sulfotransferase (SmSULT). One focus of this research is to understand why OXA does not kill S. haematobium or S. japonicum and with this information reengineer OXA to be effective against *S. haematobium* and *S. japonicum*. An alignment of the sulfotransferases (SULT) shows that SmSULT, ShSULT and SjSULT share considerable sequence identity (71% Sm/Sh; 58% Sm/Sj, and 58% Sh/Sj) and predicted structural similarity. We sought to understand how differences in the amino-acid composition of Sm-, Sh-, and SjSULTs gave rise to species-specific drug action. Using site-directed mutagenesis, we demonstrated that SmSULT modified to look like ShSULT and vice versa each could activate OXA in an in vitro assay ie the SULTs were functional. We next evaluated the transcriptional differences between the SULTs by qPCR and Digital PCR. SmSULT transcription was 100X ShSULT and 1000X SjSULT. The differences in transcription account in part for the inability of OXA to be cidal in Sh and Sj. Next we employed an iterative process which lead to the identification an OXA derivative (CIDD790) that is effective against *S. mansoni* (100% killing), *S. haematobium* (80% killing) and *S. japonicum* (80% killing). These results demonstrate that understanding the mechanism of action of a drug and its structure function relationship can lead to novel cidal drugs.

Engaging worms for health - drug discovery on live schistosomes

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Schistosomes are parasitic worms endemic in sub/tropical countries. Worldwide at least 218 million people required preventive treatment against schistosomiasis in 2015 and more than 200,000 deaths occur each year in Sub-Saharan Africa alone. The drug of choice for treating schistosomiasis is praziquantel. However, praziquantel has only limited efficacy in immunocompromised hosts, is less effective in young worms and resistance is emerging. Thus, new drugs are required. We have established an *in vitro* culture protocol that mimics the *in vivo* environment of the worms and allows large numbers of *Schistosoma mansoni* larvae to be grown into adults. Using this approach, schistosome life cycle stages (schistosomula, juveniles and adults) were exposed to compounds from various drug libraries for anti-schistosomal agents. Drug effects on the parasites were microscopically assessed and recorded at several time points. Thus, changes in morphology and motility of the worms as well as the time course of drug action could be precisely characterised and saved by photo/video documentation. This approach revealed drug/group-specific patterns of action and morphological/functional changes such as early vs. late onset of effects, hyper-activity vs. paralysis, shrinkage vs. extension, empty vs. filled guts, circular contractions vs. ballooning of the worms. As a secondary finding, compounds with anti-neoplastic/cytostatic activity became apparent due to adverse effects on the host cells present in the culture. Notably, the analysis of approved drug libraries revealed several drugs with so-far unknown activity against schistosomes with at least one of them being potentially equivalent to praziquantel.

Recent advances in pathogenesis of the carcinogenic liver fluke *Opisthorchis viverrini* and bile duct cancer

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Liver fluke infections caused by *Clonorchis sinensis* and *Opisthorchis viverrini* are major foodborne parasitic zoonotic diseases with over 30 million people infected. The infections are associated with several hepatobiliary diseases including cholangiocarcinoma (CCA), a fatal liver cancer arising from the bile duct epithelium. Opisthorchiasis caused by *Opisthorchis viverrini* infection is a major public health in Thailand and neighboring Mekong countries. The infection is associated with cholangitis, cholecystitis, gallstones, hepatomegaly, periductal fibrosis and cholangiocarcinoma (CCA). The rates of CCA in regions where the parasite is endemic are unprecedented. Host-parasite interaction by liver fluke's tegument and excretory-secretory products through endocytosis pathway drive biliary epithelial proliferation and production of inflammatory cytokines. This induces severe inflammation of the bile ducts, resulting in oxidative and nitrative DNA damage of the biliary epithelium. *Opisthorchis* specific pro-inflammatory cytokine/chemokine, specifically IL-6 and IL-8 production through biliary TLR4 activation was observed, supports inflammatory mechanism of the infected bile ducts. Elevation of IL-6 production is associated with advanced periductal fibrosis in infected individuals. IL-6 can induce inflammation, anti-apoptosis, cell transformation and eventually malignancy. Moreover, liver fluke excretory-secretory products including certain parasite antioxidant proteins and inhibitor of apoptosis protein can inhibit biliary cell apoptosis when cells underwent oxidative stress. These cells are thus stimulated to uncontrolled hyper-proliferate, providing an additional potential mechanism by which inflamed biliary epithelial cells become neoplastic in opisthorchiasis-associated CCA. In addition, our recent findings indicated that certain *Helicobacter* spp inhabited in the liver fluke may orchestrate the pathogenesis of opisthorchiasis and associated CCA.

Secreted serine protease SmSP2 from *Schistosoma mansoni* with hemostatic role during host infection.

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Hemostatic factors are known to be produced by schistosomes, but information about exact molecules involved is scarce. This the first time that characterized protease is directly identified to be participating in these processes. SmSP2 protease from the human blood flukes Schistosoma mansoni was identified in our laboratory previously. It belongs to an unusual novel trypsin-like group that is unique for platyhelminth parasites as it contains thrombospondin type 1 repeat (TSR-1) not present in other serine proteases. Protease has an endopeptidase trypsin-like activity, but with a limited ability to process protein substrates. However, interestingly SmSP2 was found to activate the key components of the fibrinolytic system, tissue plasminogen activator and plasminogen, and releases vasoregulatory kinin from kininogen. Our results suggest that secreted SmSP2 contributes to the survival of the parasite at the host-parasite interface by release of host vasodilatory factors and activation of fibrinolytic system. These critical properties make SmSP2 crucial factor for successful infection and a potential target for anti-schistosomal therapies.

Functional diversity of secreted cestode Kunitz proteins: inhibition of serine peptidases and blockade of cation channels

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We previously reported a multigene family of monodomain Kunitz proteins from *Echinococcus granulosus* (*Eg*KU-1-*Eg*KU-8), and provided evidence that some *Eg*KUs are secreted by larval worms to the host interface. In addition, functional studies and homology modeling suggested that, similar to monodomain Kunitz families present in animal venoms, the *E. granulosus* family could include peptidase inhibitors as well as channel blockers. Using enzyme kinetics and whole-cell patch-clamp, we now demonstrate that the *Eg*KUs are indeed functionally diverse. In fact, most of them behaved as high affinity inhibitors of either chymotrypsin (*Eg*KU-2-*Eg*KU-3) or trypsin (*Eg*KU-5-*Eg*KU-8). In contrast, the close paralogs *Eg*KU-1 and *Eg*KU-4 blocked voltage-dependent potassium channels (K_v); and also pH-dependent sodium channels (ASICs), while showing null (*Eg*KU-1) or marginal (*Eg*KU-4) peptidase inhibitory activity. We also confirmed the presence of *Eg*KUs in secretions from other parasite stages, notably from adult worms and metacestodes. Interestingly, data from genome projects reveal that at least eight additional monodomain Kunitz proteins are encoded in the genome; that particular *Eg*KUs are up-regulated in various stages; and that analogous Kunitz families exist in other medically important cestodes, but not in trematodes. Members of this expanded family of secreted cestode proteins thus have the potential to block, through high affinity interactions, the function of host counterparts (either peptidases or cation channels) and contribute to the establishment and persistence of infection. From a more general perspective, our results confirm that multigene families of Kunitz inhibitors from parasite secretions and animal venoms display a similar functional diversity and thus, that host-parasite co-evolution may also drive the emergence of a new function associated with the Kunitz scaffold.

Apoplastic venom allergen-like proteins of plant parasitic nematodes modulate the activation of plant innate immunity by cell surface receptors

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Despite causing considerable damage to host tissue during the onset of parasitism, nematodes establish remarkably persistent infections in both animals and plants. It is thought that an elaborate repertoire of effector proteins in nematode secretions suppresses damage-triggered immune responses of the host. However, the nature and mode of action of most immunomodulatory compounds in nematode secretions are not well understood. Here, we show that venom allergen-like proteins of plant-parasitic nematodes selectively suppress host immunity mediated by surface-localized immune receptors. Venom allergen-like proteins are uniquely conserved in secretions of all animal- and plant-parasitic nematodes studied to date, but their role during the onset of parasitism has thus far remained elusive. Knocking-down the expression of the venom allergen-like protein Gr-VAP1 severely hampered the infectivity of the potato cyst nematode *Globodera rostochiensis*. By contrast, heterologous expression of Gr-VAP1 and two other venom allergen-like proteins from the beet cyst nematode *Heterodera schachtii* in plants resulted in the loss of basal immunity to multiple unrelated pathogens. The modulation of basal immunity by ectopic venom allergen-like proteins in *Arabidopsis thaliana* involved extracellular protease-based host defenses and non-photochemical quenching in chloroplasts. Non-photochemical quenching regulates the initiation of the defense-related programmed cell death, the onset of which was commonly suppressed by venom allergen-like proteins from *G. rostochiensis*, *H. schachtii*, and *Meloidogyne incognita*. Surprisingly, these venom allergen-like proteins only affected the programmed cell death mediated by surface-localized immune receptors. Furthermore, the delivery of venom allergen-like proteins into host tissue coincides with the enzymatic breakdown of plant cell walls by migratory nematodes. We therefore conclude that parasitic nematodes most likely utilize venom allergen-like proteins to suppress the activation of defenses by immunogenic breakdown products in damaged host tissue.

Schistosoma japonicum exosome-delivered miRNAs modulate immune responses in host macrophages

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Schistosomes can survive in host vascular system for years despite a strong immune response. Deep characterization of host-pathogen interactions to support schistosome survival may open novel perspectives for developing a novel strategy against schistosomiasis. In the present study, we find that exosomes secreted from *Schistosoma japonicum* can transfer their cargo miRNAs into host macrophages to inhibit cell proliferation and promote cell apoptosis. RNA-seq analysis of murine macrophages treated with *S. japonicum* exosomes revealed the potential key role of exosomal miRNAs in the regulation of tumor necrosis factor (TNF) and toll-like receptor (TLR) signaling pathways. A reporter assay further demonstrated that some signaling molecules involved in these pathways were targeted in macrophages by exosomal bantam miRNA or miR-125b. In addition, some of these signaling molecules were also shown to downregulate in macrophages treated with *S. japonicum* exosomes as well as in lymphocytes isolated from mice infected with *S. japonicum* cercariae. Transfection of exosomal miRNAs (bantam miRNA or miR-125b mimics) in macrophages activated TNF and TLR signaling pathways by targeting CLMP and PROS1, respectively, as result of the inhibition of cell proliferation and promotion of cell apoptosis. Collectively, our findings uncover important roles of *S. japonicum* exosomal miRNAs in the modulation of immune response to schistosome infection.

Programmed DNA Elimination in Nematodes

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Programmed DNA elimination is a developmentally regulated process leading to the reproducible loss of specific genomic sequences. In ascarid nematodes, programmed DNA elimination removes specific DNA sequences from the genome during early development in somatic cells (4-16 cell stage), leaving the germline genome intact. We have generated germline and somatic reference genome sequences for *Ascaris suum*, and the horse parasite, *Parascaris univalens.* In addition, we carried out in-depth analyses of DNA elimination in the nematode parasite of humans, *Ascaris lumbricoides,* and a parasitic nematode of dogs, *Toxocara cani*s. During DNA elimination, chromosomes break, fragments of some chromosomes are not segregated and lost, whereas others are healed by telomere addition and retained. The same DNA is eliminated independently in all five different *Ascari*s pre-somatic cells that give rise to different cell lineages. In all four` species, repetitive sequences (that differ among the genera) and germline-expressed genes (~1,000-2,000 or 5-10% of the genes) are eliminated. Thirty-five percent of these eliminated genes are conserved among these nematodes, defining a core set of eliminated genes that are preferentially expressed during spermatogenesis. This supports the view that nematode DNA elimination silences germline expressed genes. No sequence motifs or other characteristics were identified that might mark or identify the conserved sites for chromosomal breakage. However, immediately preceding DNA elimination, these chromosomal breakage regions become more open or accessible based on ATAC-sequencing. We show that *Ascaris* has holocentric chromosomes in the germline evidenced by centromeres/kinetochores distributed along the length of the chromosomes. Prior to DNA elimination in the four-cell embryo, CENP-A, the epigenetic mark of centromeres, is significantly diminished in chromosome regions that will be lost. This leads to the absence of kinetochores and microtubule attachment sites necessary for chromosome segregation, resulting in loss of these chromosome regions during mitosis. These data suggest that CENP-A localization contributes to the identification of regions to be retained and lost playing a regulatory and mechanistic role in DNA elimination. Finally, we identified two worm specific Argonautes (WAGO) associated with condensed chromosomes during DNA elimination. One WAGO preferentially associates with retained DNA only during a DNA elimination mitosis. The other WAGO is enriched on DNA that will be eliminated. Thus, these WAGOs, their associated small RNAs and/or proteins may play a role in nematode DNA elimination.

Chromatin profiling of single larvae of *Schistosoma mansoni*: how to get hold of the stem cells

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Schistosomes cause significant human, economic and social losses mainly in developing countries. The intense reproductive activity and the long period of infection are the primary driver of host pathology, inducing significant morbidity by hepatic inflammations. The repertoire of stem cells in reproductive organs and parasites surface self-renewing are become more frequent. Recently, important tools to study the *Schistosome* stem cells have been reported but these approaches are technically demanding requiring a large number of parasites. Here, we present ChIP-seq data performed on ultra low cell number corresponding to single miracidia and *in-vitro* sporocysts, that are enriched in stem cells. We use these data to obtain genome-wide chromatin signatures of *bona fide* stem cells. In addition, we demonstrate also that the type of the ChIP procedure and even the origin of the antibody can influence the result. We have therefore developed an experimental strategy for quality control that will also be presented.

Genome wide expression profiling of the *Echinococcus multilocularis* stem cell system

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It has been shown that the only proliferating cells in *Echinococcus multilocularis* are undifferentiated stem cells (so called “germinative cells”). However, little is known about the overall gene expression of these germinative cells. In this work, we studied the germinative cells of the fox tapeworm *Echinococcus multilocularis* through genome wide expression profiling. Metacestode larvae depleted of germinative cells by treatment with hydroxyurea or the polo-like kinase inhibitor Bi2536 show strong down-regulation of genes specifically expressed in germinative cells. Among them are genes coding for transcription factors, proteins for DNA repair and replication, nanos, the telomerase and the non-capsid protein 1 of *Densovirus*. As treatment with hydroxyurea or Bi2536 also depletes of the direct progeny of germinative cells, genes expressed in the direct progeny are down-regulated as well. To distinguish between genes specifically expressed in germinative cells and genes expressed in the direct progeny, we compared expression of genes (down-regulated after depletion of germinative cells) in early stages of primary cell cultures with later stages. Early stages are strongly enriched in germinative cells while later stages contain less germinative cells and more progeny. Therefore, genes up-regulated in the early stages are considered to be specific for germinative cells (e.g. nanos, telomerase,…) and genes up-regulated in the later stages specific for the progeny of germinative cells. Genes with no significant up- or down-regulation in early or late primary cells could either be expressed in both germinative cells and progeny or be expressed in a subpopulation of germinative cells that is also present in later primary cells. This data was confirmed by quantitative PCR and fluorescence whole mount *in situ* hybridizations for selected genes. This resource will facilitate future research on the *Echinococcus multilocularis* stem cell system and its progeny.

Treatment of helminth infections is associated with increased insulin resistance in infected subjects- a study in Indonesia

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Emerging data suggest that helminth infections are associated with lower whole-body insulin resistance (IR). Part of this effect might be as a consequence of immunological changes induced by helminth infections. A double-blind cluster-randomized trial was conducated in an area endemic for soil transmitted helminths (STH), Flores island, Indonesia. All subjects in the study area, were randomised to receive a single dose of albendazole or matching placebo for three consecutive days under direct supervision. Four rounds of this regimen were given at three monthly intervals. In total 1669 subjects were included and it was shown that albendazole treatment was associated with significant decrease in IgE and eosinophils, including some of the eosinophil granule proteins. Treatment was also associated with a small but significant increase in IR among helminth-infected subjects as detected by microscopy. Currently, the peripheral blood mononuclear cells are being analysed using mass cytometry to assess whether the change in IR is mediated via immune or non-immune mechanisms.

Lymphatic remodelling is promoted by the initial anti-filarial Th2 response to *Brugia malayi* infection

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Lymphatic Filariasis (LF) related morbidity (lymphoedema) is the third leading cause of disability, affecting 40 million patients globally. With current treatment limited to symptom management, novel therapies to prevent, or reverse pathology are urgently required. LF causes significant lymphatic remodelling and dysfunction, however the mechanisms mediating these changes are poorly understood.
An LF pathology model was developed where 100 *B. malayi* L3 larvae were subcutaneously injected into the hindfeet of mice. An intravital imaging system was utilised to track alterations in lymphatics 2-16 weeks post infection (P.I). Intracellular cytokine staining of lymph nodes (LNs), proximal to infection was undertaken and plasma harvested for multiplex protein analysis (luminex). Concurrently, an *in vitro* human (THP-1) macrophage/ dermal lymphatic endothelial cell (LEC) coculture assay was developed.
Early, significant lymphatic remodelling with dilation, collateral tortuous lymphatics and dermal back flow was observed 2 weeks P.I., with concomitant significant expansion of IL-4/IL-13 expressing CD4 T cells in local LNs observed. Lymphatic remodelling remained unresolved 16 weeks P.I, post infection clearance. Deficiencies in T/B cells or IL-4/IL13 responses, using SCID and IL-4 receptor (IL-4Rα) KO mice resulted in; amelioration of remodelling, with reduced incidence and severity in SCID vs WT mice and no significant difference observed between sham vs infected IL-4Rα KO mice. Multiplex analysis indicated significant increases in circulating lymphangiogenic markers following infection including: VEGF-C, β-cellulin and prolactin. Both recombinant IL-4/IL-13 polarised macrophages and live L3 or L3 extract stimulated THP-1 macrophages induced similar, significant proliferation of LECs. The data indicates that IL-4R and Th2 adaptive immune responses are key in induction of lymphatic remodelling following filarial infection, persisting beyond resolution of infection in WT mice. Additionally, initial evidence suggest macrophages may be lymphangiogenic effector cells in response to infection. Dissecting this Th2-mediated lymphatic remodelling pathway may yield novel therapeutic targets against filarial disease.

Natural Killer cells are associated with innate control of filarial nematode infection

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Lymphatic filariasis and onchocerciasis are major neglected tropical diseases affecting over 140 million people worldwide with painful and profoundly disfiguring pathologies (lymphedema). Despite crucial roles of granulocytes in controlling the early stages of the infection, little is known about the induction of the innate immune response at the infection site. As innate lymphoid cells (ILCs) are known to play important roles in the initiation of the inflammation, we therefore investigated comparative ILC1, ILC2 and natural killer (NK) cell population expansion during peritoneal *Brugia malayi* and *Onchocerca ochengi* filarial infections using either immunocompetent (BALB/c) or immunodeficient (NOD.SCID and RAG2 being deficient in functional lymphocyte responses; NOD.SCID.γ and RAG2$^{-/-}$γc$^{-/-}$ being moreover deficient in ILC populations due to an absence of IL-2 gamma chain signaling) mice. Data revealed that ILC1 and ILC2 cell populations did not expand at any time point examined post-infection in BALB/c, NOD.SCID and RAG2 mice either infected with *B. malayi* larvae or implanted with *O.ochengi* adults. In addition, an expansion of NK cells at the site of filarial parasitism was evident in those mice. However, NK impairment in NOD.SCID.γ and RAG2$^{-/-}$γc$^{-/-}$ was linked to increased susceptibility in both infection models as well as a significantly altered granulocyte recruitment local to the site of infection. In addition, while peritoneal macrophage and neutrophil numbers were not particularly impacted, eosinophil recruitment was significantly impaired in ILC-deficient mice during filarial infection, indicating a potential novel role for NK cells in regulating eosinophil granulocyte filaricidal activity. In follow up experiments we are addressing expansions of NK subsets in tissues distal to the site of infection, providing more insights to the innate immune mechanisms involved in the regulation of disease pathogenesis.

Comparative analysis of the immune response induced by native and recombinant versions of the ASP-based vaccine against gastrointestinal nematodes

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Activation-associated secreted proteins (ASP) isolated from the excretory/secretory material of different nematode species have been extensively evaluated over the past years as potential vaccine candidates. In line with this, our research group has proven the vaccine potential of two native ASPs (nASP) against the cattle gastrointestinal nematodes *Ostertagia ostertagi* and *Cooperia oncophora*. However, attempts to mimic these responses with *Pichia*-produced recombinant versions of the antigens (pASP) have been unfruitful. Therefore, our aim was to unravel whether differences in immune responses elicited by both nASP and pASP could explain the differences in the vaccine-induced protection levels. Immunization with the native antigens, unlike their recombinant counterparts, elicited a stronger cellular response after *in vitro* re-stimulation with the vaccine antigens characterized by the proliferation of CD4-T cells and NK cells for *C. oncophora* and *O. ostertagia* vaccines respectively. In terms of humoral responses, vaccination with both native and recombinant vaccines resulted in an increase of ASP-specific antibody levels. However, inhibition ELISAs showed that the antibodies induced by the native vaccine markedly differed in their binding specificity and preferentially bind the native antigen in comparison to the recombinant antigen. Taken together, these data suggest a difference in the immunogenic capacities between native and recombinant ASPs, but whether these dissimilarities are caused by structural differences in the antigen structure or its associated *N*-linked glycans remains to be elucidated. Preliminary data on this matter shows that vaccine-induced immune responses were completely abolished when animals were immunized with unfolded versions of the nASPs, highlighting the importance of the protein structure. Additionally, the implication of the *N*-linked glycans on the vaccine-induced immunity is being currently investigated.

Cellular immune response profiling after the co-administered hookworm vaccine candidates *Na*-GST-1 and *Na*-APR-M74 in healthy hookworm-exposed adults in Gabon.

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Hookworm infections affect 200 million people worldwide and although there are effective drugs available, high reinfection rates prevent effective control of these parasites in endemic regions. Therefore, vaccines are urgently needed. Two vaccine candidates, which have shown to be safe and immunogenic in phase I trials, have now been tested in an endemic area in Gabon. The candidates are proteins involved in blood digestion process of *Necator americanus* (Na- GST and Na- APR M74). The vaccines were formulated with glucopyranosyllipidA (GLA) adjuvant, while the control vaccine, Hepatitis B was formulated with Alum. Through a randomised trial, the vaccines were given to 32 healthy volunteers. Vaccine boosting was on day 28 and day 180. The immunogenicity was confirmed by measuring antibody responses. Here we studied cellular immune responses by stimulating PBMC at pre and different time points after vaccination with the recombinant vaccine candidates and measuring intracellular cytokine responses. We found elevated cytokine responses two weeks after the first boost and the highest at two weeks after the third vaccine boost. The cytokines that showed this pattern were mainly TNFa and IL2. Analyses is underway to link cytokine responses to the strength of antibody production in response to vaccination.

Structural studies of worm proteins

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A main focus of my lab over the last 15 years is to characterize the structures and functions of neglected parasite proteins. Some of the proteins are vaccine candidates, immune evasion molecules, and immune suppression molecules. Characterizing these structures allows us to: identify interactions with host proteins; define mechanisms of action of novel proteins; identify minimally active fragments of vaccines; and generate preliminary data for rational drug design. Our studies have revealed structural similarity between parasite and host proteins, likely as a result of co-evolution and may be part of attempts by parasites to mimic hosts. We have also been characterizing the structures and functions of parasite proteins from Sperm-coating protein / Tpx / antigen 5 / pathogenesis related-1 / Sc7 (SCP/TAPS) superfamily. In many nematodes, the major proteins secreted upon transition to parasitism are SCP/TAPS. These proteins are characterized by a ~15 kDa cysteine-rich CAP domain, with limited sequence identity. While a majority of eukaryotic SCP/TAPS proteins only have one CAP domain, some parasite CAP proteins have two covalently linked CAP domains. Additionally, the CAP domain has been implicated in lipid binding and transport with at least three unique lipid-binding regions. Structural analysis of nematode SCP/TAPS will be presented in the context of their possible functions.

Role of Macrophage Migration Inhibitory Factor in Immunity to Helminths

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Immunity to intestinal helminths such as *Heligmosomoides polygyrus* and *Nippostrongylus brasiliensis*is known to require both innate and adaptive components of the immune system activated along the Type 2 IL-4R/STAT6-dependent pathway. We have found that macrophage migration inhibitory factor (MIF) is essential for the development of effective immunity to these parasites. MIF-deficient mice are defective in expulsion of both *H. polygyrus* and *N. brasiliensis*, even following vaccination which induces sterile immunity to *H. polygyrus* in the wild type mouse. Cellular analyses found that the adaptive arm of the immune response, including IgG1 antibody responses and Th2-derived cytokines, were intact and that the Foxp3+ T regulatory cell response was unaltered in the absence of MIF. However, MIF was found to be an essential cytokine for innate cells, particularly in the activation and commitment of macrophages, suggesting that MIF-deficient macrophages are less able to polarise to an M2 phenotype on infection. M2 macrophages are an important immune defence mechanism against parasitic infections; in particular the M2 product Arginase-1, when inhibited, increases worm and egg burden.We therefore investigated macrophages recovered from MIF-deficient and wild type BALB/c mice exposed to *H. polygyrus* larvae by oral gavage, or *N. brasiliensis* larvae subcutaneously, and harvested peritoneal exudate cells on day 3 of infection. We were able to demonstrate the MIF-deficient mouse recruited fewer macrophages into the peritoneal cavity and produced less M2 products (Relm-a and Arginase 1). In order to assess if this was a developmental abnormality in the gene-deficient mouse or solely due to the absence of MIF during infection, we exposed wild type mice to 4-IPP, an inhibitor of MIF. Mice receiving this MIF inhibitor were less able to mount an effective immune response to the parasite, and had fewer M2 phenotype macrophages. Gene expression analysis of intestinal and lymph node tissues from wild-type and MIF-deficient infected mice indicated significantly reduced levels of Arl2bp, a factor involved in nuclear localization of STAT3. We further found that mice lacking STAT3 in the myeloid compartment (LysMCre:STAT3fl/fl) were unable to reject a secondary infection with *H. polygyrus*. We thus conclude that in the context of a Type 2 infection, MIF plays a critical role in polarizing macrophages into the protective Type 2 phenotype, and that STAT3 signalling is an important and previously unrecognised player in immunity to helminths.

The pharmacology of ivermectin as an antifilarial

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Ivermectin (IVM) has revolutionized the treatment and control of nematode parasitism and arthropod infestation in veterinary and human medicine. IVM and related macrocyclic lactone endectocides act on invertebrate glutamate-gated chloride (GluCl) channels, causing hyperpolarization with consequent paralysis of nematode and arthropod neuromuscular systems. In gastrointestinal nematodes, IVM paralyzes somatic and pharyngeal muscle, but the effects of IVM on tissue-dwelling filarial nematodes are less well understood. IVM has minor effects on motility or survival of adults and larvae of filariae in culture at pharmacologically relevant concentrations and durations of exposure. IVM appears to act on microfilariae, and possibly other larval stages, by inhibiting secretion of immunomodulatory molecules that protect against host immune responses. In adult filariids, IVM causes prolonged inhibition of egg-laying, lasting far longer than the presence of the drug in the host. Our hypothesis is that IVM causes this effect by inhibiting pharyngeal pumping, needed to acquire iron from the host for production of microfilariae. Free iron is present at extremely low concentrations in mammalian biofluids, instead being carried by transport proteins such as ferritin or in hemoproteins, meaning that, unlike other nutrients, iron cannot be obtained by uptake across the cuticle. Transcriptomic and proteomics analyses of the effects of IVM on the model filariid *Brugia malayi* in culture reveal changes in abundance of transcripts and proteins (not perfectly overlapping) associated with iron-dependent pathways. Efforts to monitor pharyngeal pumping of this organism in culture have proven challenging, but these data support (although they do not prove) the hypothesis that blockade of iron acquisition may account, at least in part, for the prolonged sterilization caused by IVM in these parasites.

High throughput screening of a schistosome serotonin receptor identifies potent anthelmintic lead compounds

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Serotonergic G-protein coupled receptors (GpCRs) are attractive anti-schistosomal targets given that serotonin (5-HT) regulates parasite movement and neuromuscular function. While GpCRs are privileged drug targets, accounting for >1/3rd of all FDA approved medications, target based screens for anthelmintics have been hampered by the fact that no flatworm G-protein coupled receptor has been functionally expressed in a high throughput capable assay. Here, we report the expression of a 5-HT receptor cloned from the predominant *Schistosoma* species responsible for human infection (*S. mansoni, S. japonicum, S. haematobium*). Stable expression of these receptors alongside a bioluminescent cAMP reporter provided a live cell assay with a real-time readout of receptor activity miniaturized to 384 well format. This system allowed rapid screening of natural product libraries (>4000 compounds), with "hit" compounds clustering into groups containing either tryptamine or benzylisoquinoline ring systems. Structure-activity profiling of these pharmacophores identified potent agonists (EC_{50}<100nM) and antagonists (K_i<100nM) active against receptors from all three parasite species. These hits were also efficacious against adult schistosomes cultured *in vitro*; agonists stimulated movement (the best hits were 1000x more potent than serotonin), and antagonists blocked movement (IC_{50}s as low as 100nM). Drugs prioritized from *in vitro* screens showed *in vivo* efficacy. Intraperitoneal injection of compounds into schistosome infected mice resulted in an acute clearance of parasites from the mesenteric vasculature to the liver, and 65% reduction in worm burden when dosed over one week. Despite the fact that not all worms were eliminated during this dosing regimen, intestinal oogram analysis revealed a 97% reduction in schistosome egg production- a crucial outcome given that the pathology of schistosomiasis is driven by host Th2 response to parasite eggs. These data provide the first high throughput screen of a GpCR, validating schistosome serotonergic signaling as an anthelmintic target and identifying promising new anti-schistosomal lead compounds.

Genomics of sex, drugs, and recombination in the gastrointestinal nematode, *Haemonchus contortus*

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Gastrointestinal nematodes are responsible for significant health and economic burdens in human and animal hosts worldwide. *Haemonchus contortus* is a major pathogen of small ruminants, and is the focus of significant anthelmintic control to minimise parasite burden and reduce disease. Drug resistance is widespread, and isolates resistant to all major classes of anthelmintics (including multi-drug resistant strains) have been described. We have exploited and built upon the available genomics resources for *H. contortus* by undertaking a genome-wide approach toward understanding the mechanisms of anthelmintic resistance, with the aim of identifying genetic markers to diagnose drug resistance in the field. A genetic cross between the drug-susceptible ISE (used in WTSI genome assembly) and the multi-drug (benzimidazole, levamisole and ivermectin) resistant UGA/2004 strains was performed, from which a F1 genetic linkage map was constructed. From these data it was possible to assess recombination rate and to generate a kinship network and infer the pedigree of the F1 generation, which suggests that the female worm sequenced had mated with multiple males (as many as 6). Both recombination rate and polyandry have important implications in the development of anthelmintic resistance and will be incorporated into models of anthelmintic resistance transmission and spread. The F2 progeny of the cross were subsequently used to dissect the genetic basis of each of the individual drug classes via a bulk segregant analysis of pre- and post-drug treatment populations. This analysis has provided evidence for drug selection throughout the genome and has highlighted loci for further study. Populations of *H. contortus* from the field are being collected before and after anthelmintic treatment for population genomic analysis and loci under selection will be compared with resistance loci identified in the laboratory cross, with the aim of developing robust genetic markers for drug resistance in the field.

Global diversity and population genetic structure of the sheep parasite *Haemonchus contortus*

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We have analyzed the diversity of *Haemonchus contortus* populations, the most pathogenic of ruminant gastro-intestinal nematodes, using whole-genome sequencing of 265 individual males from 37 isolates spanning 11 countries. SNP calling yielded 6,577,423 SNP (2.78 ± 0.18 SNP/100 bp) with substantial co-segregation across populations (196,804 ± 64,967 between 5 isolates on average). A principal component analysis applied to genotypic data only explained 10.44% of the observed variance on the first component. Three clusters seemed to emerge however, one composed of sub-Saharan isolates, a second grouping both European and Australian isolates together, and a last one that connected samples from Guadaloupe, São Tomé and Brazil. This clustering was also supported by a maximum-likelihood phylogenetic tree inferred from the consensus mitochondrial sequence of each individual. Linkage disequilibrium decay with physical distance varied between populations (0.09 to 0.33 at 2 Kbp) but could be compatible with a putative high effective population size *Ne*. A 30-kbp wide region of chromosome 1 showed a strong differentiation signal (F_{ST} between 0.58 and 0.66) between ivermectin-resistant and –susceptible populations. Additional analysis will assess the amount of admixture between populations and estimate global *Ne*. Our results should help in designing and interpreting future studies on the genetics of anthelmintic resistance in parasitic nematodes.

A Helmind-Derived Inhibitor of IL-33.

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Epidemiological studies show that infection with parasitic helminths negatively correlates with the prevalence of allergic diseases. These observations led us to hypothesise that parasitic helminths secrete immune-modulatory factors which could be directly developed as novel therapeutics for diseases such as asthma. Infection with the murine parasite *Heligmosomoides polygyrus* suppresses allergic responses in mouse models of asthma. We previously showed that the excretory/secretory products of *H. polygyrus* (HES) could replicate this effect in the absence of infection, and that this suppression was through inhibition of IL-33 release. IL-33 is an "alarmin" cytokine, stored in the nucleus of epithelial cells and released under conditions of necrosis. The IL-33 pathway is strongly implicated in the development of allergic disease, and is a target for novel therapeutic agents. Using fractionation and mass spectrometry, we identified a single protein from HES with IL-33 suppressive activity: we have called this protein the *H. polygyrus* Alarmin Release Inhibitor (HpARI). Recombinant HpARI binds to both IL-33 and DNA, effectively tethering the cytokine within necrotic epithelial cells, and blocking the binding of this cytokine to its receptor. In a mouse model of asthma dependent on Alternaria allergen inhalation, we can show that HpARI is effective at suppressing IL-33 release, ILC2 activation, and recall responses to allergen, leading to reduced pathology in the lung and abrogated airway hyperresponsiveness. Furthermore, using a human lung explant system, we can show the IL-33-suppressive effects of HpARI translate to human IL-33 release, and thus has potential as a novel therapeutic in allergic disease.

Parasite cytokines: a structurally distinct immunosuppressive TGF-β mimic from an intestinal helminth that potently induces murine and human regulatory T cells.

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Helminth parasite infections often drive regulatory T cell (Treg) expansion to block protective immunity, evoking also bystander suppression of allergy and other disorders. We established that Treg expansion by the mouse intestinal helminth *Heligmosomoides polygyrus* is mediated through excretory/secretory products (HES), signaling through the TGF-β receptor to induce Foxp3 expression. We have now identified the active molecule as a novel and functional TGF-β mimic, named TGM. TGM shares no sequence homology to the TGF-β superfamily, and is active as a full-length >400 amino acid protein with no requirement for further processing. TGM induces Foxp3 expression in murine T cells at similar concentrations (approx. 1 ng/ml) as mammalian TGF-β, and induced Foxp3+ cells are functionally suppressive in vitro. An inhibitor of the TGF-β receptor II kinase ALK5 (SB431542) completely ablates TGM activity, while neutralizing antibody against mammalian TGF-beta has no effect. Biacore studies reveal TGM binds to TGF-β receptor II with a lower affinity than TGF-β, but in contrast TGM also binds directly to TGF-β receptor I. Remarkably, TGM can induce FoxP3 expression of human peripheral blood CD4+ T cells at similar concentrations to mammalian TGF-β, indicating that TGM could translate directly into human disease. We are now applying TGM to additional mouse models of inflammation (asthma, autoimmunity and colitis) to assess its therapeutic potential and/or its involvement in immune modulation through the TGF-β pathway.

The synthesis and characterisation of helminth bioactive peptides as potential therapeutic treatments for autoimmune diseases

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Hookworm infections are known to cause many severe immunopathological complications in human host. More recently however, there has been evidence to suggest a more harmonious host-parasite interplay, with the nematodes' ability to beneficially alter the host immune system. This study reports on the synthesis and characterization of hookworm-derived peptides for the future development of potential therapeutic treatments for autoimmune diseases. Several ShK-like peptides from the *Ancylostoma caninum* and *Necator americanus* hookworms were produced using solid phase peptide synthesis and characterised using nuclear magnetic resonance spectroscopy. Studies using NMR spectroscopy revealed that each peptide folded into a similar structure as the ShK toxin and this similarity was highlighted by the determination of the three dimensional structures of two of the active peptides, A1 and N1. Multiple immunological assays such as cytometric bead array, intracellular cytokine staining and functional T-cell assessment were used to investigate the biological effects of the peptides on human peripheral blood mononuclear cells. Here it is shown that A1 and N1 significantly decreased the production of inflammatory cytokines on human peripheral blood mononuclear cells. Further studies included the TNBS-induced mouse model of colitis, where various parameters were measured to assess pathology including weight loss, clinical and macroscopic scores and histological structure. A1 and N1 peptides showed significant protection against different parameters of colitic inflammation, suggesting that both may be a potential drug lead for the treatment of inflammatory bowel disease.

Ex vivo immunoproteomic analysis of *Fasciola hepatica* tegumental antigens identifies multiple exosome proteins recognized by antibody from resistant sheep

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Indonesian Thin Tail (ITT) sheep express a high level of acquired immunity to *F. gigantica* within 4 weeks of infection and antibodies in ITT sera can promote antibody-dependent cell-mediated cytotoxicity against the surface tegument of juvenile *F. gigantica in vitro*. Given the high protein sequence similarity between *F. hepatica* and *F. gigantica*, we hypothesised that antibody from *F. gigantica*-infected ITT sheep would identify the orthologous proteins in the tegument of *F. hepatica*. Purified IgG from the sera of *F. gigantica*-infected ITT sheep, collected pre-infection and 4 weeks post-infection, were incubated with live adult *F. hepatica ex vivo* and the immunoprecipitate formed was isolated and analysed using MS/MS proteomic methods. A total of 38 proteins were identified at a significantly higher abundance in the immunoprecipitate using week 4 IgG, including 8 predicted membrane proteins, 20 secreted proteins, 9 proteins predicted to be associated with the lysosomes, the cytoplasm or the cytoskeleton, and one protein with an unknown cellular localization. Three of the membrane proteins are transporters including a multidrug resistance protein, an amino acid permease and a glucose transporter. Interestingly, a total of 21 of the 38 proteins matched with proteins reported to be associated with the small exosome-like extracellular vesicles (EV) of adult *F. hepatica,* suggesting that the ITT week 4 IgG is recognising proteins released by EVs or is immunoprecipitating intact EVs during *ex vivo* incubation. Five EV membrane proteins were identified including two predicted to be associated with vesicle transport/ exocytosis (VPS4, vacuolar protein sorting-associated protein 4b; and Niemann-Pick C1 protein). RNAseq analysis of the transcription of the 38 immunosloughate proteins showed that the sequences are expressed over a wide abundance range during *F. hepatica* development. The results raise the possibility that resistance in ITT sheep may involve antibody-mediated interference in exosome function in *F. hepatica*.

Interaction between commensal gut microbiota and *Schistosoma mansoni* infection in C57BL/6 and type-1 diabetes NOD mouse models

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The hygiene hypothesis implicates both commensal gut microbiota and exposure to infectious agents as determinants of a beneficial immune homeostasis, which favours health rather than disease in terms of autoimmune and inflammatory conditions. Experimental work has demonstrated that both commensal gut microbial composition and helminth infections, such as infection with the trematode Schistosoma mansoni, can affect incidence and onset time of type-1 diabetes in the NOD mouse model. S. mansoni is a potent immunomodulator and the preventive effect of the infection has been related to egg-induced immune responses. Using oral administration of broad-spectrum antibiotics (ampicillin/vancomycin) to strongly alter the intestinal microbiota composition combined with S. mansoni infection, we investigated whether the preventive effect of helminth infection in the NOD model would be impacted by alteration of the microbiota. Our data reproduce the finding of a preventive effect on autoimmunity in S. mansoni infection and furthermore implicate stimuli from the gut microbiota as necessary for the delay in type-1 diabetes onset and in controlling the inflammatory phase of the infection. We observed changes in intestinal permeability as a result of altered gut microbiota and also address the short chain fatty acid levels in circulation in C57BL/6-NTAC models. Gut microbial composition are observed by tag-encoded 16S rRNA gene NextSeq-based high-throughput sequencing of faecal samples at baseline, pre-patent and patent infection time points. The results are relevant for deepening our understanding of the intestinal "ecosystem" where the sum of host-pathogen-commensal microbiota interactions engenders immune responses crucial to a healthy state.

Helminth infections, deworming and allergic conditions in Uganda

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We are conducting epidemiological studies in Uganda, to understand the relationship between helminths infections and allergic conditions in humans. Results from previous studies have been inconsistent. For our birth cohort, 2,500 pregnant women were enrolled (2003-05) into a double blind, placebo controlled trial of albendazole Vs placebo and praziquantel Vs placebo in a 2X2 factorial design. Their children have been followed up from birth to date, and we have prospectively collected data on allergic conditions and their own helminth infections. At age five we found that maternal hookworm during pregnancy was inversely associated with childhood eczema [aHR (95%CI) 0.71(0.51-0.99)] and that maternal albendazole during pregnancy increased the risk of childhood eczema [1.58(1.15-2.17). Childhood *T. trichiura* and hookworm were inversely associated with eczema. These effects waned by nine years. Asthma in this birth cohort was rare, so we conducted a case-control study among school children (5-17 years), 560 with and 1,100 without asthma. The prevalence of helminths is lower among asthmatics, but they are also more likely to have used deworming medication recently. What has been consistent in the two studies, and in literature, is that children born/spent early childhood in the rural areas are less likely to develop atopy, eczema, and asthma. And, the association between atopic sensitization and wheeze was stronger in the urban area [OR (95% CI), 5.6(2.9-10.7)] than in the rural area [2.49 (1.43-4.33)]. In the birth cohort, Dermatophagoides-specific IgE was positively associated with eczema if the child's mother had no hookworm during pregnancy [HR (95%CI) 2.72(1.11-6.63), but not if the mother had hookworm [0.41(0.10-1.69), interaction p-value=0.03]. Could helminths explain the observed urban/rural difference in allergy risk in developing countries?

Litomosoides sigmodontis infection interferes with vaccine induced protection against influenza virus infection

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Parasitic helminths infect 2 billion people worldwide thereby dampening the immune system of their hosts. Accordingly, several studies report a negative correlation between pre-existing helminth infection and response to vaccination in the human population. We have reiterated these findings in the mouse system by showing that concurrent infection with the parasitic nematode *Litomosoides sigmodontis* suppressed the antibody (Ab) response to model antigen vaccination (DNP-KLH). Reduced Ab response to DNP-KLH "vaccination" was observed during acute *L. sigmodontis* infection but also if vaccination was performed several months after termination of infection. In order to asses the clinical relevance of these findings, we are currently analyzing the impact of concurrent helminth infection on vaccine-induced protection against influenza. Seasonal influenza is predominantly elicted by circulating influenza virus A (presently H1N1 and H3N2) and causes up to 500.000 fatal casualties each year. Disease can be prevented by a vaccination that induces a neutralizing Ab response specific for the variable epitopes of the haemagglutinin (HA) head. Vaccination of C57BL/6 mice with the commercially available seasonal (2014/15) trivalent subunit anti-influenza vaccine Begripal (Novartis), that is licensed for humans, elicits a neutralizing HA-specific Ab response. Vaccinated mice are protected against challenge infections with the human pathogenic influenza strain A H1N1 (H1N1 A/Hamburg/NY1580/09). Mice that were infected with *L. sigmodontis* for 4 weeks at the moment of vaccination displayed reduced titres of HA-specific IgG as well as a reduced neutralizing capacity quantified by haemagglutinin infhibition assay. Reduced Ab response in vaccinated, *L. sigmodontis*-infected mice was reflected by increased weight loss and increased influenza virus burden during challenge infection compared to vaccinanted, non-helminth-infected mice. As our results suggest that anti-influenza vacciantions with non-adjuvanted vaccines may be less protective in helminth-infected humans, we are currently comparing the protective efficacy of alternative vaccination regimens using adjuvanted influenza vaccines in *L. sigmodontis*-infected mice.

Compartmentalised immune responses provide protection against type 1 pathogens challenge during helminth infection

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The counter regulation of polarised type 1 and type 2 immune responses is a well-established immunologic paradigm. However, in some common infections, such as barrier damaging gut helminths, protection against both the possible translocation of gut bacteria (requiring type 1 immunity) and further helminth invasion (requiring type 2 immunity) would be advantageous. Whether and how it is possible to generate concurrent type 1 and type 2 responses in order to allow the immune system to multitask in this way is not understood. We used *Heligmosomoides polygyrus* (*Hp*), a helminth restricted to the small intestine and associated with type 2 response, to investigate mechanisms employed by the immune system to overcome this problem. Intriguingly, we found that during *Hpb* infection substantial polarisation of immune populations occurred in a tissue specific manner at sites distal to the gut. Barrier surfaces, such as the lung, became highly type 2-polarised as evidenced by expression of canonical factors such Relm-alpha and Ym1 while circulating immune cells displayed features more typically associated with classical type 1 immune signals. In particular, we observed dramatic upregulation of Sca-1 on circulating monocytes during *Hp* infection that was lost in the absence of IFN-γ receptor signalling. Challenging the current paradigm, which links *Hp* infection to increased susceptibility to bacterial infection, we observed a reduced bacterial load in the spleen following systemic *Salmonella* challenge infection, indicating a protective function of *Hp* against bacterial translocation. These data demonstrate that tissues distal to the initial site of gut worm establishment can acquire distinct polarisation states following infection. Further exploration is required in order to fully appreciate how these responses function co-operatively to provide optimal protection against pathogenic challenge.

Novel immune mechanisms involved in protection in a coinfection model with two distinct helminth parasites

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Coinfection with multiple pathogens, including helminths, is the norm in many non-Westernised countries, and research into how the immune system copes with, and is impacted by, coinfection is growing. Helminth infection has been linked to the downregulation of immune responses against other pathogens, the dampening of vaccine responses and the alleviation of allergic diseases in both animal models and human epidemiological studies. However, some experimental helminth infections induce strong systemic Th2 responses, which could influence immunity to other pathogens at distal sites to the gut. The strictly enteric rodent helminth parasite *Heligmosomoides polygyrus* (*Hp*) has previously been shown to induce a highly regulatory immune environment, with the dampening of several systemic allergic and inflammatory disease phenotypes, including in the lung. Therefore, we expected to see a downregulation of the immune response against coinfection with a distinct helminth, *Nippostrongylus brasiliensis* (*Nb*), which migrates through the lung *en route* to the gut. In reality, we observed a striking protective effect of prior *Hp* infection, resulting in significantly lower numbers of *Nb* larvae migrating through the lung and continuing their lifecycle to the gut tissue. This protection is ablated in RAG1-KO mice and by depletion of $CD4^+$ T cells, which corresponds with a stark reduction in circulating IL-5 levels and eosinophilia in the lung, which we believe to be key factors in the protective response against *Nb*. These findings are expanding our understanding of the host immune mechanisms involved in control of *Nb*, a helminth that closely mirrors the lifecycle and physiology of human hookworms. How a helminth confined to the intestine could have such profound effects on immunity in distal tissues, and the translation to human helminth infection, is the topic of ongoing research in our laboratory.

POSTER SESSION 1

ABSTRACTS

1. Novel rapid diagnostic test kits for tropical fasciolosis by *Fasciola gigantica*

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Tropical fasciolosis caused by *Fasciola gigantica* infection is an important parasitic disease. It causes enormous economic loss in the livestock industry in the tropical regions of Asia, Africa as well as Thailand. It causes an economic loss in livestock industry in developing and underdeveloped countries for over 3.2 billion US dollars per annum. In addition, human fasciolosis is also recognized by the World Health Organization as a major public health problem. It was also reported that at least 2.4-17 million people are presently infected worldwide and about 91 million are at risk. Conventional parasitological diagnosis of fasciolosis is often unreliable and has very low sensitivity. Hence, the antigen detection is thought to be a better alternative for diagnosis of fasciolosis, as it reflects the real parasite burden. In this work, we have produced the number of monoclonal antibodies against recombinant *F. gigantica* cathepsin proteases, and developed both ELISA and immunochromatographic (IC) test for rapid detection of circulating cathepsin proteases in the sera from mice experimentally and cattle naturally infected with *F. gigantica*. Monoclonal antibodies and biotinylated rabbit anti-recombinant cathepsin protease antibody were selected due to their high reactivities and specificities. The lower detection limits of sandwich ELISA and IC test were 3pg/ml and 256 pg/ml, respectively. Sandwich ELISA and IC test could detect *F. gigantica* infection from day 1 to 35 after infection. In experimental mice, the sensitivity, specificity and accuracy were at least 95%, 100% and more than 98% (for sandwich ELISA), and 93%, 100% and more than 98% (for IC test), while in natural cattle they were more than 98%, 100% and more than 99% (for sandwich ELISA), and more than 96%, 100% and more than 99% (for IC test). These two detection methods exhibited high efficiencies and precisions for diagnosis of fasciolosis by *F. gigantica*.

2. Structural insights in the activation-associated secreted proteins of the cattle parasites *Ostertagia ostertagi* and *Cooperia oncophora* – implications for vaccine development

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Ostertagia ostertagi and *Cooperia oncophora* constitute two of the most common parasitic nematodes (*syn*. helminths) in cattle worldwide. Historically, infection treatment and prevention was carried out by using anthelmintics, however upcoming resistance issues against these anthelmintics necessitate the exploration of other routes, with protein-based vaccines being the most appealing and viable alternative. Our research group has previously shown the protective capacity of activation-associated secreted proteins (ASP) purified from the excretome-secretome (ES-fraction) of both *O. ostertagi* and *C. oncophora*. Given that natively produced antigens are not commercially viable though, efforts have been put in developing recombinant versions, which to this day unfortunately have not been able to induce similar protection levels against either *O. ostertagi* or *C. oncophora*. In an attempt to clarify why native *versus* recombinant antigens behave so differently in infection trials we sought to investigate any structural differences between natively produced and recombinant ASPs. Most strikingly, fundamental differences were observed on three levels: i) sequence analyses showed significant polymorphisms in native ASPs, ii) *N*-glycan profiling indicated subtle differences in native *versus* recombinant ASP glycosylation, and iii) crystallographic and mass spectrometric analyses demonstrated variations in disulphide bonding between native and recombinant ASPs. Currently, we are applying this knowledge to further fine-tune our recombinant versions of both *O. ostertagi* and *C. oncophora* ASPs so that they resemble their native counterparts more closely in terms of structure and, most importantly, in their ability to induce a proper immunological response leading to adequate protection levels in cattle.

3. Towards the penside detection of triclabendazole efficacy against liver fluke parasites of livestock

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Fasciola hepatica threatens global food security because of severe livestock infections and is recognised as an emerging public health concern. Current diagnostics utilised for diagnoses in the field consist of low sensitivity faecal egg counting, which is negatively influenced by irregular egg passage from bile secretions to faeces. Molecular detection from animal samples is only available under laboratory conditions and has included highly complex commercial and in-house ELISAs for antigen and antibody detection, with problematic performances and varied success. Without viable vaccine developments, fascioliasis control greatly relies on flukicide drenches, particularly the benzimidazole triclabendazole (TCBZ), as it is the only commercially available compound that is active against the damaging juvenile and adult parasites. Rapid diagnostics that can detect fluke presence and also measure drug resistance at the penside will be especially important to future control strategies, as disease modelling has predicted increasing incidences coupled with emerging TCBZ resistance. We have developed a novel proteomic approach to discover and identify secreted proteins of the adult fluke TCBZ response. Cathepsin L proteases are our control biomarkers, as they are major components of excretory/secretory products responsible for host blood and tissue breakdown and thus also indicate TCBZ-SO treatment efficacy and fluke fitness. We have discovered calreticulin and enolase proteins are released upon fluke survival of TCBZ-SO challenge, whereas triose-phosphate isomerase, gelsolin, protein deglycalase (DJ-1) and actin are biomarkers to indicate TCBZ-SO-mediated fluke death. To this end, we have cloned and recombinantly expressed calreticulin, enolase and gelsolin biomarkers and we have raised polyclonal antibodies to a recombinant cathepsin L zymogen to confirm protein recovery from experimentally-infected and natural field samples. We now report progress of our biomarker validation pipeline to develop the first field-based diagnostics for liver fluke.

4. Tetracycline antibiotics modify both filarial Th2 inflammation and inflammatory-associated lymphatic remodelling in pre-clinical lymphatic filariasis pathology models

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An anti-morbidity effect of the second-generation tetracycline, doxycycline (DOX), has been identified in filarial lymphoedema which is disassociated from active filarial infection and anti-*Wolbachia* activity. In this study, we utilise a series of *in vivo* preclinical models of filarial infection, inflammation and vascular remodelling to interrogate the effects of oral treatment with human bio-equivalent exposures of DOX or the related second generation tetracycline, minocycline (MIN). DOX or MIN oral treatments modified the early systemic Th2 response to *Brugia malayi* L3 intraperitoneal infections without adversely affecting parasite recovery. Additionally, MIN oral treatment curtailed dermal inflammation and skin thickening in response to *B. malayi* female extract. The Th2 modifying effects of DOX were also apparent in the local draining lymph node $CD4^+$ T cell response following subcutaneous hind limb infections of *B. malayi* L3. Concomitantly, extensive lymphatic remodelling in the hind limb could be traced following *B. malayi* infection, using non-invasive near infra-red bioimaging, which was significantly modified by treatment with DOX. A direct effect of modifying lymphangiogenesis by DOX or MIN was determined *in vitro* by culturing human adult dermal lymphatic endothelial cells (LEC) with specific vascular endothelial growth factors in the presence or absence of titrations of antibiotic. Therefore, we provide evidence in preclinical models of filarial infection and inflammation of an anti-inflammatory and anti-lymphangiogenic mode of action of second generation tetracyclines.

5. Helminth secretomes reflect different lifestyles and parasitized hosts

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Helminths cause a number of medical and agricultural problems and are a major cause of parasitic infections in humans, animals and plants. Comparative analysis of helminth genes and genomes are important to understand the genomic biodiversity and evolution of parasites and their hosts in terms of different selective pressures in their habitats. The interactions between helminths and their hosts (environments) are mediated in large part by secreted proteins, known collectively as the "secretome". Proteins secreted by parasites are able to modify a host's environment and modulate their immune system. The present study aimed to predict, *in silico*, the secretome in 44 helminth species including Nematoda (31 species) and Platyhelminthes (13 species) and, understand the diversity and evolution of secretomes. Secretomes from plant helminths range from 7.6% (943 proteins) to 13.9% (2,077 proteins) of the filtered proteome with an average of 10.2% (1,412 proteins) and from free-living helminths range from 4.4% (870 proteins) to 13% (3,121 proteins) with an average of 9.8% (2,126 proteins), respectively, and thus are considerably larger secretomes in relation to animal helminth secretomes which range from 4.2% (431 proteins) to 11.8% (2,419 proteins) of the proteomes, with an average of 7.1% (804 proteins). Across 44 secretomes in different helminth species, we found five conserved domains: PF00014 (Kunitz/Bovine pancreatic trypsin inhibitor domain), PF00046 (Homeobox domain), PF00188 (cysteine-rich secretory proteins), PF00085 (Thioredoxin) and PF07679 (Immunoglobulin I-set domain). Secreted proteins had higher architecture diversity compared with non-secreted proteins and the secretome was not conserved across species and the differences suggest possible evolutionary adaptations related with the ecology, lifestyle and environment. Our results detected secreted proteins associated with invasion, infection, adhesion and immunoregulation processes, among other functions. This study will contribute towards the understanding of host-parasite interactions and possibly identify new molecular targets for the treatment or diagnosis of helminthiases.

6. Using metabolic networks to probe the symbiosis between *Wolbachia* and filarial nematodes

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The filarial nematodes *Brugia malayi* and *Onchocerca volvulus* represent a leading cause of disability in the developing world, infecting 140 million people and causing lymphatic filariasis and river blindness, respectively. Currently available drugs affect the microfilariae but not the adult worms – which can survive in the host for up to 15 years – and are contraindicated in 11 Central African countries due to potentially fatal co-infection interactions. When combined with recently emerging resistance, novel treatments are urgently required. One potential target is the parasite's endosymbiotic bacteria from the genus *Wolbachia*. Killing these bacteria impedes parasite development, fecundity, and survival; this reliance along with available genome resources suggest that some of the worms' metabolic requirements have been shifted to their endosymbionts. In our first research phase, we have generated metabolic networks for *B. malayi* and *O. volvulus*, including their endosymbionts. Our initial results suggest that the bacteria appear to be contributing to fatty acid and purine metabolism, as well as haem and riboflavin biosynthesis. We are analyzing these networks using constraint-based modeling such as flux balance analysis, which predicts the activities of each metabolic enzyme such that the entire system of the cell behaves as observed. Related methods identify choke-points in the network, enzymes that result in a severe impact if perturbed. In collaboration, we are also generating dual RNA-seq data for *B. malayi* and its *Wolbachia* for a variety of tissues and life stages. These data will be incorporated into the networks, providing us with an understanding of the parasite's metabolic activities during an infection at a level unprecedented in nematodes. Beyond contributing to the fundamental understanding of eukaryotic metabolic symbiosis, the choke-point and other analyses of these models will allow us to prioritize several enzymes that may be usefully targeted for therapeutic intervention.

7. Molecular characterization of human and animal *Echinococcus* isolates in Iran

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Cystic echinococcosis (CE), caused by the larval stages of *Echinococcus granulosus*, is an important public health problem and a major economic importance in Iran. Approximately 1.1% of admission to surgery awards is due to hydatid cyst. Seropositive reported from different parts is 1.1-7.2% for human and 24.4%, 18.9%, 8.5% and 35.76% for sheep, cattle, goat and camel, respectively. This article reviews the genotype of the parasite responsible for human and animal CE in Iran. For this purpose different databases and search engines were utilized from 2010. *Echinococcus granulosus* (G1, G2 and G3 genotype) is the most frequent species responsible for most human and animal infections. It has been reported from not only human but also from sheep, goat, camel, cattle, buffalo and boar as intermediate host from all or local regions of the country. Dogs were infected with G1, G2, G3 and G6 genotypes and wild canids with G1 genotypes. These animals act as the final host in different regions. Recently *Echinococcus intermedicus* (G6, camel strain) has been reported as the second most prevalent strain from human. *Echinococcus intermedicus* (G7, goat strain) is reported from northeast part of Iran. Alveolar cyst, caused by *E. multilocularis*, has been reported in few areas.

8. Optimising liver fluke (*Fasciola hepatica*) extracellular vesicle purification to understand their role within parasite drug exposure

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Fascioliasis is a major neglected tropical disease that infects humans and ruminant species worldwide. At least 2.4 million people are currently infected in over seventy countries, with millions more at risk. Furthermore, this disease is a significant animal health and welfare issue costing the livestock industry an excess of $3 billion per annum. In the absence of vaccines, control is via anthelminthic treatment, primarily with triclabendazole (TCBZ). However, liver fluke resistance towards TCBZ has been reported, threatening future control. Recent discoveries have identified extracellular vesicles (EVs) in liver fluke excretory/secretory products. However, whilst research has begun to look at the host-EV interaction and immune modulation functions, it is unknown what role EVs undertake during parasite drug exposure.

In order to determine the effect of TCBZ on EV production by the liver fluke, we first compared differential centrifugation and size exclusion chromatography EV purification methods to optimise liver fluke EV isolation. EVs purification was assessed using transmission electron microscopy, atomic force microscopy, protein quantification, proteomics and western blotting. To understand the role of EVs during parasite drug exposure, liver flukes were exposed to TCBZ and its metabolites, TCBZ-sulphoxide and TCBZ-sulphone. EVs were purified using the optimised protocol, before being morphologically characterised and analysed for TCBZ metabolites. Experimental results have lead to a novel understanding of liver fluke EV integrity after exposure to sub-lethal and lethal drug dosage, as well as EV function in respect to parasite-drug mechanisms. This research has contributed towards sustaining the control of fascioliasis and identifying novel parasite control possibilities.

9. Using simulated anaerobiosis in *C. elegans* as a platform for anthelmintic drug discovery

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Parasitic helminth infections affect ¼ of the world, and result in thousands of deaths every year as well as economic losses in agriculture. This is in part due to increasing anthelmintic resistance, thus fueling a need for the discovery of novel anthelmintics. During host infection, parasitic helminths are in an oxygen-deprived environment, during which they use a special form of anaerobic respiration to survive – malate dismutation – which requires rhodoquinone, a ubiquinone-related electron carrier. This pathway is absent in mammals, but exists in the nematode worm *C. elegans*, making it an ideal drug target and the worm a good model of parasitic helminth metabolism. We established an image-based assay of worm movement—the acute assay—and found that worms treated with potassium cyanide (KCN) become paralyzed, however additional treatment with 2-Deoxy-D-glucose (2DG) recovers movement. KCN prevents oxidative phosphorylation in the aerobic electron transport chain (ETC), and 2DG is a glucose analog that blocks glycolysis. A key question is how worms are producing ATP when both glycolysis and the ETC are blocked. We used RNAseq to investigate the transcriptional response to KCN + 2DG, and found our treatment activates the hypoxia response. Mutant analysis of genes required for this response has shown this response is required for recovery. Furthermore, RNAseq data shows that treatment with KCN + 2DG recapitulates anaerobiosis. Interestingly, we also see inhibition of Complex I with Rotenone prevents recovery. Therefore, we conclude that recovery from paralysis requires at least two processes: a working hypoxia response, and the anaerobic electron transport chain. My project goal is to better understand these and other anaerobic pathways to uncover new anthelmintic targets. Our long-term goals include conducting a drug screen for new anthelmintics using simulated anaerobiosis in *C. elegans,* and a forward genetic screen to uncover alternative metabolic pathways required for recovery.

10. Manipulating the Host: Investigating Targets of *H. polygyrus* Excretory-Secretory Products

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Heligmosomoides polygyrus is an intestinal parasite that utilises multiple immunomodulatory mechanisms to establish chronic infections in mice. The production of a mixture of proteins termed *H. polygyrus* excretory-secretory products (HES) is believed to enable the parasite to manipulate its environment within the host. A number of these proteins are likely to bind to specific host cell surface ligands, to modulate responses or interactions of those cell types in a manner favourable to parasite survival. To identify host-interactive products, we are screening HES proteins for binding to key populations within the intestinal environment, and from peripheral immune system organs. HES binds murine B cells, in a manner dependent on CD24, a highly glycosylated surface protein also shared with dendritic cells; other cell types, in particular myeloid cells, are bound by HES products in a CD24-independent manner. HES also binds to intestinal epithelial cells, and we have identified a single protein, VAL-4, that as a recombinant stains both Paneth cells and Goblet cells. Interestingly, VAL-4 is related to the *Ancylostoma* secreted proteins (ASPs) identified in hookworm species. Taken together, these interactions suggest that parasites may target a range of innate and adaptive immune cells to shape their surroundings and prolong infection in the host.

11. Upsetting the protease/anti-protease balance could be a novel vaccine strategy against *Fasciola hepatica* infection

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The liver fluke, *Fasciola hepatica* is a pathogen of economic importance in ruminants, such as sheep and cattle worldwide and is now recognised as an important zoonosis. Due to over-reliance on anthelmintics, drug-resistant parasites have emerged meaning that new control strategies are required. Vaccine development is becoming a greater focus of current research. *F. hepatica*-expressed molecules that act at the host-parasite interface, modify host cell function, and aid parasite development and survival are therefore of major interest as vaccine targets. Based on recent genome and associated stage specific transcriptome/proteome analysis, we have identified a number of molecules as potential vaccine candidates including Cystatin and Kunitz-type protease inhibitors. We have successfully expressed these inhibitors in *Pichia pastoris*, achieving a highly purified yield. Extensive characterisation of these inhibitors has been carried out and revealed that the Cystatins are broad range inhibitors of both host and parasite cysteine proteases compared with the Kunitz-type inhibitor that shows specific inhibition of Cathepsin L type proteases. The protease/anti-protease balance may be critical in the regulation of parasite processes including penetration, feeding, development and immune evasion, as well as modulation of host innate cell proteases involved in antigen processing and presentation. The potential of upsetting this balance is currently being assessed in sheep vaccine trials.

12. High throughput chemical genomics in *C. elegans* to screen for novel anthelmintics and their targets

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Biologically active chemicals are the basis of most known therapeutics and are powerful tools to study cell biological processes. However the discovery of new bioactive compounds and characterization of their molecular targets remain a challenge. We are using small molecule and natural products to identify novel compounds that affect animal development and study their modes of action. We established a high-throughput automated platform for chemical and functional genomic screening that accommodates both cell-based and whole-organism assays. We are focusing on broad-spectrum anthelmintics using the free-living nematode models *C. elegans* and the distantly related *P. pacificus*. Given the short life cycle of the worm, our platform enables one person to screen 20,000 chemicals per week and perform one genome-wide RNAi screen every three weeks. We validated our approach in a pilot screen of an FDA-approved drug library, which confirmed the effects of known anthelmintics on *C. elegans* and/or *P. pacificus*. We screened a library of ~32,000 small molecules, selected using a computational approach to predict bioavailability in nematodes and identified numerous candidate molecules that will be assayed for toxicity in mammalian cells. To uncover molecular targets of bioactives and mechanisms of resistance, we will use forward and reverse genetic screens to identify suppressors (or enhancers) of chemical sensitivity. We have also screened a *Bacillus thuringiensis* (Bt) library of 300 uncharacterized strains isolated in Lebanon and the UAE. Bt is a spore-forming bacterium that synthesizes crystal inclusions, certain of which show species-specific toxicity against insects, nematodes (i.e. Cry5B), and cancer cells. Bt crystal toxins therefor constitute a promising alternative to chemical anthelmintics. We found 95 strains that hinder the development of worms, and among them 50 strains that act through a Cry5-independent mechanism. Virulence factors will be characterized by DNA sequencing combined with functional genomic assays to elucidate their mechanisms of action.

13. Structural basis for inhibition of the SmCB1 drug target from the blood fluke *Schistosoma mansoni*

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Schistosomiasis caused by parasitic blood flukes of the genus Schistosoma afflicts over 240 million people worldwide. *Schistosoma mansoni* cathepsin B1 (SmCB1) is a gut-associated peptidase that digests host blood proteins as a source of nutrients. In our recent work we demonstrated that SmCB1 is a drug target for vinyl sulfone peptidomimetic inhibitors. Now we performed a detailed analysis with a unique set of 30 vinyl sulfone derivatives with diverse substituents. The inhibitors were screened *in vitro* against recombinant SmCB1 and *ex vivo* against *S. mansoni*. Two most effective inhibitors in terms of IC_{50} values and parasite suppression were complexed with SmCB1, and high resolution crystal structures were determined. Analysis of 3D structures and inhibition profiling identify key binding interactions and provide insight into SmCB1 inhibition specificity. Our work provides a footing for the rational design of anti-schistosomal chemotherapeutics.

14. Characterisation of *Schistosoma mansoni* Larval Extracellular Vesicle protein-1 (SmLEV-1) an immunogenic, schistosome-specific, protein exhibiting developmentally regulated alternative splicing

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As an integral component of cellular communication, Extracellular Vesicles (EVs) have been described in both protozoa and metazoan parasites. Both larval schistosomula and mature adult *Schistosoma mansoni* worms release pre-packaged EVs, but to what purpose? Identifying components of this critical parasitic data transfer (between parasites or parasite-to-host) will aid the development of potential schistosomiasis control strategies. To this end, this project aims to characterise the most abundant EV protein in the tissue-migrating schistosomula stage - *Schistosoma mansoni* Larval Extracellular Vesicle protein (SmLEV-1). Comparative sequence analysis demonstrates that while SmLEV-1 has orthologs in *Schistosoma haematobium,* and *Schistosoma japonicum,* it lacks any homologs outside of the genus, nor has any characterised protein domains. By employing qRT-PCR, we discovered differential expression of SmLEV-1 across the schistosome lifecycle, with peak expression in cercariae as well as male biased expression in sexually reproductive adults. Importantly, SmLEV-1 exhibits developmentally regulated alternative splicing during infection of the mammalian host. Specifically, when compared with adult worms, cercariae displayed over twice the amount of transcripts containing exon-5; but only two-thirds the amount of transcripts containing exon-8. Recombinant expression of SmLEV-1.3, the most abundant isoform in cercariae, has enabled investigation of the host's response to SmLEV-1, in both the mouse model and endemic human populations. Interestingly, preliminary serological analysis from *S. mansoni* infected individuals shows a strong IgG1 response against SmLEV-1 with minimal antigen-specific IgG4 and IgE measured; this finding is congruent to antibody responses generated against other surface/secreted schistosome proteins (e.g. SmLy6A, D, and SmTSP-2). Collectively, these results point to SmLEV-1 being an abundant, novel schistosome-specific, secreted protein (within EVs). As such, future work is directed towards localising the population of parasite cells containing SmLEV-1 transcripts by whole-mount *in situ* hybridisation (WISH) as well as understanding the function of SmLEV-1 by RNAi and mechanistic immunological investigations in murine models.

15. Lentiviral delivery of artificial miRNAs to Nippostrongylus brasiliensis infective larvae

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The ability to assign biological functions to genes of parasitic nematodes is critical to understanding development and survival in the mammalian host, yet most attempts to reliably trigger the RNAi pathway using exogenous dsRNA have failed or been inconsistent. Following the recent success of viral delivery systems for endogenous expression of the RNAi trigger (miRNA or siRNA) in trematodes, we tested whether a similar approach could be applied to *Nippostrongylus brasiliensis*. Uptake of DiD-labelled virus particles by activated third-stage larvae was confirmed by confocal imaging following *in vitro* exposure. No loss of infectivity was observed as a result of *in vitro* manipulation, and parasites were able to complete their life cycle in the mammalian host. Detection of proviral DNA by PCR of genomic DNA from infective larvae and adult worms isolated following infection indicated chromosomal integration of the provirus. Functionality of the transgene expression cassette was validated by detection of mCherry transcripts over a time course of up to 5 days of *in vitro* culture. Preliminary investigations into transcriptional silencing of secreted acetylcholinesterase following lentiviral delivery of a miRNA-adapted shRNA expression cassette resulted in >60% knockdown at 72 hr post virus exposure. We are currently optimising the delivery system for knockdown efficiency and consistency, and testing its applicability for *in vivo* experimentation. Viral transduction of *N. brasiliensis* infective larvae allows for endogenous expression of RNAi trigger molecules, circumventing problems associated with conventional methods to address RNAi in parasitic nematodes.

POSTER SESSION 2

ABSTRACTS

16. The search for novel anthelmintic targets: Characterizing alternative metabolic pathways in *Caenorhabditis elegans*

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Around a quarter of the human population is infected by parasitic helminths and they place a large economic burden on the agricultural industry. Unfortunately anthelmintic resistance is a growing problem. Although helminths are able to survive long periods of time of hypoxia in their hosts, their anaerobic metabolism has yet to be fully characterized. These alternative pathways are a promising, selective target for new drugs. The Fraser lab has developed a movement assay that allows for quantitive response to drugs and genetic perturbations to be measured in Caenorhabditis elegans, a free living relative who can also survive hypoxia. When the electron transport chain (ETC) is blocked by high doses of cyanide (KCN), worms are unable to move. However, if glycolysis is blocked at the same time by 2-deoxyglucose (2DG), the worms are able to recover. This two drug combination recapitulates the hypoxia response on a transcriptional level and this recovery requires the key hypoxia transcription factor, HIF-1. Furthermore, Complex I, an established hallmark of anaerobic metabolism and a target of existing anthelmintics, has been shown to be essential for this recovery. Together this strongly suggests that C. elegans recovers from KCN-induced paralysis in the presence of 2DG using anaerobic metabolism. Steady state LCMS has already revealed that the addition of 2DG allows for a buildup of succinate in worms treated with KCN alone to be depleted while the glycolytic shunt and the propionate shunts have been found to be nonessential for recovery. Two metabolic mutants in opposing pathways in the rate-limiting step TCA cycle show hyper- and hypo-recovery (idhg-2 and idh-2 respectively) from KCN in the presence of 2DG which demonstrates that this system will allow us to find inhibitors of anaerobic metabolism and so, hopefully anthelmintic targets.

17. DNA Sequencing within "Russian nested dolls": Large Fragment Targeted Enrichment Capture of *Wolbachia* genomes from filarial nematodes

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Approximately half of all studied filarial nematodes (Nematode; Onchocercidae) contain the endosymbiotic alpha-proteobacteria *Wolbachia*. In particular, most human parasitic filarial nematodes harbor *Wolbachia*. While the symbiosis is considered to be mutualistic as antibiotic treatments lead to decreased fertility and eventual death of adult worms, the symbiotic mechanism(s) remain unclear and have largely been inferred from genomic analysis. Currently, 3 complete and 3 draft genomes of *Wolbachia* from filarial nematodes have been produced. Additional Genomic data, for a variety of *Wolbachia* clades might help to characterize the nature of the symbiosis. However, a major challenge concerns the presence of host DNA (nematode and vertebrate host) in the sample, often complicating the assembly of the *Wolbachia* symbiont genome. To eliminate this "Russian nested dolls" effect, we are using an approach based on biotinylated probes to capture small fragments of *Wolbachia* DNA for Illumina sequencing and large fragments for PacBio sequencing. We have used this approach to capture and sequence *Wolbachia* from several clades and have successfully captured 2-3 kb *Wolbachia* fragments from *Brugia malayi* filarial nematode (as a known sequence control) and from *Aedes albopictus* mosquitoes (as a divergent *Wolbachia*). From *B. malayi*, PacBio sequencing of the captured DNA results in sequence mapping to 99.99% of the known *Wolbachia* sequence.

18. First insights into helminth-induced activation of tuft cells at mucosal barrier surfaces

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In the 1950s, tuft cells were first discovered as a potentially distinct epithelial cell sub-type in multiple mucosal tissues due to their unique morphological structure. Since then, tuft cell expansion has been noted in biopsies collected from human patients undergoing chronic bronchitis. Tuft cells have also been shown to express taste receptors linked through a conserved signalling pathway. A closer characterisation of mucosal tuft cells, however, was lacking until 2011. In their respective study, Jay and colleagues confirmed tuft cells as the fourth distinct secretory epithelial cell type, based on transcription factor profile. Interestingly, three succeeding publications independently emphasized the pivotal function of tuft cells and taste receptor signalling in innate immune reactions. Tuft cells are an endogenous source of IL-25, the release and expression of which has been shown to rely on the taste receptor signalling cascade. Furthermore, intestinal parasite-driven release of IL-25 from tuft cells leads to ILC2 and Th2-cell infiltration and consequently IL-13 mediated tuft cell hyperplasia. Although it has been underlined that both the helminthic and protozoan parasites, respectively *Nippostrongylus brasiliensis* and *Tritrichomonas muris* are efficient in inducing tuft cell proliferation, it is so far unknown if a molecular mediator secreted by the invading parasites is responsible for inducing the observed effects. To examine this, a first metabolomics screen was exercised on tissue culture media harvested from *N.brasiliensis.* It was evident that *N.brasiliensis* predominantly secreted various amino acids, purine derivatives and phosphocholine into the supernatant. This is an interesting observation as both amino acids and purines were shown previously to stimulate taste receptor signalling. We will further investigate the effects of different secreted metabolites of *N.brasiliensis* on tuft cell proliferation in intestinal organoids and characterise the taste receptor expression profile of FACS sorted tuft cells.

19. Long term *in vitro* culture of adult *Brugia malayi* parasites to evaluate drug response and host-parasite interactions

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Filarial parasites cause lymphatic filariasis and onchocerciasis. *In vivo* models are required since it is not currently possible to generate adult parasites from infectious stage larvae *in vitro.* In addition, reproductively active adult female parasites have a very limited life-span in culture. The current *in vitro* systems are maintained for less than 1 week. Arguably, this period of time is not sufficient to accurately simulate effects of *in vivo* drug exposures and may not accurately inform *in vivo* preclinical testing. Additionally, host-pathogen relationships and pathology, caused as a result of parasite modulation of the immune system, cannot be accurately evaluated *in vitro*. Instead, there is a heavy reliance on animal models to further knowledge in these areas. Here, we have developed an *in vitro* co-culture system with human lymphatic endothelial and myeloid cells. This model more accurately replicates the environment in which lymphatic parasites inhabit, making it possible to maintain parasite 'fitness' for a period of 2-3 weeks, comparable to those isolated from animal hosts. The longevity of this culture system should facilitate the more accurate *in vitro* assessment of host-parasite interactions, making it possible to study how the parasites can induce myeloid cell proliferation and how the immune system may be modulated by drugs which reduce lymphatic proliferation, ultimately improving disease pathology. As the *in vitro* co-culture system is more representative of the parasitic niche, it may be used as an alternative to animal models, thereby significantly obviating the need for animals for this purpose, or as a first stage anti-morbidity drug screen. Additionally, our *in vitro* co-culture system allows for further investigation of parasite biology to be explored in further detail, again reducing the need for animal experiments.

20. Evaluation of clinical status, diagnosis and treatment of suspicious patients to fascioliasis referred to Shahid Mofatteh clinic of Yasuj, Iran since Feb 2015 to July 2016

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Fasciola hepatica is one of the liver bile duct and gallbladder trematodes in animals. In the life cycle of the worm, snails are intermediate host and parasitic infection occurs by eating aquatic vegetables contaminated with metacercaria. Humans also can be infected with this worm. According to the previous study, infection of slaughtered livestock is significant in our country and also in Yasuj. This study is a cross sectional study. A total of 56 suspicious cases had been collected. Stool samples in laboratory tested by standard formalin ether precipitation method. Sediment was studied with an optical microscope at magnifications of ×10. Also serum sample had been assessment with indirect ELISA test. All stool samples that collecting before treatment was negative and 5out of 56 serum samples were positive that all of them were female and had positive history of eating aquatic vegetables. Hypereosinophilia was the most sign and observed in 53 patients, but majority of them had anemia, in 69% abdominal pain presented and other symptoms of fascioliasis such as headache and urticaria and epigastric pain was seen in few cases. This study showed that, patient that refer with fascioliasis symptom such as abdominal pain, chronic gastrointestinal symptom, low grade fever, jaundice, itching, with positive history of eating aquatic vegetables, should assessment for fascioliasis with some laboratory data such as stool exam and serological test. If final diagnosis was fascioliasis definitely, treatment start with single dose of triclabendazole. Prevention and on time diagnose can significant decrease mortality and economical charges.

21. The role of a parasite protein termed ''*Fasciola hepatica* transfroming growth factor-like molecule (FhTLM)'' on T cell activation and immuno-suppression.

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Fasciola hepatica (Liver fluke) is a helminth parasite which causes disease called fasciolosis in livestock with more than 300 million cattle and 250 million sheep infected worldwide. It is also an emerging zoonotic disease infecting more than 17 million people infection globally. *F. hepatica* infection often elicit a non-protective Th2 response favouring parasite persistence, suggesting that *F. hepatica* might be capable of secreting molecule able to modifying host immunological environment to their survival. Previously, a protein termed '*F. hepatica* TGF-β-like molecule (FhTLM)' has been identified in *F. hepatica* genome and this molecule was shown to have potential in ligating host receptor and initiating TGF- β signalling pathway, suggesting a role for this molecule at host immuno-regulation. Here, functional assays were carried out to determine the role of this molecule on T cell activation and generation of T regulatory cells or molecules associated with T cell hypo-responsiveness. Stimulation of murine (C57BL/6J) splenic naïve CD4 T cells with FhTLM resulted in increased expression of immunomodulatory cytokine IL-10. In addition, FhTLM, converted naïve CD4 T cells to $CD4^{+}CD25^{+}$ cells but do not express FoxP3. Expression of programme death ligand and its receptor (PD-L1 and PD-1) was also slightly upregulated in FhTLM stimulated CD4 cells when compared to control. Taken together, this findings suggests that FhTLM secretion might be one of the mechanism by which *F. hepatica* evade the immune response of its host and subsequently generation of down moulted host immune response. Thus, FhTLM might have implication in the development of new strategies for the treatment of this infection.

22. Chemokines and cytokines Levels in healthy adults and children co infected with multiple intestinal Parasites in Bayelsa state, Nigeria

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This study determined serum concentration of IL-4, IFN-γ, CCL-4 and CCL-24 in healthy volunteers infected with intestinal helminths prior to and after antiparasitic treatment. The study was carried out between May –June 2016 in four rural communities- Otuegela, Immiringi, Otuesega and Ibelebiri which are known to be endemic for intestinal helminths and malaria. Ethical approval was obtained from the Ethical committee of College of Medicine, University of Benin, Nigeria.Both stool and blood samples was collected during active surveillance from 829 volunteers, age range 4-80 years.Intestinal parasites were diagnosed by microscopy and infected volunteers were treated with antiparasitic drugs. Chemokines and cytokines levels were measured using ELISA protocols from sera obtained from 88 helminths and plasmodium infected volunteers, before and 18 days post antiparasitic therapy. The volunteers were coinfected with multiple intestinal helminths. Double infection was 1.1% and they involve Schistsosoma and hookworm and Schistsosoma and *A.lumbricoides*.Single infections was 23.7% . Seven species of intestinal helminths were identified microscopically; *Schistsosoma intercalatum* (10.4%), *A.lumbricoides* (6.5%), *S.mansoni*(4.2%), *Trichuris trichiura* (2.5%) hookworm (2.0%), tapeworm (0.2%). *S.haematobium* which was diagnosed in non- urine contaminated faeces of an 8- year old boy. Asymptomatic infection of intestinal helminths generated an increase in responses of IL-4, IFN-γ and CCL-24 .While IL-4, IFN-γ and CCL24 levels were reduced after antihelminthic treatment,CCL-4 seral concentration remained elevated.

23. Use of antigens from *Strongyloides* papillosus instead of *S. ratti* increases ELISA specificity for human strongyloidiasis

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Human infection by *Strongyloides stercoralis* can manifest with dermatological, intestinal and pulmonal symptoms frequently passing into a chronic disease. Low parasitic loads and discontinuous larvae excretion hamper diagnosis by coproscopy. To detect infection, serological test systems are much more sensitive, however, assays commonly based on native antigens from *Strongyloides ratti* larvae and lack specificity. Lysate from *S. papillosus* was used for the Anti-Strongyloides ELISA IgG. ELISA performance was evaluated by participation in an external quality assessment scheme (NEQAS, UK) encompassing three positive and three negative samples, a correlation with commercial Bordier-ELISA (Strongyloides ELISA kit based on *S. ratti* antigens) through testing of 58 pre-characterized sera and the comparison with an inhouse developed *S. ratti*-based ELISA determining specificities with respect to a cross reactivity panel (193 samples from patients with other parasitic or bacterial infections) and a control panel (samples from 500 blood donors, 100 pregnant women and 88 children). Results obtained with Anti-Strongyloides ELISA were in 100% agreement with the quality assessment target values. Furthermore, in 48 of 58 samples (82.7%), the result of the Anti-Stronygloides ELISA correlated with the pre-characterization by Bordier-ELISA. Serological analysis of discrepant cases (positive with Bordier-ELISA but negative with Anti-Strongyloides ELISA) indicated infections with *Plasmodium ssp*. as well as with *Schistosoma ssp*. The *S. ratti*-based ELISA was reactive in 13.9% of sera in the cross reactivity panel and in 10.6% of the samples from healthy individuals, yielding a combined specificity of 88.6%. In comparison, reactivities of 6.2% (cross reactivity panel) and 3.5% (healthy controls) were detected with the Anti-Strongyloides ELISA. The use of native antigens from *S. papillosus* increases assay specificity.

24. Impact of Human gene, Tumor Necrotic Factor Super Family 13B, and environmental risk factors on the prevalence and intensity of *Ascaris lumbricoides* infection in Igbo-Ora, Nigeria.

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Soil Transmitted Helminths (STH) remain one of the major health challenges in the developing world. The greatest number of soil transmitted helminth infections occurs in the tropical and subtropical regions of Asia as well as sub-saharan Africa. These infections have been found to aggregate in families, host genetic factor (relatedness) and domestic environmental factors have been shown to have significant involvement on susceptibility and infection intensity. TNFSF13B gene, found on chromosome 13 has been demonstrated to be candidate gene influencing susceptibility to *Ascaris* infection. The aim of this study is to identify the impact of TNFSF13B gene on human susceptibility and domestic environmental effect on the prevalence and intensity of *Ascaris lumbricoides* infection within households in Igbo-Ora, a semi urban community in Nigeria. The prevalence of *A. lumbricoides* was 16.7% in the community, 296 family members from 91 families participated in this analysis. The expression of TNFSF13B gene was observed in 218 (73.7%) of the participants. The total absence or non-expression of TNFSF13B gene in all members of a family was observed in 3 families and the presence in all family members was observed in 41 families. In 47 families, there was the absence of the gene in at least one or more members of the family. The heritability of this gene was estimated at 32.9% within related family member (Chi= 0.573, deg =1, Std. Error= 0.45, p=0.224, Kullback-Leibler R-Squared =0.00813). Variance component analysis assessing the genetic effect of TNFSF13B on individuals with STH infection within family members. For *Ascaris* infection, the additive genetic host factors (***h^2***) accounted for 42% of the total prevalence, (h^2=0.4164, Chi-0.525, deg = 1, p= 0.234, Std. Error 0.44). It is however observed, in this study, that host genetic factor (relatedness) and TNFSF13B has greater and significant effect on the prevalence and intensity of *A. lumbricoides* than the shared common household. Meanwhile, the common shared environment also has significant effect on the intensity of *A. lumbricoides.*

25. Urinary metabolite investigation in response to hookworm vaccine administrated to healthy adults in Gabon.

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Metabolomics provides a phenotype of an individual that is influenced by genetic and environmental factors. It reflects the metabolic activity of various organs and their response to external and internal stimulations. It is possible that by studying the metabolic profiles before and after vaccination, it will be possible to identify markers that predict if a vaccine is likely to be successful in large scale studies. Human hookworm infection caused by intestinal nematodes, *Necator americanus* and *Ancylostoma duodenale,* is one of the most common diseases in sub-Saharan Africa and affect more than 700 million people worldwide. Novel vaccine candidates adjuvanted with glucopyranosyl lipidA (GLA), have recently been tested in Gabon in a trial to assess safety and immunogenicity. We used Metabolomics to characterize metabolic change in response to these vaccine candidates. We performed a metabolic profiling before and after vaccination using a nuclear magnetic resonance ^{1}H NMR spectroscopy followed by the exploratory and univariate data analysis. Healthy Gabonese (32) were enrolled in the trial and received three immunizations of the experimental vaccine or Hepatitis B as a control vaccine. Urine samples were collected before and after each immunization over a one year period. Metabolite profiles generated were analysed to investigate the effect of vaccination on the metabolic profile. Antibody responses show that the hookworm vaccine candidates are immunogenic. The highest levels are seen after the third vaccination, however the responses are not sustained at a high level. Comparing the metabolite profiles before and one day after vaccination by univariate analysis shows significant shifts in some metabolites, which are being annotated. Further analysis is needed to assess whether metabolic changes can predict the degree of immunogenicity of the vaccines used.

26. New whipworm genomes and annotations

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The human whipworm (*Trichuris trichiura*) is a soil-transmitted helminth that infects an estimated 700 million people worldwide. The genomes of *Trichuris trichiura* and its mouse model, *Trichuris muris*, were first assembled in 2014. Here, we present an improved *T. muris* genome, reflecting the incorporation of long read sequencing data. Over 80% of the genome is now assembled into 3 main scaffolds (N50 = 28.9 Mb). We have annotated the new genome assembly using a combination of transfer of gene models from the previous assembly, automated gene prediction and manual annotation. We welcome suggestions from the *Trichuris* community on which genes or gene families should be prioritised for targeted curation efforts. We also present an improved *T. trichiura* assembly. We are using the *T. trichiura* annotation as the basis for a school outreach project.

27. Genetic Diversity of Antigen B1 from Sheep, Cattle and Human Isolates of *Echinococcus granulosus* in South of Iran

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Cystic echinococcosis (CE), known as hydatid cyst or hydatid disease, is a zoonotic parasitic infection caused by the larval stage of *Echinococcus granulosus*. Hydatid cyst, with its significant economic and medical impact, constitutes an important public health problem in many developing countries. Antigen B as a major component of the hydatid cyst fluid is encoded by a multigene family with a high degree of genetic variability in different hosts. The present study aimed to evaluate the genetic diversity of gene encoding antigen B1 (AgB1) among different Iranian isolates of *Echinococcus granulosus*. A total of 28 isolates were collected from human (9 isolates), sheep (10 isolates) and cattle (9 isolates). DNA from either protoscolices or germinal layer was extracted from each cyst. PCR followed by DNA sequencing was used to find out the sequence variation and polymorphism of AgB1 among different isolates. The AgB1sequences were aligned and compared with those of existing related sequences available in the GenBank, using MEGA 7 software. Phylogenic tree was constructed by maximum likelihood and estimates of evolutionary divergence between sequences were conducted using the Maximum Composite Likelihood model. After the PCR amplification, using AgB1 primers, an almost 315 bp band was amplified in all of the isolates. Analysis of phylogenetic tree revealed that the isolates from human, sheep and cattle are all clustered in one group and were homologous to M1 allele of AgB1 described earlier. Polymorphism between our isolates was 0.0, while polymorphism between our isolates and related antigen in the GenBank was 0.5 (with AF 143813) and 2.6 (with DQ137836). The sequence data for the 28 antigen of B1 sequences obtained in this study were deposited in GenBank with accession numbers of KY709266 to KY709293. Findings of this study revealed the differences in the sequences of AgB1 between the Iranian isolates of *E. granulosus* and those from other CE-endemic areas. These differences may affect the performance of any diagnostic test which uses AgB for the diagnosis of hydatid cyst.

28. Seroepidemiological study and associated risk factors of Toxocara canis infection among preschool children in Osun State, Nigeria.

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Human toxocariasis is one of the zoonotic diseases caused by the nematode, *Toxocara canis*. In Nigeria, seroepidemiological studies have not been previously carried out among the most vulnerable group, the preschool aged children. A cross-sectional study was conducted in pre-school children in four communities from Osun State, Nigeria between January and July, 2016 . A total of 308 children aged 9 months and 5 years were studied comprising 53.2% (164/308) male and 46.8% (144/308) female. Blood samples were collected and screened for the presence of anti-*Toxocara* IgG antibodies by Western blot analysis based on the excretory-secretory antigens of larva *T. canis* (TcES). Questionnaires were given to parents/guardians of the studied children to collect data on this infection. The overall seroprevalence of *Toxocara* infection was 37.34%. The seroprevalence in the preschool children ranged from 18.18% in children less than one year old to 57.61% in children aged 3 years and above. The logistic regression analysis of risk factors showed that children's age, odds ratio (OR) = 6.12, 95% confidence interval (CI) = 1.25 – 29.90, p = 0.02, contact with dogs (OR = 3.17, 95% CI = 1.40 – 7.20, p = 0.01) and parent's religion (OR = 0.54, 95% CI= 0.32 – 0.91, p=0.02) were the risk factors associated with *Toxocara* infection. Preschool children were exposed early in life to *T. canis* infection as 18.18% of children less than one year old were infected. This is the first serological investigation of *T. canis* infection among preschool children in Nigeria and shows the presence of *T. canis* infection with a high seroprevalence among the studied group. It indicates high transmission with the consequent of visceral or ocular larva migrans. The results also provide baseline data for effective prevention strategies of toxocariasis in Southwest Nigeria.

29. Helminth secretomes reflect different lifestyles and parasitized hosts

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Helminths cause a number of medical and agricultural problems and are a major cause of parasitic infections in humans, animals and plants. Comparative analysis of helminth genes and genomes are important to understand the genomic biodiversity and evolution of parasites and their hosts in terms of different selective pressures in their habitats. The interactions between helminths and their hosts (environments) are mediated in large part by secreted proteins, known collectively as the "secretome". Proteins secreted by parasites are able to modify a host's environment and modulate their immune system. The present study aimed to predict, *in silico*, the secretome in 44 helminth species including Nematoda (31 species) and Platyhelminthes (13 species) and, understand the diversity and evolution of secretomes. Secretomes from plant helminths range from 7.6% (943 proteins) to 13.9% (2,077 proteins) of the filtered proteome with an average of 10.2% (1,412 proteins) and from free-living helminths range from 4.4% (870 proteins) to 13% (3,121 proteins) with an average of 9.8% (2,126 proteins), respectively, and thus are considerably larger secretomes in relation to animal helminth secretomes which range from 4.2% (431 proteins) to 11.8% (2,419 proteins) of the proteomes, with an average of 7.1% (804 proteins). Across 44 secretomes in different helminth species, we found five conserved domains: PF00014 (Kunitz/Bovine pancreatic trypsin inhibitor domain), PF00046 (Homeobox domain), PF00188 (cysteine-rich secretory proteins), PF00085 (Thioredoxin) and PF07679 (Immunoglobulin I-set domain). Secreted proteins had higher architecture diversity compared with non-secreted proteins and the secretome was not conserved across species and the differences suggest possible evolutionary adaptations related with the ecology, lifestyle and environment. Our results detected secreted proteins associated with invasion, infection, adhesion and immunoregulation processes, among other functions. This study will contribute towards the understanding of host-parasite interactions and possibly identify new molecular targets for the treatment or diagnosis of helminthiases.

30. The role of autophagy in anti-*Wolbachia* antibiotic therapy

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Lymphatic filariasis and onchocerciasis are significant global public health problems affecting more than 157 million people. Previous studies have proven that eliminating *Wolbachia*, a mutualistic obligatory bacterial endosymbiont, with antibacterial drugs leads to potent antifilarial effects including macrofilaricidal activity. We have examine the role of autophagy in antibiotic elimination of *Wolbachia* to determine if targeting this pathway can enhance antibiotic efficacy. A range of chemical inhibitors targeting different stages of the autophagy pathway were evaluated in *Wolbachia* infected cell lines and adults and microfilariae of *Brugia malayi.* Markers of autophagy activation and suppression (LC3B-II and P62) were monitored using immunofluorescence confocal microscopy or immunoblotting to confirm activity of the selected inhibitors (3-MA, Wortmannin, LY294002 and L-asparagine). The suppression of autophagy during antibiotic treatment of *Wolbachia* was assessed for the impact on the reduction in *Wolbachia* loads and the viability of residual bacteria using RNA expression analysis. Results have shown that in *Brugia malayi* microfilariae and adult females, inhibition of autophagy leads to a block in the reduction in *Wolbachia* loads following exposure to doxycycline and rifampicin. Furthermore, the reduced viability of the residual population suggests that autophagy makes a direct contribution to the antibiotic mode-of-action rather than simply having a role in the clearance of dead bacteria. The outcomes of the study will further enhance our understanding of the therapeutic mode-of-action of anti-*Wolbachia* drugs to improve future drug design for the treatment of filarial diseases.

31. Identification of schistosome molecules that drive splenic regulatory B cell development

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During chronic schistosome infections, a complex regulatory network is induced to regulate the host immune system, in which IL-10-producing regulatory B cells (Breg) play a significant role. Schistosomal egg antigens (SEA) are bound and internalized by CD1Dhi-marginal zone B cells and induce IL-10 production. IPSE/alpha-1 protein, one of the major antigens in SEA, was identified as one of the molecules able to induce IL-10 producing B cells, contrary to other major schistosomal antigens like omega-1 and kappa-5. (Haeberlein *et al*, Plos Pathogens, 2017). Interestingly, SEA depleted from IPSE was still capable of inducing IL-10-producing Breg cells, suggesting that more molecules are present with a similar activity. Both heat and trypsin treatment of SEA abolished their IL-10-inducing capacity, suggesting that at least some of the critical Breg molecules present are proteins. To isolate the proteins of interest, we have applied fractionations based on size or charge by HPLC in parallel. The anion exchange separation showed 4 active fractions (out of 12) containing IL-10-inducing molecules, while the size separation showed 8 active fractions (out of 20) in both the high and low MW range. The high MW fractions were rich in glycosylated molecules, while the lower MW fractions were relatively devoid of glycans and contained a relative low number of proteins. On those, we performed proteomics analysis and compared the content to a similar fraction with lower IL-10 inducing activity. In this strategy, we obtained only few unique proteins, which is probably due to the size of the proteins, which were quite similar in the tested fractions. Although proteomics is mostly semi-quantitative, we did observe differences in the number of peptides obtained for several proteins. Currently, the activity of some of these proteins is tested on B cells and processed for recombinant expression in our mammalian or plant systems.

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