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ABSTRACTS

SESSION V

Physiology and immunology of the host-parasite relationship

Heligmosomoides polygyrus infection alters percentage of CD11b+Gr1+ cells in mice

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Immunosuppressive mechanisms provoked by nematode infections in the host are not yet well understood. Some have already been identified; these include regulatory T cells, regulatory B cells and alternatively activated macrophages and related regulatory cytokine *milieu*. Recently, a new population of suppressor cells has been suggested to play a role in the escape of the parasite from the host immune response; myeloid derived suppressor cells (MDSCs), a population of granulocyte immature progenitors, dendritic cells, macrophages and other myeloid cells. Although heterogeneous, they share the ability to suppress activated T and B cells. Murine MDSCs are characterised by monocyte morphology, an immature state and the presence of CD11b and Ly6G/Ly6C (recognised by Gr-1 monoclonal antibody) markers. Cells with such characteristics are present in steady state in the bone marrow, where they constitute a myeloid reservoir and differentiate into granulocytes, monocytes or dendritic cells when needed. In pathology, these cells migrate to secondary lymphoid organs, their differentiation is blocked but activation occurs. They mediate their tolerogenic effects by cytokine production, reactive oxygen species secretion and manipulation of L-arginine metabolism, which alters the functions of cells associated with both adaptive and innate immunity. First observed in a cancer environment, this cell population has now been shown to appear during infections with tapeworms, which makes MDSCs a new player in parasite-induced immunosuppression.

The aim of the study was to establish whether *H. polygyrus* infection in mice is associated with the activation and response of MDSCs. Mice infected with *H. polygyrus* were studied at 6 and 21 days post infection (dpi) and cells double positive for CD11b and Gr-1 markers were identified in the peritoneum, blood, spleen and bone marrow. The percentage of CD11b+ Gr1+ cells was found to change after infection; the number of these cells was diminished in the blood and peritoneum, but increased in the bone marrow. These results may suggest that cells of MDSCs characteristics are recruited to the site of infection (the intestine) where they mediate their suppressive effect. Research aimed at confirming the presence of immune regulation by MDSCs in the intestine is in progress.

This study was funded by a grant of the National Science Centre no. 2012/05/N/NZ6/1028.

A proteomic analysis of *Heligmosomoides polygyrus* antigens

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Most parasitic nematodes induce a state of immunosuppresion to promote their survival within the host. Nowadays, there are many studies aimed at identifying the parasite-derived products that mediate this effect. Both structural or secreted proteins of different nematode stages might cause immunomodulation. In our previous studies, we observed that several fractions of somatic antigen of the adult stage of *H. polygyrus* differentially affected the apoptosis of lymphoid cells. It is likely that larvae and adult stages may use similar factors for immunoregulation in the host. In this study, we characterised excretory/secretory products and somatic extracts of *H. polygyrus* larvae and adults, a parasitic nematode of mice that elicits strong immunosuppression.

Somatic antigens from different stages of the nematode (adult males and females, L4 larvae, eggs) and excretory-secretory (ES) products of adult worm were analysed with HPLC using a ProteinPak column, which separates proteins based on molecular size. Fractions characteristic of particular stages were identified. We showed that protein profile differs depending on the life form of the worm, both qualitatively and quantitatively. We observed peaks present in all isolates and those that were present only in the one life stage. We established that the somatic antigen of L4 larvae resembles that of adult males, whereas adult females contain unique fractions that are not present even in egg isolate. In the case of ES products some peaks were observed to be the same as in the somatic antigen but also noticed changes in the composition of the medium in which the worms were cultured.

The presented results constitute an introduction to identification and characterisation of parasitic proteins that may mediate immunosuppression. The exact effect of different fractions from different stages of the nematode on the activity of immune cells will be studied.

Evaluation of IL-6 in patients with toxoplasmosis

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Toxoplasma gondii is an obligate intracellular protozoan parasite. IL-6 is a critical pro-inflammatory cytokine involved in inflammatory response, which may be present during infection with variety organisms, including *T. gondii.*

The aim of this study was the evaluation of IL-6 level in the serum of patients with acute and chronic phase of toxoplasmosis and to compare of IL-6 concentration in patients and healthy controls.

The examined group consisted of 22 patients with toxoplasmosis (6 women and 16 men); 12 patients were in the acute and 10 in the chronic phase of the disease, 13 persons were included in the control group (12 women and one man). IL-6 concentration was assessed in blood samples in ELISA (Human sIL-6 instant ELISA, eBioscience).

The highest level of Il-6 was observed in the group 1, with an acute phase of toxoplasmosis (41.45 pg/ml), lower in the group 2, with a chronic phase of the disease (19.24 pg/ml) and lowest in the control group (7.56 pg/ml). The differences in the level of IL-6 between group 1 and the control group, and between group 2 and the control group were statistically significant (respectively $p<0.000$ and $p<0.013$).

Results indicate the presence of increased production of cytokine Il-6 in patients infected with *T. gondii.*

The pattern of TEM alterations in the lung parenchyma during *Trichinella spiralis* or *Toxocara canis* infection

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Trichinella spiralis and *Toxocara canis* have a tissue-migratory larval stage, which passes through the lung microvascular system on the way to skeletal muscles or different tissues of host bodies. Both *T. spiralis* and *T. canis* induce many alterations in the lung parenchyma.

The purpose of this report is to describe the pattern of changes at the electron microscope level in the lung parenchyma of mice experimentally infected with *T. spiralis* or *T. canis*. The aim of the studies was to evaluate the pattern of lung histopathology resulting from mechanical damage provoked by larvae, or caused by a local inflammatory reaction evoked by antigens deposited during larval migration through the lung.

The lung tissue samples were fixed with a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 20 hrs. The tissue sections were additionally postfixed in 1% OsO_4 and 0.8% K_4FeCN_6. After dehydration in ethanol and infiltration with propylene oxide, the lung parenchyma samples were embedded in Spurr resin (Polysciences LTD. USA). Ultrathin sections (~ 50 nm) cut with an ultramicrotome were examined using a JEM 1200 EX transmission electron microscope (TEM).

Our ultrastructural studies demonstrate that the tissue-migratory *T. spiralis* larval stage evoked mainly destruction of type I epithelial cells, disintegration of the lamellar bodies of epithelial-type cells or the extracellular alveolar lining layer, depending on the number of infective larvae present (400 or 800 *T. spiralis* larvae) probably as a result of mechanical damage to the lung parenchyma. However, infection with *T. canis* larvae was found to initiate mainly eosinophilic pervasculitis and vasculitis, as well as macrophage accumulation as a result of an inflammatory reaction, which were additionally impacted by a large number of crystalloid inclusions in the macrophages.

Heligmosomoides polygyrus larvae exposed to triterpenoid saponins differentially activate a dendritic cell line

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The pathways of immune response strictly depend on the parasitic antigens; larval factors secreted by excretory/secretory glands and cuticle are sampled and processed by innate immune cells including dendritic cells (DC). After activation, DCs produce cytokines and intensively-expressed antigen-presenting molecules such as (MHC-II) and co-stimulatory molecules belonging to the cluster of differentiation e.g. CD80, CD86 and CD40. Antigen presentation by immature DCs induces immune tolerance, whereas mature DCs induce antigen-specific protective immunity through polarization of Th1 immune responses.

H. polygyrus larvae were cultured on agar with *C. officinals* or *B. vulgaris* triterpenoid saponins (glucuronides of ursolic and oleanolic acid, GlcUAOA) and ethanol as a control. Infective L3 larvae were harvested and co-cultured with an immortalized and immature JAWS II dendritic cell line. The production of cytokines was evaluated in culture after 48 hours. The expression pattern of receptors and the percentage of activated cells were measured by flow cytometry. The expression of MHC II receptors was enhanced in cell populations cultured with larvae. Although CD40 receptor expression increased, the percentage of CD40-positive cells halved when activated by larvae exposed to GlcUAOA. Larvae exposed to ethanol promoted cell death; the percentage of CD40 positive cells reduced to a third. The percentage of CD86-positive cells doubled in all cell populations, and enhanced expression of CD86 coexisted with CD80 receptors in cells cultured with larvae exposed to *C. officinalis* GlcUAOA. Larvae exposed to *C. officinalis* GlcUAOA more effectively activated JAWSII cells toward Th1 related response than larvae exposed to *B. vulgaris*. Larvae cultured on agar with ethanol induced production of proinflammatory cytokines such as MCP-1, TNF-a, IL-1b and IL-6. Interestingly, larvae developed in a medium with *Beta vulgaris* GlcUAOA inhibited production of MCP-1 and IL-1b and larvae cultured with *C. officinalis* inhibited production of IL-1b and TGF-b. Unprimed cells produced very low levels of cytokines and strongly responded to LPS stimulation. Triterpenoid saponins impair immunogenicity of parasitic nematodes. *H. polygyrus* larvae developed in agar supplemented with ethanol, and GlcUAOA of marigold or beet root may acquire antigens which induce different patterns of receptor expression and cytokine production.

Heligmosomoides polygyrus antigens affect expression of apoptosis related proteins

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There is growing evidence that nematodes prevent some immune-mediated diseases. The development of immunologically well-defined laboratory models of intestinal nematode infection has allowed significant advances to be made in understanding the immunological basis of effector mechanisms operating during infection under controlled laboratory conditions. The *Heligmosmoides polygyrus* – mouse system is used for studies of parasite immunomodulation. *H. polygyrus* causes a chronic, asymptomatic intestinal infection and effectively maintains both local and systemic tolerance to reduce allergic and autoimmune inflammation during *colitis* and experimental autoimmune encephalomyelitis. However, exposure of mice to *H. polygyrus* antigen induces spontaneous and glucocorticoid-induced apoptosis of CD4-positive T cells in mesenteric lymph node (MLN). The survival of activated CD4 T cells in infected mice is strictly dependent on inhibitors of apoptosis Bcl-2 and FLICE-like inhibitory protein (FLIP) overexpression, which are transcriptionally regulated by the transcription factor NF-kB. Upon stimulus by *H. polygyrus* antigens NF-kB is released and translocated to the nucleus, where it regulates gene transcription.

To explore the functional changes and possible oncogenic potential of *H. polygyrus* antigen fractions, we evaluated the long term proliferation, cytokine secretion, cell cycle progression and expression of apoptosis related genes such as tumor suppressor p27*Kip1*, survivin, caspases, Bcl-2/Bax protein, cyclin D1 and P-glycoprotein in MLN CD4 T cells of uninfected and *H. polygyrus* infected mice *ex vivo* and *in vitro* after restimulation with parasite excretory secretory antigen (ESAg), somatic antigen (SAg) and fraction 9 (F9Ag) of somatic antigen.

H. polygyrus antigens affect the intrinsic pathway of apoptosis. We found that proliferation provoked by fraction 9 and the inhibition of apoptosis was dependent on a low Bax/Bcl-2 ratio, dramatic upregulation of survivin, D1 cyclin, P-glycoprotein, and loss of p27*Kip1* protein with inhibition of active caspase-3 but not caspase-8. Therapy with living helminths appears to be effective in several immunological diseases but has disadvantages. Some of the nematode molecules may modify host-cell homeostasis and increase the risk of malignant transformation or susceptibility to oncogenic factors.

The effect of an extremely low-frequency magnetic field (elfmf) on larvae production in the parasite-host system: *Fasciola hepatica* L. – *Lymnaea truncatula* L.

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The biological effect of magnetic fields depends on many conditions, including the radiation dose and the sensitivity of the studied organisms. In this study, we aimed to find how exposure of the fluke *Fasciola hepatica* and the host snail *Lymnaea truncatula* to an extremely low-frequency magnetic field (ELFMF) in various stages of development of the parasite (i.e. during the embryogenesis and larval stages in the intermediate host) influences the production of *F. hepatica* larvae. *F. hepatica* eggs were incubated in tap water at 24°C for 14 days. *Lymnaea truncatula* snails were raised in the authors' own culture according to Taylor and Mozley (1948). Each snail was exposed to contact with 5 miracidia. The experimental cultures of *F. hepatica* eggs and *F. hepatica*-infected *L. truncatula* snails were placed under an extremely low-frequency 50 Hz magnetic field of 2 mT, produced by a solenoid connected to a power network. The infected snails were divided into 4 groups, 10 specimens each. The snails in groups I and II were infected with miracidia obtained from the control cultures, while the snails of group III and IV were infected with miracidia reared from eggs that had been incubated in the ELFMF. After infection, the snails of groups II and IV were placed within the ELFMF, while those of group I and III – outside the ELFMF. On the 36th day post infection (dpi) the number of larvae (cercariae and metacercariae) obtained from each snail were examined. The number of metacercariae encysting on the walls of crystallizers in which the snails lived were counted at 35 and 36 dpi in each group of snails.

The conducted studies showed varied effects of exposure of the host and parasite to ELFMF. Exposure during embryogenesis (group III) stimulated the production of larvae, while exposure of snails in which larval development took place (group II) had a lesser effect. No stimulating effect of an ELFMF on larva production was observed for *F. hepatica* both during embryogenesis and the subsequent larval stages in the snails (group IV).

The nature of the commensal intestinal microbiota during hymenolepidosis in rats

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Hymenolepis diminuta is a tapeworm of the small intestine of rodents (mostly mouse and rat) as well as other mammals including humans [1]. This non-tissue invasive parasite-host interaction is characterized by alternations in host motility [2]. The gastrointestinal epithelium is exposed to intestinal commensal microflora, and also to intestinal pathogens and their metabolites [3].

The aim of this study was to describe the composition and amount of commensal intestinal microbiota in rats infected with *H. diminuta*.

The jejunum and colon of infected rats contained Gram-negative bacteria (*E. coli*), Gram-positive bacteria (*Enterococcus, Streptococcus, Staphylococcus, Bacillus, Lactobacillus*) and *Candida*. The total number of intestinal bacteria was higher in *H. diminuta* infected rats than in the control rats.

In this study, we observed changes in the composition of the intestinal microbiota rats infected with *H. diminuta*.

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Analysis of expression of Toll-like receptor (TLR2 and TLR4) genes in small and large intestines during hymenolepidosis in rats

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Toll-like receptors in the gastrointestinal tract can influence intestinal homeostasis and play a role in the repair and restitution of the intestinal epithelium following tissue damage. In our previous study on rats in early stages of hymenolepidosis, a statistically significant increase in the level of TLR4 and TLR2 gene expression was observed [1]. Moreover the immunopositive cell number and the intensity of immunohistochemical staining, indicating the presence of TLRs within intestinal epithelial cells, increased over the infection period.

In this study, we determined changes in the expression of toll-like receptor genes TLR 2 and 4 in rats infected with *Hymenolepis diminuta*. In the isolated jejunum of infected rats at 16 days post infection (dpi), mRNA for TLR4 and TLR2 were expressed significantly more strongly in comparison with the uninfected rats. In the colon, significantly increased expression of gene TLR2 was observed from 16 dpi to 40 dpi, and gene TLR4 from 16 dpi to 60 dpi.

Toll-like receptors play a role in maintaining epithelial barrier functions in response to enteric pathogens and parasites. In our study, the alteration of TLR2 and TLR4 expression in the infected rats supports the potential implication of an innate immune system in the pathomechanism of this infection.

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Remission of experimental autoimmune encephalomyelitis in mice infected with *Heligmosomoides polygyrus*

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Multiple sclerosis (MS) is a chronic inflammatory disease that affects the central nervous system. Pathogenesis of the disease is caused by CD4 lymphocytes and macrophages hyperactive to myelin. The ongoing inflammation results in damage to neurons and axons. Animal model of neurodegenerative disease is an experimental autoimmune encephalomyelitis (EAE). Both MS and EAE are associated with elevated production of proinflammatory cytokines in the blood and cerebrospinal fluid (CSF); during relapse of MS, changes in the level of regulatory cytokines are observed. It is also that low dose naltrexone therapy (LDN) inhibits MS symptoms, what suggests that endogenous opioid could be involved in the immunergulation. In our earlier studies *Heligmosmoides polygyrus* reduced pathogenesis of ongoing inflammatory bowel disease (IBD) and EAE. The effect was enhanced between 2 and 6 days post infection when L4 larvae inhibited inflammatory reaction by mechanism associated with endogenous opioid production.

The aim of the study was to estimate changes in leukocyte populations associated with reduced EAE pathogenesis during histotropic phase of *H. polygyrus* infection. We used $C_{57}Bl_6$ mice with EAE, induced by 200 μg myelin oligodendrocyte glycoprotein peptide 35–55 emulsified in complete Freund's adjuvant containing 400 μg/ml of *Mycobacterium tuberculosis*. During experiment clinical scores of the disease were estimated in mice. After 22 days mice were infected with 300 L_3 larvae of *H. polygyrus*. Every 2 days blood smears were collected and 6 days post infection magnetic resonance was performed. With RT-PCR we examined expression of POMC and opioid receptors: MOR, DOR, KOR in leukocytes from the blood and CSF. With ELISA-test the level of: b-endorphin, dynorphin and enkephalin in the blood and CSF samples were measured. The reduction of EAE symptoms was observed 3 days post infection and these completely disappeared 6 days post infection. The inhibition was associated with changes in leukocyte populations and endogenous opioids production both in the blood and CFS. The inhibition of EAE 6 days post infection was confirmed by magnetic resonance.

The importance of immune and inflammatory responses during *Sarcoptes scabiei* infestation

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Sarcoptes scabiei infestation has been known throughout the centuries as an illness of humans and animals. Sarcoptic mange is most common among people of low economic status. In breeding animals *S. scabiei* infestation is usually observed at a low prevalence; however, over the decades scabies has become a serious plaque among some wildlife species, for example in foxes, wombats, and some ungulates, occasionally resulting in complete extermination of the infested population.

In humans sarcoptic mange causes irritation at localized areas (with the exception of Nordic scabies), but in animals it often leads to death. Pathological lesions that cause symptoms in fatal cases are not well recognized. During its initial successful development in the stratum corneum of the skin, *S. scabiei* may not be recognized by the host immune system. The substances produced by mites and materials that derive from the bodies of dead mites degraded by host enzymes affect inflammatory and immune responses after primary infestation.

Cells responsible for the pathological process of scabies are present in the skin and its vasculature. Post-capillary venule endothelial cells control cell diapedesis during the host's inflammation and immune responses. They are responsible for regulation of the migration of cells involved in immunosuppression during initial development and the following lack of inflammation.

The experiments done in vitro provided an insight into some important phenomena that occur during sarcoptic mange infestation. They are a very efficient method of physiopathological examinations.

Assessment of the levels of chosen cytokines in *giardiosis* and *toxoplasmosis*

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IL-12 stimulates T-cell cytotoxicity and NK cells, IFN-γ and TNF-α, inhibits secretion of IgE. IL-13 regulates antiparasitic response and acts on B cells. TNF-α is a major cytokine in inflammatory responses and activates cytotoxic reactions against protozoa. The study was carried out to evaluate the concentration of IL-12, IL-13 and TNF-α in patients infected with *Giardia intestinalis* before treatment (G1 – 75 patients) and 2 weeks after antiparasitic treatment (G2 – 45 patients), as well as in patients infected with *T. gondii* (T – 50 patients). Comparing all treatment groups and the control group (Table 1), the level of IL-12 was lower in G1, G2 and T. In patients infected with *T. gondii* IL-13 production exceeded fivefold the control level. Clearly, the antiparasitic activity of IL-13 in the course of *toxoplasmosis* can be observed. In the course of *giardiosis* IL-13 was also elevated and increased three times. However, the concentration of IL-13 in patients infected with *G. intestinalis* in both groups (before and after treatment) was comparable. It seems that the antiparasitic treatment does not change the level of IL-13. No change was observed in the production of TNF-α regardless of the type of parasite.

Table 1. The concentration of IL-12, IL-13 and TNF-α in *giardiosis* (G1-before antiparasitic treatment, G2 – after treatment) and in *toxoplasmosis* (T)

Parameter [pg/ml]	**IL-12** Mean +/- SD Median	**IL-13** Mean +/- SD Median	**TNF-α** Mean +/-SD Median
Study group G1 N=75	X= 63.15 ± 25.57 M=63.5	X= 45.25 ± 27.44 M=38.2	X= 1.09 ± 2.24 M=0.5
Study group G2 N=45	X= 62.43 ± 39.24 M=46.3	X= 49.46 ± 33.90 M=37.8	X= 0.71 ± 1.00 M=0.4
Study group T N=50	X= 88.82 ±23.62 M=57.85	X= 67.26 ± 27.31 M=64.6	X= 0.85 ± 1.62 M=0.4
Control group C N=30	X= 98.16 ± 26.24 M=94.8	X= 13.12 ± 5.65 M=12.4	X= 0.88 ± 0.49 M=0.9
P	**G1:C p<0.001* G2:C p<0.01***	**G1:C p<0.001* G2:C p<0.01* G1:T p<0.001* T:C p<0.001***	**G2:C p<0.05* T:C p<0.05***

The antiparasitic activity of *Calendula officinalis* triterpenoid saponins against *Heligmosomoides polygyrus* L3 stage larvae

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Several products of plants have been getting more attention because of their anti-parasitic activity. *Calendula officinals* is known to be effective as an antibacterial, antifungal and anticancer agent. It is likely that such a broad range of activity by the marigold relies on apoptosis deregulation. In our previous studies, *C. officinalis* triterpenoid saponins were found to have very strong anti-nematode activity against the L3 stage of *H. polygyrus*. In the present study, we examine the alternation evoked by marigold glucuronides of ursolic and oleanolic acid (GlcUAOA) in the integument and muscle cells of L3 *H. polygyrus.*

The structural changes induced in the larvae were assessed using Transmission Electron Microscopy. The cross and longitudinal sections of the whole larvae were compared and analysed. Changes were observed in the ultrastructure of cuticle, hypodermis, muscle cells, nuclei and mitochondria.

In larvae cultured with ethanol, the hypodermis was seen to have shrunk away from the cuticle. The space between the exo- and endo-cuticle was mostly expanded, more lucent than in control larvae and the cuticle was swollen in a few places. Aggregated and condensed heterochromatin appeared in the nucleus of the hypoderm cells. Compared with the controls, mitochondrial disruption was also observed with dilation of the mitochondrial cristae. In the control larvae, the arrangement of myofibrils was regular and parallel to the long axis of the muscle fiber, but after ethanol treatment, the myofibrils were irregularly shifted and discontinuously arranged. In larvae treated with GlcUAOA, the layers of the cuticle adhered tightly and changes in the position of cuticle were observed. The nucleus of hypoderm cells was destroyed, with changes similar to apoptosis. The mitochondria were very large and swollen with amorphous matrix and prominent lucent vesicles; the mitochondrial cristae were disintegrated and deleted. The regular arrangement of myofibrils was completely lost; in one muscle cell, the myofibril orientation was both diagonal and transverse patterned. Additionally, the nucleus of the muscle cell was shrunken and the chromatin was strongly condensed. The cytoplasm of muscle cells was vacuolated and filled with prominent myelinated structures which were probably mitochondria under autophagy.

The antiparasitic activity of *C. officinalis* was reflected in the hardly affected ultrastructure of *H. polygyrus* L3 stage larvae.

Modulation of the immune response by Baikal skullcap root (*Scutellaria baicalensis* Georgi) in mice infected with *Trichinella spiralis*

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Baikal skullcap (*Scutellaria baicalensis* Georgi) is a popular traditional Chinese herb widely used for the treatment of various inflammatory diseases in Asia, but is still a little-known plant in Europe. The high level of physiological and therapeutic activities of skullcap root are caused by the presence of almost 70 flavonoids, of which the most abundant are wogonin, baicalein and baicalin, and their glycosides (mainly glucuronides). These compounds are known as modifiers of inflammatory processes, having antibacterial, antiviral, antitumor and antioxidative properties.

The aim of this study was to investigate the influence of aqueous extracts of Baikal skullcap root on the local immune response during *T. spiralis* infection. CFW mice were infected with 200 larvae of *T. spiralis*/mouse. Aqueous baikal skullcap root extract was orally administered between 5th day prior to infection and 28th day post infection (dpi) with *T. spiralis*. The lymphocytes obtained from the spleen and mesenteric lymph nodes (MLN) on 7th, 14th, 21th, 28th dpi were counted and $CD4^+$ and $CD8^+$ subpopulations were analyzed by flow cytometry. The $CD4^+/CD8^+$ ratio and weight index of spleen and MLN was also calculated.

Aqueous baikal skullcap root extract exerted the stimulating effect on the subpopulation of $CD4^+$ lymphocytes in the spleen and MLN in *T. spiralis*-infected mice ($p<0.001$). The $CD8^+$ lymphocytes were stimulated by this extract only in the spleen ($p<0.05$). In MLN the $CD4^+/CD8^+$ ratio was higher in mice treated with skullcap root extract ($p<0.001$). Significant $CD4^+$ and $CD8^+$ lymphocyte stimulation was observed in the spleen on the 14th and 21th dpi, whereas the MLN lymphocyte stimulation, which concerned only $CD4^+$ subpopulation, was noticed on the 7th and 14th dpi.

These results suggest that Baikal skullcap root extract stimulates murine cellular immune response during the intestinal phase of *T. spiralis* infection.

Modelling parasite control in grazing animals

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Gastrointestinal nematodes cause disease and death in both humans and livestock. In the UK alone, nematodes cost the sheep industry about Ł100 million per year. Anthelmintics are commonly used, but parasites are developing resistance. A potential control method is selective breeding.

However, the response to selection for disease resistance cannot be predicted by purely genetic methods because culling infected animals reduces parasite transmission. Therefore, we created a dynamic, mechanistic model of the infection process in the sheep – *Teladorsagia circumcincta* system. This model was driven by the wealth of data on the genetic architecture of host resistance, the immunological mechanisms and pathological processes. Advanced Bayesian computation was used to fit means, variances, distributions and variance components such as heritability.

The results from this model support the view that the immune response is relatively simple, involving two main mechanisms and is under very strong genetic control. The model was then used to predict the response to selection. Selection for low FEC reduced the mean egg count to less than one-third of the starting values within 10 generations. The rapid reduction in faecal egg count is compatible with the results from experimental farms. In addition, we compared the response to selection on faecal egg count and parasite-specific IgA activity. Somewhat surprisingly selection on IgA activity was better at reducing faecal egg count than direct selection on faecal egg count itself. Although this result contradicts the widely held assumption that indirect selection is slower than direct selection on a trait itself, it is compatible with our understanding of the underlying biology.

This analysis indicates that the response to selection for parasite resistance will be relatively rapid and also that the protective immune response will provide better markers for diagnosis and control than parasitological measurements.

Evaluation of the cellular immune response of male and female rats vaccinated with cDNA encoding a phosphoglycerate kinase of *Fasciola hepatica* (cDNAFhPGK)

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Studies have noted gender associated differences in the course of immune responses following parasitic infections. However, there are not many studies that document sex-specific effects in vaccine efficacy and differences between the immune responses of males and females following immunization and infection. The overall objective of the present study was to characterize the immune responses of rats after vaccination with cDNA*Fh*PGK and subsequent challenge infection with liver fluke metacercariae, and to evaluate differences in immune response between males and females.

The trial involved 4 experimental groups (6 males and 6 females each). Prior to infection, animals from the 1st group were immunized with cDNA*Fh*PGK/pCMV, while animals from the 2nd group were immunized with an empty vector (pCMV); animals from the 3rd group were only infected, whereas animals from the 4th group remained non-immunized and uninfected.

Rats were bled on the infection day and at 2, 4, 6, 8 and 10 weeks post infection to collect blood samples which were subjected to flow cytometric analysis. Neutrophil, eosinophil, monocyte, CD4+ T cell and CD8+ T cell counts were estimated.

In vaccinated females the number of isolated flukes decreased by 52% when compared to the infection control group, whereas in vaccinated males 11% more flukes were recovered from the livers. Males and females differed in their monocyte, eosinophil and CD8+ T cell responses. Moreover, females exhibited lower counts of all above-mentioned cell populations in comparison with males.

It is evident that males and females respond differently to cDNA*Fh*PGK vaccination both in terms of vaccine effectiveness and the quality/quantity of the immune response. Since successful immunization can depend on gender more detailed immunological studies have to be carried out when designing a vaccine

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Study of the local immune response in the peritoneal fluid and lymph nodes of rats vaccinated with cDNA encoding a phosphoglycerate kinase of *Fasciola hepatica* (cDNA-FhPGK)

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Characterization of the local immune response in the peritoneal fluid and lymph nodes of rats after vaccination with cDNA*Fh*PGK and subsequent infection with liver fluke metacercariae was the aim of the following study. The vaccine trial involved 4 experimental groups (each containing 9 males). Prior to infection, animals from the 1st group were immunized with cDNA-*Fh*PGK/pCMV, while animals from the 2nd group were immunized with an empty vector (pCMV); animals from the 3rd group were only infected, whereas animals from the 4th group remained non-immunized and uninfected. We conducted three autopsies– at four days, four weeks and ten weeks post-infection, as those terms represent key-moments during fasciolosis (liver capsule penetration, acute and chronic phase, respectively). In order to determine changes in the peritoneal fluid cytology, the percentages of monocytes, neutrophils, eosinophils, basophils, and CD4+ and CD8+ lymphocytes were estimated. In the case of hepatic lymph nodes, analysis of CD4+ and CD8+ T cell percentages was conducted.

In males vaccinated with cDNA*Fh*PGK/pCMV or pCMV more flukes were recovered when compared to the infection control group, an 11% and 85% increase, respectively. In the peritoneal fluid the total number of recovered cells was highest 4 days after infection. We observed a robust neutrophil, eosinophil and CD8+ T cell response during the study in all immunized groups; alternatively, monocyte and CD4+ T cell percentages were lower. In the lymph nodes of vaccinated rats we also observed a lower percentage of CD4+ T cells, and higher CD8+ T cell percentages.

The cDNA*Fh*PGK/pCMV vaccine in males promoted an excessive inflammatory response which had a detrimental effect on the host, and was not successful in combating the infection. Heightened immunity led to more adverse events following vaccination.

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Molecular cloning and immunomodulatory effect of the cathepsins L3 from *Fasciola hepatica*

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Fasciolosis causes significant global problems in veterinary and human medicine. *Fasciola hepatica* is a liver fluke capable of infecting a wide range of mammals, including humans (2.4 million people infected and 180 million at risk). During host infections flukes express cysteine proteases, termed cathepsins, which play pivotal roles in parasite feeding, migration through host tissues and immune evasion. Expression of cathepsins L is developmentally regulated. Excystment of the infective larvae is dependent on FhCB and FhCL3 and together these enzymes account for over 80% of total protease activity in *F. hepatica* newly excysted juvenile (NEJ).

We focus on members of the cathepsin L gene family, belonging to the CL3 clade. The cDNA of two novel CL3 proteases – Fh-CL3-1 and Fh-CL3-2 were cloned. The inactive form of Fh-CL3-1 was obtained by mutation of the enzyme active site. The recombinant protein was expressed in *Escherichia coli* and *Pichia pastoris*. The recombinant FhCL3 proteins (rFhCL3) were purified by affinity chromatography.

In order to study the immunomodulatory properties of recombinant cathepsins, the human macrophage line (THP-1) was treated with purified recombinant Fh-CL3-1 and Fh-CL3-2 from *E. coli* and *P. pastoris* and inactive Fh-CL3-1 from *P. pastoris.* The results show the influence of these proteins on the profile of cytokines secreted by LPS-activated and not activated cells.

The assesment of *in vitro* immune response induced by the native antigens from *Fasciola hepatica*

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Fasciolosis is a significant problem in veterinary medicine since it causes economic losses to livestock production and also poses risk to human health. Disease control is currently based almost exclusively on drug treatment. However, this approach is not satisfactory as drug resistant parasites have appeared. Research on immunology of *Fasciola hepatica* infection is necessary so as to improve our knowledge about immunological mechanism evoked by the parasite and to develop new control strategies against liver fluke.

In this study we estimated the suitability of human macrophages line (THP-1) for preliminary testing of response against native parasite antigens. The cells were stimulated using excretory/secretory material from adult and NEJ stage. We assessed the TNFα, IL-1β, IL-6, IL-10 level upon stimulation. The results show the influence of these proteins on the profile of cytokines secreted by LPS-activated and not activated cells. We have also identified differences in the profile of secreted cytokines depending on the used excretory/secretory products.