The immunomodulatory capacity of helminths on inflammation: Impact of eosinophils on *E. coli*-induced sepsis and genome-wide transcriptome profiling of human monocytes stimulated with helminth extract and LPS implicate immune functions and diseases

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Erklärung

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Preamble

Nowadays, there are antithetic perceptions of the distribution of pathogenic organisms worldwide. Based on increased hygiene standards, parasitic infections are no longer considered as a common health problem in industrial nations. Instead, during the last decades allergies, autoimmune and metabolic disorders like diabetes are new representatives of our modern life. This situation is in part explained by the extended hygiene hypothesis. In industrialized countries, parasitic infections are mostly limited to children infected with head lice or pin worms and to domestic animals. However, parasitic infections represent enormous health problems in tropical and subtropical regions in Africa, South America and Asia. Thus, programs to eradicate different parasitic infections like lymphatic filariasis or onchocerciasis have been launched by the World Health Organization (WHO) to intensify the efforts in the development of new treatments on these long neglected tropical diseases.

Furthermore, helminth parasites modify the immune system to ensure their long-term survival and distribution in the hosts. This kind of mechanism is termed immunomodulation and comprises nearly all fields of immunology but also genetics. The immunoregulatory and immunogenetic mechanisms of the host protection but also the regulatory protective effects of helminth infections on inflammatory disorders are not fully understood yet.

Motivated by the fascinating contrary impact of helminth infections on inflammation this work is not limited to experimental results on the protective effect of eosinophil granulocytes against *E. coli* induced sepsis in mice. We also performed a genome-wide transcriptional profiling of human non-endemic monocytes to analyze the impact of a human pathogenic filarial crude antigen extract on the following LPS stimulation. We identified immunomodulatory effects that are linked to IL-17 driven diseases like psoriasis, arthritis or allergy but also atherosclerosis.

Therefore, the work of this thesis provides not only new insights to filarial host-pathogen-interactions but also more information on the protective effects and cutting points on inflammatory immune responses and genomics termed immunomics. Further investigations are needed to analyze the genetic impact on the expression profiles in response to helminth products that are necessary to personalize treatments.

Yours sincerely,

Benedikt Bürfent

Summary

Human filarial nematode infections cause lymphatic filariasis and onchocerciasis in more than 150 million people worldwide. An infection can result in debilitating clinical manifestations like visual impairment or lymphedema that lead to socioeconomic problems and is therefore listed by the World Health Organization for elimination. Principally, filariae modulate cellular immune responses for long-term survival in their host and this modulation of the host's immune response also dictates the occurrence of host pathology. Helminth infections and helminth-derived products can furthermore modify host immune responses to protect against inflammatory and autoimmune diseases. For instance, we previously provided evidence that infection with the rodent filarial nematode *Litomosoides sigmodontis*, a well-accepted model to study immune responses during filariasis, improves sepsis outcome without worsen immune paralysis and protects against autoimmune and type 2 diabetes.

Since eosinophil granulocytes are a hallmark of helminth infections we examined the impact of murine eosinophils against *Escherichia coli*-induced sepsis and the effect of the specific chemoattractants eotaxin-1 and 2 (CCL11 and CCL24) on the release of sepsis-relevant cytokines and chemokines of bone-marrow-derived eosinophils (bmEos). We identified eotaxin-2 (CCL24) to modify the interleukin (IL)-4 response after LPS, P3C and *L. sigmodontis* crude extract stimulation and to alter the expression of the eotaxin receptor C-C chemokine receptor type 3 (CCR3). The intercellular adhesion molecule 1 (ICAM-1) was not affected by CCL24 treatment. In vivo relevance of eosinophils during the course of a bacterial sepsis was demonstrated using eosinophil-deficient ΔdblGATA mice. Those mice had an exacerbated sepsis outcome with increased pro-inflammatory IL-6 and CXCL2 levels, worsened hypothermia and reduced numbers of adaptive and innate immune cells six hours after *E. coli* challenge compared to wildtype controls. Co-cultivation of bmEos and *E. coli* highlighted the potential to reduce of the bacterial burden in vitro via the formation of NET-like extracellular structures and phagocytosis. Initial results of a transcriptome-wide analysis of sorted SiglecF+ eosinophils revealed a *L. sigmodontis*-dependent but *E. coli*-independent effect on eosinophil modulation.

We furthermore investigated the immunomodulatory effect of the crude extract of the human pathogenic filaria *Brugia malayi* (BmA) on the transcriptome of purified human CD14+ monocytes derived from twenty healthy male European non-endemic volunteers. Based on a genome-wide transcriptional profiling more than 47.000 transcripts of monocytes stimulated with BmA, *E. coli* lipopolysaccharide ultrapure (LPS) or a successive stimulation of both were analyzed. Several genes were identified that were differentially expressed upon BmA priming before LPS re-stimulation including genes like PTX3↓, MMP9↑, CXCL5/ENA-78↑, CXCL6/GCP-2↑, CXCL7/PPBP↑, and CCL20/MIP3α↓. Quantification of CXCL5 and CXCL6 protein levels confirmed our results. Lower expression levels of HLA-DR and CD86 on BmA primed monocytes were further detected by flow cytometry. BmA-only stimulation reduced the frequency of apoptotic cells whereas pre-stimulation did not alter apoptosis after LPS stimulation. Accordingly, Ingenuity Pathway Analysis (IPA) © of the transcriptome of monocyte LPS responses in dependence on BmA-priming depicted associations in immune functions and inflammatory disorders like granulocyte and agranulocyte adhesion, diapedesis and atherosclerosis. Notably, BmA treatment affected several IL-17-dependent pathways like signaling in psoriasis, arthritis and allergic inflammatory airway diseases.

Thus, exposure of human monocytes and murine eosinophils to filarial extracts modifies inflammatory immune responses and affects the response to subsequent stimuli. This immunomodulation may not only impact the development of filaria-caused pathology in humans, but may also affect bystander immune responses that contribute to dysregulated immune responses as they occur during autoimmunity or inflammation during sepsis. Since earlier results indicate that specific gene variants are associated with the severity of pathology, genome-wide analysis of expression quantitative trait loci (e²QTL) will further point out regulatory gene variants that are correlated with differential expressed transcripts and may reveal new targets for treatment strategies against lymphatic filariasis and dysregulated immune responses.

Introduction

Parasites are pathogenic organisms that are evolved in different phylogenetic taxonomies with variable phenotypes and are *per se* creatures that live at the expense of a host. Most parasitic infections are a phenomenon of optimal adaption to the prevailing conditions, which allows their survival and distribution. In general, parasites can be divided by their lifestyles into the two groups of ectoparasites and endoparasites. Human parasitic infections present a major health problem and are under particular advertency of the World Health Organization (WHO). Human bacterial infections that lead to sepsis are furthermore a leading cause of critical illness and death worldwide. Analyzing immunomodulatory mechanisms from parasitic helminths may contribute to the identification of novel targets and strategies to eliminate filarial infections and to treat autoimmune and inflammatory diseases.

1.1 Human pathogenic parasites

In humans, two main groups of endoparasites are described that can induce pathology: protozoa (*Sarcodina* like *Entamoeba spp.*, *Mastigophora* like *Giardia spp. or Leishmania spp., Sporozoa* like *Plasmodium spp.*) and helminths like flatworms (*Platyhelminthes* including *Trematoda* (flukes) and *Cestoda* (tapeworms)), thorny-headed worms (*Acanthocephala*) and roundworms (*Nemathelminthes*). Common members of nemathelminths are rotifer (*Rotifera*), acanthocephalans (*Acanthocephala*), horsehair worms (*Nematomorpha*) and threadworms (*Nematoda*) (Lucius et al., 2017). Basically, protozoans comprise pathogens like *Leishmania tropica* (leishmaniasis), *Trypanosoma cruzi, T. brucei* (trypanosomiasis), *Toxoplasma gondii* (toxoplasmosis) as well as *Trichomonas vaginalis* (trichomoniasis) and infections can result in a strong humoral immune response of the host (Baron, 1996). Particularly, infections by *Plasmodium falciparum,* a member of *Plasmodiidae* that causes malaria, induce strong inflammation with non-cyclic fever and cerebral malaria with severe neurological symptoms that often result in death (Bartoloni and Zammarchi, 2012). In addition, *Entamoeba histolytica* infections can induce abscesses in the lung and the liver, diarrhea and ulceration (Baron, 1996). Helminth infections lead to chronic infections and fatal cases are e.g. described for infections by

Schistosoma spec. (schistosomiasis) and *Echinococcus multilocularis* (Burke et al., 2009; Eckert and Deplazes, 2004). Helminth is a medical term describing worms of different phylogenies with a multi-faceted phenotype. Infections with gastrointestinal helminths like *Ascaris lumbricoides* (ascariasis), *Trichuris trichiura* (trichuriasis) and hookworms belong to the soil-transmitted helminth infections and can cause diarrhoea, abdominal pain, malnutrition and anemia (Guyatt and Bundy, 1991; Mishra et al., 2014). Infections with filarial nematodes can cause severe pathologies like skin-disease and blindness (*Onchocerca volvulus* infection, onchocerciasis) or chronic lymphedema (*Wuchereria bancrofti*, *Brugia malayi* or *Brugia timori* infection, elephantiasis or hydrocele) (Taylor et al., 2010). Helminth infections often last over several years and dependent on the species, the pathogens can persist either in the gastrointestinal tract, blood, lymphatic system or subcutaneous tissues of their hosts.

Several arthropods are representatives of ectoparasites that can live temporarily or permanently on the host (Lucius et al., 2017). Blood-feeding ectoparasites serve as vectors for the transmission of endoparasites such as *Plasmodium* or filariae. Due to co-evolutionary adaption filarial nematodes like *Mansonella perstans* are closely adapted to their hosts and environments. This interdependency and host-specificity ensures long-time survival, reproduction and transmission and is based on a host-immunity manipulation termed immunomodulation. Accordingly, poorly adapted parasites like *P. falciparum* or *E. multilocularis* cause severe pathologies in humans. Parasitic infections are a major global health problem that may cause life threatening diseases, severe morbidity and chronic debilitating diseases.

1.1.1 Helminth-driven human diseases – lymphatic filariasis and onchocerciasis

More than 150 million people are infected worldwide by human pathogenic filarial nematodes causing lymphatic filariasis and onchocerciasis (WHO, 2016a, b). *Wuchereria bancrofti, Brugia malayi and Brugia timori* are transmitted by bloodsucking mosquitos causing lymphatic filariasis (LF) in approx. 120 million people. In addition, *Onchocerca volvulus* leads to approximately 30 million cases of onchocerciasis. Co-endemicity was reported also for *Loa Loa* and *Mansonella perstans* (Gardon et al., 1997; Keiser et al., 2003). Onchocerciasis mainly occurs in sub-Saharan Africa and LF is distributed in tropical and sub-tropical regions worldwide, representing a global health problem of common developing countries with a low economic standard and causing socioeconomic problems (Fig. 1). With an incidence of almost 90% *Wuchereria bancrofti* infections represent the most prominent cause of LF (Michael et al., 1996) especially in Africa but also in Middle and South America. *Brugia malayi* infections are described for south and south-west Asia that represent nearly 10% of all LF cases followed by *Brugia timori* infections, which are found in Indonesia.

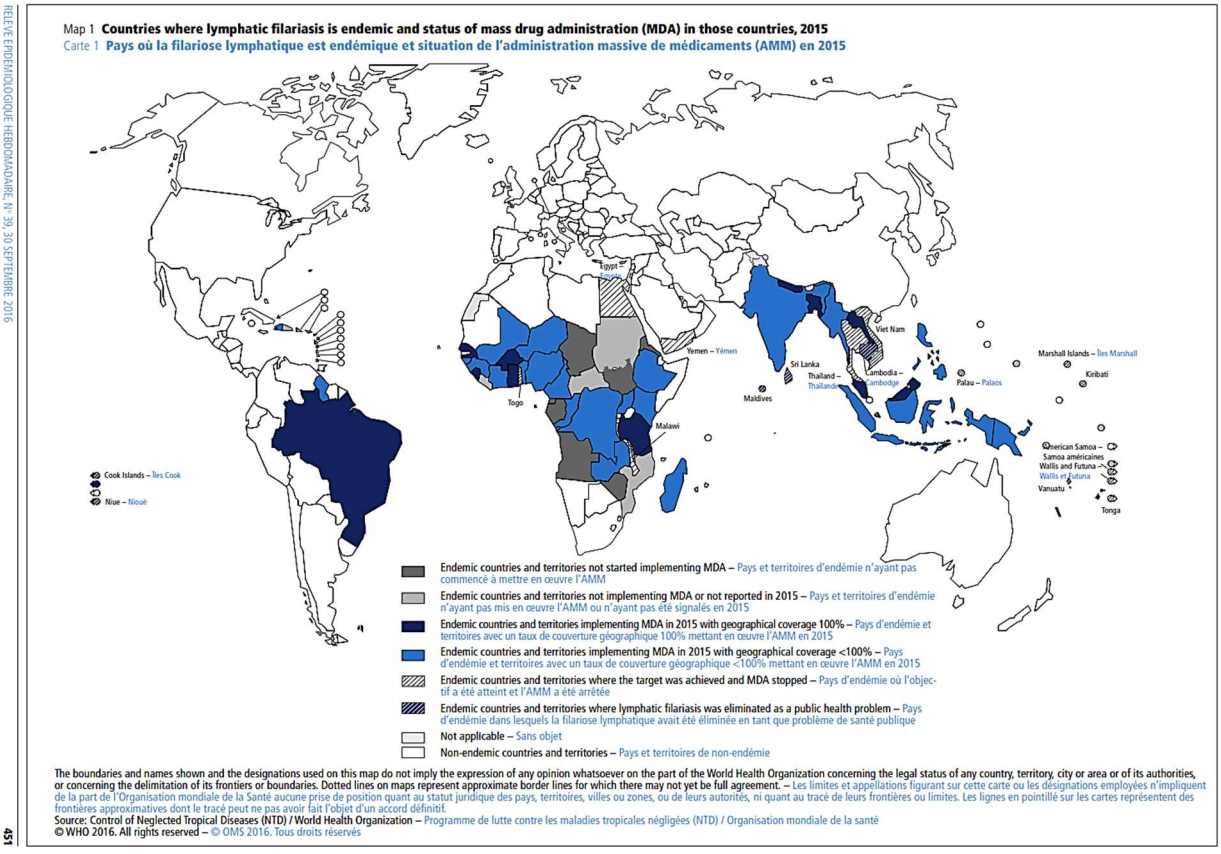

Figure 1: Global programme to eliminate lymphatic filariasis: *progress report, 2015. Countries where lymphatic filariasis is endemic and status of mass drug administration (MDA) is shown (WHO 2016).*

The life cycle of Wuchereria bancrofti *and* Brugia *spp. (Lymphatic filariasis)*

Published by Patrick Manson in 1878, infectious stage-3 larvae (L3 larvae) of *W. bancrofti* or *Brugia spp.* are transmitted to humans during the blood meal of mosquitos into humans (*Culicidae*; e.g. *Anopheles* spp. in Africa, *Culex* spp. in America and *Aedes* spp. in Asia and pacific areas) (Manguin et al., 2010; Manson, 1878). Following two molting steps the larvae become adult and persist in the lymphatics where they produce microfilariae (mf) and cause pathology. When the microfilariae reach the blood stream, mosquitos can take them up. Within the mosquito the microfilariae penetrate the midgut and migrate to the thoracic muscles, where they moult from stage-1 larvae (microfilariae) to infectious L3 larvae. Afterwards, they migrate to the proboscis of the mosquitos for transmission during the next blood meal (Fig. 2).

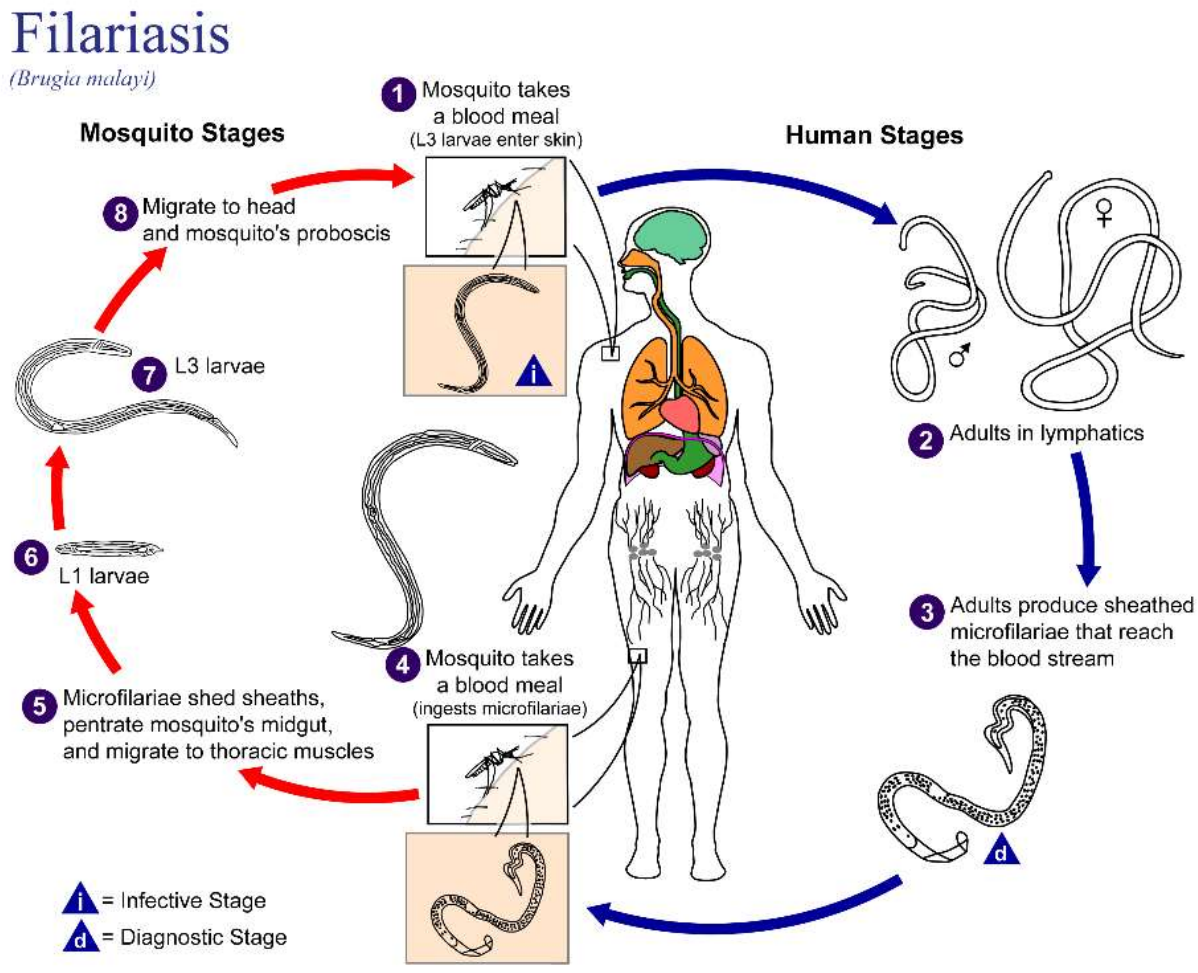

Figure 2:* Brugia malayi *life cycle as example for human filarial infection. *After L3 larvae transmission during the blood meal of mosquitos, the larvae become adult in the lymphatics and produce microfilariae that are transmitted with the next blood meal of the vector. Taken up by the vector, the microfilariae migrate to the thoracic muscles and become L3 larvae. The L3 larvae migrate to the mosquito's proboscis and infect the host during the next blood meal. Illustration by CDC/Alexander J. da Silva, PhD/Melanie Moser, 2002, ID# 3379.*

Infections with *Brugia* spp. and *W. bancrofti* can result in lymphedema pathology, termed elephantiasis, with a deformation of the lower extremities caused by adult filaria-induced dilatation of the lymphatics by e.g. matrix metalloproteinases (MMP) and disordered lymph fluency (Bennuru and Nutman, 2009). The majority of filarial nematodes, including *W. bancrofti* or *Brugia spp.* contain endosymbiotic *Wolbachia* spp. bacteria that promote inflammatory responses and thus pathogenesis in filarial nematode diseases (Nutman, 2013; Pfarr et al., 2009; Taylor et al., 2000). The filaria can also migrate to the lymphatics in the genitals of male patients resulting in hydrocele, the testicular disfiguration of the male genital tract. The life cycles of lymphatic filariasis and onchocerciasis are similar and infections often occur during childhood leading to severe disability of the adult patients. Consequently, infected patients often cause socio-economic problems in the endemic countries and are excluded from their society.

The life cycle of Onchocerca volvulus *(Onchocerciasis)*

In onchocerciasis infectious L3 larvae of *Onchocerca volvulus* are transmitted by blood-feeding blackflies (*Simulium* spp.). In contrast to LF, in *O. volvulus* infection the transmitted L3 larvae molt into adults in subcutaneous tissues leading to nodules, termed onchocercoma, that contain the adult filariae. Fertile female worms can produce microfilariae for approximately nine to fourteen years (Schulz-Key, 1990; WHO, 1995). Remarkably, chronic *O. volvulus* infections manifest clinically in dermatitis and chronic hyperreactive onchodermatitis (leopard-skin; sowda). The released microfilariae migrate to the sub-dermal layer of the host skin. During the next blood meal, the ingested microfilariae penetrate the midgut of the blackflies and migrate to the thoracic muscles and develop from first-stage larvae to L3 larvae. The pathology of Onchocerciasis is linked to the microfilarial stage. If microfilariae migrate from the nodules to the eye and die, a local inflammation occurs that can gradually lead to visual impairment and finally blindness driven by the disposal of *Wolbachia* spp. bacteria (Pearlman, 2003). As the transmitting *Simulium* flies require rivers for their development, onchocerciasis is always located close to rivers in endemic regions and is therefore termed as river blindness.

LF and onchocerciasis are classified by the WHO as neglected tropical diseases (NTDs). Beside LF and onchocerciasis, the NTDs also comprise worm infections like soil-transmitted helminthiasis caused by roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*), hookworm infections (*Necator americanus* and *Ancylostoma duodenale*), foodborne trematode infections (*Clonorchis sinensis*, *Fasciola hepatica* and *Fasciola gigantica)*, schistosomiasis (*Schistosoma* spp.), and echinococcosis caused by tapeworms (*Echinococcus granulosus* and *E. multilocularis*). Non-helminth-based diseases of NTDs include human African trypanosomiasis and Chagas disease (*Trypanosoma* spp.) and viral infections like dengue haemorrhagic fever and chikungunya infection. Several programs have been implemented to eradicate lymphatic filariasis and onchocerciasis by preventive chemotherapy through mass drug administration (MDA). Moreover, the WHO initiated the elimination of the distribution of lymphatic filariasis by the year 2020 (Ichimori et al., 2014).

Therefore, the Global Programme to Eliminate Lymphatic Filariasis (GPELF) has been started in 2000 to eliminate lymphatic filariasis in all 80 worldwide endemic countries (Ottesen, 2000). Up until the year 2014 the GPELF has delivered 5.6 billion treatments to over 763 million people (Turner et al., 2016). Because of an overlap of endemic areas for filarial diseases the annual or bi-annual MDA treatment options had to be adapted to prevent potential life threatening severe adverse events. Since disseminations of *Loa loa* and *O. volvulus* are limited to Africa, the MDA was conducted to diethylcarbamazine citrate (DEC) + albendazole (ALB) in endemic areas outside of Africa (de Kraker et al., 2006). Because of co-endemicity of lymphatic filariasis and onchocerciasis the MDA of ivermectin (IVM) + ALB was performed in Africa. Accordingly, severe adverse events had to be prevented in co-endemic areas for loasis (encephalitis promoted by treatment with DEC as well as IVM) and onchocerciasis (mazzotti reaction and blindness promoted by treatment with DEC).

Because lymphatic filariasis and onchocerciasis are vector-transmitted diseases, vector-control programs were additionally started (Bockarie et al., 2009; Hougard et al., 2001).

Accordingly, with the aid of the African Programme for Onchocerciasis Control (APOC), the Onchocerciasis Elimination Program for the Americas (OEPA) and the Onchocerciasis Control Programme in West Africa (OCP), the distribution of onchocerciasis in South America is largely eliminated (CDC, 2013). Initial successes were achieved in Africa shown by epidemiological evaluations (2008-2014) initiated by APOC. In 32 affected areas (25.4 million people) onchocerciasis has almost been eradicated by the annual ivermectin mass drug treatment (MDA) (Tekle et al., 2016). Interestingly, the treatment in 18 areas (17.4 million people) depict more years of treatment but in eight areas (10.4 million people) the treatment was in this period unsuccessful.

Furthermore, chemotherapy against LF and onchocerciasis targeting endosymbiotic *Wolbachia* is possible. The most common *Wolbachia spp.* are *Wolbachia pipientis*, *W. melophagi* and *W. persica* and are described for insects (*Arthropoda*), spiders (*Chelicerata*) and most human pathogenic filarial nematodes except e.g. *Loa loa (McGarry et al., 2003).* Furthermore, *Wolbachia* endosymbionts are not confirmed for all *Mansonella* spp. (Gehringer et al., 2014; Grobusch et al., 2003). Interestingly, *Wolbachia* spp. are transferred to the ovule of the next generation of the host organism via vertical transmission and are essential for filarial fertility and survival (Hoerauf, 2008). As a consequence, depletion of *Wolbachia* spp. with antibiotics leads to sterile filariae and thus prevent transmission of filarial infection. Furthermore, anti-*Wolbachia* therapy is the only safe treatment that has a macrofilaricidal effect, i.e. kills the adult worms (Hoerauf et al., 2008; Hoerauf et al., 2000). Notably*, Wolbachia* spp. constitute ligands e.g. for Toll-like-receptor (TLR) 2 and 4 as well as the nucleotide-binding oligomerization domain containing 1 and 2 (NOD) (Ajendra et al., 2016b; Brattig et al., 2004; Pearlman, 2003). Thus, *Wolbachia* spp. trigger activation and recruitment of host immune cells expressing these receptors, e.g. neutrophils, eosinophils and macrophages (Tamarozzi et al., 2011).

1.1.2 Immunopathogenesis of human lymphatic filariasis and onchocerciasis

The interplay between filariae and the human host may induce immunopathology. The severity of the pathology is dependent on the ratio between pro-inflammatory and anti-inflammatory responses, the level of immunomodulation induced by the parasite and the genetic background of the host. In *O. volvulus* infection, suppressive CD4+ T cells producing interleukin (IL)-10/ transforming growth factor (TGF)β and expressing elevated levels of cytotoxic T-lymphocyte-associated Protein (CTLA)-4, known as cluster of differentiation (CD)152, expand and are associated with reduced pathology (Doetze et al., 2000; Satoguina et al., 2002). In onchocercomas of hyporeactive patients altered TGF-β1 expression of T cells, platelets, plasma/B cells, macrophages and increased levels of IgG4 are mediating immunosuppression and inducing microfilariae tolerance (Adjobimey and Hoerauf, 2010; Korten et al., 2009; Korten et al., 2010). In contrast, in hyperreactive human onchocerciasis reduced levels of TGF-β1 and IgG4, increased HLA-DR expression and elevated type 2 immune responses including increased numbers of eosinophil granulocytes result in severe dermatitis and lymphadenitis, but promote defense against parasites (Korten et al., 2011).

Moreover, *O. volvulus* infected patients with the genetic IL-13 variant Arg110Gln have increased levels of IL-13, which is associated with a higher risk to develop hyperreactive onchocerciasis (Hoerauf et al., 2002). In hyperreactive patients type 2 immune responses and Th17 immune responses are increased, whereas regulatory T cells are reduced (Katawa et al., 2015). Furthermore, in lymphatic filariasis the TGF-β1 Leu10Pro polymorphism leads to reduced levels of TGF-β1 and is associated with a lack of microfilariae in the blood of the infected patients (Debrah et al., 2011). Interestingly, polymorphisms in plasma Vascular Endothelial Growth Factor-A (VEGF-A) are associated with an increased risk of developing hydrocele (Debrah et al., 2007; Pfarr et al., 2009). Moreover, effector CD4+ and CD8+ T cells are discussed to trigger the development of the pathogenesis in lymphatic filariasis and onchocerciasis by supporting pro-inflammatory Th1, Th9 and Th17 type immune responses (Anuradha et al., 2014; Babu et al., 2009a; Babu and Nutman, 2014).

Human monocytes mediate an essential function in the immunopathogenesis during filarial infection. An alternative activation of those monocytes with an increased expression of arginase-1 (ARG1) and mannose receptor C type 1 (MRC1) was shown in asymptomatic *B. malayi* infected individuals (Babu et al., 2009b). Monocytes of asymptomatically infected people develop a regulatory and anti-inflammatory phenotype with increased anti-inflammatory TGFβ and IL-10 expression and decreased pro-inflammatory IL-12 and IL-18 expression, as was described after re-stimulation with *B. malayi* crude extract (BmA) (Babu et al., 2009b). Furthermore, the lysate of *B. malayi* microfilariae can modulate the cytokine and chemokine production of naïve monocytes and suppress adaptive and innate immune responses by the expression of regulatory PD-L1 (CD274) and IL-10 as was shown by *W. bancrofti* asymptomatically infected patients (O'Regan et al., 2014). Subsequently, IL-10 induces microRNA-187 that negatively regulates tumor necrosis factor (TNF), IL-6, and IL-12p40 production in TLR4-stimulated monocytes (Rossato et al., 2012). Live *B. malayi* mf induce the expression of PD-L2 (CD273) and the downregulation of TLR3 (detects double-stranded RNA, poly I: C), TLR5 (detects profilin and bacterial flagellin), and TLR7 (detects among others single-stranded RNA) expression (Semnani et al., 2011). Accordingly, the chemokines CCL15, CCL17, CCL18, CCL22 are upregulated and involved in immune cell trafficking. Live *B. malayi* mf induce autophagy in human dendritic cells by inhibiting the mTOR pathway and thus IL-12 and IL-10 production and the capacity to activate CD4+ T cells (Narasimhan et al., 2016; Semnani et al., 2003). Thus, monocytes play a pivotal role during filarial infection due to their capacity to change their phenotype and to develop into specialized phagocytes and antigen presenting macrophages or dendritic cells (Guilliams et al., 2014; Semnani, 2013). Furthermore, monocytes produce cytokines and chemokines but also matrix metalloproteinases (MMPs) that contribute to the development of lymphangiogenesis and thus the pathology of a filarial infection.

1.1.3 Immunomodulation by helminths and helminth-derived molecules

Helminths modulate cellular functions for their long-time survival and reproduction. Dependent on the host's immunity, the clinical picture varies during infection. Filariae and filariae-derived products possess a high potential to modify innate and adaptive immune responses of the host (Allen and Maizels, 2011). Based on the co-evolution of parasites and their host, the hygiene hypothesis was postulated, which explains the increased rates of allergy and autoimmune diseases in developed countries by an improved hygiene and the resulting loss of infections (Cooke, 2009; de Ruiter et al., 2017; Maizels, 2016). Helminth–derived products and infections can improve e.g. experimental autoimmune type 1 diabetes and diet-induced glucose intolerance (Ajendra et al., 2016a; Berbudi et al., 2016b; Hübner et al., 2012b; Hübner et al., 2009a). Hence, immunomodulation by helminth infections plays an important role in a balanced immune system (Helmby, 2015).

Helminth infections induce a type 2 immunity with increased systemic levels of Th2 cytokines IL-4, IL-5, IL-13, and B cell derived immunoglobulins IgE, IgG1 in mice and IgG4 in humans (Maizels et al., 2004). Accordingly, Th2 cytokine-producing CD4+ T cells expand during helminth infections. CTLA-4 expression is altered in regulatory B and T cells that release anti-inflammatory IL-10 and TGFβ and are supporting regulatory, anti-inflammatory immune responses during infection. During chronic filarial infection alternatively activated macrophages (AAM) that express resistin-like molecule alpha (RELMα), but also basophils, mast cells and neutrophil granulocytes, are induced. Eosinophil granulocytes are a hallmark of helminth infections (Behm and Ovington, 2000; Huang and Appleton, 2016). Type 2 innate lymphoid cells (ILC2) are also characteristically increased by filarial infections promoting Th2 responses (Boyd et al., 2015). IL-13+ neutrophil granulocytes (N2) were characterized in secondary *Nippostrongylus brasiliensis* infections (Chen et al., 2014). Since lymphatic filariasis patients have an increased risk to acquire HIV (human immunodeficiency virus)-infections (Kroidl et al., 2016), immunomodulation by helminth may have a chitinase-like 3 (YM1)-dependent promoting effect on the impairment of antiviral immunity (Buerfent et al., 2015; Osborne et al., 2014).

Filarial nematodes release various immunomodulatory components during the different stages of infection. Excretory/secretory proteins can interfere with host cells and affect antigen processing and presentation to reduce inflammatory pathogenic immune responses. Consequently, the phosphorylcholin containing glycoprotein excretory/secretory (ES)-62 and its analogs derived from the rodent filarial nematode *Acanthocheilonema vitae* reduce the pathogenesis in different inflammatory-driven diseases like allergies (Rzepecka et al., 2014), rheumatoid arthritis (Pineda et al., 2012) and atherosclerosis (Aprahamian et al., 2015).

ES-62 interacts with the pattern recognition receptor (PRR) TLR 4 (CD284) on macrophages and dendritic cells by targeting the myeloid differentiation factor 88 (MyD88) (Goodridge et al., 2005; Goodridge et al., 2007). ES-62 treatment also protects against kidney damage in an MRL/lpr mouse model of systemic lupus erythematosus (SLE) (Rodgers et al., 2015a). Besides macrophages and dendritic cells, ES-62 modifies B cells, mast cells and T cells (Al-Riyami and Harnett, 2012). In addition, filarial-derived *Brugia malayi* adult worm extracts and the *Litomosoides sigmodontis* crude extract were shown to induce macrophage tolerance in a TLR dependent manner that counter-regulates inflammatory responses (Gondorf et al., 2015; Turner et al., 2006). E/S products from the intestinal helminth parasites *A. viteae* and *Heligmosomoides polygyrus* induce Th2 immune responses, IL-10 producing regulatory T cells and inhibit T cell proliferation and macrophage nitric oxide production (Hewitson et al., 2009). However, the protection of experimental *H. polygyrus* infection against colitis-induced intestinal inflammation is due to suppression of the mucosal IL-17 production, but independent of IL-10 (Elliott et al. 2008; Elliott et al. 2004).

Furthermore, filarial nematodes produce cysteine protease inhibitors termed cystatins (Klotz et al., 2011a). Cystatins are essential pathogenicity factors and interfere with antigen processing and presentation that leads inter alia to a reduction of T cell responses (Hartmann and Lucius, 2003). In humans, the cystatin homolog derived from *B. malayi* (Bm-CPI-2) inhibits multiple host cysteine protease activities in the endosomes/lysosomes and reduces MHC class II -restricted antigen processing (Manoury et al., 2001). Cystatin derived from *A. viteae (*AvCystatin*)* or *O. volvulus* (Onchocystatin) modulate macrophages and induce both pro- and anti-inflammatory

cytokines like TNF and IL-10. (Klotz et al., 2011b; Schonemeyer et al., 2001). AvCystatin induces regulatory macrophages and modulates via dual specificity phosphatase (DUSP) 1 and 2, thus inducing IL-10 expression. Interferon (IFN) γ cultured macrophages respond to cystatins with an up-regulation of nitric oxides (Hartmann et al., 2002; Verdot et al., 1996). Consequently, recombinant cystatin (rBmCys) and recombinant abundant larval transcript 2 (rBmALT2) of *B. malayi* are effective therapeutics in the experimental model of dextran sulfate sodium (DSS)-induced colitis (Khatri et al., 2015a; Khatri et al., 2015b; Kumar et al., 2016). RBmALT2 is an effective anti-inflammatory therapeutic against streptozotocin (STZ)-induced type 1 diabetes in mice (Reddy et al., 2017).

Correspondingly, abundant larval transcripts (ALT) are highly conserved filaria E/S products and are found in *A. viteae* (Pogonka et al., 1999)*, W. bancrofti* and *O. volvulus* (Madhumathi et al., 2010; Sakthidevi et al., 2014). Immunomodulatory ALT are the most abundant E/S products from infectious *L. sigmodontis* third stage larvae (Allen et al., 2000; Hewitson et al., 2009). Soluble antigens of *Schistosoma* spp. are also described as immunomodulators. Soluble schistosomal egg antigens (SEA) bind to Dectin-2/FcγR (Fc receptor γ chain) complex and modify host immune responses by NLRP3 (NLR family, pyrin domain containing 3) inflammasome activation and IL-1β production (Ritter et al., 2010) by suppressing TLR-dependent responses (Kane et al., 2004; Kane et al., 2008). Subsequently, Omega (Ω)-1, a glycoprotein and single component secreted by *S. mansoni* eggs, drives Th2 responses in human dendritic cells (Everts et al., 2009; Steinfelder et al., 2009).

The administration of *Heligmosomoides polygyrus* derived exosomes suppresses allergen-induced Type 2 innate responses and eosinophilia by suppressing DUSP1 and IL-33R genes (Buck et al., 2014). Similarly, filarial nematodes secrete extracellular vesicles like exosomes with a size of 30-100nm that contain small RNAs (microRNA and Y RNAs) and nematode Argonaute proteins, which are internalized by host cells and modulate innate immunity (Marcilla et al., 2014; Marcilla et al., 2012). Exosome-like vesicles secreted by infectious L3 larvae were described for the human pathogenic filaria *B. malayi* (Zamanian et al., 2015) and the inhibition of inflammatory

responses and the induction of regulatory immune cells by helminth-derived exosomes containing miRNA is discussed for allergy and autoimmune diseases (Siles-Lucas et al., 2015). In contrast, exosome-like vesicles derived by *Schistosoma japonicum* adult worms (blood fluke) mediate M1-type immune responses of macrophages in vitro (Wang et al., 2015).

1.1.4 Murine *Litomosoides sigmodontis* infection as model for human filarial infections

Since *Litomosoides sigmodontis* establishes patent infections (occurrence of microfilaremia) in rodents and induces immune responses that are similar to human filarial nematode infections, experimental *L. sigmodontis* infection is an accepted model for human filariasis (Allen et al., 2008; Hoffmann et al., 2000). *L. sigmodontis* is transmitted by the tropical rat mite *Ornithonyssus bacoti* to the natural rodent host *Sigmodon hispidus* (cotton rat) in different tropical and subtropical parts of America. *L. sigmodontis* can also infect different strains of (semi)-susceptible mice like C57BL/6 (only prepatent infection) and BALB/c (patent infection) laboratory inbred mice. Similar to human pathogenic filarial nematodes, *L. sigmodontis* L3 larvae are the infectious stage for the rodent host (Fig. 3) (Hübner et al., 2009b). Two to six days post infection (dpi) the transmitted L3 larvae reach the thoracic cavity and molt to juvenile L4 worms. The L4 worms become adult around 30 dpi and start to release microfilariae (L1 worms) that are transmitted to the intermediate host during the blood meal 50 days after the infection. In *O. bacoti,* the mf mature via L2 to L3 infective-stage larvae that are transmitted to the rodent host during the next blood meal.

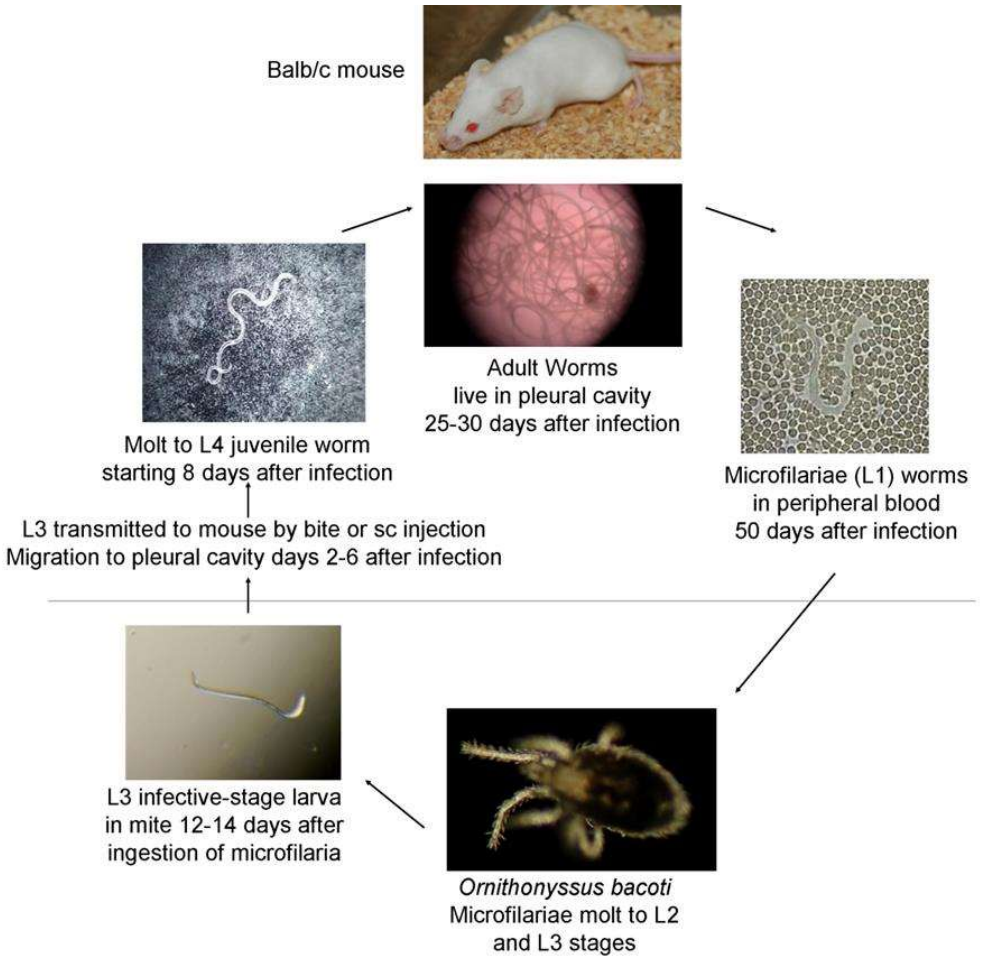

***Figure 3: Life cycle of the rodent filaria nematode* Litomosoides sigmodontis.** *After L3 infective-stage larvae transmission during the blood meal of the tropical rat mite* Ornithonyssus bacoti*, the larvae become adult in the pleural cavity and produce microfilariae (L1 larvae) that are transmitted with the next blood meal. The microfilariae develop into L3 larvae within the rodent vector and infect the host during the next blood meal (Hübner et al., 2009b).*

1.2 Helminth-mediated protection against LPS-driven immune diseases

The capability of helminth and helminth antigens to modulate host immune responses revealed protective effects on inflammatory disorders (Soares and Araújo, 2008). The induction of a regulatory and anti-inflammatory milieu suppresses and counter-balances exacerbated pro-inflammatory immune responses caused by systemic bacterial infections or LPS-induced endotoxemia (Hübner et al., 2013). Sepsis is a complex dysregulation of the immune system caused by the coincidence of an infection by microorganisms like bacteria or fungi inducing a systemic (pro-)inflammatory response syndrome (SIRS) that leads to fever (≥38°C) or hypothermia (≤36°C), leukocytosis (≥12.000/mm³) and/or an increased heart rate (≥90/min) (S2k guidelines) (Reinhart et al., 2010).

The SIRS phase is followed by a compensatory anti-inflammatory response syndrome (CARS) dominated by immunosuppression and increased risk of secondary infections (Bone et al., 1992). Severe sepsis and septical shock can lead to multiple organ failure, hypoxia (≤75mmHg) and cell apoptosis (Buras et al., 2005). The succeeded alternation of SIRS to CARS is termed mixed antagonistic response syndrome (MARS) (Weigand et al., 2003). Due to a mortality of 50% in patients with septic shocks (Morrell et al., 2009) and mortality rates of severe septic patients in intensive care units from 40 to 66%, sepsis is one of the leading causes of death worldwide (Beale et al., 2009; Martin et al., 2003).

1.2.1 Immunopathogenesis of bacterial-induced inflammation

Microbial invaders are primarily detected by innate immune cells like macrophages and dendritic cells by pattern recognition receptors, e.g. Toll-like-receptor 2 and 4 (Akira et al., 2006). After the detection of the pathogen-associated molecular pattern (PAMP) e.g. the TLR2 ligand lipoprotein (LP) from gram- positive or the TLR4 ligand lipopolysaccharide (LPS) of gram- negative bacteria, the activation results in an excessive production of pro-inflammatory immune responses, initiating the SIRS phase (Danner et al., 1991; Oliveira-Nascimento et al., 2012). The binding of LPS to the TLR4/MD2 complex activates both the signal cascades of the adapter molecule myeloid differentiation primary response gene (MyD)88 resulting in pro-inflammatory Th1 cytokines TNF, IL-6 and IL-12 release and the adapter molecule TRIF (interferon-inducible genes like IFNα and IFNβ) signaling (Van Amersfoort et al., 2003). Additionally, the LPS binding initiates the cleavage and thus activation of cytosolic IL-1β (Martinon et al., 2009). The activation of macrophages is accompanied by the upregulation of the major histocompatibility complex (MHC) class II surface receptor, CD80 and CD86 to induce the activation and the production of Th1 cytokines by CD4+ T cells that promote cytotoxic CD8+ T cells for pathogen killing in humans and mice (Hotchkiss and Opal, 2010). The exacerbated production of pro-inflammatory mediators can result in an excessive activation and recruitment of neutrophil granulocytes, which improve bacterial clearance but also increase tissue damage by the release of proteases (Cohen, 2002).

The exacerbated inflammation is counter-balanced by the induced homoeostasis during sepsis progression. Subsequently, the CARS phase is accompanied by T cell anergy (Venet et al., 2009) and characteristically increased numbers of IL-10 and TGFβ producing regulatory and suppressive T cells and increased CTLA-4 expression by T cells (Hotchkiss and Opal, 2010; Venet et al., 2008). During the SIRS phase the expression of type I interferons by splenic macrophages results in immunosuppression and thus impaired adaptive immune responses against secondary infections after severe sepsis (immune paralysis) (Schwandt et al., 2012).

1.2.2 Helminth-mediated immunomodulation against bacterial-induced sepsis

During the last decades several studies have investigated whether the administration of regulatory mediators IL-10 (Howard et al., 1993; Schulte et al., 2013), IL-7 (Unsinger et al., 2012; Unsinger et al., 2010), IL-15 (Inoue et al., 2010), neutralizing antibodies against pro-inflammatory IL-1β (Fisher et al., 1996) and TNF (TNF receptor bound to Fc portion of IgG1 (TNFR:Fc)) (Fisher et al., 1996), the blocking of the TLR4/MD2 complex (eritoran tetrasodium (E5564)) (Kalil et al., 2011; Paramo et al., 2015; Roger et al., 2009) or TLR2 antagonists improve sepsis survival (Meng et al., 2004). Although several experimental results revealed a beneficial effect by impairing the uncontrolled inflammation, those results were not confirmed in human clinical trials and are probably due to the diverse clinical picture of sepsis, the diversity of microorganisms and the necessity to improve both the SIRS and CARS phase (Riedemann et al., 2003a).

Similar to experimental helminth infections and helminth product administrations that revealed an immunomodulatory protective effect on autoimmune diseases like diabetes (Berbudi et al., 2016a), initial experiments depicted a protective effect of helminths to reduce exacerbated pro-inflammatory immune responses without impairing bacterial control. The helminth defense molecule of *Fasciola hepatica* (FhHDM-1) directly binds to LPS and reduces its interaction with LPS-binding protein on the surface of macrophages, thus protecting mice against LPS-induced endotoxemia (Robinson et al., 2011).

Similarly, the tegument antigen of *F. hepatica* suppresses serum IL-12p70 and IFNγ levels by impairing dendritic cell maturation and function (Hamilton et al., 2009). The chito-oligosaccharide chitohexaose blocks LPS-induced endotoxemia and pro-inflammatory TNF, IL-1β and IL-6 production via TLR4 receptor interaction and induces alternative Arginase-1 and IL-10 expression in murine macrophages and human monocytes (Panda et al., 2012). Accordingly, an infection with the murine intestinal helminth *Nippostrongylus brasiliensis* improves the survival of *Klebsiella pneumoniae*-induced peritonitis that correlates with a reduced peritoneal bacterial load (Sutherland et al., 2011).

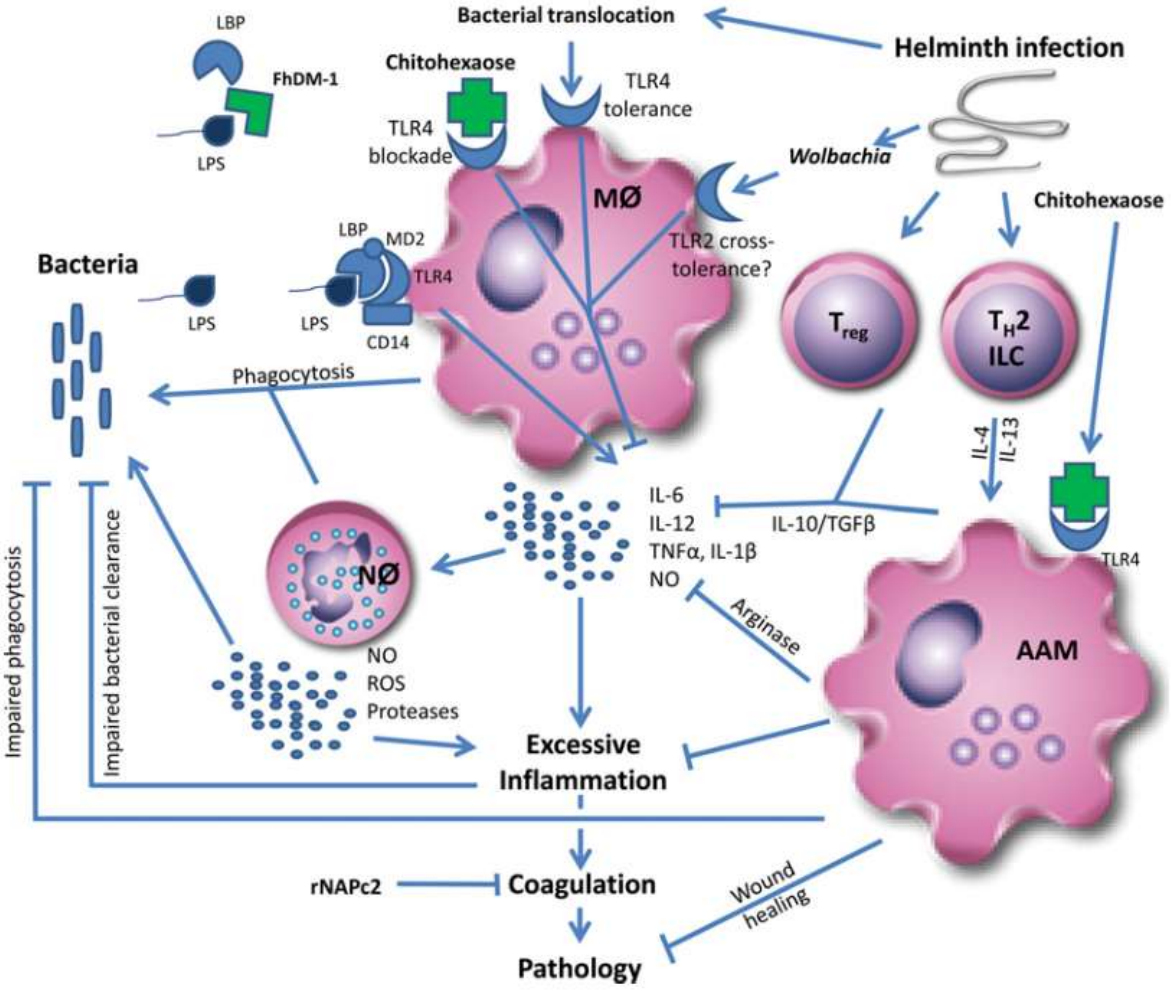

Figure 4: Helminth-mediated protection against bacterial induced sepsis. *Helminth infection and the release of mediators like endosymbiotic* Wolbachia spec. *or components like Chitohexaose interfere with pathogen-recognition receptors like TLR2/4. Induced regulatory T cells, T helper 2 cells, innate lymphoid cells (ILCs) and alternatively activated macrophages (AAM) may reduce excessive inflammation amongst others by the release of anti-inflammatory mediators IL-10 and TGFβ and by re-programming macrophages. The immunomodulation by helminths improves phagocytosis, bacterial control and sepsis pathology (Hübner et al., 2013).*

Chronic *L. sigmodontis* infection reduces pro-inflammatory immune responses and ameliorates *Escherichia coli*-induced hypothermia (Gondorf et al., 2015). The protective effect is *Wolbachia spp.* and TLR2-dependent and associated with an increased phagocytic activity and downregulation of genes involved in TLR signaling that increases sepsis survival (Fig. 4). My own work demonstrated hereby that the *E. coli*-induced immune paralysis during the immunosuppressive CARS phase is not exacerbated in chronic *L. sigmodontis* infected mice (Buerfent et al., 2015). Correspondingly, *Mycobacterium tuberculosis* pathology and control is not worsened by chronic filarial infection (Hübner et al., 2012a). Whereas the implantation of *L. sigmodontis* female adult worms from prepatent infections protect mice, microfilariae injection exacerbates LPS-induced endotoxemia through the induction of increased pro-inflammatory responses (Hübner et al., 2008).

Eosinophil granulocytes are a hallmark of parasitic helminth infections and provide anti-parasitic properties (Huang and Appleton, 2016). Principally, eosinophils are required for the long-term maintenance of plasma cells in the bone marrow by their IL-6 and APRIL (a proliferation-inducing ligand) release (Chu et al., 2014; Chu et al., 2011), whereas the role of eosinophils on bacterial sepsis is controversially discussed. Eosinophils have the capacity to support the clearance of bacterial infections. IL-5, the main inducer of eosinophils and produced by eosinophils themselves, was shown to be protective in sublethal cecal ligation and puncture (CLP) sepsis in an eosinophil-independent manner (Linch et al., 2012). Several publications indicate anti-bacterial properties of eosinophils in vitro and in vivo (Linch et al., 2009; Pulido et al., 2012). Accordingly, LPS triggers eosinophil recruitment and accumulation via C-C chemokine receptor type (CCR) 3 activation (Penido et al., 2001) and spontaneously released extracellular structures of eosinophil-derived mitochondrial DNA and proteins reduce bacterial burden in vitro (Yousefi et al., 2008). Hence, the eosinophil count is discussed to be a diagnostic marker for sepsis survival in humans (Merino et al., 2012; Terradas et al., 2012).

1.3 Objectives of the thesis

Chronic helminth infections modulate immune responses, thus protecting against bacterial-induced systemic inflammation. The immunomodulatory capacity of helminth products and helminth infections were mainly focused on the detection of immunomodulatory mediators (McSorley et al., 2013) and their capacity to improve diseases that are due to dysregulated immune responses (El-Malky et al., 2011; Gondorf et al., 2015).

Part A - Impact of eosinophil granulocytes on E. coli-*induced sepsis*

The first aim of my thesis was to investigate the impact of the hallmark cell population of parasitic infections (Rosenberg et al., 2013), eosinophil granulocytes, on the development of an experimental *E. coli*-induced sepsis. Consequently, I investigated whether transfer of bone-marrow derived eosinophils is protective against *E. coli*-induced sepsis and if the reduction of systemic inflammation during the SIRS phase is eosinophil-dependent. In this context, genome-wide transcriptional profiling comparing eosinophils of *L. sigmodontis*-infected mice, naïve mice and eosinophils obtained during sepsis in *L. sigmodontis*-infected and uninfected mice was performed to identify transcriptional changes in eosinophils that may reveal diverse functions in different immunological settings.

Part B - Genome-wide transcriptome analysis of human monocytes stimulated with helminth extracts and LPS reveal a link to IL-17 associated diseases

Since helminth extracts of the human pathogenic filaria *Brugia malayi* (BmA) are known to modulate inflammatory responses, the second aim of this thesis was to perform a genome-wide transcriptional profiling of BmA crude extract primed and LPS re-stimulated monocytes of human male non-endemic donors. Based on differentially expressed genes we performed a transcriptome-wide association analysis of relevant pathways by IPA® analysis to reveal immunomodulation by filarial extracts and their potential impact on autoimmune diseases and the development of filarial pathology.

Material & Methods

2.1 Murine studies

2.1.1 Ethics statement and mice

All mice were housed in the animal facility of the Institute for Medical Microbiology, Immunology and Parasitology of the University Hospital Bonn, Germany, with access to food and water ad libitum. All performed experimental procedures were approved by the Landesamt für Natur, Umwelt und Verbraucherschutz, Cologne, Germany and performed according to the European Union animal welfare guidelines. Female homozygous ΔdblGATA-1 gene deficient mice were purchased from The Jackson Laboratory, Bar Harbor, USA. The age-matched female BALB/cJ wild type (WT) controls were obtained from Janvier Labs, Le Genest-Saint-Isle, France.

2.1.2 Chronic filarial infection model – *Litomosoides sigmodontis*

As previously described by Ajendra et al. (Ajendra et al., 2014), healthy 6 to 8 week old mice were naturally infected with the rodent filarial nematode *Litomosoides sigmodontis* (L. s.) via the tropical rat mite *Ornithonyssus bacoti.* For maintenance of the life cycle, *L. sigmodontis* infected cotton rats (*Sigmodon hispidus*) were exposed to mites. Ninety days post infection (p.i.) the experiments were performed. The chronic filarial infection was confirmed by the identification of adult worms within the thoracic cavity and the presence of microfilariae in the peripheral blood (20µl blood + 1mL red blood lysis buffer (eBioscience/Affymetrix, Santa Clara, USA)) following necropsy.

2.1.3 Bacterial sepsis model

Chronic *L. sigmodontis*-infected mice and controls were intraperitoneally (i.p.) challenged with $1x10^8$-$1x10^9$ colony forming units [cfu] of gram-negative *Escherichia coli* K12 (ATCC 25922) in a 200µL sterile lysogeny broth medium and monitored for six hours. Mice developing severe septical symptoms like hematuria and body temperature <27°C were prematurely euthanized according to humane endpoint criteria by an overdose of isoflurane (AbbVie Deutschland GmbH & Co. KG, Wiesbaden, Germany). The body temperature decrease was measured hourly by

infrared measurement as previously described (Gondorf et al., 2015). The mice were euthanized after six hours and the peritoneal and pleura lavage was performed by injecting 1 and 4mL (total 5mL) of cold PBS. The first mL of the peritoneal lavage was used to determine the bacteria concentration (plated as serial dilutions on Luria–Bertani (LB) agar plates and incubated overnight at 37°C) as well as the cytokine and chemokine concentrations after centrifugation at 300 g for 10 min (stored at -20°C). The combined cell pellet was used to determine peritoneal cell populations and numbers. Cells were counted using the CASY TT (Roche, Pensberg, Germany). The systemic cytokine and chemokine concentration was determined in the peripheral blood after centrifugation at 1000 g for 10 min and storage at -20°C.

2.1.4 Eosinophil culture

Eosinophil granulocytes were generated using an optimized in vitro differentiation protocol from the bone marrow of BALB/cJ mice (Dyer et al., 2008). In brief, the bone marrow was cultured (at 37°C, 5% CO_2 and 95% humidity) with 100 ng/mL recombinant murine SCF and FLT3-L for 4 days, followed by 20ng/mL recombinant murine IL-5 for 8 days (all three from Peprotech, St. Louis, USA) in an RPMI 1640 advanced medium containing 20% fetal calf serum, 25 mM HEPES, 1X GlutaMAX (all from Gibco Technologies / Thermo Fisher Scientific, Waltham, USA) and 100 U/ml penicillin + 10 µg/mL streptomycin (both from PAA / GE Healthcare, Pasching, Austria) in Advanced TC™ flasks with filter caps (Greiner Bio-One, Kremsmünster, Austria). The purity was checked by flow cytometry and always exceeded 95% $SSC^{hi}SiglecF^{+}$ eosinophil granulocytes.

2.1.5 In vitro experiments of bone-marrow-derived eosinophil granulocytes

The in vitro experiments were performed using the previously mentioned sterile medium. The bone-marrow-derived eosinophil granulocytes (bmEos) were incubated at 37°C and 5% CO_2 for 24 hours with CCL11 or CCL24, followed by a 24h stimulation with LPS *E. coli* ultrapure [300 ng/mL], Pam3CSK4 [100 ng/mL] (both Invivogen, San Diego, USA) and *L. sigmodontis* crude adult worm extract (LsAg) [25 µg/mL]. Single stimulated cells served as controls. The supernatant of the in vitro experiments was stored at -20°C until measurement by ELISA. The in vitro bacteria killing assays were done in medium lacking antibiotics. The bacteria were suspended in PBS

($1x10^7$ bacteria in 10 µL) and co-incubated at 37°C for 3h (Yousefi et al., 2008). The suspension was plated on LB agar plates and after incubation for 18 hours at 37°C, the number of CFUs was determined optically.

2.1.6 Flow cytometry and immunoassays of murine experiments

The cells of the in vivo and in vitro experiments were first blocked with PBS containing 1% bovine serum albumin and 0.1% rat immunoglobulin (Sigma-Aldrich, St. Louis, USA) for 30 min at 4°C. After a washing step with cold PBS at 300 g for 10 min, cells were stained for 30min with combinations of anti-mouse antibodies SiglecF PE or AL647 (Clone: E50-2440) (both BD Pharmingen), F4/80 APC or PerCP-Cy5.5 (Clone: BM8), Ly6G PE (Clone: 1A8), Ly6C PerCP-Cy5.5 or APC-Cy7 (Clone HK1.4) (all BioLegend) and CD11b FITC or PE-Cy7 (Clone: M1/7; eBioscience). For intracellular staining the cells were first incubated with fixation and permeabilization buffer overnight (eBioscience). Afterwards, the cells were stained in two steps in permeabilization buffer with rabbit anti-murine RELMα (Peprotech), followed by donkey anti-rabbit IgG Alexa Fluor 647 (Clone: Poly4064) (BioLegend) or goat anti-rabbit IgG (H+L) Alexa Fluor 488 (Thermo Fisher Scientific). For activation analysis, cells were stained with CD69 PE and CD86 PE (eBioscience, San Diego, USA). The cells were analyzed on a BD Canto flow cytometer combined with Diva 6.0 software (BD Bioscience, San Jose, USA) and the data was subsequently analyzed by FlowJo v10 software (FLOWJO LLC, Ashland, USA).

The cytokine concentrations of interleukin-4, interleukin-6, CXCL1 (KC) and CCL5 (RANTES) as well as tumor necrosis factor α, interferon γ, interleukin-10 and CXCL2 (MIP-2) were determined from cell culture supernatants and plasma by Enzyme Linked Immunosorbent Assay (ELISA) (eBioscience and R&D) according to kit protocols on a SpectraMAX 190 with SoftMAX Pro 6.5 software (Molecular Devices, Sunnyvale, USA).

2.1.7 RNA sequencing and transcriptome profiling of sorted eosinophil granulocytes

Viable FSC^{hi}SSC^{hi}Hoechst33258^{neg}CD11b^{+}F4/80^{+}SiglecF^{+} eosinophil granulocytes (Fig. 5) were sorted directly after peritoneal and pleural lavage with 10 ml RPMI 1640 containing 10% FCS of *L. sigmodontis* infected and non-infected BALB/c mice six hours after *E. coli* or PBS injection (BD FACSAria™ III, BD Biosciences). Additionally, eosinophils were sorted from the intestine of non-infected mice (in collaboration with Prof. Dr. Christoph Wilhelm, University Hospital of Bonn).

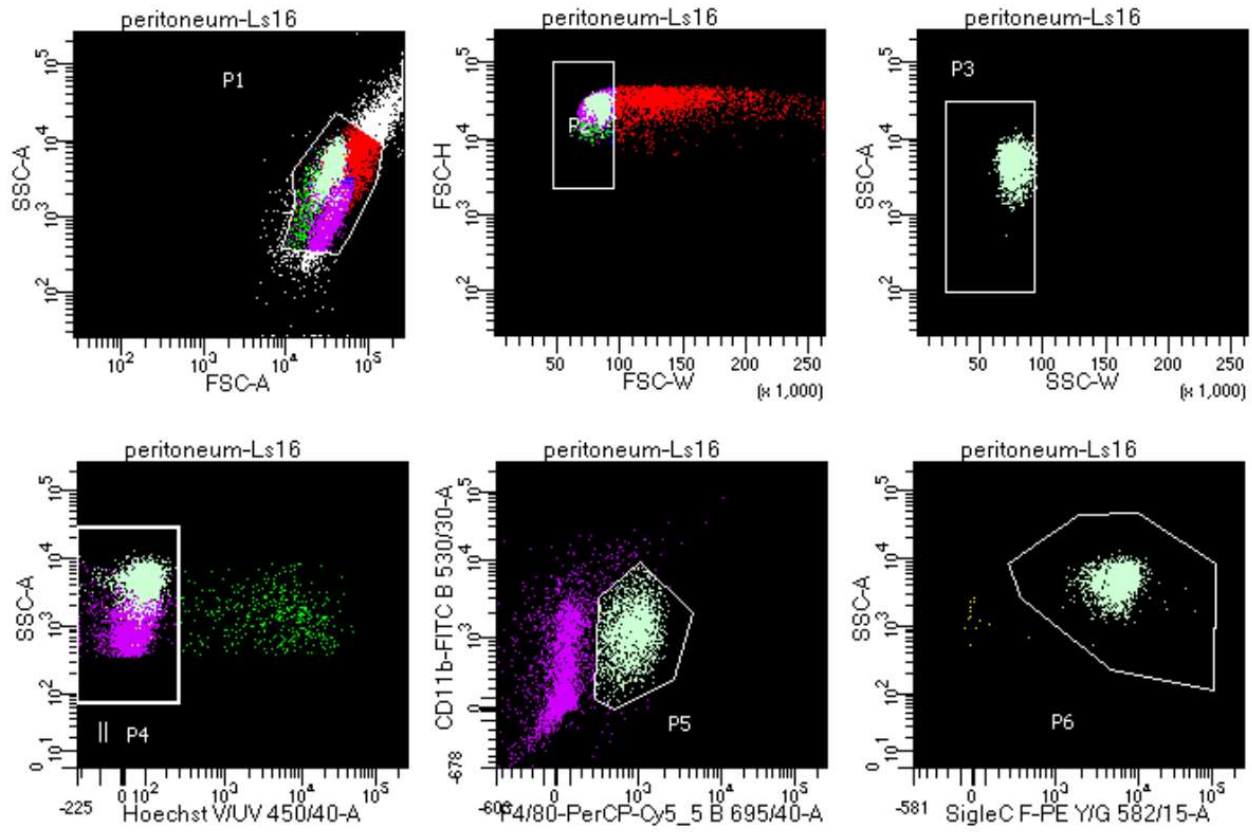

Figure 5: Representative gating-strategy of sorted murine eosinophils for subsequent RNA sequencing.

In brief, the intestine and the Payer Patches were removed. Afterwards, the intestine was cut into small pieces and digested with Liberase TM (Roche, Basel, Switzerland) under continuous stirring for 30 min. Afterwards, the intestinal pieces and cell suspension were homogenized through a 70 µm cell strainer, centrifuged for 8 min at 330 g at 4°C and passed through a 40 µm cell strainer. The single cell suspension was then blocked with a medium containing 3% FCS and 0.1% rat immunoglobulin for 30 min and stained with the same fluorescence-coupled antibodies that were

used as previously mentioned. The sorted cells were centrifuged at 300 g for 10 min, lysed in RLT Buffer Plus (QIAGEN, Venlo, Netherlands) and stored at -80°C until RNA preparation.

Subsequently, total RNA was extracted using the RNeasy Plus Micro Kit from QIAGEN following the manufacturer`s instruction (QIAGEN). For quality control, RNA concentrations were measured using NanoDrop (PeqLab, Erlangen, Germany) and degradation via Qubit® RNA HS Assay Kit (Thermo Fisher Scientific, Waltham, USA). The RNA sequencing was performed using QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen, Vienna, Austria) and HiSeq 2500 (1x50bp, single-read, Rapid Modus; Illumina, San Diego, USA). Data analysis was performed using ShinyOmics and ShinyDGEA (Jan Gehlen, Human Genetics of University Hospital of Bonn, Germany), a browser based compilation of the packages Subread aligner 1.5.1 (http://subread.sourceforge.net/), multiQC 0.8 (https://github.com/ewels/MultiQC) and limma 3.30.11 (https://bioconductor.org/packages/ release/bioc/html/limma.html).

2.2 Human study

2.2.1 Human study population

The human study was approved by the ethics committee of the University of Bonn. 20 healthy male Caucasian volunteers of European origin at age of 18 to 35 years were recruited and peripheral blood samples were taken. The inclusion criteria were the following: at least three generations of grandparents in Central Europe, non-smoker, no acute or chronic infection, no surgery or vaccination four weeks prior to blood withdrawal and C-reactive protein (CRP) levels < 2.5 mg/dl. Female donors were excluded due to hormonal factors which could possibly have influenced transcriptional expression patterns. The patients gave their consent to the anonymous publication of the results.

2.2.2 Monocyte isolation

Peripheral blood mononuclear cells were purified using a Ficoll-Plaque density gradient from heparinized whole blood (Gölz et al., 2016a). Afterwards, $CD14^+$ monocytes were isolated by using CD14-microbeads and the AutoMACS Pro Separator system according to the manufacturer`s instruction (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). The cell purity of CD14+ cells was determined by flow cytometry (see section 2.2.4). After separation, the cells were re-suspended in RPMI 1640 media, supplemented with GlutaMAX™, 10% heat-inactivated FCS (Gibco® Life Technologies, Waltham, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin (both PAA Laboratories/GE Healthcare, Pasching, Austria).

2.2.3 Monocyte stimulation

The purified monocytes (> 95% purity) were cultured in 96-well round bottom wells at a density of 500.000 cells/well in 100 µl for 2 h after separation. Afterwards, the cells were stimulated with 10 µg/ml *Brugia malayi* adult worm crude extract (BmA) for 18 h and re-stimulated for an additional six hours with 200 ng/ml LPS *Escherichia coli* ultrapure (InvivoGen, San Diego, USA). All experiments were performed using one batch of BmA that was prepared according to the *L. sigmodontis* crude extract (Ziewer et al., 2012). BmA was tested for detectable endotoxin levels

by QCL-1000™ Endpoint Chromogenic LAL assay (Lonza, Basel, Switzerland). The cell viability of BmA stimulated monocytes was tested by the Colorimetric Cell Viability Kit III (XTT; PromoKine, Heidelberg, Germany). Unstimulated and BmA- or LPS-only stimulated monocytes served as controls. After stimulation the supernatants were collected and the cells were lysed in RLT Plus buffer (QIAGEN, Hilden, Germany) and stored at -80°C. In additional experiments, monocytes were stimulated (after a resting period of 2 h) for 18 h or 24 h with BmA followed by 24 h or 18 h of LPS stimulation (total 42 h), respectively. Additionally, a long-term treatment of 48 h, including a 24 h and 42 h priming with BmA followed by 24 h or 6 h of LPS re-stimulation, was performed.

2.2.4 Flow cytometry and immunoassays of human monocyte experiments

The overnight monocyte survival was checked microscopically by trypan blue staining and confirmed by Annexin V and propidium iodide analysis (eBioscience, San Diego, USA) via flow cytometry (Gölz et al., 2016a). For the analysis of monocyte activation, the cell suspension was centrifuged at 300 g for 10 min. The cells were incubated in PBS/1% BSA, including 0.1% IgG from rat serum for 30 min (Sigma-Aldrich, St. Louis, USA). Afterwards, the cells were stained with anti-CD14 FITC (Clone: 62D3), anti-CD16 APC (Clone: eBioCD16), anti-HLA-DR PerCP-Cy5.5 (Clone: LN3, eBioscience), and anti-CD86 PE (Clone: 2331 (FUN-1), BD Bioscience, San Diego, USA). The flow cytometry analysis was performed using BD FACS Canto, followed by FACS Diva software (BD Biosciences) and FlowJo V10 software (FLOWJO LLC, Ashland, USA). The cytokine and chemokine concentrations in the cell culture supernatant were measured by ELISA (CXCL5/ENA-78 and CXCL6/GCP-2; both R&D Systems, Minneapolis, USA) and Multiplex Immunoassay TNF, IL-1β, IL-6, IL-10 (all R&D Systems).

2.3 RNA extraction

RNA from lysed monocytes was extracted using the AllPrep DNA/RNA Mini Kit from QIAGEN following the manufacturer`s instruction. For quality control RNA concentrations were measured using NanoDrop (PeqLab, Erlangen, Germany) and degradation via Bioanalyzer (RIN 7; Agilent Technologies, Santa Clara, USA).

2.4 Transcriptome analysis

The Illumina TotalPrep RNA Amplification Kit (Life Technologies) was used to amplify and biotinylate the RNA. Whole transcriptome profiling was performed on Illumina's HT-12v4 bead arrays (Illumina, San Diego, USA), comprising 47.231 transcripts.

The statistical analysis was performed using functions implemented in the statistical software R (version 3.1.0) and Bioconductor packages. Expression data was normalized using the limma package (Ritchie et al., 2015) and subsequently log-transformed. Only probes that obtained a detection P value <0.05 in at least 5% of all samples were taken into account. The selected expression probes were then filtered for a perfect or good probe quality, as reported in the Bioconductor package illuminaHumanv4.db (Barbosa-Morais et al., 2010). After quality control and filtering, data for 18.452 expression probes from up to 20 individuals was included in the analysis. Differentially expressed probes were calculated using limma (Ritchie et al., 2015), applying a paired design in the ANOVA analysis to identify the contrast between controls and stimulated samples. All nominal *P value*s *P* were adjusted for multiple testing using Benjamini-Hochberg correction in order to control for false discovery if not reported otherwise. Only probes with a fold-change (FC) ≥ 1.5 were selected in line with the microarray sensitivity of 1.35. The fold-change was calculated by dividing the mean intensity of the expression of a target gene of one group by the expression intensity of the same gene from the group it was compared with. If this number was <1, the negative reciprocal was used. The hierarchical cluster analysis was performed using the cluster method in R on z-transformed probes. Distances of the samples were calculated using Pearson correlation and clusters were formed by taking the average of each cluster.

2.5 Pathway analysis

Data evaluations were performed using Ingenuity Pathway Analysis (IPA® Systems Inc., Redwood City, USA). Each gene is represented in a global molecular network designed by information and provided by the Ingenuity Pathways Knowledge Base. "Networks" were generated algorithmically based on their connectivity concerning activation, expression and

transcription. Molecular relationships between genes are visible as connecting lines between nodes supported by published data stored in Ingenuity Pathways Knowledge Base and/or PubMed. Enriched GO and KEGG pathways, Protein-Protein-Interaction networks and associated diseases were calculated using the WEB-based GEne SeT AnaLysis Toolkit (WebGestalt, (Wang et al., 2013)), using all analyzed probes (n=18,452) as the background reference, a minimum of 3 genes per category and a FDR of 1% as the threshold for significance.

2.7 Data management and statistical analysis

The flow cytometry analysis was performed by BD FACS Canto and BD FACS Diva 6.0 (BD Bioscience) and analyzed by FlowJo v10 software (Tree Star, Ashland, USA). The ELISA was performed according the manufacturer`s instructions using SpectraMAX 190 and SoftMax Pro 6.5 (Molecular Devices, Sunnyvale, USA). The Multiplex Immunoassays were performed using a xMAP MAGPIX system according to the manufacturer`s instruction (Luminex Corporation, Austin, USA). The statistical analysis was performed using Prism GraphPad 5.01 (GraphPad Software, San Diego, USA).

All murine experiments with multiple groups were tested for significance by the Kruskal-Wallis test, followed by Dunn post hoc multiple comparisons. The differences between two unpaired groups were tested for statistical significance with the two-tailed Mann–Whitney U test. The differences between two paired groups were tested for statistical significance with the paired t-test. The two-way ANOVA and Bonferroni post hoc test was used to test for significances of multiple groups over time. Significance is defined as P value < 0.05 (*<0.05; **<0.01, ***<0.001) and the error bars represent means ± SEM.

Secreted protein and surface marker expression profiles of the human study were analyzed by ANOVA, followed by the Bonferroni Comparison Test. The data are shown as mean with SEM and P value of 5% was considered as statistically significant.

Results

3.1 Impact of eosinophil granulocytes on *E. coli*-induced sepsis

Helminths modulate inflammatory responses, thus providing protection against sepsis (Hübner et al., 2013). Eosinophil granulocytes are a hallmark of parasitic infections (Huang and Appleton, 2016) and reveal anti-bacterial properties in vitro and in vivo (Linch et al., 2009; Yousefi et al., 2008). Eosinophil numbers have been discussed as a marker of sepsis severity in the past. The challenges of anti-sepsis therapies are the control of systemic inflammation, especially during severe sepsis or septic shock, and the need to provide anti-bacterial properties without worsening the compensatory phase. Accordingly, in our recent publication we presented both improved bacterial killing and reduced systemic inflammation during the SIRS phase and thus increased the survival of *E. coli*-induced sepsis in chronic *L. sigmodontis* infected mice (Gondorf et al., 2015). Consequently, one aim of this thesis was to explore the impact of eosinophil granulocytes on the outcome of an *E. coli*-induced sepsis during the SIRS phase. Additionally, TGFβ independent protection of *L. sigmodontis* infection on *E. coli*-induced sepsis is presented in complimentary project A.

3.1.1 Absence of eosinophil granulocytes exacerbates *E. coli*-induced sepsis in ΔdblGATA mice

Since earlier reports showed evidence that eosinopenia is a diagnostic marker of sepsis mortality in humans (Merino et al., 2012; Terradas et al., 2012) and because eosinophils respond to bacteria, the outcome of an *E. coli*–induced sepsis in eosinophil-deficient ΔdblGATA mice was compared to age-matched BALB/cJ controls. The development of hypothermia was monitored for six hours. Afterwards, the peritoneal bacterial load, sepsis-related peripheral blood chemokine and cytokine concentrations, relative numbers of T cells and myeloid derived cells including granulocytes, monocytes, macrophages and dendritic cells were analyzed. Shown data are pooled from two independent experiments (Fig. 6).

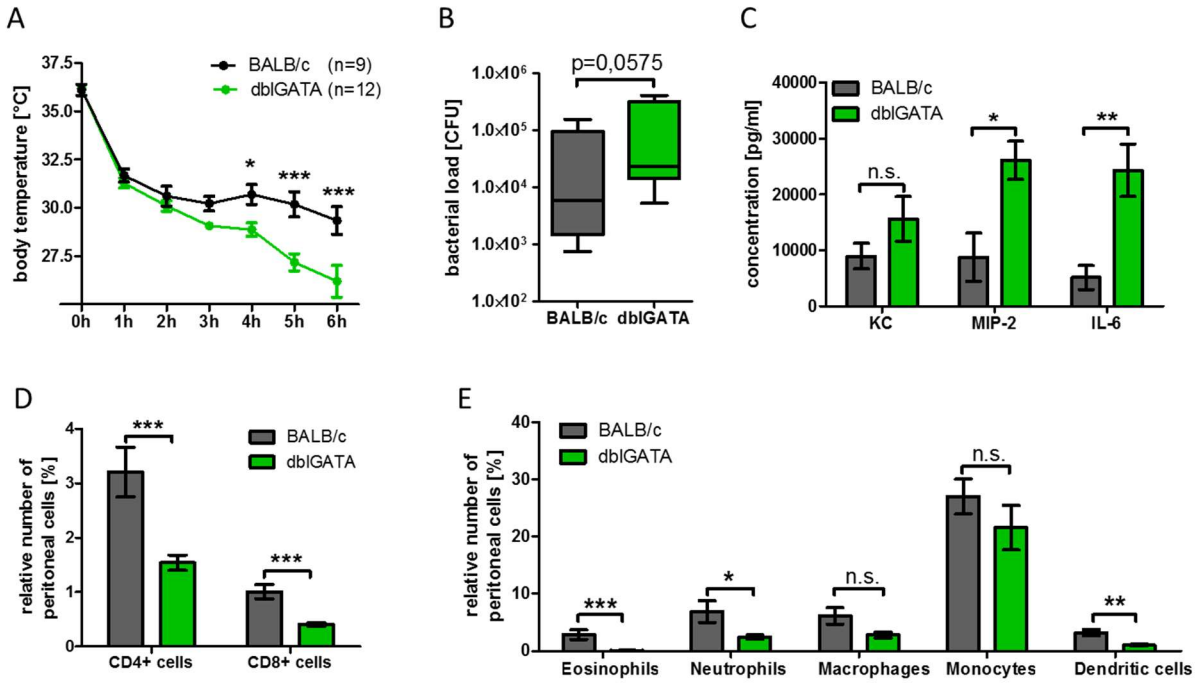

Figure 6: Absence of eosinophils in ΔdblGATA mice exacerbates sepsis outcome compared to controls. *Development of hypothermia (A), bacterial load (B) and the concentration of systemic levels of CXCL1 (KC), CXCL2 (MIP-2) and IL-6 (C) six hours after injection of 3x10^7 CFU* E. coli *in wildtype and eosinophil-deficient ΔdblGATA mice (n=9-12 per group) are presented. The relative numbers of CD4$^+$ and CD8$^+$ cells are shown in (D) and the relative numbers of SiglecF$^+$ eosinophils, Ly6G$^+$Ly6C$^+$ neutrophils, CD11b$^+$F4/80$^+$ macrophages, Ly6C$^+$Ly6G$^-$ monocytes and CD11b$^+$CD11c$^+$ dendritic cells are displayed in (E). Statistical significances were tested by Mann-Whitney-test (B-E) or Two-way ANOVA (A) (*p<0.05, **p<0.01, ***p<0.001).*

Starting four hours after the *E. coli* injection, the body temperature was significantly reduced in ΔdblGATA mice (n=12) compared to wild type controls (n=9) (Fig. 6A). Accordingly, the peritoneal bacterial load of ΔdblGATA mice was by tendency increased (Fig. 6B; p=0.0575). Systemic chemokine and cytokine concentrations of CXCL2 (MIP-2; p=0.0171) and IL-6 (p=0.0050) were significantly increased in ΔdblGATA mice compared to the controls (Fig. 6C), whereas differences in CXCL1 (KC) levels did not reach statistical significance (p=0.4550). As expected, the relative and absolute numbers of SiglecF+ eosinophils were significantly reduced in ΔdblGATA mice (Fig. 6E; p=0.0001). Moreover, the frequency of peritoneal CD4+, CD8+ cells (Fig. 6D; both p=0.0007) and Ly6G+Ly6C+ neutrophils (Fig. 6E; p=0.0142) were significantly reduced in ΔdblGATA mice six hours after *E. coli* injection compared to controls. However, absolute numbers of peritoneal CD4+ (p=0.2883), CD8+ (p=0.1452) as well as neutrophils (p=0.1886) were not significantly decreased in eosinophil-deficient mice.

In contrast, absolute, but not relative, numbers of CD11b+F4/80+ macrophages were significantly decreased (p=0.0302) in ΔdblGATA mice (Fig. 6E). Both relative (Fig. 6E, p=0.4555) and absolute numbers of Ly6C+ monocytes (p=0.2410) were not reduced, whereas the relative (Fig. 6E, p=0.0012) and absolute numbers of CD11c+ dendritic cells (p=0.0062) were significantly reduced in eosinophil-deficient mice six hours after *E. coli* injection. These data indicate that eosinophils are contributing to a protective effect during a bacterial sepsis by lessening sepsis induced inflammation and the development of hypothermia. Furthermore, eosinophils seem to support anti-bacterial responses and the recruitment and maintenance of innate and adaptive cell types to the site of inflammation.

3.1.2 Ex vivo generation of bone-marrow-derived eosinophil granulocytes

To analyze whether an adoptive eosinophil transfer can improve sepsis outcome and since eosinophils are a hallmark of Th2 driven immune responses as they occur during parasitic helminth infections and allergies, a modified method to generate high numbers of bone-marrow derived and IL-5-induced functionally competent eosinophil granulocytes (bmEos) in high purity ex vivo was established (Dyer et al., 2008). Bone-marrow cells of naïve BALB/c mice were cultured with 100 µg/mL stem cell factor (SCF), 100 µg/mL FMS-like tyrosine kinase 3 ligand (FLT3L) for four days, followed by a 20 µg/mL IL-5 stimulation for eight additional days.

Twelve days after the cultivation analysis by scanning electron microscopy, light microscopy using H&E staining and flow cytometry revealed a homogenous single-cell population of mature IL-5-induced eosinophil granulocytes (Fig. 7A-C) with a purity of more than 98% SiglecF+CCR3+ cells (Fig. 7D) and simultaneously less than 4% Annexin V or Propidium iodide positive apoptotic and necrotic cells (Fig. 7E).

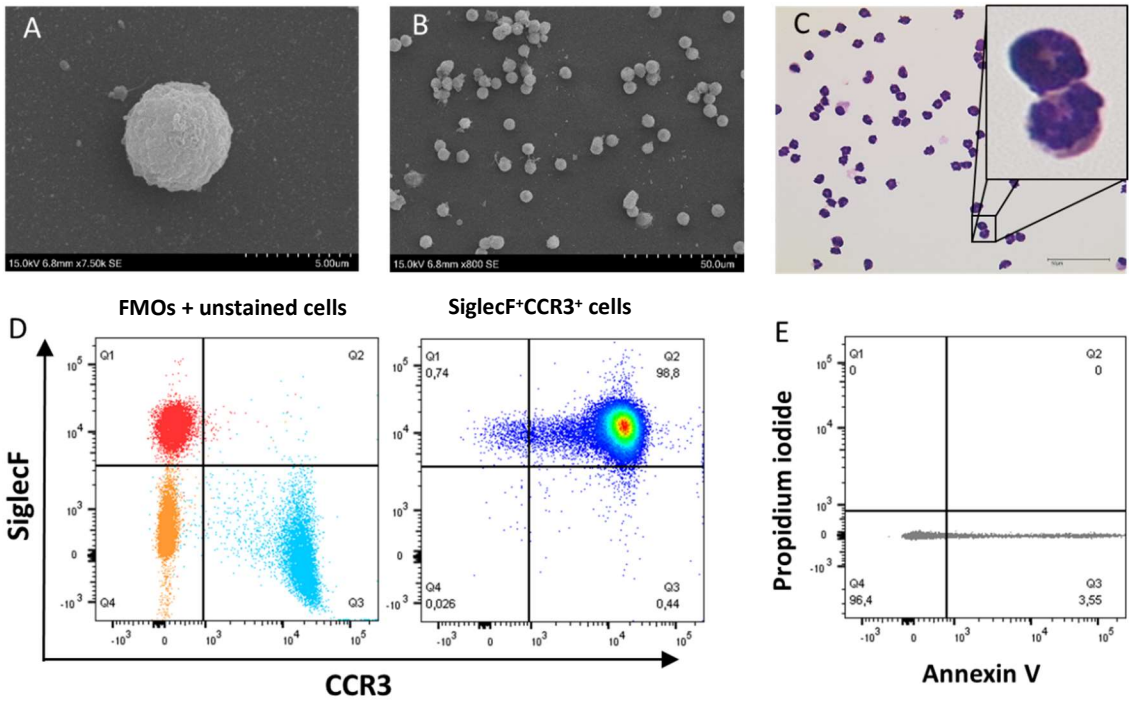

Figure 7: Representatives of ex vivo generated bone-marrow derived eosinophil granulocytes. *Scanning electron microscopy (SEM) picture of a single cell (A) and the homogenous cell population (B) (SEM Pictures were taken by Alexandra Ehrens (AG Hübner) in the lab of Dr. Coralie Martin at the Muséum National d'Histoire Naturelle, Paris, France). Representative light microscopy picture of hematoxylin and eosin stained cell culture at day 12 (C). Additionally, the cells were stained for flow cytometry and analyzed for SiglecF and CCR3 (D) as well as for Annexin V and Propidium iodide (E) demonstrating a high purity (>95%) and low apoptosis (<5%).*

3.1.3 Impact of chemoattractant eotaxins on TLR4 and TLR1/2-driven responses of bmEos

Eosinophil granulocytes are predominantly guided by chemokines like eotaxins via the blood stream to the place of inflammation and infection (Huang and Appleton, 2016). To investigate whether bmEos require priming or pre-activation before an adequate anti-bacterial response, we examined the consequences of chemoattractants on the responsiveness to TLR1/2, TLR4 ligands and filarial extracts in vitro.

BmEos were treated by either CCL11 (eotaxin-1) or CCL24 (eotaxin-2) for 24 hours, followed by TLR4 agonist LPS *E. coli* ultrapure, TLR 1/2 agonist Pam3CSK4 (P3C) and the crude extract of murine filarial pathogenic *Litomosoides sigmodontis* (LsAg) re-stimulation for another 24 hours. Single stimulations and unstimulated bmEos served as controls and the secreted mediators were measured in the supernatant by ELISA. Pooled data of two independent experiments with the same outcome were statistical analyzed by Kruskal-Wallis test (Fig. 8).

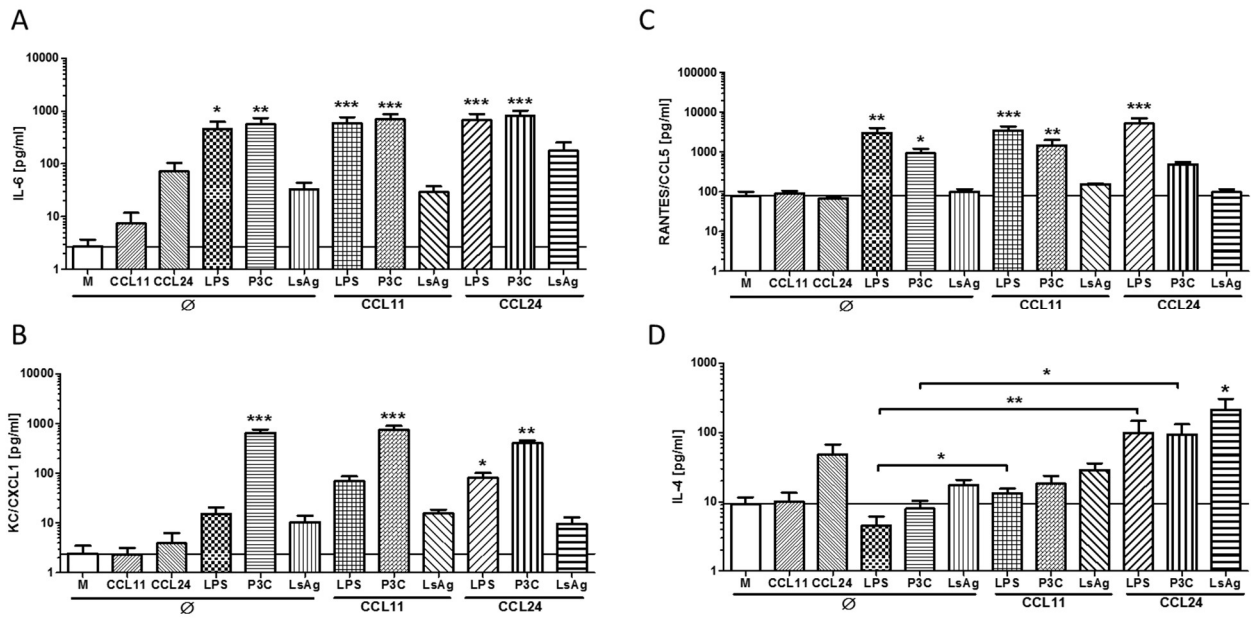

Figure 8: Impact of recruitment factors CCL11 and CCL24 on the TLR1/2, TLR 4 and response to crude filarial antigen extract of bmEos. *The levels of IL-6 (A), KC/CXCL1 (B), RANTES/CCL5 (C) and IL-4 (D) in the supernatant after stimulation are shown. BmEos were treated by eotaxin-1/CCL11 or eotaxin-2/CCL24 for 24 hours followed by 24h re-stimulation with LPS, Pam3CSK4 (P3C) or the crude* Litosomoides sigmodontis *extract (LsAg). Untreated cells served as controls. Data of two independent and pooled in vitro experiments with a total of seven replicates are shown. Data is presented on logarithmic scales as mean + SEM and analyzed for statistical significance using Kruskal-Wallis test (*p<0.05, **p<0.01, ***p<0.001).*

As shown in Figure 8, the P3C stimulation of bmEos resulted in elevated levels of pro-inflammatory IL-6 ($p<0.01$), CXCL1 ($p<0.001$) and CCL5 ($p<0.05$) compared to unstimulated controls (Fig. 8A-C). The levels of IL-4 were not induced after P3C-only stimulation (Fig. 8D; $p>0.05$). The LPS stimulation increased the levels of IL-6 ($p<0.05$) and CCL5 ($p<0.01$) compared to unstimulated control (Fig. 8A+C). The priming of CCL11 and CCL24 did not alter the significantly increased release of IL-6, CXCL1 and CCL5, as well as IL-6 and CCL5 after P3C or LPS re-stimulation, respectively. Of notice, the pre-stimulation with CCL11 and CCL24 resulted in increased levels of CXCL1 after LPS re-stimulation that reached statistical significance for priming with CCL24 (Fig. 8B). Similarly, the combination of CCL24 and LsAg resulted in an increased release of IL-6 ($p>0.05$). Priming with CCL11 ($p<0.05$) and CCL24 ($p<0.01$) altered the release of IL-4 after LPS re-stimulation compared to LPS alone (Fig. 8D). Moreover, this effect was also observed for IL-4 concentrations measured from CCL24-primed and P3C re-stimulated compared to P3C-only stimulated bmEos ($p<0.05$). CCL24 treatment and LsAg re-stimulation significantly increased the release of IL-4 compared to unstimulated controls ($p<0.05$).

The pre-stimulation by the eotaxins CCL11 and CCL24 did not alter the release of the pro-inflammatory neutrophil-recruitment factors IL-6, CXCL1 and CCL5, with the exception of LPS and CXCL1. The IL-4 levels were affected by CCL11 and CCL24 priming before re-stimulation with LPS and P3C. Accordingly, our results provide evidence that the TLR ligands P3C and LPS are potent eosinophil activators for the release of chemotactic mediators and previous eotaxin stimulation has no unique effect on type 1 and type 2 immune responses, but may be more important for type 2 immune responses.

As CCL11 and CCL24 are bound to C-C chemokine receptor type (CCR) 3 (Sturm et al., 2013), it was further investigated whether 24 h of CCL24 treatment could alter the CCR3 expression of bmEos after six hours of P3C re-stimulation, which may indicate changes in the local recruitment of eosinophils. Additionally, Intercellular Adhesion Molecule 1 (CD54; ICAM-1) expression, which is involved in eosinophil adhesion and degranulation (Horie et al., 1997; Reimert et al., 1998), was examined. Consequently, bmEos were analyzed by flow cytometry for the expression of CCR3, ICAM-1 for cell recruitment and adhesion and CD69 for cell activation.

As shown in Figure 9, the treatment with CCL24 resulted in a significant downregulation of CCR3 expression on SiglecF+ cells independent of P3C stimulation (Fig. 9A+B; $p<0.01$). Furthermore, CD54/ICAM-1 expression was significantly induced after P3C stimulation, but independent of CCL24 priming (Fig. 9C+D; $p<0.01$). However, changes in CD69 expression were not observed (Fig. 9E+F; $p>0.05$).

In summary, the stimulation with bacterial LPS and P3C altered the release of pro-inflammatory cytokines and chemokines of bmEos in vitro. Furthermore, especially CCL24 treatment selectively altered the responsiveness to LPS and P3C and reduced the expression of CCR3 on bmEos, whereas CCL24 priming did not alter ICAM-1 expression.

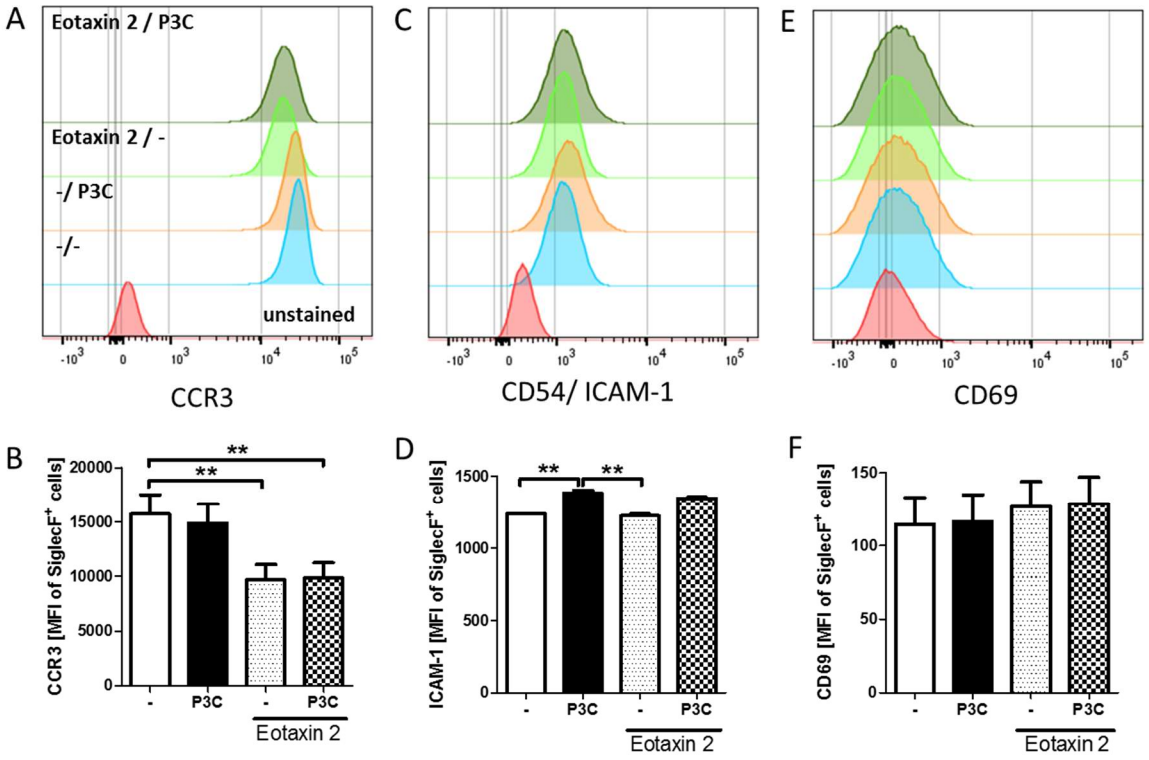

Figure 9: P3C stimulation and eotaxin-2/CCL24 priming alter CD54 and CCR3 expression of bmEos, respectively. *Bone-marrow derived eosinophil granulocytes were treated by CCL24 for 24h followed by six hours stimulation with Pam3CSK4 (P3C). Histograms and MFI of CCR3 positive (A+B), CD54/ ICAM-1 positive (C+D) and CD69 positive (E+F) SiglecF+ eosinophil granulocytes are shown. Data of two independent and pooled in vitro experiments with a total of six replicates are shown and is presented as mean + SEM and analyzed for statistical significance using Kruskal-Wallis test (n=18, *p<0.05, **p<0.01, ***p<0.01).*

3.1.4 Anti-bacterial properties of bone-marrow-derived eosinophil granulocytes

Since our experiments revealed that ex vivo differentiated bmEos respond to LPS and P3C with the release of pro-inflammatory mediators, we further investigated whether bmEos reduce the bacterial burden in vitro. Eosinophils reduce the bacterial burden by a spontaneous deposition of extracellular structures like extracellular mitochondrial DNA combined with eosinophil granule proteins in a process termed ETosis (Yousefi et al., 2008). Previous data demonstrate that human eosinophil granulocytes phagocytose bacteria, but with a lower capacity and more slowly compared to neutrophils (Cline et al., 1968; Yazdanbakhsh et al., 1986). Therefore, we examined the capability and mechanism of bmEos to reduce the bacterial burden one hour after the in vitro co-cultivation by scanning electron microscopy (SEM) (Fig. 10).

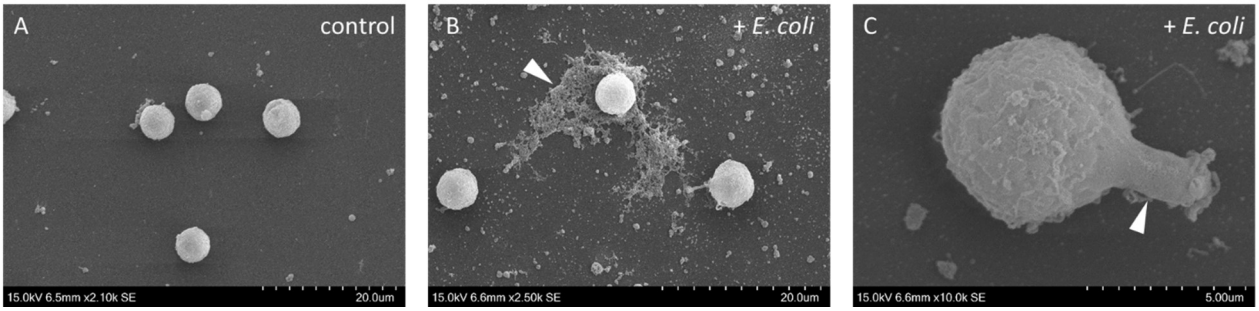

Figure 10: BmEos phagocytose* E. coli *and release extracellular structures. *Scanning electron microscopy (SEM) representatives of bmEos incubated for one hour with* E. coli *ATCC 25922. Unstimulated controls (A), the released extracellular structures (white wedge) (B), phagocytosis of one bacteria (white wedge) (C) are presented. The pictures were taken by Alexandra Ehrens (AG Hübner) in the lab of Dr. Coralie Martin at the Muséum National d'Histoire Naturelle, Paris, France).*

Co-cultivation of bmEos and *E. coli* led to eosinophil-derived extracellular net-forming structures and phagocytosis-related cell arrangements compared to non-*E. coli* exposed controls (white wedges, Fig. 10A-C). The capacity of bmEos to reduce the bacterial burden in vitro was examined. $1x10^7$ bmEos were co-cultured with $5x10^8$ CFU *E. coli* for three hours. Generally, bmEos significantly reduced the number of *E. coli* in vitro (Fig. 11) (p=0.005), independent of prior treatment with the Golgi inhibitors Monensin and Brefeldin A or interferon γ (data not shown). Those data indicate that eosinophils are implemented in protective immune responses against bacteria and provide a direct bactericidal effect which may contribute to the observed protective effect during a sepsis in vivo.

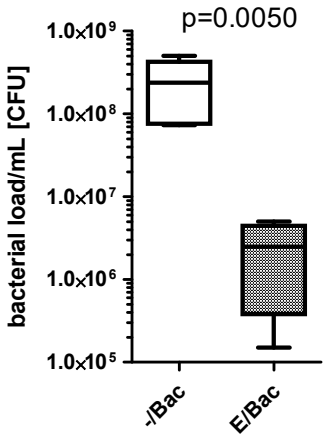

Figure 11: BmEos reduce the number of bacteria in vitro. *$1x10^7$ bmEos were co-cultured with $5x10^8$* E. coli *bacteria for three hours. Pooled data of two independent experiments are shown (n=6 replicates). Data were analyzed by Mann-Whitney-test for significance (p=0.005).*

3.1.5 Genome-wide transcriptome profiling of filarial-induced and non-induced murine eosinophil granulocytes in the absence or presence of an *E. coli* challenge

In this thesis we demonstrated that pathogen-associated molecular patterns like LPS and P3C generates pro-inflammatory responses in bmEos and that the lack of eosinophils in ΔdblGATA mice worsen sepsis outcome. Thus, we further investigated whether a chronic filarial infection modifies the gene expression of murine eosinophils and whether eosinophil gene expression is affected during the acute phase of sepsis. Furthermore, we examined whether the local environment provides a specific transcriptional profile. Consequently, FSC^{hi} SSC^{hi} Hoechst 33258^{neg} $CD11b^{+}F4/80^{+}SiglecF^{+}$ peritoneal and pleural eosinophil granulocytes from *L. sigmodontis*-infected BALB/c mice and uninfected controls were FACS sorted six hours after *E. coli* challenge and genome-wide mRNA sequencing was performed. Unchallenged mice served as controls.

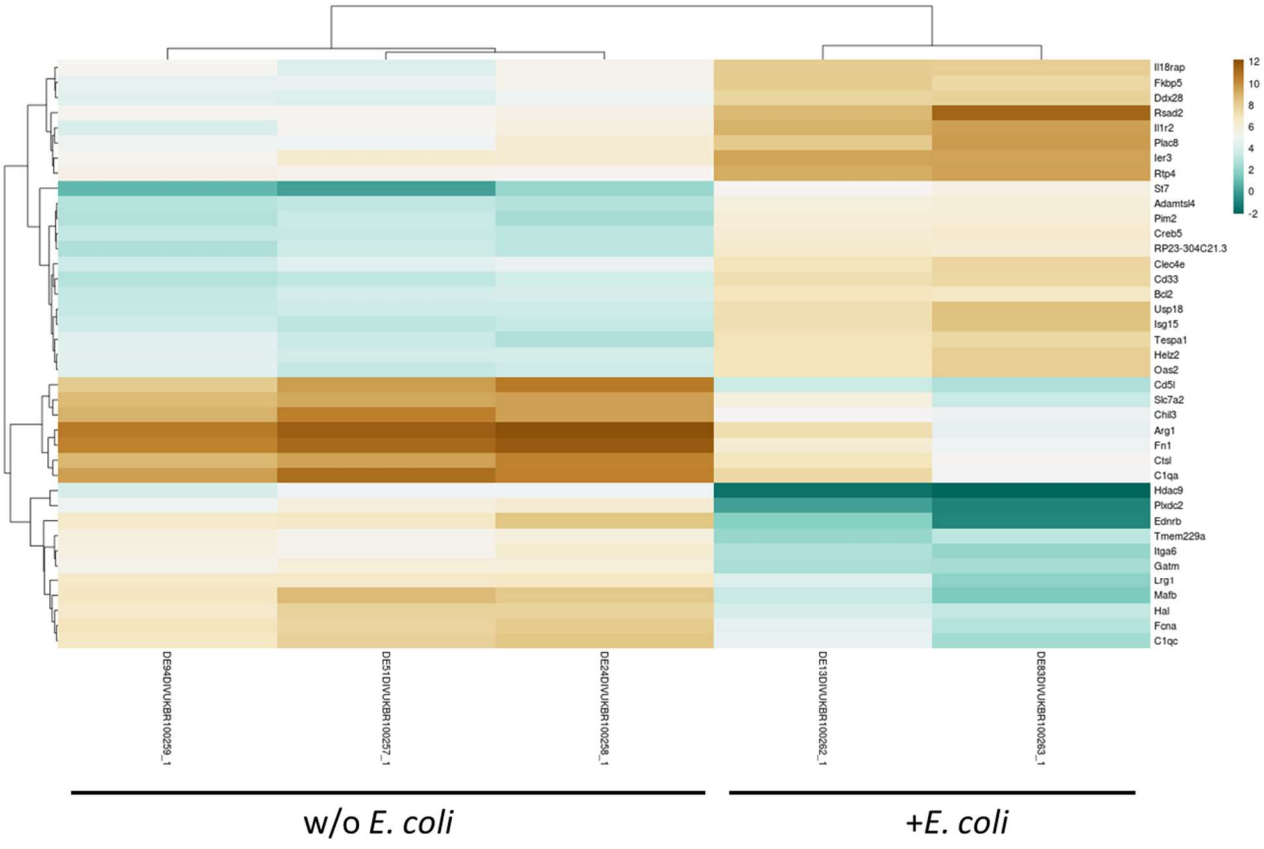

Figure 12: Hierarchical cluster analysis of FACS sorted $FSC^{hi}SSC^{hi}Hoechst33258^{neg}CD11b^{+}F4/80^{+}SiglecF^{+}$ pleural eosinophil granulocytes from* L. sigmodontis*-infected BALB/c mice six hours after $5x10^{8}$ E. coli challenge or PBS injection (adj. P value < 0.05 and FC ≥ 3; n=2-3 mice per group). *Columns represent individual mice, whereas rows represent transcripts. Brown indicates high relative expression levels and blue indicates low relative expression levels. Cluster analysis was performed using Pearson's correlation as distance measurement and average linkage.*

Our initial results suggest that eosinophils of non-infected mice revealed a locus specific and *E. coli* treatment specific transcriptional profile. The *E. coli* challenge induced 39 differentially expressed transcripts in in eosinophils from the pleura of *L. sigmodontis* infected mice compared to eosinophils of non-*E. coli* challenged, *L. sigmodontis* infected mice (adjusted P value < 0.05 and logarithmic fold change ≥ 3 with two to three mice per group; Fig. 12). The differential gene expression analysis revealed an up-regulation of genes (Rsad2↑, USP18↑ and ISG15↑) that are associated with interferon and interleukin 1 responses (Il1r2↑; Fig. 13 and Tab. 1). Moreover, the expression of transcripts of the apoptosis suppressor Bcl2↑, the pathogen and damaged cell detecting cell-surface receptor Clec4↑ and the interleukin-18 receptor accessory protein (Il18rap↑) were increased. Remarkably, Arg1↑, Chil3↑ and Resistin-like alpha (Retnla)↑ were induced in pleural eosinophils by the *L. sigmodontis* infection compared to gut-derived eosinophils (Fig. 14, Fig. 15 and Tab. 2). However, following *E. coli* challenge, the transcripts Arginase-1 (Arg1)↓ and Chitinase-like protein 3 (Chil3)↓ were downregulated in comparison to eosinophils from non-*E. coli* challenged, *L. sigmodontis*-infected mice (Fig. 12 and Tab. 1).

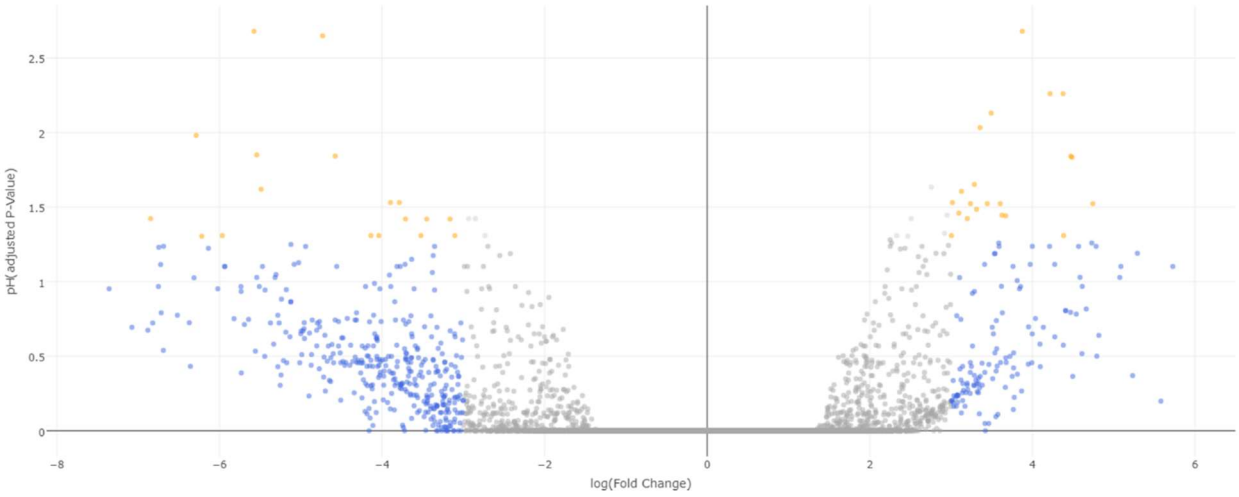

Figure 13: Eosinophils derived from* L. sigmodontis *infected versus* L. sigmodontis *infected and* E. coli *challenged mice reveal 39 differentially expressed genes. *The fold change (x-axis) versus the P value (y-axis) is shown in a volcano plot analysis. Orange dots represent differentially expressed genes (FC ≥ 2.5 and adjusted P value < 0.05) while blue dots represent genes that were non-significant altered (FC ≥ 2.5 and adjusted P value > 0.05).*

Table 1: 39 differentially expressed genes were detected in pleural eosinophils of* E. coli *challenged,* L. sigmodontis *infected mice compared to* L. sigmodontis *infected mice (L.s. inf. +*E. coli *vs. L.s. inf. w/o* E. coli*) (P value < 0.05; FC ≥ 3).

ID/ Transcript	*Symbol*	*logFC*	*adj. P Value*
ENSMUSG00000020641	Rsad2	4,74340631	0,02994141
ENSMUSG00000030107	Usp18	4,4867181	0,01465119
ENSMUSG00000035692	Isg15	4,47297817	0,01436471
ENSMUSG00000029534	St7	4,38342525	0,0491068
ENSMUSG00000026073	Il1r2	4,37682175	0,00547947
ENSMUSG00000004609	Cd33	4,21757108	0,00547947
ENSMUSG00000033355	Rtp4	3,87545246	0,00208824
ENSMUSG00000029322	Plac8	3,67152593	0,03614274
ENSMUSG00000032690	Oas2	3,62550806	0,03578469
ENSMUSG00000034833	Tespa1	3,60452583	0,02994141
ENSMUSG00000003541	Ier3	3,49370319	0,00738762
ENSMUSG00000027580	Helz2	3,44470097	0,02994141
ENSMUSG00000045538	Ddx28	3,35553482	0,00924954
ENSMUSG00000112895	RP23-304C21.3	3,31287212	0,03267535
ENSMUSG00000057329	Bcl2	3,28684254	0,02224129
ENSMUSG00000030142	Clec4e	3,23737771	0,02994141
ENSMUSG00000031155	Pim2	3,19923808	0,0377336
ENSMUSG00000026068	Il18rap	3,12729791	0,02474535
ENSMUSG00000053007	Creb5	3,0955683	0,03464101
ENSMUSG00000024222	Fkbp5	3,01589631	0,02945313
ENSMUSG00000015850	Adamtsl4	3,00350131	0,0491068
ENSMUSG00000048022	Tmem229a	-3,10413747	0,0491068
ENSMUSG00000027199	Gatm	-3,16401518	0,03799554
ENSMUSG00000027111	Itga6	-3,45003611	0,03799554
ENSMUSG00000021477	Ctsl	-3,52138468	0,0491068
ENSMUSG00000037095	Lrg1	-3,71127403	0,03799554
ENSMUSG00000020017	Hal	-3,78738844	0,02945313
ENSMUSG00000026938	Fcna	-3,89618837	0,02945313
ENSMUSG00000036887	C1qa	-4,03892091	0,0491068
ENSMUSG00000036896	C1qc	-4,13610053	0,0491068
ENSMUSG00000031596	Slc7a2	-4,57621844	0,01436471
ENSMUSG00000040809	Chil3	-4,73038329	0,00224281
ENSMUSG00000074622	Mafb	-5,48708854	0,02395874
ENSMUSG00000019987	Arg1	-5,54046603	0,01411056
ENSMUSG00000026193	Fn1	-5,5746639	0,00208824
ENSMUSG00000026748	Plxdc2	-5,9641843	0,0491068
ENSMUSG00000004698	Hdac9	-6,21727061	0,04953091
ENSMUSG00000015854	Cd5l	-6,28659083	0,01044002
ENSMUSG00000022122	Ednrb	-6,84677601	0,0377336

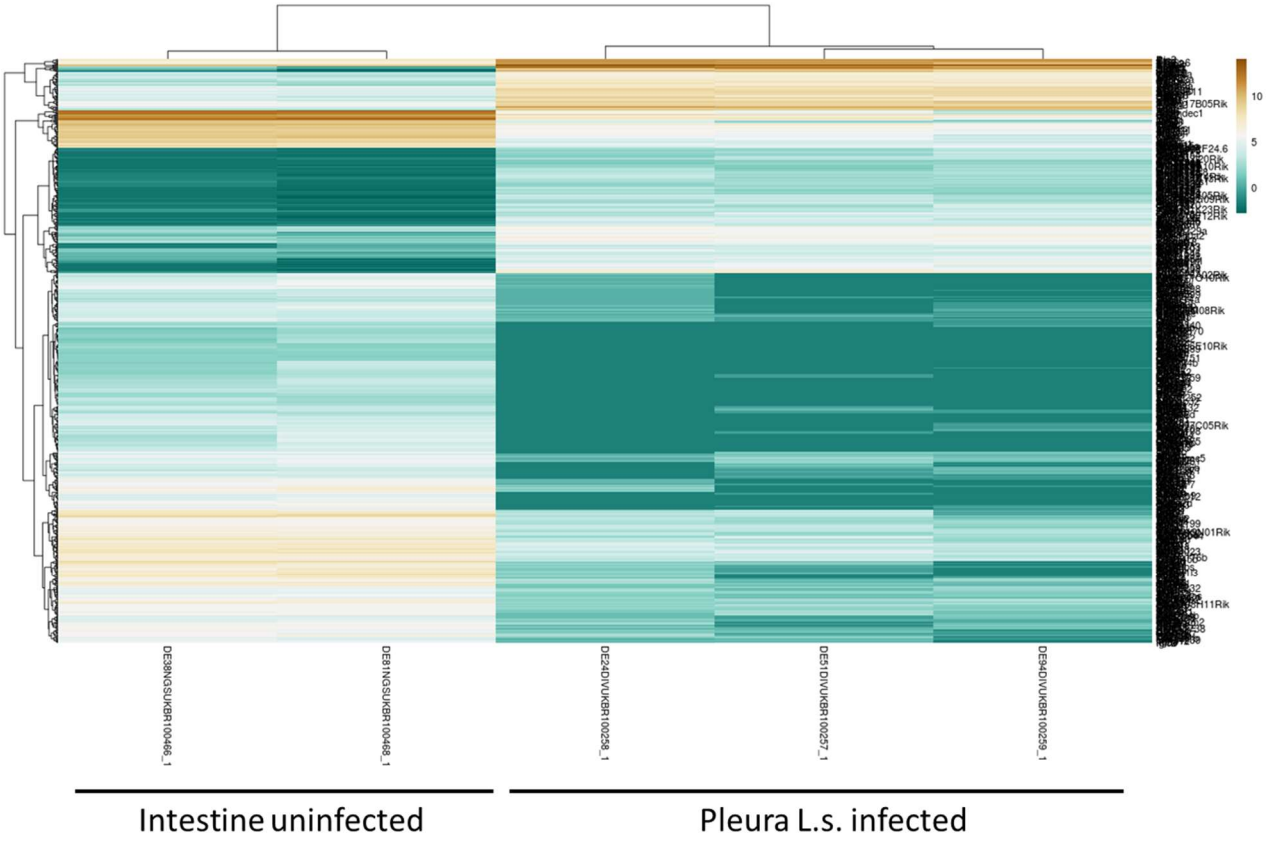

Figure 14: Hierarchical cluster analysis of FACS sorted FSC^{hi}SSC^{hi}Hoechst33258^{neg}CD11b^{+}F4/80^{+}SiglecF^{+} pleural eosinophil granulocytes from* L. sigmodontis*-infected BALB/c mice compared to uninfected intestinal eosinophils without* E. coli *challenge (adj. P value < 0.05 and FC ≥ 3; n=2-3 mice per group). *Columns represent individual mice, whereas rows represent transcripts. Brown indicates high relative expression levels and blue indicates low relative expression levels. Cluster analysis was performed using Pearson's correlation as distance measurement and average linkage.*

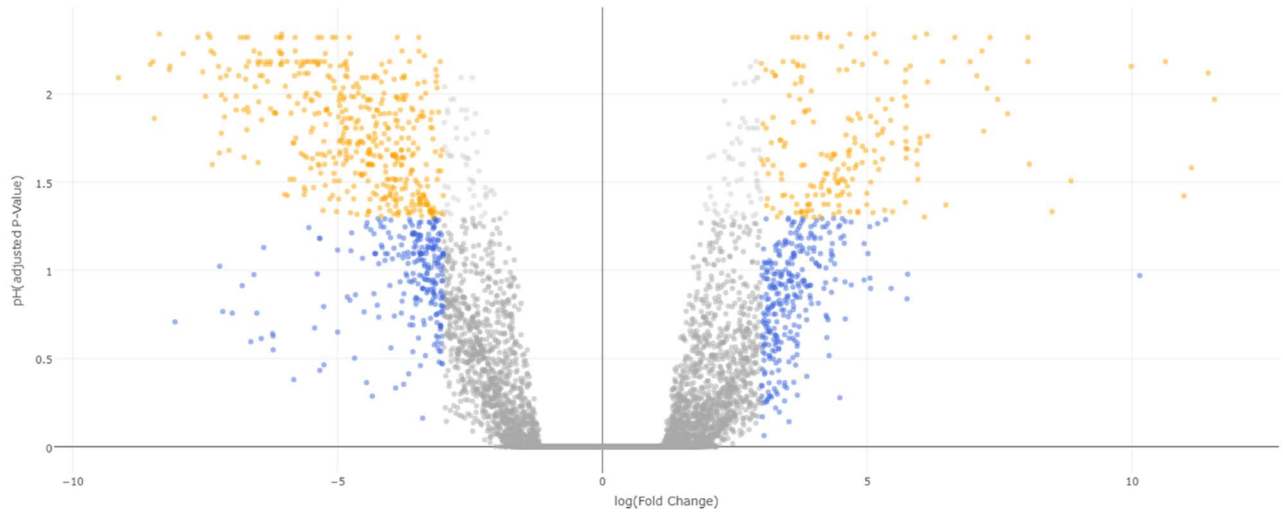

Figure 15: Eosinophils derived from the pleura of* L. sigmodontis *infected versus intestinal eosinophils of uninfected controls revealed 647 differentially expressed genes. *The fold change (x-axis) versus the P value (y-axis) is shown in a volcano plot analysis. Orange dots represent differentially expressed genes (FC ≥ 3 and adjusted P value < 0.05) while blue dots represent genes that were non-significant altered (FC ≥ 3 and adjusted P value > 0.05).*

Table 2: The* L. sigmodontis *infection induced 647 differentially expressed genes in pleural eosinophils compared to intestinal eosinophils from uninfected controls (L.s. inf. pleura vs. uninfected intestine) (P value < 0.05; FC ≥ 3). The top 5 up-regulated and downregulated transcripts are presented.

ID/ Transcript	*Symbol*	*logFC*	*adj. P Value*
ENSMUSG00000019987	Arg1	11,55457936	0,010746584
ENSMUSG00000040809	Chil3	11,43566765	0,0076202
ENSMUSG00000015854	Cd5l	11,11875599	0,02626452
ENSMUSG00000040026	Saa3	10,97756454	0,037889887
ENSMUSG00000061100	Retnla	10,63012459	0,006565505
ENSMUSG00000030017	Reg3g	-8,367517561	0,004581137
ENSMUSG00000049350	Zg16	-8,462132772	0,013788715
ENSMUSG00000020405	Fabp6	-8,487285808	0,006565505
ENSMUSG00000071356	Reg3b	-8,535966189	0,006808938
ENSMUSG00000095079	Igha	-9,13657624	0,00809737

Our results indicate that the transcriptome of eosinophils is altered upon experimental *L. sigmodontis* infection and differs from eosinophils derived from the gut of naïve mice as well as pleural eosinophils from *L. sigmodontis* infected animals after *E. coli* challenge. Future studies should hereby compare the impact of systemic and local modulation of eosinophil responses by the filarial infection. Therefore, gut-derived eosinophils should be analyzed for their modulation in response to the *L. sigmodontis* infection. Furthermore, experiments comparing pleural and peritoneal eosinophil granulocytes from non-*L. sigmodontis* infected and *E. coli*-treated or untreated mice as well as peritoneal eosinophils from *L. sigmodontis* and *E. coli* challenged mice have to be repeated, as those cells had to be excluded from the analysis due to the obtained low quantity of mRNA (<250 pg/µl).

3.2 Genome-wide transcriptional profiling of human monocytes stimulated with *Brugia malayi* crude extract and LPS reveals link to IL-17 associated pathways and diseases

More than 150 million people worldwide are infected with filarial nematodes causing LF and onchocerciasis (WHO, 2015). Helminth parasites modulate cellular immune responses to ensure their survival and reproduction. Consequently, human monocytes from twenty healthy male, non-endemic Caucasian volunteers were exposed to the crude extract of human pathogenic filaria *B. malayi* (BmA) before *Escherichia coli* LPS ultrapure stimulation. A genome-wide transcriptional profiling of more than 47.000 transcripts was performed by Illumina® HumanHT-12 v4 Expression BeadChip technology. Those results were supplemented with flow cytometry analysis and ELISA. Gene Set Enrichment Analysis using Ingenuity IPA© analysis, Gene Ontology enrichment analysis and Protein-Protein Interaction Analysis were performed to identify associations to immune functions and inflammatory disorders that potentially contributes to the development of filaria-caused pathology.

3.2.1 Experimental design and human CD14+ monocyte purification

To examine the immunomodulatory effect of BmA on subsequent LPS stimulation, CD14+ monocytes were purified using a Percoll gradient, followed by magnetic cell separation (MACS). Two hours after separation the cells were stimulated by BmA for 18 h followed by a subsequent stimulation with LPS for an additional 6 h (Fig. 16A). Corresponding BmA or LPS ultrapure *E. coli* only stimulated samples served as controls. 24 hours after stimulation the cells were harvested, lysed and an RNA purification was performed. MACS of human monocytes reached more than 95% purity, the overnight monocyte apoptosis was less than 8% Annexin V+ or Propidium iodide + cells (Fig. 16B+C).

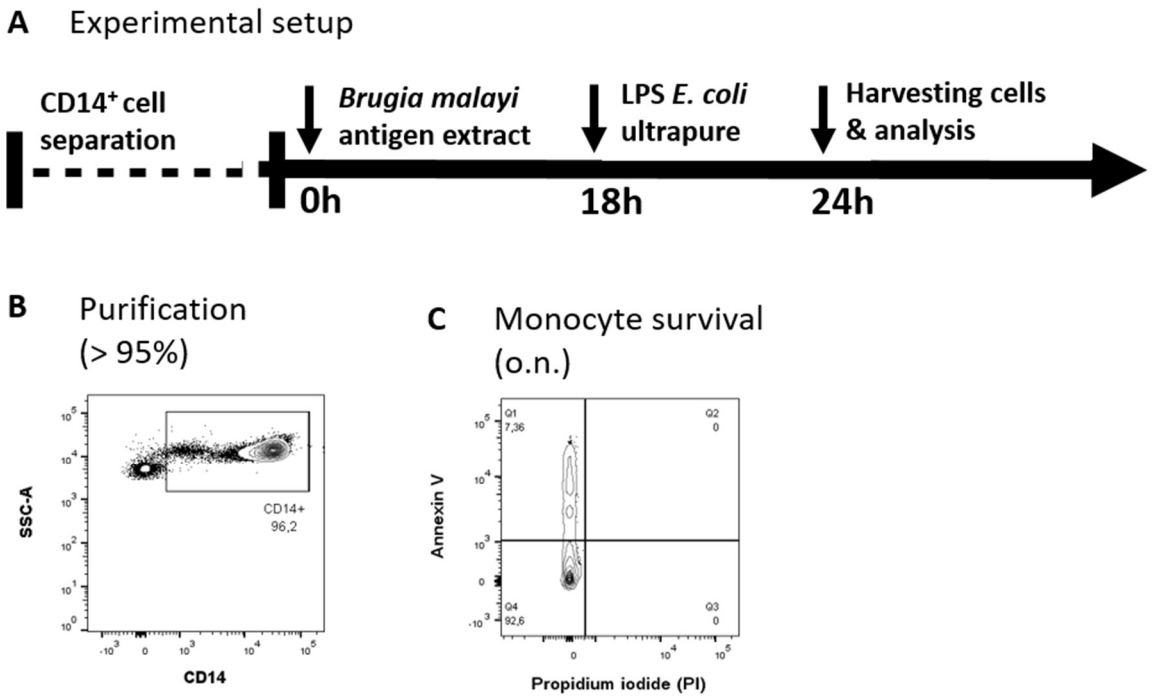

Figure 16: Experimental setup and stimulation scheme of the transcriptomic profiling approach *is presented in A. The purification after CD14+ monocyte separation of healthy human non-endemic volunteers is shown in (B) and the overnight viability of separated monocytes is displayed in (C).*

3.2.2 BmA stimulation alone significantly induced transcripts in human monocytes compared to unstimulated controls

To examine whether BmA can induce differentially expressed genes (P value < 0.05; FC ≥ ±1.5), BmA-only stimulated human monocytes were analyzed 24 h after stimulation and compared to unstimulated controls (BmA vs. control). 42 transcripts were nominally significant induced by BmA treatment, but did not withstand correction for multiple testing.

Members of the metallothionein family↑, indoleamine 2,3-dioxygenase 1 (INDO/IDO1)↑, TNF alpha induced protein 6 (TNFAIP6)↑, leukocyte immunoglobulin like receptor A3 (LILRA3)↑, C-C motif chemokine ligand 23 (CCL23)↑, interleukin 8, C-X-C motif chemokine ligand 8 (IL8/CXCL8)↑ and signal transducer and activator of transcription 1 pathway (STAT1)↑ were upregulated upon BmA treatment. In contrast, albumin (ALB)↓, CAMPATH-1 antigen (CD52)↓, ribonuclease P RNA component H1 (RPPH1)↓, and Fc fragment of IgE receptor 1α (FCER1A)↓ and genes of the SNOR- and RNU-family↓ were differentially downregulated (Tab. 3).

Table 3: Stimulation with* Brugia malayi *crude extract (BmA) induced 42 significantly expressed transcripts in human monocytes 24 h after treatment compared to unstimulated controls (P value < 0.05; FC ≥ 1.5).

ID/Transcript	*Gene*	*Definition*	*Adj. P Value*	*FC*
ILMN_1715401	**MT1G**	metallothionein 1G	2,03E-05	3,04
ILMN_2124802	**MT1H**	metallothionein 1H	3,10E-05	2,99
ILMN_1718766	**MT1F**	metallothionein 1F	3,16E-05	2,43
ILMN_2173611	**MT1E**	metallothionein 1E	0,0004463	2,32
ILMN_1686664	**MT2A**	metallothionein 2A	0,00071025	2,14
ILMN_1691156	**MT1A**	metallothionein 1A	0,00068149	2,09
ILMN_1775170	**MT1X**	metallothionein 1X	0,00060765	2,05
ILMN_1656310	**INDO**	indoleamine 2,3-dioxygenase 1	0,01480863	1,96
ILMN_1657435	**MT1M**	metallothionein 1M	0,00474874	1,94
ILMN_3239965	**IDO1**	indoleamine 2,3-dioxygenase 1	0,02005119	1,91
ILMN_1686109	**CCL23**	C-C motif chemokine ligand 23	0,00030124	1,77
ILMN_1769388	**GJB2**	gap junction protein beta 2	6,09E-05	1,75
ILMN_3237878	**LOC100133875**	similar to Leukocyte immune-globulin-like receptor, subfamily A (without TM domain), member 3	0,01584309	1,68
ILMN_1785732	**TNFAIP6**	TNF alpha induced protein 6	0,00256657	1,68
ILMN_1786303	**LILRA3**	leukocyte immunoglobulin like receptor A3	0,01752226	1,66
ILMN_1776188	**MAP1LC3A**	microtubule associated protein 1 light chain 3 alpha	0,00252913	1,61
ILMN_1662640	**C20orf127/MT1P3**	metallothionein 1 pseudogene 3	0,00360858	1,61
ILMN_3307868	**CHI3L1**	chitinase 3 like 1	0,00016417	1,61
ILMN_1764030	**CCL23**	C-C motif chemokine ligand 23	0,00169947	1,56
ILMN_1691364	**STAT1**	signal transducer and activator of transcription 1	0,02646289	1,56
ILMN_1690105	**STAT1**	signal transducer and activator of transcription	0,03108525	1,54
ILMN_1661631	**LILRA3**	leukocyte immunoglobulin like receptor A3	0,04043949	1,54
ILMN_1695316	**SLC39A8**	solute carrier family 39 member 8	0,00504967	1,51
ILMN_2184373	**IL8 / CXCL8**	interleukin 8, C-X-C motif chemokine ligand 8	0,00468001	1,50
ILMN_1713832	**SNORD15B**	small nucleolar RNA, C/D box 15B	0,0015155	-1,51
ILMN_2082762	**SNORD68**	small nucleolar RNA, C/D box 68	0,00135061	-1,52
ILMN_3309759	**VTRNA1-1**	vault RNA 1-1	0,00228141	-1,52
ILMN_1684982	**PDK4**	pyruvate dehydrogenase kinase 4	7,96E-06	-1,53
ILMN_3244640	**SNORD96A**	small nucleolar RNA, C/D box 96A	0,00084078	-1,57
ILMN_3249167	**SNORA63**	small nucleolar RNA, H/ACA box 63	0,00244951	-1,59
ILMN_3240594	**RNU4ATAC**	RNA, U4atac small nuclear (U12-dependent splicing)	0,00561025	-1,59
ILMN_2208903	**CD52**	CD52 molecule	0,00280334	-1,62
ILMN_1782939	**ALB**	albumin	0,00672508	-1,62
ILMN_3238435	**SNORA12**	small nucleolar RNA, H/ACA box 12	0,02534893	-1,63
ILMN_1901419	**RNU11**	RNU11, RNA, U11 small nuclear	0,00292359	-1,64
ILMN_3246209	**SCARNA10**	small Cajal body-specific RNA 10	0,00383962	-1,66
ILMN_1908824	**RNU6ATAC**	RNA, U6atac small nuclear (U12-dependent splicing)	0,01291778	-1,66
ILMN_3241878	**RNU6ATAC**	RNA, U6atac small nuclear (U12-dependent splicing)	0,00870081	-1,68
ILMN_1704056	**RPPH1**	ribonuclease P RNA component H1	0,00643212	-1,75
ILMN_3245103	**RNU11**	RNA, U11 small nuclear	0,00188116	-1,78
ILMN_1768139	**RNU12**	RNA, U12 small nuclear	0,00262698	-1,78
ILMN_3242405	**RMRP**	RNA component of mitochondrial RNA processing endoribonuclease	0,00048553	-1,81
ILMN_1688423	**FCER1A**	Fc fragment of IgE receptor Ia	8,21E-05	-1,82
ILMN_1682402	**SNORD46**	small nucleolar RNA, C/D box 46	0,00268996	-1,89

3.2.3 High similarity in the transcriptional profiles of LPS-only and BmA+LPS re-stimulated monocytes compared to unstimulated controls

Similarities in the transcriptional profiles of LPS-only and combined BmA+LPS stimulated monocytes (LPS vs. control and BmA+LPS vs. control) were further analyzed. Our results supported previous findings of LPS-only stimulated human monocytes (Kim et al., 2014). Accordingly, differentially expressed genes (P value < 0.05; FC ≥ ±1.5) were involved in inflammation and inflammasome activation. Furthermore, increased levels of C-X-C motif chemokine ligand 1 (CXCL1/ NAP-3)↑, C-C motif chemokine ligand 19 (CCL19/ MIP3B)↑, tumor necrosis factor superfamily member 12 (TNFSF12)↑, but also cytokines like interleukin 1 beta (IL-1B)↑ and interleukin 6 (IL-6)↑ were induced.

The BmA treatment and LPS re-stimulation resulted in 1,979 differentially expressed genes (BmA+LPS vs. control), whereas LPS alone stimulation resulted in 2,215 differentially expressed genes (LPS vs. control) compared to unstimulated controls. By comparing both conditions, a high overlap of 1,862 differentially expressed genes with a high concordance across fold-changes (r^2=0.98) was identified (Fig. 17A). 335 transcripts were regulated exclusively in LPS-only stimulated cells in contrast to 117 transcripts in BmA primed and LPS re-stimulated cells compared to control (Fig. 17B).

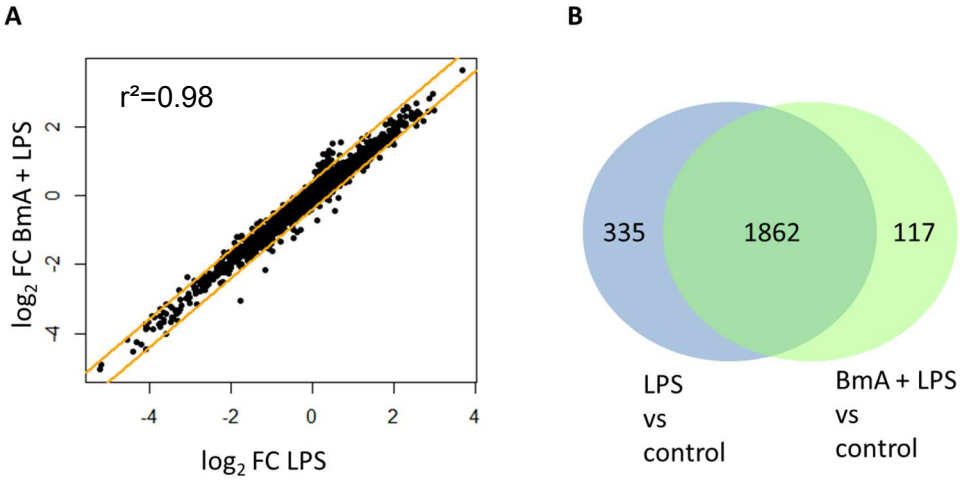

Figure 17: High overlap of 1,862 differentially expressed genes and concordance across fold-changes in human monocytes after stimulation with BmA+LPS re-stimulation and LPS-only stimulation versus unstimulated controls.

The Log_2 fold-change (FC) for LPS versus unstimulated control against the log_2 FC for B. malayi *extract (BmA) plus LPS re-stimulation versus unstimulated control is shown in A (FC ≥ 1.5 and corrected P value < 0.05). Orange lines show a fold-change ≥ 1.5 and the correlation of both FCs is r^2=0.98. In addition, the data sets are presented as Venn diagram of differentially expressed transcripts presenting the high overlap of LPS and BmA+LPS versus control.*

3.2.4 BmA treatment modulates gene expression in human monocytes after LPS stimulation

It was further examined whether BmA treatment of human monocytes can modify TLR4-responses. Evidence was previously reported for soluble *Schistosoma mansoni* egg antigens (Kane et al., 2004; Kane et al., 2008), *Fasciola hepatica* defense molecule 1 (FhDM-1) (Robinson et al., 2011) and Chitohexaose derived from *Setaria digitata* (Panda et al., 2012).

Consequently, the immunomodulatory effect of BmA priming on pro-inflammatory LPS responses was analyzed by comparing human monocytes primed with BmA for 18 h and LPS re-stimulation for six hours directly to LPS-only stimulated monocytes (BmA+LPS vs. LPS).

After multiple test correction only pentraxin 3 (PTX3/TSG-14)↓, a member of the pentraxins superfamily and a multifunctional soluble pattern recognition receptor, was significantly reduced (P value < 0.05; FC ≥ ±1.5). 29 downregulated and 19 upregulated transcripts were altered at nominal significance, resulting in 49 differentially expressed transcripts overall (Tab. 4, Fig. 18A). In addition, a hierarchical cluster analysis of differentially expressed genes was performed to analyze subjects and transcripts using Pearson's correlation as a distance measurement and average linkage (Fig. 18B).

In line with our previous findings (BmA vs. control), members of the metallothionein family and members of the SNOR C/D box↓, SNOR H/ACA box↓ and RNU-family genes↓ were down-regulated by BmA priming before LPS *E. coli* stimulation. ALB↓, TNFRSF21↓, CD52↓, and CCL20/MIP3α↓ were reduced.

In contrast, the transcripts of the C-X-C motif chemokine ligands CXCL5/ENA-78↑, CXCL6/GCP-2↑, and CXCL7/NAP-2/PPBP↑ were up-regulated. Additionally, the expression of C-type lectin domain family 4 member A (CD367/CLEC4A), as well as the matrix metalloproteinases MMP7↑ and MMP9↑, were up-regulated.

Table 4: Stimulation with* Brugia malayi *crude extract (BmA) before LPS re-stimulation induced 49 significantly expressed transcripts in human monocytes 24 h after treatment compared to LPS-only stimulated (P value < 0.05; FC ≥ 1.5).

ID/Transcript	*Symbol*	*Definition*	*Adj. P Value*	*FC*
ILMN_2171384	**CXCL5**	C-X-C motif chemokine ligand 5, ENA-78, SCYB5	0,16580403	2,56
ILMN_2124802	**MT1H**	metallothionein 1H	0,21755233	2,10
ILMN_1752562	**CXCL5**	C-X-C motif chemokine ligand 5, ENA-78, SCYB5	0,19664078	2,08
ILMN_1767281	**PPBP**	pro-platelet basic protein, CXCL7; NAP-2	0,24213693	1,90
ILMN_1657435	**MT1M**	metallothionein 1M	0,36481335	1,80
ILMN_1718766	**MT1F**	metallothionein 1F	0,31856643	1,74
ILMN_1796316	**MMP9**	matrix metallopeptidase 9,	0,07680070	1,70
ILMN_1715401	**MT1G**	metallothionein 1G	0,51871992	1,70
ILMN_1779234	**CXCL6**	C-X-C motif chemokine ligand 6, GCP2	0,10022298	1,62
ILMN_1709204	**CLEC4A**	C-type lectin domain family 4 member A, CD367	0,14503500	1,58
ILMN_1733579	**EVI2A**	ecotropic viral integration site 2A	0,18209529	1,57
ILMN_1780671	**PLEKHG3**	pleckstrin homology and RhoGEF domain containing G3	0,13891895	1,56
ILMN_1690342	**LTA4H**	leukotriene A4 hydrolase	0,37794065	1,55
ILMN_2161577	**CXCL6**	C-X-C motif chemokine ligand 6, GCP2	0,07680070	1,55
ILMN_1740015	**CD14**	CD14 molecule	0,45457167	1,55
ILMN_1689518	**PECAM1**	platelet and endothelial cell adhesion molecule 1	0,12248916	1,54
ILMN_1735910	**VMO1**	vitelline membrane outer layer 1 homolog	0,33937674	1,53
ILMN_1763452	**EVI2B**	ecotropic viral integration site 2B	0,13891895	1,53
ILMN_1690125	**PDLIM7**	PDZ and LIM domain 7	0,06134147	1,52
ILMN_3246869	**SCARNA21**	small Cajal body-specific RNA 21	0,12483774	-1,51
ILMN_2208903	**CD52**	CD52 molecule	0,41519934	-1,51
ILMN_1801842	**PTX3**	pentraxin 3, TSG-14; TNFAIP5	0,03244278	-1,53
ILMN_1691846	**G0S2**	G0/G1 switch 2	0,12483774	-1,53
ILMN_3241021	**RNY4**	RNA, Ro-associated Y4	0,27347828	-1,54
ILMN_3236049	**SNORD12**	small nucleolar RNA, C/D box 12	0,14863846	-1,54
ILMN_1704730	**CD93**	CD93 molecule	0,45819779	-1,57
ILMN_1689378	**CCRN4L**	CCR4 carbon catabolite repression 4-like	0,07680070	-1,57
ILMN_1699695	**TNFRSF21**	TNF receptor superfamily member 21	0,19664078	-1,58
ILMN_2082762	**SNORD68**	small nucleolar RNA, C/D box 68	0,13891895	-1,60
ILMN_1810462	**C10orf115**	chromosome 10 open reading frame 115 / Ba215c7.4	0,14863846	-1,60
ILMN_3244640	**SNORD96A**	small nucleolar RNA, C/D box 96A	0,14503500	-1,62
ILMN_3309759	**VTRNA1-1**	vault RNA 1-1	0,14863846	-1,62
ILMN_1713832	**SNORD15B**	small nucleolar RNA, C/D box 15B	0,12972666	-1,63
ILMN_3240594	**RNU4ATAC**	RNA, U4atac small nuclear (U12-dependent splicing)	0,24872237	-1,65
ILMN_1713751	**ADAM19**	ADAM metallopeptidase domain 19	0,30719616	-1,69
ILMN_3249167	**SNORA63**	small nucleolar RNA, H/ACA box 63	0,13849379	-1,73
ILMN_1657234	**CCL20**	C-C motif chemokine ligand 20	0,31270144	-1,74
ILMN_1737709	**RPL10L**	ribosomal protein L10 like	0,07680070	-1,76
ILMN_3246209	**SCARNA10**	small Cajal body-specific RNA 10	0,17434420	-1,78
ILMN_1901419	**RNU11**	RNA, U11 small nuclear	0,13891895	-1,79
ILMN_3242405	**RMRP**	RNA component of mitochondrial RNA processing endoribonuclease	0,10022298	-1,90
ILMN_3241878	**RNU6ATAC**	RNA, U6atac small nuclear (U12-dependent splicing)	0,17434420	-1,91

ILMN_3245103	**RNU11**	RNA, U11 small nuclear	0,13849379	-1,91
ILMN_3238435	**SNORA12**	small nucleolar RNA, H/ACA box 12	0,22461734	-1,91
ILMN_1704056	**RPPH1**	ribonuclease P RNA component H1	0,18209529	-1,92
ILMN_1908824	**RNU6ATAC**	RNA, U6atac small nuclear (U12-dependent splicing)	0,18003597	-1,94
ILMN_1782939	**ALB**	albumin	0,10022298	-1,96
ILMN_1768139	**RNU12**	RNA, U12 small nuclear	0,12248916	-2,00
ILMN_1682402	**SNORD46**	small nucleolar RNA, C/D box 46	0,12248916	-2,11

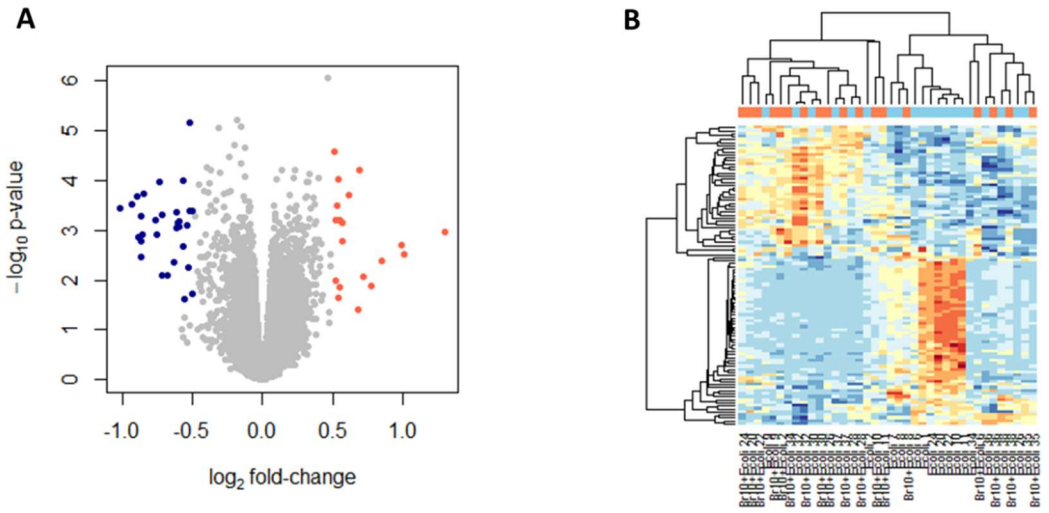

Figure 18: BmA+LPS re-stimulation versus LPS-only stimulation of human monocytes resulted in 49 differentially expressed genes. *The Log_2 fold-change (x-axis) versus the log_{10} P value (y-axis) for* B. malayi *antigen (BmA) plus LPS re-stimulation versus LPS alone is shown in a volcano plot analysis (A) (see Table 2). Orange dots represent up-regulated while blue dots represent down-regulated differentially expressed genes (FC ≥ 1.5 and corrected P value < 0.05). The hierarchical cluster analysis of differentially expressed genes is shown in (B). Columns represent subjects, whereas rows represent transcripts. Red indicates high relative expression levels and blue indicates low relative expression levels. Cluster analysis was performed using Pearson's correlation as distance measurement and average linkage.*

The overlap of transcriptional changes of BmA-only stimulated (BmA vs. control) and BmA primed and LPS re-stimulated cells (BmA+LPS vs. LPS) was analyzed to determine the differentially expressed transcripts that were individually altered (double detected transcripts were excluded) (Fig. 19). BmA+LPS compared to LPS stimulation resulted in 25 transcripts including CCL20, CXCL5-7, PTX3 and TNFRSF12. Members of the metallothionein family and the SNOR and RNU-family in addition to albumin and CD52 were found in the overlay of both groups (BmA vs. control and BmA+LPS vs. LPS). Moreover, IL-8 and CCL23 beside STAT1 expression were found in BmA-only stimulated cells compared to unstimulated controls.

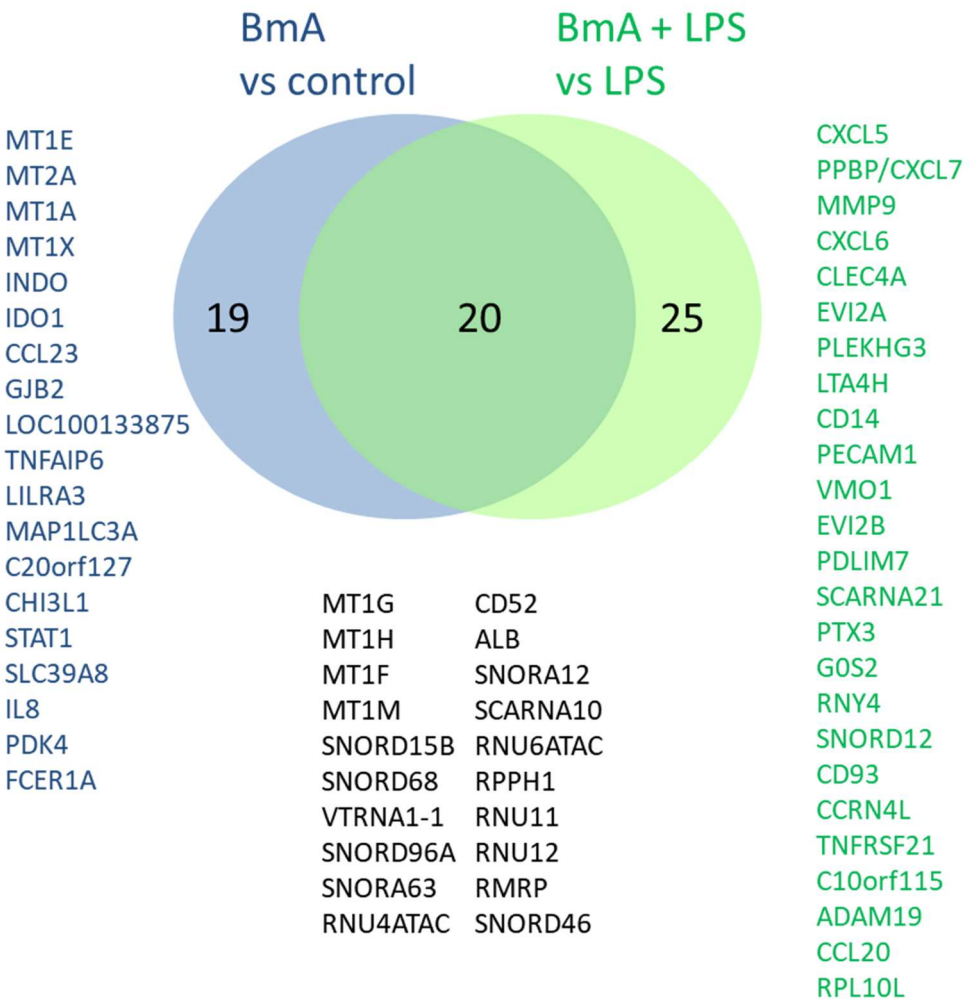

Figure 19: High correspondence of transcripts of* B. malayi *extract (BmA) only (BmA vs. control) compared to BmA+LPS stimulated human monocytes (BmA+LPS vs. LPS). *Venn diagram showing the comparison of differentially expressed transcripts (FC ≥ 1.5 and P value < 0.05).*

3.2.5 BmA priming reduces CD86 and HLA-DR expression of LPS-stimulated human monocytes and triggers CXCL5 and CXCL6 release

Since an *L. sigmodontis*–mediated protection against *E. coli*-induced sepsis is associated with reduced peritoneal macrophage activation (Gondorf et al., 2015), expression profiles of the co-stimulatory cell activation marker B7-2 (also known as CD86) and the human leukocyte antigen - antigen D related (HLA-DR) on human monocytes were investigated by flow cytometry (n=8) (Fig. 20). To confirm part of the data of the transcriptome analysis, released protein levels in the supernatants were measured by ELISA or Multiplex Assay (n=20).

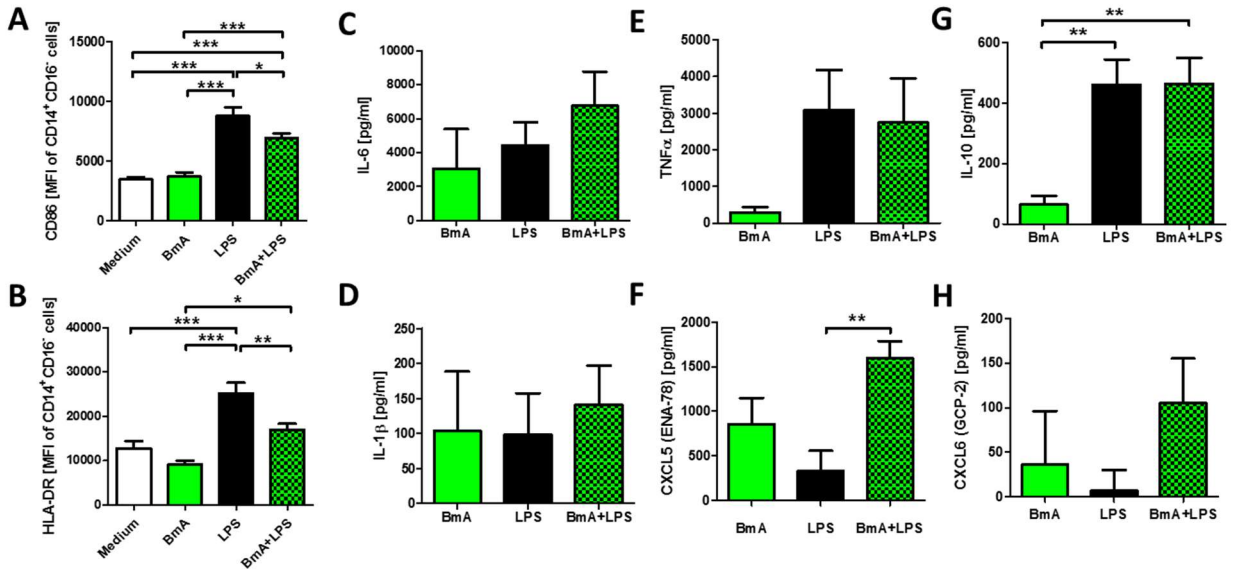

Figure 20: BmA priming reduces CD86 and HLA-DR expression on human CD14⁺CD16⁻ monocytes after LPS stimulation and increases CXCL5 and CXCL6 levels. *The mean fluorescence intensity (MFI) of CD86 (A) and HLA-DR (B) of monocytes stimulated with BmA and/or LPS as well as unstimulated controls (n=8). Mean concentrations of IL-6 (C), IL-1ß (D), TNF (E), CXCL5 (F), IL-10 (G), and CXCL6 (H) in the supernatant after stimulation (n=20). Baseline concentrations of unstimulated controls were subtracted for each donor. Data are presented as mean + SEM and analyzed for statistical significance using ANOVA followed by Bonferroni Comparison Test (*p<0.05, **p<0.01, ***p<0.01).*

As shown in Figure 20A+B, LPS stimulation caused a significant increase of CD86 and HLA-DR expression ($p<0.001$). The BmA pre-exposure followed by LPS stimulation significantly reduced the CD86 ($p<0.05$) and HLA-DR ($p<0.01$) expression levels compared to LPS alone. BmA stimulation alone did not result in an altered expression of CD86 and HLA-DR ($p>0.05$). Furthermore, the release of IL-1β and IL-6 was not significantly altered upon BmA priming followed by LPS stimulation ($p>0.05$) (Fig. 20C+D). An increased release of TNF ($p>0.05$) and IL-10 ($p<0.01$) was observed following LPS stimulation, but was not affected by BmA pre-treatment ($p>0.05$) (Fig. 20E+G). Increased levels of CXCL5 ($p>0.01$) and CXCL6 ($p>0.05$) caused by BmA priming before LPS stimulation confirmed our transcriptional profiles of up-regulated differentially expressed transcripts (BmA+LPS vs. LPS).

Furthermore, the apoptosis of human monocytes was reduced by the BmA treatment compared to unstimulated monocytes (Fig. 21A, p=0.0257, n=12). LPS-only stimulation also reduced the frequency of apoptotic cells (Fig. 21B) and previous BmA treatment did not affect the frequency of apoptotic cells upon LPS-restimulation compared to LPS-only stimulated cells (Fig. 21C, p=0.7051).

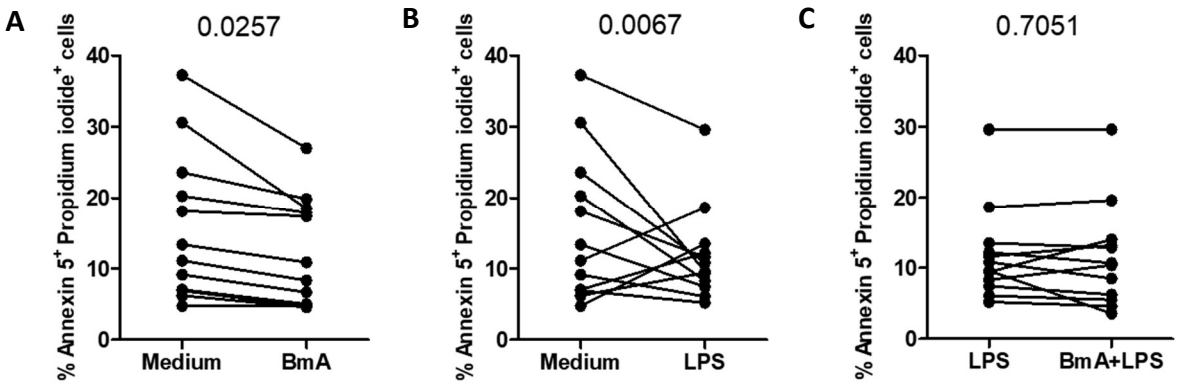

Figure 21: The BmA treatment reduces apoptosis of human monocytes and does not alter reduced apoptosis after LPS stimulation. *The frequency of Annexin5+ Propidium iodide+ cells after BmA-only stimulation (A), LPS-only (B) and in combination of BmA-priming and LPS stimulation of twelve donors is shown. The data were analyzed by Paired t test for significance.*

3.2.6 Pathway analysis of transcriptome profiles of BmA primed and LPS stimulated human monocytes reveals link to IL-17 associated pathways and diseases

KEGG chemokine signaling pathway analysis

To clarify whether BmA-induced differentially expressed genes are involved in biological pathways and diseases, gene enrichment analyses were performed. First, both sets of differentially expressed genes (BmA vs. control and BmA+LPS vs. LPS) were analyzed by Kyoto Encyclopedia of Genes and Genomes (KEGG) chemokine signaling pathway analysis (Tab. 5). BmA vs. control affected five pathways: chemokine signaling pathway, inflammation, bronchial diseases, bronchiolitis and immune system diseases. Pathway analysis of BmA+LPS vs. LPS revealed thirteen affected pathways, including the identified pathways of BmA vs. control except for immune system diseases. The analysis revealed pathways (BmA+LPS vs. LPS) that included arteriosclerosis and cardiovascular diseases like infarction.

Table 5: Overview of pathways affected by BmA treatment alone (BmA vs. control) or followed by LPS stimulation (BmA+LPS vs. LPS) determined by KEGGs chemokine signaling pathway analysis. Differentially expressed genes (P value < 0.05; FC ≥ 1.5) of monocytes of human non-endemic controls (n=20) were analyzed.

Comparison	*Category*	*#Gene*	*Symbol*	*Statistics*
BmA vs. control	**Chemokine signaling pathway**	**3**	STAT1, CXCL8, CCL23	C=145;O=3;E=0.46;R=6.49;rawP=0.0109;adjP=0.0109
	Inflammation	**7**	IDO1, TNFAIP6, FCER1A, CCL23, CXCL8, CHI3L1, STAT1	C=278;O=7;E=0.89;R=7.90;rawP=2.30e-05;adjP=9.20e-05
	Bronchial Diseases	**5**	IDO1, FCER1A, CCL23, CXCL8, CHI3L1	C=139;O=5;E=0.44;R=11.29;rawP=7.28e-05;adjP=0.0001
	Bronchiolitis	**5**	IDO1, FCER1A, CCL23, CXCL8, STAT1	C=142;O=5;E=0.45;R=11.05;rawP=8.06e-05;adjP=0.0001
	Immune System Diseases	**5**	IDO1, FCER1A, CD52, CXCL8, CHI3L1	C=407;O=5;E=1.30;R=3.85;rawP=0.0089;adjP=0.0089
BmA+LPS vs LPS	**Cytokine-cytokine receptor interaction**	**5**	CXCL5, CCL20, TNFRSF21, PPBP, CXCL6	C=161;O=5;E=0.64;R=7.80;rawP=0.0004;adjP=0.0012
	Rheumatoid arthritis	**3**	CXCL5, CCL20, CXCL6	C=71;O=3;E=0.28;R=10.61;rawP=0.0028;adjP=0.0028
	Chemokine signaling pathway	**4**	CXCL5, CCL20, PPBP, CXCL6	C=145;O=4;E=0.58;R=6.92;rawP=0.0026;adjP=0.0028
	Inflammation	**8**	MMP9, CXCL5, CCL20, PTX3, CXCL6, CD14, PECAM1, PPBP	C=278;O=8;E=1.11;R=7.22;rawP=1.19e-05;adjP=9.06e-05
	Bronchial Diseases	**6**	MMP9, CXCL5, CCL20, CXCL6, CD14, LTA4H	C=139;O=6;E=0.55;R=10.83;rawP=1.71e-05;adjP=9.06e-05
	Respiratory Tract Infections	**6**	MMP9, CXCL5, CXCL6, CD14, PECAM1, PPBP	C=144;O=6;E=0.57;R=10.46;rawP=2.09e-05;adjP=9.06e-05
	Infarction	**5**	MMP9, PTX3, CD14, PECAM1, LTA4H	C=117;O=5;E=0.47;R=10.73;rawP=9.61e-05;adjP=0.0002
	Arteriosclerosis	**5**	MMP9, PTX3, CD14, PECAM1, PPBP	C=121;O=5;E=0.48;R=10.37;rawP=0.0001;adjP=0.0002
	Arterial Occlusive Diseases	**5**	MMP9, PTX3, CD14, PECAM1, PPBP	C=123;O=5;E=0.49;R=10.20;rawP=0.0001;adjP=0.0002
	Myocardial Infarction	**5**	MMP9, PTX3, CD14, PECAM1, LTA4H	C=124;O=5;E=0.49;R=10.12;rawP=0.0001;adjP=0.0002
	Common Cold	**5**	MMP9, CXCL5, CXCL6, CD14, PPBP	C=140;O=5;E=0.56;R=8.96;rawP=0.0002;adjP=0.0003
	Bronchiolitis	**5**	CXCL5, CXCL6, CD14, PECAM1, PPBP	C=142;O=5;E=0.57;R=8.84;rawP=0.0002;adjP=0.0003
	Myocardial Ischemia	**5**	MMP9, ALB, PTX3, CD14, PECAM1	C=142;O=5;E=0.57;R=8.84;rawP=0.0002;adjP=0.0003

Gene Ontology enrichment analysis (GO)

Furthermore, a Gene Ontology enrichment analysis (GO) and a Protein-Protein-Interaction networks (PPI) analysis was performed to link related genes to their functions (using WebGestalt) and to identify (highly) connected modules, respectively.

As shown in Figure 22, BmA treatment before LPS stimulation (BmA+LPS vs. LPS) resulted in differentially expressed genes that were involved in biological processes like responses to inorganic substances (6 genes; adjusted P value (adjP)=1.20e-03), cellular response to cadmium (3 genes; adjP=1.00e-04) or zinc (4 genes; adjP=5.81e-07), phagocytosis (4 genes; adjP=6.00e-04), but also immune system processes (11 genes; adjP=3.40e-03). In addition, BmA-only stimulation resulted in effects on molecular functions like carbohydrate binding (3 genes; adjP=2.20e-02), zinc ion binding (11 genes; adjP=9.30e-03), chemokine activity (4 genes; adjP=4.29e-05), and metallopeptidase activity (3 genes; adjP=6.50e-03).

The Gene Ontology enrichment analysis also revealed effects on cellular components like extracellular space (8 genes; adjP=2.00e-04) and platelet alpha granule (3 genes; adjP=1.00e-03).

Similarly, BmA-only stimulation (BmA vs. control) resulted in effects on inorganic substances (9 genes; adjP=1.88e-07), including cadmium and zinc as well as carbohydrate derivate binding (3 genes; adjP=5.80e-03). Effects on cellular components like extracellular space (4 genes; adjP=1.37e-01) were also determined. Effects on the perinuclear region of cytoplasm (8 genes; adjP=5.05e-06) were uniquely identified by BmA-only stimulation (data not shown).

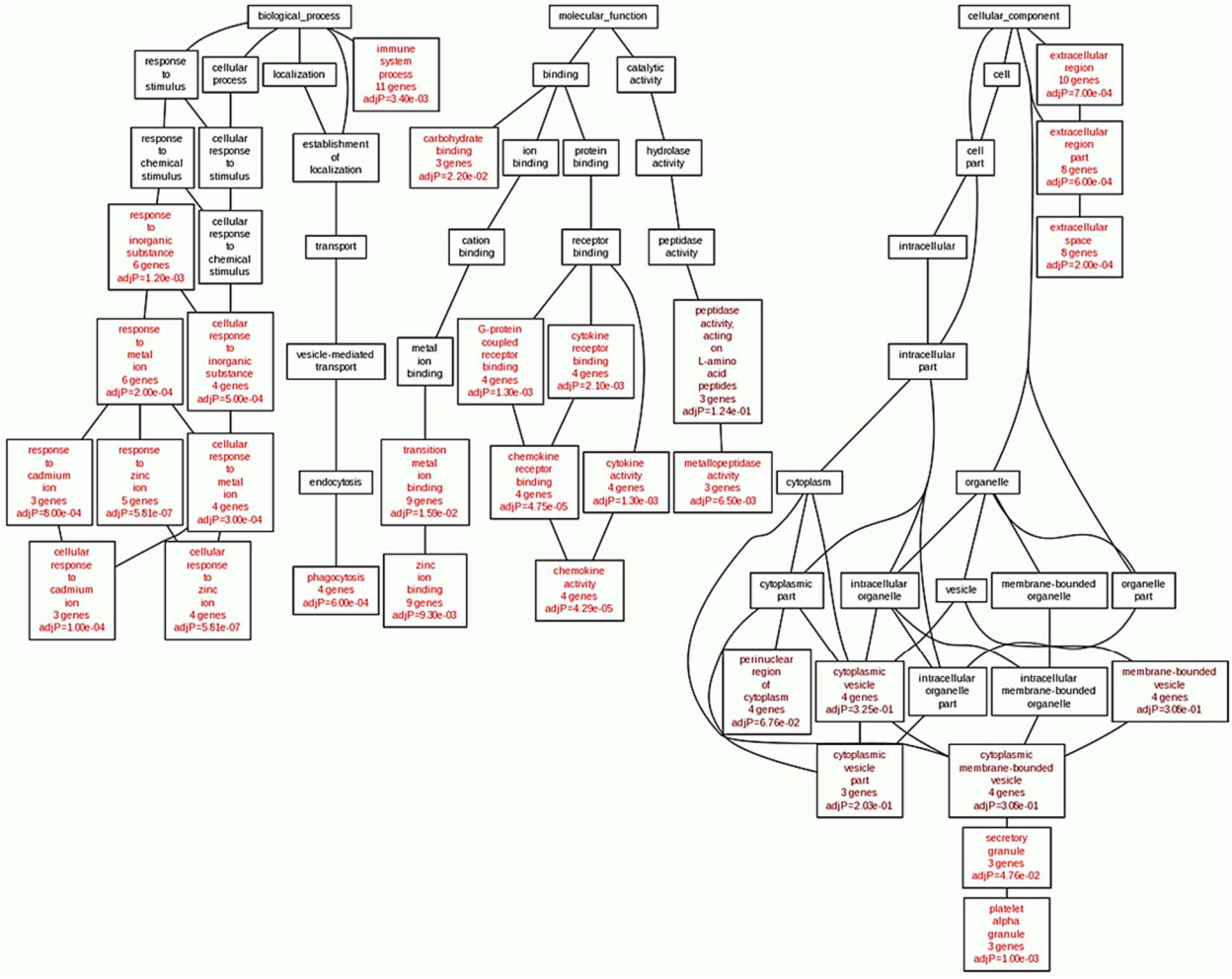

Figure 22: Gene Ontology (GO) enrichment analysis of differentially expressed genes (BmA+LPS vs. LPS) reveal associations to biological processes, molecular functions and cellular components with a minimum of 3 genes per GO term.

Protein-Protein Interaction analysis (PPI)

A Protein-Protein Interaction analysis (PPI) of the 45 differentially induced genes by BmA treatment and LPS stimulation (BmA+LPS vs. LPS) revealed three highly connected nodules (CCL20, CXCL5 and CXCL6) that affected a diverse list of genes including chemokine receptors CCR2 or CXCR1 and chemokine ligands CXCL1 (Fig. 23).

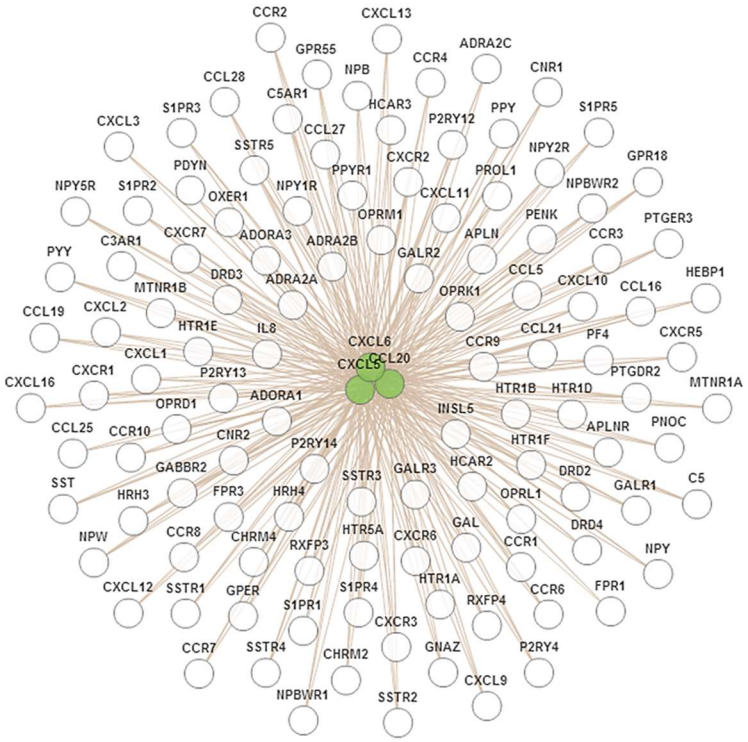

Figure 23: Protein-Protein Interaction analysis (PPI) of BmA and LPS vs. LPS stimulated differentially expressed genes discovered three highly connected modules (highlighted in green). *The connected interaction partners are shown in white.*

Ingenuity® Pathway Analysis (IPA®)

To identify disease-relevant pathways that were affected by *B. malayi* crude extract priming in the context of LPS stimulation, a Gene Set Enrichment Analysis using Ingenuity® Pathway Analysis was performed to identify canonical pathways with genes that were disproportionately highly enriched compared to randomly enriched genes. Consequently, 73 canonical pathways were identified with BmA alone stimulated human monocytes (BmA vs. control, Tab. 6), compared to 55 canonical pathways by BmA treatment before LPS stimulation (BmA+LPS vs. LPS, Tab. 7).

BmA treatment alone revealed effects on Oncostatin M signaling (MT2A, CHI3L1, STAT1). In addition, pathways with two enriched genes include IL-6 signaling (CXCL8, TNFAIP6), atherosclerosis signaling (CXCL8, ALB), IL-12 signaling and production in macrophages (ALB, STAT1), Tec kinase signaling (FCER1A, STAT1), granulocyte and agranulocyte adhesion and diapedesis (CXCL8, CCL23), the production of nitric oxide and reactive oxygen species in macrophages (ALB, STAT1) and hepatic fibrosis / hepatic stellate cell activation (CXCL8, STAT1) and glucocorticoid receptor signaling (CXCL8, STAT1). Furthermore, associations to psoriasis, allergic inflammatory airway diseases, arthritis, and airway pathology in chronic obstructive pulmonary disease were detected (all CXCL8 only). In addition, an impact on IL-22, IL-15, IL-9, and interferon signaling was determined (all STAT1 only).

Table 6: Ingenuity Canonical Pathways® analysis of differentially expressed genes of human monocytes that were treated with BmA only (BmA vs. control). List of pathways, P values and identified molecules are displayed.

Ingenuity Canonical Pathways	*-log(P value)*	*Molecules*
Oncostatin M Signaling	5,04E00	MT2A,CHI3L1,STAT1
IL-6 Signaling	2,06E00	CXCL8,TNFAIP6
Airway Pathology in Chronic Obstructive Pulmonary Disease	2,02E00	CXCL8
Tryptophan Degradation to 2-amino-3-carboxymuconate Semialdehyde	2,02E00	IDO1
Atherosclerosis Signaling	2,01E00	CXCL8,ALB
IL-12 Signaling and Production in Macrophages	1,95E00	ALB,STAT1
Tec Kinase Signaling	1,81E00	FCER1A,STAT1
Role of IL-17A in Psoriasis	1,81E00	CXCL8
NAD biosynthesis II (from tryptophan)	1,74E00	IDO1
Granulocyte Adhesion and Diapedesis	1,71E00	CXCL8,CCL23
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	1,7E00	ALB,STAT1
Hepatic Fibrosis / Hepatic Stellate Cell Activation	1,69E00	CXCL8,STAT1
Agranulocyte Adhesion and Diapedesis	1,66E00	CXCL8,CCL23
Tryptophan Degradation III (Eukaryotic)	1,6E00	IDO1
IL-22 Signaling	1,54E00	STAT1
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	1,54E00	STAT1
IL-17A Signaling in Gastric Cells	1,52E00	CXCL8
Role of JAK family kinases in IL-6-type Cytokine Signaling	1,52E00	STAT1
IL-15 Production	1,49E00	STAT1
IL-9 Signaling	1,39E00	STAT1
Role of JAK2 in Hormone-like Cytokine Signaling	1,39E00	STAT1
Interferon Signaling	1,37E00	STAT1
Autophagy	1,37E00	MAP1LC3A
Glucocorticoid Receptor Signaling	1,36E00	CXCL8,STAT1
Role of PKR in Interferon Induction and Antiviral Response	1,32E00	STAT1
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	1,29E00	CXCL8
Role of IL-17F in Allergic Inflammatory Airway Diseases	1,28E00	CXCL8

iNOS Signaling	1,28E00	STAT1
Hematopoiesis from Pluripotent Stem Cells	1,26E00	CXCL8
CNTF Signaling	1,21E00	STAT1
Role of Cytokines in Mediating Communication between Immune Cells	1,2E00	CXCL8
Role of IL-17A in Arthritis	1,2E00	CXCL8
Thrombopoietin Signaling	1,19E00	STAT1
EGF Signaling	1,18E00	STAT1
Activation of IRF by Cytosolic Pattern Recognition Receptors	1,14E00	STAT1
Role of JAK1 and JAK3 in γc Cytokine Signaling	1,14E00	STAT1
GM-CSF Signaling	1,14E00	STAT1
IL-15 Signaling	1,13E00	CXCL8
Growth Hormone Signaling	1,09E00	STAT1
Caveolar-mediated Endocytosis Signaling	1,08E00	ALB
IL-3 Signaling	1,08E00	STAT1
T Helper Cell Differentiation	1,08E00	STAT1
IL-17 Signaling	1,08E00	CXCL8
JAK/Stat Signaling	1,08E00	STAT1
Prolactin Signaling	1,07E00	STAT1
FLT3 Signaling in Hematopoietic Progenitor Cells	1,07E00	STAT1
TREM1 Signaling	1,06E00	CXCL8
PDGF Signaling	1,05E00	STAT1
Role of BRCA1 in DNA Damage Response	1,04E00	STAT1
Bladder Cancer Signaling	9,99E-01	CXCL8
UVA-Induced MAPK Signaling	9,94E-01	STAT1
Communication between Innate and Adaptive Immune Cells	9,89E-01	CXCL8
Pancreatic Adenocarcinoma Signaling	9,18E-01	STAT1
Fc Epsilon RI Signaling	9,1E-01	FCER1A
Renin-Angiotensin Signaling	9,06E-01	STAT1
phagosome formation	9,06E-01	FCER1A
Type I Diabetes Mellitus Signaling	9,02E-01	STAT1
Role of Tissue Factor in Cancer	9,02E-01	CXCL8
p38 MAPK Signaling	8,77E-01	STAT1
HMGB1 Signaling	8,67E-01	CXCL8
LXR/RXR Activation	8,64E-01	ALB
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	8,51E-01	CXCL8
FXR/RXR Activation	8,47E-01	ALB
Hepatic Cholestasis	7,5E-01	CXCL8
Acute Phase Response Signaling	7,3E-01	ALB
Role of NFAT in Regulation of the Immune Response	7,26E-01	FCER1A
Dendritic Cell Maturation	7,12E-01	STAT1
IL-8 Signaling	6,97E-01	CXCL8
Clathrin-mediated Endocytosis Signaling	6,95E-01	ALB
ERK/MAPK Signaling	6,91E-01	STAT1
Systemic Lupus Erythematosus Signaling	6,39E-01	RNU11
Colorectal Cancer Metastasis Signaling	6,02E-01	STAT1
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	5,18E-01	CXCL8

Finally, the immunomodulatory impact of BmA treatment before LPS re-stimulation on canonical pathways was investigated (BmA+LPS vs. LPS, Tab. 7). Granulocyte and agranulocyte adhesion and diapedesis (both CXCL5, CXCL6, CXCL7, CCL20, PECAM1, and MMP9), IL-17A in psoriasis, arthritis and in airway cells (all CCL20, CXCL5, and CXCL6) and LXR/RXR activation (ALB, CD14 and MMP9) were identified. Associations to IL-17-driven diseases including the role of IL-17F in allergic inflammatory airway diseases (CXCL5, CXCL6), IL-17A signaling in gastric cells (CCL20) and IL-17A signaling in fibroblasts were found beside IL-17 signaling (CXCL5). Of notice, at least 2 gene associations to atherosclerosis signaling (ALB, MMP9) and hepatic fibrosis (CD14, MMP9) were identified.

Table 7: Ingenuity Canonical Pathways® analysis of differentially expressed genes of human monocytes that were BmA treated and LPS re-stimulated (BmA+LPS vs. LPS). List of pathways, P value and identified molecules are displayed.

Ingenuity Canonical Pathways	***-log(P value)***	***Molecules***
Granulocyte Adhesion and Diapedesis	6,74E00	CXCL5,CXCL6,CXCL7,CCL20, PECAM1, MMP9
Agranulocyte Adhesion and Diapedesis	6,57E00	CXCL5,CXCL6,CXCL7,CCL20, PECAM1, MMP9
Role of IL-17A in Psoriasis	6,1E00	CCL20,CXCL5,CXCL6
Role of IL-17A in Arthritis	4,18E00	CCL20,CXCL5,CXCL6
IL-17A Signaling in Airway Cells	3,96E00	CCL20,CXCL5,CXCL6
LXR/RXR Activation	3,15E00	ALB,CD14,MMP9
Role of IL-17F in Allergic Inflammatory Airway Diseases	2,73E00	CXCL5,CXCL6
Airway Pathology in Chronic Obstructive Pulmonary Disease	1,94E00	MMP9
Atherosclerosis Signaling	1,85E00	ALB,MMP9
Leukotriene Biosynthesis	1,67E00	LTA4H
Hepatic Fibrosis / Hepatic Stellate Cell Activation	1,54E00	CD14,MMP9
Leukocyte Extravasation Signaling	1,48E00	PECAM1,MMP9
IL-17A Signaling in Gastric Cells	1,45E00	CCL20
MIF-mediated Glucocorticoid Regulation	1,33E00	CD14
Inhibition of Angiogenesis by TSP1	1,32E00	MMP9
Cell Cycle Regulation by BTG Family Proteins	1,3E00	NOCT
IL-17A Signaling in Fibroblasts	1,3E00	CXCL5
Inhibition of Matrix Metalloproteases	1,26E00	MMP9
Neuroprotective Role of THOP1 in Alzheimer's Disease	1,25E00	MMP9
MIF Regulation of Innate Immunity	1,24E00	CD14
iNOS Signaling	1,21E00	CD14
Glioma Invasiveness Signaling	1,1E00	MMP9
Eicosanoid Signaling	1,05E00	LTA4H
IL-10 Signaling	1,03E00	CD14
GDNF Family Ligand-Receptor Interactions	1,03E00	PDLIM7
Macropinocytosis Signaling	1,03E00	CD14
Caveolar-mediated Endocytosis Signaling	1,01E00	ALB

IL-17 Signaling	1,00E+00	CXCL5
LPS-stimulated MAPK Signaling	9,96E-01	CD14
Toll-like Receptor Signaling	9,9E-01	CD14
VDR/RXR Activation	9,69E-01	CD14
Bladder Cancer Signaling	9,24E-01	MMP9
Death Receptor Signaling	9,01E-01	TNFRSF21
Axonal Guidance Signaling	8,83E-01	ADAM19,MMP9
HIF1α Signaling	8,59E-01	MMP9
Pancreatic Adenocarcinoma Signaling	8,44E-01	MMP9
IL-6 Signaling	8,08E-01	CD14
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	7,78E-01	PTX3
FXR/RXR Activation	7,75E-01	ALB
Ovarian Cancer Signaling	7,59E-01	MMP9
IL-12 Signaling and Production in Macrophages	7,53E-01	ALB
Adipogenesis pathway	7,5E-01	NOCT
Relaxin Signaling	7,47E-01	MMP9
Tec Kinase Signaling	6,88E-01	TNFRSF21
Hepatic Cholestasis	6,79E-01	CD14
Acute Phase Response Signaling	6,6E-01	ALB
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	6,36E-01	ALB
IL-8 Signaling	6,27E-01	MMP9
Regulation of the Epithelial-Mesenchymal Transition Pathway	6,27E-01	MMP9
Clathrin-mediated Endocytosis Signaling	6,25E-01	ALB
ILK Signaling	6,25E-01	MMP9
Systemic Lupus Erythematosus Signaling	5,71E-01	RNU11
Actin Cytoskeleton Signaling	5,67E-01	CD14
LPS/IL-1 Mediated Inhibition of RXR Function	5,6E-01	CD14
Colorectal Cancer Metastasis Signaling	5,34E-01	MMP9

Moreover, the ten highest differentially expressed genes that were affected by BmA priming before LPS stimulation were further analyzed by IPA® analysis to determine the common superordinate affected function in vivo (BmA+LPS vs. LPS). All identified top transcripts CD14, TNFRSF21, PECAM-1, ALB, MMP9, CCL20, CXCL5, CXCL6, CXCL7, and PTX3 were related to cell movement of myeloid cells (Fig. 24).

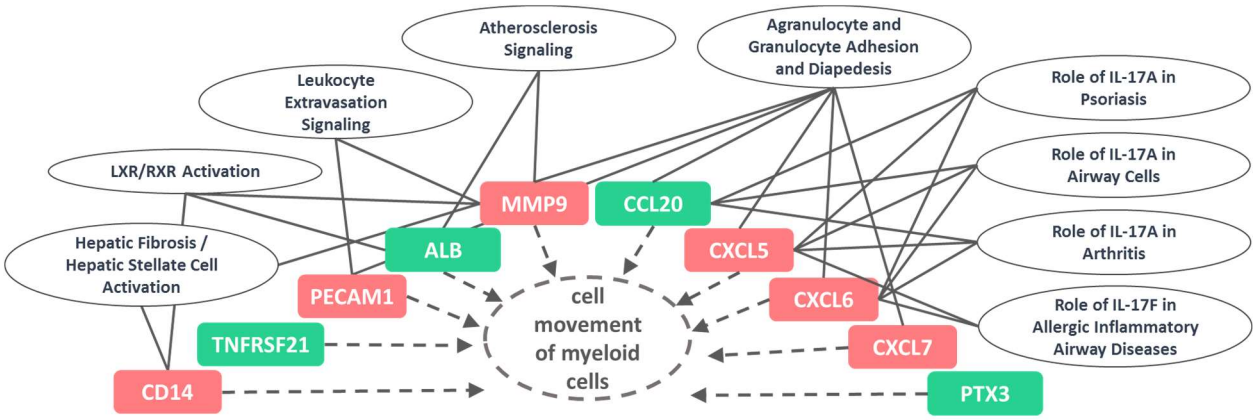

Figure 24: BmA treatment before LPS stimulation affects cell movement of myeloid cells. *Analysis of top 10 differentially expressed genes of BmA primed LPS re-stimulated compared to LPS-only stimulated monocytes (BmA+LPS vs. LPS) revealed interrelation to cell movement of myeloid cells and impact on associated canonical pathways (FC ≥ 1.5 and P value < 0.05). Upregulated differentially expressed genes are indicated in red and downregulated genes in green.*

In summary, BmA treatment before LPS stimulation compared to LPS alone resulted in several differentially expressed genes that were enriched in canonical pathways like granulocyte and agranulocyte adhesion, diapedesis and atherosclerosis. Furthermore, the IPA® analysis revealed associations to several IL-17-dependent pathways and diseases including arthritis, psoriasis and allergic inflammatory airway diseases. The BmA treatment showed evidence for the immunomodulatory effect of filarial extracts on human non-endemic monocyte responses with and without subsequent pro-inflammatory stimulation. Consequently, our results revealed transcripts and associated pathways that may be involved in the development of filaria-caused pathology and dysregulated immune responses as they occur e.g. during autoimmunity.

Discussion

Despite constant efforts in intensive care units (ICU), severe sepsis and septic shock remain a leading cause of hospital mortality worldwide (Beale et al., 2009). The SepNet Critical Care Trials Group identified a 40.4% mortality rate of patients with severe sepsis or septic shock in German ICUs (SepNet Critical Care Trials, 2016). Novel classifications (SEPSIS-3) of septic shock patients even increase this number to a mortality of 50%. A strongly increased number of hospitalizations for severe sepsis patients were determined in the United States between 2003 and 2007, however with a reduction in mortality (Lagu et al., 2012; Suarez De La Rica et al., 2016). Hence, improved sepsis treatments are needed.

Eosinophils play a pivotal role in clearance of helminth infections and the development of allergy, but the clinical relevance of eosinopenia during sepsis is controversially debated, in part due to the lack of experimental data (Shaaban et al., 2010; Smithson et al., 2009). Previously we showed that an experimental *L. sigmodontis* infection protects against *E. coli*-induced sepsis by leading to a milder hypothermia, improving bacterial clearance and reducing systemic inflammation without worsening immune paralysis (Buerfent et al., 2015; Gondorf et al., 2015). Hence, the first objective of this thesis was to determine eosinophil responses against bacterial pathogens and the impact of eosinophils on the outcome of sepsis in the mouse model. A locus and filarial-infection dependent transcriptome profiling of eosinophils was performed in the absence or presence of an *E. coli* challenge.

As a second objective, the immunomodulatory properties of a crude filarial extract of the human pathogenic filaria *Brugia malayi* on subsequent LPS *E. coli*-induced responses were investigated. A transcriptome-wide expression analysis and subsequent pathway analysis of stimulated human monocytes from non-endemic healthy male volunteers was performed. Identified transcripts may be candidates that are involved in the development of filarial pathology. Moreover, the pathway analysis may reveal bystander immune responses that affect autoimmune diseases.

4.1 Eosinophil granulocytes are involved in the protection against sepsis

4.1.1 Eosinophil-deficiency exacerbates inflammation during *E. coli*-induced sepsis

Using a well-accepted animal model for clinical sepsis (Hubbard et al., 2005), the lack of eosinophils in ΔdblGATA mice significantly increased the hypothermia four to six hours after the *E. coli* challenge compared to WT BALB/c controls. In addition, levels of systemic pro-inflammatory mediators were increased and the numbers of adaptive und innate immune cells were reduced in ΔdblGATA mice six hours after the *E. coli* injection. This was accompanied by an increased peritoneal bacterial load in ΔdblGATA mice (p=0.0575). Thus, our results propose that eosinophils are involved during inflammatory responses against pathogens and the outcome of sepsis. In agreement to this, an increased bacterial load was observed 18 h after the induction of *Pseudomonas aeruginosa*-induced peritonitis in PHIL mice, a mouse line lacking eosinophil-promyelocytes (Lee et al., 2004; Linch et al., 2009). However, the *Pseudomonas aeruginosa* infection led to unaltered systemic levels of pro- and anti-inflammatory cytokines. The discrepancy between this study and the results of my thesis could be due to the differences in the timing of the analysis, as well as to the pathogen investigated**.** An overexpression of IL-5 in transgenic NJ.1638 mice also exhibited an improved sepsis outcome, which was nevertheless independent of eosinophils (Linch et al., 2012). This suggests that bystander immune responses induced by IL-5 overexpression may compensate for the lack of mature eosinophils as the IL-5 receptor is present on other immune cells like B cells (Hitoshi et al., 1990). During parasite infection, IL-5 is mainly produced by CD4+ helper T cells promoting eosinophilia for parasite clearance (Cadman et al., 2014; Lee et al., 1997). In addition, Specht et al. previously demonstrated that eosinophil peroxidase (EPO) and major basic protein (MBO) are essential for the defense against experimental *L. sigmodontis* infections (Specht et al., 2006). Furthermore, a MBP deficiency altered the IL-10 production of thoracic macrophages, whereas an EPO-deficiency-induced eosinophilia was based on increased IL-5 levels derived from CD4+ T cells that both may compensate for the impaired anti-filarial defense. Future studies should therefore further decipher to which degree eosinophils and IL-5 are implemented in protective immune responses during sepsis. Moreover, eosinophils are important for gut immune homeostasis because eosinophils

promote the TGFβ-dependent development of mucosal IgA producing plasma cells in the Peyer's patches (Chu et al., 2014). Chu et al. also presented evidence for eosinophil-derived IL-6 and APRIL production in bone marrow to promote plasma cell maintenance (Chu et al., 2011). In addition, the lack of eosinophils diminishes the numbers of regulatory T cells and dendritic cells that contributes to changes of the microbiota composition in the gut lumen. Generally, the absence of eosinophils induces IgG1 producing plasma cells in the Peyer's patches that are associated with an affected control of microorganisms and inflammation. Thus, previous publications and the results of this thesis suggest that eosinophil granulocytes exhibit various regulatory functions and are involved in the control of local inflammatory responses like gut homeostasis or pathogen-induced inflammation as it occurs during bacterial-induced peritonitis and respiratory viral infection (Percopo et al., 2014).

4.1.2 Eosinophils release pro-inflammatory recruitment factors to TLR2 and TLR4 ligands

Not only can eosinophils reduce the bacterial burden in vitro via the spontaneous release of mitochondrial DNA and eosinophil granule proteins like the eosinophil cationic protein (ECP) forming bactericidal extracellular networks (Pulido et al., 2012; Yousefi et al., 2008), but also release chemokines like CXCL1 (KC) and CCL5 (RANTES) as well as cytokines IL-4 and IL-6 after exposure to bacterial-pathogen patterns, which may essentially modulate granulocyte recruitment and the local cytokine milieu. In the murine asthma model, lipopolysaccharide was previously shown to induce eosinophil migration (Penido et al., 2001). Penido et al. demonstrated that eosinophil accumulation after LPS injection is independent of eotaxin and CCL5 but dependent on CCR3. Purified human eosinophils also express the CD14 co-receptors TLR2 and TLR4 and a stimulation with LPS resulted in a dose-dependent release of ECP and TNF (Plötz et al., 2001). Furthermore, LPS in presence of IL-5 and IL-33 was shown to induce IL-4 and IL-13 responses of bmEos and that autocrine granulocyte macrophage colony-stimulating factor (GM-CSF) production mediates eosinophil survival (Willebrand and Voehringer, 2016).

The results of this thesis indicate that a stimulation of bmEos with the TLR4-ligand *E. coli* LPS ultrapure or the TLR1/2-ligand Pam3CSK4 induces a substantial CXCL1 and CCL5 response that is known to be chemotactic for neutrophils, monocytes, T cells and eosinophils, respectively. Moreover, the exposure to LPS resulted in a strong increase of IL-6 levels (Hunter and Jones, 2015). Pre-exposure to CCL11 and CCL24 did not affect the increased release of CCL5 and IL-6 but CXCL1, which may suggest a specific role in neutrophil recruitment. Elevated levels of IL-6 were previously associated with severe cases of sepsis and septic shock (Hack et al., 1989). Accordingly, sepsis induced development of hypothermia was worsened in IL-6 deficient mice (Remick et al., 2005). The application of anti-IL-6 antibodies, which impair neutrophil recruitment, in B10/D2 nsnJ mice results in a reduced expression of the complement factor 5a receptor and an improved sepsis outcome (Riedemann et al., 2003b). Similarly, reduce serum IL-6 levels and improved sepsis outcome were observed in chronic *L. sigmodontis* infected BALB/c mice (Gondorf et al., 2015). Since eosinophils release various chemoattractants like CXCL1 and IL-6 in response to TLR1/2 and TLR4 stimulation, eosinophils may promote neutrophil recruitment and activation to improve early anti-bacterial responses during sepsis (Fielding et al., 2008; Sawant et al., 2016). Platelet-derived CCL5 was moreover previously described to promote neutrophil recruitment in experimental colitis and septical lung injury (Hwaiz et al., 2015; Yu et al., 2016). Thus, induced recruitment of neutrophils by increased levels of CCL5, CXCL1 and IL-6 of eosinophils may indicate an up to now unknown protective mechanism during the early phase of sepsis. Consequently, further studies should investigate whether altered levels of eosinophil-derived neutrophil chemoattractants correlate with the severity of sepsis since early improved neutrophil recruitment in experimental sepsis was shown to improve bacterial clearance and thus sepsis survival (Craciun et al., 2010).

The release of IL-4 by bmEos was enhanced by CCL24 pre-stimulation. Eosinophil-derived IL-4 is known to promote local alternative activation of adipose tissue macrophages (Wu et al., 2011). Moreover, adipokine/myokine meteorin-like (Metrnl) was identified to trigger IL-4 and IL-13 production by eosinophils (Rao et al., 2014) that leads to a catecholamine release of AAMs, promoting UCP1-expressing “beige” adipocytes (Lee and Tontonoz, 2014; Qiu et al., 2014).

Thus, CCL24 priming of eosinophils may have an essential impact on several eosinophil-derived IL-4 associated immune responses. Dixon et al. provided evidence for increased mRNA CCL24 levels in gastrointestinal *Trichinella spiralis* and *Trichuris muris* infection (Dixon et al., 2006) and Zimmermann et al. additionally demonstrated that CCL24 expression is induced by IL-4 overexpression in transgenic mice and after intranasal administration (Zimmermann et al., 2000). Accordingly, our results suggest that increased levels of CCL24 caused by filarial infection may additionally improve the induction of a regulatory Th2 response firstly during the chronic filarial infection and secondly during the early phase of sepsis. Although Lechner et al. demonstrated that CCL24 levels are not altered in humans during an occult *O. volvulus* infection (Lechner et al., 2012), further studies should focus on the dependence of CCL24 and IL-4 levels and additionally they should determine whether a CCL24 deficiency or an overexpression correlate with an increased pathology in experimental filarial infection and further influence sepsis survival in filarial infected and sepsis protected mice.

4.1.3 Eosinophils independently regulate CCR3 receptor and ICAM-1 expression

Besides its role in cell adhesion, ICAM-1 is an important survival factor for eosinophils (Pazdrak et al., 2008). The CCR3 receptor binds eotaxin and is therefore required for eosinophil recruitment to the place of inflammation. Moreover, eosinophils detect pathogens via Toll-like-receptors TLR1, TLR2 and TLR4 and secrete mediators, thus mediating protection during sepsis. Kobayashi et al. and Czech et al. showed that the pro-inflammatory cytokine TNF, released by human monocytes after LPS stimulation, results in the up-regulation of the intercellular adhesion molecule 1 (ICAM-1) on human eosinophils (Czech et al., 1993; Kobayashi et al., 2009). Since bmEos respond to the TLR2 ligand Pam3CSK4, eosinophils may respond to *Wolbachia* endosymbionts and hereby support anti-filarial responses (Nfon et al., 2006). The pre-treatment with CCL24 reduced the CCR3, but not the ICAM-1 expression, suggesting two independent mechanisms. CCR3-dependent recruitment of eosinophils via eotaxins was previously shown for *Mycobacterium bovis* BCG pleural infection in mice (D'Avila et al., 2007). Thus, we hypothesized

that in *E. coli*-induced sepsis eosinophils are recruited via chemoattractants like eotaxins, e.g. produced by inflammatory monocytes guided to the place of infection (Gentil et al., 2014; Travers and Rothenberg, 2015). The activation via TLR2/(TLR6), TLR4 or/and pro-inflammatory cytokines induces ICAM-1 expression, thus increasing the adherence and cross-talk to other leucocytes (Rothlein et al., 1986). In addition, the subsequent down-regulation of CCR3 by increased levels of CCL24 and hypothetically by CCL11 may lead to the local abidance of the recruited eosinophils. Increased eotaxins level generally induce chemotaxis of eosinophils and may accordingly induce the remaining of eosinophils at the place of inflammation. Thus, the induction of ICAM-1 by pathogen recognition via TLR may improve cell-cell-communication and immigration into inflammatory tissue.

4.1.4 Eosinophil granulocytes execute important roles during sepsis – a hypothesized model

It can be hypothesized that eosinophil granulocytes are essentially involved in immune responses against gram-negative bacteria during sepsis. Not only can eosinophils reduce the bacterial burden in vitro, but their release of chemotactic mediators may help to recruit additional phagocytes. The results of this thesis showed that eosinophil-deficient mice had an increased peritoneal bacterial load during sepsis and reduced numbers of innate immune cells at the site of inflammation. Principally, *E. coli*-injections result in an excessive inflammatory response leading to systemic inflammation (Denk et al., 2012). However, depending on the pathogen load and virulence, the activation of leucocytes leads to a rapid pro-inflammatory response affecting antigen-presenting cells like dendritic cells and macrophages, but also neutrophils (Hotchkiss and Karl, 2003). Subsequently, CD4+ and CD8+ T cells are activated (Kasten et al., 2010a; Kasten et al., 2010b). The release of inflammatory products enhances the adaptive and innate cell recruitment to the place of inflammation, but also increases the likelihood for uncontrolled systemic inflammation that can lead to apoptosis, hypoxia, multiple organ failure and consequently death (Bosmann and Ward, 2013; Buras et al., 2005; Hotchkiss and Karl, 2003).

Hence, in our proposed sepsis model eosinophils may be guided by the release of increased levels of the CCR3 ligands and the presence of TLR ligands to the place of infection.

Our initial transcriptional analysis of filariae-induced eosinophils further revealed an upregulation of interferon response-associated transcripts. Further analysis should determine whether chronic filarial infection also modulates the expression of TLRs and the impact of chemoattractants on TLR expression and parasite and bacterial clearance. Such a modulation by helminth infections was previously reported to impair anti-viral responses (Buerfent et al., 2015; Kroidl et al., 2016; Osborne et al., 2014).

Furthermore, the down-regulation of CCR3 and increase of ICAM-1 may result in a cross-talk to leucocytes and remaining in inflammatory tissue. Local Th2 cell-derived or autocrine IL-5 may subsequently pro-long eosinophil survival and functionality. Subsequently, with the secretion of extracellular bactericidal structures eosinophils may not only reduce the bacterial burden, but additionally induce neutrophil influx by the release of chemokines. An early increased neutrophil influx was previously shown to improve bacterial clearance and survival (Craciun et al., 2010). Thus, development of eosinopenia may indeed be an unspecific marker for the severity of sepsis.

4.1.5 Outlook: Immunomodulatory impact of helminths on eosinophils

In line with previous data from our group and others our recent results reveal that eosinophil granulocytes are essentially involved in pro-inflammatory immune diseases like sepsis, diabetes and cancer (Carretero et al., 2015; Wu et al., 2011). Further analysis should be based on our initial results of transcriptome-wide profiling of eosinophils and analyze the impact of eosinophil modulation in the context of a filarial infection and sepsis modulation. The proteins arginase-1, chitinase-like protein 3 and Resistin-like alpha were differentially expressed by experimental *L. sigmodontis* infection compared to eosinophils derived from the intestine of naïve mice and were previously associated with alternatively activated macrophages induced by nematode infection (Loke et al., 2000). Thus, further transcriptome-wide analysis may also determine different locus-specific subtypes of eosinophil granulocytes that may have a specific role during parasitic and bacterial infection but also other eosinophil-associated diseases like allergy.

4.2 Genome-wide transcriptional profiling of human monocytes stimulated with filarial extract and LPS reveals link to IL-17 associated pathways and diseases

Helminth and helminth-derived products modulate cellular functions of host immune cells. Filarial nematodes basically induce a regulatory Type 2 immune response that counter-regulates pro-inflammatory Type 1 responses (Maizels and McSorley, 2016). Experimental filarial infections and filariae-derived products were shown to exhibit protective effects on inflammatory disorders. The experimental *L. sigmodontis* infection revealed protective effects on *E. coli*-induced sepsis by reducing inflammation during the SIRS phase without worsening the anti-inflammatory CARS phase and the risk of secondary infections (Buerfent et al., 2015; Gondorf et al., 2015). In addition, the *L. sigmodontis* infection and crude extract administration improves metabolic disorders like diabetes, hence autoimmune and diet-induced glucose intolerance (Ajendra et al., 2016a; Berbudi et al., 2016b; Hübner et al., 2012b; Hübner et al., 2009a). Furthermore, filariae-derived excretory/secretory products like ES-62 and analogues were shown to protect against inflammatory diseases like experimental atherosclerosis, systemic lupus erythematosus, rheumatoid arthritis and allergy (Aprahamian et al., 2015; Pineda et al., 2014; Rodgers et al., 2015b; Rzepecka et al., 2014). The recombinant cystatin of the human filaria *Brugia malayi* (rBmCys) ameliorates experimental colitis in mice (Khatri et al., 2015a; Khatri et al., 2015b). Interestingly, in human lymphatic filarial disease, a severe pathology with clinical symptoms like lymphedema, hydrocele or elephantiasis develop in patients that fail to down-regulate such inflammatory responses (Babu and Nutman, 2012), whereas asymptomatic infected humans are associated with a strong immune tolerance against circulating microfilariae or circulating parasite antigens.

Consequently, the immunomodulatory effect of the crude filarial extract of *Brugia malayi* (BmA) on purified human non-endemic male CD14+ monocytes was analyzed. 47.000 transcripts were analyzed and 49 differentially expressed transcripts were identified ($FC \geq 1.5$; $p \leq 0.05$) that are specific for immunomodulation by BmA pre-exposure to subsequent LPS ultrapure *E. coli* stimulation (BmA+LPS vs. LPS). Principally, the LPS stimulation alone resulted in 2.215

differentially expressed genes that are involved in inflammation, thus confirming recent findings (Kim et al., 2014). Since TLR4 signaling is essentially involved in autoimmune diseases, sepsis but also cancer, immunomodulation of LPS-driven responses by helminth products may affect bystander immune responses and autoimmunity as well (Rakoff-Nahoum and Medzhitov, 2009).

The exposure of human monocytes to the crude *B. malayi* extract alone (BmA vs. control.) revealed 42 differentially expressed genes that were associated with inflammation, immune system diseases, chemokine signaling pathway, bronchial diseases and bronchiolitis. BmA pre-exposure before LPS stimulation resulted in 49 differentially expressed genes compared to LPS-only stimulated monocytes (BmA+LPS vs. LPS). In addition to the enriched pathways by BmA alone, the BmA pre-exposure before LPS stimulation resulted in differentially expressed genes that were linked to rheumatoid arthritis, respiratory tract infections, infarction and arteriosclerosis.

4.2.1 BmA induces cellular protective mechanisms in human non-endemic monocytes

The BmA treatment of monocytes of non-endemic donors induced the expression of several members of the metallothionein family, small nucleolar and nuclear RNAses and chemokines. Similarly, the BmA pre-treatment before LPS re-stimulation resulted in altered levels of the metallothioneins MT1F-H and MT1M. Metallothioneins (MTs) are small cysteine-rich proteins that are involved in the cellular detoxification as a consequence of elevated metal ion concentrations like zinc or cadmium (Suhy et al., 1999). Since MTs are principally induced in eukaryotic cells to prevent apoptosis, MTs were previously described as a cellular protective mechanism to reduce chemotherapy-induced apoptosis in acute leukemia patients and were discussed as potential reason for drug resistance and tumor development (Takahashi, 2012; Tsangaris et al., 2000). Moreover, in helminth infection an up-regulation of MT1 was described in regulatory CD4+CD25hi T cells in *Schistosoma mansoni* infection (Layland et al., 2010). The nematode *Caenorhabditis elegans* releases the stress-related metallothioneins CeMT-1 and CeMT-2 in presence of Zn (II) or Cd (II) (Roh et al., 2006; You et al., 1999; Zeitoun-Ghandour et al., 2010). Accordingly, our data suggest that the crude filarial extract BmA contains active immunomodulatory components

and reduces apoptosis in the absence of a LPS- re-stimulation. Such a reduced apoptosis was also observed in macrophages of *L. sigmodontis*-infected animals upon *E. coli*-challenge and during *Heligmosomoides polygyrus* infection (Doligalska et al., 2007; Gondorf et al., 2015). Further experiments should investigate the impact of metallothioneins during filarial infection, infection-associated diseases and bystander immune responses. Elevated levels of MT may be part of the induced protection in inflammatory disorders by supporting cell survival and functionality of regulatory cell populations, but may also lead to an increased risk for tumor growth and consequently carcinogenesis (Brindley et al., 2015; Oikonomopoulou et al., 2014). Herrera and Ostrosky-Wegman suggested that helminth-induced carcinogenesis may be promoted further by an inflammation-induced genetic instability, thus DNA damage and also epigenetic events (Herrera and Ostrosky-Wegman, 2001; Neeshma Jaiswal and Sandeep K, 2015). Furthermore, induced MTs may also be involved in the pathogenesis during filarial infection since metallothionein 2 (MT2) is an essential regulator of vascular endothelial growth factor (VEGFC) and thus angiogenic processes (Schuermann et al., 2015). In this line, Debrah et al. provided evidence that plasma VEGF-A and VEGF-A gene polymorphisms are associated with hydrocele development in lymphatic filariasis (Debrah et al., 2007).

BmA pre-exposure before LPS re-stimulation elevated the levels of matrix metalloproteinases 9 (MMP-9; Gelatinase B). MMP-9 alters the expression of VEGF in retinal pigment epithelial (RPE) cells under hypoxic conditions (Hollborn et al., 2007) and MMP-9 is acting as a zymogen and is involved in the breakdown of the extracellular matrix (Egeblad and Werb, 2002). MMP-9 synthesis by monocytes is induced by TNF and negatively regulated by TGF-β (Vaday et al., 2001). Anuradha et al. provided evidence that MMPs are essentially involved in regulating tissue pathology of bancroftian filariasis with increased ratios of MMP-1/tissue inhibitor of metalloproteinases (TIMP)-4 and MMP-8/TIMP-4 (Anuradha et al., 2012). Furthermore, elevated levels of MMP-9 and VEGF are associated with angiogenesis and a progression of carcinogenesis like gastric carcinomas (Bergers et al., 2000; Zheng et al., 2006) and could be an association for cancer promotion. Thus, confirming previous findings, BmA induces cellular protective mechanism, but further experiments are needed to determine the regulatory character

of MT and MMP-9 in lymphatic filariasis, autoimmune diseases and apoptosis-dependent diseases like cancer.

Moreover, BmA treatment prior to LPS stimulation resulted in a suppression of pentraxin-3 (PTX3) in non-endemic monocytes, a soluble pathogen recognition receptor that provides anti-microbial recognition to several pathogenic structures and is mainly found in the muscle tissue promoting regulation of inflammations (Alles et al., 1994; Garlanda et al., 2002; Garlanda et al., 2016; Introna et al., 1996). PTX3 can recognize LPS and it modulates the release of tissue factors (TF) in human monocytes that are involved in thrombogenesis and wound healing (Napoleone et al., 2004). In addition, PTX3 activity is reduced in the presence of pro-inflammatory mediators like TNF or IL-1β . PTX3 was identified by Bonavita et al. as an extrinsic oncosuppressor that is epigenetically repressed in human tumors such as malignant smooth muscle tumors and colorectal cancer and regulates complement activation by interacting with C1q affecting tumor cell recruitment (Bonavita et al., 2015). PTX3 repression therefore leads to an increased cancer-related inflammation and promotes M2 macrophage recruitment and tumor growth. Interestingly, *Brugia malayi*-derived Calreticulin (BmCRT) similarly interacts with the first component C1q of the human host to prevent classical complement pathway activation that may reduce pathogen clearance and clearance of apoptotic-cells by antigen-presenting cells as was previously shown for tissue PTX3 (Baruah et al., 2006; Yadav et al., 2014). Consequently, PTX3 may be a target to improve parasite clearance and tissue homeostasis.

4.2.2 BmA modulates noncoding RNA expression with diverse cellular functions

BmA exposure to human non-endemic monocytes altered the gene expression of the small nucleolar RNA families SNOR C/D box, SNOR H/ACA box, small Cajal body-specific RNA SCARNA, as well as small nuclear RNA families like RNUs. SnoRNAs are 60 to 300 nucleotides long, localized in the nucleolus and the most abundant group of metabolically stable noncoding RNAs that regulate gene expression at the level of transcription (Matera et al., 2007). Basically, box C/D snoRNAs mediate methylation of 2'-hydoxyl groups and are involved e.g. in the site-

specific ribose methylation and thus in the maturation of pre-ribosomal RNA. Box H/ACA snoRNAs conduct the conversion of uridine into pseudouridine that provide regulatory functions in the suppression of translation termination (Karijolich and Yu, 2011; Kiss-Laszlo et al., 1998). Moreover, snoRNAs also provide microRNA functionality in post-transcriptional gene silencing (Ender et al., 2008; He and Hannon, 2004). The transcriptome profiling after BmA pre-exposure and subsequent LPS stimulation reveals new regulatory post-transcriptional candidates that were not previously associated with filarial diseases. Associations to inflammatory disorders were previously shown for SNORD12 as a potential marker for breast cancer (Askarian-Amiri et al., 2011). Furthermore, previous investigations provide evidence that the small nuclear RNA component of mitochondrial RNA processing endoribonuclease (RMRP) is related to carcinogenesis in gastric cancer, immunodeficiency and cartilage-hair hypoplasia (Ip et al., 2015; Ridanpaa et al., 2001; Shao et al., 2016). BmA pre-exposure modulates changes in the gene expression of the RNU4ATAC gene that were previously associated with the Roifman Syndrome, a rare genetic disease that causes dysgammaglobulinemia, skeletal dysplasia, developmental delay and retinal dystrophy (Merico et al., 2015; Roifman, 1999). Thus, immunomodulation by helminths and helminth products may have a profound impact on regulatory cell mechanisms. Consequently, further investigations should focus on the regulatory impact of long non-coding RNA in the context of etiopathogenesis because of their alterative influence on gene expression and post-transcriptional processes (Engreitz et al., 2016).

4.2.3 BmA priming before LPS treatment affects chemotaxis and cell movement of myeloid cells

As another consequence of BmA priming before LPS re-stimulation, an upregulation of the neutrophil CXC chemoattractants and activators CXCL5 (ENA-78), CXCL6 (GCP-2) and CXCL7 (PPBP) (Turner et al., 2014) were detected. CCL20, also known as Macrophage Inflammatory Protein-3 (MIP3A), was downregulated. CXCL5-7 signal via CXC receptor 2 (CXCR2) and are pro-angiogenic. Thus, they may contribute to the development of lymphedema in lymphatic filarial diseases since MMP-9 was additionally elevated due to BmA priming before LPS re-stimulation

(Couto et al., 2011). CXCL5 is a potent neutrophil recruitment factor and is additionally associated with tumor growth and carcinogenesis (Zhou et al., 2012). Zhou et al. further demonstrated that an increased CXCL5 expression in hepatocellular carcinoma cells (HCC) promotes tumor growth and metastasis. Correspondingly, the hypoxia-inducible factor (HIF)-1α similarly promotes CXCL6 expression in HCC (Tian et al., 2014). Since an upregulation of CXCL6 through the tetraspanin (TSPAN) 12–β-catenin pathway promotes cancer cell invasion and proliferation, reduced levels of CXCL6 beside TSPAN12 were recently discussed as effective therapeutic option against epithelial cancer progression and lung cancer (Otomo et al., 2014; Zhu et al., 2006). Moreover, CXCL5 is associated with colorectal cancer progression and consequently presented as a predictive prognostic marker for survival (Kawamura et al., 2012). CXCL7 was shown to induce cell proliferation in lung, colon, kidney and bile duct cancer (Desurmont et al., 2015; Grepin et al., 2014; Guo et al., 2017; Unver et al., 2015). Furthermore, CXCL5-induced neutrophil infiltration in the murine model of LPS-induced keratitis suggest that increased CXCL5 expression may also contribute to the pathogenesis in onchocerciasis and promote vision loss (Lin et al., 2007). The expression of MMP7, Claudin-7 (CLDN7) and CXCL6 was also upregulated in human chronic schistosomiasis (Gobert et al., 2015). In contrast, Rousselle et al. revealed a protective role of CXCL5 in atherosclerosis by a regulation of macrophage foam cell formation, limiting the cholesterol content of macrophages. However, in experimental colitis-associated colorectal cancer (CAC), a *Taenia crassiceps* infection reduces the CXCR2 expression in colon tissue (Leon-Cabrera et al., 2014). Consequently, CXCR2 neutralization antibodies may inhibit proliferation and thus the progression of human cancer.

Since BmA treatment and LPS re-stimulation alter the release of the CXCR2 ligands CXCL5, CXCL6 and CXCL7, further context-specific analyses are needed to investigate the impact of CXCR2-dependent pathways on the development of filariae-driven diseases. The impact of filariae infection on cancer is still unclear (Hall et al., 2001).

BmA priming before LPS re-stimulation reduced the CCL20 expression. CCL20 was previously shown to be characteristically induced in human inflammatory bowel disease (Kaser et al., 2004)

and the experimental *Trichuris muris* infection revealed chemotactic properties for dendritic cells for the recruitment to the colonic mucosa for CCL20 in combination with CCL5 (Cruickshank et al., 2009).

Immunomodulation by BmA did not only alter the chemotactic behavior of monocytes of human non-endemic donors, but also reduced the expression of cell surface proteins CD86 (B7-2) and Human Leukocyte Antigen - antigen D Related (HLA-DR). Changes in the expression of major histocompatibility complex 2 and the co-stimulator for T cell priming were observed and were in line with our previous results of filariae-modulated macrophages that mediated protection against *E. coli*-induced sepsis (Gondorf et al., 2015). The expression of CD86 and HLA-DR in BmA-primed and LPS-restimulated monocytes compared to LPS alone was significantly reduced. Furthermore, BmA stimulation alone reduced the expression of the glycoprotein CAMPATH 1 (CD52). CD52 is targeted by the pharmaceutical drug Alemtuzumab, a common treatment for multiple sclerosis, chronic lymphocytic leukemia, transplantation and rheumatoid arthritis (Coles et al., 2012; Cooles et al., 2016; Fiegl et al., 2014). Multiple lines of evidence suggest that helminths and their products can protect against autoimmune diseases. For instance Correale et al. and Fleming showed that helminths mediate protection against multiple sclerosis (Correale, 2014; Correale and Farez, 2009; Fleming, 2013), but further investigations are needed to determine whether a CD52 downregulation is involved in this protective effect (Baker and Isaacs, 2014). Interestingly, tumor necrosis factor receptor superfamily member (TNFRSF) 21, a surface receptor and induced by NF-κB activation and known as death receptor (DR) 6, serves as a marker for adult sarcomas and was reduced by BmA priming, followed by LPS re-stimulation (Kasof et al., 2001; Yang et al., 2012). Further investigation should determine whether filarial antigens reduce sarcoma development based on TNFRSF21 reduction. Moreover, altered levels of chemoattractants in combination with the altered expressions of ALB, PECAM-1, TNFRSF21 and CD14 are associated with cell movement of myeloid cells analyzed by Ingenuity Pathway Analysis (IPA®).

4.2.4 BmA priming before LPS re-stimulation reveals link to inflammatory disorders including IL-17 dependent diseases

The Ingenuity® Canonical Pathways Analysis of BmA-altered transcripts revealed several associations with inflammatory disorders, mainly IL-17-dependent diseases, cell adhesion and migration, as well as autoimmune diseases. Granulocyte and agranulocyte adhesion and diapedesis pathways were the mostly affected pathways with six altered genes CCL20, CXCL5, CXCL6, CXCL7, MMP9, and PECAM1 in BmA-primed and LPS-restimulated compared to LPS-alone stimulated monocytes. Changes in the expression of CCL20, CXCL5 and CXCL6 were subsequently associated with IL-17 dependent diseases, in particular the role of IL-17A in psoriasis, arthritis and airway cells (CCL20, CXCL5 and CXCL6), the role of IL-17F in allergic inflammatory airway diseases (CXCL5 and CXCL6), IL-17A signaling in gastric cells (CCL20), and IL-17A signaling in fibroblasts and IL-17 signaling alone (CXCL5). Such a modulation of IL-17 pathways was previously shown to be part of the helminth-mediated protection against autoimmune diseases and the failure to modulate these IL-17 pathways was correlated to filarial pathogenesis. Elliot et al. demonstrated that intestinal *Heligmosomoides polygyrus* infection suppresses mucosal IL-17 production and protects against intestinal inflammation in piroxicam-induced colitis, a model for inflammatory bowel disease (Elliott et al., 2008). With regard to filarial pathology, filarial infected *O. volvulus* and *B. malayi* patients that developed a severe pathology like lymphedema or hyperreactive onchocerciasis revealed increased Th17 responses with reduced numbers of regulatory T cells (Babu et al., 2009a; Katawa et al., 2015). Furthermore, evidence was provided that the development of Th17 responses in filarial infections is mainly established by IL-1 and IL-23 and associated with pathogenesis in the case of schistosomiasis (Anuradha et al., 2014; Mbow et al., 2013; Shainheit et al., 2011). *F. hepatica*-derived molecules protease cathepsin L1 (rFhCL1) and sigma class glutathione transferase (rFhGST-si) were shown to induce suppressive dendritic cells that dampen Th17 cells (Dowling et al., 2010). Consequently, these studies suggest that IL-17-dependent mechanisms contribute to the development of severe pathologies of helminth infections. Further experiments on the particular role of CCL20, CXCL5

and CXCL5 during the pathogeneses in filarial infections have not been conducted yet. Angiogenic chemoattractants like CXCL5 regulate IL-17 production in arthritis and neutrophil homeostasis (Mei et al., 2012; Pickens et al., 2011). Consequently, the dampening of IL-17 dependent pathways may counter-regulate inflammatory autoimmune disorders like rheumatoid arthritis and systemic lupus erythematosus, as was recently shown for filariae-infected patients (Panda and Das, 2017). Thus, further investigations should focus on the immunomodulation of IL-17-dependent pathways by helminths and helminth-derived molecules, especially in autoimmune diseases and inflammatory disorders (Helmby, 2015).

Moreover, with at least two altered genes the pathways of LXR/RXR activation (ALB, CD14 and MMP9), atherosclerosis signaling (ALB, MMP9), hepatic fibrosis / hepatic stellate cell activation (CD14, MMP9), leukocyte extravasation signaling (PECAM1, MMP9), and axonal guidance signaling (ADAM19, MMP9) were affected after BmA priming and LPS re-stimulation. Most of the BmA-affected pathways after BmA priming and LPS re-stimulation were also found after BmA-only stimulation. However, genes of the Oncostatin M signaling (MT2A, CHI3L1 and STAT1) and IL-6 signaling (CXCL8 and TNFAIP6) were mostly specifically enriched in BmA-only stimulated monocytes compared to unstimulated controls. Oncostatin M signaling was shown to be a potent inducer of IL-33 in lung and liver epithelial cells and to be involved in the tumor growth of non-malignant human breast epithelial cells (HBECs) (Arshad et al., 2015; Grant et al., 2001; Richards et al., 2016). Furthermore, Oncostatin M (OSM), a member of the IL-6/LIF cytokine family, induces IL-33 receptor (ST2) expression on CD4+ T cells (Arshad et al., 2015). This ST2 receptor is required for the splenic clearance of microfilariae from peripheral blood in *L. sigmodontis* infections (Ajendra et al., 2014). Thus, a variation in the OSM pathway may affect the transmission of filariae, but the role of OSM in helminth infection needs to be examined further. Langdon et al. provided evidence that OSM induces eotaxin expression in mouse lung fibroblasts and thus is involved in the eosinophil infiltration and may contribute, in combination with IL-17A, to inflammatory airway diseases (Kwofie et al., 2015; Langdon et al., 2003). Hence, our findings suggest that the modulation of the OSM pathway may affect the clearance of helminth infections.

Furthermore, since OSM promotes the dysfunction of the mucosal epithelial barrier, OSM may also have an impact on the development of eosinophil-dependent diseases (Pothoven et al., 2015). Thus, the modulation of OSM pathways may modify inflammatory responses like allergies.

BmA treatment is also associated with IL-6 signaling. Confirming our results, Smith et al. provided evidence that IL-6 counter-balances Th2 responses, induces regulatory T cells and thus promotes host susceptibility to helminth infection in mice (Smith and Maizels, 2014). Thus, the modulation of IL-6 pathways by filariae may be an essential host response, but the pathogenic character of CXCL8 and TNFAIP6 that were enriched in altered IL-6 signaling has to be analyzed further.

Our genome-wide transcriptional analysis of BmA-primed and LPS-restimulated human non-endemic monocytes reveals associations to granulocyte and agranulocyte adhesion and diapedesis, atherosclerosis and several IL-17-dependent inflammatory pathways and diseases like psoriasis, arthritis and allergic inflammatory airway diseases. Moreover, BmA treatment before subsequent LPS re-stimulation resulted in a reduced MHC2 expression, suggesting a diminished antigen presentation and a lower expression of the co-stimulatory molecule CD86 that may contribute to a modulated cell movement and an expression of angiogenic chemoattractants. BmA-only treatment may also affect IL-6-dependent host-protective mechanisms. The immunomodulation of human non-endemic monocytes by helminths and helminth products may subsequently influence autoimmunity and inflammatory disorders. Thus, immunomodulation is not limited to disease-specific immune responses but may also affect bystander immune responses.

4.2.5 Expression quantitative trait loci (e²QTLs) analysis of human monocytes to determine DNA variations that contribute to different gene expressions of anti-filarial responses and helminth-induced immunomodulation

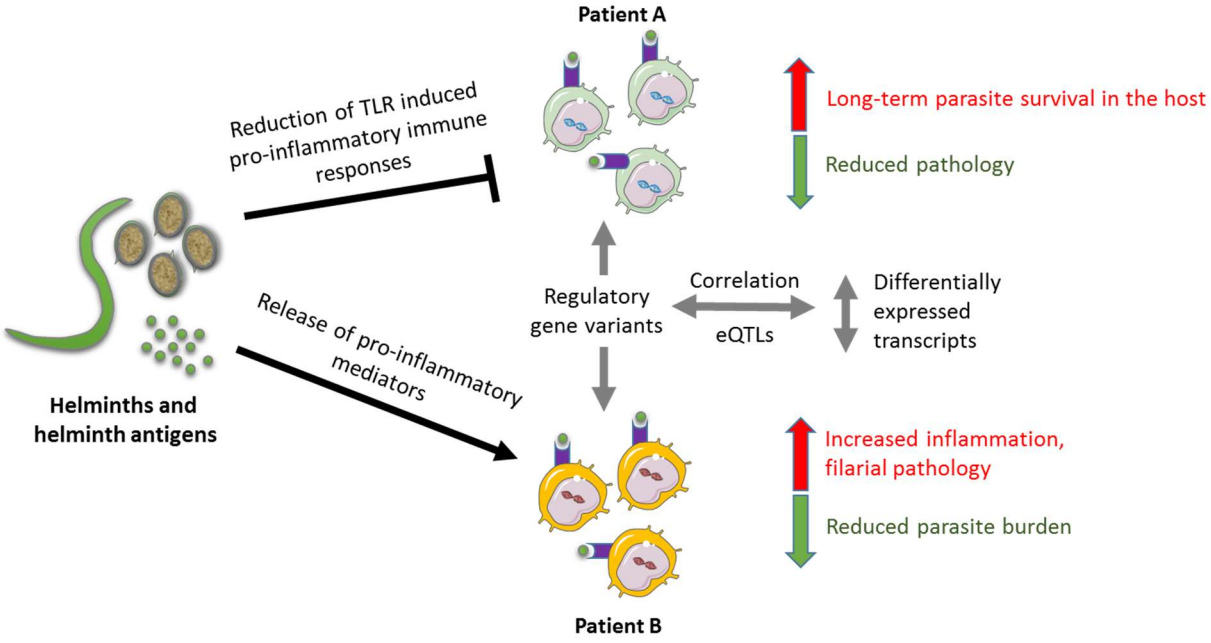

Figure 25: e²QTL analysis to determine regulatory gene variants that may be linked to the development of filarial pathology. *Illustrations are taken from Servier Medical Arts (http://servier.com/Powerpoint-image-bank; June 28, 2017).*

The genome-wide transcriptional analysis of human non-endemic monocytes stimulated with the adult worm extract of *Brugia malayi* and subsequently with LPS revealed a link to several IL-17 associated pathways and diseases as well as dysregulated immune responses that may lead to metabolic diseases like atherosclerosis. Using an e²QTL approach, genetic variants will be correlated in future analyses with the quantitative strength of transcript expression (Fig. 25). This will allow the identification of transcripts that are differentially expressed between individuals due to regulatory gene variants. Associations of single nucleotide polymorphisms (SNPs) will be further aligned with the transcriptome of human monocytes stimulated with BmA. Accordingly, genetic variants and new pathways may be identified that could be linked to the development of filarial pathology and present new treatment targets for filariasis. Understanding the underlying genetic mechanisms associated with differential expression after an antigenic stimulation of monocytes may also lead to new strategies for treating diseases that result from uncontrolled pro-inflammatory responses like autoimmune diseases.

Complimentary project A

Helminth-mediated protection against *E. coli*-induced sepsis is independent of TGFβ

Since helminth and helminth-derived products induce a regulatory anti-inflammatory milieu in their hosts, it was investigated whether the *L. sigmodontis*-mediated protection against *E. coli*-induced sepsis during the SIRS phase is dependent on the transforming growth factor (TGF) β. TGFβ is induced during the CARS phase of sepsis and by chronic filarial infection (Buerfent et al., 2015; Ince et al., 2009). Moreover, Hübner et al. provided evidence that TGFβ is essential for the *L. sigmodontis*-mediated protection against type 1 diabetes (Hübner et al., 2012b). TGFβ includes three isoforms and is mainly produced by myeloid-derived cells, platelets and suppressive regulatory T cells and contributes to an immunosuppressive milieu (Borish and Steinke, 2003; Chen et al., 2001). Thus, systemic TGFβ1-3 was depleted in chronic *L. sigmodontis*-infected female BALB/cJ mice and uninfected age-matched controls by an intraperitoneal injection of 100µg/mouse depletion antibody (Clone: 1D11.16.8) on day -3 and -1 before the injection of $8.5x10^8$ CFU *E. coli* K12. Controls were administered by 100µg/mouse IgG1 isotype control (Clone: MOPC-21, both BioXCell, West Lebanon, USA).

TGFβ depletion does not alter L. sigmodontis *improvement of* E. coli*-induced hypothermia, reduced peritoneal bacterial load and increased myeloid cell composition six hours after* E. coli *challenge*

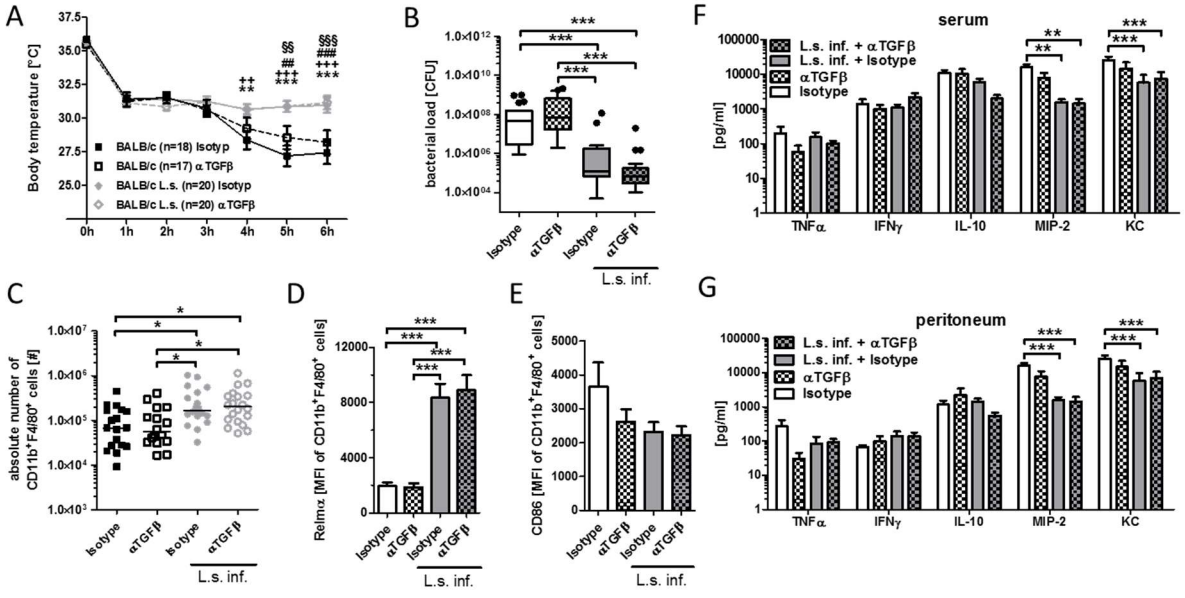

Figure 26:* L. sigmodontis *induced protection against* E. coli*-induced sepsis is independent of TGFβ depletion during the SIRS phase. *(A) Kinetic of the body temperature for six hours after intraperitoneal injection of 8.5x10⁸ cfu* E. coli *K12 in chronic* L. sigmodontis *(L.s.)-infected BALB/c mice (L.s. Isotype: n = 20; L.s. αTGFβ: n=20) and uninfected controls (Isotype: n = 18; αTGFβ: n=17; significant differences were shows as: * = BALB/c + Isotype vs. BALB/c L.s. + Isotype, + = BALB/c + Isotype vs. BALB/c L.s. + αTGFβ, # = BALB/c L.s. + Isotype vs. BALB/c + αTGFβ and § = BALB/c + αTGFβ and BALB/c L.s. + αTGFβ). (B) Peritoneal bacterial load [cfu], (C) absolute cell number of CD11b+F4/80+ cells as well as (D) the RELMα and (E) CD86 expression (mean fluorescence intensity) on CD11b+F4/80+ cells six hours after* E. coli *injection. (F) Serum cytokine/chemokine and (G) peritoneal cytokine/chemokine profile six hours after* E. coli *injection. Pooled data of three independent experiments with at least 6 mice per group. Data is shown as median or mean ± SEM and was tested for statistical significance by 2-way ANOVA and Bonferroni post hoc test (A, F+G) and 1-way ANOVA followed by Kruskal-Wallis test and Dunn's Multiple Comparison post hoc test (B-E). Significance is indicated as *p<0.05; **p<0.01; ***p<0.001 (B-G).*

The *L. sigmodontis* infection resulted in a reduced *E. coli*-induced hypothermia, which confirmed our previous findings (Fig. 26A+B) (Gondorf et al. 2015). Generally, the *E. coli*-only induced hypothermia was highly correlated with an increased peritoneal bacterial load (r_s= -0.7074; p< 0.0001; n=74 pooled individuals of three independent experiments). The reduced hypothermia in *L. sigmodontis* infected mice was further correlated with a reduced bacterial load. Importantly, TGFβ depletion did not influence the *L. sigmodontis* improved body temperature and reduced bacterial load. Moreover, the experimental *L. sigmodontis* infection led to significantly altered absolute and relative numbers of peritoneal macrophages, but not to an altered RELMα expression (Fig. 26C+D). The CD86 expression was by tendency reduced but unaffected by TGFβ depletion (Fig. 26E).

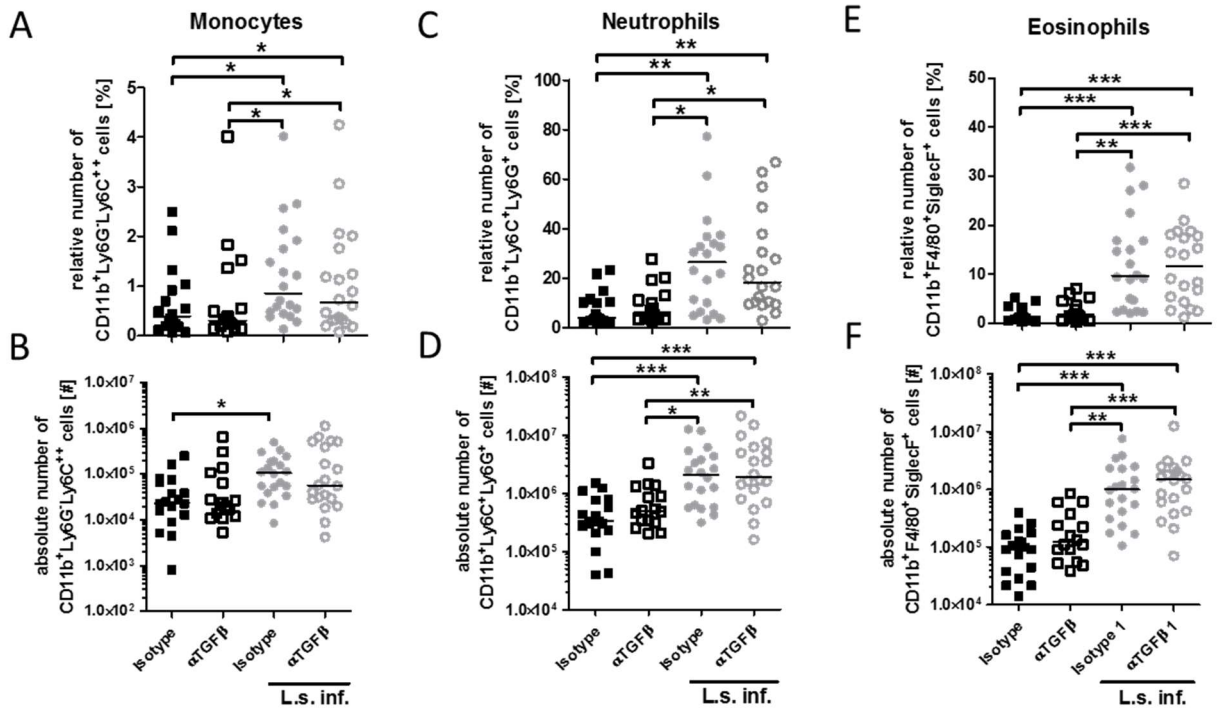

Figure 27: The increased peritoneal numbers of monocytes, neutrophil and eosinophil granulocytes six hours after i.p.* E. coli *injection in chronic* L. sigmodontis*-infected BALB/c mice is not altered by anti-TGFβ treatment. *(A+B) Relative and absolute number of peritoneal CD11b+Ly6G-Ly6C++ cells (monocytes), (C+D) CD11b+Ly6G+Ly6C+ cells (neutrophils) and CD11b+F4/80(med)SiglecF+ (eosinophils) (E+F) six hours after* E. coli *injection. Data represent three pooled independent experiments with at least six mice per group. Data are shown as median and was tested for statistical significance by Kruskal-Wallis test followed by Dunn's Multiple Comparison post hoc test (*p<0.05; **p<0.01; ***p<0.001).*

No changes in systemic and peritoneal cytokine and chemokine levels of L. sigmodontis*-infected animals six hours after* E. coli *challenge*

Since our previous findings provided evidence that a chronic filarial infection reduces the *E. coli*-induced release of pro-inflammatory mediators like pro-inflammatory IL-6, the cytokine and chemokine profile was analyzed six hours after the *E. coli* injection (Gondorf et al., 2015). Confirming previous results, the *L. sigmodontis*-infected animals had lower concentrations of pro-inflammatory cytokines/chemokines following the *E. coli* challenge compared to *E. coli*-injected controls (Fig. 26F+G). Interestingly, while almost no changes were observed with regard to IL-10 and IFNγ production, the concentration of CXCL1 and CXCL2 was significantly reduced in *L. sigmodontis*-infected animals independent of previous TGFβ depletion. In *L. sigmodontis*-uninfected controls the TGFβ depletion led to a trend in reduced peritoneal and serum TNF levels.

TGFβ depletion does not alter increased numbers of monocytes, neutrophils and eosinophils caused by L. sigmodontis *infection and six hours after* E. coli *injection*

L. sigmodontis increased the numbers of monocytes, neutrophil and eosinophil granulocytes six hours after the *E. coli* challenge compared to *E. coli*-only challenged controls (Fig. 27). Subsequently, TGFβ depletion did not affect the capacity of the *L. sigmodontis* infection to improve sepsis outcome and sepsis-relevant cell populations were not affected by the absence of TGFβ during the early phase of sepsis either.

Accordingly, our results suggest that TGFβ is not essential for the *L. sigmodontis*-mediated protective effect during the initial SIRS phase of sepsis. Previous results of Ahmad et al. provided evidence that elevated levels of TGFβ are found one day after induced sepsis (Ahmad et al., 1997). TGFβ is also known to induce TGFβ-induced proteins (TGFβIp), an integrin-interacting

extracellular matrix protein that is associated with an increased vascular inflammation and thus with the severity of the sepsis (Bae et al., 2014). Regulatory T cells are described to produce TGFβ and also IL-10 to counter-regulate pro-inflammatory responses to pathogens and hence contribute to immunosuppression and an increased risk for secondary infections (Monneret et al., 2003; Wisnoski et al., 2007). Moreover, Hiraki et al. demonstrated that the depletion of both TGFβ and IL-10 can reduce suppressive T cell populations and improve sepsis survival in mice (Hiraki et al., 2012). Since the TGFβ depletion did not impair the *L. sigmodontis*-mediated improved sepsis outcome, our results suggest that the filariae-mediated protection against sepsis is not dependent of counter-regulatory TGFβ levels. Our previous work revealed that *L. sigmodontis* modulated macrophages are essential in the protection against *E. coli* induced sepsis. We here demonstrate that the protective mechanism is independent of TGFβ, despite helminth induced regulatory T cells and increased levels of peripheral TGFβ (Dittrich et al., 2008). Furthermore, during the compensatory CARS phase a chronic *L. sigmodontis* infection did not enhance TGFβ production and had no negative impact on sepsis outcome (Buerfent et al., 2015). In contrast to those results, the *L. sigmodontis*-mediated protection against autoimmune diabetes was dependent on TGFβ (Hübner et al. 2012). Consequently, our results suggest that dependent of the local context, several mechanisms of filarial immunomodulation may protect against inflammatory diseases (Helmby, 2015; Maizels and McSorley, 2016).

Complimentary project B

Transcriptome-wide analysis of human monocytes stimulated with *Porphyromonas gingivalis* LPS and metal ions and its impact on systemic and autoimmune diseases

In cooperation with the Institute of Human Genetics (Prof. Dr. Markus M. Nöthen and PD Dr. Johannes Schumacher) and the Department of Orthodontics of the University of Bonn (Prof. Dr. Andreas Jäger and PD Dr. Lina Gölz) we conducted studies about the impact of LPS from the periodontopathogen *Porphyromonas gingivalis* (P.g.), nickel and cobalt metal ions on human monocytes. A genome-wide transcriptional analysis and an IPA® analysis was performed to further determine differentially expressed transcripts and pathways that are linked to periodontal destruction and systemic and autoimmune diseases. The transcriptome-analysis of LPS P.g. revealed 902 transcripts with several new candidates that are linked to autoimmune diseases like atherosclerosis, arthritis and cancer (Gölz et al., 2016a). The stimulation of human monocytes by nickel ions (Ni^{2+}), signaling via cross-linking of two TLR4 monomers (imidazole groups) (Rothenberg, 2010), resulted in a total of 1385 differentially expressed transcripts in a dose-dependent manner (Gölz et al., 2016b). The IPA® analysis of nickel-stimulated human monocytes revealed associations to colitis, cancer and encephalitis. Two publications with co-authorships in renowned peer-reviewed journals resulted out of those studies so far. These studies are the basis for future e²QTL analysis.

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List of abbreviations

AAM	alternatively activated macrophages
APC	allophycocyanine
BmA	*Brugia malayi* crude antigen extract
BSA	bovine serum albumin
°C	degree Celsius
CCL / CCR	chemokine (c-c motif) ligand / receptor
CD	cluster of differentiation
CFU	colony forming units
CXCL	chemokine C-X-C motif ligand
CCL	CC-chemokine ligand
DAMP	damage-associated molecular pattern
DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
Dpi	days post infection
E/S	excretory/secretory
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FACS	Fluorescence activated cell sorting
FCS	fetal calf serum
FITC	Fluorescein isothiocyanate
FMO	fluorescence minus one
FSC	forward scatter
Hi	high expressing
IFN	interferon
IL	Interleukin
i.p.	Intra-peritoneal
LF	lymphatic filariasis
Lo	low expressing
LPS	lipopolysaccharide
LsAg	*Litomosoides sigmodontis* crude antigen extract
MACS	magnet activated cell sorting

min	minute(s)
mf	microfilariae
MFI	mean fluorescence intensity
MHC	mayor histocompatibility complex
ml	milliliter
MMP	matrix metalloproteinase
MyD88	myeloid differentiation primary response protein 88
o/n	over night
P3C	Pam3CSK4; Synthetic triacylated lipoprotein
PAMP	pathogen-associated molecular pattern
PBMC	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PE	phycoerythrin
PI	propidium iodide
PRR	pattern recognition receptor
RBC	red blood cell
RELM	resistin-like molecule
RNA	ribonucleic acid
rpm	rounds per minute
RT	room temperature
SEM	standard error of mean
SIRS	systemic inflammatory response syndrome
SSC	side scatter
Treg	regulatory T cell
TGFβ	transforming growth factor beta
Th	T helper cell
TLR	Toll-like receptor
TNF	tumor necrosis factor
WHO	World Health Organisation
WT	wild type

Curriculum Vitae

Benedikt Christian Bürfent

EDUCATION AND SCIENTIFIC CAREER

SCHOLARSHIPS AND MEMBERSHIPS

03/2017	**Travel grant** for "1st Winter School Immunology meets Genomics and Bioinformatics" of the **Excellence Cluster Immunosensation.**
04/2016 – 04/2017	Extension of the scholarship of the **Jürgen Manchot Stiftung**, Düsseldorf.
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CONFERENCES

Poster presentation at "**1st Winter School: Immunology meets Genomics and Bioinformatics**" of the "Immunosensation Excellence Cluster", Bonn, 3th-7th November 2017; (Abstract title: „Transcriptome wide analysis of human monocytes stimulated with filarial antigens and LPS reveals a link to IL-17 associated pathways and diseases")

Oral and poster presentation at "**Science Day 2016**" of the "Immunosensation Excellence Cluster", Bonn, 7th-8th November 2016; (Abstract title: „Transcriptome wide analysis of human monocytes stimulated with filarial antigens and LPS reveals a link to IL-17 associated pathways and diseases")

Poster presentation at "**Science Day 2015**" of the "Immunosensation Excellence Cluster", Bonn, 2th-3th November 2015; (Abstract title: „Impact of genetic factors on immune responses after monocyte stimulation with helminth antigens and LPS")

Poster presentation at "**11th Spring School on Immunology March Ettal – Bavaria**" of the "German Society of Immunology (DGfI)", Bonn, 8th-13th March 2015; (Abstract title: "Impact of a filarial infection on the function of CD4+ and CD8+ cells after *E. coli* induced sepsis"

Poster presentation at "**Science Day 2014**" of the "Immunosensation Excellence Cluster", Bonn, 3th-4th November 2014; (Abstract title: "The influence of genetic factors on immune responses after monocyte exposure to helminth antigens")

Poster presentation at "**44th Annual Meeting of German Society for Immunology**" (DGfI) 2014, Bonn, 17th-20th September 2014; (Abstract title: "Impact of a filarial infection on the function of CD4+ and CD8+ cells after *E. coli* induced sepsis")

ADDITIONAL PROFESSIONAL QUALIFICATIONS

09/2016	"**GPM Young Crew Workshop 2016**" of the **German Society for Project Management** (GPM) in Kassel, Germany.
06/2016	**Basic course for good clinical practice following pharmaceutical law (ICH-GCP E6)** at the University Hospital Bonn (Dr. med. Martin Coenen).
03/2014	**Advanced training in SPSS and Statistics** at the Institute for Medical Biometry, Informatics and Epidemiology of the University of Bonn.
03/2013	**Course in Basics of Laboratory Animal Science according to FELASA B** after the guidelines of the Federation of European Laboratory Animal Science (House for experimental therapy; Dr. vet. med. Wolfgang Eichelkraut & Andrea Lohmer) at the University of Bonn.

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Dipl. Biol. Benedikt Christian Bürfent

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