EMYH 2014

1st European Meeting for Young researchers on soil-transmitted Helminths:

"Host-Helminth Interactions from a Global Health Perspective"

23-26 March 2014

Jongny, Switzerland

This meeting was made possible thanks to the generous support of:

SCIENTIFIC PROGRAM

Sunday, March 23rd

14.00 – 18.00	Arrival, registration, reception
17.00 – 17.30	Coffee break
18.00 – 18.15	**Nicola Harris, Lausanne** Welcome address
	Julia Esser-von Bieren and Ilaria Mosconi, Lausanne Practical infos
18.15 – 19.00	***Keynote lecture - supported by EPFL WISH foundation***
	Maria Yazdanbakhsh, Leiden University Medical Center Immune-epidemiology of human helminth infections
20.00 – 21.30	***Dinner***

Monday, March 24th

7.00 – 8.30	Breakfast
8.30 – 10.15	***Human and veterinary helminth infections from a Global Health Perspective***
8.30 – 9.30 *Chaired by Susanna Fleurkens, Zürich*	**Peter Geldhof, Ghent University** Immunity to Ostertagia and Ascaris **Ayola Akim Adegnika, Centre de Recherches Médicales de Lambaréné (Special support from the Global Health Institute, EPFL)** Burden of helminths infection in Lambaréné
9.30 – 10.15 *Chaired by Duncan Sutherland, Lausanne*	**S01 - Sanne de Jong, Leiden University Medical Center** Immunoglobulin G galactosylation as a marker for inflammation and urbanisation of human populations **S02 - Frederik Van Meulder, Ghent University** Using transcriptomics to analyze vaccine-induced immunity against the bovine parasite *Cooperia oncophora* **S03 - Linda Wammes, Erasmus Medical Center Rotterdam** Repeated albendazole treatment alleviates helminth-induced immune hyporesponsiveness in a household-based RCT in in Indonesia
10.15 – 10.30	Coffee break
10.30 – 11.30	Poster pitches
11.30 – 16.00	***Sandwiches for lunch, social activity***
16.00 – 17.00	Discussion groups, coffee available
17.00 – 19.00	***Innate immune responses to soil-transmitted helminths***
17.00 – 18.00 *Chaired by Andrea Kemter, Edinburgh*	**Judith Allen, University of Edinburgh** Macrophages in helminth infection **Cornelis Hokke, Leiden University Medical Center** Innate and adaptive immune responses to glycan antigens
18.00 – 19.00 *Chaired by Beatrice Volpe, Lausanne*	**S04 - Katherine Smith, Cardiff Institute of Infection and Immunity** Alarmin immunity to chronic parasite infection **S05 - Nicole Driessen, Leiden University Medical Center** Schistosome eggshell-linked glycans induce granuloma formation **S06 - Julia Esser-von Bieren, École Polytechnique Fédérale de Lausanne** Local regulation of arginase-1 and nitric oxide synthase expression determines early immunity during intestinal helminth infection
19.15 – 20.45	***Dinner***
21.00 – 23.00	Poster session

Tuesday, March 25th

7.00 – 9.00	Breakfast
9.00 – 11.00	***Adaptive immunity against helminths***
9.00 – 10.00 *Chaired by* *Janice Murray,* *Edinburgh*	**Nicola Harris, École Polytechnique Fédérale de Lausanne** Interactions between intestinal helminths and the microbiota – consequences for the host **Rick Maizels, University of Edinburgh** Harnessing adaptive immunity for new vaccinations against helminthes
10.00 – 11.00 *Chaired by* *Lalit Dubey,* *Lausanne*	**S07 - Haeberlein Simone, Leiden University Medical Center** Schistosome egg antigens directly activate B cells for IL-10 production **S08 - Vicky Hunt, University of Bristol** The molecular basis of parasitism in the nematode *Strongyloides ratti* **S09 - Kelly Hayes, University of Manchester** *Trichuris muris* infection is associated with exacerbated intestinal tumours
11.00 – 11.15	Coffee break
11.15 – 12.00	Poster session
12.00 – 14.00	***Lunch***
14.00 – 16.00	***Immune-modulation and helminth-microbiota interactions***
14.00 – 15.00 *Chaired by* *Mario Zaiss,* *Lausanne*	**Mark Wilson, National Institute for Medical Research (MRC), London** Regulatory T-cell development during helminth infection **Richard Grencis, University of Manchester** Helminth-microbiota interactions in *Trichuris* infection
15.00 – 16.00 *Chaired by* *Luc Lebon,* *Lausanne*	**S10 - Gillian Cockley, University of Edinburgh** Secreted exosomes from *Heligmosomoides polygyrus* modulate cellular responses of the murine host **S11 - Ilaria Mosconi, École Polytechnique Fédérale de Lausanne** Differential role of Peyer's patches and mesenteric lymph nodes in driving T cell responses following helminth infection **S12 - Chris Johnston, University of Edinburgh** A role for helminths in achieving immunological tolerance
16.00 – 16.15	Coffee break
16.15 – 18.00	Presentation of discussion groups
19.00	***Departure for dinner***

Wednesday, March 26th

7.00 – 9.00	Breakfast
9.00 – 10.15	***Preventive and therapeutic approaches against soil-transmitted helminths***
9.00 – 10.00 *Chaired by* *Nicole Driessen, Leiden*	**Jennifer Keiser, Swiss Tropical and Public Health Institute, Basel** Drug development against human soil-transmitted helminth infection **Ronald Kaminsky, Novartis Animal Health, Saint Aubin** Drugs against veterinary helminths – from candidate to market
10.00 – 10.15 *Chaired by* *Nicole Driessen, Leiden*	**S13 - Ana González-Hernández, University of Ghent** Unravelling the role of natural killer cells in the vaccine-induced immune response against bovine gastrointestinal parasites: using mice as a model
10.15 – 10.30	Coffee break
10.30 – 11.00	Concluding remarks, poster and short talk prizes
11.00	***Pick up sandwiches for lunch and departure***

This meeting was made possible thanks to the generous support of:

FNSNF Fonds national suisse Schweizerischer Nationalfonds Fondo nazionale svizzero Swiss National Science Foundation

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POSTER LIST

P01 - Géraldine Bastien, Foundation Edmund Mach, San Michele all'Adige
Composition of the gut microbiota of the yellow-necked mouse, *Apodemus flavicollis*

P02 – Lalit Kumar Dubey, École Polytechnique Fédérale de Lausanne
Mesenteric lymph node organization during helminth infection

P03 – Susanna Fleurkens, ETH Zürich
Carbohydrate-based vaccines against *Haemonchus contortus*

P04 - Andrea Kemter, University of Edinburgh
Dendritic Cell modulation by *Heligmosomoides polygyrus* excretory/secretory products

P05 – Luc Lebon, École Polytechnique Fédérale de Lausanne
Interactions between intestinal helminths and bacteria and their impact on the mammalian host

P06 – Janice Murray, University of Edinburgh
Excretory/ secretory products from *Heligmosomoides polygyrus*: the VAL proteins

P07 – Duncan Sutherland, École Polytechnique Fédérale de Lausanne
Eosinophils: new players in intestinal homeostasis

P08 – Emilia Vendelova, Insitute of Parasitolgy, SAS, Kosice
Impairment of dendritic cell activation by excretory-secretory products of the model tapeworm *Mesocestoides vogae*

P09 – Beatrice Volpe, École Polytechnique Fédérale de Lausanne
Macrophage function in the innate immune response to helminth infection

P10 – Mario Zaiss, École Polytechnique Fédérale de Lausanne
Helminthic infections increase short-chain fatty acid (SCFA) levels and modulate inflammatory responses

SHORT TALK LIST

S01 - Sanne de Jong, Leiden University Medical Center
Immunoglobulin G galactosylation as a marker for inflammation and urbanisation of human populations

S02 - Frederik Van Meulder, Ghent University
Using transcriptomics to analyze vaccine-induced immunity against the bovine parasite *Cooperia oncophora*

S03 - Linda Wammes, Erasmus Medical Center Rotterdam
Repeated albendazole treatment alleviates helminth-induced immune hyporesponsiveness in a household-based RCT in Indonesia

S04 - Katherine Smith, Cardiff Institute of Infection and Immunity
Alarmin immunity to chronic parasite infection

S05 - Nicole Driessen, Leiden University Medical Center
Schistosome eggshell-linked glycans induce granuloma formation

S06 - Julia Esser-von Bieren, École Polytechnique Fédérale de Lausanne
Local regulation of arginase-1 and nitric oxide synthase expression determines early immunity during intestinal helminth infection

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

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Differential role of Peyer's patches and mesenteric lymph nodes in driving T cell responses following helminth infection

S12 - Chris Johnston, University of Edinburgh
A role for helminths in achieving immunological tolerance

S13 - Ana González-Hernández, University of Ghent
Unravelling the role of natural killer cells in the vaccine-induced immune response against bovine gastrointestinal parasites: using mice as a model

BURDEN OF HELMINTHS INFECTION IN LAMBARÉNÉ

Ayola Akim ADEGNIKA, CERMEL-ASH

Helminthiases are considered neglected tropical diseases (NTD) and mainly affect developing countries where water resources and poor sanitation allow development an infection of vectors and snails. In spite of WHO resolutions (WHA.54.19), schistosomiasis and soil helminths (SHTs) infections remain a major health problem worldwide.

Despite a recommendation for MDA campaign in endemic country, Gabon a central Africa, rain forest tropical humid and hot geographical area where helminthes are endemic, failed to undertake an appropriate action. This implies the current burden of these infections which continue to afflict population at the risk including school aged children and pregnant women.

In literature few data are available for Gabon. The majorities of published data are from Lambaréné, Gabon and indicate prevalence of helminthes ranged from 9% for filariasis to 71% for *T. trichiura.* Nevertheless, ongoing surveys indicate reduction of the burden of these infections, which correlate with economical growth and sanitation improvement rather a National Program action.

MACROPHAGES IN HELMINTH INFECTION

Tara Sutherland, Dominik Rückerl, Lucy Jones & Stephen Jenkins & Judith Allen
Institute Immunology & Infection Research, University of Edinburgh, Edinburgh, EH9 3JT, United Kingdom

Macrophages activated by the Th2 cytokines IL-4 and IL-13 are found in high numbers at the site of helminth infection, with a signature gene expression profile that includes arg1, retnla (RELMalpha) and chi3l3 (YM1). We have demonstrated that macrophage accumulation in this setting can occur by proliferative expansion rather than recruitment from the blood. The full spectrum of macrophage function during helminth infection remains to be elucidated and despite the very high abundance of Ym1 and RELMalpha at the sites of helminth infection, their specific functions remain to be elucidated. Our recent work suggests that the function of these molecules is highly context dependent. Ym1 and RELMa, for example, both contribute to the control of parasite numbers during *N. brasiliensis* infection but also are important for damage repair. These dual functions are particularly striking for Ym1, because in the early stages of infection it promotes damage to the lung in a trade off for enhanced parasite control.

COMPOSITION OF THE GUT MICROBIOTA OF THE YELLOW-NECKED MOUSE, *APODEMUS FLAVICOLLIS*

Géraldine Bastien[1], Jakub Kreisinger[1], Emily Pascoe[1], Heidi Hauffe[1] and Sarah E. Perkins[1,2]

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The gut microbiota plays a key role in the development of vital host functions, such as the immune system. Emerging evidence indicates that pathogens and parasitic helminths also interact with the microbiota to affect host development. However, to date, the interaction between host, microbiota and helminths is not well studied. Wild rodents, such as the yellow-necked mouse, are naturally parasitized, and thus represent a promising model to start to address this question. As such, we have investigated the variability of the gut microbiota in five distinct locations of the gut, where parasites are commonly found.

We analysed the variability of the gut microbial community in the small intestine, caecum, proximal and distal colon within fifteen yellow-necked mouse *Apodemus flavicollis*. A higher proportion of reads were assigned to the order *Bacteroidales* and *Clostridiales* in faecal samples and the caecum, whereas the small intestine and intestinal membrane were dominated by *Lactobacilliales*. These results at the class and order level are consistent with previous analyses of other mammal's gastrointestinal microbiota. Most interestingly, our results at a lower taxonomic level suggest considerable variation in the composition of the microbiota colonizing the digestive tract, and further analyses will allow us to establish to what extent this result is related to the presence of parasites colonizing those locations. To our knowledge this study is the first providing insight into compartment-dependent variation of gastrointestinal microbiota composition in a wild rodent non-captive population.

SECRETED EXOSOMES FROM *HELIGMOSOMOIDES POLYGYRUS* MODULATE CELLULAR RESPONSES OF THE MURINE HOST

Gillian Coakley , Fabio Simbari, Henry McSorley, Rick Maizels & Amy Buck[1]

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University of Edinburgh, Edinburgh, UK

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

IMMUNOGLOBULIN G GALACTOSYLATION AS A MARKER FOR INFLAMMATION AND URBANISATION OF HUMAN POPULATIONS

Sanne E. de Jong[1], Maurice H. Selman[2], John Raynes[3], Philip J. Cooper[4], Jon Genuneit[5], Erika von Mutius[6], André C. Knulst[7], Manfred Wuhrer[2], Maria Yazdanbakhsh[1]

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IgG Fc glycosylation has been described to affect antibody activity and to vary with age, sex, and pregnancy, as well as in autoimmune diseases, infectious diseases, and cancer. However, whether IgG glycosylation varies between populations worldwide remains unknown. Therefore, we analysed IgG Fc N-glycosylation in children from different areas in Ghana, Gabon, Ecuador, Germany and the Netherlands, as well as in adults from Gabon and the Netherlands. Serum/plasma IgG was purified by affinity chromatography, and Fc N-glycosylation profiles were determined by fast nanoliquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS). IgG galactosylation followed an urban-to-rural gradient, with higher levels in higher income countries and more urbanised areas within countries. The galactosylation gradient might be partly related to inflammatory activity, as children with higher levels of parasitic infections or with allergic asthma were located at the rural side of the gradient. Galactosylation also correlated moderately with C-reactive protein levels. Other glycosylation features, like fucosylation, sialylation and the incidence of bisecting N-acetylglucosamines, did not follow an urban-to-rural gradient. While the functional consequences of the observed differential galactosylation require further investigation, these data show that IgG Fc galactosylation could be used as a marker for urbanisation and possibly inflammation to compare human populations between and within countries, which could provide a valuable tool for public health studies.

SCHISTOSOME EGGSHELL-LINKED GLYCANS INDUCE GRANULOMA FORMATION

Nicole N. Driessen, Lara van der Laan, Cornelis H. Smit,
Hermelijn H. Smits, Maria Yazdanbakhsh, and Cornelis H. Hokke

Department of Parasitology, Leiden University Medical Center,
Leiden, The Netherlands

Approximately 200 million people in (sub-)tropical areas suffer from infections with schistosomes. The main pathological symptoms of schistosomiasis are caused by the deposition of parasite eggs into organs of the host, such as the liver, where they induce granuloma formation. Several studies indicated that glycoconjugates released by the egg contribute to the regulation of the hosts' immune response. However, the immature eggs that the female worms produce, do not yet produce excretory/secretory glycoproteins and the initial interaction of the host with the newly produced egg is with the outer eggshell. We recently found that in contrast to the complex glycosylation pattern of soluble egg antigens, the eggshell carries only a single dominant glycan structure, a tri-antennary N-glycan with terminal LacNAc (Galβ1-4GlcNAc) motifs. Van de Vijver et al. (2006), showed that a LacNAc-conjugate coated to egg-size beads is able to induce Th2-type granulomas after implantation in the mouse liver. Triggered by this, we studied the role of the eggshell-linked glycans in vivo and found that stripped eggshells, purified of soluble antigens, were able to induce granulomas. Currently, we're aiming to identify the cell types and lectin receptors involved in this process and thereby gain new insights on the structure-function relationship of glycans regulating the immune response.

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MESENTERIC LYMPH NODE ORGANIZATION DURING HELMINTH INFECTION

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The lymph node stroma is composed of non-haematopoietic cells, which can be divided into Fibroblastic reticular cells (FRCs) ($gp38^{+}CD31^{-}$), follicular dendritic cells (FDCs) ($gp38^{+/-}CD31^{-}$), lymphatic endothelial cells (LECs) ($gp38^{+}CD31^{+}$) and blood endothelial cells (BECs) ($gp38^{-}CD31^{+}$) based on their surface markers expression and function. Amongst these FRCs organize themselves to form a conduits that can transport small antigens and provide a scaffold for efficient cellular interactions between DCs and T cells. Furthermore FRCs can regulate antigen-specific T cell priming through the secretion of soluble mediators. To date the structural and functional properties of the stromal network during type2 immunity following intestinal helminth infection is not known. In the present study we investigated the structural changes in the stromal and lymphoid compartments of the draining mesenteric lymph nodes (MLN) following intestinal helminth infection caused by *Heligmosomoides polygyrus bakeri (Hp).*

By visualizing the complete length of MLN using fluorescent scanning we identified a dramatic changes in the MLN cellularity and structure following *Hp* infection. Infection resulted in complete re-organization of the well-defined B cell/T cell zones with an increased number of B cell follicles. This was accompanied by an extension of the lymphatic network into the T cell zone along with an increased number of FRCs aggregating around the newly formed B cell follicles. All of these effects were partially dependent on IL-4Rα signalling. These defined histological observations were supported by FACS analysis and indicate that parasitic infection actively influences non-haematopoietic cells and their organization within the MLN. We are currently investigating the molecular process by which IL-4Rα signalling promotes such diversified yet distinctive changes. In addition we are examining the impact of stromal cells on Th2 immune responses and protective immunity following Hp infection.

LOCAL REGULATION OF ARGINASE-1 AND NITRIC OXIDE SYNTHASE EXPRESSION DETERMINES EARLY IMMUNITY DURING INTESTINAL HELMINTH INFECTION

Julia Esser-von Bieren[1], Beatrice Volpe[1], Ilaria Mosconi[1], Romain Guiet[2], J. Sjef Verbeek[3], Nicola L. Harris[1]

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[3]Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

We have recently shown that anti-helminth antibodies induce the rapid trapping of helminth larvae by reprogramming macrophages to an alternatively activated phenotype whereby they express Arginase-1 (Arg1). Helminth-antibody-mediated induction of macrophage Arg-1 expression occurred independently of IL-4Rα signaling and was partially dependent on activating Fc receptors. While Arg1 has an established role in the immune response against the murine parasite *Heligmosomoides polygyrus bakeri* (*Hp*), the role of its competing enzyme inducible nitric oxide synthase (iNOS) remains unclear. Whilst helminth-induced Arg-1 expression by macrophages *in vitro* required the addition of immune serum we noted that the presence of helminth larvae alone promoted the strong expression of iNOS. However, during *Hp* challenge infection *in vivo*, iNOS was undetectable in intestinal granulomas, whereas Arg1 was highly abundant. In contrast, early during primary *Hp* infection, Arg1 and iNOS were both expressed in the close vicinity of invading larvae within the submucosa. Strikingly mice genetically deficient in iNOS or treated with the iNOS inhibitor L-NIL during primary *Hp* infection harbored lower numbers of tissue larvae despite of an unaltered T_H2 response. We are currently investigating the triggers and signaling pathways governing Arg1 and iNOS expression during primary *Hp* infection *in vivo*. Our findings suggest that local iNOS induction by helminth larvae during primary *Hp* infection may constitute an immune evasion mechanism, overcoming Arg1-dependent larval trapping.

CARBOHYDRATE-BASED VACCINES AGAINST HAEMONCHUS CONTORTUS

Susanna Fleurkens[1,2], Alex Butschi[1], Chia-wei Lin[2], Christine Neupert[1], Markus Aebi[2], Bruno Oesch[1]

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[2]Institute for Microbiology, ETH Zürich, Switzerland

The blood sucking stomach worm of sheep *Haemonchus contortus* causes major economic damage reducing meat and wool quality. The parasite is currently controlled by chemical treatments yet the advent of resistant strains necessitates new strategies such as an effective vaccine. It is well known that extracts of *H. contortus* are able to confer immunity thereby reducing the worm burden however, attempts to make a recombinant vaccine have failed up to now which might be due to the lack of glycosylation. We therefore have undertaken the production of various antigens of *H. contortus* containing the parasite specific glycosylation. Here we report the results of expression of the major antigen H11 using insect cells and including the co-expression of a nematode-specific galactosidase. Glycan analysis of native or recombinantly expressed H11 shows that the type of glycans as well as the site-specific glycosylation is comparable in native and recombinant preparations of H11. This indicates that we have been able to produce a potential immunogen for a *H. contortus* vaccine in large quantities in order to test the hypothesis of the importance of glycosylation.

ANALYSIS OF THE NATURAL AND VACCINE INDUCED IMMUNE RESPONSES AGAINST GASTROINTESTINAL PARASITES IN CATTLE AND PIGS

Peter Geldhof[1], Ana Gonzalez Hernandez[1], Frederik Van Meulder[1], Dries Masure[1], Belgacem Mihi[1], Stefanie Van Coppernolle[1], Jozef Vercruysse[1], Edwin Claerebout[1]

[1]Laboratory of Parasitology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

Parasitic helminth infections pose a massive burden on human and animal health worldwide. Control of these parasites relies completely on treatment with anthelmintics. However, with the increasing incidence of anthelmintic resistance, the question arises whether this approach is sustainable in the long term. The aim of our research is to provide essential information needed for the development of anti-parasitic vaccines. The main focus lies on the bovine parasites *Ostertagia ostertagi* and *Cooperia oncophora* and the porcine parasite *Ascaris suum*. For these bovine parasites, two experimental vaccines were developed that, after intramuscular immunisation, raise an effective protective immune response in cattle. Analysis of the vaccine induced immune response indicated an antigen-induced proliferation of natural killer (NK) cells. This NK response was not only observed following vaccination but also appears in naturally infected cattle. How the NKs are involved in this anti-parasitic immune response is still unclear and forms the basis of our current research. Our work on *A. suum* is aimed at unravelling the natural intestinal immune responses against the penetrating L3 larvae, the pre-hepatic barrier, and the L4 larvae, the self-cure reaction. Our research showed that the development of the pre-hepatic barrier coincides with an influx of eosinophils that degranulate after contact with the larvae, leading to the death of the larvae. The self-cure reaction on the other hand coincides with increased peristaltic movements of the intestine, likely triggered by eosinophils and intra-epithelial T cells. Our current research is aimed at elucidating how these responses can be mimicked by vaccination.

UNRAVELLING THE ROLE OF NATURAL KILLER CELLS IN THE VACCINE-INDUCED IMMUNE RESPONSE AGAINST BOVINE GASTROINTESTINAL PARASITES: USING MICE AS A MODEL

González-Hernández A., Van Coppernolle S., Claerebout E., Geldhof P.

Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University, Belgium

In recent years, our research group has developed protective experimental vaccines against two of the most important gastrointestinal parasites in cattle, *Ostertagia ostertagi* and *Cooperia oncophora*, based on activation-associated secreted proteins (ASP) purified from the excretory-secretory material of adult worms. In order to understand how the protective mucosal immune response is achieved, bovine vaccination trials were conducted where we observed that in vitro re-stimulation of peripheral blood mononuclear cells of animals vaccinated with the protective native antigen (nASP) resulted in a marked proliferation of NK cells, which was not observed in animals vaccinated with the recombinant non-protective antigens (rASP). Based on the results obtained in cattle, we investigated whether the observed NK cell response following vaccination would also be triggered in other species such as mice. In a trial with the *O. ostertagi* vaccine, 3 groups of animals were vaccinated with QuilA adjuvant, the native vaccine or the recombinant combined with QuilA. Interestingly, injection of mice with nASP+QuilA resulted in an antigen specific NK cell proliferation after *in vitro* re-stimulation. This NK cell response was not present in mice vaccinated with the recombinant vaccine. In addition to the NK cell response, T and B cell proliferation was also observed. Currently we are investigating the responses in mice following vaccination with the *Cooperia* nASP vaccine. The results will be analysed and compared with the results obtained in cattle. In summary, the results so far indicate the existence of a conserved NK cell response after vaccination with these ASP worm antigens.

HELMINTH - MICROBIOTA INTERACTIONS IN TRICHURIS INFECTION

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Trichuris is a successful genera of gastrointestinal nematodes where component species have radiated out to infect a wide variety of hosts including mouse and man. Their preferred intestinal infection site is the caecum and the parasites occupy an intraepithelial niche in all life cycle stages. Naturally infection is long lived and prevalence in endemic regions high characterized by relatively low levels of infection.
T. muris readily infects inbred mouse strains presenting a manipuable experimental system. The caecum is a major site of intestinal microflora and our work has shown that *T. muris* eggs are induced to hatch by caecal microflora providing a major stimulus for hatching in this location. *In vitro* studies have shown that many different microbes (but not all) can induce hatching including *E. coli*. In the case of the latter, attachment to the eggs fimbrae play critical role. Moreover, low-level chronic infection with *T. muris* causes a profound change in the microbiome of the caecum. Significant changes are evident by day 21 post infection with marked change seen by day 35 (by which time a patent infection is established) and continued increased dysbiosis up until even day 91 post infection. Following removal of patent infection with anthelmintic, the microbiome returns to that seen in uninfected animals, but gradually. Treatment of uninfected mice with anthelmintic also induces transient dysbiosis. Thus, chronic infection with *T. muris* causes a major modification of the intestinal microflora. The data will be discussed in relation to host immunity and parasite biology.

SCHISTOSOME EGG ANTIGENS DIRECTLY ACTIVATE B CELLS FOR IL-10 PRODUCTION

Haeberlein Simone[1], van der Vlugt Lucien EPM[1], Driessen Nicole N[1], Ozir-Fazalalikhan Arifa[1], Langhans Kristina[2], Schramm Gabriele[2], Haas Helmut[2], Hokke Cornelis H[1], Yazdanbakhsh Maria[1], Smits Hermelijn H[1]

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Infection with the helminth *Schistosoma mansoni* induces the development of interleukin (IL)-10 producing regulatory B cells (Breg cells) in mice and men. We have recently demonstrated that splenic $CD1d^+$ Breg cells produce IL-10 during chronic *S. mansoni* infection and suppress allergic airway inflammation in a mouse model. Therefore, it is of great interest to study the conditions in which these Breg cells are induced in order to develop novel therapies against allergic disorders through enhancing Breg cell activity. Here we investigated whether schistosomal antigens are able to induce IL-10 production in B cells *in vitro* and *in vivo*. Repeated *in vivo* injection of schistosome eggs or soluble egg antigen (SEA) into C57BL/6 mice, without the context of natural infection, did lead to potent IL-10 production by splenic B cells. Immunohistochemistry and flowcytometry showed that splenic B cells directly bind fluorescently labeled SEA *in vivo*. Also *in vitro*, naive sorted B cells were able to bind and internalize SEA, and to produce IL-10 in response to SEA which was further increased upon co-stimulation with anti-CD40 antibody. IL-10 was also induced if anti-CD40 was combined with IPSE, a well-characterized antigen within SEA. In conclusion, schistosomal antigens are able to directly induce IL-10 production in B cells. Whether an interaction of CD40 ligand-expressing cell-types with B cells is involved *in vivo* during egg or SEA treatment is still open. Current work focuses on the identification of cellular interaction partners and the antigen binding receptors to provide new insights in the generation Breg cells.

INTERACTIONS BETWEEN INTESTINAL HELMINTHS AND THE MICROBIOTA – CONSEQUENCES FOR THE HOST

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We have used the model intestinal helminth, *Heligmosomoides polygyrus bakeri* (*Hp*), to determine the impact of helminth infection on intestinal bacterial communities and to investigate the consequences of such interactions for the murine host. To this end we developed a model for hatching of infective axenic larvae that can be used to infect germ-free or gnotobiotic mice. Infection of gnotobiotic mice harboring a minimal and benign flora (the Altered Schadlers Flora, ASF) we showed that Hp infection alters bacterial communities resulting in the outgrowth of *Lactobacillus* spp in both the small and large intestine. In turn the presence of intestinal bacteria impacted on the number and fecundity of helminth parasites. Lastly we show data indicating that helminth-bacterial interactions contribute to the ability of these large parasites to modulate immune-mediated diseases. Hp-induced attenuation of house-dust mite (HDM) allergy required the presence of intestinal bacteria as determined in animals receiving Hp together with a course of antibiotics to reduce intestinal bacteria numbers and by fecal transfer experiments in which bacteria but not parasites were transmitted to naive hosts. The ability of Hp-altered bacterial communities to modulate HDM-induced allergic airway inflammation correlated with an increased production of short chain fatty acids (SCFA) in Hp infected mice and SCFA ligation of the host receptor GPR41 was required for disease modulation. Together these studies reveal a close and complex interplay between intestinal helminths, intestinal bacteria and their mammalian hosts.

TRICHURIS MURIS INFECTION IS ASSOCIATED WITH EXACERBATED INTESTINAL TUMOURS

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Trichuris muris is a natural gut-dwelling parasitic nematode of mice that can stimulate a protective Th2 response or a non-protective Th1 response, depending on the strain of host mouse infected. Chronic *T. muris* infection is associated with the production of IFN gamma. However, this response is strongly regulated with the additional production of IL-10. The induction of a regulatory response has been exploited in new and novel treatment of IBD (inflammatory bowel disease) with *T. suis*. As IBD patients bear an increased risk of developing colorectal cancer with IBD ranking as one of the top three risk factors in intestinal neoplasia we investigated the effects of a chronic *T. muris* infection, and the resulting regulatory response, in two murine models of bowel neoplasia.

In both models of bowel neoplasia, infected animals had significantly more tumours than non-infected animals. This increase in tumour number was not restricted to the site of infection, the large intestine, but apparent throughout the intestinal tract. Additionally, infected mice had an increased number of small tumours suggesting that *T. muris* infection was initiating tumour development. Enhanced proliferation and apoptosis associated with *T. muris* infection was only evident in the caecum of infected mice suggesting no causative relationship with tumour development.

Though more work is needed to dissect out the cause of the enhanced tumour genesis, this study highlights that Trichuriasis is associated with increased tumour development, an important consideration for chronic helminth infection *per se* and when these helminths are used as immunotherapeutic agents.

INNATE AND ADAPTIVE IMMUNE RESPONSES TO GLYCAN ANTIGENS

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All parasitic helminths expose or secrete immunogenic glycolipids and glycoproteins to which the mammalian host mounts immune responses. These may include innate responses that modulate or regulate the host's reaction to infection, but also anti-glycan antibody responses potentially related to immunity are induced.

Although not all parasitic helminths have been studied to the same extent with respect to their glycan repertoire it is clear that the glycome of a helminth can be very complex with numerous N-glycan, O-glycan and lipid-glycan motifs present. In the past decade, many trematode- or nematode-derived molecules or crude preparations have been identified to which glycan-dependent immune responses *in vitro* and *in vivo* are observed. These observations underline the importance of glycan motifs in the host-pathogen interaction and control of infection and inflammation, and they have raised interest in helminth-derived glycoconjugates as therapeutic modulators for immune disorders.

Glycans of soil-transmitted nematodes also harbor important immunobiological properties, but the best studied helminth species in terms of glycomics and glycobiology so far is the trematode *Schistosoma mansoni*. In this presentation the complexity and life-stage specificity of the schistosome glycome in relation to that of other helminth glycomes will be highlighted. Molecular aspects of the glycan-dependent induction of Th2 responses by schistosome secretions will be discussed, and novel glycan microarray data on the presence of specific anti-glycan antibodies during schistosome infection will be presented.

THE MOLECULAR BASIS OF PARASITISM IN THE NEMATODE *STRONGYLOIDES RATTI*

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The *Strongyloides* life cycle includes a parasitic female-only stage, which inhabits the small intestine of its host, and a facultative, dioecious free-living adult generation. These adult life-cycle stages are genetically identical and so comparing parasitic and free-living stages offers an almost unique opportunity to investigate and address questions about the molecular adaptations required to be a successful parasitic nematode. We have used quantitative mass spectrometry and RNAseq analyses to compare the proteome and transcriptome of parasitic and free-living females of *S. ratti*. This found that 15% of genes in the genome are differentially expressed between these life stages. Many of the genes only upregulated in the parasitic stage of the life-cycle are clustered in the genome. These clusters of 2-18 adjacent genes mostly comprise genes with likely similar functions. Approximately 20% of genes that make up these clusters code for astacins of the zinc metalloproteases family, implying these may have a key role in parasitism in nematodes.

A ROLE FOR HELMINTHS IN ACHIEVING IMMUNOLOGICAL TOLERANCE

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

DRUGS AGAINST VETERINARY HELMINTHS – FROM CANDIDATE TO MARKET

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

DRUG DEVELOPMENT AGAINST HUMAN SOIL-TRANSMITTED HELMINTH INFECTION

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Infections with soil-transmitted helminths (STH; *Ascaris lumbricoides*, hookworm, and *Trichuris trichiura*) are responsible for a considerable public health burden. For these widespread helminth infections, the large scale administration of safe, single-dose treatments is the front-line intervention to control morbidity ("preventive chemotherapy"). Albendazole or mebendazole are most widely used, but both drugs have limitations and show a particularly low efficacy against *T. trichiura*. New broad spectrum therapeutic products should be developed to improve control and potentially achieve local elimination of STH infections. We have developed a simple and cost-effective drug sensitivity assays using *T. muris* first-stage larvae (L_1) and *A. ceylanicum* L_3. The assays are routinely used in our laboratory and provide accurate and reproducible drug effect data *in vitro*. In collaboration with the Drugs for Neglected Diseases initiative (DND*i*) several libraries of marketed drugs and advanced drug candidates have been tested in this model and first results will be shown. Following a "low hanging fruit" approach we also tested several veterinary anthelminthics. Oxantel pamoate revealed an excellent trichuricidal activity *in vitro* and in mice and performed superior than the standard drugs albendazole or mebendazole. In view of these findings and following a bench to field approach a randomized controlled trial was conducted on Pemba Island, Tanzania with oxantel pamoate and oxantel pamoate-albendazole. We evaluated the efficacy and tolerability of an oxantel pamoate (20 mg/kg)-albendazole (400 mg) combination in school-aged children infected with *T. trichiura*. Oxantel pamoate-albendazole showed a higher cure rate and egg reduction rate against *T. trichiura* compared to the standard treatments. Hence this combination could have a significant impact within global STH control programs. Nonetheless, drug discovery efforts to develop novel drugs for STH infections should receive high priority.

DENDRITIC CELL MODULATION BY HELIGMOSOMOIDES POLYGYRUS EXCRETORY/SECRETORY PRODUCTS

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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, 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 five 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. Finally, we could show that the phosphorylation levels of JNK1/2 and ERK1/2 were equal in LPS and LPS+HES treated BMDCs, indicating no effect of HES on the activation of these MAP kinases. 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.

INTERACTIONS BETWEEN INTESTINAL HELMINTHS AND BACTERIA AND THEIR IMPACT ON THE MAMMALIAN HOST

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Intestinal helminth infection is well recognized to lead to a generalized immuno-suppression of the mammalian host[1], and it was recently shown that helminth infection can alter the composition of bacterial communities[2]. Yet whether this impact on microbiota contributes to the helminth immuno-modulatory potential has not been investigated. To better understand this complex interplay, we developed a model of axenic hookworm infections using the murine nematode *Heligmosomoides polygyrus*.

Helminths are typically hatched from faeces or soil containing contaminating bacteria. To avoid bacterial contamination, we successfully developed a protocol to generate infective axenic larvae from purified eggs hatched in the presence of an auxotrophic *E. coli*[3] which cannot grow within germ-free or minimally-colonized mice.

We then confirmed the ability of helminth infection to alter bacterial communities by infecting altered Schaedler flora (ASF) mice (which harbour a limited number of benign bacterial species[4]) with axenic larvae. Infection resulted in a selective outgrowth of *Lactobacillus* species. We also observed that infection results in increased bacterial translocation across the intestinal wall, as determined by sCD14 levels in peritoneum and serum. We thus hypothesized that altered exposure of host to bacteria during helminth infection may impact on the immune response. Indeed, an analysis of worm burdens in germ-free or antibiotic-treated mice versus SPF mice indicated that an absence of intestinal bacteria results in increased numbers and fecundity of adult parasites.

We now plan to investigate how bacterial exposure alters host immunity against parasites and whether intestinal bacteria impact on helminth-induced modulation of allergic and autoimmune diseases.

[1] Maizels *et al.*, *Curr. Op. Immunol.* 24, 459-466, 2012.

[2] Walk *et al.*, *Inflamm. Bowel Dis.* 16, 1841-1849, 2010.

[3] Hapfelmeier *et al.*, *Science* 328, 1705-1709, 2010.

[4] Dewhirst *et al.*, *Appl. Environ. Microbiol.* 65, 3287-3292, 1999.

STERILISING IMMUNITY AGAINST GASTROINTESTINAL HELMINTH INFECTION ELICITED BY A VACCINATION WITH EXCRETORY SECRETORY ANTIGENS IS MEDIATED BY SPECIFIC IGG1 ANTIBODIES AND IL-4RA/IL-25-DEPENDENT MYELOID EFFECTOR CELLS

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In the absence of effective vaccines, helminth infections remain a pervasive global health problem. We have shown, however, that 100% protective immunity can be induced to the murine helminth *Heligmosomoides polygyrus* by vaccination with parasite excretory/secretory (HES) products. Protection requires cognate B cells acting through IgG1 antibodies and immune cell activation by Type 2 cytokines (through IL-4R, and IL-25), and macrophage migration inhibitory factor, acting to trap larvae in the early days of infection. Passive transfer of IgG1 from vaccinated mice confers a high level of protection to naïve recipients, but not sterile immunity. Significantly, mice lacking IL-25 or MIF are unable to clear parasites despite high antibody titres to HES, again arguing that antibody requires complementation by a cytokine-activated effector cell population. Mice deficient in FcRγ chain or C3 activation remain fully immune, indicating antibodies directly neutralise parasite molecules. As few as 3 secreted proteins of the dominant VAL family induce immunity, which is associated with greatly enhanced arrest and extravasation of LysM+ myeloid cells visualised by intra-vital imaging. Hence a distinct Type 2 effector cell mediates vaccine-induced immunity alongside IgG1 antibodies directed against secreted proteins. Such a model is consistent with data from human gastrointestinal infections and may facilitate the rational design of future vaccines to eradicate helminth diseases globally.

DIFFERENTIAL ROLE OF PEYER'S PATCHES AND MESENTERIC LYMPH NODES IN DRIVING T CELL RESPONSES FOLLOWING HELMINTH INFECTION

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Intestinal helminth infections represent a major cause of morbidity among populations living in developing countries with an estimated 2 billion people currently infected. Helminth infections are typically chronic in nature, however the molecular mechanisms by which these organisms promote or thwart host immunity remain unclear. Immune expulsion requires the differentiation of $CD4^+$ T cells into Th2 cells whilst regulatory T cells act to dampen the extent of the Th2 response. Priming of T cells requires drainage or capture of antigens within lymphoid tissues and in the case of intestinal helminthes such sites include the mucosal associated PPs or MLN. To gain insight into where and when the activation of the various T cell subsets takes place following intestinal helminth infection we analyzed Th2 and regulatory T cell responses in the PPs and MLN following infection with murine intestinal helminth, *Heligmosomoides polygyrus bakeri*. A greater expansion of Tregs within the PPs compared to the MLN was noted and PP-derived Tregs showed a more activated phenotype. Conversely, protective Th2 responses were observed to be largely restricted to the MLN. Taken together, these data indicate that the PPs and MLN play differential roles during immune responses following intestinal helminth infection with the MLN acting as the main site of protective type 2 immunity whilst the PPs dampen the immune response and thus likely favor the establishment of chronicity. We are currently investigating the molecular mechanisms by which these two organs support differential T cell responses.

EXCRETORY/ SECRETORY PRODUCTS FROM *HELIGMOSOMOIDES POLYGYRUS*: THE VAL PROTEINS

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The intestinal parasite *Heligmosomoides polygyrus* maintains itself in mice for many months, imposing a broad immunosuppressive effect. Interference with host immunity is believed to be mediated by the release of a potent cocktail of molecules termed excretory/ secretory products (HES). These have been studied at both transcriptomic and proteomic levels identifying a spectrum of molecules with potential immunomodulatory function. The most predominant are a set of >20 VAL proteins (**V**enom allergen, ***A****ncylostoma* secreted protein **L**ike), belonging to a larger family of proteins called SCP/TAPS. These proteins are prominent across the Nematode phylum from plant parasites to the free-living *C.elegans,* and are also represented as extracellular proteins in insect venom, mammalian sperm coats and even in the leaves of infected tomato plants. First described from the dog hookworm *Ancylostoma caninum*, the VAL proteins are often expressed at critical points in the parasite's lifecycle such as during the transition to parasitism. Given the prevalence of these molecules, their structural variety and the extent to which they are expressed we hypothesise that they are intimately involved in manipulation of host/parasite interactions. To examine the biological function of VAL molecules, we have expressed soluble recombinant proteins of Hp-VAL-1 and -4, representing respectively a double- and single-domain form as found throughout the SCP/TAPS family. Labelled VAL proteins have been used in confocal and whole-mount immunofluorescence microscopy to demonstrate binding of these and other HES proteins to distinct gut epithelial cell types, providing early insights into the molecular and cellular targets of *H. polygyrus* secreted products.

ALARMIN IMMUNITY TO CHRONIC PARASITE INFECTION

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Recently alarmin cytokines such as IL-25 have been reported to play a vital role in the generation and amplification of innate and adaptive type-2-immune responses. IL-25 is released and produced in the epithelium in response to tissue damage and effectively accelerates clearance of acute parasitic infections, such as *Nippostrongylus brasiliensis* from the host. However, it is not yet clear how this alarmin impacts on the immune response to a chronic helminth infection, and hence the role of IL-25 was tested following *Heligmosomoides polygyrus* infection.

Using IL-25R-deficient BALB/c mice, we find that Th2 responses and adult worm burdens were equivalent to wild-type BALB/c mice at the peak of Th2 inflammation, however, by day 28 post-infection, adult worm expulsion was significantly delayed in IL-25R-deficient mice. Injection of rIL-25 at the peak, but not the initiation, of Th2 inflammation was able to enhance parasite expulsion in more susceptible C57BL/6 mouse strains. Construction of bone-marrow chimeras demonstrated that the IL-25 responsive cell was of a hematopoietic lineage and use of immune-deficient $RAG^{-/-}$ mice, provided with an exogenous source of IL-4, demonstrated that the IL-25 responsive cell was innate and that it required IL-4Rα signaling in order to effectively mediate helminth clearance.

This work generates the novel and fascinating hypothesis that IL-25R signaling may be redundant during the early phases of chronic helminth infection and that it is most effective in driving parasite expulsion subsequent to initial IL-4Rα signaling.

EOSINOPHILS: NEW PLAYERS IN INTESTINAL HOMEOSTASIS

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The abundance of eosinophil granulocytes in intestinal tissues even in the absence of infection or commensal bacteria has long been a puzzle as to their function. We find eosinophils constitute 10-20% of immune cells present in the healthy upper small intestine of mice, a higher level than found either in the circulation or in any other peripheral tissue. The ratio of eosinophils declines along the intestinal tract as bacterial density increases and the mucus layer becomes thicker. To date no function for eosinophils in modulating intestinal homeostasis has been reported. Here we show that eosinophil deficient mice maintained in non-SPF housing have vastly reduced T cell-dependent IgA production in Peyer's patches and reduced IgA+ cells in intestinal lamina propria suggesting eosinophils may play a key role in intestinal IgA homeostasis.

USING TRANSCRIPTOMICS TO ANALYZE VACCINE-INDUCED IMMUNITY AGAINST THE BOVINE PARASITE COOPERIA ONCOPHORA

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Cooperia oncophora, a bovine intestinal nematode, is becoming increasingly economically important, not in the least because of its rapidly emerging levels of resistance against chemical anthemintics. In an attempt to find alternative control strategies for *Cooperia*, the laboratory of parasitology in Merelbeke has developed an experimental vaccine based on excretory-secretory material (ES) of the parasite. *Cooperia*-infected calves vaccinated with this experimental vaccine, showed a reduction in terms of eggs per gram faeces (EPG) of 91% compared to non-vaccinated controls. To further optimize this vaccine, however, a thorough understanding of the vaccine-induced immune responses, both systemic as mucosal, is essential. On a systemic level, we surprisingly showed the main immune cell population triggered by the vaccine to be NK-cells. Moreover, these NK-cells seem to be capable of holding an 'immune memory' and presumably also transfer its protective signal to the site of infection. On a mucosal level, we could not observe changes in classical immune markers. Therefore, we applied a broader whole-transcriptomic approach using RNA-seq in which we compared 7 vaccinated animals to an equal-sized control group. Per animal, approximately 15 million reads were generated. Differential analysis of the dataset yielded a list of genes that were significantly impacted in the vaccinated group. Several of them, such as BOLA (Bovine Lymphocyte Antigen), have previously been associated with helminth immunity. A smaller selection of these genes that also correlated with parasitological parameters, is potentially involved in direct effector mechanisms targeting the parasite. These results will be presented and discussed.

IMPAIRMENT OF DENDRITIC CELL ACTIVATION BY EXCRETORY-SECRETORY PRODUCTS OF THE MODEL TAPEWORM *MESOCESTOIDES VOGAE*

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Dendritic cells (DC) are critically involved in stimulation and modulation of the host immune response by helminths (nematodes, trematodes and cestodes). Despite the fact that cestodes cause debilitating chronic disease with long asymptomatic state, their immunomodulatory potential is highly understudied as compared to nematodes and trematodes. Moreover, the influence of this class of helminth on host DC is poorly understood. Using an *in vitro* stimulation systems and an *in vivo* model of intraperitoneal injection of the model cestode *Mesocestoides vogae* (syn. *M. corti*), we investigated the influence of released products of larval cestode on host DC. Although considerably recruited to the site of infection, host DC failed to produce elevated amounts of IL-12p70 in *M. vogae* injected mice when compared to mock-injected controls. *In vitro*, we found that neither excreted/secreted (E/S) products nor somatic extracts of the parasite were able to induce conventional maturation of DC (as judged by MHC II class and CD86 surface marker expression and cytokine production). Moreover, whereas the parasite secretions significantly suppressed LPS-induced production of IL12p70, somatic extracts failed to do so. A bio-assay guided biochemical analysis of the parasite secreted product(s) revealed that the observed effect was most likely driven by a glycoprotein. Finally, an analysis of the protein profiles of parasite secreted (active) and somatic (inactive) antigens provided us with several differentially expressed proteins that could constitute efficient anti-inflammatory factors. The identification strategy of such factor(s) and the possible implication of our findings for cestode immunomodulation will be discussed.

MACROPHAGE FUNCTION IN THE INNATE IMMUNE RESPONSE TO HELMINTH INFECTION

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Chronic helminth infections are mainly caused by the parasites ability to evade the immune response and to exert their immunomodulatory properties. While the mechanisms of protective immunity are not yet fully defined, our group has recently used a model of gastrointestinal helminth infection with *Heligmosomoides polygyrus* to show that antibodies can activate macrophages in immune mice to trap tissue migrating larvae[1]. This involved the ability of antibodies to induce expression of Arginase1, which contributed to macrophage-mediated larval immobilization. However the impact of this cell type on parasite burdens during primary infection needs to be clarified.

Preliminary data indicate that macrophages have a simultaneous expression of Arginase1 and its enzymatic counterpart, the inducible form of nitric oxide synthase (iNOS) during primary infection. Additional *in vivo* data demonstrate that $iNOS^{-/-}$ mice are more protected against the infection, with lower parasite burdens despite normal Th2 immune responses. We are now investigating the mechanisms that regulate the expression and balance of the two enzymes and the means by which each pathway impacts on parasite burden.

[2]Esser von Bieren, J., *et al.*, PLoS Pathog, 2013, 9(11): e1003771.

REPEATED ALBENDAZOLE TREATMENT ALLEVIATES HELMINTH-INDUCED IMMUNE HYPORESPONSIVENESS IN A HOUSEHOLD-BASED RCT IN INDONESIA

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In cross-sectional studies, chronic helminth infections have been shown to be associated with cellular immune hyporesponsiveness, which can also affect immune responses to unrelated antigens, mediated by regulatory cells and molecules.

To disentangle the impact of helminths on the immune system from other influences, we conducted a randomized controlled deworming trial in a geohelminth-endemic area in Flores, Indonesia. We assessed cellular immune responses in 1059 subjects, at 9 and 21 months after three-monthly treatment with albendazole or placebo. In a subset of the population, proportions of regulatory T-cells (Treg; $CD4^+CD25^{hi}FOXP3^+$) and regulatory molecules CTLA-4 and PD-1 in peripheral blood were measured by flow cytometry.

This intensive treatment resulted in significant increases in *Ascaris*-specific IL-2 responses and in malaria-specific TNF and IFN-γ responses. The frequency of T-reg cells did not change after anthelmintic treatment. However, the expression of inhibitory molecule CTLA-4 on $CD4^+$ T-cells decreased significantly in albendazole-treated individuals, in line with the enhanced immune responses after deworming.

This trial provides evidence for the ability of geohelminths to downregulate immune responses to bystander antigens, which may help to understand the detrimental and the possible beneficial effects of these parasites in rural areas of the world.

REGULATORY T CELL DEVELOPMENT DURING HELMINTH INFECTION

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Switching the immune system OFF is arguably as important as switching the immune system ON. Although multiple negative feedback loops exists within innate and adaptive effector immune responses, specialized regulatory cells are an essential non-redundant division of the adaptive T cell repertoire, termed regulatory T cells (T_{REG}). T_{REG} cells critically prevent the development of autoimmunity and calibrate the magnitude of de novo responses to foreign antigens.

It has been widely reported that negative feedback loops and immuno-regulatory pathways are over-activated during helminth infection, preventing the development of a proficient anti-helminth immune response. These observations led to the hypothesis that chronic helminth infections hijack these inhibitory pathways for their own survival; a hypothesis supported by several studies identifying the induction of T_{REG} cells by helminth-derived products.

Several years ago, we identified the expansion of T_{REG} cells during infection with the blood-dwelling trematode, *Schistosoma mansoni*, or the intestinal nematode, *Heligmosomoides polygyrus*; both of which have the capacity to inhibit allergen-reactive Th cell-mediated airway inflammation. These studies, along with many others, suggested that helminth-elicited regulatory responses contribute to the inverse relationship between chronic helminth infection and allergic and autoimmune diseases. We have since embarked on multiple lines of investigation interrogating 1) The molecular make-up of T_{REG} cells during helminth infection identifying an important role for microRNA-182 (miR-182) in Th2-associated T_{REG} cells; 2) The functional plasticity of T_{REG} cells during proficient anti-helminth immunity; 3) The ability of T_{REG} cells to inhibit Th cell-mediated inflammation employing a non-cell-autonomous gene-silencing mechanism.

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IMMUNE - EPIDEMIOLOGY OF HUMAN HELMINTH INFECTIONS

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In the rural areas of the developing countries, parasitic infections in general and helminth infections, in particular, are highly endemic. These infections are associated with profound changes to the immune system, namely the expansion of TH2 type immune responses and an increase in regulatory T and B cells. These immune profiles are thought to affect responses to unrelated antigens and affect disease outcomes. For example, helminth infections are often negatively associated with allergic responses and it is thought that in areas where helminths and malaria are co endemic, the responses to malaria antigens may be altered. In order to go beyond association studies, we conducted a placebo controlled anthelminthic drug trial and assessed immunological responses to allergens and malaria antigens. Albendazole was given once every 3 months for 21 months which reduced Necator americanus and Ascaris lumbricoides in the population but not Trichuris trichiura and resulted in a significant reduction in total IgE. The cellular immune responses were altered by treatment, in particular responses to malaria antigen. There was a borderline effect on skin prick test reactivity to allergens and a temporary significant increase in malaria parasitemia. These results will be discussed in relation to future studies.

HELMINTHIC INFECTIONS INCREASE SHORT-CHAIN FATTY ACID (SCFA) LEVELS AND MODULATE INFLAMMATORY RESPONSES

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It was shown that infection with intestinal helminths result in significant changes in the gut microbiota. Interestingly, helminths and the gut microbiota impact on and share metabolic pathways with their mammalian hosts. Thus enabling both the helminths and the microbiota to influence their host immune responses by intestinal metabolites. Here, we investigate if the intestinal helminth *Heligmosomoides polygyrus bakeri* (Hp) changes any intestinal metabolites and if those changes might contribute to the known immunomodulatory capacities of Hp.

First, we observed that chronic Hp infection increased cecal levels of the SCFAs acetate and butyrate. SCFAs are known end products of fermentative metabolism of complex plant polysaccharides by intestinal bacteria. Second, we found that Hp infection ameliorates the severity of house dust mite (HDM)-induced allergic airway eosinophilia in wild-type mice, as did the treatment with acetate or butyrate by itself. Thus, we hypothesized that altered SCFAs levels after Hp infection might contribute to the amelioration in the HDM model. SCFAs show a high binding affinity for the mammalian G protein coupled orphan receptor (GPR) 41. GPR41$^{-/-}$ mice did not show similar amelioration in the HDM model as observed in wild-type mice. These results indicate that increased SCFA levels, mainly acetate and butyrate, resulting from chronic Hp infections may modulate the immune response of their hosts to inflammatory diseases.

Next, we want to unravel the source of the SCFAs, as it was shown that helminths could also produce acetate, and the potential of the changed microbiota by it self to modulate inflammatory diseases.

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