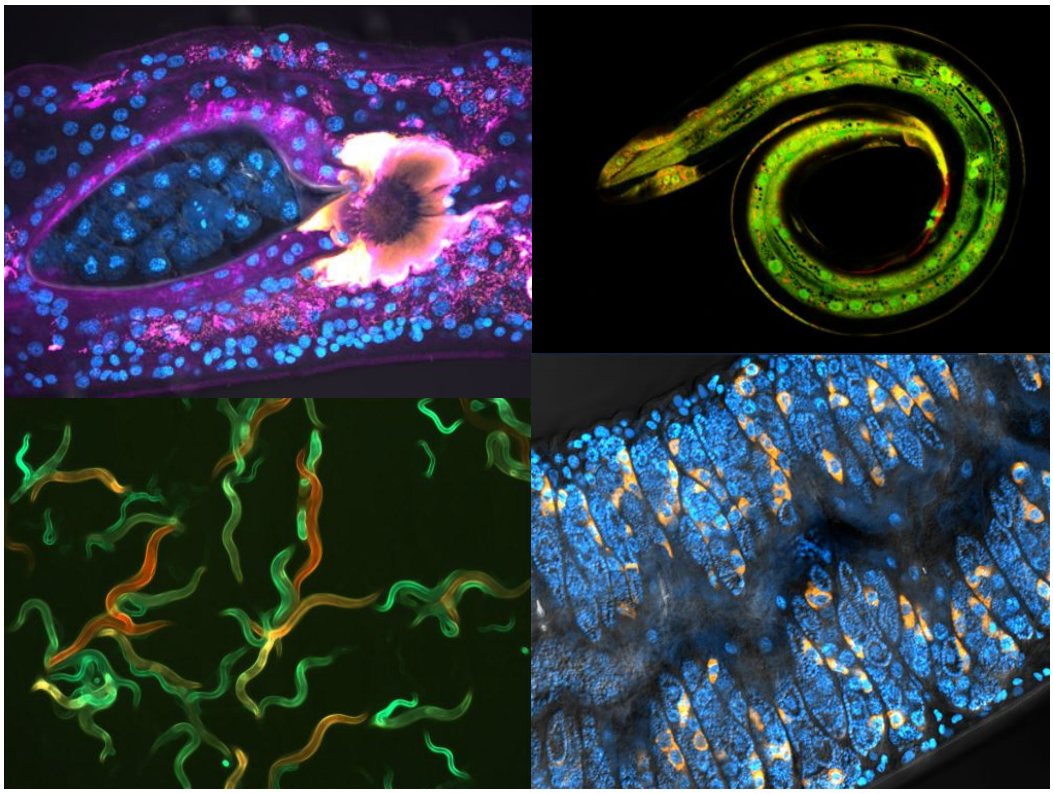
Functional characterization of the nicotine-sensitive AChR from Oesophagostomum dentatum

Claude Charvet, Elise Courtot, Hadile Gabaj, Nicolas Peineau, Richard J. Martin, Alan P. Robertson, Thomas Boulin, Robin Beech, Cédric Neveu

Molecular and Cellular Biology of Helminths IX



31st August - 5th September 2015

Bratsera Hotel, Hydra, Greece

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'

Dates of MCBHP-X Meeting: 5-10 September 2016

ORGANISERS, 2015

Kleoniki Gounaris (Imperial College, UK)
Rick Maizels (University of Edinburgh, UK)
Murray Selkirk (Imperial College, UK),

ADMINISTRATION FOR 2015

Jennifer Hurst
Centre for Immunity, Infection and Evolution & Edinburgh Infectious Diseases
Ashworth Laboratories, Charlotte Auerback Road
University of Edinburgh, Edinburgh, EH9 3FL
Tel: (+44) 131 651 3688
E-mail: jennifer.hurst@ed.ac.uk

Cover photos, clockwise from top left, *Schistosoma egg (James Collins), C. elegans (Leo Kurz and Jonathan Ewbank); Schistosome vitellaria (James Collins); C. elegans (Nathalie Pujol and Jonathan Ewbank).*

	Monday 31 August	Tuesday 1 September	Wednesday 2 September	Thursday 3 September	Friday 4 September	Saturday 5 September
	ARRIVE	**Development and Behaviour**	**Immunology of helminths**	**Mini Symposium: Small RNAs**	**Molecular Immunology**	DEPART
09:00		**James Collins**	**Graham Le Gros**	Eric Miska (*)	**Jonathan Ewbank**	
09:20						
09:40		Aziz Aboobaker	Bill Horsnell	Julie Claycomb (*)	Emma Ringqvist	
10:00		Sung-Jong Hong	Cornelis Hokke	Peter Sarkies	Allison Bancroft	
10:20		Elissa Hallem	Sandra O'Neill	Jose Tort	Allison Aldridge	
10:40-11:10	Coffee break			* 30 min talks		
		Chemotherapy and Drug Development	**Molecular interactions with the host**	**Mini Symposium: Small RNAs**	**Genomes and systems Biology**	
11:10		Richard Martin	Patrick Skelly	Cei Abreu-Goodger	Annette Dougall	
11:30		Alex Blanchard-Letort	Murray Selkirk	Eileen Devaney	Matt Berriman	
11:50		Robin Beech	Michael Smout	Guofeng Cheng	**Genomics Workshop** Bruce Bolt	
12:10		Jurgen Krucken	Gillian Coakley	Juan Quintana		
12:30		Peter Fischer	Sara Lustigman	Amy Buck		
12:50-4:30	Afternoon break					
		Cellular immunology	**Immunomodulation**	**Molecular Biology of helminths**	**Immunity and repair**	
4:30	**Registration Opens at Bratsera Hotel**	Andrew MacDonald	**Maria Yazdanbakhsh**	**Richard Davis**	De'Broski Herbert	
4:50		Mark Siracusa			Wiebke Hartmann	
5:10		Meera Nair	Alex Loukas	Gabriel Rinaldi	Alex Phythian-Adams	
5:30		**Poster Pitches, 17 x 2 minutes**	Bill Harnett	**Poster Pitches 17 x 2 minutes**	Maria Duque-Correa	
5:50			Conor Finlay		Rick Maizels	
		6:10 Break/End of Sessions				
6:30	**Pre-lecture drinks**	**Poster Session 1**		**Poster Session 2**		
7:30	**Keynote Lecture: Marie-Anne Felix**		**Vlychos Taverna Dinner (Boat leaves 7:00 PM)**		**Bratsera Farewell Dinner (8:30 PM)**	
8:30	**Bratsera Welcome Dinner (8:45 PM)**	**End of Session**		**End of Session**		

NOTES

Monday 31 August

Chair: **Rick Maizels**, University of Edinburgh	
19:30	Keynote Lecture: **Marie-Anne Félix**, Institut de Biologie de l'Ecole Normale Supérieure, CNRS-ENS-Inserm ***C. elegans* in an Ecological and Evolutionary Context**
20:30	**Welcome Reception and Dinner, Bratsera Hotel**

Tuesday 1 September

09:00 - 10:40 Session 1: Developmental and Behaviour

Chair: **Rick Maizels**, University of Edinburgh		
09:00	**James Collins,** *UT Southwestern Medical Center, Dallas, Texas*	It's no Fluke! Using free-living planarians to guide our understanding of schistosomes.
09:40	**Aziz Aboobaker,** *University of Oxford*	Can we use planarian pluripotent stem cells as a model for studying cancer stem cell behaviour?
10:00	**Sung-Jong Hong,** *Chung-Ang University, Korea*	Bile-chemotactic factors, signal transducer and behaviours of *Clonorchis sinensis.*
10:20	**Elissa Hallem,** *University of California, LA*	Odor-driven host seeking in parasitic nematodes.

11:10 – 12:50 Session 2: Chemotherapy and Drug Development

Chair : **Niki Gounaris**, *Imperial College London*		
11:10	**Richard Martin,** *Iowa State University*	Asu-ACR-16: A dispersed nAChR drug target in muscle, intestine and other tissues of *Ascaris*.
11:30	**Alex Blanchard-Letort,** *French National Institute for Agricultural Research*	In vivo approach to elucidate the functionality of nicotinic receptors in *Haemonchus contortu*s using RNA interference.
11:50	**Robin Beech,** *McGill University*	Evolutionary mechanisms and functional implications for ion-channel anthelmintic drug targets.
12:10	**Jürgen Krücken,** *Freie Universitat Berlin*	Interaction of different macrocyclic lactones and ketoconazole with *Cylicocylus* elongates P-glycoprotein 9 in a yeast growth assay.
12:30	**Peter Fischer,** *Washington University School of Medicine in St Louis*	Effects of short-term antibiotic treatment on *Wolbachia* and its filarial host *B.malayi*.

16:30 - 18:10 Session 3: Cellular immunology

Chair : **Graham Le Gros,** *Malaghan Institute for Medical Research*		
16:30	**Andrew MacDonald,** *University of Manchester*	A dominant role for the methyl-CpG-binding protein Mbd2 in controlling dendritic cell induction of type-2 inflammation.
16:50	**Mark Siracusa,** *Rutgers New Jersey Medical School*	In situ hematopoiesis-derived mast cells promote protective immunity to *Trichinella spiralis*.
17:10	**Meera Nair,** *University of California Riverside*	Delineating the function of human resistin using transgenic mice and clinical samples from helminth infected individuals.
17:30	**Pitches for Poster Session 1**	

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

Chair: **Murray Selkirk,** Imperial College London			
1	**Nicola Beesley**	University of Liverpool	Ploidy and population genetic structure of *Fasciola hepatica* in sheep and cattle
2	**Rita Berkachy**	Imperial College London	Comparative analysis of secreted nucleotide-metabolising enzymes in parasitic nematodes
3	**Alejandro Cabezas-Cruz**	Lille University	Effect of glucose on the structure and transcriptional regulation of glucose transporters in *Schistosoma mansoni*.
4	**Claude Charvet**	National Institute of Agronomical Research	Functional investigation of nematode parasite specific acetylcholine receptors
5	**Franklin Chow**	University of Edinburgh	Investigation of extracellular RNA in *Heligmosomoides polygyrus*: Are we witnessing cross-species communication?
6	**Thomas Crellen**	Imperial College London/ Wellcome Trust Sanger Institute	Population and comparative genomics of African *Schistosoma mansoni*
7	**Janina Demeler**	Freie Universitat Berlin	Development and field application of pyrosequencing assays for analysis of three *Trichostrongylus colubriformis* β-tubulin single nucleotide polymorphisms associated with benzimidazole resistance
8	**Katarzyna Donskow-Łysoniewska**	University of Warsaw	Early phase of live intestinal nematode therapy mediated regulatory cell recruitment to the Central Nervous System in EAE model of Multiple Sclerosis
9	**Thomas Duguet**	McGill University, Canada	Functional diversification of levamisole receptors in the trichostrongylid nematode *Haemonchus contortus*
10	**Lewis Entwistle**	The Francis Crick Institute	Endogenous phospholipase A_2 group 1B (PLA_2g1B) has direct anti-helminth properties and is essential for immunity to *Heligmosomoides polygyrus*
11	**Gisela Franchini**	Universidad de la Planta, Argentina	Identification and characterization of lipid binding proteins from the parasitic nematode *Dioctophyma renale.*
12	**Geoffrey Gobert**	QIMR Berghofer Medical Research Institute, Australia	Characterising Host Gene Expression during Recovery from Hepatic Schistosomiasis Japonica
13	**Alessandra Guidi**	National Research Council, Rome	Development and validation of a luminescence-based, medium-throughput assay for drug screening in *Schistosoma mansoni*
14	**Jana Hagen**	University of Melbourne	OVA-transgenic schistosome eggs as a traceable model of host-parasite interaction
15	**Martin Horn**	Academy of Sciences of the Czech Republic	SmCB1 drug target from the *Schistosoma mansoni* blood fluke: structural basis for inhibition and activation
16	**Francesca Jarero**	National History Museum	Heads or tails: Functional investigations of gene regulatory networks controlling planarian AP patterning in the model tapeworm *Hymenolepis microstoma*
17	**Maria Kaisar**	Leiden University Medical Center	Conditioning of dendritic cells for Th2 polarization by helminth-derived molecules: exploring a role for lipids

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

Wednesday 2 September

09:00 - 10:40 Session 4: Immunology of helminths

Chair: **Maria Yazdanbakhsh,** *Leiden University Medical Center*		
09:00	**Graham Le Gros,** *Malaghan Institute for Medical Research*	The biology underpinning helminth parasite induced Type 2 immune responses related to host immunity, tissue repair and pathology.
09:40	**Bill Horsnell**, *University of Cape Town*	Natural and vaccine-mediated immunity to *Salmonella typhimurium* is Impaired by the helminth *Nippostrongylus brasiliensis*
10:00	**Cornelis Hokke,** *Leiden university Medical Center*	Analysis of antibodies to all individual glycan antigens of the schistosome glycome in human and animal sera in relation to immunity.
10:20	**Sandra O'Neill,** *Dublin City University*	High mannose glycoproteins present on *Fasciola hepatica* surface coat exhibit immune modulatory properties independent of the mannose receptor.

11:10 – 12:50 Session 5: Molecular interactions with the host

Chair: **Richard Davis,** *University of Colorado School of Medicine*		
11:10	**Patrick Skelly**, *Tufts University*	The schistosome surface and host hemostasis.
11:30	**Murray Selkirk,** Imperial College London	Nematode acetylcholinesterases and cholinergic regulation of immunity
11:50	**Michael Smout,** James Cook University	A granulin growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini*, and its role in wound healing and carcinogenesis.
12:10	**Gillian Coakley,** *University of Edinburgh*	Secreted exosomes from *Heligmosomoides polygyrus* modulate cellular responses of the murine host.
12:30	**Sara Lustigman,** *Lindsley F. Kimball Research Institute*	Functional interplay between *Brugia* and its *Wolbachia* symbiont.

16:30 – 18:10 Session 6: Immunomodulation

Chair: **Andrew MacDonald,** University of Manchester		
16:30	**Maria Yazdanbakhsh,** *Leiden University Medical Center*	Helminth infections, the immune system and metabolism
17:10	**Alex Loukas,** James Cook University	The therapeutic hookworm.
17:30	**Bill Harnett,** *University of Strathclyde*	The *Acanthocheilonema viteae*-derived immunomodulator ES-62 acts as a molecular switch to promote IL-22-mediated resolution of joint inflammation in a model of rheumatoid arthritis.
17:50	**Conor Finlay,** *Trinity College Dublin*	Helminths protect against autoimmunity through IL-5 and IL-33.

Thursday 3 September

09:00 - 10:40 Mini Symposium: Small RNAs

Chair: **Amy Buck,** University of Edinburgh		
09:00	**Eric Miska** *University of Cambridge*	Evolution of non-coding RNA pathways in nematodes
09:30	**Julie Claycomb** *University of Toronto*	Functional genomic characterization of endogenous small RNA pathways in *Caenorhabditis* nematodes.
10:00	**Peter Sarkies,** *Imperial College London*	The cost of silence: Insights from the evolution of DNA methylation in nematodes.
10:20	**Jose Tort,** *Universidad de la Republica, Uruguay*	Small RNA pathways in *Fasciola hepatica*

11:10- 12:50 Mini Symposium: Small RNAs

Chair: **Richard Davis,** *University of Colorado School of Medicine*		
11:10	**Cei Abreu-Goodger,** *Langebio-Cinvestav*	Inferring microRNA function: from model organisms to parasitic interactions.
11:30	**Eileen Devaney,** *University of Glasgow*	MicroRNAs in parasitic nematodes: a role in anthelmintic resistance?
11:50	**Guofeng Cheng,** *Chinese Academy of Agricultural Sciences*	Schistosome miRNAs play a regulatory role in sexual maturation and gametogenesis.
12:10	**Juan Quintana,** *University of Edinburgh*	Secreted filarial small RNAs – localization in biofluids and biomarker potential for onchocerciasis.
12:30	**Amy Buck,** *University of Edinburgh*	An extracellular RNA interference pathway as a mechanism of parasite-host communication.

16:30 – 8:10 Session 7: Molecular Biology of helminths

Chair: **Eileen Devaney,** University of Glasgow		
16:30	**Richard Davis,** *The University of Colorado School of Medicine*	Programmed DNA elimination in nematodes
17:10	**Gabriel Rinaldi,** *George Washington University*	Retroviral-based functional genomic tools for schistosomes.
17:30	**Pitches for Poster Session 2**	

Thursday 3 September

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

Chair: **Niki Gounaris**, *Imperial College London*			
18	**Gregory Karadjian**	Museum National d'Histoire Naturelle-ANSES	*Litomosoides sigmodontis* filarial infective larvae migrate through the lungs increasing s100A9 in neutrophils
19	**Andrea Kemter**	University of Edinburgh	Dendritic cell modulation by *Heligmosomoides polygyrus* excretory/secretory products
20	**Marije Kuipers**	Leiden University Medical Center	Various life stages of *Schistosoma mansoni* release extracellular vesicles
21	**Dominik Laetsch**	University of Edinburgh	Inter- and intra-specific analyses of the effector complement in potato cyst nematodes
22	**Silvia Libro**	New England Biolabs	Characterization of immune response genes in the parasitic nematode *Brugia malayi*
23	**Coralie Martin**	Museum National d'Histoire Naturelle	Unravelling the filarial larvae migrations: lessons from *Litomosoides sigmodontis* mouse model
24	**Jun Matsumoto**	Nihon University, Japan	Characterization of glucose transporter genes from fox tapeworm *Echinococcus multilocularis*
25	**Cecile Menez**	National Institute of Agronomical Research	Comparison of ivermectin and moxidectin resistance profiles in the nematode *Caenorhabditis elegans*
26	**Carlos Minutti**	University of Edinburgh	T1/ST2-EGF-R co-expression in T_H2 cells is essential for IL-13-induced worm expulsion
27	**Eyitayo Oluwadare**	Edinburgh Napier University	Evaluation of a protease-dependent prodrug in the potential treatment of parasitic helminth infections
28	**Christian Owusu**	Wellcome Trust Sanger Institute	Temporal transcriptional profiling of livers from *Schistosoma mansoni*-infected mice
29	**Jan Pankrác**	Charles University in Prague	Embryonic muscle development in redia and cercaria of *Fascioloides magna* (Trematoda: Digenea)
30	**Franca Ronchese**	Malaghan Institute, New Zealand	Transcriptomic profiling of skin dendritic cells in the immune response to *Nippostrongylus brasiliensis*
31	**Danielle Smyth**	University of Edinburgh	Identification of a functional TGFβ mimic secreted by *Heligmosomoides polygyrus*.
32	**Tao Wang**	Sichuan Agricultural University, China	Proteomic analysis of the excretory-secretory products of adult *Ascaris suum*
33	**Xuhang Wu**	University of Edinburgh	Functional analysis of Bm-SPN-2, the major secreted product of *Brugia malayi* microfilariae
34	**Ruud Wilbers**	Wageningen University, The Netherlands	Engineering of plants for the expression of helminth glycoproteins with their native N-glycan structures

Friday 4 September

09:00 - 10:40 Session 8: Molecular Immunology

Chair: **Bill Harnett,** *University of Strathclyde*		
09:00	**Jonathan Ewbank,** *Centre d'Immunologie de Marseille-Luminy*	How *C. elegans* fights fungal infection
09:40	**Emma Ringqvist,** *Karolinska Instituet, Sweden*	Changes in the immunological landscape during a *Nippostrongylus brasiliensis* infection does not affect the emphysematous long term pathology.
10:00	**Allison Bancroft,** *University of Manchester*	Structure and function of the major Excreted Secreted protein of *Trichuris muris*.
10:20	**Allison Aldridge,** *Dublin City University*	*Fasciola hepatica* tegumental antigens induce anergenic T-cells in vivo and in vitro via dendritic cells in a mannose receptor dependent manner.

11:10 – 12:50 Session 9: Genomes and systems Biology

Chair: **Alex Loukas,** James Cook University		
11:10	**Annette Dougall,** University of Liège	Helminth-induced inflammation controls murine γ-herpesvirus replication in the lung
11:30	**Matthew Berriman,** *Sanger Institute*	WornBase-Parasite a new resource for helminth genomics.
11:50	**Genomics Workshop with Bruce Bolt and Matt Berriman**	

16:30 – 18:10 Session 10: Immunity and repair

Chair : **Murray Selkirk,** Imperial College London		
16:30	**De'Broski Herbert,** *University of California San Francisco*	Myeloid restricted AMPKα1 regulates Type 2 immunity and lung tissue repair following hookworm infection.
16:50	**Wiebke Hartmann,** *Bernhard Nocht Institute for Tropical Medicine*	*Litomosoides sigmodontis* induces TGF-β receptor responsive, IL-10 producing T cells that suppress bystander T cell proliferation in vivo.
17:10	**Alex Phythian-Adams,** *University of Manchester*	Defining the importance of dendritic cell subsets in promotion and regulation of immunity to *Schistosoma mansoni*.
17:30	**Maria Duque-Correa,** *Wellcome Trust Sanger Institute*	Role of receptors of IL-10 superfamily members during *Trichuris muris* infection.
17:50	**Rick Maizels,** *University of Edinburgh*	Innate cytokines required for the effector phase of helminth expulsion

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

ABSTRACTS

KEYNOTE LECTURE

***C. elegans* in an Ecological and Evolutionary Context**

MARIE-ANNE FÉLIX

INSTITUT DE BIOLOGIE DE L'ECOLE NORMALE SUPERIEURE, CNRS-ENS-INSERM

Biological processes are generally studied in the laboratory under one environmental condition and in one reference genetic background. This is in particular true for studies using the nematode *Caenorhabditis elegans* as a model organism. We try to widen this horizon and provide an ecological and evolutionary context for *C. elegans* as well as study the relationship between genetic, environmental and phenotypic variation. Natural populations of *C. elegans* and its relatives can be found in rotten vegetal material such as rotting stems, flowers and fruits. These food sources are ephemeral, forcing a boom-and-bust lifestyle on *C. elegans*: after a first population proliferation stage, young larvae in large populations develop into diapausing dauer larvae. We surveyed *Caenorhabditis* populations in an orchard and a wood over several years, so as to also probe *C. elegans* (meta)population structure and outcrossing rate. Natural pathogens provide strong and changing selection pressures and are thus relevant to study the defense systems of *C. elegans* and their potentially rapid evolution. Several natural pathogens of *C. elegans* were isolated, including the first viruses that infect *C. elegans* or *C. briggsae*. A genome-wide association study of Orsay RNA virus load after infection of a worldwide set of *C. elegans* isolates indicates one major locus segregating in the species. We found that this major locus corresponds to a widespread deletion inactivating the homolog of vertebrate RIG-I viral sensors, thus allowing viral replication. Another focus of your work is vulva cell fate specification, which provides a model developmental system to study the evolution of developmental processes at different evolutionary scales. I will report on our ongoing work characterizing vulva mutations in the rhabditid nematode *Oscheius tipulae* using genetic mapping and genome sequencing.

It's No Fluke! Using free-living planarians to guide our understanding of schistosomes.

JAMES COLLINS

UT SOUTHWESTERN MEDICAL CENTER, DALLAS, TEXAS, USA

Schistosomiasis is among the most prevalent human parasitic diseases, affecting more than 200 million of the world's poorest people. The etiological agents of schistosomiasis are parasitic flatworms (*Schistosoma*) that live and lay eggs within the vasculature of the host. These eggs lodge in host organs, causing inflammatory responses that are the primary cause of morbidity. Since these parasites can live within human hosts for decades, elucidating the mechanisms promoting their longevity is of fundamental importance. Previously, we demonstrated that schistosomes possess a population of somatic stem cells that we refer to as neoblasts given their similarity to the stem cells found in free-living planarian flatworms. To characterize the cellular roles for neoblasts in adult schistosomes, we examined the transcriptional profile of parasites 2-3 weeks following neoblast ablation. These studies, coupled with EdU pulse-chase experiments, indicate that a large fraction of differentiating neoblasts are destined to give rise to cells associated with the parasite's syncytial epidermal coat, a structure known as the tegument. To define factors required for the differentiation of neoblasts towards this tegumental lineage, we conducted an RNAi screen and identified a novel zinc finger protein as required for the commitment of neoblasts to a tegumental lineage. Strikingly, a similar factor has been previously shown to be essential for the commitment of planarian neoblasts towards epidermal fates. Thus, a conserved molecular program may drive the differentiation of neoblasts to epidermal fates in both free-living and parasitic flatworms. Since the tegument represents a distinguishing factor between the parasitic and free-living flatworms, unraveling this conserved molecular program may provide clues about the developmental underpinnings that allowed for the evolution of parasitism among the Platyhelminthes.

Can we use planarian pluripotent stem cells as a model for studying cancer stem cell behaviour?

AZIZ ABOOBAKER

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF OXFORD, SOUTH PARKS ROAD, OXFORD OX1 3PS, UK.

We are interested in studying the basic mechanisms that control regeneration and the underpinning adult stem cell biology that fuels this process. The mechanisms underpinning the behavior of stem cells that drive regeneration, namely proliferation, self-renewal, differentiation and migration, are relevant to many human disease processes, but in particular cancer. The model organisms we work with, highly regenerative planarians, have the potential to reveal new important physiological functions for conserved genes and gene networks. Our previous research has already demonstrated this for the PIKK TOR-related kinase SMG1 (Suppressor with Morphological effect on Genitalia) which when knocked down by RNAi in planarians leads to the formation of tumor like outgrowths that eventually kill the animals. SMG1 has since been confirmed as a novel tumor suppressor gene in mice. Building on these findings we have designed improved assays in planarians that employ Ionizing Radiation (IR) to allow us to i) Perform expression studies capturing the molecular profile of key stem cell behaviours such as proliferation, migration and response to stress ii) track key stem cell behaviours using FISH and confocal microscopy and iii) identify regulatory mechanisms for these processes using RNAi based loss of function screens. These approaches will allow is use the planarian model system to assign physiological function to genes implicated in human cancer expression studies and to discover potentially novel regulatory processes.

Bile-chemotactic factors, signal transducer and behaviors of *Clonorchis sinensis*

TAE IM KIM, SHUNYU LI, WON GI YOO, **SUNG–JONG HONG**

DEPARTMENT OF MEDICAL ENVIRONMENTAL BIOLOGY, CHUNG-ANG UNIVERSITY COLLEGE OF MEDICINE, SEOUL, KOREA

Clonorchis sinensis metacercariae excyst in duodenum and migrate into bile duct. Bile attracts the *C. sinensis* newly excysted juvenile (CsNEJ) to migrate into the bile duct. The CsNEJs migrated toward 0.01-0.1% bile in vitro, but not to higher bile. Of bile components, cholic acid was only attractive to the CsNEJs and stimulated them to move quickly. While the CsNEJs moved repellently from lithocholic acid. In the common bile duct, the CsNEJs are avoid from gall bladder containing condense bile of higher lithocholic acid and migrate to bile capillaries. The second and third addition of cholic acid to the assay chamber re-activated chemotactic migration of the CsNEJs, saying their sensing and responding to cholic acid gradient. Bile chemotactic migration was inhibited sensitively in vitro by dopamine-receptor antagonists D1, D2 and D3 and a dopamine uptake inhibitor, but less by glutaminergic, serotonininergic and cholininergic neuron inhibitors. Migration of the CsNEJs in a mammalian host was traced by *in vivo* imaging techniques. The CsNEJs were radio-labeled with ^{18}F-fluorodeoxyglucose (^{18}FDG) by incubating in a maintaining media containing 74 MBq ^{18}FDG at 37°C. A catheter was positioned in middle duodenum of rabbits under anesthesia and cholecystokinin was injected to contract gall bladder. After 12 minutes of cholecystokinin injection, bile was released from gall bladder. The ^{18}FDG-labeled CsNEJs were inoculated at middle duodenum through a catheter. Photon signals of the ^{18}FDG-labeled CsNEJs were collected using positron emission tomography-computed tomogram. Signals of the CsNEJs were detected from 9 minutes and increased rapidly in the liver until 21 minutes. Collectively, it was elucidated that the CsNEJs sensed cholic acid of bile in the duodenum and migrated up chemotactically and quickly into the bile duct, and that dopaminergic neurons play a major role of bile-chemotactic behavior of the CsNEJs.

Odor-driven host seeking in parasitic nematodes

MICHELLE CASTELLETTO, SPENCER GANG, AREZOO BARNIA, and **ELISSA HALLEM**

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

The infective juveniles (IJs) of many parasitic nematodes are thought to search for hosts to infect, yet their host-seeking behavior is poorly understood. We conducted an in-depth analysis of host seeking in parasitic nematodes, with a focus on the skin-penetrating human threadworm *Strongyloides stercoralis*. We found that *S. stercoralis* is highly motile relative to other parasitic nematodes and appears to actively cruise for hosts. It is attracted to a diverse array of human skin and sweat odorants, including some that are considered human-specific. The strongest attractants for *S. stercoralis* also attract mosquitoes, suggesting that mosquitoes and worms target humans using many of the same olfactory cues. Carbon dioxide is not a long-range attractant for skin-penetrating nematodes, but is an essential developmental cue required for IJ activation. Olfactory responses of *S. stercoralis* are life-stage specific, with IJs responding differently from other life stages. A comparison of odor-driven behavior in *S. stercoralis* and six other nematode species revealed that parasite olfactory preferences reflect host specificity and infection mode rather than phylogeny, suggesting an important role for olfaction in the host selection process. We are now investigating the neural circuits and signaling pathways that mediate host seeking in skin-penetrating nematodes. We are conducting a comparative analysis of sensory neural circuit function in *S. stercoralis*, the closely related rat parasite *Strongyloides ratti,* and the free-living nematode *C. elegans*. We are also identifying genes required for host seeking and host infection by *S. stercoralis* and *S. ratti*. Our results will provide insight into how sensory neural circuits differ in free-living versus parasitic species so as to support parasite-specific behaviors, and may enable the development of odor-based traps or repellents for parasitic nematodes.

Asu-ACR-16: A dispersed nAChR drug target in muscle, intestine and other tissues of *Ascaris*

RICHARD J. MARTIN[1], MELANIE ABONGWA[1], SAMUEL K. BUXTON[2], ELISE COURTOT[2 3], CLAUDE CHARVET [2 3], & ALAN P. ROBERTSON [1]

[1]DEPARTMENT OF BIOMEDICAL SCIENCES, COLLEGE OF VETERINARY MEDICINE, IOWA STATE UNIVERSITY, USA; [2]INRA, UR1282 INFECTIOLOGIE ANIMALE ET SANTÉ PUBLIQUE, F-37380 NOUZILLY, FRANCE; [3]UNIVERSITÉ FRANÇOIS RABELAIS DE TOURS, UMR1282 INFECTIOLOGIE ET SANTÉ PUBLIQUE, F-37000 TOURS, FRANCE

Older, 'classic,' cholinergic anthelmintics, levamisole and pyrantel, and more recent 'modern,' anthelmintics, tribendimidine and derquantel, act on heterogeneous pentameric nAChRs (composed of: UNC-29, UNC-38, ACR-8 and UNC-63) while monepantel acts on nAChRs composed of DEG-3-like subunits. Resistance to these anthelmintics is of concern and evidence suggests that the resistance may involve altered subunit sequences and subunit truncation. If we can identify suitable, novel, and vital nAChR subunits as targets, we can develop screens for selective compounds for discovery of 'resistance-busting' anthelmintics. We cloned *Asu*-ACR-16 from *Ascaris suum,* a clade III nematode parasite, and found its expression in *Ascaris* intestine, oviduct & uterus, head region and body muscle strips. Single-cell PCR, using intracellular pipettes, showed expression in single-muscle cells but not in pharyngeal muscle cells. We expressed *Asu*-ACR-16 in *Xenopus* oocytes, as a functional homomeric receptor channel, using *Asu*-ACR-16 cRNA co-injected with cRNA for *Asu*-RIC-3, a chaperone protein. We tested effects on currents under voltage-clamp, of different agonists, modulators and antagonists on these expressed receptors. The receptors were not sensitive to levamisole or pyrantel: the rank order of potency series for agonists and anthelmintics when normalized to the control 100 µM ACh was: nicotine> acetylcholine> cytisine> 3bromocytisine> epibatidine> DMPP> oxantel >>> choline = betaine = lobeline=morantel=levamisole=methyridine=thenium=bephenium=tribendimidine=pyrantel. Based on agonist potency series, this receptor is unlike the distinctive levamisole receptors of nematodes. We also tested a series of antagonists including derquantel and found that the antagonist potency series was also unlike the levamisole receptors. Positive allosteric modulators including ivermectin and genistein and PNU120596 were also tested but found to be without effect. The wide distribution in different tissues of the nematode is interesting and the different pharmacology of *Asc*-ACR-16 supports the suggestion that this receptor could be used as a suitable screen for novel nicotinic anthelmintics that are not likely to show cross-resistance to existing cholinergic anthelmintics.

In vivo approach to elucidate the functionality of nicotinic receptors in _Haemonchus contortus_ using RNA interference

ALEXANDRA BLANCHARD-LETORT[1,2], FABRICE GUEGNARD[1,2], CHRISTINE SAUVE[1,2], CEDRIC NEVEU[1,2]

[1] NATIONAL INSTITUTE OF AGRONOMICAL RESEARCH (INRA), UMR1282 INFECTIOLOGIE ET SANTE PUBLIQUE, F-37380 NOUZILLY, FRANCE; [2] UNIVERSITE DE TOURS, UMR1282 INFECTIOLOGIE ET SANTE PUBLIQUE, F-37380 NOUZILLY, FRANCE

Gastro-intestinal nematodes (GIN) are a major threat to ruminant production systems throughout the world. The control of GIN has been largely based on the use of anthelminthics but the massive over-use of these drugs has led to the emergence and the wide scale spread of resistant worms. Research is currently underway to develop new, efficient and sustainable strategies to control the GIN including the development of new drugs. In order to preserve the efficacy of new and existing drug molecules, their targets need to be clearly identified to better understand their mode of action and the molecular basis of resistance. Numerous receptors to the main drugs have already been identified in nematodes such as acetylcholine receptors (AChR). These receptors are composed of 5 subunits (homo or heteropentamers) involved in fast neuro-transmission. The drugs act as an agonist to the muscular AChR inducing paralysis of the worms. The rearrangement and stoichiometry of the subunits confers various degrees of susceptibility to the drugs. To date, the involvement of the subunits in the receptor constitution and the consequent susceptibility/resistance to the drugs has only been demonstrated in heterologous expression systems (e.g. *Xenopus laevis* oocytes) as no stable transformation was available for GIN. Using the ruminant GIN *Haemonchus contortus* larval stages we were able to silence for the first time different AChR subunits. The invalidation of these subunits was found to either induce a motility default or modulate the degree of drug sensitivity in the worms. This original work offers promising opportunities to aid in the future characterization of drug targets *in vivo.*

Evolutionary mechanisms and functional implications for ion-channel anthelmintic drug targets.

ROBIN BEECH[1], THOMAS DUGUET[1], CLÉMENCE ACKERMANN[1], CLAUDE CHARVET[2] AND CÉDRIC NEVEU[2]

[1]INSTITUTE OF PARASITOLOGY, MCGILL UNIVERSITY, MONTREAL, CANADA. [2]INRA, TOURS, FRANCE.

Pentameric ligand-gated ion-channels (pLGICs) trace back to before the eukaryote/prokaryote split more than 2 billion years ago.Their structural organization and physical arrangement of individual subunits has remained essentially unchanged over this time frame. The original channel was likely a homopentamer that subsequently diversified through gene duplication and loss, changes in expression and functional characteristics to produce an enormous repertoire of receptors in almost all eumetazoans that enable synaptic signalling, regulate immune cells and even control calcium carbonate deposition in mollusk shells. The pLGICs are of particular interest as important helminth drug targets. Characterization in the model nematode, *Caenorhabditis elegans*, and related parasites provides detailed information on receptors including those for glutamate and GABA, targeted by ivermectin, and acetylcholine targeted by nicotine, levamisole, monepantel, bephenium, tribendimidine and others. Comparison of cognate receptors from parasitic nematodes and *C. elegans* reveals a surprising degree of plasticity in receptor composition. Implications of this for anthelmintic sensitivity and resistance are far from clear. Our understanding of pLGIC evolution provides concrete guidance on limits of receptor composition change and what to expect from different species. Loss of the glc-2 glutamate receptor in *Loa loa* and duplication of the unc-29 levamisole receptor subunits in *Haemonchus contortus* provide examples that reveal general mechanisms involved in these fundamental processes. Comparison with nematode unc-49 GABA-, acc-1 acetylcholine- and trematode glutamate-receptors reveal how new pharmacological targets can derive from subunit diversification. Newly reconstituted acetylcholine receptors containing acr-26 or acr-21 demonstrate features expected for subunits that co-assemble in vivo and provide guidance for future experimental reconstitution of new target receptors. Although we are still at the infancy of understanding the pLGICs of parasitic helminths, inclusion of evolutionary information provides practical assistance for this maturing field.

Interaction of different macrocyclic lactones and ketoconazole with *Cylicocylus elongatus* P-glycoprotein 9 in a yeast growth assay

JÜRGEN KRÜCKEN[1], MAXIMILIANE KASCHNY[1], JANINA DEMELER[1], I. JANA I. JANSSEN[1], TETIANA A. KUZMINA[2], BRUNO BESOGNET[3], THEO KANELLOS[4], DOMINIQUE KERBOEUF[5], GEORG VON SAMSON-HIMMELSTJERNA[1]

[1]INSTITUTE FOR PARASITOLOGY AND TROPICAL VETERINARY MEDICINE, FREIE UNIVERSITÄT BERLIN, BERLIN, GERMANY, [2]SCHMALHAUSEN INSTITUTE OF ZOOLOGY, NAS OF UKRAINE, KYIV, UKRAINE, [3]ZOETIS, FRANCE, [4]ZOETIS INTERNATIONAL SERVICES, PARIS, FRANCE, [5]INRA, ISP, ANIMAL INFECTION AND PUBLIC HEALTH, NOUZILLY, FRANCE

Macrocyclic lactones (MLs) are widely used nematocidal drugs but resistance is a major problem in parasites of horses and livestock. Reports of resistance or decreased susceptibility of human and canine filaria also become more frequent. In the absence of any well-established ML resistance mechanisms related to target-site modifications, involvement of non-specific mechanisms and in particular of P-glycoproteins (Pgps) has frequently been investigated. Transport of MLs was demonstrated for mammalian Pgps, Pgps on nematode egg shells and very recently for *H. contortus* Pgp-2. In this study, expression of Pgp-9 from the equine parasite *Cylicocyclus elongatus* in a *Saccharomyces cerevisiae* strain lacking seven endogenous efflux transporters was used to analyse transporter function. Using the monoclonal antibody UIC2, which recognizes an epitope present only on Pgps during transport, active Pgp was detected on these yeasts by flow cytometry and chemiluminescence. In a growth assay, Pgp-9 decreased susceptibility to the fungicides ketoconazole, actinomycin D, valinomycin and daunorubicin but not to the fungicidal anthelmintic thiabendazole. Since MLs have no strong fungicidal activity, their interaction with Pgp-9 was investigated in an assay involving two drugs. In the presence of the highest ketoconazole concentration not affecting growth, yeasts were incubated with increasing ML concentrations to determine modulation of ketoconazole transport. Equimolar concentrations of ivermectin and eprinomectin with ketoconazole efficiently inhibited yeast growth and growth was abolished at fourfold higher ML concentrations. In contrast, selamectin and doramectin had no effect on susceptibility to ketoconazole, although doramectin has been shown to strongly interact with mammalian Pgps. Moxidectin showed an intermediate interaction with ketoconazol. Binding of UIC was increased in the presence of ivermectin, moxidectin, daunorubicin and ketoconazole but not selamectin confirming specific activation of Pgp-9. These results demonstrate direct effects of MLs on a recombinant nematode Pgp in an ML-specific manner.

Effects of short-term antibiotic treatment on *Wolbachia* and its filarial host *B. malayi*

PETER U FISCHER, KERSTIN FISCHER, KURT C CURTIS, GARY J WEIL

INFECTIOUS DISEASES DIVISION, DEPARTMENT OF INTERNAL MEDICINE, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE, ST. LOUIS, MO 63110, USA

Wolbachia endosymbionts are crucial for growth, reproduction, and survival of most medically important filarial parasites. Tetracycline (TET) antibiotics inhibit bacterial protein synthesis, prevent bacterial reproduction and clear *Wolbachia* over a period of weeks and eventually kill the worms. Antibiotics such as gentamicin (GEN) or penicillin G (PEN) neither clear *Wolbachia* nor kill the worms. *Wolbachia* release polymorphic outer membrane vesicles (OMVs) that may be essential for their mutualistic relationship with filarial worms. OMVs may transport bacterial products that are required for parasite growth and development. We assessed the early effects of TET treatment on the morphology of *Wolbachia* and filarial worms. Gerbils with i.p. *Brugia malayi* infections were treated with TET, GEN or PEN on days 19 and 20 post-infection. Immature worms recovered on day 21 were examined by immunohistology and electron microscopy. Significantly fewer developing worms were recovered from TET treated gerbils compared to GEN or PEN treated or untreated animals. Diverse *Wolbachia* forms and abundant OMVs were present in the lateral chords of GEN, PEN or untreated worms. *Wolbachia* of GEN treated worms showed increased numbers of ribosomes and nucleoids while PEN treated *Wolbachia* appeared to be larger and more clustered. In contrast, in the lateral chords of TET treated worms OMVs were decreased and *Wolbachia* were rarely associated with OMVs. In addition, the bacteria were often surrounded by membranes that appeared to have come from the endoplasmic reticulum. Lateral chords in TET treated worms were heavily vacuolated with increased glycogen granules compared to untreated worms. Staining pattern of the exosome marker EXOSC4 was not affected by treatment. These results suggest that TET treatment blocks OMVs production by *Wolbachia* and promotes encapsulation of the bacteria by internal host cell membranes. This provides further evidence that OMVs play an important role in the relationship between *Wolbachia* and filarial worms.

A dominant role for the methyl-CpG-binding protein Mbd2 in controlling dendritic cell induction of type-2 inflammation

PETER C. COOK,[1,2] HEATHER OWEN,[3] AIMÉE M. DEATON,[3] SHEILA BROWN,[1] GARETH-RHYS JONES,[1,2] LUCY H. JONES,[2] RACHEL J. LUNDIE,[2] ANGELA K. MARLEY,[1,2] ALEXANDER T. PHYTHIAN-ADAMS,[1,2] FREYA R. SVEDBERG,[1] LAUREN M. WEBB,[1,2] TARA E. SUTHERLAND,[2] GRAHAM D. THOMAS,[2] JOHN R. GRAINGER,[1,2] ANDREW N.J. MCKENZIE,[4] IRMGARD FÖRSTER[5], JUDITH E. ALLEN,[2] RICK M. MAIZELS,[2] ALASDAIR C. IVENS,[2] ADRIAN BIRD[3] AND **ANDREW S. MACDONALD[1,2]**.

[1]MANCHESTER COLLABORATIVE CENTRE FOR INFLAMMATION RESEARCH, UNIVERSITY OF MANCHESTER, MANCHESTER, U.K. [2]INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH, CENTRE FOR IMMUNITY, INFECTION AND EVOLUTION, SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF EDINBURGH, EDINBURGH, U.K. [3]WELLCOME TRUST CENTRE FOR CELL BIOLOGY, UNIVERSITY OF EDINBURGH, EDINBURGH, U.K. [4]MEDICAL RESEARCH COUNCIL LABORATORY OF MOLECULAR BIOLOGY, CAMBRIDGE, U.K. [5]IMMUNOLOGY AND ENVIRONMENT, LIFE AND MEDICAL SCIENCES (LIMES) INSTITUTE, UNIVERSITY OF BONN, BONN, GERMANY.

Dendritic cells (DCs) play a critical role in priming type-2 inflammation against helminths and allergens, yet the mechanism by which they initiate Th2 responses remain poorly understood. Since Th2 inducing DCs display minimal transcriptional activation, we investigated whether Th2 priming by DCs is dependent on epigenetic regulation of gene transcription via methyl-CpG binding domain protein-2 (Mbd2), which links CpG methylation to repressive chromatin structure. We found that global *Mbd2*$^{-/-}$ mice mount an impaired Th2 response following injection of eggs from the parasitic helminth *Schistosoma mansoni*. Then, to determine the impact of restriction of Mbd2 deficiency to DCs alone, we generated mice with conditional deletion of Mbd2 in CD11c+ cells (*CD11c*^{cre}*Mbd2*$^{fl/fl}$). These animals displayed significantly impaired Th2 development in response to egg challenge, illustrating that Mbd2 has a central role in controlling Th2 priming by DCs. To further address this possibility, we generated bone marrow derived DCs from global Mbd2$^{-/-}$ mice. *Mbd2*$^{-/-}$ DCs activated with soluble egg antigen (SEA) from *S. mansoni* were less able to prime OT-II T cell proliferation and Th2 polarisation *in vitro*. Furthermore, *Mbd2*$^{-/-}$ DCs transferred into naïve recipient mice exhibited severely impaired Th2 induction ability against either SEA or the allergen house dust mite, compared to WT DCs. These data demonstrate that epigenetic regulation of DCs, via the action of Mbd2, can be critical for Th2 induction and development both *in vitro* and *in vivo*. Ongoing work is investigating which genes are targeted by Mbd2, with the aim being identification of specific mechanisms employed by DCs that are fundamental for Th2 promotion.

In situ hematopoiesis-derived mast cells promote protective immunity to _Trichinella spiralis_

EVERETT K. HENRY[1], CHANDLER B. SY[1], AND **MARK C. SIRACUSA[1]**

[1]CENTER FOR IMMUNITY AND INFLAMMATION, DEPARTMENT OF MEDICINE, NEW JERSEY MEDICAL SCHOOL, RUTGERS-THE STATE UNIVERSITY OF NEW JERSEY, NEWARK, NEW JERSEY,

In situ hematopoiesis (ISH) refers to the differentiation of hematopoietic stem/progenitor cells (HSPCs) into effector cells that occurs at active sites of inflammation. Previous studies have demonstrated that ISH contributes to the population expansion of basophil and mast cell populations in the context of multiple helminth infections. However, whether ISH-derived effector cell populations are critical regulators of T_H2 cytokine-mediated inflammation and protective immunity to helminth parasites remain unknown. Here, we demonstrate a previously unrecognized role for ISH in promoting the population expansion of intestinal mast cell populations, T_H2 cytokine responses and protective immunity to *Trichinella spiralis*. Further, discovery based-studies suggest that helminth-induced HSPCs exhibit distinct transcriptional profiles compared to their bone marrow-resident counterparts. These data demonstrate that ISH contributes to the development of protective immunity to helminth parasites and that helminth-induced HSPCs are phenotypically distinct cells that may represent viable therapeutic targets.

Delineating the function of human resistin using transgenic mice and clinical samples from helminth-infected individuals

JESSICA C JANG[1], GRAHAM LE GROS[2], PHILIP J COOPER[3], **MEERA G NAIR[1]**

[1]SCHOOL OF MEDICINE, UNIVERSITY OF CALIFORNIA RIVERSIDE; [2]MALAGHAN INSTITUTE OF MEDICAL RESEARCH; [3]LABORATORIO DE INVESTIGACIONES, ECUADOR

Resistin-like molecules (RELM) belong to a family of secreted proteins that are expressed in multiple helminth infections with important effects on the host immune response. However the importance of human RELM proteins in helminth infections is less well understood. To investigate this, we utilize transgenic mice in which the human resistin gene along with its transcriptional regulatory elements was inserted. We recently showed that infection with the hookworm *Nippostrongylus brasiliensis* caused significantly increased human resistin expression the infected lung and intestine. Human resistin expression was detrimental to the host and provoked a monocyte-rich inflammatory response in the lung, increased expression of inflammatory cytokines such as TNFα and impaired parasite expulsion. Supportive of these mouse studies, *Ascaris*-infected children from Ecuador had elevated serum resistin levels, which were positively correlated with parasite egg counts in the stool and with serum inflammatory cytokines. Together, these studies identify a detrimental role for human resistin in instigating a non-protective inflammatory response following helminth infection. In ongoing studies, we are investigating the genetic determinants that regulate resistin gene expression including single nucleotide polymorphisms in the gene and putative transcription binding sites in the promoter. Preliminary analysis has identified two STAT6 binding sites in the human resistin promoter implicating the Th2 cytokine pathway in promoting human resistin expression. Identifying the cellular and molecular signals that regulate resistin expression may have important diagnostic and therapeutic implications for helminth infection.

The biology underpinning Helminth parasite induced Type 2 immune responses related to host immunity, tissue repair and pathology.

GRAHAM LE GROS

MALAGHAN INSTITUTE OF MEDICAL RESEARCH, NEW ZEALAND

Helminth parasites are an extremely successful group of organisms infecting over one billion people, with some able to parasitise a host for several decades. Helminths are phylogenetically diverse, with a broad range of migration patterns and life cycles, and are spread across three phyla: Nematodes, Trematodes and Cestodes. Helminths are credited with being the major selective force driving the evolution of the so called "type 2" immune responses in vertebrate animals, with their size and infection strategies presenting unique challenges to the immune system. Originally, type 2 immune responses were defined by the presence and activities of the $CD4^+$ T helper 2 subset producing the canonical cytokines IL-4, IL-5 and IL-13. This picture is now being challenged by the discovery of a more complex pattern of $CD4^+$ T helper cell subsets that appear during infection, including Tregs, Th17, Tfh and more recently Th22, Th9, and ThGM. In addition to these archetypal adaptive immune cell subsets a series of innate lymphocyte populations (ILC1, ILC2, ILC3) have also been described that have many of the functional properties of T cells but do not respond through a MHC restricted antigen receptor. The mechanisms by which helminths and their products selectively prime the $CD4^+$ T cell and Innate lymphocyte subsets is emerging and in this talk I will focus on recent new data concerning the selective priming, differentiation and functional role of $CD4^+$ T helper cell and Innate cell subsets in the context of helminth infection. We argue for a re-evaluation of the original Th2 paradigm and discuss how the observed plasticity of the immune cell subsets may enable the parasitized host to achieve an appropriate compromise between elimination, tissue repair, containment and pathology.

Natural and vaccine-mediated immunity to *Salmonella typhimurium* is impaired by the helminth *Nippostrongylus brasiliensis.*

SAEEDA BOBAT[1], MATTHEW DARBY[2], DUNJA MRDJEN[2], CHARLOTTE COOK[1], ERIN LOGAN[2], JENNIFER AURET[2], ELIZABETH JONES[1], CORRINNA SCHNOELLER[3], ADRIANA FLORES-LANGARICA[1], EWAN A. ROSS[1], ALYKHAN VIRA[2], CONSTANTINO LOPEZ-MACIAS[4], IAN R. HENDERSON[1], JAMES ALEXANDER[5], FRANK BROMBACHER[2], **WILLIAM G HORSNELL[2]**, ADAM F CUNNINGHAM[1]

[1]MRC CENTRE FOR IMMUNE REGULATION, INSTITUTE OF MICROBIOLOGY AND INFECTION, SCHOOL OF IMMUNITY AND INFECTION, COLLEGE OF MEDICAL AND DENTAL SCIENCES, UNIVERSITY OF BIRMINGHAM, BIRMINGHAM, B15 2TT, UK. [2]INSTITUTE OF INFECTIOUS DISEASE AND MOLECULAR MEDICINE, FACULTY OF HEALTH SCIENCES, UNIVERSITY OF CAPE TOWN, ANZIO ROAD, OBSERVATORY 7925, CAPE TOWN, SOUTH AFRICA. [3]DEPARTMENT OF LIFE SCIENCES, SIR ERNST CHAIN BUILDING, SOUTH KENSINGTON CAMPUS, IMPERIAL COLLEGE LONDON, SW7 2AZ LONDON UK. [4]MEDICAL RESEARCH UNIT ON IMMUNOCHEMISTRY, SPECIALTIES HOSPITAL, NATIONAL MEDICAL CENTRE "SIGLO XXI" MEXICAN INSTITUTE FOR SOCIAL SECURITY (IMSS), MEXICO CITY, MEXICO. [5]STRATHCLYDE INSTITUTE OF PHARMACY AND BIOMEDICAL SCIENCES, UNIVERSITY OF STRATHCLYDE, GLASGOW, UK, G4 0NR.

The impact of exposure to multiple pathogens concurrently or consecutively on immune function is unclear. We have recently found that infection with the helminth N. brasiliensis (Nb) alters host natural and vaccine mediated immunity to a systemic Salmonella infection (Bobat et al. 2014). We found that impaired natural immunity to STm following related to reduced secretion of IFNg by non-T cells, an increased regulatory T cell response and a reduced anti-STm anti-body response. Impaired vaccine mediated protection against STm in Nb infected mice also related to reduced anti-STm anti-body titres. Here we present data demonstrating how Nb infection alters the ability of key immune cells ability to respond to a subsequent STm challenge.

Analysis of antibodies to all individual glycan antigens of the schistosome glycome in human and animal sera in relation to immunity

ANGELA VAN DIEPEN[1], CORNELIS SMIT[1], LINH NGUYEN[1], KARL HOFFMANN[2], ALAN WILSON[3], DAVID DUNNE[4], **CORNELIS HOKKE[1]**

[1]DEPARTMENT OF PARASITOLOGY, LEIDEN UNIVERSITY MEDICAL CENTER, THE NETHERLANDS. [2]IBERS, ABERYSTWYTH UNIVERSITY, UNITED KINGDOM. [3]CENTRE FOR IMMUNOLOGY & INFECTION, UNIVERSITY OF YORK, YORK, UNITED KINGDOM. [4]DEPARTMENT OF PATHOLOGY, UNIVERSITY OF CAMBRIDGE, CAMBRIDGE, UNITED KINGDOM

During infections with schistosomes, humoral and cellular immune responses against numerous parasite antigens expressed by larvae, worms and egg are raised. Major targets of the antibody response are the antigenic glycan motifs of glycoproteins and glycolipids, raising the question what the role of these anti-glycan antibodies is in relation to immunity. First, using a mass spectrometric glycomics approach we have investigated the expression profiles and structural identity of a large number of glycans expressed by the schistosome life stages. A number of striking shifts and switches in the expression of antigenic glycan motifs during the maturation of the worm and the egg were identified that appear to be linked to specific parasite-host interactions. Subsequently, we have generated a microarray of the hundreds of isolated N-, O-, and lipid-glycans covering the entire glycome of *S. mansoni*. We have used the array to determine IgG and IgM titers to each glycan in a number of human and animal cohorts. Cross-sectional studies of human *S. mansoni* infection sera were performed in relation to subject age, infection intensity, treatment and resistance to reinfection. In addition, we performed longitudinal analyses of anti-glycan antibodies in *S. japonicum* infected macaques that self-cure the infection, and in baboons vaccinated with irradiated *S. mansoni* cercariae that are protected from challenge infection. The spatial expression of several characteristic glycan antigens during parasite development was investigated by microscopy using monoclonal antibodies characterised by glycan array analysis. Integrated analyses of these studies suggesting that multifucosylated glycan motifs expressed on schistosome larvae may be targets of an effective immune response will be presented. The possibility of exploring such glycans for immunisations and induction of antibodies will be discussed.

High mannose glycoproteins present on *Fasciola hepatica* surface coat exhibit immune modulatory properties independent of the mannose receptor.

ALESSANDRA RAVIDÀ[1], ALLISON M. ALDRIDGE[1], NICOLE N. DRIESSEN[2], CORNELIS H. HOKKE[2] AND **SANDRA M. O'NEILL[1],**

[1]PARASITE IMMUNE MODULATION GROUP, SCHOOL OF BIOTECHNOLOGY, FACULTY OF SCIENCE AND HEALTH, DUBLIN CITY UNIVERSITY, GLASNEVIN, DUBLIN 9, IRELAND; [2]PARASITE GLYCOBIOLOGY GROUP, DEPARTMENT OF PARASITOLOGY, LEIDEN UNIVERSITY MEDICAL CENTER, LEIDEN, THE NETHERLANDS

Fascioliasis, caused by the liver fluke *Fasciola hepatica*, is a neglected tropical disease infecting over 1 million individuals annually with 17 million people at risk of infection. Like other helminths, *F. hepatica* employs mechanisms of immune suppression in order to evade its host immune system. In this study the N-glycosylation of *F. hepatica's* tegumental coat (FhTeg) and its carbohydrate-dependent interactions with bone marrow derived dendritic cells (BMDCs) were investigated. Mass spectrometric analysis demonstrated that FhTeg N-glycans comprised mainly of oligomannose and to a lesser extent truncated and complex type glycans. The expression of these glycans on the surface of the fluke was confirmed by microscopy and lectin blots. The interaction of FhTeg with both carbohydrate-binding domains of the mannose receptor (MR) was investigated. Binding of FhTeg to MR-transfected CHO cells and BMDCs was blocked when pre-incubated with mannan and GalNAc-4S, carbohydrate ligands of the CRDs and the cysteine-rich domain of MR respectively. The expression of SOCS3, a negative regulator of the TLR and STAT3 pathway, was inhibited by pre-incubation with GalNAc-4S prior to FhTeg stimulation of BMDCs while cytokine suppression by FhTeg was reversed with the addition of mannan. We further elucidated the role played by MR in the immunomodulatory mechanism of FhTeg and demonstrated that while FhTeg's binding was significantly reduced in BMDCs generated from MR knockout mice, the absence of MR did not alter FhTeg's ability to induce SOCS3 or suppress cytokine secretion from LPS activated BMDCs. We conclude that *F. hepatica* high mannose glycoproteins are crucial in modulating its host's immune system. However, although MR on BMDCs binds to these glycoproteins, other CLRs are involved in the immunomodulatory mechanism of FhTeg.

The schistosome surface and host hemostasis

AKRAM DA'DARA[1], BARBARA C. FIGUEIREDO[2], RITA BHARDWAJ[1], SERGIO C. OLIVEIRA[2], **PATRICK J. SKELLY**[1].

[1] DEPARTMENT OF INFECTIOUS DISEASE AND GLOBAL HEALTH, CUMMINGS SCHOOL OF VETERINARY MEDICINE, TUFTS UNIVERSITY, NORTH GRAFTON, MASSACHUSETTS, USA. [2] DEPARTAMENTO DE BIOQUÍMICA E IMUNOLOGIA, INSTITUTO DE CIÊNCIAS BIOLÓGICAS, UNIVERSIDADE FEDERAL DE MINAS GERAIS, BELO HORIZONTE, MINAS GERAIS, BRAZIL.

The surface of the intravascular schistosome tegument constitutes a major site of interaction with the host. Among the proteins identified in the surface membranes are a collection of enzymes some of which likely help regulate host immunity and hemostasis. Using RNA interference (RNAi) to suppress the expression of *Schistosoma mansoni* genes encoding selected surface enzymes, we have shown that one tegumental enzyme, ATPdiphosphohydrolase1 (SmATPDase1), can cleave the pro-inflammatory Damage Associated Molecular Pattern (DAMP) ATP as well as the pro-thrombotic molecule ADP. By cleaving exogenous ADP, we hypothesize that SmATPDase1 helps to inhibit platelet activation and aggregation around the worms within the vasculature. A second schistosome ATPdiphosphohydrolase enzyme - SmATPDase2 - does not hydrolyze exogenous ATP or ADP. Schistosome alkaline phosphatase (SmAP) can hydrolyze exogenous AMP to generate the potent anti-inflammatory mediator, adenosine. Other schistosome surface proteins include housekeeping enzymes such as enolase (SmEno). While best known as a glycolytic enzyme, we have shown that SmEno can additionally bind the plasma zymogen plasminogen and promote its activation (by tissue plasminogen activator (tPA)) to generate plasmin – a serine protease that cleaves fibrin and dissolves blood clots. Live parasites likewise mediate this effect. Thus, acquiring host proteases by binding them to e.g. surface enolase may represent another mechanism that schistosomes employ to limit blood clot formation around them. This work on the functional characterization of schistosome tegumental enzymes is designed to generate a comprehensive understanding of the role of these surface proteins in promoting parasite survival by controlling host hemostasis.

Nematode acetylcholinesterases and cholinergic regulation of immunity

MURRAY E. SELKIRK[1], LUKUS ROBERTS[1], RACHEL VAUX[1], WILLIAM G. HORSNELL[2], CORINNA SCHNOELLER[1]

[1]DEPARTMENT OF LIFE SCIENCES, IMPERIAL COLLEGE, LONDON, UK; [2]DIVISION OF IMMUNOLOGY, IIDMM, UNIVERSITY OF CAPE TOWN. SOUTH AFRICA;

Many parasitic nematodes which colonise mucosal surfaces secrete acetylcholinesterases (AChEs), although the function of these enzymes is not known. Recently we showed that cholinergic signalling through the M3 muscarinic acetylcholine receptor was required for optimal immunity to nematode and bacterial infection, and production of both type 1 and type 2 cytokines, suggesting that acetylcholine (ACh) may act as a co-stimulatory signal for T cell activation. We now demonstrate that a major non-neuronal source of ACh in the lungs during infection with *Nippostrongylus brasiliensis* are group 2 innate lymphoid cells (ILC2s) and activated CD4+ T cells. Synthesis and release of ACh by ILC2s occurs rapidly in response to parasite infection, intranasal administration of *Alternaria alternata* extract, and the alarmin cytokines IL-33 and IL-25. We are working on which downstream effector functions are influenced by ACh, focussing on cellular recruitment and alternative activation/polarisation of macrophages, and the role of nematode secreted AChEs in regulating these events. We have developed a system for heterologous gene expression in *Trypanosoma musculi*, a natural parasite of mice, and are using this as an in vivo vehicle for screening potential immunoregulatory activities of helminth proteins. *N. brasiliensis* AChE is secreted by this vehicle in active form, and can be detected in the serum of infected mice. Transgenic *T. musculi* expressing AChE show an altered course of parasitaemia, and we have defined effects on the host immune response which suggest a role for these enzymes.

A granulin growth factor secreted by the carcinogenic liver fluke, *Opisthorchis viverrini*, and its role in wound healing and carcinogenesis

MICHAEL JAMES SMOUT[1]; JAVIER SOTILLO[1]; THEWARACH LAHA[2]; BANCHOP SRIPA[2]; MULVENNA JASON MULVENNA[3]; LAI YUE CHAN [4]; GABRIEL RINALDI [5]; PAUL BRINDLEY[5]; ALEX LOUKAS[1].

[1]QUEENSLAND TROPICAL HEALTH ALLIANCE AND CENTRE FOR BIODISCOVERY & MOLECULAR DEVELOPMENT OF THERAPEUTICS, JAMES COOK UNIVERSITY, CAIRNS, QLD, AUSTRALIA; [2] DEPARTMENT OF PARASITOLOGY, KHON KAEN UNIVERSITY, KHON KAEN, THAILAND; [3] QUEENSLAND INSTITUTE OF MEDICAL RESEARCH, BRISBANE, QLD, AUSTRALIA; [4] INSTITUTE OF MOLECULAR BIOSCIENCE, BRISBANE, QLD, AUSTRALIA; [5] GEORGE WASHINGTON UNIVERSITY, WASHINGTON DC, USA

The human liver fluke, *Opisthorchis viverrini*, infects 9 million people throughout South-East-Asia and is a major cause of cholangiocarcinoma (bile duct liver cancer). The mechanisms by which the parasite causes cancer are multi-factorial, but one process is the secretion of mitogenic parasite proteins into the bile ducts, driving cell proliferation, wound healing and contributing to a tumourigenic environment. When the *O. viverrini* secretome was characterized we identified *Ov*-GRN-1, a homologue of the human growth factor, granulin. The granulin family has wide ranging potent effects across species and cell type, and *Ov*-GRN-1 has proved no different. Previously we demonstrated potent nanomolar mitogenicity (induces proliferation) of recombinant *Ov*-GRN-1 on human biliary cells. We will present our current work exploring this intriguing fluke secretion, including dramatically improved *in vivo* wound closure *Ov*-GRN-1 treatment with mouse wounding models. Additionally we are exploring three other aspects of *Ov*-GRN-1 treatment that promote a carcinogenic environment: stimulation of angiogenesis, mitogenesis, and modulation of immune cell recruitment. Underlying these four aspects are the signaling pathways stimulated by *Ov*-GRN-1 and we explore these with qPCR arrays, proteome arrays and temporal whole cell quantitative proteomics. Why *Ov*-GRN-1 is secreted by the fluke is unknown, but we suspect in the short term the parasite minimizes its impact on the host by healing wounds with *Ov*-GRN-1. But, over many decades this never ending cell replication results in carcinogenic mutations and deadly cholangiocarcinoma in as many as one-sixth of infected patients. As we uncover the wide ranging potent impact of *Ov*-GRN-1 we contribute to the understanding of host-parasite interactions, and begin to address the mechanisms by which this parasite influences the host and causes such a devastating form of cancer.

Secreted exosomes from *Heligmosomoides polygyrus* modulate cellular responses of the murine host

GILLIAN COAKLEY , FABIO SIMBARI, HENRY MCSORLEY, MARISSA LEAR, RICK MAIZELS & AMY BUCK

INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH, ASHWORTH LABORATORIES, UNIVERSITY OF EDINBURGH, SCOTLAND, UK

Exosomes are nanovesicles (50-100nm), which facilitate cellular communication through the transfer of small RNAs, lipids and proteins. It has been shown that parasites' secretome can play a key role in both pathogenicity and host immunoregulation. During *Trypansoma cruzi* and *Leishmania donovani* infection, parasite-derived exosomes are able to modulate the host inflammatory immune response, an adventitious strategy promoting parasite survival. In this study, we demonstrate that secreted vesicles from the murine gastrointestinal nematode *Heligmosomoides polygyrus* exhibit a range of immunosuppressive and regulatory properties on murine cells and *in vivo*. We will also discuss how this contributes to their role in host-helminth interactions. Through microarray of *H.polygyrus* exosome-treated small intestinal epithelial cells, we see significant gene changes, including those involved in the regulation of signaling and the immune response, such as DUSP1 (dual-specificity phosphatase) and IL1RL1 (the receptor for IL-33). We have investigated these genes in various cell types, in order to clarify the signaling cascade initiated upon parasitic exosome interaction with recipient cells. Furthermore, there is evidence that *H.polygyrus*-derived exosomes can affect the cytokine and gene signatures in both classical and alternatively activated bone-marrow derived macrophages and mixed monocyte populations. Using a model of lung inflammation, *in vivo* studies demonstrate that in both prophylactic and co-administration experiments, exosomes modulate the innate cellular response. This is represented by changes in innate lymphoid cells, bronchoalveolar lavage eosinophils and type-2 cytokine output. Finally, we are able to show that immunization of mice using an exosome/alum conjugate may contribute to protection from a subsequent *H.polygyrus* infection, as seen through a reduction in egg counts and worm burden. This work suggests that exosomes secreted by nematodes could mediate the transfer and uptake of parasitic products into host cells, establishing cross-species communication to suppress the host 'danger' or inflammatory response.

Functional interplay between *Brugia* and its *Wolbachia* symbiont

SARA LUSTIGMAN[1], DENIS VORONIN[1], THOMAS R UNNASCH[2], ELODIE GHEDIN[3]

[1]LINDSLEY F. KIMBALL RESEARCH INSTITUTE, NEW YORK BLOOD CENTER, NEW YORK, NY 10065 USA; [2]DEPARTMENT OF GLOBAL HEALTH, UNIVERSITY OF SOUTH FLORIDA, TAMPA, FL 33612 USA; [3]DEPARTMENT OF BIOLOGY, CENTER FOR GENOMICS AND SYSTEMS BIOLOGY, NEW YORK UNIVERSITY

Brugia malayi, like other filarial parasites, harbors an endosymbiotic intracellular bacterium, *Wolbachia* (*w*Bm), required for the development and reproduction of the worm. Because of its crucial function in worm survival, anti-*Wolbachia* chemotherapeutic approaches are being sought for the treatment of human filarial infections. Our studies are aimed at identifying proteins that play an essential function in this endosymbiotic relationship. We found that *B. malayi* proteins involved in glycolysis were bound to two outer membrane proteins at the surface of the bacteria. We hypothesized that this surface localization of glycolytic enzymes is to increase the local concentration of glycolytic products, such as pyruvate, to be used by the endosymbiont as an energy source. Notably, the glycolysis pathway in *w*Bm is defective and is replaced by host gluconeogenic enzymes. Gene expression and protein levels of *B. malayi* aldolase—an enzyme in the glycolysis pathway—are increased during parasite development; suppression of the enzyme by siRNA reduced bacteria load and initiated programmed cell death in the developing embryos. We also found that *B. malayi* cathepsin L-like cysteine proteases—specifically *Bm-cpl-3* and *Bm-cpl-6*—have a bimodal pattern of regulation in tetracycline-treated worms. Moreover, reduction in *Bm-cpl-3* and *Bm-cpl-6* transcript levels by RNAi resulted in hindered embryogenesis, decreased the release of microfilariae *in vitro*, and led to lower *Wolbachia* DNA levels. Similar outcomes were obtained when the activity of the CPLs was inhibited in female adult worms with K11777, a synthetic cysteine protease inhibitor. This drug blocked embryogenesis and development of mature microfilariae as well as reduced *Wolbachia* loads in the treated adult female worms. Taken together, these studies confirm the importance of the glycolytic pathway and cysteine proteases in the maintenance of *Wolbachia-Brugia* symbiosis. As both pathways may be involved in the bacteria-host mutualistic relationship, they represent an interesting potential target for anti-filarial drugs.

Helminth infections, the immune system and metabolism

MARIA YAZDANBAKHSH

DEPT OF PARASITOLOGY, LEIDEN UNIVERSITY MEDICAL CENTER, LEIDEN, THE NETHERLANDS

Helminths along with other infectious agents are highly prevalent in rural areas of developing countries. These infections are associated with considerable alteration of the immune system, studied by comparing responses in populations residing in rural areas and in urban centres. The contribution of helminths to the altered immune responses can be studied by examining responses pre and post treatment. Taking this approach it has been possible to analyse the changes in immune responses induced by helminth infections but also changes in metabolic parameters. In addition to population studies, experimental animal models have allowed us to examine in more detail the mechanisms behind the effect that helminths have on a number of metabolic processes.

The therapeutic hookworm

ALEX LOUKAS

CENTRE FOR BIODISCOVERY AND MOLECULAR DEVELOPMENT OF THERAPEUTICS, AUSTRALIAN INSTITUTE OF TROPICAL HEALTH AND MEDICINE, JAMES COOK UNIVERSITY, CAIRNS, QUEENSLAND, AUSTRALIA

Hookworms possess potent immunoregulatory properties. Support for this notion comes from immuno-epidemiological observations in hookworm-endemic countries, clinical trials involving experimental infection of volunteers with hookworms, and studies with animal models of inflammatory diseases. As proof-of-concept for the therapeutic benefits of helminth therapy, we have completed a small open label clinical trial using trace gluten consumption coupled with hookworm as an immunoregulatory agent to treat coeliac disease. Beyond our expectations, the treatment resulted in improved gluten tolerance, improved coeliac disease activity measurements and increased regulatory T cell (Treg) numbers in the intestinal epithelium. Despite the promising efficacy of live helminth therapy, the approach has major drawbacks for wide-spread implementation. Central to the hookworm's ability to modulate inflammation is the excretory/secretory (ES) component, the parasite's public face of the host-pathogen interactome. We have characterised the hookworm secretome using genomics and targeted proteomics of the ES proteins. At least one family of abundant ES proteins with therapeutic properties in mouse models of asthma and colitis has been identified, and one of these proteins (AIP-2) is undergoing further development in partnership with large pharma as a novel biologic for treating IBD.

The *Acanthocheilonema viteae*-derived immunomodulator ES-62 acts as a molecular switch to promote IL-22-mediated resolution of joint inflammation in a model of rheumatoid arthritis

WILLIAM HARNETT[1], MIGUEL A PINEDA[2], MARLENE CORBET[2], FELICITY LUMB[1] AND MARGARET M HARNETT[2]

[1]STRATHCLYDE INSTITUTE OF PHARMACY AND BIOMEDICAL SCIENCES, UNIVERSITY OF STRATHCLYDE, 161 CATHEDRAL STREET, GLASGOW G4 0RE, UK AND [2]INSTITUTE OF INFECTION, IMMUNITY AND INFLAMMATION, GLASGOW BIOMEDICAL RESEARCH CENTRE, UNIVERSITY OF GLASGOW, 120 UNIVERSITY PLACE, GLASGOW G12 8TA, UK

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that displays an inverse global distribution to helminth infection. Consistent with this, ES-62, an immunomodulator secreted by the filarial nematode *Acanthocheilonema viteae*, protects against development of collagen-induced arthritis (CIA), a mouse model of RA. ES-62 acts by targeting pathogenic IL-17 responses generated via a network of dendritic cells, Th17 and gd T cells. IL-22 cooperates with IL-17 during initiation of pathogenesis in CIA yet counter-regulates its actions to effect resolution of inflammation in the joint during established disease: ES-62 also acts to suppress the early IL-17/22 synergy and promote the later tissue repair abilities of IL-22. Reflecting its targeting of non-haemopoietic cells, IL-22 mediates resolution of joint inflammation by desensitising synovial fibroblast (SF) responses that otherwise result in SF hyperplasia, chronic pro-inflammatory mediator (IL-6 and MCP-1) release and osteoclastogenesis. Moreover, desensitisation of inflammatory SF responses is afforded by ES-62 and neutralising anti-IL-22 antibodies block this. During CIA pathogenesis, SF become "rewired" to a stably aggressive phenotype indicative of their epigenetic modification and this conversion appears to be promoted by IL-17/IL-22-associated STAT3 and ERK MAPK signalling. Indeed SF from CIA-mice show enhanced IL-17 stimulated ERK signalling relative to those from normal mice. By contrast, SF from CIA mice treated with ES-62 show "normal"-like ERK responses and this is mirrored in SF from CIA mice administered rIL-22 in the footpad during established disease. The molecular switch underpinning ES-62 promotion of differential IL-22 signalling in CIA-SF versus normal-SF remains to be fully defined. However of note, it has been reported that exposure to IFNb, production of which is elevated in CIA-SF by ES-62, can rewire coupling of IL-22 from STAT3 to STAT1 signalling and the latter is employed by this type of IFN to limit joint inflammation.

Helminths protect against autoimmunity through IL-5 and IL-33.

CONOR M FINLAY[1], ANNA M STEFANSKA[4], KEVIN P WALSH[1], BENJAMIN DOYLE[1], LOUIS BOON[2], ED C. LAVELLE[3], PATRICK T WALSH[4] AND KINGSTON H G MILLS[1]

[1]IMMUNE REGULATION RESEARCH GROUP, SCHOOL OF BIOCHEMISTRY AND IMMUNOLOGY, TRINITY BIOMEDICAL SCIENCES INSTITUTE, TRINITY COLLEGE DUBLIN, DUBLIN 2, IRELAND, [2]BIOCEROS BV, UTRECHT, THE NETHERLANDS. [3]ADJUVANT RESEARCH GROUP, SCHOOL OF BIOCHEMISTRY AND IMMUNOLOGY, TRINITY BIOMEDICAL SCIENCES INSTITUTE, TRINITY COLLEGE DUBLIN, DUBLIN 2, IRELAND, [4]DEPARTMENT OF CLINICAL MEDICINE, SCHOOL OF MEDICINE, TRINITY COLLEGE DUBLIN, DUBLIN, IRELAND.

Epidemiology studies in humans have demonstrated that infection with helminth parasites is are associated with a reduced risk of developing autoimmune diseases. Mechanistic studies in mice have linked this to the suppressive effects of helminth-induced regulatory T (Tregs) or Th2 cells. Although treatment with live helminth is under clinical evaluation for autoimmune disease in humans, helminth products may be a more acceptable therapeutic approach. Here, we demonstrate that treatment of mice with *Fasciola hepatica* excretory/secretory products (FHES) attenuated the clinical signs of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. Protection was associated with a significant reduction in the infiltration of pathogenic Th1 and Th17 cells into the brain. Although FHES enhanced anti-inflammatory cytokine and Th2 responses, protection against EAE was independent of IL-4, IL-10 and Tregs. However, administration of FHES induced production of the type-2 cytokines IL-33 and IL-5, which promoted expansion of eosinophils. FHES-induced expansion of eosinophils and protection against EAE was lost in IL-$33^{-/-}$ and in IL-$5^{-/-}$ mice. Furthermore, transfer of FHES-induced eosinophils conferred protection against EAE. This study is the first to report that helminth-induced IL-5 and IL-33 and eosinophils in protection against autoimmunity.

Ancient and novel Small RNA pathways compensate for thelLoss of piRNAs in multiple independent nematode lineages

PETER SARKIES [1], MURRAY E. SELKIRK [2], JOHN T. JONES [3], VIVIAN BLOK [3], THOMAS BOOTHBY [4], BOB GOLDSTEIN [4], BEN HANELT [5], ALEX ARDILA-GARCIA [6], NAOMI M. FAST [6], PHILLIP M. SCHIFFER [7], CHRISOPHER KRAUS [7], MARK J. TAYLOR [8], GEORGIOS KOUTSOVOULOS [9], MARK L. BLAXTER [9], **ERIC A. MISKA [10]**

[1]MRC CLINICAL SCIENCES CENTRE, IMPERIAL COLLEGE LONDON, LONDON, UK, [2]DEPARTMENT OF LIFE SCIENCES, IMPERIAL COLLEGE LONDON, LONDON, UK, [3] THE JAMES HUTTON INSTITUTE, INVERGOWRIE, DUNDEE, UK, 4 DEPARTMENT OF BIOLOGY, UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL, CHAPEL HILL, NORTH CAROLINA, USA, 5 CENTER FOR EVOLUTIONARY AND THEORETICAL IMMUNOLOGY, DEPARTMENT OF BIOLOGY, UNIVERSITY OF NEW MEXICO, ALBUQUERQUE, NEW MEXICO, USA, 6 DEPARTMENT OF BOTANY, UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, BRITISH COLUMBIA, CANADA, 7 ZOOLOGISCHES INSTITUT, UNIVERSITÄT ZU KÖLN, COLOGNE, NRW, GERMANY, 8 MOLECULAR AND BIOCHEMICAL PARASITOLOGY, LIVERPOOL SCHOOL OF TROPICAL MEDICINE, LIVERPOOL, UK, 9 INSTITUTE OF EVOLUTIONARY BIOLOGY, UNIVERSITY OF EDINBURGH, EDINBURGH, UK, 10 WELLCOME TRUST/CANCER RESEARCH UK GURDON INSTITUTE, CAMBRIDGE, UK

Small RNA pathways act at the front line of defence against transposable elements across the Eukaryota. In animals, Piwi interacting small RNAs (piRNAs) are a crucial arm of this de- fence. However, the evolutionary relationships among piRNAs and other small RNA path- ways targeting transposable elements are poorly resolved. To address this question we sequenced small RNAs from multiple, diverse nematode species, producing the first phylum-wide analysis of how small RNA pathways evolve. Surprisingly, despite their prominence in *Caenorhabditis elegans* and closely related nematodes, piRNAs are absent in all other nematode lineages. We found that there are at least two evolutionarily distinct mecha- nisms that compensate for the absence of piRNAs, both involving RNA-dependent RNA polymerases (RdRPs). Whilst one pathway is unique to nematodes, the second involves Dicer-dependent RNA-directed DNA methylation, hitherto unknown in animals, and bears striking similarity to transposon-control mechanisms in fungi and plants. Our results high- light the rapid, context-dependent evolution of small RNA pathways and suggest piRNAs in animals may have replaced an ancient eukaryotic RNA-dependent RNA polymerase path- way to control transposable elements.

Functional genomic characterization of endogenous small RNA pathways in *Caenorhabditis* nematodes

SHIKUI TU[1], MONICA WU[2], JIE WANG[3], ASHER CUTTER[4], ZHIPING WENG[1], **JULIE M. CLAYCOMB[2]**

[1]PROGRAM IN BIOINFORMATICS AND INTEGRATIVE BIOLOGY, UNIVERSITY OF MASSACHUSETTS MEDICAL SCHOOL, WORCESTER MA USA; [2]DEPT. OF MOLECULAR GENETICS, UNIVERSITY OF TORONTO, TORONTO ON CAN; [3]DEPT. OF BIOCHEMISTRY, UNIVERSITY AT BUFFALO, BUFFALO NY USA; [4]DEPT. OF ECOLOGY AND EVOLUTIONARY BIOLOGY, UNIVERSITY OF TORONTO, TORONTO, CANADA

RNA interference (RNAi) and related endogenous small RNA (sRNA) pathways entail a potent and widely conserved means of regulating gene expression. Such pathways are characterized by Argonaute proteins (AGOs) and sRNAs, in which the sRNAs guide the AGO to target transcripts via complementarity. *C. elegans* has been a champion of sRNA research since the initial discoveries of RNAi and microRNA pathways 20 years ago. From this early foundation, small RNA biology in the worm has flourished to encompass four types of sRNAs, including microRNAs, piRNA/21U-RNAs, and endogenous sRNAs (22G-RNAs and 26G-RNAs), along with 27 Argonaute proteins. Our understanding of small RNA biology in many organisms has been shaped from the identification and characterization of these pathways in *C. elegans*. The only essential *C. elegans* Argonaute, CSR-1 (Chromosome Segregation and RNAi deficient), plays novel nuclear roles in modulating chromatin and promoting germline gene expression via associated 22G-RNAs. CSR-1 is conserved amongst diverse nematodes (Clades III and V), thus to characterize the functions of the CSR-1 pathway, its targets, and their evolution across various species, we recently performed a comparative functional genomic analysis of the CSR-1 pathway between *C. elegans* and *C. briggsae*. We find that *C. briggsae* CSR-1-associated sRNAs include both 22A-and 22G-RNA species, which overlap with sRNAs depleted in *cbr-csr-1* RNAi-treated worms. By comparing 22G-RNAs and target genes between species, we defined a set of CSR-1 target genes with conserved germline expression, enrichment in operons, and more slowly-evolving sequences than other germline-expressed genes, along with a group of evolutionarily labile targets that appear to be the targets of different AGOs in each species. We demonstrate that the association of CSR-1 with chromatin is preserved, and show that depletion of *cbr-csr-1* leads to chromosome segregation defects and embryonic lethality, highlighting its key role in germline gene regulation across multiple nematode species.

The cost of silence: Evolution of DNA methylation in nematodes

SILVANA ROSIC[1], RACHEL AMOUROUX[1], PETRA HAJKOVA[1], MURRAY SELKIRK[2], **PETER SARKIES[1]**

[1]MRC CLINICAL SCIENCES CENTRE, IMPERIAL COLLEGE LONDON. [2]DEPARTMENT OF LIFE SCIENCES, IMPERIAL COLLEGE LONDON

Transposons are genomic parasites that, left unchecked, represent a major threat to genome integrity. As a result, organisms have evolved several mechanisms in order to restrain transposons. These mechanisms, however, must themselves evolve extremely rapidly to keep up with their elusive targets. We have previously shown that nematodes display an extreme example of the diversity of transposon silencing mechanisms as the piRNA pathway, fundamental to transposon control across the animal kingdom, has been lost altogether in several independent nematode lineages (Sarkies et al., 2015). Here we explore the evolution DNA methylation, another key component of the transposon silencing machinery. Through a detailed analysis of the conservation of DNA methylation machinery across nematodes, we show that, despite being absent altogether from the model nematode *C. elegans*, multiple different DNA methylation pathways exist within the phyla implying that, similarly to piRNAs, DNA methylation evolved extremely rapidly in nematodes. We use this diversity to study the essential functions of the mammalian DNA methyltransferases DNMT1, DNMT2 and DNMT3 by characterising DNA methylation in nematodes using mass spectrometry and bisulfite sequencing. Additionally, through analysis of pathways that co-evolve with DNA methylation, our work uncovers a surprising link between DNA methylation and DNA repair, offering a novel explanation for the frequent loss of DNA methylation in nematodes and indeed across animals more generally. Overall, our analyses provide further insight into the rapid evolution of epigenetic pathways and the potentially deleterious consequences of overexuberant silencing mechanisms.

Small RNA pathways in *Fasciola hepatica*

SANTIAGO FONTENLA[1], NICOLÁS DELL'OCA[1], PABLO SMIRCICH[1,2], MARIA DEL MAR SILES[3]
MAKEDONKA MITREVA[4], **JOSÉ F.TORT[1]**

[1]DEPARTAMENTO DE GENÉTICA, FACULTAD DE MEDICINA (UDELAR);
[2]LAB. DE INTERACCIONES MOLECULARES, INSTITUTO DE BIOLOGÍA, FACULTAD DE CIENCIAS (UDELAR)
[3]INSTITUTO DE RECURSOS NATURALES Y AGROBIOLOGÍA DE SALAMANCA (IRNASA), SALAMANCA, ESPANA
[4] THE GENOME INSTITUTE, WASHINGTON UNIVERSITY SCHOOL OF MEDICINE, ST LOUIS MISSOURI, USA

Fasciola hepatica is responsible for one of the most widespread food borne trematodiasis worldwide, with economic impact in livestock production estimated in billions. Increasing reports of human cases and the emergence of resistance to available drugs have renewed the interest in liver fuke biology searching for novel targets towards its control. Small RNAs have emerged as relevant modulators of gene expression playing essential roles in development. For this reason we investigated their role in the liver fluke life cycle using different approaches. We mined the recently available genomes of *F.hepatica* and other flatworms identifying proteins involved in small RNA-mediated regulatory pathways. While canonical piwi proteins are absent in parasitic flatworms, a novel flatworm specific subfamily of argonaute proteins was detected. No orthologs to most of the nematode genes involved in RNAi amplification and spreading were found, although experimental evidence suggests that these processes take place in trematodes. By massive sequencing we analyzed the small RNAs expressed in different stages of the liver fluke life cycle. We found a reduced mirnome with notorious absence of several ancient miRNAs conserved in all metazoans and protostomes, while others are highly expressed throughout the cycle. Novel flatworm and *Fasciola* specific miRNAs were identified and stage-dependent differences were observed. Although miRNAs have been recognized as highly conserved during evolution, flatworm miRNAs are highly divergent with conservation restricted mostly to seed regions, posing interesting questions regarding target identification and recognition. A set of tRNA derived molecules, mainly 5´ halves of selected tRNAs were enriched in the small RNAs samples. Differential expression of these molecules was evident with more variants identified in the invasive stages. Taken together, our data shows interesting variations in small RNA pathways and mediators in flatworms, potentially useful as biomarkers and targets for control strategies.

Inferring microRNA function: from model organisms to parasitic interactions

CESARÉ OVANDO-VÁZQUEZ, ROBERTO ÁLVAREZ-MARTÍNEZ AND **CEI ABREU-GOODGER**

LANGEBIO-CINVESTAU, KM 9.6 LIBRAMIENTO NORTE CARRETERA IRAPUATO-LEON. IRAPUATO, GTO, MEXICO.

MicroRNAs (miRNAs) are small regulatory RNAs that act as post-transcriptional repressors of gene expression by directly binding to target transcripts. In our group we are developing computational methods that take advantage of experimental data to systematically explore genes that are functionally connected to miRNAs. We are particularly interested in being able to predict indirect regulation caused by miRNAs. In order to infer affected genes and processes, we use profiling experiments of miRNA overexpression or knockdown in cell lines or model organisms. Genes that are down-regulated in response to a miRNA include both direct and indirect targets, while up-regulated genes represent indirect targets. Many available target prediction methods are well suited to predict direct targets. We use machine-learning algorithms to build on these methods, by combining their scores with gene expression measurements. We find that adding expression information improves the predictions, making our results more relevant to particular organs or cell-types. We also generate co-expression networks from hundreds or thousands of publically available expression profiles. With these networks, we predict two kinds of indirect targets: those that correlate and those that anti-correlate with the direct targets. By including indirect regulation, our results can expand our understanding of miRNA function, beyond what could be predicted from the direct targets. We are currently evaluating how to use our methods in the context of non-model organisms, such as parasitic nematodes. Some of these parasites secrete small RNAs within exosomes during infection, and we are particularly interested in understanding the role of these small RNAs.

microRNAs in parasitic nematodes: a role in anthelmintic resistance?

EILEEN DEVANEY[1], VICTORIA GILLAN[1], ROZ LAING[1], KIRSTY MAITLAND[1], HENRY GU[1], ALAN WINTER[1], DAVE BARTLEY[2], ALLISON MORRISON[2], PHILP SKUCE[2], AXEL MARTINELLI[3], JOHN GILLEARD[4], ANDREW REZANSOFF[4], COLLETTE BRITTON[1]

[1]UNIVERSITY OF GLASGOW, [2]MOREDUN RESEARCH INSTITUTE, [3]WELLCOME TRUST SANGER INSTITUTE, [4]UNIVERSITY OF CALGARY

microRNAs are small non-coding RNAs that play a role in most biological systems by modulating levels of gene expression. We have studied their potential role in anthelmintic resistance using the model nematode *Caenorhabditis elegans* and the parasitic species, *Haemonchus contortus*. In *H. contortus,* miRNA expression levels were compared by microarray in a susceptible isolate (MHco3, ISE) and in two anthelmintic resistant isolates (MHco4, WRS and MHco10, CAVR). In addition, we had access to a unique resource: two lines of *H. contortus* generated by a backcross between the susceptible isolate and each of the resistant isolates. A single miRNA, Hco-miR-9551, was up-regulated in each of the resistant isolates compared to the susceptible isolate. In susceptible worms, this miRNA is only detectable in female worms and not in male worms or L3. However, it is expressed in males and L3 of the resistant isolates. A variety of computational methods were used to identify potential miRNA binding sites using an *in silico* library containing predicted 3'UTRs of all *H. contortus* genes. A large number of potential targets were identified which were narrowed down by searching for predicted binding sites which overlapped using all three programmes. This resulted in 68 potential target mRNAs, the expression of which was then compared in transcriptomic data originating from adult female worms of the susceptible MHco3 (ISE) isolate and both resistant parental isolates (MHco4, WRS and MHco10, CAVR). Six mRNAs with the expected expression profile were identified, i.e. significantly down-regulated in the resistant worms. These mRNAs are currently being further analysed to determine whether they may play a novel role in anthelmintic resistance.

Schistosome miRNAs play a Regulatory Role in Sexual Maturation and Gametogenesis

JIANGPING ZHAO[1§], CHAO HU[1§], JIANBIN WANG[2§], CHUNJING ZHOU[1], JINBIAO PENG[1], RONG LUO[1],YOUXIN JIN[3], RICHARD E DAVIS[2*], **GUOFENG CHENG[1*]**

[1]SHANGHAI VETERINARY RESEARCH INSTITUTE, CHINESE ACADEMY OF AGRICULTURAL SCIENCES KEY LABORATORY OF ANIMAL PARASITOLOGY, MINISTRY OF AGRICULTURE, CHINA, 200241; [2]DEPARTMENTS OF BIOCHEMISTRY AND MOLECULAR GENETICS, UNIVERSITY OF COLORADO SCHOOL OF MEDICINE, AURORA, COLORADO, USA 80045; [3]SCHOOL OF LIFE SCIENCES, SHANGHAI UNIVERSITY, CHINA; § EQUALLY CONTRIBUTED TO THIS STUDY

MicroRNAs (miRNAs) are key regulators of many biological processes including development, cell proliferation, metabolism, and signal transduction. Schistosomes, blood flukes, are an important global public health concern. Adult female schistosomes produce large numbers of eggs that are primarily responsible for the disease pathology and critical for dissemination. Consequently, understanding schistosome development and egg production are important in both the pathology and in disease transmission. Here, we identified *Schistosoma japonicum* (*S. japonicum*) miRNAs in the key stages of male-female pairing, gametogenesis, and egg production using small RNA deep sequencing. We identified 38 highly confident miRNAs, including 10 previously unknown miRNAs, many of these miRNAs are differentially expressed between male and female schistosomes and during different stages of development. Then, we identified 29 target genes for 15 of the *S. japonicum* miRNAs using antibody-based pull-down assays and bioinformatics analyses and further validated some of these target genes using either *in vitro* luciferase assays or *in vivo* miRNA suppression experiments. In addition, suppression of several female enriched miRNAs (bantam and miR-31) led to morphological alternation of ovaries and aberrant expression of eggshell proteins in female schistosomes. These findings uncover key roles for miRNAs in schistosome sexual maturation and egg production that may facilitate the development of new interventions for the control of schistosomiasis.

Secreted filarial small RNAs – localization in biofluids and biomarker potential for onchocerciasis

JUAN F. QUINTANA[1], BENJAMIN L. MAKEPEACE[2], SIMON A. BABAYAN[3], ALASDAIR IVENS[1], KENNETH M. PFARR[4], MARK BLAXTER[1], ALEXANDER DEBRAH[5], SAMUEL WANJI[6], HENRIETTA F. NGANGYUNG[7], GERMAIN S. BAH[7], VINCENT N. TANYA[8], DAVID W. TAYLOR[9], ACHIM HOERAUF[4], AMY H. BUCK[1].

[1]CENTRE FOR IMMUNITY, INFECTION AND EVOLUTION, ASHWORTH LABORATORIES, UNIVERSITY OF EDINBURGH, WEST MAINS ROAD, EDINBURGH, UK, [2]INSTITUTE OF INFECTION AND GLOBAL HEALTH, UNIVERSITY OF LIVERPOOL, LIVERPOOL, MERSEYSIDE, UK, [3]INSTITUTE OF BIODIVERSITY, ANIMAL HEALTH AND COMPARATIVE MEDICINE, COLLEGE OF MEDICAL, VETERINARY AND LIFE SCIENCES, UNIVERSITY OF GLASGOW, UK, [4]INSTITUTE OF MEDICAL MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY, UNIVERSITY HOSPITAL OF BONN, BONN, GERMANY, [5]KUMASI CENTRE FOR COLLABORATIVE RESEARCH (KCCR) AND KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA, [6]RESEARCH FOUNDATION IN TROPICAL DISEASES AND ENVIRONMENT AND UNIVERSITY OF BUEA, CAMEROON, [7]INSTITUT DE RECHERCHE AGRICOLE POUR LE DÉVELOPPEMENT, REGIONAL CENTRE OF WAKWA, NGAOUNDÉRÉ, CAMEROON, [8]CAMEROON ACADEMY OF SCIENCES, YAOUNDÉ, CAMEROON, [9]DIVISION OF PATHWAY MEDICINE, SCHOOL FOR BIOMEDICAL SCIENCES, UNIVERSITY OF EDINBURGH

Extracellular small RNAs, in particular miRNAs, are found in a wide variety of bodily fluids and have been proposed as biomarkers for diseases[1,2]. More recently, reports have suggested that parasitic nematodes secrete specific miRNAs in exosomes and these can be found in serum of infected patients, with major implications for diagnosis and evaluation for treatment efficacy**.** We have identified microRNAs derived from three different filarial nematodes (Clade III): *Litomosoides sigmodontis* (murine filariasis), *Onchocerca volvulus* (human filariasis) and *Onchocerca ochengi* (bovine filariasis), in serum or nodule fluids obtained from their definitive hosts. Specifically, miRDeep revealed a total of 62 mature miRNAs from 52 distinct pre-miRNA candidates in nodule fluids from cattle infected with *O. ochengi* of which 59 are identical in the genome of the human parasite *O. volvulus*. Six of the extracellular miRNAs were also identified in sequencing analyses of serum and plasma from humans infected with *O. volvulus* from endemic regions in Cameroon and Ghana. Also, fourteen parasite-derived miRNAs were found in mouse serum during the patent stage of the infection, all of which were detected in either human serum or bovine nodule fluid samples from endemic geographical regions in Cameroon. These results suggest that common miRNAs are secreted by filarial parasites and we have carried out an initial assessment of the ability of these miRNAs to detect infection in the serum of mice infected with *L. sigmodontis*, suggesting high sensitivity and specificity (80/100). Interestingly, among all of the secreted miRNAs described to date, whether in secretory-excretory products or detected in host body fluids, there are common secreted miRNA such as miR-71 and miRNA families including miR-100 and bantam, as well as specific differences across the clades. These results confirm the conserved nature of RNA secretion by nematodes and also suggest that there might be specific secreted signatures depending on each parasite, their life cycle, developmental stage and the niche that they occupy within the final host.

An extracellular RNA interference pathway as a mechanism of parasite-host communication

AMY BUCK

INSTITUTES OF EVOLUTIONARY BIOLOGY AND IMMUNOLOGY & INFECTION RESEARCH, UNIVERSITY OF EDINBURGH, ASHWORTH LABORATORIES, CHARLOTTE AUERBACH ROAD, EDINBURGH, UK

A new frontier in RNA biology has emerged in the last 7 years with the discovery that small RNAs (sRNAs) exist outside of cells and can be transferred from one cell to another in a functional form. This has been proposed as a mode of cell-to-cell communication and, in theory, could also enable communication between different organisms. Yet the basic mechanics of extracellular sRNAs are not understood, including how specificity in secretion is achieved and how sRNAs enter a functional RNA interference (RNAi) pathway in recipient cells. We have identified several classes of small RNA (microRNAs, Y RNAs, 22-G RNAs) in the secretion product of *Heligomosides polygyrus,* a gastrointestinal nematode that infects mice. Some of these RNAs (microRNAs, Y RNAs) are protected from degradation by encapsulation within vesicles whereas others are found outside of vesicles, protected from degradation by proteins. We are currently investigating whether this reflects two separate secretion pathways for RNA or whether lysed vesicles contribute to the extracellular RNA populations detected. We have also identified a newly-evolved Argonaute protein in the secretion product and postulate that this could be one of the key protein-binding partners of secreted RNA. We are currently characterizing the Argonuate protein to identify the RNAs to which this binds inside and outside of the nematode and determine whether this can be functional in mammalian cells.

Programmed DNA elimination in nematodes

JIANBIN WANG, YUANYUAN KANG, ASHLEY NEFF, and **RICHARD E. DAVIS**

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR GENETICS, UNIVERSITY OF COLORADO SCHOOL OF MEDICINE, USA

Genomes rarely change. However, a few organisms undergo a programmed process that eliminates specific DNA sequences from the genome during development (chromatin diminution). In the parasitic nematode *Ascaris suum,* we found that 13% of the genome is eliminated in somatic cell lineages during early embryonic divisions, while the germline genome remains intact. The eliminated DNA consists of specific repetitive and unique sequences, including ~700 genes. The eliminated genes are primarily expressed in the germline and early embryo leading us to suggest that DNA elimination in *Ascaris* is an essential, irreversible mechanism for silencing a subset of germline and early embryo expressed genes in somatic tissues. How specific *Ascaris* chromosomal regions are targeted and selected for elimination or retention remain unknown. We recently found that worm specific Argonautes, WAGO-2 and -3, are highly enriched on retained (WAGO-2) or eliminated (WAGO-3) condensed chromosomes during diminution mitoses. We hypothesize that these Argonautes and their associated small RNAs play a role in DNA elimination. We will describes experiments that define these worm specific Argonaute small RNAs, the location of these Argonautes on the genome, and proteins associated with these Argonautes to gain insight into their contributions to the mechanism of chromatin diminution. The histone H3 variant CENP-A epigenetically defines centromeres and is required to nucleate kinetochore assembly for microtubule attachment to facilitate chromosome segregation. Nematodes have holocentric chromosomes with multiple centromeres distributed along the length of the chromosome. Our data suggest that only retained chromosome regions have extensive deposition of CENP-A during As*caris* DNA elimination; chromosome regions destined for elimination have little associated CENP-A. This suggests a mechanism for how specific portions of chromosomes are retained or eliminated. We will describe studies that define the genome-wide distribution of CENP-A and examine whether CENP-A deposition is regulated to facilitate differential chromosome segregation during DNA elimination.

Retroviral-based functional genomic tools for schistosomes

GABRIEL RINALDI, VICTORIA H. MANN, PAUL J. BRINDLEY

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY & TROPICAL MEDICINE, AND RESEARCH CENTER FOR NEGLECTED DISEASES OF POVERTY, SCHOOL OF MEDICINE AND HEALTH SCIENCES, THE GEORGE WASHINGTON UNIVERSITY, WASHINGTON, DC 20037, USA

Functional genomic studies will facilitate the characterization of the role and essentiality of newly available genome sequences of the major human schistosomes, *Schistosoma mansoni, S. japonicum* and *S. haematobium*. To develop transgenesis for these pathogens, we have previously demonstrated that VSVG-pseudotyped murine leukemia virus (MLV) can transduce eggs of schistosomes leading to chromosomal integration and germline transmission of reporter transgenes and short hairpin RNA cassettes, facilitating the development of stable transgenic lines. However, a sustained and robust transgene expression has been a challenge. We have previously employed insulator sequences to protect the expression of MLV transgenes preventing chromatin silencing/ positional effects, and more recently demonstrated that the expression of endogenous (retro) transposable elements increased when epigenetic marks, e.g. cytosine methylation, were manipulated. Notably, the expression of reporter transgenes also increased in parasites transduced with MLV following culture with DNA methyltransferase inhibitors. In addition, exogenous cis-regulatory elements including conditional promoters, schistosome tissue-specific promoters, codon optimization to improve transgene translation, and antibiotic selection of retroviral-transduced parasites are being evaluated. Recently we investigated whether lentiviruses, including VSVG-pseudotyped human immunodeficiency virus type 1 (HIV-1) might be utilized for transgenesis of schistosomes. Early steps of lentivirus infection of schistosomes including attachment of virions to the schistosome tegument, reverse transcription to synthesize proviral cDNA, and genome integration were observed. Anchored-PCR and high throughput sequencing analyses revealed widespread integration of HIV provirus into schistosome chromosomes with a slight preference for non-coding regions. Bias towards actively transcribed genes as occurs with HIV-1 in human T cells was not evident. It is expected that these retroviral-based approaches, including lentivirus will facilitate transgenesis and functional genomics investigation of this neglected tropical disease pathogen.

How *C. elegans* fights fungal infection

JONATHAN EWBANK

CENTRE D'IMMUNOLOGIE DE MARSEILLE-LUMINY, MARSEILLE, FRANCE

The nematode *C. elegans* has become an important model for the study of host-pathogen interactions. In its natural environment, rotting fruit, *C. elegans* encounters a wide range of potential pathogens, including bacteria, fungi, viruses and microsporidia. It has developed a range of strategies to defend itself against infection. The first is pathogen evasion. Through chemosensation, *C. elegans* is able to detect specific microbial products, such as surfactants and quorum sensing molecules, and has the capacity to learn to avoid particular pathogens. If *C. elegans* is infected, this generally triggers the expression of defence programmes, involving the transcriptional up-regulation of hundreds of genes. The exact repertoire of host genes that is induced depends on the nature of the pathogen and the site of infection. Infection of the epidermis by the natural fungal pathogen *Drechmeria coniospora* leads to the production of antimicrobial peptides. These peptides are subject to complex patterns of regulation, characterised by a cross-talk between multiple pathways and between tissues. Our current state of knowledge of this innate immune response will be presented.

Changes in the immunological landscape during a *Nippostrongylus brasiliensis* infection does not affect the emphysematous long term pathology.

EMMA RINGQVIST[1], TARA SUTHERLAND[2], RICK M. MAIZELS[2], JUDITH E. ALLEN[2].

[1]RESPIRATORY MEDICINE, DEPARTMENT OF MEDICINE, KAROLINSKA INSTITUTET, SOLNA, SWEDEN. [2]INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH, UNIVERSITY OF EDINBURGH, EDINBURGH, SCOTLAND, UK.

Emphysema, enlargement of airway spaces, can be idiopathic or have environmental, genetic or pathogenic causes. After infection with the hookworm *Nippostrongylus brasiliensis*, mice develop progressive emphysema long after the infection has been cleared and larval antigens are no longer present in the lung parenchyma. The infection causes substantial damage as migrating larvae passage through the lung. The damage and accompanied bleeding is enhanced by neutrophil influx within the first days of infection, and limited by a strong Type 2 immune response within the first week of infection. In immune competent animals, the infection is self-limiting and adult worms are cleared from the intestine after the first week of infection. Emphysema appears about 20 days later. The emphysematic pathology after *N. brasiliensis* infection is characterized by the presence of alternatively activated interstitial macrophages (AAM) and micro-bleeding. Previous studies have highlighted the increase of macrophage transcripts and the alternatively activated phenotype in total lung, with potential effects of neutrophils early in the emphysematic phase. Here we show that the emphysema is not characterized by ongoing neutrophilia, nor is linked to the early neutrophil influx. We also show that the extent of emphysema formation is independent of general inflammation, eosinophil numbers or the level of Type 2 activation. No alternatively activated profile was detectable on protein or transcript levels 60 days post-infection in total lung or bronchoalveolar lavage. Some alternatively activated macrophages (AAM) were detected within the parenchyma by immunohistochemistry, but were not localized to emphysemateous foci. The underlying and driving mechanisms of *N. brasiliensis* caused emphysema remain elusive, and seem uncoupled from the inflammatory response.

Structure and function of the major Excreted Secreted protein of *Trichuris muris*

ALLISON BANCROFT, KELLY HAYES, EDWARD MCKENZIE, MATTHEW BALL, COLIN LEVY AND RICHARD GRENCIS

FACULTY OF LIFE SCIENCES, UNIVERSITY OF MANCHESTER, UK

The major excreted/secreted (ES) protein (43 kDa; P43) protein of the intestinal helminth *Trichuris muris* has previously been shown to have a low level of host immune reactivity in a number of immunological assays. This was particularly surprising given that this protein comprises approximately 90% of *T. muris* ES and the host and parasite are in intimate contact throughout infection. Both purified native P43 protein and an expressed selenomethionine P43 mutated protein were crystallized. The structure was determined by Se Methionine, single wavelength anomalous dispersion (SAD) in the first instance. This model was then subsequently used as the basis for molecular replacement against the native data revealing a novel disulphide rich protein with a putative protein binding cleft. P43 also has two thrombospondin type 1 repeat regions in addition to a natural heavy metal binding tail. The function of the protein is still as yet unknown although genomic studies suggest the gene is more highly expressed in the rear end of the worm which extends free within the lumen of the large intestine in comparison to the front end of the worm which invades the caecal epithelium. A combination of immunocytochemistry and whole mount *in situ* hybridization studies are being used to determine where in the worm the gene and protein are expressed together with protein pull down assays to reveal potential protein-protein interactions between P43 and worm homogenate, caecal contents and intestinal epithelial cells. The data generated should add to our functional understanding of P43 and analogous molecules secreted by other *Trichuris* species.

***Fasciola hepatica* tegumental antigens induce anergenic T-cells *in vivo* and *in vitro* via dendritic cells in a mannose receptor dependent manner**

ALLISON ALDRIDGE AND SANDRA M. O'NEILL

PARASITE IMMUNE MODULATION GROUP, SCHOOL OF BIOTECHNOLOGY, FACULTY OF SCIENCE AND HEALTH, DUBLIN CITY UNIVERSITY, GLASNEVIN, DUBLIN 9, IRELAND

Studies examining T-cell responses associated with helminth infection report two main regulatory phenotypes, FoxP3^{+} T-reg cells and anergic T-cells. Here we examine the T-cell responses during *F. hepatica* infection to its tegumental antigens that are shed from the fluke every 2-3 hours. FhTeg comprises of a rich source of glycoproteins that are comprises mainly of oligomannose N-glycans and to a lesser extent truncated and complex type glycans, including a sulphated subset. This study demonstrated a novel mechanism for the T-cell unresponsiveness observed during *F. hepatica* infection and after injection with FhTeg. During infection anergy is the main T-cell response observed when examining adaptive immune responses to FhTeg as a lack of cytokine responses and proliferation is observed and this can be reversed with the addition of IL-2 to culture. Markers of T-cell anergy such as CTLA4 and PD1 are also increased and we also have an up-regulation of genes relating to anergy induction and maintenance, RNF128, EGR2, ICOS and ITCH. The injection of FhTeg over the sternum can mimic the effect of infection. Dendritic cells treated with FhTeg induce a novel phenotype characterised by SOCS3^{high}, CD80^{low}, CD86^{low} and CD11c^{low} and MR^{high}. The Dc phenotype drives anergenic T-cells *in vitro* as measured by enhanced RNF128 and CTLA4 by RNA and supressed cytokine expression in anti-CD3 stimulated CD4^{+} cells. This study has also shown a role for the mannose receptor in dendritic cell communication with CD4^{+} cells. This study furthers our understanding about how helminths modulate the host's immune system during infection.

Helminth-induced inflammation controls murine γ-herpesvirus replication in the lung

A. M. DOUGALL[1], M. ROLOT[1], A. VANDERPLASSCHEN[1], B. DEWALS.[1]

[1]IMMUNOLOGY-VACCINOLOGY (B43B), DEPARTMENT OF INFECTIOUS AND PARASITIC DISEASES, FACULTY OF VETERINARY MEDICINE, UNIVERSITY OF LIÈGE, B-4000 LIÈGE, BELGIUM

Traditionally, host-pathogen interactions are studied in isolation. Although in reality hosts are often subject to concurrent infections in the natural environment. Schistosomiasis overlaps geographically with human γ-herpesviruses such as Kaposi's sarcoma-associated herpesvirus, also responsible for severe malignancies. A recent study indicated that the helminth Th2 dominant environment could be beneficial to viruses. Reactivation of latent murine γ-herpesvirus (MuHV-4) was stimulated with either *Heligmosomoides polygyrus* natural infection or *Schistosoma mansoni* eggs *in vivo*. In M2 macrophages IL-4 was found to increase viral replication of MuHV-4 by promotion of the reactivation gene 50 via a STAT6 pathway. Here, we sought to determine how acute γ-herpesvirus infection and host colonization could be affected by preliminary helminth infection. We used the lung model to generate *S. mansoni* egg-induced inflammation and typical Th2-type responses in mice, this was followed by intranasal infection with a MuHV-4-luc virus. We observed that the parasite egg-induced inflammation caused a significant reduction of MuHV-4 replication in the lung at day 5 and 7 post-infection (p.i.) using *in vivo* imaging and viral titration. Additionally, the egg-induced inflammation in the lung protected from the weight loss caused by the acute MuHV-4 infection. Using a MuHV-4-eGFP virus, we further observed that the egg-induced inflammation was associated with significantly reduced numbers of alveolar macrophages supporting viral infection. At day 7 p.i. with MuHV-4, flow cytometry analyses revealed that the presence of *S. mansoni* eggs in the lung was associated with reduced numbers of neutrophils and eosinophils, while increased RELM-α^+ and Ym-1^+ macrophages, $CD4^+$ effector T-cells and importantly $CD8^+$ T-cells. These results demonstrate that *S. mansoni* egg induced-inflammation controls acute MuHV-4 infection in the lung. Whether IL-4-dependent Th2-type responses and/or type-I interferon responses are involved are currently investigated.

WormBase-Parasite a new resource for helminth genomics

BRUCE BOLT[1], KEVIN HOWE[1], JANE LOMAX[2], ALESSANDRA TRAINI[2], PAUL STERNBERG[3], PAUL KERSEY[1], **MATT BERRIMAN**[2] AND THE WORMBASE CONSORTIUM

[1]EUROPEAN BIOINFORMATICS INSTITUTE (EMBL-EBI), WELLCOME GENOME CAMPUS, HINXTON, CAMBRIDGE, CB10 1SD, UK. [2]THE WELLCOME TRUST SANGER INSTITUTE, WELLCOME GENOME CAMPUS, HINXTON, CAMBRIDGE, CB10 1SA, UK. [3]CALIFORNIA INSTITUTE OF TECHNOLOGY, 1200 E. CALIFORNIA BLVD., PASADENA, CA 91125, USA

For more than 90 helminth species, genome sequences are now available, but with more than a million new genes to analyse, there is critical need for the data to be organised and presented to enable analysis by the research community. Building on expertise within the WormBase Consortium, WormBase-Parasite (parasite.wormbase.org) was launched twelve months ago to extend the scope of the intensively curated WormBase model organism database into a rapidly expanding universe of draft genomes for flatworms and nematodes. The new site provides access to both published and unpublished data; the majority of genomes within WormBase-ParaSite are currently unpublished and include all data from the Helminth Genomes Initiative – an international collaboration led from the Sanger Institute and The Genome Institute (St. Louis). WormBase-ParaSite is based on the well-established Ensembl infrastructure and provides several tools for exploring and analyzing data, including a BLAST tool for sequence comparisons against user-configurable species lists; a data-mining tool (BioMart) and phylogeny-based gene trees for comparative genomics analysis. In this presentation, I will highlight the major features of WormBase-Parasite and discuss the roadmap for future developments. The presentation will be followed by a drop-in workshop.

Myeloid restricted AMPKα1 is both essential and sufficient for Type 2 immunity and lung tissue repair following hookworm infection

WILDALIZ NIEVES[1], LI-YIN HUNG[1], TAYLOR K. ONISKEY[1], KOSHIKA YADAVA[1], MAX MURPHY[2] LOUIS BOON[3], MARC FORTEZ[4], BENOIT VIOLLET[4], AND **DE'BROSKI R. HERBERT[1]**

[1]DIVISION OF EXPERIMENTAL MEDICINE AND [2] DIVISION OF INFECTIOUS DISEASES, UNIVERSITY OF CALIFORNIA, SAN FRANCISCO, CA 94110; , [3]BIOCEROS, BV 3584 CM UTRECHT, THE NETHERLANDS, AND [4]INSTITUT COCHIN INSERM U1016, CNRS UMR 8104, UNIVERSITÉ PARIS DESCARTES, DEPT ENDOCRINOLOGY, METABOLISM AND CANCER 24 RUE DU FAUBOURG SAINT-JACQUES 75014 PARIS, FRANCE

How the metabolic demand of parasitism affects host physiology and immune resistance is unclear. Although immunity against parasitic helminths requires M2 cells and interleukin-13 (IL-13) secretion from $CD4^+$ T_H2 and ILC2 lymphocytes, it is presently unknown whether metabolic enzyme dysregulation in myeloid cells controls infectious disease outcome. This study demonstrates that AMP-activated protein kinase (AMPK), an essential regulator of cellular energy, shapes myeloid cell function to promote tissue repair and Type 2 responses. Mice lacking the AMPK catalytic α1 subunit in alveolar macrophages and conventional DC ($CD11c^{cre}$AMPKα1) lack pulmonary tissue repair and show defective immunity against the hookworm parasite *Nippostrongylus brasiliensis*. Defective immunity was due to IL-12/23p40-associated inflammation and reduced M2 polarization, which in turn, blocked the expansion of IL-13-producing $CD4^+$ T_H2 and ILC2. Moreover, transgenic over-expression of a constitutively active AMPK mutant solely in tissue macrophages restored pulmonary gas exchange function and accelerated the canonical features of Type 2 immunity. This demonstrates that metabolic enzyme regulation in macrophages and DC is a critical determinant of Type 2 immunity and pulmonary tissue repair following infectious injury.

Litomosoides sigmodontis* induces TGF-β receptor responsive, IL-10 producing T cells that suppress bystander T cell proliferation *in vivo

WIEBKE HARTMANN [1], CHRISTOPH SCHRAMM[2] and MINKA BRELOER [1]

[1]BERNHARD NOCHT INSTITUTE FOR TROPICAL MEDICINE, HAMBURG, GERMANY
[2]UNIVERSITY HOSPITAL HAMBURG EPPENDORF, HAMBURG, GERMANY

Helminths suppress immune responses to prolong their survival within their mammalian host. Thereby not only helminth-specific but also non-helminth-specific bystander immune responses are downregulated. Here, we dissect the chain of events leading from the presence of the parasitic nematode *Litomosoides sigmodontis* in the thoracic cavity of infected mice to systemic suppression of T cells with different antigen specificity. OVA-specific OT-II T cells displayed reduced antigen-induced *in vivo* proliferation upon adoptive transfer into *L. sigmodontis*-infected mice compared to transfer into naïve mice. Suppression of this bystander T cell proliferation *in vivo* required adaptive immunity as suppression was not established in helminth-infected RAG $1^{-/-}$ mice and replenishment of the host with T and B cells restored suppression. Suppression was independent of functional TGF-ß receptor in the suppressed OT-II T cells but required TGF-ß receptor signaling in host-derived T cells. Neutralization of mammalian TGF-ß did not abrogate suppression, suggesting that parasite-derived molecules engaged the TGF-ß receptor on T cells within *L. sigmodontis*-infected mice. Suppression was abrogated by IL-10 receptor blockade during antigen-specific stimulation of OT-II T cells and strictly dependent on the ability of host-derived T cells to produce IL-10. Thus, our results suggest that helminth-induced suppression of bystander T cell responses is established via a two-staged process. Presence of *L. sigmodontis* larvae is first sensed by TGF-ß receptor-dependent signaling on host-derived T cells that leads to subsequent IL-10 production. This host T cell-derived IL-10 mediates suppression of bystander OT-II T cell proliferation. responding to OVA-specific stimulation in *L. sigmodontis*-infected mice.

Defining the importance of dendritic cell subsets in promotion and regulation of immunity to *Schistosoma mansoni*

ALEXANDER PHYTHIAN-ADAMS[1], ANGELA MARLEY[2], SHEILA BROWN[1], PETER COOK[1], GARETH-RHYS JONES[1], LUCY JONES[2], LAUREN WEBB[1], RICK MAIZELS[2] AND ANDREW MACDONALD[1]

[1]MANCHESTER COLLABORATIVE CENTRE FOR INFLAMMATION RESEARCH, UNIVERSITY OF MANCHESTER, M13 9NT, UK. [2]INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH, SCHOOL OF BIOLOGICAL SCIENCES, ASHWORTH LABORATORIES, UNIVERSITY OF EDINBURGH, EH9 3JT, UK.

Although dendritic cells (DCs) are both sufficient and necessary for induction of Th2 immune responses against *Schistosoma mansoni*, the relative contributions of particular DC subsets to this process are poorly understood. CD8α$^+$ cDCs have been shown to play a critical role in Th1 settings and in cross presentation of bacterial antigens, but their role in Th2 immunity has yet to be addressed. We have used mice that are deficient in the expression of the transcription factor Batf3 (B6-*Batf3*$^{-/-}$), which lack most CD8α$^+$ lineage cDCs to tackle this question. *Batf3*$^{-/-}$ mice injected with *S. mansoni* eggs displayed dramatically enhanced Th2 cytokine production, along with increased IL-17 and impaired IFNγ, in comparison to WT controls. Similarly, *S. mansoni* infected *Batf3*$^{-/-}$ exhibited exaggerated Th2 cytokines and reduced IFN-γ in the liver and mesenteric lymph nodes. These novel data suggest that CD8α$^+$ cDCs are an integral part of a mechanism that controls the extent of Th2 responses to egg antigens. Culture of murine bone marrow with Flt3-L generates CD24^{hi} FLDCs (equivalent to CD8α$^+$ cDCs) and CD11b^{hi} FLDCs (equivalent to CD11b$^+$ cDCs). To enhance our understanding of the role of CD8α$^+$ and CD11b$^+$ cDC subsets in the initiation of adaptive immune responses against *S. mansoni*, we sorted CD24^{hi} and CD11b^{hi} FLDCs, stimulated them overnight with soluble egg Ag (SEA) and transferred them to B6 recipients. Whilst CD24^{hi} cDCs induced the production of only IFNγ, CD11b^{hi} cDCs initiated a potent Th2 response in recipient mice. Sorted FLDC subsets in co-culture with CD4$^+$ T cells promoted cytokine responses that where similarly biased. As well as identifying that CD8α$^+$ DCs are dispensable for Th2 induction against *Schistosoma mansoni*, our data support the emerging consensus that CD11b$^+$ conventional DCs are the most effective DC subset at priming Th2 responses *in vitro* and *in vivo.*

Role of receptors of IL-10 superfamily members during *Trichuris muris* infection

MARÍA A. DUQUE-CORREA[1], ALLISON J. BANCROFT[2], CORDELIA BRANDT[1], SIMON CLARE[1], MATTHEW BERRIMAN[1], RICHARD K. GRENCIS[2], GORDON DOUGAN[1].

[1]WELLCOME TRUST SANGER INSTITUTE, WELLCOME TRUST GENOME CAMPUS, HINXTON, CB10 1SA, UK. [2]FACULTY OF LIFE SCIENCES, UNIVERSITY OF MANCHESTER, MANCHESTER M13 9PT, UK.

Whipworms (*Trichuris trichuira*) are soil-transmitted helminths that infect about 700 million people in the tropics and sub-tropics and cause the human disease, trichuriasis. Whipworms live preferentially in the cecum of their hosts where they tunnel through epithelial cells and cause inflammation potentially resulting in colitis. Despite extensive research, the role of whipworm interactions with epithelial and immune cells in triggering parasite expulsion remains unclear, hindering the development of anti-parasite therapies. Here, using *T. muris,* a mouse model of *T. trichuira* infection in humans, we studied the role of the receptors of IL-10 superfamily members on epithelial cells during whipworm infection and immunity. Thus, mutant mouse lines for interleukin 10 (IL-10) receptor beta, IL-22 receptor alpha and IL-28 receptor alpha were challenged with *T. muris* and the influence of these mutations on anti-parasite immunity and expulsion was evaluated. While high dose infection with *T. muris* does not result in chronic infection in IL-22 receptor alpha and IL-28 receptor alpha mutant mice, IL-10 receptor beta mutant mice succumbed to whipworm infection at day 21 post infection. Interestingly, we found the susceptibility of IL-10 receptor beta mutant mice to *T. muris* is related to a leaky gut syndrome that allows the colonization of the liver by members of the gut microbiota. On-going experiments focus on understanding the role of IL-10 in the regulation of epithelial cells and immune cells that promote the immunity and expulsion of whipworms. These results highlight the importance of IL-10 regulation on intestinal epithelial cells during *T. muris* infection for the maintenance of the mucosal barrier that physically contains the microbiota in the gut preventing the systemic colonization by opportunistic bacteria and the development of inflammatory responses that result in immunopathology.

Innate cytokines required for the effector phase of helminth expulsion

KATHERINE A SMITH[1], KARA J FILBEY[2], JAMES P HEWITSON[3], YVONNE HARCUS, HENRY J MCSORLEY[4]
RICK M MAIZELS

INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH, UNIVERSITY OF EDINBURGH, EH9 3JT, EDINBURGH UK. CURRENT ADDRESSES : [1]CARDIFF UNIVERSITY, WALES; [1] MALAGHAN INSTITUTE, WELLINGTON, NZ; UNIVERSITY OF YORK; QUEENS MEDICAL RESEARCH INSTITUTE, EDINBURGH, UK

The type-2 immune response that co-ordinates the expulsion of intestinal helminths comprises key innate cytokines such as IL-25, IL-33 and TSLP, as well as signals from dendritic cells, which induce T cells to produce IL-4 and IL-13, and drive B cell antibody production. Recently we have found that two innate signals are essential components in enhancing the effector response for elimination of the mouse model intestinal nematode *Heligmosomoides polygyrus*. One of these is IL-25R signalling, which is dispensible for the induction of a Th2 response to the parasite, but is required for both elimination of chronic infection and for sterile immunity following challenge infection of vaccinated mice. Furthermore, as IL-25R together with IL-4R signalling will induce expulsion in T cell-deficient RAG-/- mice, the effector mechanism targetted by IL-25 is an innate, rather than an adaptive, cell type. The second essential component is Macrophage Migration Inhibitory Factor (MIF), as mice deficient in this mediator, or receiving a pharmacological inhibitor of MIF, are impaired in expulsion of both *H. polygyrus* and *Nippostrongylus brasiliensis* in both settings of primary infection and following vaccination. MIF-deficient animals show a marked reduction in eosinophilia and the induction of alternatively activated macrophages (AAMs); gene expression analysis reveals that a number of AAM-associated transcripts induced by MIF administration in vivo are compromised in MIF-deficient mice. Since we also found that eosinophil-deficient mice are able to clear infection normally, we hypothesise that an AAM population driven by IL-4/IL-13, IL-25 and MIF act in the intestinal tract to debilitate and destabilise nematode parasites and promote protective immunity.

POSTER SESSION 1

ABSTRACTS

1. Ploidy and population genetic structure of *Fasciola hepatica* in sheep and cattle

NICOLA J BEESLEY[1], KRYSTYNA CWIKLINSKI[1], DIANA. J. L. WILLIAMS[1], STEVE PATERSON[2], JANE E. HODGKINSON[1]

[1]*VETERINARY PARASITOLOGY, INSTITUTE OF INFECTION AND GLOBAL HEALTH, UNIVERSITY OF LIVERPOOL.*
[2]*CENTRE FOR GENOMIC RESEARCH, UNIVERSITY OF LIVERPOOL*

Fasciola hepatica infection causes parasitic disease of economic and welfare importance in sheep and cattle and is also an important zoonosis. Information on ploidy and population structure is key to understanding the biology of *F. hepatica*, including its complex reproductive biology, and to improve our knowledge of gene flow in populations. The ploidy and presence of sperm in 715 wild British adult *F. hepatica,* collected from the livers of 66 sheep and 35 cattle, was determined by aceto-orcein squash. A panel of eight polymorphic microsatellites, previously validated by our laboratory, was used to produce a multilocus genotype (MLG) for 950 *F. hepatica* adults from 44 sheep and 629 *F. hepatica* adults from 31 cattle. All *F. hepatica* adults studied were diploid and contained sperm. The eight loci under study showed alleles (including private alleles) ranging from 10 to 39 and between 21 and 178 genotypes. The average heterozygosity across all parasites and loci was 0.75247 (SD±0.12995). No significant difference was detected between the heterozygosity of parasites from sheep and cattle. Of the 1579 samples, 1424 distinct MLGs were observed, which supports high genotypic diversity in the population as a whole. However, some hosts harboured multiple, genotypically identical parasites (clones). The F_{ST} value across all loci was 0.0286 in parasites from cattle and 0.0143 in parasites from sheep. In conclusion, all parasites studied contained sperm, suggesting sexual reproduction predominates in *F. hepatica*. The majority of livers contained clonal *F. hepatica* with 96 MLGs observed multiple times, most likely as a result of clonal expansion of the parasite within the snail intermediate host and aggregation of metacercariae with the same MLG on pasture prior to ingestion by a definitive host. However, overall the F_{ST} suggests high gene flow and low population structure within *F. hepatica* populations.

2. Comparative analysis of secreted nucleotide-metabolising enzymes in parasitic nematodes

RITA BERKACHY, RACHEL VAUX, MURRAY E. SELKIRK and KLEONIKI GOUNARIS

DEPARTMENT OF LIFE SCIENCES, IMPERIAL COLLEGE LONDON, LONDON SW7 2AY, UK

Tissue damage results in the release of molecules which activate elements of the immune system. One such class of these molecules is extracellular nucleotides, which can also be released in a regulated manner, exerting a variety of effects via signalling through purinergic receptors. In general, ATP and ADP are pro-inflammatory, whereas adenosine is anti-inflammatory, and recent data suggest that adenosine promotes the development of M2 macrophages. Mice lacking the A2B adenosine receptor exhibit impaired immunity to *Heligmosomoides polygyrus*, implicating adenosine as a contributing factor to parasite expulsion. Nematode parasites are known to secrete nucleotide-metabolising enzymes, which would be predicted to affect the availability and local concentrations of extracellular nucleotides and ensuing cellular responses. We have screened secreted products of *Trichinella spiralis* and *H. polygyrus* for nucleotide-metabolising enzymes, and found differences in the types of enzymes present and their substrate specificity, which may reflect altered requirements for different stages of the parasite life cycles. We have also developed a natural parasite of mice, *Trypanosoma musculi*, as a vehicle for heterologous expression and in vivo delivery of helminth secreted proteins. Nucleotide-metabolising enzymes from *T. spiralis* and *H. polyyrus* are thus being expressed in *T. musculi* in order to assess their potential roles in modulating immune responses.

3. Effect of glucose on the structure and transcriptional regulation of glucose transporters in *Schistosoma mansoni*.

ALEJANDRO CABEZAS-CRUZ[1 +], JAMES VALDÉS[2 +], JULIEN LANCELOT[1] AND RAYMOND PIERCE[1]*.

[1] CENTER FOR INFECTION AND IMMUNITY OF LILLE (CIIL), INSERM U1019 – CNRS UMR 8204, UNIVERSITÉ LILLE, INSTITUT PASTEUR DE LILLE, LILLE, FRANCE.. [2] INSTITUTE OF PARASITOLOGY, BIOLOGY CENTRE OF THE ACADEMY OF SCIENCES OF THE CZECH REPUBLIC, 37005 ČESKÉ BUDĚJOVICE, CZECH REPUBLIC + JOINT FIRST AUTHORSHIP

One of the most striking evolutionary adaptations of the trematode *Schistosoma mansoni* is its capacity to reversibly switch from oxidative phosphorylation (OXPHOS) to glycolysis (GLY) in the presence of glucose. Glucose transport in schistosomes occurs mainly via facilitated diffusion. The genome of *S. mansoni* presents four glucose transporters, SGTP1, SGTP2, SGTP3 and SGTP4. Although, glucose uptake is an essential activity for *S. mansoni* during its parasitic stages within the human host, we lack knowledge regarding the structural basis of glucose transport by these transporters. In addition, little is known regarding the transcriptional regulation of *S. mansoni* glucose transporters upon vertebrate host infection. By using phylogenetic and bioinformatics analyses we first classified SGTP2 and SGTP3 as class I glucose transporters. The other two, SGTP1 and SGTP4, fall in a cluster separated from class I, II and III glucose transporters, but together with glucose transporters from other Platyhelminthes, suggesting that they form a platyhelminth-specific class of glucose transporters. Nevertheless, SGTP1 and SGTP4 share several structural properties with class I and II glucose transporters. Secondly, we used molecular dynamics to investigate GLUT1 D-glucose induced fit docking to compare tertiary predicted models of SGTP1, SGTP2, SGTP3 and SGTP4 with an experimental crystal structure. These computational results revealed important amino acid residues putatively responsible for glucose binding and translocation in *S. mansoni* glucose transporters, providing a structural basis for further design of *S. mansoni*-glucose transporter-specific drugs. Thirdly, using quantitative RT-PCR, we showed that the transcripts levels of SGTP1, SGTP2 and SGTP4 are differentially regulated in schistosomula in the presence of glucose. SGTP1 and SGTP4 are down-regulated, while SGTP2 is upregulated. Interestingly, several transcript variants of SGTP2 were identified. Altogether, these results open new avenues of research for glucose transport in trematode.

4. Functional investigation of nematode parasite specific acetylcholine receptors

CLAUDE L. CHARVET [1,2], ELISE COURTOT [1,2], HADILE GABAJ [1,2], NICOLAS PEINEAU [3], RICHARD J. MARTIN [4], ALAN P. ROBERTSON [4], THOMAS BOULIN [5] , ROBIN N. BEECH [6] AND CÉDRIC NEVEU [1,2]

[1]INRA, UMR1282 INFECTIOLOGIE ET SANTE PUBLIQUE, F-37380, NOUZILLY, FRANCE. [2]UNIVERSITE FRANÇOIS RABELAIS DE TOURS, UMR1282 INFECTIOLOGIE ET SANTE PUBLIQUE, F-37000, TOURS, FRANCE. [3]UNIVERSITE FRANÇOIS RABELAIS DE TOURS, DEPARTEMENT DE PHYSIOLOGIE ANIMALE, F-37000, TOURS, FRANCE. [4]DEPARTMENT OF BIOMEDICAL SCIENCES, COLLEGE OF VETERINARY MEDICINE, IOWA STATE UNIVERSITY, AMES, IA. USA. [5]CNRS, UMR5534 – UNIVERSITE CLAUDE BERNARD LYON 1, 69622, VILLEURBANNE, FRANCE [6]INSTITUTE OF PARASITOLOGY, MCGILL UNIVERSITY, MACDONALD CAMPUS, STE. ANNE DE BELLEVUE, QUEBEC H9X 3V9, CANADA

The acetylcholine receptors (AChRs) are major targets for anti-nematodal drugs. In the free-living model nematode *Caenorhabditis elegans* the prototypical nicotine-sensitive AChR (N-AChR) that mediates the fast neurotransmission at the neuromuscular junction is composed of five identical subunits encoded by the *Cel-acr-16* gene. In the present study we have identified the N-AChR from the pig parasite *Oesophagostomum dentatum* and performed a detailed pharmacological characterization of the *O. dentatum* and *C. elegans* N-AChRs. Here, we have cloned the homologue of the *Cel-acr-16* gene in *O. dentatum* and expressed it in *Xenopus laevis* oocytes. Full dose-response experiments were performed to explore the respective *O. dentatum* and *C. elegans* N-AChR sensitivities to acetylcholine (ACh) and nicotinic analogues using two-electode voltage-clamp. First, we found that the *O. dentatum* ACR-16 sequence is highly similar to Cel-ACR-16. Interestingly, injection of *Ode-acr-16* cRNAs gave rise to a functional homopentameric channel having an ACh EC_{50} value very similar to that of the *C. elegans* N-AChR. Surprisingly, nicotine was less potent than ACh in activating the *O. dentatum* N-AChR, unlike *C. elegans* N-AChR. Finally, the pharmacological analysis of nicotine derivatives reveals a potency series of ACh > nic > anabasine = cytisine > nornicotine for the *O. dentatum* N-AChR different from that of the *C. elegans* N-AChR. These results allowed us to refine a computer model of the N-AChR 3D structure able to predict ligand-receptor interactions *in silico* that will be used for drug optimization. We have characterized for the first time the functional N-AChR of a Clade V parasitic nematode and showed that its pharmacology differs from that of the *C. elegans* N-AChR. This work advances the understanding of N-AChR structure-function relationships in parasitic nematodes and opens the way for molecular screening of novel antiparasitic pharmaceuticals.

5. Investigation of extracellular RNA in *Heligmosomoides polygyrus*: Are we witnessing cross-species communication?

FRANKLIN WN CHOW[1], C.ABREU-GOODGER[2], JULIE CLAYCOMB[3], AMY BUCK[1]

[1]INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH, THE UNIVERSITY OF EDINBURGH [2]LABORATORIO NACIONAL DE GENOMICA PARA LA BIODIVERSIDAD, CENTRO DE INVESTIGACION Y DE ESTUDIOS AYANZADOS DEL INSTITUTO POLITECNICO NACIONAL, MEXICO [3]DEPARTMENT OF MOLECULAR GENETICS, UNIVERSITY OF TORONTO, CANADA

Extracellular small RNA is an emerging field in RNA biology and it has been proposed as a means of cell-to-cell communication within an organism and a mechanism of cross-species communication between organisms. We previously showed that an Argonaute protein (HpWAGO) and small RNAs including microRNAs (miRNAs) and Y RNAs are secreted in exosome-like vesicles produced by *Heligomosomoides polygyrus,* a gastrointestinal nematode that infects mice. Instranasal administration of the *H.polygyrus* to mice suppresses a Type 2 innate response in an airway allergy model and our work suggests that nematodes-derived small RNAs can suppress host gene expression based on *in vitro* reporter assays. Interestingly we find that the nematode Ago protein is found both inside and outside of vesicles. Here I extend the analyses of extracellular RNA in *H.polygyrus* towards answering two questions: 1) Are the microRNAs or other RNAs directly bound to the secreted Ago protein? 2) Are the populations of exRNA inside and outside of vesicles related, or do these likely represent two separate modes of secretion? I will present unpublished data based on size exclusion chromatography and small RNA sequencing, which suggest that large amounts of 5'-triphosphate small RNAs are present in Ago fractions when compared with the 5'-monophosphate libraries, but this is not observed in fractions not containing Ago. Together these results suggest further diversity in the exRNA that is secreted by parasites and will shed light on the properties of the proteins to which these bind.

6. Population and comparative genomics of African *Schistosoma mansoni*

THOMAS CRELLEN[1 2], SOPHIA DAVID[2], THOMAS HUCKVALE[2], NANCY HOLROYD[2], FIONA ALLEN[3], JOANNE WEBSTER[4], MATTHEW BERRIMAN[2], JAMES COTTON[2]

[1]IMPERIAL COLLEGE, LONDON . [2]WELLCOME TRUST SANGER INSTITUTE, HINXTON. [3]NATURAL HISTORY MUSEUM, LONDON. [4]ROYAL VETERINARY COLLEGE, UNIVERSITY OF LONDON

Schistosomiasis is among the most important parasitic diseases, with over 200 million people infected and 300,000 deaths annually across Africa, Asia, South America and the Caribbean caused mainly by three closely related species. Around 90% of cases are in sub-Saharan Africa, where *Schistosoma mansoni* is one of the two most clinically important species, and the principal cause of intestinal schistosomiasis. A draft reference genome is available for *S. mansoni* and is being actively curated and improved, based on an isolate from Puerto Rico that has been maintained in research labs for many years. Here, we present genome sequence data and assemblies from seven adult male *S. mansoni* that were recently collected from the field with minimal lab passage, including six diverse African isolates - the first genomic data from the region of greatest public health interest. We confirm that the *S. mansoni* reference sequence is a suitable substrate for genomic analysis of African populations. We use this genomic diversity data to investigate signatures of natural selection on the *S. mansoni* genome, and apply two coalescent-based models to infer the population history of *S. mansoni* on two continents. Our results show that the New World strains have smaller past effective population sizes (Ne) than African strains, suggesting the possible occurrence of a past population bottleneck. We estimate the divergence time between the African and New World populations, finding support for the hypothesis that *S. mansoni* colonised the New World via the 16-19th century West African slave trade. In the light of this potential population bottleneck, we investigate systematic differences between South American populations and African populations in both genome structure (copy number variants) and single nucleotide polymorphisms (SNPs).

7. Development and field application of pyrosequencing assays for analysis of three *Trichostrongylus colubriformis* β-tubulin single nucleotide polymorphisms associated with benzimidazole resistance

JANINA DEMELER [1], PHILIP SKUCE [2], LAURA RINALDI [3], SABRINA RAMUNKE [1], LYNSEY MELVILLE [2], THEO DE WAAL [4], HUBERTUS HERTZBERG [4], GEORG VON SAMSON-HIMMELSTJERNA [1], JURGEN KRUCKEN [1]

[1]INSTITUTE FOR PARASITOLOGY AND TROPICAL VETERINARY MEDICINE, FREIE UNIVERSITAT BERLIN, BERLIN, GERMANY, [2]MOREDUN RESEARCH INSTITUTE, EDINBURGH, SCOTLAND, UK, [3]DEPARTMENT OF VETERINARY MEDICINE AND ANIMAL PRODUCTIONS, UNIVERSITY OF NAPLES FEDERICO II, CREMOPAR, ITALY, [4]SCHOOL OF VETERINARY MEDICINE, DUBLIN, IRELAND, [5]INSTITUT FUR PARASIROLOGIE, UNIVERSITAT ZURICH, ZURICH, SWITZERLAND.

Resistance to benzimidazoles (BZs) in trichostrongylid nematodes is a worldwide problem for livestock production, particularly in farming of small ruminants. Sensitive, reliable and cost efficient methods are required to assess the resistance status of at least the most important nematode species. Besides currently available *in vivo* or *in vitro* methods for detection of BZ-resistance, molecular tools become increasingly important. Several single nucleotide polymorphisms (SNPs) in the β-tubulin isotype 1 gene of various nematodes correlate with BZ-resistance. Accordingly, conventional PCRs, real time PCRs as well as RFLP methods and pyrosequencing assays, which have the advantage of enabeling the analysis of pooled larval samples, have been described. While PCR-based methods have been reported for the three economically most important nematodes *Trichostrongylus*, *Haemonchus* and *Teladorsagia*, pyrosequencing assays are so far only available for the latter two. This led to the design and evaluation of a pyrosequencing assay for both β-tubulin genes, isotype 1 and isotype 2 of *Trichostrongylus colubriformis*. For either of the two alleles respective PCR-fragments were combined in defined ratios to evaluate the assay and the correlation between the given and the measured allele frequencies of the respective SNPs was very high for all three SNPs (Codon 167, 198 and 200). Additionally, pyrosequencing assays for all three species were used for a BZ resistance survey, which was carried out in the three European countries Italy, Ireland and Switzerland. Larval cultures obtained from field samples were used for DNA extraction and pyrosequencing was applied when at least 10% of the target species were present in a sample. In isotype 1 only at codon 200 BZ-resistance associated alleles were found to be significantly increased in all three species. Results for *T. colubriformis* isotype 2 showed a few farms with high BZ-resistance associated allele frequencies in at least one of the three codons.

8. Early phase of live intestinal nematode therapy mediated regulatory cell recruitment to the Central Nervous System in EAE model of Multiple Sclerosis

KATARZYNA DONSKOW-ŁYSONIEWSKA

UNIVERSITY OF WARSAW, DEPARTMENT OF PARASITOLOGY, WARSAW, POLAND

Targeting the helminths represents a potential new paradigm in the treatment of an autoimmune human disease, including human MS that could significantly ameliorate patients comfort. Helminths use various immunomodulatory and anti-inflammatory mechanisms, to evade destruction by the host immune system and they are capable of altering disease course for MS and other autoimmune disease. However, the exact mechanisms of this active process at early phase of live intestinal nematode therapy remain elusive. We have previously demonstrated that the development of clinical signs of ongoing experimental autoimmune encephalomyelitis *(*EAE) inflammation in mice can be curtailed by the infection with live *Heligmosomoides polygyrus* intestinal nematode and larvae infection promotes the full recovery of mice with EAE from clinical signs of the disease as soon as 2 days post infection. To elucidate the mechanisms of intestinal nematode *H. polygyrus* intervention on autoimmunity in the CNS we have established an *in vivo* MS prototype animal model of monophasic EAE induced by myelin oligodendrocyte glycoprotein $(MOG)_{35-55}$ in mice. We analyzed the composition of infiltrating cells and the expression of different adhesion molecules, as well as the functioning of blood brain barrier BBB, brain lesions, the expression of neurotrophins and inflammatory/anti-inflammatory factors implicated in the pathogenesis of EAE. Our results demonstrated that early pre-patent phase of intestinal nematode infection dramatically diminishes EAE symptoms and attenuate the neuropathology of EAE as a consequence of altering anti-inflammatory/ regulatory leukocytes migration to the CNS target organ by enhanced permeability of BBB. The extensive infiltration of immune cells in the cerebral cortex of mice promoted axon regeneration and support remyelination according to the restored expression of nerve growth factor- NGF.

9. Functional diversification of levamisole receptors in the trichostrongylid nematode *Haemonchus contortus*

THOMAS DUGUET[1], CLAUDE L. CHARVET[4], SEAN G. FORRESTER[2], CLAUDIA M. WEVER[3], JOSEPH A. DENT[3], CEDRIC NEVEU[4], ROBIN N. BEECH[1]

[1]*INSTITUTE OF PARASITOLOGY, MCGILL UNIVERSITY, CANADA.* [2]*FACULTY OF SCIENCE, UNIVERSITY OF ONTARIO INSTITUTE OF TECHNOLOGY, CANADA.* [3]*DEPARTMENT OF BIOLOGY, MCGILL UNIVERSITY, CANADA.* [4]*INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE, NOUZILLY, FRANCE*

The cholinergic agonist Levamisole is widely used to cure parasitic nematode infections. This drug activates a class of acetylcholine receptors (L-AChRs) expressed at the neuromuscular junction. The L-AChR of the free-living nematode model *Caenorhabditis elegans*, is composed of five subunits encoded by *unc-63*, *unc-38*, *lev-8*, *lev-1* and *unc-29*. In contrast, the parasitic nematode of small ruminants, *Haemonchus contortus,* does not require *lev-1*, replaced *lev-8* by *acr-8* and contains four copies of *unc-29*. We examine the functional diversification of these subunits and aim to characterize novel, parasite specific, receptors produced. Reconstitution of the *H. contortus* L-AChR in *Xenopus* oocytes identified channels produced by UNC-29.1, UNC-29.3 and UNC-29.4 with identical affinity for acetylcholine but different sensitivities to levamisole. No responsive channel could be produced substituting UNC-29.2 for UNC-29.1 in *Xenopus*, although immunolocalization showed that the UNC-29.2 was present at the surface. Transfection of a levamisole resistant *C. elegans unc-29* mutant strain with each gene copy restores levamisole sensitivity showing these subunits, including UNC-29.2*,* are functional in *C. elegans*. Specific antibodies allowed us to observe identical expression of UNC-29.1 and UNC-29.2 in the body muscle of both sexes and uterine muscles in the female. UNC-29.4 expression is harder to detect and may be expressed in non-body muscle tissues. The anti-UNC-29.3 antibodies recognize the peptide antigen but fail to recognize the protein. The *unc-29* copies of *H. contortus* are functionally divergent and produce receptors with distinct pharmacology. In addition, the unusual properties of UNC-29.2 suggest that this may provide an experimental platform to investigate the evolution of subunit interaction and assembly. Taken as a whole, this work establishes critically that evolution between nematode species must be taken into account when using *C. elegans* as a model and that it is essential to examine the molecular mechanisms of drug action in specific parasites in detail.

10. Endogenous phospholipase A_2 group 1B (PLA_2g1B) has direct anti-helminth properties and is essential for immunity to *Heligmosomoides polygyrus*

LEWIS J. ENTWISTLE[1], STEPHANIE M. COOMES[1], VICTORIA S. PELLY[1], JIMENA PEREZ-LLORET[1], NIKOLAY NIKOLOV[1], DAVID HUI[2] AND MARK S. WILSON[1]

[1]*THE FRANCIS CRICK INSTITUTE, MILL HILL LABORATORY, LONDON, UNITED KINGDOM.*
[2] *DEPARTMENT OF PATHOLOGY, METABOLIC DISEASES INSTITUTE, UNIVERSITY OF CINCINNATI COLLEGE OF MEDICINE, CINCINNATI, USA.*

With emerging evidence of drug-resistant helminths, it is of paramount importance to understand the mechanisms of anti-helminth immunity to provide new avenues of therapeutic intervention. To identify novel mechanisms of immunity we compared the small intestinal transcriptome of mice that were susceptible (primary infected, *H. p.* 1^o) or resistant (secondary challenge infected, *H. p.* 2^o) to the evolutionally adapted murine intestinal helminth *Heligmosomoides polygyrus*. We identified distinct clusters of genes in resistant mice, some of which have previously been described, and many that have not. In particular, we identified elevated expression of lipid metabolism pathways and the lipid catabolising enzyme, Phospholipase A_2 Group 1B (*Pla2g1b*), in resistant, but not susceptible mice. Elevated expression of *Pla2g1b* was dependent upon drug-mediated killing of *H. polygyrus*, required *Rag* and common gamma chain-dependent immune compartments and was restricted to the $CD45^-$ fraction of the small intestine. Importantly, elevated expression of *Pla2g1b* was critical for immunity to *H. polygyrus*, as *Pla2g1b*$^{-/-}$ mice failed to expel a challenge infection with *H. polygyrus*. The failure to expel *H. polygyrus* in *Pla2g1b*$^{-/-}$ mice was not due to an ineffectual or aberrant immune response as $CD4^+$ T cells, antibodies and innate immune pathways were all intact in *Pla2g1b*$^{-/-}$ mice. Instead we show that Phospholipase A_2 Group 1B had a direct effect on *H. polygyrus* larvae, with *in vitro* treatment of L3 larvae compromising their ability to establish *in vivo*. We hypothesise that activation of immune pathways upregulates endogenous *Pla2g1b*, which is required for direct killing of the invading larvae in resistant mice. We are currently exploring the precise source and regulation of *Pla2g1b* expression in resistant mice as well as the mechanism by which PLA_2g1B kills *H. polygyrus* larvae.

11. Identification and characterization of lipid binding proteins from the parasitic nematode *Dioctophyma renale.*

NAHILI GIORELLO [1,2], MARCOS BUTTI [2], NILDA A. RADMAN [2], MALCOLM W. KENNEDY [3], BETINA CORSICO [1] AND **GISELA FRANCHINI** [1]

[1]INIBIOLP-CONICET, FAC.DE CIENCIAS MEDICAS, UNIVERSIDAD DE LA PLANTA, ARGENTINA , [2]LABORATORIO DE PARASITOSIS HUMANAS Y ZOONOSIS PARASITARIAS, CATEDRA DE PARASITOLOGIA COMPARADA, FAC. DE VETERINARIA, UNIVERSITDAD DE LA PLATA, ARGENTINA, [3]INSTITUTE OF BIODIVERSITY, ANIMAL HEALTH AND COMPARATIVE MEDICINE, UNIVERSITY OF GLASGOW, UK

Dyoctophyma renale, the giant kidney worm, is probably the largest parasitic nematode by mass of land animals described so far and is distributed worldwide. These parasites locate in the kidney of their definitive hosts (carnivores, mainly piscivores) and have an indirect life cycle with an annelid as the main intermediate host. Humans are rarely affected, but in those that are, one or both kidneys are destroyed. In Argentina, *D. renale* is found mostly in dogs that live close to rivers and the infection is diagnosed by urine analysis, ultrasonography, surgery, or at necropsy. Changing climatic conditions, environmental degradation, and compromised sanitation are increasing the risk to humans. Having incomplete lipid metabolisms, helminth parasites rely on their hosts for fatty acids, cholesterol and complex lipids. The acquisition and transport of these hydrophobic molecules is crucial to these organisms, and the proteins and receptors involved in lipid transport and exchange provide potential targets for chemo- and immunotherapy. Nematodes in particular produce and secrete a wide range of novel lipid binding proteins (LBPs), many of which are structurally distinct from those of their hosts. These proteins bind several lipid classes such as fatty acids, retinoids, eicosanoids, triglycerides, phospholipids and cholesterol. The main objective of this work is to identify and characterize novel LBPs from *D. renale* using biophysical techniques, and to explore their value as diagnostic markers. Using the fluorescent probe DAUDA, we have detected an abundant LBP in the pseudocoelomic fluid from these organisms and we are working on its characterization. The results obtained from this work should shed light on the lipid metabolism of this unusually large nematode parasite of mammals and begin to understand its particular survival mechanisms.

12. Characterising Host Gene Expression during Recovery from Hepatic Schistosomiasis Japonica

CANDY CHUAH[1,2,3], MALCOLM K JONES[1,2], MELISSA L. BURKE[4], DONALD P. MCMANUS[1], GRANT A. RAMM[1], **GEOFFREY N. GOBERT[1]**

[1]QIMR BERGHOFER MEDICAL RESEARCH INSTITUTE, AUSTRALIA. [2]SCHOOL OF VETERINARY SCIENCES, THE UNIVERSITY OF QUEENSLAND, AUSTRALIA. [3]SCHOOL OF MEDICAL SCIENCES, UNIVERSITI SAINS MALAYSIA, MALAYSIA. [4]DIVISION OF MYCOBACTERIAL RESEARCH, NATIONAL INSTITUTE FOR MEDICAL RESEARCH, UK

In schistosomiasis japonica and mansoni, the egg-induced granulomatous response and the development of extensive hepatic fibrosis is the main pathology. Information regarding the specific mechanisms associated with granuloma regression and the subsequent recovery events in the host liver are still limited. In this study, a murine model of schistosomiasis japonica was used to characterise the multicellular pathways occurring during liver regeneration. *Schistosoma japonicum*-infected C57BL/6 mice were treated with the drug praziquantel (PZQ), on a daily basis for five consecutive days to eliminate all adult parasites and stop the deposition of new eggs. Cellular infiltration and pathological changes in the liver were examined after 3, 6 and 7 weeks of PZQ treatment. PZQ treatment significantly reduced the degree of splenomegaly, granuloma density and liver fibrosis. The infiltration of inflammatory cells, including neutrophils, eosinophils and macrophages to the liver were also significantly decreased. Transcriptomic analysis revealed the significant up-regulation of fatty acid metabolism genes and the identification of peroxisome proliferator-activated receptor alpha (PPAR-α) as the upstream regulator during the process of liver recovery. Aryl hydrocarbon receptor (AhR) signalling pathway that is involved primarily in the regulation of hepatic enzymes responsible for xenobiotic metabolism was also differentially up-regulated. These findings confirm prior work suggesting that schistosome egg-induced fibrogenesis process is reversible, and provide a better understanding of the mechanisms associated with regression of hepatic schistosomiasis. These results hold important implications for the future alleviation of this and other fibrotic diseases of clinical significance.

13. Development and validation of a luminescence-based, medium-throughput assay for drug screening in *Schistosoma mansoni*

CRISTIANA LALLI[1], **ALESSANDRA GUIDI[1]**, NADIA GENNARI[2], SERGIO ALTAMURA[2], ALBERTO BRESCIANI[2]*¶, GIOVINA RUBERTI[1]*¶

[1]NATIONAL RESEARCH COUNCIL, INSTITUTE OF CELL BIOLOGY AND NEUROBIOLOGY. CAMPUS A. BUZZATI-TRAVERSO, MONTEROTONDO (ROMA), ITALY [2]IRBM SCIENCE PARK SPA, POMEZIA, ITALY¶These authors contributed equally to this work

Schistosomiasis, one of the world's greatest neglected tropical diseases, is responsible for over 280,000 human deaths per annum. Praziquantel, developed in the 1970s, has high efficacy, excellent tolerability, few and transient side effects, simple administration procedures and competitive cost and it is currently the only recommended drug for treatment of human schistosomiasis. The use of a single drug to treat a population of over 200 million infected people appears particularly alarming when considering the threat of drug resistance. Quantitative, objective and validated methods for the screening of compound collections are needed for the discovery of novel anti-schistosomal drugs. We have developed and validated a luminescence-based, medium-throughput assay for the detection of schistosomula viability through quantitation of ATP, a good indicator of metabolically active cells in culture. This validated method is demonstrated to be fast, highly reliable, sensitive and automation-friendly. The optimized assay was used for the screening of a small compound library on *S. mansoni* schistosomula, showing that the proposed method is suitable for a medium-throughput semi-automated screening. Interestingly, the pilot screening identified hits previously reported to have some anti-parasitic activity, further supporting the validity of this assay for anti-helminthic drug discovery. In conclusion, this luminescence-based assay for detecting schistosomula viability was proven successful and suitable for the identification of novel compounds potentially exploitable in future schistosomiasis therapies.

14. OVA-transgenic schistosome eggs as a traceable model of host-parasite interaction

MUSAMMAT NAHAR, JEAN-PIERRE SCHEERLINCK AND **JANA HAGEN**

FACULTY OF VETERINARY AND AGRICULTURAL SCIENCES, THE UNIVERSITY OF MELBOURNE, AUSTRALIA

Schistosoma mansoni has been widely adopted as a model to examine immune responses to parasitic helminths. In particular, soluble egg antigen (SEA) of *S. mansoni* is widely used as an experimental tool for immunomodulation and the polarisation of TH2 responses. However, a major drawback in the investigation of immunomodulation by parasitic helminths *in vivo* and the identification of critical structures involved in host-parasite interaction has been the lack of a traceable model system. We have addressed this shortcoming by expression of model antigen chicken ovalbumin (OVA) in eggs of *S. mansoni*. An OVA-expression cassette was introduced into *S. mansoni* eggs by lentiviral transduction. Our data confirm transgene expression in transduced eggs on the mRNA level and OVA protein was present in egg homogenate as shown by RT-PCR and western blotting, respectively. Excitingly, transduced schistosome eggs were able to induce OVA-specific immune responses in vivo following injection into BALB/c mice. We are currently optimising OVA-expression in *S. mansoni* eggs and characterising the OVA-specific immune response induced in mice. The utilisation of OVA as a model antigen will allow, for the first time, researchers to ask specific questions on the kinetics, quality and modulation of immune responses induced by *S. mansoni* eggs *in vivo* and *in vitro*. Therefore, our transduction system will provide access to a broad range of state-of-the-art techniques and allow testing hypotheses derived from *in vitro* experiments in the much more complex scenario *in vivo*.

15. SmCB1 drug target from the *Schistosoma mansoni* blood fluke: structural basis for inhibition and activation

MARTIN HORN[1], ADÉLA JÍLKOVÁ[1], PAVLÍNA ŘEZÁČOVÁ[1], CONOR R. CAFFREY[2], AND MICHAEL MAREŠ[1].

[1]INSTITUTE OF ORGANIC CHEMISTRY AND BIOCHEMISTRY, ACADEMY OF SCIENCES OF THE CZECH REPUBLIC, PRAGUE, CZECH REPUBLIC; [2]CENTER FOR DISCOVERY AND INNOVATION IN PARASITIC DISEASES, DEPARTMENT OF PATHOLOGY, UNIVERSITY OF CALIFORNIA SAN FRANCISCO, USA.

Blood flukes of the genus *Schistosoma* cause the parasitic disease schistosomiasis that infects over 230 million people worldwide. *Schistosoma mansoni* cathepsin B1 (SmCB1) is a critical digestive peptidase that has been validated in a murine model of schistosome infection as a molecular target for therapy. We determined crystal structures of SmCB1 in complex with the peptidomimetic vinyl sulfone inhibitors to describe the binding mode. Also, we demonstrated that the severity of phenotypes induced in the parasite by vinyl sulfone inhibitors correlated with enzyme inhibition. Further structural analysis was on the activation of the SmCB1 zymogen and the processing through which the pro-peptide (an intra-molecular inhibitor) is removed. Crystal structures of three molecular forms of SmCB1 along the activation pathway were determined, namely the inactive SmCB1 zymogen, an activation intermediate, and fully processed mature SmCB1. The interaction regions within the SmCB1 pro-peptide represent potential scaffolds for the development of inhibitory mimetics. The structure-function data provide a footing for the continued rational design of anti-schistosomal inhibitors targeting SmCB1.

16. Heads or tails: Functional investigations of gene regulatory networks controlling planarian AP patterning in the model tapeworm *Hymenolepis microstoma*

FRANCESCA JARERO[1, 2], DR PETER OLSON[1]

[1]NATURAL HISTORY MUSEUM, DEPARTMENT OF LIFE SCIENCES, CROMWELL ROAD, LONDON, SW7 5BD.
[2]DEPARTMENT OF GENETICS, ENVIRONMENT AND EVOLUTION, UCL, DARWIN BUILDING, GOWER STREET, LONDON, WC1E 6BT

Tapeworms are a medically and economically important group of flatworms for which, like most parasitic animals, we remain ignorant of their basic developmental pathways. By contrast, an increasingly sophisticated and instructional model of developmental gene regulation has formed over the last decade from studies of free-living planarians. Gene regulatory networks (GRN) controlling anteroposterior (AP) patterning during planarian growth and regeneration are centred on *Beta-catenin*-dependent *Wnt* signalling, showing that continual expression of *βcat* results in posteriorisation, whereas suppression of *βcat* production via RNA interference, small molecule inhibition, or through the expression of *Wnt* antagonists results in anteriorisation of tissues. In turn, *Wnt* signalling is found to be regulated by *Hedgehog* signals, while downstream *Wnt* targets includes *Hox* and other genes. We examine quantitative and spatial expression of *Wnt* and *Hedgehog* signalling components throughout the complex life cycle of the model tapeworm *Hymenolepis microstoma*. In addition, we present functional investigations of *Wnt* repression during larval development by injecting inhibitors directly into the haemocoel of the beetle intermediate host. Initial results of these studies indicate that tapeworm AP patterning, although highly modified, is controlled by the same underlying GRN found in free-living flatworms, and is most broadly comparable to other animals during larval development, while being co-opted and modified during strobilar, adult growth.

17. Conditioning of dendritic cells for Th2 polarization by helminth-derived molecules: *exploring a role for lipids*

MARIA MM KAISAR[1], BART EVERTS[1], MARTIN GIERA[2], MARIA YAZDANBAKHSH[1]

LEIDEN UNIVERSITY MEDICAL CENTER, THE NETHERLANDS: [1]DEPT.PARASITOLOGY; [2]CENTER OF PROTEOMICS AND METABOLOMICS

Helminth-derived molecules (HDMs) are well known for their ability to induce T helper 2(Th2) polarization via functional modulation of Dendritic Cells (DCs). Yet the molecular mechanisms through which HDMs condition DCs for Th2 polarization are still incompletely understood. We here explored the role of lipids in this process by characterizing lipids present in *Schistosoma* soluble egg antigen (SEA), a potent Th2-polarizing antigen mixture, as well as by profiling lipid production by SEA conditioned DCs. To this end, human monocyte-derived DCs were stimulated with one of the following: LPS (neutral DC activator), LPS + IFNγ (Th1 inducer) or SEA (Th2 inducer). Supernatants were collected at various time-points following stimulation to examine the relative quantity of extracellular polyunsaturated fatty acids using Ultra Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS). We found that SEA contains various intermediates of arachidonic acid (AA) metabolism, including 9-Hydroxyoctadecadienoic (HoDE), 13-HoDE, 5-Hydroxyeicosatetraenoic (HETE), 8-HETE, 11-HETE and 15-HETE. Stimulation of DCs with SEA resulted in rapid disappearance of these lipids from the culture supernatant, suggesting they were internalized and/or metabolized by these cells. Interestingly, we observed that secondary to the disappearance of AA-derived lipids, the lipid Prostaglandin E2 was generated specifically in DCs conditioned by SEA. Since other studies have linked some of these AA-derived metabolites to induction of a Th2-promoting DC phenotype it is tempting to speculate that these lipids play a role in SEA-driven Th2 polarization. Additional studies are currently under way to test this hypothesis. Taken together, these findings may provide new clues about the molecular mechanisms that underpin conditioning of DCs for Th2 polarization by HDMs.

NOTES

POSTER SESSION 2

ABSTRACTS

18. *Litomosoides sigmodontis* filarial infective larvae migrate through the lungs increasing s100A9 in neutrophils

GREGORY KARADJIAN[1,2], FRÉDÉRIC FERCOQ[1], NATHALY VALLARINO-LHERMITTE[1], SABINE SPECHT[2], CORALIE MARTIN[1]

[1]UNITE MOLECULES DE COMMUNICATION ET ADAPTATION DES MICROORGANISMES (MCAM, UMR 7245), MUSEUM NATIONAL D'HISTOIRE NATURELLE, PARIS, FRANCE. [2]UMR BIOLOGIE MOLECULAIRE ET IMMUNOLOGIE PARASITAIRES (BIPAR, ANSES-ECOLE VETERINAIRE DE MAISONS ALFORT, FRANCE. [3]INSTITUTE FOR MEDICAL MICROBIOLOGY, IMMUNOLOGY & PARASITOLOGY (IMMIP), UNIVERSITY HOSPITAL OF BONN, BONN, GERMANY

After entering their host many parasitic nematodes undergo tissue migration to a greater or lesser extent. Their life cycles can involve a more or less long stage of pulmonary migration ranging from a transient passage (i.e. *Ascaris lumbricoides, Nippostrongylus brasiliensis, Strongyloides* spp.) to a permanent presence in lungs (i.e *Dictyocaulus* spp.). Here we analyzed the early stage of filarial infection in the mouse model *L. sigmodontis*. The migratory route of *L. sigmodontis* infective larvae from the skin to the pleural cavity remains scarcely delineated. We showed that the infective larvae passed through the cardiopulmonary blood system then through the lungs to finally arrive in the pleural cavity where they will moult twice. The kinetics of their arrival takes hours to days. The passage of infective larvae through the lungs induces local petechiae and cell recruitment, including neutrophils. Finally we characterized a transient inflammation defined by a recruitment of neutrophils into the lungs associated with the overexpression of s100a8 and s100a9.

19. Dendritic cell modulation by *Heligmosomoides polygyrus* excretory/secretory products

ANDREA M. KEMTER, BLAISE DAYER, RICK M. MAIZELS

INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH, UNIVERSITY OF EDINBURGH, EDINBURGH, SCOTLAND, UK

Heligmosomoides polygyrus is a gastrointestinal nematode of rodents exhibiting a wide spectrum of immunomodulatory effects, mediated in part by soluble molecules released by adult worms in vitro, the excretory/secretory products (HES). Among other properties HES is a potent inhibitor of dendritic cell (DC) activation by Toll-like receptor (TLR) ligands. Following this finding, we aim to identify the modulatory molecule and its mechanism of action. Here, we show that the modulatory molecule is heat labile, indicating a modulatory protein. Therefore, HES was fractionated by gel filtration and anion exchange chromatography and the active fractions analysed by Mass Spectrometry. Identification of the proteins enriched in these fractions resulted in a shortlist of six candidates for further study. Furthermore, the modulatory molecule does not act via MyD88 or TRIF, since there was no difference in inhibition of activation of bone marrow-derived DCs (BMDCs) from $MyD88^{-/-}TRIF^{-/-}$ mice and wild type mice. Treatment of BMDCs with an inhibitor of PI3K did not protect from the effects of HES, just as treatment of BMDCs with a Syk inhibitor or blocking of Dectin-1 and 2 by antibodies did not alter DC inhibition. The phosphorylation levels of JNK1/2 and p38 were equal in LPS and LPS+HES treated BMDCs, indicating no effect of HES on the activation of these MAP kinases. Finally, we could show that while phosphorylation levels of ERK1/2 in LPS+HES treated DCs were significantly elevated 20 to 30 minutes after stimulation, treatment of DCs with U0126, a MEK1/2 inhibitor, did not abolish the inhibitory effect of HES. In conclusion, this work narrows down the list of potential DC modulators in HES and excludes a number of signalling pathways with important roles in DC activation as targets of DC inhibition by HES.

20. Various life stages of *Schistosoma mansoni* release extracellular vesicles

MARIJE KUIPERS[1], ALWIN VAN DER HAM[1], ARIFA OZIR-FAZALALIKHAN[1], MARCA WAUBEN[2], RON HOKKE[1], ESTHER NOLTE-'T HOEN'[2], HERMELIJN SMITS'[1]

[1]*DEPT. OF PARASITOLOGY, LEIDEN UNIVERSITY MEDICAL CENTER (LUMC), LEIDEN, THE NETHERLANDS* [2]*DEPT. OF BIOCHEMISTRY & CELL BIOLOGY, UTRECHT UNIVERSITY, UTRECHT, THE NETHERLANDS *JOINT SUPERVISION*

The parasite *Schistosoma mansoni* ensures its own survival by regulating the immune system of the mammalian host. Molecules released by *S. mansoni* are known to modulate dendritic cells (DCs) to skew the development of naïve T cells towards T helper 2 and/or regulatory T cells. In addition to soluble factors, some parasites also have the ability to release nano-sized vesicles called extracellular vesicles (EVs). We investigated whether *S. mansoni* eggs, schistosomulae and schistosome adult worms release EVs. Indeed, EVs could be isolated from culture supernatants of all life stages following high speed centrifugation and density gradient ultracentrifugation. The quantity of released EV was determined by high-resolution flow cytometry and differed substantially between the life stages. Western blot detection of parasite-specific glycans showed dissimilarities in protein content of the EVs released by the different life stages. In addition, crude EV preparations were added to human monocyte-derived (mo-)DCs to investigate their immunomodulatory properties. Mo-DCs incubated with schistosomulae-derived EVs expressed both pro- and anti-inflammatory cytokines, but did not influence the T cell polarizing capacity of mo-DCs substantially. DCs pulsed with egg-derived EVs increased interleukin (IL)-4 expressing T cells, suggesting the promotion of T helper 2 responses. In contrast, DCs exposed to adult worm-derived EVs showed a small dose-dependent capacity to increase T cell IL-10 production. In conclusion, we demonstrate for the first time that different life stages of *S. mansoni* release EVs which may have unique features in terms of molecular composition and modulation of DC function.

21. Inter- and intra-specific analyses of the effector complement in potato cyst nematodes

DOMINIK R LAETSCH[1,2,3], SEBASTIAN EVES-VAN DEN AKKER[4], VIVIAN C BLOK[3], PETER J COCK[2], JOHN T JONES[3], THE GLOBODERA GENOME SEQUENCING CONSORTIUM, MARK L BLAXTER[1]

[1]*INSTITUTE OF EVOLUTIONARY BIOLOGY, UNIVERSITY OF EDINBURGH, UK* [2]*INFORMATION AND COMPUTATIONAL SCIENCE GROUP, THE JAMES HUTTON INSTITUTE, UK.* [3]*CELL AND MOLECULAR SCIENCE GROUP, THE JAMES HUTTON INSTITUTE, UK.* [4]*COLLEGE OF LIFE SCIENCES, UNIVERSITY OF DUNDEE, UK*

Potato cyst nematodes (PCNs) are sedentary endoparasites of *Solanum tuberosum* (potato) crops and are estimated to cause annual crop losses of £50 million in the UK. The biology of their life cycle, which makes control of infestations through crop rotation impractical, paired with recent tighter legislation regarding the use of nematicides, makes an understanding of the host-parasite interaction imperative for sustainable and competitive potato production. The *Globodera* genome project has produced genome and transcriptome data for different species and pathotypes of PCNs, which are now being investigated to understand the basis of host and parasite specificities. In addition to the published *G. pallida* reference strain Lindley Pa2/3, an assembly was produced for *G. rostochiensis* strain Ro1. Structural and functional annotation of the *G. rostochiensis* genome was carried out using a custom gene-finding pipeline followed by a collaborative annotation jamboree held at the University of Edinburgh. During this event 11.5% of the predicted gene-models were manually checked and subsequently used as training set in a second round of annotation. Genetic variation between pathotypes of *G. pallida*, which display virulence against different host genotypes, was explored using whole genome sequencing. Here, we present analyses of the inter- and intra-specific genomic variation within PCNs, with particular focus on the "effectorome", proteins suspected of being secreted from the parasite into the host in order to manipulate the host cell and establish the permanent feeding site. The effectors are also putative factors recognized by the host in nonspecific or specific (resistance) incompatible responses.

22. Characterization of immune response genes in the parasitic nematode *Brugia malayi*

SILVIA LIBRO, JEREMY FOSTER, BARTON SLATKO

NEW ENGLAND BIOLABS, USA

The filarial nematode *Brugia malayi* is one of the causative agents of lymphatic filariasis, a neglected tropical that affects 120 million people worldwide. Due to the limited effectiveness of the drugs available and the absence of a vaccine, extensive efforts have been made in order to understand the basic biology of *B. malayi* and its symbiotic association with *Wolbachia* endobacteria. At present, little is known about the mechanisms underlying *Brugia*`s immune system. This overlooked area of research is of high interest, as and it can help to advance our understanding on the interaction between *Brugia* and *Wolbachia*. It has been suggested that in order to reside within the nematode`s tissue, *Wolbachia* may evade the host immune system. Therefore, understanding what mechanisms are involved in *Brugia* immunity can help in identifying targets for the development of new drugs and vaccines. In particular, small regulatory RNAs are potentially good candidate targets, as they are likely to be specific, decreasing the chances of off-target interactions. Several studies highlighted the key role of small regulatory RNAs during anti-viral immunity in *Caenorhabditis elegans* and suggest that *Brugia*, that possesses the genes encoding several components of the RNAi machinery, may use similar mechanisms to avoid parasites. In order to characterize the main genetic pathways involved in *B. malayi* immunity, we exposed adult male worms to four different immune elicitors: Gram + and Gram- bacterial lysates (*Escherichia coli* and *Bacillus sp*), dsRNA and dsDNA. We performed transcriptome and small RNA sequencing of worms exposed to each immune elicitors for two different exposure times (24 hours and 3 days). Differential gene expression analysis of untreated and immune challenged worms were used to characterize gene expression patterns associated to each type of immune insult and selected candidate immune genes were further validated using quantitative RT-PCR.

23. Unravelling the filarial larvae migrations: lessons from *Litomosoides sigmodontis* mouse model

GREGORY KARADJIAN[1], FRÉDÉRIC FERCOQ[1], NATHALY VALLARINO-LHERMITTE[1], SABINE SPECHT[2], WITOLD W. KILARSKI[3,4], MELODY SWARTZ[3,4], **CORALIE MARTIN**[1]

[1]UNITE MOLECULES DE COMMUNICATION ET ADAPTATION DES MICROORGANISMES (MCAM, UMR 7245), MUSEUM NATIONAL D'HISTOIRE NATURELLE, PARIS, FRANCE. [2]INSTITUTE FOR MEDICAL MICROBIOLOGY, IMMUNOLOGY & PARASITOLOGY (IMMIP), UNIVERSITY HOSPITAL OF BONN, BONN, GERMANY. [3]INSTITUTE OF BIOENGINEERING AND SWISS INSTITUTE FOR EXPERIMENTAL CANCER RESEARCH (ISREC), ECOLE POLYTECHNIQUE FÉDÉRALE DE LAUSANNE (EPFL), LAUSANNE, SWITZERLAND. [4]INSTITUTE FOR MOLECULAR ENGINEERING, UNIVERSITY OF CHICAGO, CHICAGO, ILLINOIS

After entering their host many parasitic nematodes undergo tissue migration to a greater or lesser extent. Here we analyzed the early stage of filarial infection in the mouse model *L. sigmodontis*. The migratory route of infective larvae from the skin to the pleural cavity remains scarcely delineated. We showed absolute requirement of functional lymphatic for invasion of filariae into the host that occurred at the level of collecting vessels within minutes after inoculation. Worms entered the lymphatic collecting vessel by physically disrupting the lymphatic basement membrane. Within lymphatics, filaria were guided by the system of valves and rapidly migrated to subcapsular sinus of draining lymph node. From there the infective larvae reached the thoracic duct and passed through the cardiopulmonary blood system then through the lungs to finally arrive in the pleural cavity where they will moult twice. We also characterized a transient inflammation defined by a recruitment of neutrophils into the lungs associated with the overexpression of s100a8 and s100a9.

24. Characterization of glucose transporter genes from fox tapeworm *Echinococcus multilocularis*

TAKUYA KASHIIDE[1], SHINGO KIKUTA[2], MISAKI YAMAGUCHI[1], TORU KATO[1], TAKAO IRIE[3], HIROKAZU KOUGUCHI[3], KINPEI YAGI[3], **JUN MATSUMOTO[1]**

[1] NIHON UNIVERSITY COLLEGE OF BIORESOURCE SCIENCES, KANAGAWA, JAPAN. [2] TOKYO UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, TOKYO, JAPAN. [3] HOKKAIDO INSTITUTE OF PUBLIC HEALTH, HOKKAIDO, JAPAN

Alveolar echinococcosis (AE) is a life-threatening zoonotic parasitosis caused by larval stage of fox tapeworm, *Echinococcus multilocularis* (Em). Em is distributed widely in northern hemisphere, causing serious health problems in various animals and humans. Em, like other cestodes, lacks digestive tract and absorbs essential nutrients including glucose across syncytial tegument on its external body surface. Therefore, Em is supposed to use membrane transporters on its surface to gain glucose as previously reported for a closely-related species, *Taenia solium* (Rodríguez-Contreras, 1998). Based on this speculation, we tried to clone and characterize glucose transporter homologues from Em. As a result, we obtained full-length sequence of putative 2 glucose transporter genes from Em, designated as EmGLUT1 and EmGLUT2. *In silico* analysis revealed that these were both facilitated diffusion transporter classified in solute carrier family 2. Transcriptional expression of each transporter was confirmed by realtime RT-PCR both in larval and adult stages of the parasite. Functional expression analysis using *Xenopus* oocytes demonstrated clear uptake of 2-deoxy-D-glucose (2-DG) by EmGLUT1, but not by EmGLUT2 in this experimental system. 2-DG uptake of EmGLUT1 was significantly inhibited by cytochalasin B, a known inhibitor for glucose transporters. Kinetic values of EmGLUT1 for 2-DG were calculated as follows: Km = 6.5±1.8mM, Vmax = 41.8±5.8mM, showing that EmGLUT1 has relatively high activity in glucose transport. Further analyses using the *Xenopus*-oocyte system revealed that 2-DG uptake of EmGLUT1 did not depend on presence/concentration of Na^+ nor H^+, respectively. Based on above-mentioned findings, we have concluded that EmGLUT1 is a simple facilitated glucose transporter and possibly plays an important role in glucose uptake by Em throughout its life cycle.

25. Comparison of ivermectin and moxidectin resistance profiles in the nematode *Caenorhabditis elegans*

CÉCILE MÉNEZ, DALIA KANSOH, MÉLANIE ALBERICH, ANNE LESPINE

INRA UMR1331, UNIVERSITÉ DE TOULOUSE, INP, TOXALIM, F-31027 TOULOUSE, FRANCE

The anthelmintic macrocyclic lactones ivermectin (IVM) and moxidectin (MOX) are the most widely administrated drugs to treat nematode infections. Their widespread use has led to the emergence of resistance to these two drugs. Although they have identical modes of action, differences in terms of pharmacokinetics, spectrum and toxicity have been reported between MOX and IVM. Moreover, they do not have the same ability to select for drug resistance. The objective of this study was to investigate and compare the mechanisms underlying the development of MOX and IVM resistance in the nematode model *Caenorhabditis elegans*. For this purpose, we have evaluated the capacity of *C. elegans* to become resistant to MOX through stepwise exposure. Sensitivity to IVM and MOX of the MOX-selected worms was determined and compared with wild-type N2B and IVM-resistant strains with a larval development assay. *C. elegans* developed resistance to MOX but, after 40 weeks, worms were able to grow only on 5.7 nM of MOX, which was twice lower than the IVM concentration on which IVM-selected worms were able to survive. While, in wild-type strain, IVM was more effective than MOX (IC50 of 1.55±0.09 and 2.21±0.41 nM, for IVM and MOX, respectively), MOX had higher efficiency in both IVM-selected (12.57±1.34 and 2.82±0.20 nM, for IVM and MOX, respectively) and MOX-selected (12.76±0.87 and 4.12±0.34 nM, for IVM and MOX, respectively) strains. The effect of co-administration of MDR-reversing agents on drug sensitivity was evaluated in the different strains and discussed regarding the transcriptional profiles of *C. elegans* detoxification system. We have, for the first time, selected and characterized a MOX-resistant *C. elegans* strain. We show that there are significant differences in the development of resistance to IVM and MOX in *C. elegans*. This may help to understand the differences in IVM and MOX resistance development observed in parasitic nematodes.

26. T1/ST2-EGF-R co-expression in T_H2 cells is essential for IL-13-induced worm expulsion

CARLOS MINUTTI, NATALIE BLAIR, RICK MAIZELS AND DIETMAR ZAISS.

CENTRE FOR IMMUNITY, INFECTION AND EVOLUTION, AND THE INSTITUTE FOR IMMUNOLOGY AND INFECTION RESEARCH, SCHOOL OF BIOLOGICAL SCIENCES, UNIVERSITY OF EDINBURGH, EDINBURGH, EH9 3FL, UK.

For a number of worm infections, it has been shown that the protective capability of the response is based, to a major part, on the activity of CD4 T-cells that have differentiated into T_H2 cells. Upon infection, these cells are primed in an antigen-dependent manner by dendritic cells in the draining lymph nodes (dLN), proliferate and differentiate, and then migrate to the site of inflammation where they release large quantities of effector cytokines, such as Interleukin (IL)-13. Similar to other types of leukocytes (mast cells or basophils) also T_H2 cells express the IL-33 receptor T1/ST2. IL-33 is considered to be an "alarmin" that is released upon worm-induced necrosis of cells in the mucosal barrier. Recently, it has been shown that in mast cells T1/ST2 can efficiently mediate an IL-33-induced signal only, if it interacts with the activated receptor tyrosine kinase (RTK) c-kit. We have found that another RTK, the epidermal growth factor receptor (EGF-R) is expressed by activated T_H2 cells, and that T1/ST2 can interact with the EGF-R. We have also observed that *in vitro* differentiated T_H2 cells that don't express EGF-R (isolated from CD4crexEGF-$R^{fl/fl}$ mice) do not induce IL-13 upon IL-33 exposure. Furthermore, CD4crexEGF-$R^{fl/fl}$ mice express less *Il13* but not *Il4* nor *Il5* mRNA in the duodenum in response to worm infection. Consequently, CD4crexEGF-$R^{fl/fl}$ mice are more susceptible to *Heligmosomoides polygyrus* and *Nippostrongylus brasiliensis* infection, as determined by increased worm burden and egg-output at different time points after infection. We thereby hypothesize that T_H2 cells that are primed in an antigen-dependent manner by dendritic cells in the dLN, up-regulate both the EGF-R and T1/ST2. This co-expression allows activated T_H2 cells then to sense the presence of IL-33 and to secrete cytokines, including IL-13, in an antigen independent way as they migrate to the site of inflammation.

27. Evaluation of a protease-dependent prodrug in the potential treatment of parasitic helminth infections

EYITAYO OLUWADARE, YAO DING, AGNES TURNBULL, EVA MALONE, DAVID MINCHER, LORNA PROUDFOOT

SCHOOL OF LIFE, SPORT AND SOCIAL SCIENCES, EDINBURGH NAPIER UNIVERSITY, EDINBURGH, UK

Globally, billions of livestock are infected with helminth parasites, accompanied by significant morbidity and mortality. Although anthelmintic drugs are available, the development of resistance poses a threat to their continuous use requiring the need to develop more effective and better targeted anthelmintics. Proteases of *Caenorhabditis elegans* (as a parasite model), *Haemonchus contortus* and *Teladorsagia circumcincta* were investigated as novel targets for the delivery of anthelmintic drugs. We propose that the action of a specific protease on prodrug-delivered anti-parasite drugs could expose this new 'Achilles heel'. We employed the use of a novel synthetic molecular probe to detect the presence of a specific protease in the afore-mentioned helminths. The probe consists of a rhodamine fluorophore and an asparagine-containing peptide attached to a quencher. Cleavage by the protease separates the quencher from the probe triggering fluorescence. Relative fluorescence intensity was used as a measure of protease activity detected in helminth lysates exposed to 10µM probe after 2 hours incubation (20^{o}C). All helminth lysates tested exhibited fluorescence under the above conditions. Subsequently, using differential solubilization techniques, we suggest that the *C.elegans* protease is membrane-bound following observation of its activity in somatic fractions exposed to the probe under the same conditions as described above. The presence of the protease in helminth membrane fractions may impact on enhanced efficiency of drug delivery. Furthermore, prodrug delivery could take us a step further in being able to repurpose existing drugs as the high cost of developing new drugs hinders progress in this area.

28. Temporal transcriptional profiling of livers from *Schistosoma mansoni*-infected mice

CHRISTIAN OWUSU[1] ALEXANDER PHYTHIAN-ADAMS[2], ANDREW MACDONALD[2] AND MATTHEW BERRIMAN[1]

[1]*PARASITE GENOMICS, WELLCOME TRUST SANGER INSTITUTE, WELLCOME TRUST GENOME CAMPUS, HINXTON, UK*
[2]*MANCHESTER COLLABORATIVE CENTRE FOR INFLAMMATION RESEARCH, THE UNIVERSITY OF MANCHESTER, MANCHESTER, UK*

The immune response to *Schistosoma mansoni* infection has been well-characterised in experimental mouse models. Early in the infection, migrating larvae trigger a mixed type 1 (Th1)and 2 (Th2) inflammatory response. This changes to a dominant Th2 response after mated worm pairs begin producing eggs around week 5 post infection. The Th2 response peaks around week 8, after which the response gradually weakens, corresponding to the chronic phase of the disease. While much is known about the major effector cells involved in immunopathological changes in the liver, we are still largely ignorant of the exact molecular triggers leading to the observed pathology. In this study, we profile changes in gene expression in the livers of infected mice across 6 time points. The time points were chosen to reflect the progression of the immune response, beginning prior to the onset of egg deposition, and extending all the way through to the chronic stage of the disease. We identify putative mRNA targets of differentially expressed miRNAs through the integrated analysis of miRNA and mRNA expression profiles. In addition, we show evidence for changes in expression of receptors and ligands involved in antigen presentation and T cell activation. These data provide clues regarding possible mechanisms underlying T cell hyporesponsiveness seen during the later time points. We also show temporal changes in the expression of genes involved in extracellular matrix organisation and corresponding to changes in liver tissue architecture as the granulomatous response becomes fibrotic. Finally, cluster analysis reveals several genes which share similar patterns of expression. In many clusters, genes grouped together either act on the same pathway or identify effector cell types showing coordinated recruitment to the liver across the different time points.

29. Embryonic muscle development in redia and cercaria of *Fascioloides magna* (Trematoda: Digenea)

JAN PANKRÁC[1], MARTIN KAŠNÝ[1,2], JANA BULANTOVÁ[1], PETR HORÁK[1]

[1]DEPARTMENT OF PARASITOLOGY, FACULTY OF SCIENCE, CHARLES UNIVERSITY IN PRAGUE, VINICNA 7, 128 00 PRAGUE, CZECH REPUBLIC. . [2]DEPARTMENT OF BOTANY AND ZOOLOGY, FACULTY OF SCIENCE, MASARYK UNIVERSITY BRNO, KAMENICE 753/5 625 00 BRNO BOHUNICE, CZECH REPUBLIC.

Fascioloides magna (Trematoda: Digenea) is a parasite having a two-host life cycle and causing severe destruction of the liver tissue of large mammals. The intermediate hosts are freshwater snails of the family Lymnaeidae. Development in the intermediate host includes asexual reproduction of two morphologically different developmental stages - sporocysts and rediae, and final production of the third life stage - cercariae. Digenean developmental stages may substantially differ in their body plans. Even the stages produced within the snail intermediate hosts (sporocysts, rediae and cercariae) show differences in morphogenesis, including formation of body musculature. In order to disclose the differences, we compared myogenesis of cercariae and rediae. Daughter rediae and cercariae of different age/developmental phase were isolated from living larvae of the previous generation, and structures containing F-actin were labelled with phalloidin-FITC. Myogenesis was observed from an early embryo (also called germinal ball) to the fully developed individuals. The most prominent structures containing F-actin recorded during embryonic development of rediae and cercariae are muscle fibers of the body wall. In both developmental stages delay in morphogenesis of longitudinal muscle fibers in comparison to circular muscle fibers was recognized. Except this similarity, the process of formation of body wall musculature seems to be substantially different between cercariae and rediae since the early embryos. For instance, embryonic form of longitudinal muscle fibers is replaced by definitive longitudinal muscle fibers during the development of cercariae. No such process was observed in rediae. Pronounced differences were also recorded during morphogenesis of circular muscle fibers. Finally, formation of diagonal muscle fibers is restricted to cercariae. Our data suggest that myogenesis in rediae and cercariae of *F. magna* is considerably different. More research needs to be done to characterize differences in development in case of other body systems.

30. Transcriptomic profiling of skin dendritic cells in the immune response to *Nippostrongylus brasiliensis*

LISA CONNOR[1], ALEX SMITH[1], SHIAU CHOOT TANG[1], DAVID ECCLES[1], GRAHAM LE GROS[1], MELANIE McCONNELL[2], **FRANCA RONCHESE**[1]

[1]*MALAGHAN INSTITUTE OF MEDICAL RESEARCH, WELLINGTON, NEW ZEALAND* [2]*VICTORIA UNIVERSITY, WELLINGTON, NEW ZEALAND*

Nematode parasites entering the body via the skin come into contact with a complex population of dendritic cells that comprises at least four different subsets with distinct phenotypes and properties. Experiments using gene KO or diphtheria toxin receptor transgenic mice to eliminate specific dendritic cell subsets in vivo have provided information on which populations are necessary or dispensable for the induction of Th2 immune responses. In addition, we have shown that purified, total skin-derived dendritic cells from the lymph node of mice exposed to non-viable *N. brasiliensis* larvae are able to initiate Th2 immune response upon transfer into naive mice, suggesting that dendritic cells can also provide a sufficient set of signals for Th2 induction. However, it is currently unclear whether the ability to induce Th2 responses requires the cooperation of multiple dendritic cell subsets, or whether one specific population might be sufficient for Th2 induction. To better define the properties of dendritic cell populations in mice exposed to *N brasiliensis*, we are using RNAseq to characterize the transcriptomic profile of dendritic cells from naïve mice or mice injected with non-viable *N brasiliensis* larvae. Initial analyses suggest a shared set of transcriptomic changes that involve multiple dendritic cell populations in the lymph node. In addition to these shared changes, dendritic cell subset-specific changes are also apparent, which affect more profoundly some subsets compared to others. Further characterization of these sets of differentially expressed genes is ongoing and will provide valuable information on the signalling pathways that are activated in dendritic cells upon exposure to *N brasiliensis*, and that may be key to the initiation of Th2 immune responses.

31. Identification of a functional TGFβ mimic secreted by *Heligmosomoides polygyrus*.

DANIELLE SMYTH, HENRY MCSORLEY, CHRIS JOHNSTON, ANNA KILDEMOES, YVONNE HARCUS, JAMES HEWITSON, RICK MAIZELS

INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH AND CENTRE FOR INFLAMMATION RESEARCH, UNIVERSITY OF EDINBURGH, UK

It has been previously shown that the helminth parasite *H. polygyrus*, and its excretory-secretory antigens (HES), can directly induce Foxp3-expressing regulatory T cells from naïve murine Foxp3-negative $CD4^+$ T cells. Furthermore, data from an in vitro TGFβ bioassay (cell line MFB-11) indicates that HES contains a mimic which signals through the mammalian TGFβ receptor. To identify the TGFβ mimic, HES was fractionated by both ion exchange and gel filtration chromatography, with all fractions being tested for TGFβ-like activity using the in vitro TGFβ bioassay. The proteins present in each fraction were identified using characterisation by mass spectrometry (Orbitrap), matching hits to a protein database derived from deep transcriptomic sequencing. Proteins common in positive fractions from both chromatography techniques were cloned and transfected into mammalian HEK293T cells and recombinant molecules were tested using the TGFβ bioassay. From this screen of candidates, we identified a novel functional TGFβ mimic (TGM). Hp-TGM shares no homology to mammalian TGFβ (and is not a member of the TGFβ superfamily), is acid stable (to pH 3), remains fully active after 28 days at 37°C, and even retains residual activity after 5 minutes at 100°C. Hp-TGM induces Foxp3 expression in murine T cells at similar concentrations (~1 ng/ml) as mammalian TGFβ, and induced Foxp3+ cells are functionally suppressive in vitro. An inhibitor of the TGFβR signalling kinase ALK5 (SB431542) completely ablates Hp-TGM activity, while neutralizing antibody against mammalian TGFβ has no effect. Remarkably, Hp-TGM can induce FoxP3 expression and suppress proliferation of human peripheral blood CD4+ T cells at similar concentrations to mammalian TGFβ, indicating that Hp-TGM could translate directly into human disease. We are now applying Hp148 to several mouse models of inflammation (asthma, colitis, autoimmunity and transplant tolerance) to assess its therapeutic potential and/or its involvement in immune modulation through the TGFβ pathway.

32. Proteomic analysis of the excretory-secretory products of adult *Ascaris suum*

TAO WANG, XIAOBING GU, GUANGYOU YANG

DEPARTMENT OF PARASITOLOGY, COLLEGE OF VETERINARY MEDICINE, SICHUAN AGRICULTURAL UNIVERSITY, CHINA

Ascaris lumbricodis and *Ascaris suum* are widespread parasites of humans and pigs, respectively. Recent prevalence data suggested approximately 1.2 billion people are infected mainly in the tropics and subtropics. After the hepato-pulmonary migration, the adult females and males establish and develop in the small intestine. The adult worm can reside within host gut up to 1 year. Strategies employed by *Ascaris* to establish infections are poorly understood. The excretory-secretory (ES) products are the proteins presented at the parasite-host interface. These molecules are likely to play critical roles in the induction and development of protective immune and other host responses. The aim of this study was to identify the ES products of adult *Ascaris suum* by liquid chromatography-tandem mass spectrometry. 20 adult female and male *A. suum* were cultured separately *in vitro* for 3 days. ES products were collected and concentrated for SDS-PAGE separation. Using LC-MS/MS, 387 different proteins were detected in the ES products of adult stage *A. suum*, 301 proteins in female worm and 182 proteins in male worm. Although several proteins (n=96) were shared between ES products of female and male, the protein composition of ES products was gender-specific. Interestingly, comparison of the adult ES products and the intestinal L4 larvae ES identification list (previous study) showed much more similar than that of other larval stages. This could indicate that there is a strong niche dependence of ES products content, probably reflecting functional roles of ES proteins in modulating local host responses. In conclusion, this proteomic analysis extends our knowledge of biology of *A. suum* and provides important information on the host-parasite interaction and the biology of this parasite.

33. Functional analysis of Bm-SPN-2, the major secreted product of *Brugia malayi* microfilariae

XUHANG WU, HENRY J. MCSORLEY, YVONNE HARCUS, RICK M. MAIZELS

INSTITUTE OF IMMUNOLOGY AND INFECTION RESEARCH, UNIVERSITY OF EDINBURGH

Some parasites have evolved to release products that inhibit host defence mechanisms such as enzymes in the mammalian host in order to promote and sustain their survival within the host. The human filarial nematode *Brugia malayi* produces larval microfilariae, which circulate in the blood stream. Their most abundant secreted product is a serine protease inhibitor Bm-SPN-2. Serine protease inhibitors (Serpins) are reported to be involved in how the nematodes avoid host immune defences. Bm-SPN-2 protein was found to specifically inhibit the enzymatic activity of human neutrophil elastase and cathepsin G in a dose-dependent manner. Recently, these enzymes have been linked to the activation of a major innate cytokine IL-33, which is stored as a full-length protein in the cell nucleus, and released as the C-terminal domain upon stimulation. Thus, Lefrançais et al. demonstrated that full-length human IL-$33_{1–270}$ is cleaved into mature forms IL-$33_{95–270}$, IL-$33_{99–270}$, and IL-$33_{109–270}$ by cathepsin G and elastase. In this study, we aim at detecting whether Bm-SPN-2 blocks human and mouse full-length IL-33 cleavage by inhibiting cathepsin G and elastase. Soluble Bm-SPN-2 protein was readily expressed by in *E.coli* and purified by ATKA purification. Soluble full-length mouse and human IL-33 were produced in transfected HEK 293T cells, following mutation of the nuclear binding motif which retains IL-33 in the nucleus, suggesting IL-33 DNA binding site should be removed. Western blot analysis indicates that the full-length IL-33 cleavage by Cathepsin G is reduced with increasing concentration of Bm-SPN-2, suggesting Bm-SPN-2 is promising inhibitor for Cathepsin G to block IL-33 cleavage. Studies are also underway with mouse models of IL-33 release in vivo following allergen exposure in the airways.

34. Engineering of plants for the expression of helminth glycoproteins with their native N-glycan structures

RUUD H.P. WILBERS[1], LOTTE B. WESTERHOF[1], BART EVERTS[2], KIM VAN NOORT[1], DEBBIE R. VAN RAAIJ[1], DIEU-LINH NGUYEN[2], MARIA YAZDANBAKHSH[2], CORNELIS H. HOKKE[2] AND ARJEN SCHOTS[1]

[1]*LABORATORY OF NEMATOLOGY, WAGENINGEN UNIVERSITY, WAGENINGEN, THE NETHERLANDS*
[2]*DEPARTMENT OF PARASITOLOGY, LEIDEN UNIVERSITY MEDICAL CENTER, LEIDEN, THE NETHERLANDS*

Schistosoma mansoni is a parasitic trematode that, like other helminths, secretes immunomodulatory proteins. These secreted proteins are main topics of research as they are possible vaccine candidates or may have therapeutic potential to treat inflammatory disorders. Many helminth secretory proteins carry complex N-glycans, but the exact role of these N-glycans on immunomodulatory properties remains to be elucidated. As the purification of a single glycoprotein from S. mansoni is inefficient and unsustainable, a platform is required that enables production of such glycoproteins. Here we show that S. mansoni-derived glycoproteins can be efficiently produced in plants. Furthermore, we have engineered the plant glycosylation machinery to synthesise N-glycans carrying structures like Lewis X or LDNF. Altogether, our results demonstrate that plants are an excellent platform for the expression of helminth glycoproteins with their native N-glycans. This opens up a new field of research and might lead to the identification of novel therapeutic targets.

NOTES

DELEGATE LIST

LIST OF PARTICIPANTS

Aziz	**Aboobaker**	University of Oxford	Aziz.Aboobaker@zoo.ox.ac.uk
Cei	**Abreu-Goodger**	Langebio - Cinvestav	cei@langebio.cinvestav.mx
Allison	**Aldridge**	Dublin City University	allison.aldridge2@mail.dcu.ie
Allison	**Bancroft**	University of Manchester	allison.j.bancroft@manchester.ac.uk
Robin	**Beech**	McGill University	robin.beech@mcgill.ca
Nicola	**Beesley**	University of Liverpool	nbeesley@liv.ac.uk
Rita	**Berkachy**	Imperial College London	r.berkachy14@imperial.ac.uk
Matthew	**Berriman**	Wellcome Trust Sanger Institute	mb4@sanger.ac.uk
Alexandra	**Blanchard**	National Institute of Agronomical Research	alexandra.blanchard-letort@tours.inra.fr
Bruce	**Bolt**	EMBL European Bioinformatics Institute	bruce.bolt@ebi.ac.uk
Amy	**Buck**	University of Edinburgh	a.buck@ed.ac.uk
Alejandro	**Cabezas-Cruz**	Université Lille, Institut Pasteur de Lille	cabezasalejandrocruz@gmail.com
Claude	**Charvet**	National Institute of Agronomical Research	claude.charvet@tours.inra.fr
Guofeng	**Cheng**	Chinese Academy of Agricultural Sciences	chenggfeng@shvri.ac.cn
Franklin Wang Ngai	**Chow**	University of Edinburgh	cwn5810@gmail.com
Julie	**Claycomb**	University of Toronto	julie.claycomb@utoronto.ca
Gillian	**Coakley**	University of Edinburgh	g.coakley@sms.ed.ac.uk
James	**Collins**	University of Texas Southwestern Medical Center	JamesJ.Collins@utsouthwestern.edu
Thomas	**Crellen**	Imperial College London/ Wellcome Trust Sanger Institute	tc13@sanger.ac.uk
Richard	**Davis**	University of Colorado School of Medicine	richard.davis@ucdenver.edu
Eileen	**Devaney**	University of Glasgow	eileen.devaney@glasgow.ac.uk
Janina	**Demeler**	Freie Universität	j.demeler@fu-berlin.de
Benjamin	**Dewals**	University of Liege	bgdewals@ulg.ac.be
Katarzyna	**Donskow-Łysoniewska**	University Of Warsaw	zuzia@biol.uw.edu.pl
Annette	**Dougall**	University of Liege	Annette.Dougall@ulg.ac.be
Thomas	**Duguet**	McGill University	thomas.duguet@mail.mcgill.ca
Maria Adelaida	**Duque Correa**	The Wellcome Trust Sanger Institute	md19@sanger.ac.uk
Lewis	**Entwistle**	The Francis Crick Institute	lewis.entwistle@crick.ac.uk
Jonathan	**Ewbank**	Centre d'immunologie de Marseille-luminy	ewbank@ciml.univ-mrs.fr
Marie-Anne	**Félix**	Institut de Biologie de l'Ecole Normale Superieure, CNRS-ENS-Inserm	felix@biologie.ens.fr
Conor	**Finlay**	Trinity College Dublin	finlaycm@tcd.ie

Peter	**Fischer**	Washington University School of Medicine in St. Louis	Pufische@dom.wustl.edu
Gisela Raquel	**Franchini**	Instituto de Investigaciones Bioquimicas de La Plata (CONICET-UNLP)	gfranchini@conicet.gov.ar
Geoffrey	**Gobert**	QIMR Berghofer Medical Research Institute	geoffG@qimr.edu.au
Niki	**Gounaris**	Imperial College London	k.gounaris@imperial.ac.uk
Richard	**Grencis**	University of Manchester	richard.k.grencis@manchester.ac.uk
Alessandra	**Guidi**	CNR (National Research Council)	alessandra.guidi@ibcn.cnr.it
Jana	**Hagen**	The University of Melbourne	hagen.j@unimelb.edu.au
Elissa	**Hallem**	University of California Los Angeles	ehallem@microbio.ucla.edu
William	**Harnett**	University of Strathclyde	w.harnett@strath.ac.uk
Wiebke	**Hartmann**	Bernhard Nocht Institute for Tropical Medicine	hartmann@bni-hamburg.de
DeBroski	**Herbert**	University of California San Francisco	debroski.herbert@ucsf.edu
Cornelis	**Hokke**	Leiden University Medical Center	c.h.hokke@lumc.nl
Sung-Jong	**Hong**	Chung-Ang University	hongsj@cau.ac.kr
Martin	**Horn**	Academy of Sciences of the Czech Republic	horn@uochb.cas.cz
William	**Horsnell**	University of Cape Town	wghorsnell@gmail.com
Francesca	**Jerero**	Natural History Museum, London	f.jarero@nhm.ac.uk
Maria	**Kaiser**	Leiden University Medical Center	M.Kaisar@lumc.nl
Gregory	**Karadjian**	Museum national d'Histoire naturelle - ANSES	karadjian@mnhn.fr
Andrea	**Kemter**	University of Edinburgh	a.m.kemter@sms.ed.ac.uk
Malcolm	**Kennedy**	University of Glasgow	malcolm.kennedy@glasgow.ac.uk
Jürgen	**Krücken**	Freie Universität Berlin	juergen.kruecken@fu-berlin.de
Marije	**Kuipers**	Leiden University Medical Center	m.e.kuipers@lumc.nl
Dominik R	**Laetsch**	University of Edinburgh	dominik.laetsch@gmail.com
Graham	**Le Gros**	Malaghan Institute of Medical Research	glegros@malaghan.org.nz
Silvia	**Libro**	New England Biolabs	libro@neb.com
Alex	**Loukas**	James Cook University	alex.loukas@jcu.edu.au
Sara	**Lustigman**	Lindsley F. Kimball Research Institute	slustigman@nybloodcenter.org
Andrew	**MacDonald**	University of Manchester	andrew.macdonald@manchester.ac.uk
Rick	**Maizels**	University of Edinburgh	Rick.maizels@ed.ac.uk
Coralie	**Martin**	MNHN	cmartin@mnhn.fr
Richard	**Martin**	Iowa State University	rjmartin@iastate.edu
Jun	**Matsumoto**	Nihon University College of Bioresource Sciences	matsumoto.jun82@nihon-u.ac.jp
Cecile	**Menez**	National Institute of Agronomical Research	cecile.menez@toulouse.inra.fr

Carlos	**Minutti**	University of Edinburgh	carlos.minutti@gmail.com
Eric	**Miska**	University of Cambridge	eam29@cam.ac.uk
Meera	**Nair**	University of California Riverside	Meera.nair@ucr.edu
Sandra	**O'Neill**	Dublin City University	sandra.oneill@dcu.ie
Eyitayo	**Oluwadare**	Edinburgh Napier University	10006584@napier.ac.uk
Christian	**Owusu**	Wellcome Trust Sanger Institute	co7@sanger.ac.uk
Jan	**Pankrác**	Charles University in Prague	pankra@seznam.cz
Alex	**Phythian-Adams**	The University of Manchester	alexander.phythian-adams@manchester.ac.uk
Juan	**Quintana**	University of Edinburgh	jquintan@staffmail.ed.ac.uk
J. Gabriel	**Rinaldi**	Wellcome Trust Sanger Institute	gr10@sanger.ac.uk
Emma	**Ringqvist**	Karolinska Institutet, Sweden	emma.ringqvist@ki.se
Franca	**Ronchese**	Malaghan Institute of Medical Research	fronchese@malaghan.org.nz
Giovina	**Ruberti**	CNR (National Research Council)	giovina.ruberti@cnr.it
Peter	**Sarkies**	MRC Clinical Sciences Centre, Imperial College London	psarkies@imperial.ac.uk
Irma	**Schabussova**	Medical University Vienna	ischabuss@gmail.com
Murray	**Selkirk**	Imperial College London	m.selkirk@imperial.ac.uk
Mark	**Siracusa**	Rutgers New Jersey Medical School	mark.siracusa@rutgers.edu
Patrick	**Skelly**	Tufts University	Patrick.Skelly@tufts.edu
Michael	**Smount**	James Cook University	michael.smout@jcu.edu.au
Danielle	**Smyth**	University of Edinburgh	danielle.smyth@ed.ac.uk
Jose	**Tort**	Universidad de la Republica, UDELAR	jtort@fmed.edu.uy
Tao	**Wang**	Sichuan Agricultural University	wangtao0614@gmail.com
Lotte	**Westerhof**	Wageningen University	lotte.westerhof@wur.nl
Ruud	**Wilbers**	Wageningen University	Ruud.Wilbers@wur.nl
Mark	**Wilson**	The Francis Crick Institute	Mark.wilson@crick.ac.uk
Xuhang	**Wu**	University of Edinburgh	s1349970@sms.ed.ac.uk
Maria	**Yazdanbakhsh**	Leiden University Medical Center	M.Yazdanbakhsh@lumc.nl
Dietmar	**Zaiss**	University of Edinburgh	Dietmar.Zaiss@ed.ac.uk

NOTES