Fakultät für Medizin

Institut für Medizinische Mikrobiologie, Immunologie und Hygiene

Chronic *Schistosoma mansoni* Infection during Pregnancy: Effects on Offspring's T Cell Differentiation Capacity, Epigenetics and Memory T Cell Compartment

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Vollständiger Abdruck der von der Fakultät für Medizin der Technischen Universität München zur Erlangung des akademischen Grades eines Doktors der Medizinischen Wissenschaft (Dr. med. sci.) genehmigten Dissertation.

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Die Dissertation wurde am 12.05.2020 bei der Fakultät für Medizin der Technischen Universität eingereicht und durch die Fakultät für Medizin am 02.12.2020 angenommen.

Acknowledgement

Throughout the writing of my medical thesis I received a great deal of support. First of all I would like to thank my supervisor Dr. rer. nat. Kathrin Klar and my advisor Prof. Dr. med. Clarissa Prazeres da Costa for their continuous support, for their motivation and guidance. Their immense knowledge helped me in all the time of my research and writing of this dissertation.

I would like to thank Prof. Dr. Dirk Busch for giving me the possibility to do my medical thesis at the Institute of Medical Microbiology, Immunology and Hygiene.

In addition my sincere thanks goes to my colleagues in our laboratory, especially Sabine Paul and Marija Ram for excellent technical assistance, Ulla Henn and Stephanie Fetzer for taking care of the schistosome cycle and Matthew Lacorcia for the discussion and proof reading. I would also like to thank Dr. Hani Harb and Dr. Rouzanna Istvanffy for the cooperation and discussions.

Last but not least I cannot find words to express my gratitude to my family and friends for their love, patience and encouraging words.

Abstract

In strong contrast to third world countries, the prevalence of allergic diseases, such as asthma, continues to rise in industrialized countries. In addition to viral and bacterial infections, parasitic infections are thought to play a major role in suppressing bystander immune responses, such as allergies. Several studies have demonstrated an inverse relationship between the prevalence of helminth infections and the development respectively incidence of allergies. Currently, over 250 million people are infected with schistosomiasis, a non-transplacental helminth infection. The infection with *Schistosoma mansoni* is immunologically characterized by an initial T_H1 type response, followed by an egg induced T_H2 type inflammation and eventually a long-term immunosuppressive (Reg) phase to protect the host and the parasite against perilous inflammatory responses, but also against bystander responses. Interestingly, besides the allergen-preventing effect within the host, schistosomiasis during pregnancy can influence the offspring's susceptibility to allergies. Indeed, our group has previously shown, that the progeny of female mice suffering from chronic schistosomiasis is protected from the onset of allergic airway inflammation (AAI). With regard to the underlying mechanisms, this study investigated the effects of *in utero* exposure to chronic schistosomiasis (Reg phase) on T cell differentiation capacity and immune cell development in the offspring. While the early development of B and T cells in thymus and bone marrow was unmodified, the immune cell composition in peripheral blood showed specific variations: increased numbers of granulocytes and higher percentages of naïve $CD4^+$ and $CD8^+$ T cells in peripheral blood, and decreased percentages of natural killer (NK) cells in splenocytes. Furthermore, *in vitro* T cell differentiation studies exposed naïve T cells of offspring of schistosome-infected mothers having a strong capacity to differentiate in T_H1 cells, whereas their ability to differentiate in T_H2 cells was impaired. As potential underlying mechanisms, epigenetic changes such as histone acetylation of naïve T cells were analyzed. In accordance with the restricted T_H2 differentiation, the promoter regions of IL-4 and IL-5 revealed a decreased H4 acetylation in naïve T cells, while the H4 acetylation at FoxP3 and RORγT promoter regions was increased. Interestingly, $CD4^+CD62L^+$ naive T cells of offspring of schistosome-infected mothers spontaneously produced IL-4 *in vitro*, pointing towards a functional T_H2 immunophenotype within the $CD4^+CD62L^+CD44^+$ memory T cell compartment. Taken together, this transgenerational mouse model study revealed distinct epigenetic changes within the naïve T cell compartment affecting T_H1 and T_H2 cell differentiation in offspring of chronic schistosome-infected mothers. These findings will help

to understand how the maternal immune status during pregnancy alters the offspring's development of the immune system.

Kurzfassung

Im starken Gegensatz zu Entwicklungsländern steigt die Prävalenz von allergischen Erkrankungen wie Asthma in Industrienationen kontinuierlich an. Zusätzlich zu viralen und bakteriellen Infektionen werden auch Infektionen mit Parasiten immer mehr als potentielle Schutzfaktoren gegenüber dem zunehmenden Auftreten von Allergien miteinbezogen. Mehrere Studien haben eine umgekehrte Relation zwischen der Prävalenz von Helminthinfektionen und der Entwicklung bzw. Inzidenz von allergischen Erkrankungen gezeigt. Derzeit sind über 250 Millionen Menschen weltweit mit Bilharziose, einer nichttransplazentaren, chronischen Trematodeninfektion, infiziert. Die Infektion mit *Schistosoma mansoni* ist charakterisiert durch drei aufeinanderfolgende Immunphasen: die beginnende T_H1 Immunantwort geht in eine T_H2 Typ Inflammation über, und resultiert schließlich in einer dauerhaften, immunsuppressiven (Reg) Phase, um den Wirt und den Parasiten vor einer gefährlichen Entzündungsreaktion zu schützen. Nicht nur der an Bilharziose erkrankte Wirt selbst ist vor Allergien geschützt, eine Erkrankung während der Schwangerschaft beeinflusst zudem Anfälligkeit und Auftreten von Allergien bei den Nachkommen. Unsere Arbeitsgruppe hat in der Tat zuvor gezeigt, dass Nachkommen von Mäusen mit chronischer Bilharziose vor allergischen Atemwegserkrankungen (AAI) geschützt sind. Diese Studie wurde in Hinsicht auf die zugrundeliegenden Mechanismen konzipiert, um die Effekte der *in utero* Exposition von chronischer Bilharziose auf die T Zell Differenzierungskapazität und Immunzellentwicklung bei den Nachkommen zu erforschen. Während die frühen Entwicklungsstadien von B- und T-Zellen aus Thymus und Knochenmark keinerlei Unterschiede zeigten, so wies die Zusammensetzung der Immunzellen im peripheren Blut spezifische Unterschiede auf: eine erhöhte Anzahl von Granulozyten und naiven $CD8^+$ and $CD4^+$ T-Zellen im peripheren Blut und einen niedrigeren Anteil an natürlichen Killerzellen in Milzzellen. In-vitro-T-Zellen-Differenzierungsstudien zeigten, dass naive T-Zellen aus Nachkommen von chronisch schistosomen-infizierten Mäusen sich stärker in T_H1-Zellen differenzieren lassen konnten, während ihre Fähigkeit, sich in T_H2-Zellen zu differenzieren, erheblich beeinträchtigt war. Als mögliche zugrunde liegende Mechanismen wurden epigenetischen Veränderungen analysiert, wie zum Beispiel die Histon-Acetylierungen naiver T-Zellen. In Übereinstimmung mit der eingeschränkten T_H2-Differenzierung zeigten naive T-Zellen eine verminderte Histon-H4-Acetylierung an den Promoterregionen IL-4 und IL-5, während die H4-Acetylierung an den Promoterregionen FoxP3 und RORγt erhöht war. Interessanterweise produzierten $CD4^+CD62L^+$ T-Zellen von Nachkommen mit Müttern, die chronisch an Schistosomiasis

während der Schwangerschaft erkrankt waren, auch spontan IL-4 *in vitro* und gaben somit Hinweise auf einen T_H2 umfassenden Immunphänotyp innerhalb des $CD4^+CD62L^+CD44^+$ T-Zell-Kompartiments. Zusammengefasst wurde in dieser Mausstudie deutlich, dass epigenetische Veränderungen innerhalb des naiven T-Zell-Kompartiments die T_H1- und T_H2-Zell-Differenzierungskapazität stark beeinflussen. Die Ergebnisse tragen wesentlich zum Verständnis bei, wie der mütterliche Immunstatus während der Schwangerschaft die Entwicklung des Immunsystem der Nachkommen beeinflusst.

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Abbreviations

	Abbreviation	Description
A	AAM	Alternatively activated macrophages
	ACT	Ammoniumchloride-tris
	AGM	Aorto-gonado-mesonephros
	AHR	Airway hyper-responsiveness
	Al(OH)3	Aluminium (hydroxide (alum)
	APC	Antigen presenting cells
	APC	Allophycocyanin (Fluorescent dyes)
	Arg1	Arginase 1
B	BAL	Bronchoalveolar lavage
	Bcl-2	B cell lymphoma 2
	BCG	Bacillus Calmette Guerin
	BM	Bone marrow
	Bp	Base pair(s)
	Breg	Regulatory B cells
	BSA	Bovine serum albumin
C	CBMC	Cord blood mononuclear cells
	CD	Cluster of differentiation
	CTL	C-type lectin
	CTLA	Cytotoxic T lymphocyte antigen
D	DC	Dendritic cell
	DEREG	Depletion of regulatory T cells
	DMEM	Dulbecco's modified eagle medium
	DMSO	Dimethyl sulfoxide

	Abbreviation	Description
	DNA	Deoxyribonucleic acid
	dNTPs	Deoxynucleoside triphosphate
	DOI	Degree of infection
	dsRNA	Double-stranded RNA
	DTT	Dithiothreitol
E	EDTA	Ethylenediaminetetraacetic acid
	ELISA	Enzyme linked immunosorbent assay
F	FACS	Fluorescence-activated cell sorting
	Fc	Fragment crystallizable
	FcεRI	Fc epsilon receptor I
	FcRγ	Fc receptor gamma chain
	FCS	Fetal calf serum
	FITC	Fluorescein isothiocyanate
	FIZZ	Found in inflammatory zone
	Foxp3	Forkhead box P3
G	GATA	Globin transcription factor
	GM-CSF	Granulocyte-macrophage colony-stimulating factor
H	HAT	Histone acetylases
	HCl	Hydrogen chloride
	HDAC	Histone deactylases
	HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
	H3K9	Histone H3 lysine 9
	HIV	Human immunodeficiency virus
	HRP	Horseradish peroxidase

	Abbreviation	Description
	HP1α	Heterochromatin protein 1α
	HSC	Hematopoietic stem cell
	H2O	Water
	H2SO4	Sulfuric acid
I	ICOS	Inducible costimulatory molecule
	IFN	Interferon
	Ig	Immunoglobulin
	IGF	Insulin-like-growth factor
	IL	Interleukin
	i.p.	Intraperitoneal
	IL-1R	IL-1 receptor
	ILC2	Type II innate lymphoid cells
	IVF	In vitro fertilization
K	Kb	Kilobase
	KCl	Potassium chloride
	kDa	Kilo Dalton
	KO	Knockout
	KOH	Potassium hydroxide
L	LPS	Lipopolysaccharide
	LiCl	Lithium chloride
M	MHC	Major histocompatibility complex
	MIH	Institute of medical Microbiology, Immunology and Hygiene
	MLN	Mesenteric lymph node
	MRI	München Klinikum rechts der Isar

	Abbreviation	Description
	MTCT	Mother-to-child-transmission
	MyD88	Myeloid differentiation primary response protein 88
N	NaCl	Sodium chloride
	NF-κB	Nuclear factor-κB
	NH4Cl	Ammonium chloride
	NK	Natural killer
	NKT	Natural killer T cell
O	OVA	Ovalbumin
P	PAMPs	Pathogen-associated molecular pattern molecules
	PBMCs	Peripheral blood mononuclear cells
	PBS	Phosphate buffered saline
	PCR	Polymerase chain reaction
	PE	Phycoerythrin
	PFA	Paraformaldehyde
	PGE2	Prostaglandin E2
R	RAG	Recombination activating gene
	Reg	Regulatory
	Relmα	Resistin-like molecule α
	RNA	Ribonucleic acid
	RORγT	Retinoid-related orphan receptor gamma T
	RPMI	Roswell Park Memorial Institute medium
	RWE	Ragweed extract
S	SCID	Severe combined immunodeficiency

	Abbreviation	Description
	SD	Standard deviation
	SEA	Soluble egg antigen
	SEM	Standard error of the mean
	SMAC	Supramolecular activation complex
	S. mansoni	*Schistosoma mansoni*
	SmTAL-2	*Schistosoma mansoni* tegumental-allergen-like protein
	SP	Single positive
	STAT	Signal transducer and activator of transcription
	Suv39h1	Suppressor of variegation 3-9 homolog 1
T	TAE	Tris acetate EDTA
	T-bet	T-box expressed in T cells
	TBST	Tris-buffered saline and Tween 20
	TCR	T cell receptor
	TGF	Transforming growth factor
	T_H	T helper
	TLR	Toll-like receptor
	TNF	Tumor necrosis factor
	TRAM	TRIF-related adaptor molecule
	Tris	Tris(hydroxymethyl)aminomethane
	Treg	Regulatory T cells
V	VDR	Vitamin D receptor
	v/v	Volume per volume
W	WHO	World Health Organization
	wt	Wild type

Abbreviation	Description
w/v	Weight per volume

1 Introduction

1.1 Schistosomiasis

1.1.1 Global burden of schistosomiasis

Schistosomiasis, also known as bilharzia, is a common and severe disease. 250 million infected humans worldwide (Stensgaard et al., 2013) and 280,000 deaths annually in Africa (Hotez et al., 2006) demonstrate the severity of the disease. 85% of the infections are located in sub-Saharan Africa, Asia, and South America (Engels et al., 2002; Hotez et al., 2007). Due to poor hygiene standards, such as contaminated drinking water and unclean sanitation facilities, humans there are constantly exposed during daily routines of agricultural, occupational or domestic activities to the infection with schistosomiasis (Hürlimann et al. 2011). Schistosome infections are correlated with higher morbidity and mortality mainly caused by malnutrition and significant anemia (Harris et al., 2011). Further physical symptoms such as abdominal pain, diarrhea, and reduced exercise tolerance (Savioli et al., 2005) often occur and can result in socio-economic disadvantages (Sleigh et al., 1998). Since helminth infections play a crucial role in social inequality (Sleigh et al., 1998) by causing 4,5 million disability-adjusted life years (DALYs) (Hotez et al., 2006), the World Health Organization (WHO) declared a large-scale anthelmintic treatment with praziquantel of infected humans and at-risk populations. In 2017, approximately 99 million people got treated. Including the humans treated in 2016 and 2015, almost 68% of the at-risk population was covered with the prophylaxis, of which 57% humans lived in the high-endemic areas of Africa (WHO, 2017). Despite this progress and global support, the burden of disease still continues to rise (Fenwick, 2012), since more than 600 million humans live in endemic areas and new transmission areas may become suitable for *Schistosoma mansoni* and its intermediate host Biomphalaria (Stensgaard et al. 2013). Future scenarios show that due to climate changes snails will move to cooler places in the south and east of Africa. Additionally, the increased migration of humans to urban areas causes new environmental modifications, such as construction of dams, that facilitate transmission, and, thus, the infection with schistosomiasis (Stensgaard et al., 2013). Consequently, not only nature, but also human habitats largely contribute to the current distribution of helminth infections (Stensgaard et al., 2013). The

main targets to dampen the spreading of the disease is to improve the safe access to clean water and sanitation facilities, to teach hygiene and to control the distribution of fresh water snails in schistosome-endemic areas (King et al., 2005; World Health Organization, 2002). In 2020, at least 75% of all school-age children in endemic areas are expected to have been treated with praziquantel (WHO, 2017). Taken together, the infection with schistosomes is a high-endemic disease in developing countries with severe, physical symptoms, resulting in significant socio-economic disadvantages. The distribution of helminths and the spreading of the disease can be reduced by human habitats and the anthelmintic treatment with praziquantel.

1.1.2 **Human species of helminths**

Schistosomiasis is a worm disease caused by the larvae of trematodes of the genus schistosoma. The majority of human helminths belongs to two phyla, the nemathelminths which include the nematodes (roundworms), such as hookworms, ascarids, and filarial worms, and secondly, the phylum of platyhelminths. Within the platyhelminths, the class of cestodes (tapeworms) includes the genera of taenia and echinococcus, while the class of trematodes (flukes) includes schistosomes, of which *S. mansoni*, *S. japonicum* and *S. haematobium* are the main human species. *S. mansoni* and *S. japonicum* cause intestinal schistosomiasis, while *S. haematobium* causes urogenital schistosomiasis. (Gryseels, 2012)

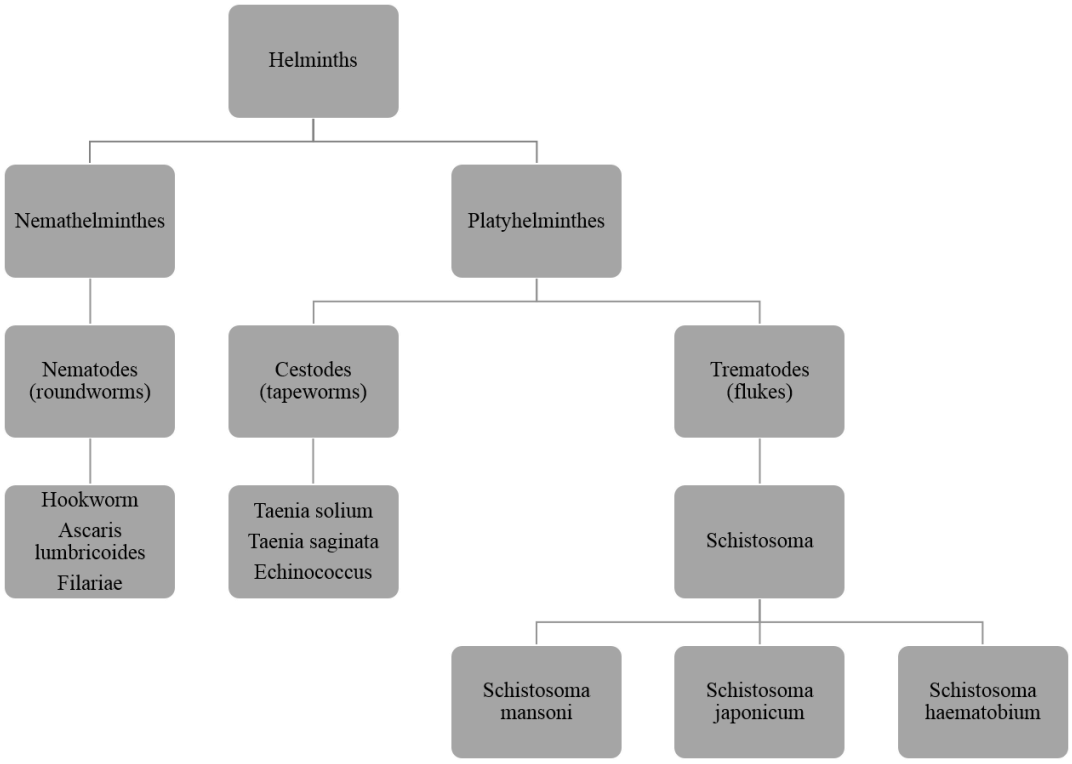

Figure 1: **Classification of helminths**

The illustration provides an overview of different helminth species. The phylum of nemathelminths includes nematodes (roundworms), such as hookworms, ascarids, and filarial worms. Within the phylum of platyhelminths, the class of cestodes (tapeworms) includes taenia solium and saginata and echinococcus. The class of trematodes (flukes) includes schistosomes, of which *S. mansoni*, *S. japonicum* and *S. haematobium* are the main human species. The figure is adopted from (Gibson, 2010).

1.1.3 Immune responses during schistosomiasis infection

One of the most important functions of parasites is to guarantee their transmission to a new definitive host. While transmission rates of schistosomes are relatively low, parasites require a prolonged time where the parasite is available to the host, resulting in strategies by which schistosomes prevent immune elimination (reviewed in Yazdanbakhsh et al., 2010). The infection with *Schistosoma mansoni* is characterized by three typical phases: an acute T_H1 driven phase, followed by an egg-induced T_H2 phase, and eventually a long-term immunosuppression (Reg phase). The acute phase of infection is accompanied by a proinflammatory response caused by infective schistosomes before the egg production starts, the so-called pre-patent phase of infection (Pearce et al., 1991). Cercariae and the newly transformed schistosomula activate the complement cascade (Rujeni et al., 2012) and elicit proinflammatory responses (Jenkins et al., 2005; Paveley et al., 2011). The antigen-specific T_H1 cells further start to produce Interleukin (IL)-2 and Interferon (IFN)-γ

(Grzych et al., 1991). Furthermore high amounts of eosinophils, tumor necrosis factor (TNF)-α and Immunoglobulin E (IgE) were detectable in patients with acute schistosomiasis (de Jesus et al., 2002). Nevertheless an overshooting T_H1 and T_H17 cell mediated inflammation might injure the host and needs to be dampened by a distinct T_H2 response (Grzych et al., 1991; Pearce et al., 1991). After female worms start to produce eggs the initial T_H1 phase is replaced by an egg-induced T_H2 phase, the so-called post-patent phase of infection (Grzych et al., 1991). Schistosome-derived eggs, which cause the primary lesion of the infection of the egg-surrounding granulomas in the host's liver and intestine, especially trigger T_H2-mediated immune responses: the production and release of IL-4, IL-5, IL-9, IL-13 and IL-21 and high levels of eosinophils, basophils and mast cells (Chensue et al., 1997; Wynn et al., 1995; Wynn et al., 1993). Soluble egg antigen further inhibits dendritic cells to produce IL-12, which together with regulatory T cells successfully limit the T_H1 response (Pearce et al., 2004). Moreover, IL-4 receptor signaling and CD40-mediated co-stimulation by $CD4^+$ effector T cells induce B cells to switch their antibody class to IgG1 and IgE. Unrestricted T_H2 responses can potentially harm the host and the parasite by causing liver cirrhosis and hepatosplenic disease, therefore helminths are able to initiate a regulatory immunosuppressive environment (Thomas A. Wynn, 2007). The chronic phase of infection is mainly dominated by the cytokine IL-10, which is produced by T_H2 cells, macrophages, regulatory T (Treg) and B cells, which leads to the downregulation of proinflammatory T cell proliferation and cytokine production (Harris et al., 2011; Maizels et al., 2003). Immunoregulatory cells, such as alternatively activated macrophages (AAMs), which produce arginase-1 (Arg-1) and resistin like molecule α (RELMα), as well as regulatory B and T cells secure the survival of the infected host (Harris et al., 2011; Maizels et al., 2003) and regulate the immune-mediated pathology (Hesse et al., 2004). Taken together the immune responses during *S. mansoni* infection are complex (shown in Fig. 3) and imply an accurate balance between host and parasite. The development of an immunosuppressive environment secures the parasite's survival, and controls host pathologies.

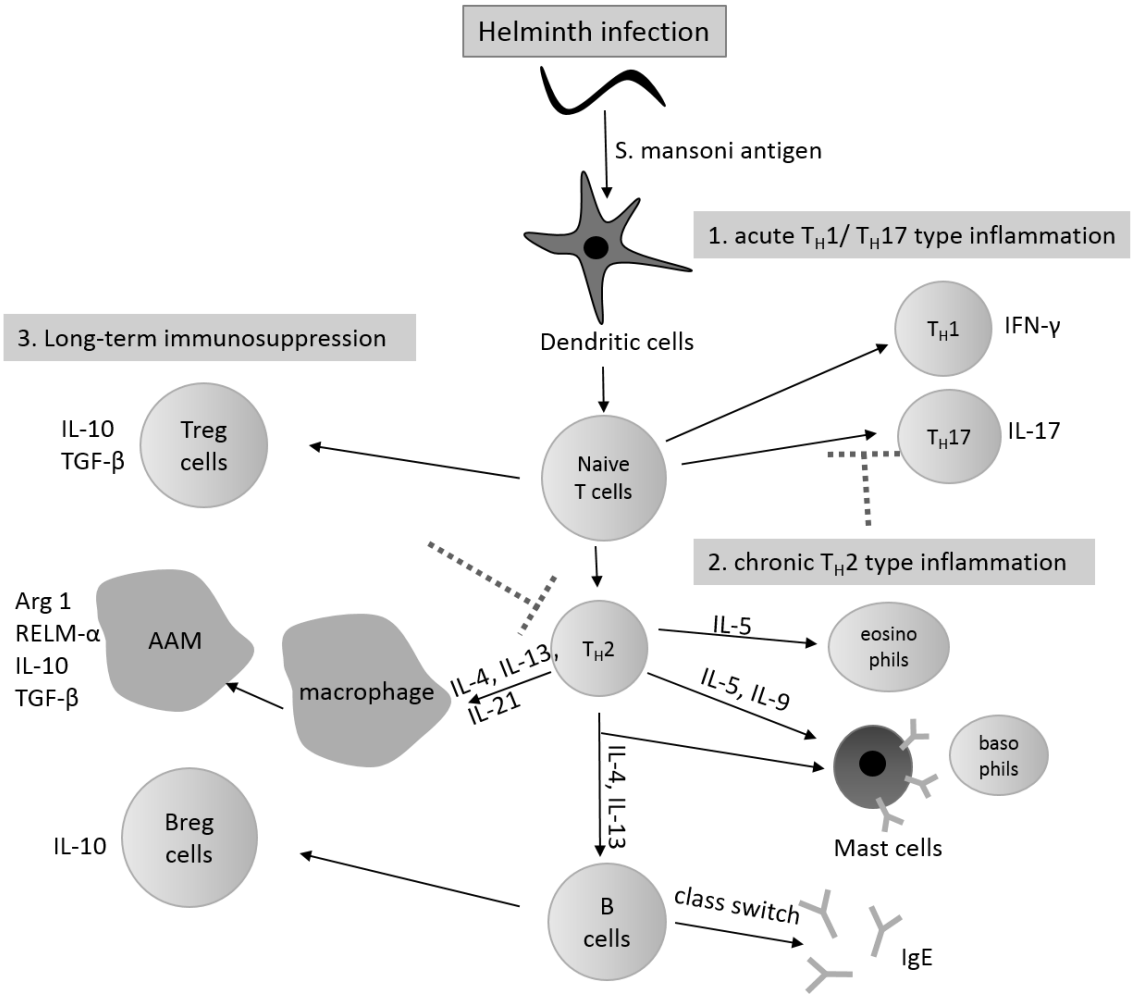

Figure 2: **Immune responses during *S. mansoni* infection**

The infection with *Schistosoma mansoni* is characterized by three immunological phases: an acute T_H1 driven phase, followed by an egg-induced T_H2 phase and eventually a long-term immunosuppression (Reg phase). After antigen-presentation of dendritic cells, naïve T cells differentiate into T_H1 and T_H17 cells and start to produce proinflammatory cytokines, such as IFN-γ and IL-17. After female worms start to produce eggs, the initial T_H1 phase of infection is replaced by an egg-induced T_H2 phase. The T_H2 type inflammation is characterized by elevated cytokine levels of IL-4, IL-5, IL-9, IL-13, IL-21 and high amounts of eosinophils, basophils and mast cells. Moreover, IL-4 receptor signaling induces B cells to switch their antibody class to IgE. Since unrestricted T_H2 responses can potentially harm the host, helminths are able to initiate a regulatory immunosuppressive phase. The chronic phase of infection is mainly dominated by macrophages, regulatory B and T cells producing IL-10 and TGF-β. The figure is adapted from (Daniłowicz-Luebert et al., 2011)

1.2 *Schistosoma mansoni* and allergies

1.2.1 Global burden of asthma and the hygiene hypothesis

The prevalence of immune-mediated diseases including autoimmune (multiple sclerosis, Crohn's disease, Type 1 diabetes) (Bach 2002) and allergic diseases (asthma, atopic eczema, rhinoconjunctivitis) (Aït-Khaled et al., 2009; Williams et al., 1999) continuously rises in industrialized countries and urban areas of developing countries (Rujeni et al., 2012), which stands in strong contrast to developing countries. In terms of allergies it is most interesting that humans living in schistosome-endemic areas have a lower risk to develop allergic diseases as people living in industrialized countries (Asher et al., 2006). Since immune disorders play a crucial role in global mortality and morbidity (Thomas et al., 2010) and negatively influence economic growth due to their immense costs of treatment (Gupta et al., 2004), it is particularly concerning that 300 million people worldwide suffer from asthma (Pawankar, 2014). Worldwide, 40-50% of school aged children are sensitized to one or more common allergies (Pawankar et al., 2013). Increasing rates of allergies have long been mysterious. In 1989, Strachan et al. have published that the rate of hay fever and eczema were negatively associated with small family sizes and reduced number of older children in the household. Furthermore allergies were overrepresented among first-born, and less frequent in children attending day care (Krämer et al., 1999), suggesting that a frequent exchange of infections may have a protective effect (Strachan et al., 1989). These data have led to the so-called 'hygiene hypothesis', in which the balance between T_H1 immune responses (viral, bacterial infections) and T_H2 immune responses (associated with helminth infections and allergic diseases) is essential (Matricardi et al., 2000). The limited exposure to viral and bacterial infections during early childhood due to improved hygiene standards, vaccination, and use of antibiotics results in a higher risk of allergic diseases, since T_H1 cells are not sufficiently stimulated and can therefore not counterbalance the expansion of T_H2 cells (Shaheen et al., 1996; Yazdanbakhsh et al., 2002). Indeed, in a cross-sectional survey Riedler and his colleagues have shown that the early-life exposure to stables and farm milk significantly reduces the susceptibility to develop asthma, hay fever, and atopic sensitization (Riedler et al., 2001). Moreover, also breast feeding protects the offspring of developing allergic diseases (Lucas et al., 1990). Besides the high impact of viral and bacterial infections also parasite infections play a major role in suppressing bystander immune responses, such as allergens. Epidemiological studies have shown that the rates of allergic diseases are less frequent in developing

countries, where humans still suffer from chronic helminth infections (Hartgers et al., 2008). One reason might be that chronic helminth infections have immunosuppressive effects on the host's immune system and therefore counterbalance allergic T_H2 responses. Although helminths induce a strong T_H2 response, they have recently been accepted in the 'expanded hygiene hypothesis' and complete the classical T_H1 inducers (Cooper, 2009). In accordance, 107 children living in the schistosome-endemic area of Caracas in Venezuela got anthelmintic treatment with Oxantel-Pyrantel for 22 months, which effectively eliminated the intestinal helminth infection. In comparison to children, who declined treatment, the treated children showed increased immediate-hypersensitivity skin-test reactivity and elevated serum levels of specific IgE antibody against environmental allergens. These findings indicate that the protection against allergic diseases obtained during helminth infection is reversible by anthelmintic treatment. Experimentally shown in schistosome-infected C57/Bl6 mice is, that only the chronic infection, which provides an immunosuppressive environment within the host, but not the acute infection with *S. mansoni*, reduces the development of allergic airway inflammation, shown by decreased lung eosinophilia, reduced peribronchial inflammation and OVA-specific T_H2 cytokine levels in chronically infected mice (Smits et al., 2007). Smits and contributors have further revealed that in chronic *S. mansoni*–infected animals, both B cells and $CD4^+$ T cells are essential for the adoptive transfer of suppression of allergic airway inflammation in recipient mice and that this inhibition is mediated through IL-10. In conclusion, allergic disorders have become a worldwide health issue, the inverse relation of parasite infections and allergic diseases is therefore from particular interest to understand the immunological mechanism of allergy-preventing and -inducing effects.

1.2.2 **Allergic inflammation and schistosome life-cycle stages**

Interestingly, different schistosome life-cycle stages result in different mechanisms to regulate the allergic phenotype. Cercarial dermatitis or swimmer's itch occurs while cercariae penetrate the host's skin (Lambertucci, 2010). This inflammatory reaction is only occasionally reported in schistosome-endemic populations, indicating that immunosuppressive responses result from multiple infections (Cook et al., 2011). The underlying mechanism of such regulatory responses may be the induction of skin-stage schistosomula-derived molecules such as prostaglandin E_2 (PGE_2), which upregulates the IL-10 production during skin penetration (Ramaswamy et al., 2000). Lung-stage schistosomula also secret IL-10 during migration through the capillary vessels of the lungs, and this

might reduce inflammatory infiltrates around the parasites caused by eosinophils (Angeli et al., 2001). These anti-inflammatory mechanisms of the lung may be one reason for the reduced severity of asthma symptoms in schistosome-infected asthmatic patients (Medeiros et al., 2003). Since helminths are the most potent natural inducers of T_H2 responses, this T_H2 polarization corresponds with the onset of eggs, of which a chemokine binding protein with anti-inflammatory activity is secreted. In detail, the so-called *S. mansoni* egg-secreted chemokine binding protein (smCKBP) inhibits the specific binding of chemokines such as CXCL8 (IL-8) and CCL-3 (MIP-1α), and therby blocks the chemokine-elicited migration of neutrophils and macrophages during granuloma formation. The specific blocking of smCKBP *in vitro* results in increased granuloma size and inflammation, measured by increased neutrophils and macrophages, indicating that the inhibition of smCKBP results in inflammatory cell activation and migration (Smith et al., 2005). This chemokine binding protein may have potential as an anti-inflammatory agent in several diseases. Moreover, a variety of studies explored the role of regulatory T cells and the production of IL-10 by regulatory B and T cells. It is hypothesized that helminth-induced immunoregulatory cells inhibit the production of allergen-specific IgE and, subsequently, the symptoms induced by IgE-mediated receptor cross-linking on allergic effector cells, such as mast cells (Nagler-Anderson., 2006). In this context, three *S. mansoni* antigens Sm22.6 (soluble protein associated with the *S. mansoni* tegument), PIII (a multivalent antigen of *S. mansoni* adult worms) and Sm29 (*Schistosoma mansoni* tegument protein 29) have been described to suppress airway inflammation by reduced eosinophils and decreased OVA-specific IgE levels. Interestingly, Sm22.6 particularly induces higher levels of IL-10, while both Sm22.6 and PIII result in a higher proliferation of $CD4^+Foxp3^+$ T cells (Cardoso et al., 2010). This concept of regulatory T cells was further supported by Layland et al. indicating that asthma protection needs the presence of viable eggs and the infection-driven Treg cells. In detail, schistosome-infected mice showed reduced leukocytes and eosinophils in bronchoalveolar lavage (BAL) and reduced inflammation score when sensitized and challenged during the patent phase of infection, suggesting the requirement of egg production of female worms. In contrast, no protection was observed in schistosome-infected mice when both sensitization and challenge was performed in the pre-patent phase of infection, six weeks after the treatment with praziquantel nor with the depletion of regulatory T cells (Layland et al., 2013). Besides immunoregulatory T cells, a certain subset of B cells, which are $CD1d^{high}$, produce also high amounts of IL-10 and play a major role in the prevention of allergies in helminths-infected individuals. After

the transfer of $CD1d^{high}$ B cells from spleens of helminth-infected mice into OVA-sensitized mice, the established airway inflammation in OVA-sensitized mice was reversed by $CD1d^{high}$ B cells via the secretion of IL-10 and the induction of $Foxp3^{+}$ Tregs TGF-β independently (Amu et al., 2010). Furthermore, Mangan and his colleagues have revealed that *S. mansoni* infected mice were protected from anaphylaxis via IL-10 producing B cells (Mangan et al., 2004). Another interesting hypothesis for suppressing allergic inflammation are the high levels of IgG4 in helminth-infected humans, since IgG4 is capable of blocking IgE-allergen interaction (Pinot de Moira et al., 2013). In detail, Pinot de Moira and her colleagues have revealed, that pre-school aged children in helminth-endemic areas showed IgG4-dependent IgE desensitization to constitutively expressed SmTAL-2 (*Schistosoma mansoni* tegumental allergen-like protein). Interestingly, this desensitization occurred earlier in children living in the village with higher transmission rates of schistosomiasis. Taken together, different parasite life-cycle stages starting from cercariae penetrating the host's skin, lung-stage schistosomula and different proteins of schistosoma antigens are linked to various molecules and cell subsets, which induce an anti-inflammatory environment, and, thus, induce the prevention of allergies in schistosome-infected individuals (Rujeni et al., 2012). Key players in the suppression of allergic diseases are the helminth-derived activation of Treg and Breg cells and the production of IL-10.

1.2.3 **Immunological principles in allergic airway inflammation**

While the human immune system must differentiate between dangerous pathogens and ubiquitous environmental allergens, it is expected to trigger defensive responses to pathogens while tolerating allergens (Rujeni et al., 2012). However, some people show immune responses against environmental allergens and develop allergic reactions such as atopic dermatitis, allergic rhinitis and asthma (Rujeni et al., 2012). Chronic allergic airway inflammation and linked asthmatic disease are often considered a result of aberrant activation of type 2 T helper cells towards innocuous environmental allergens. In the early stages, a type 1 hypersensitivity reaction against an allergen occurs, which results in a T_H2-mediated production of IgE antibodies. Secreted IgEs circulate in the blood and bind to the IgE specific receptor FcεR on mast cells, basophils and eosinophils, which are involved in the acute inflammatory response. If later these sensitized IgE-coated cells are exposed to the same allergenic molecule, the allergen can directly bind on IgE with the

release of histamine and other inflammatory chemical mediators into the surrounding tissue causing several systemic effects, such as vasodilatation, mucus secretion, and smooth muscle contraction (Janeway CA Jr, 2001). In detail, pulmonary epithelium, which serves as a barrier between the external environment and internal milieu, dictates the initial response of the lung to both infectious and non-infectious stimuli (Brune et al., 2015). Epithelial cells express pattern recognition receptors to recognize type-2-cell-mediated immune insults like proteolytic allergens or helminths, which induce their activation and the production of chemokines, innate cytokines, such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) (Allakhverdi et al., 2007; Fallon et al., 2006; Schmitz et al., 2005). These prototypical responses subsequently initiate naïve T cells to differentiate into T_H2 cells (Bredo et al., 2015), which exert their effects through production of several interleukins, such as IL-3, IL-4, IL-5, IL-9 and IL-13 (Broide, 2001). IL-4 is not only critical to the development of T_H2 cells, but also triggers together with IL-13 B cells to switch their antibody class to IgE, which is the major risk factor for the development of asthma (Del Prete et al., 1988). Additionally, IL-5 arranges the recruitment of eosinophils in the bone marrow and recruits along with NK cells and $CD8^+$ cells eosinophils into the lung (Clutterbuck et al., 1989; Lloyd et al., 2010), which are mainly responsible for the typical symptoms of asthma. Indeed, eosinophilic inflammation in the bronchial mucosa has been revealed as the most potential inducer of the pathology in bronchial asthma (Barnes, 2008). Allergens bound to the allergen-specific IgE antibody connect to the IgE receptor FcεR on mast cells, basophils and eosinophils, which results in their activation and the release of histamine, leukotrienes and prostaglandin D2 (Rujeni et al., 2012). Furthermore, the chemokines and innate cytokines of respiratory epithelial cells program dendritic cells to mount T_H2-cell-mediated immunity by the release of CCL17 and CCL22 and in so doing boost type II innate lymphoid cells (ILC2), basophil and mast cell function. This allergic inflammatory cascade, which is shown in Fig. 3, results in mucus overproduction, eosinophilia, bronchoconstriction, thickening of smooth-muscle, smooth-muscle contraction and airway remodeling (Dani et al., 2011). Although this immune process has been viewed in the context of the inappropriate T_H2 cell response to environmental allergens, findings from murine and human studies have revealed the participation of other T cell dependent pathways, such as regulatory T cells. Indeed, there is evidence that Tregs play a major role in preventing allergic airway inflammation. A human study has revealed that numbers and function of $CD4^+CD25^{hi}$ T cells are deficient and mRNA levels of FoxP3, the transcription factor of regulatory T cells, are decreased in the lungs

of asthmatic children in comparison to healthy controls (Hartl et al., 2007). In contrast, another study has demonstrated that patients with asthma have normal numbers of $CD4^+CD25^{hi}$ and $CD4^+CD25^{hi}$ $FoxP3^+$ Tregs in peripheral blood compared to healthy individuals, although the expression of the FoxP3 protein was attenuated (Provoost et al., 2009). In murine models the importance of Treg cells became clearer. The selective depletion of FoxP3 Tregs during the sensitization phase with ovalbumin (OVA) in DEREG mice by diphtheria toxin injection led to a dramatic exacerbation of allergic airway inflammation, suggesting an essential role of Treg cells in regulating immune responses against allergens (Baru et al., 2010). The suppressive functions of Treg cells were considered to be mediated by multiple mechanisms that involve either the release of suppressive cytokines (IL-10 and TGF-β) (Nakamura et al., 2004; Presser et al., 2008), the downmodulation of antigen-presenting cells through expression of inhibitory molecules such as CTLA-4 (CD 152) and LAG-3 (CD 223) (Liang et al., 2008) or the deprivation of trophic cytokines (IL-2 through CD25) (Thornton et al., 1998). In addition, accumulating evidence suggests that the severity of airway hyperresponsiveness (AHR) depends on IL-17 producing T cells (T_H17), which are increased in sputum, bronchoalveolar lavage and bronchial biopsy specimens obtained from asthmatic subjects and non-asthmatic control subjects (Harrington et al., 2005; Schmidt-Weber, 2008). Taken together, the immunological response during the sensitization and manifestation of allergic airway inflammation depends on multiple factors, of which the T_H2-driven IgE-production and subsequent activation of eosinophils, mast cells and basophils are key players. The regulation of immune responses against allergens includes the maintenance of a functional tolerance, mainly mediated by IL-10 producing regulatory T and B cells.

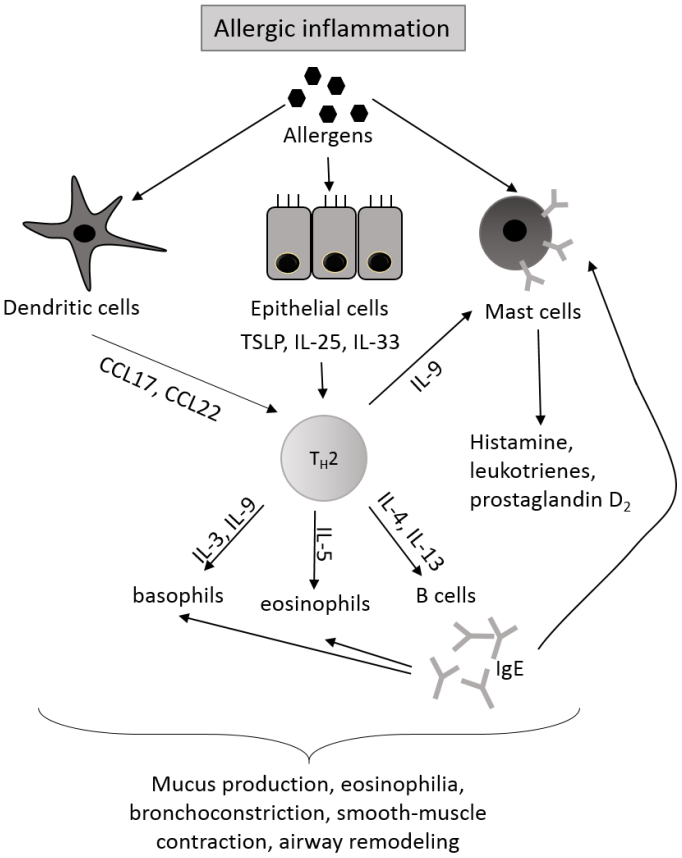

Figure 3: **Immune responses during allergic airway inflammation**

Barrier epithelial cells activated by allergens are stimulated to produce thymic stromal lymphopoietin (TSLP), IL-25 and IL-33, and, consequently, induce the differentiation of T_H2 cells, which produce elevated cytokine levels of IL-3, IL-4, IL-5, IL-9 and IL-13 to stimulate eosinophils and basophils. IL-4 together with IL-13 stimulate B cells to switch their antibody class to IgE. Allergens bound to the allergen-specific IgE antibody connect to the IgE receptor FcεR on mast cells, basophils and eosinophils, which result in their activation and the release of histamine, leukotrienes and prostaglandin D2. Furthermore, the chemokines and innate cytokines of respiratory epithelial cells program dendritic cells to mount T_H2-cell-mediated immunity by the release of CCL17 and CCL22 and in so doing boost basophil and mast cell function. This allergic airway cascade leads to mucus overproduction, eosinophilia, bronchoconstriction, smooth-muscle contraction and airway remodeling. The figure is adapted from (Daniłowicz-Luebert et al., 2011).

1.3 T cells

1.3.1 T cell subsets and differentiation

The disturbed balance between T_H1 towards an aberrant T_H2 type inflammation activated by ubiquitous allergen molecules plays a crucial role in the development of allergic airway inflammation. T cell activation is mainly mediated by an essentially unique T cell receptor (TCR), which is generated by recombination of DNA sequences during T cell

development in the thymus and is responsible for the specificity of each T cell and antigen presenting cell (APC) (Germain, 2002). The maturation of dendritic cells (DCs) depends on Toll-like receptor ligation and is characterized by the upregulation of MHC class II and co-stimulatory molecules resulting in increased antigen presentation and cytokine secretion (Kaisho et al., 2002). After recombination of a functional TCR composed of two chains (α and β), the naïve T cell is resting and migrating through secondary lymphoid tissues (lymph nodes and spleen) without being able to produce a proper immune response to protect the host from challenging infections. The activation of naïve T cells depends on the presentation of processed antigenic material non-covalently bound to a major histocompability complex (MHC) class I or II molecule by highly specialized antigen-presenting cells (APC), which determines the differentiation of naïve T cells into T-helper (T_H)1, T_H2, T_H9, T_H17, and T_H 22 effector T cell subsets via the release of cytokines and other co-factors (Akdis et al., 2009). On the T cell, the TCR links with a complex of proteins known as CD3, of which the cytosolic region is accountable for initiating an intracellular pathway after TCR ligation. To stabilize the interaction between the T cell and APC, each TCR also associates with either a CD4 or CD8 co-receptor, which binds to MHC class I for CD8 and MHC class II for CD4 cells. Besides the TCR, other costimulatory molecules are hired to support the TCR-MHC interaction, forming a large multimolecular structure known as the supramolecular activation complex (SMAC) (Monks et al., 1998) resulting in the phosphorylation of CD3 components (Samelson et al., 1986). The TCR together with co-stimulatory molecules initiates the transcription for IL-2 an autocrine factor that stimulates T cells to proliferate. Interestingly, there is evidence that T cells need an additional stimulus to become fully activated in form of CD28 (Jenkins et al., 1990). Indeed, murine experiments have shown, that the blockade of CD28 (or its ligand CD80 and CD86 on APCs) leads to an anergic T cell response (Harding et al., 1992). While $CD8^+$ cells are responsible for the elimination of pathogen-infected cells by cytotoxic means, $CD4^+$ T helper cells support the immune response by producing cytokines to activate other cells and chemokines to recruit new immune cell subsets. The T_H1 cell development as a response to viruses or intracellular bacteria depends on cell-extrinsic and cell-intrinsic factors, including signal transducer and transcription activator 1 (STAT 1), the transcription factor t-bet, IL-12 and STAT 4, whereas the T_H2 development initiated in response to parasites is coupled to IL-4, STAT 6 and the transcription factor GATA-3. Notably, their own developmental program can be strengthened by cytokines produced by mature effector cells of each lineage through positive and negative

feedback loops. IFN-γ produced by mature T_H1 cells and IL-27, an IL-12 family member generated by innate immune cells, initiate the development of T_H1 cells through the up-regulation of t-bet via STAT 1. The antigen-activated, naïve $CD4^+$ T cells leads to the upregulation of the IL-12 receptor (IL-12R) on developing T_H1 cells and suppresses T_H2 development by blocking the T_H2-related transcription factor GATA-3 (Ouyang et al., 1998; Szabo et al., 1997). Similarly, IL-4 produced by mature T_H2 cells induces STAT 6 signaling and GATA-3 expression, which inhibits via upregulation of STAT 6 the expression of t-bet (via STAT 4) and blocks the expression of IL-12R (Zheng et al., 1997). Finally, GATA-3 is also able to induce its own autoactivation through a positive feedback loop, which represents a potent mechanism for rapidly stabilizing T_H2 development (Ouyang et al., 2000). As a result of these robust counter-regulatory pathways, T_H1 and T_H2 cell development differ rapidly after antigen priming to produce mature effector cells with stable, mutually exclusive of IFN-γ and IL-4, respectively. T_H2 cell predominantly mediate the production of IgE in allergic inflammation and are also involved in the immunity to parasites (Romagnani, 2006). Recently, some other T-cell subsets appeared, including T_H9, T_H17 and T_H22, which control local tissue inflammation through upregulation of proinflammatory cytokines and chemokines. In response to extracellular bacteria and fungi, innate immune cells produce high amounts of IL-6 and TGF-β (Infante-Duarte et al., 2000). Together with IL-21 and IL-23 they stimulate naïve T cells to become T_H17 cells under the differential control of transcription factor retinoid-related orphan receptor gamma T (RORγT) (Yang et al., 2008). T_H17 cells subsequently produce IL-17, which activates neutrophils to combat fungal and bacterial infections. Regarding allergies, IL-17 producing cells are critical for the severity of the symptoms and have a key role in the induction and maintenance of autoimmune diseases by triggering secretion of pro-inflammatory cytokines and chemokines (Bettelli et al., 2006; Harrington et al., 2005). To suppress overwhelming immune responses, a regulatory subset of T cells (Treg) is produced by the immune system under the transcriptional control of FoxP3 (Fontenot et al., 2003). Unlike other T cells, Tregs can directly be produced by thymic selection (so-called natural Tregs) as well as by differentiation (induced Tregs) under the influence of environmental factors, including TGF-β and retinoid acid (Chen et al., 2003). Tregs play a key role in impairing T cell proliferation and cytokine production of other T cell subsets with the production of suppressive cytokines, such as IL-10, TGF-β or IL-35. T_H9 cells represent a novel population of effector T-helper cells involved in tissue inflammation characterized by IL-9 and IL-10 secretion (Akdis et al., 2009) and have been associated with

asthma and allergy (McLane et al., 1998). Another novel T cell subset has been described in T_H22 cells, which IL-17 independently express IL-22 and play a role in atopic dermatitis (Nograles et al., 2009). Taken together, the regulation and differentiation of naïve T cells depend on several factors that involve the intensity and specificity of the T-cell receptor (Constant et al., 1995), the strength of costimulatory factors (Gause et al., 1997), and the cytokine milieu in which T cells are primed (O'Garra et al., 1996). The imbalance between the different T cell effector subsets is responsible for different immune pathologies, including atopy and asthma.

1.3.2 **Induction of central and effector memory T cells**

During a second encounter with the antigen, memory T cells are immediately able to trigger a stronger and faster immune response than the first time the immune system responded to the antigen. Memory T cells result from the clonal expansion and differentiation of antigen-specific lymphocytes and persist life-long. Recently, numerous populations of memory T cells were discovered including central memory (T_{CM}), effector memory (T_{EM}), tissue-resident memory (T_{RM}), stem memory (T_{SCM}) and virtual memory T cells (Jameson et al., 2015). The mutual topic of the wide-ranging subsets is their immediate and lifelong expansion to effector T cells upon re-exposure to their known antigen. T_{CM} are phenotypically characterized as expressing high levels of the IL-7 receptor (CD127), by the expression of adhesion markers like $CD45R0^+$ in human and $CD44^+$ in mice and L-selectin (CD62L), as well as the lymph node-homing receptor (CCR7). CD62L and CCR7 are two T cell receptors that are also characteristic for naïve T cells, which are required for cell extravasation through high endothelial venules (HEV) and migration to T cell areas of secondary lymphoid organs (Campbell et al., 1998; Forster et al., 1999). In comparison to naïve T cells, T_{CM} are more sensitive to antigenic stimulation, depend less on co-stimulation, and have a stronger upregulation of CD40L, which results in a more effective stimulatory feedback to dendritic cells (DCs) and B cells. Following TCR triggering, T_{CM} produce mainly IL-2, but after proliferation they efficiently proliferate to effector cells with the production of high amounts of IFN-γ and IL-4, respectively. T_{EM} are characterized by the expression of $CD45R0^+$ in human or $CD44^+$ in mice, respectively, but lack the expression of the lymph node-homing receptor CCR7 and generally express low levels of CD62L and CD127 (Koch et al., 2008). The relative proportions of T_{CM} and T_{EM} cells vary in the CD4 and CD8 compartments: T_{CM} cells are predominant in CD4 and enriched in secondary lymphoid organs due to the high expression of CD62L

and CCR7, whereas T_{EM} are largely contributed to CD8 and are trafficking around non-lymphoid tissue due to their lack of CCR7 and CD62L expression (Reinhardt et al., 2001). While T_{CM} cells function as a potential stimulator of proliferation after antigen reencounter, T_{EM} cells replay a rapid effector function, including the secretion of granzyme B and IFN-γ and have a limited proliferative potential. Relating to infections, the naturally acquired resistance to reinfection with various infectious pathogens including *Leishmania, Plasmodium, Mycobacterium,* and parasites, typically coincides with an ongoing primary infection, suggesting an antigen specific memory compartment, which immediately combat the infectious pathogen during a subsequent encounter (Peters et al., 2014). Regarding helminths infections, Nausch et al. have revealed that the infection with *Schistosoma haematobium* is associated with a non-specific modulation of the $CD4^+$ effector/ memory T cell compartment, but not the $CD8^+$ pool. However, anthelmintic treatment is associated with the decline of $CD4^+$ memory T cell proportions (Nausch et al., 2012). The priming of memory T cells is considered to depend on several mechanisms, including the immunization with the antigen, a distinct cytokine milieu, predominantly IL-2 (Williams et al., 2006), and TLR ligation, such as TLR 2 agonists (Chandran et al., 2009). Taken together the memory T cell pool operates as a dynamic repository of antigen-experienced T lymphocytes that accumulate over the lifetime of individuals and is essential for the immunity to re-infections (Pennock et al., 2013).

1.4 Infections during pregnancy and their impact on offspring's immune system

1.4.1 Maternal infections during pregnancy

As mentioned in the chapters before almost one-third of humans worldwide are infected with at least one helminth and therefore infections during pregnancy are frequent with prevalence rates ranging from 10% up to 64% among pregnant women living in endemic countries (Adegnika et al., 2010; Ajanga et al., 2006; Woodburn et al., 2009). It is often considered that a poor maternal health status leads to higher maternal and fetal mortality and low birth weight (Kramer, 2003). Especially in developing countries poor nutrition and anemia, which both can be caused by the infection with helminths, often occur and have severe implications for pregnancy outcomes and neonatal health (Blackwell, 2016). Infections during pregnancy with bacteria and viruses, which are able to conquer the pla-

centa-blood barrier and transferred transplacentally to the fetus, are often caused by teratogenic pathogens designated as the 'TORCH' complex, including *Toxoplasma gondii*, *Treponema pallidum*, *Listeria monocytogenes*, Rubella virus, Cytomegalovirus, and Herpes simplex virus, but also other pathogens, such as Parvovirus B19, and Varicella zoster virus. This group of pathogens can induce several embryo and fetal pathologies, such as intrauterine death (Horn et al., 2004), fetal hydrops (Fairley et al., 1995), cataract and several heart failures (Santis et al., 2006), and plays a major role in causing placental dysfunction and intrauterine growth restriction (Adams-Waldorf et al., 2013). Besides the transplacental transfer of pathogens *in utero*, some pathogens can also be transferred perinatally (via vaginal secretions or blood during birth), e.g. group B streptococcus (GBS) or human immunodeficiency virus (HIV) (Money et al., 2016; Rogers et al., 2010), or postnatally (from breast milk or other origins), of which Cytomegalovirus (CMV) is a major risk factor for pre-term delivered newborns (Chiavarini et al., 2011). Besides transplacental infections caused by vertical transmission of pathogens via the placenta-blood barrier, most infections affecting the mother do not cause systemic infections of the fetus, suggesting that the placenta has an essential role as immune-regulatory interface protecting the fetus from congenital infections (Mor et al., 2005). However, the so-called non-transplacental infections, which include parasite infections of the mother, cannot cross the placental-blood barrier, but still affect the fetus by inducing abortion, reduced litter size and fetal weight loss (Kurtis et al., 2011). In developing countries pregnant women are at higher risk to develop single or multiple parasite infections. Indeed, 22% of pregnant women living in Lambaréné, Gabon, developed two or more parasite infections during pregnancy (Adegnika et al., 2010). A cross-sectional study showed that co-infections with helminth-*Plasmodium falciparum* (versus helminth only) significantly increased the risk of anemia, low birth weight and small-for-gestational-age infants (Yatich et al., 2010). In accordance, the infection with *Plasmodium falciparum* during pregnancy can contribute to fetal rejection, severe maternal malaria attacks and death in low transmission areas, whereas intrauterine growth retardation, preterm delivery, low birth weight and maternal anemia are of major concern in high transmission areas (Steketee et al., 2001). In addition, Gallagher et al. have demonstrated the increased risk for mother-to-child-transmission (MTCT) of HIV in women co-infected with one or more helminths versus women without helminths, possibly by mechanism in which parasite antigens activates lymphocytes *in utero* (Gallagher et al., 2005). This has led to the recommendation of

deworming treatment during pregnancy to reduce the risk of mother-to-child-transmission of HIV. HIV-transmission from mother to child and placental malaria are two important examples, which are influenced by maternal helminth infection. Both of them predominate in sub-Saharan Africa. However, the effects of maternal parasite infections on the offspring's immune system varies considerably across different species, such as geohelminths, filarial worms and schistosomes. Hookworm infections during pregnancy significantly lower the levels of hemoglobin, resulting in an increased risk of low birth weight for the new-born (reviewed in Brooker et al., 2008). For this reason, Christian and his colleagues recommended a deworming treatment with albendazole together with iron supplementary during pregnancy (Christian et al., 2004). Regarding the geohelminth *Ascaris lumbricoides,* there was recent evidence, that the maternal infection with *Ascaris lumbricoides* rises the frequencies of IFN-γ and IL-4 expressing $CD4^+$ T cells, measured in stimulated cord blood of newborns (Guadalupe et al., 2009). Beside geohelminths, studies on maternal filarial infections revealed a major impact on the offspring's developing immune system. The sensitization with filariasis occurs already *in utero* by materno-fetal transmission and children born to mothers with filarial infections are at higher risk to subsequent filarial infections (Das et al., 1997; Elson et al., 1996). The maternal transfer of parasites or their antigens released within the placenta could lead to hyporesponsiveness to filarial antigens in the offspring (Eberhard et al., 1993; Haque et al., 1982; Steel et al., 1994). An observational study among 99 pregnant women in the Philippines revealed the severe impact of the maternal infection with *Schistosoma japonicum* on the development of the fetus. Namely, the maternal infection led to increased inflammation in maternal, placental and fetal compartments (detected by increased pro-inflammatory cytokines in peripheral blood, placental-tissue samples and cord blood) and was further associated with acute subchorionitis and decreased birth weight (250 g less compared to new-born with healthy mothers) (Kurtis et al., 2011). Additionally, Siegrist and contributors demonstrated that the maternal infection with *Schistosoma haematobium* significantly increased the risk of preterm deliveries and reduced birth weight detected in 200 pregnant women living in Bawku District, Ghana (Siegrist et al., 1992). In conclusion, infections during pregnancy, which can be transmitted transplacentally by crossing the placental-blood barrier, perinatally during birth or postnatally via breast milk, can cause severe pathologies for the fetus. Due to the endemicity of parasite infections and their major impact on fetal development and health, parasite infections during pregnancy

need to be taken seriously and prevented with appropriate measures or treated accordingly. Although a wide range of studies examined the effects of maternal parasite infections, the interaction of helminth antigens with the maternal immune system ranges and modulates the fetal immune system in different ways.

1.4.2 **Immunity of offspring to infections with *S. mansoni* and allergies**

Besides the effects of maternal *Schistosoma japonicum* and *haematobium* infection, the infection with *Schistosoma mansoni* during pregnancy can alter the fetal immunity, which influences the offspring's immune responsiveness to schistosome infection and schistosomiasis-associated morbidity. So far, several epidemiological studies revealed that the majority of people exposed to schistosomiasis stays asymptomatic in endemic areas, while humans living in non-endemic areas develop more severe symptoms (Nash et al., 1982), pointing towards the possible explanation that the perinatal exposure to schistosomiasis during pregnancy modulates the offspring's immune system lifelong (da Paz et al., 2017). *In-utero* sensitization to schistosome antigens occurs in up to 50% of offspring born to women with schistosomiasis (King et al., 1998). Indeed, the fetal cytokine profile measured in cord blood cells showed similar patterns of cytokine production in comparison to maternal peripheral blood mononuclear cells (PBMCs) (Malhotra et al., 1997). It is further known that the maternal infection with *S. mansoni* induces a potential immunosuppressive response to antigens in the offspring, associated with higher levels of IL-10 in human cord blood samples in response to schistosome antigens (Tweyongyere et al., 2011). Interestingly, murine experiments have shown, that this immunosuppressive phenotype is only obtained in offspring born to schistosome-infected mice and were suspended in offspring born to naïve mothers and raised by schistosome-infected mothers (Santos et al., 2010). Besides the induction of an immunosuppressive environment, experimental murine studies revealed that schistosomiasis during pregnancy and the subsequent postnatal schistosome infection of the offspring result in protection by dampening the T_H2 response with IL-12 and TGF-β cytokines (Othman et al., 2010). Further murine studies have demonstrated, that the postnatal infection with schistosomiasis of offspring with schistosome-infected mothers showed reduced sizes and numbers of liver granuloma and worm burden (Attallah et al., 2006). These findings were associated with the transmission of circulating parasite antigens via the FcγR at the placental-blood barrier or directly via mother milk or the transfer of maternal parasite specific antibodies to the fetus (Attallah et al., 2003; Charnaud et al., 2016; Gill et al., 1983). In this regard, oral delivery

of a 63-kD *S. mansoni* antigen as well as specific IgG1 antibodies to this antigen from *S. mansoni* infected mothers to newborns via breast milk have been reported (Attallah et al., 2003). Besides oral delivery, the direct deposition of parasite eggs in the placenta has been investigated in up to 22% of 322 placentas in women living in the schistosome endemic area (Ivory Coast) using a digestion/sedimentation approach (Renaud et al., 1972). However, it is still unclear, whether the transmission of parasite specific antigens or the direct deposition of parasite eggs in the placenta can induce memory T cells within the fetus or the new-born and, hereby, alleviate symptoms of a subsequent infection with helminths. Even the nutrition of the mother during breastfeeding has a major impact of the offspring's immune system to the infection with *S. mansoni*, since malnutrition with a lack of proteins lead to increased egg elimination and extended liver granulomas when infected with schistosomiasis (Corrêa et al., 2011). These data revealed that a low protein diet during lactation decreases the protective nature of immunization via breastfeeding. Taken together, the exposure to *S. mansoni* infection during pregnancy and breastfeeding modulates the immunity against the homologous antigen in postnatal infections via the direct antigen contact or immunity factors acquired from the mother (Attallah et al., 2003; Lenzi et al., 1987). However, the influence on the immune response to heterologous antigens, such as allergens, in offspring is still poorly defined. So far, there is evidence that the anthelmintic treatment with praziquantel of pregnant women increases the risk of infantile eczema (Mpairwe et al., 2011), indicating a declined susceptibility to allergies by maternal helminth infection. As recently published by our group and shown in Fig. 4, female BALB/c mice mated during T_H1 phase and regulatory phase (Reg phase) of infection showed protection against OVA-induced allergic airway inflammation, while offspring of mothers mated during T_H2 phase of infection showed aggravated symptoms of allergic airway inflammation. Offspring of mothers mated during T_H1 and regulatory phase of infection showed reduced levels of OVA-specific IgE antibodies (Straubinger et al., 2014). Taken together, it has often been considered that prenatal priming of the fetal immune system can be caused by helminth derived antigens and such primary immune sensitization may have an impact on the maturation of postnatal immune responses, such as immunity to schistosoma infection in postnatal life. Eventually, this undeniable impact was extended to unrelated antigens, such as allergens. The allergenic potential of an individual is not only associated with the exposure to environmental factors in postnatal life,

but also relates to the immune status of the mother during pregnancy. The *in utero* exposure to schistosoma-derived antigens is one of the protective factors in offspring for the reduced susceptibility to allergies (Gebreegziabiher et al., 2014).

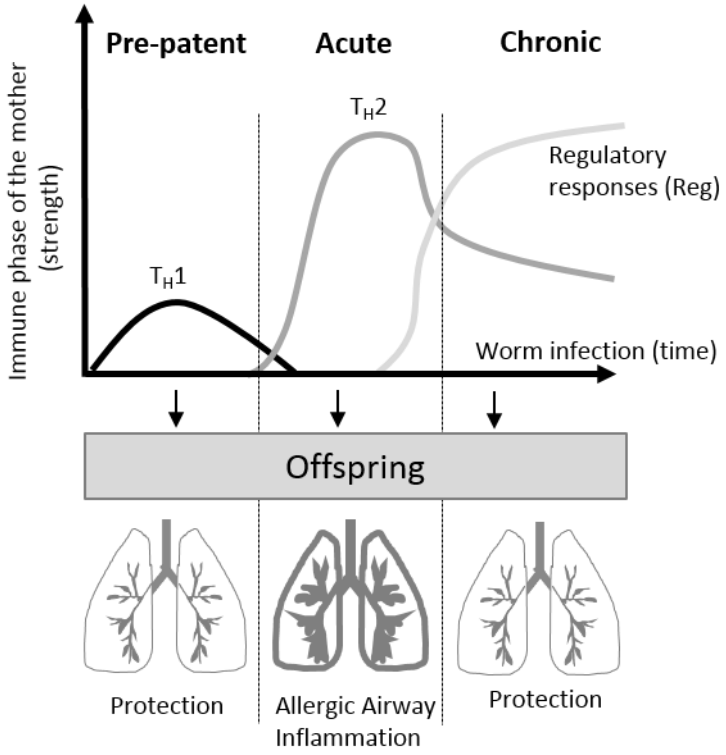

Figure 4: **Immune phases during maternal schistosomiasis influences the offspring's susceptibility to allergies**

The initial T_H1 phase of infection is characterized by elevated levels of IFN-γ, followed by an egg-induced T_H2 phase with high amounts of IL-4, IL-5 and IL-10. After 16 weeks of infection a regulatory, immunosuppressive phase (Reg phase) of infection is reached and the cytokine IL-10 is predominant. Female BALB/c mice were mated during each phase of infection with naïve BALB/c males. Offspring of female BALB/c mice mated during T_H1 phase and regulatory phase (Reg phase) of infection showed protection against OVA-induced allergic airway inflammation, while offspring of mothers mated during T_H2 phase of infection showed aggravated symptoms of allergic airway inflammation. The illustration is adopted from (Straubinger et al., 2014)

1.4.3 **Role of epigenetic modifications**

Besides the materno-fetal transfer of antigens and antibodies against *S. mansoni* via the placenta-blood barrier or directly via breastfeeding and the induction of specific immunosuppressive cytokine responses in the fetus, transgenerational epigenetic inheritance might be crucial in the distinct immunological phenotype acquired by the offspring of *S.*

mansoni infected mothers during pregnancy. Within the last years epigenetics has become of major importance, since they can alter the activity state and function of genes due to modifications without changing the primary structure of the DNA. By covalent epigenetic modifications, such as DNA methylations, histone acetylations and non-coding RNAs, the accessibility of DNA and chromatin is varied, and, thus, the transcription rate and possible pathways of differentiation (Avni et al., 2002; Fields et al., 2002). In detail, histones are part of nucleosomes that undergo different post-translational modifications on their N-terminal tails, including acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation (reviewed in Rando et al., 2009). The acetylation status of histones is regulated by two groups of enzymes: histone acetyl transferases (HAT) and histone deacetylases (HDACs). HATs catalyze the transfer of acetyl groups, which lead to a weakened interaction between histones and DNA, less compact chromatin and thus more accessible to the transcriptional machinery (Alaskhar Alhamwe et al., 2018). The removal of acetyl groups results in repressed chromatin and decreased transcription. DNA methylation and histone modifications are known to play a major role in the regulation of gene expression and the differentiation of T cell subsets, including T_H1 and T_H2 cells (Wilson et al., 2009). Transgenerational inheritance of epigenetic modifications is extremely interesting from an epidemiologic point of view as it provides a new hypothesis for the persistently increasing prevalence of allergy and related disorders (Bégin et al., 2014). It is often considered that the predisposition towards an allergic phenotype is tightly regulated by DNA methylation and histone acetylation at the T_H2 locus control region (Bégin et al., 2014). A variety of alterations in epigenetics have been detected in T cells from asthmatic patients and the protection against asthma is thought to be mediated epigenetically by maternal exposure to environmental microbes. Indeed, the daily exposure of pregnant mice to *Acinetobacter lwoffii* led to increased histone acetylation of IFN-γ promoter regions in $CD4^+$ cells in exposed offspring, resulting in more active chromatin and pronounced transcription of IFN-γ cytokines (Brand et al., 2011). The consequent T_H1 bias within the exposed offspring might counterbalance T_H2 responses later in life and reduces the risk of developing allergic diseases (Brand et al., 2011). Besides histone acetylation, also DNA methylation on IFN-γ promoter regions correlated with an altered asthma phenotype. According to these findings, an experimental study in mice revealed that neonates with asthmatic mothers have significantly increased DNA methylation in neonatal dendritic cells (Su et al., 2009). The DNA methylation occurs at CpG nucleotides and is initiated by DNA methyltransferases (DNMTs). In the following study, increased

DNA methylation on IFN-γ promoters, which leads to repressed chromatin and less transcription, was reversed with DNA methyltransferase (DNMT) inhibitors, leading to increased transcription of IFN-γ, and thereby providing beneficial influence on the allergic phenotype (Brand et al., 2012). The underlying mechanism by which allergic phenotypes and environmental exposures could be associated with epigenetic modifications is still poorly investigated. So far, a genome-wide study of posttranslational modifications of histones, including the epigenetic regulator trithorax, revealed disease-associated enhancers in $CD4^+$ T cells in asthmatic patients (Watanabe et al., 2014). Suv39h1 (suppressor of variegation 3-9 homolog 1), a genetic repressor that functions via methylation of the histone H3 lysine 9 (H3K9), has been associated with the development of asthma-like symptoms, while Suv39h1 gene-*deficient* mice showed reduced allergic pathologies (Allan et al., 2012). The methyltransferase, Suv39h1, enhances the T_H2 stability by promoting transcriptional silencing of T_H1 gene loci. In detail, Suv39h1 mediates the trimethylation of histone H3K9 which is recognized by the heterochromatin protein 1α (HP1α) leading to histone deacetylation on T_H1 gene loci and, thus, silencing it. The Suv39h1-mediated trimethylation of histone H3K9 is one example for the influence of epigenetic modifications in the differentiation of naïve T cells towards T_H1 and T_H2 cells. However, naïve T cells from Suv39h1 gene-*deficient* mice when re-cultured under T_H1 conditions were driven towards differentiation into T_H1 cells. This inability to repress T_H1 gene expression is caused by the incapacity of Suv39h1 gene-*deficient* mice to silence T_H1-related transcription factor t-bet. In accordance, deletion of histone deacetylase 1 (HDAC) in T cells results in higher levels of histone acetylation and increased transcription, leading to enhanced airway inflammation and increased T_H2 cytokine production (Grausenburger et al., 2010). In conclusion, it is becoming clear that the influence of epigenetic programming *in utero* and subsequent epigenetic regulation are critical pathways, through which environmental changes could alter gene expression, and, thus, T cell differentiation and cytokine responses. Since the maternal exposure to environmental pollutants and bacteria (Conrad et al., 2009; Prescott et al., 2009; Sugiyama et al., 2007) can affect gene expression in the offspring by epigenetic modifications, parasite infections during pregnancy might also induce epigenetic reprogramming in the offspring. Epigenetics are thought to be the link between environment and gene expression, and, thus, from particular interest in parasite infections during pregnancy and represents a novel model for transgenerational inheritance.

1.5 Early development of immune cells and critical window of vulnerability

The impact of environmental factors, such as maternal infections, malnutrition, toxins and stress, on the fetal development depends on the gestational age. The fetal immune system is especially vulnerable to environmental harmful circumstances when tissue is seeded by precursors of immune cells, which is defined as sensitive window of vulnerability (Marques et al., 2013). Different developmental stages of the fetal immune system are differentially vulnerable to immunotoxic agents. Dietert and his colleagues showed five critical time points of potential vulnerability of the rodent immune system (Dietert et al., 2000). The first critical window (gestational day 7-9) is the start of the hematopoiesis from undifferentiated mesenchymal cells (Fehling et al., 2003), followed by the migration of stem cells and expansion of lineage-specific progenitor cells for leukocytes and organogenesis of secondary lymphoid organs (gestational day 9-16) (Cumano et al., 2001). From day 13 of gestational age until birth the third critical window occurs by colonization of bone marrow and thymus (Dietert et al., 2000). After birth, two critical phases arise, the maturation of immunocompetence and the onset of normal mature immune responses (K. S. Landreth, 2002). In general, the earlier the infection during pregnancy occurs, the more severe are the consequences for the unborn child. The initial understanding of critical prenatal developmental stages of the immune system is essential to understand the potential of environmental harmful agents. Rodents are the best animal model for investigations regarding the developmental stages of immune system, since the ontogeny in human and mice are similar (Yoder, 2002). In the following chapter, the differences between human and murine immune cell development are described and highlighted. During the 9^{th} day of murine pregnancy hematopoietic stem cells (HSC) appear within the yolk sac and the intraembryonic splanchnopleuric mesenchyme surrounding the heart, a tissue also defined as aorto-gonado-mesonephros (AGM) (Weissman, 2000). At approximately day 10 of gestational age, the HSC migrate from the AGM to the developing fetal liver organ (Cumano et al., 2001) and generate more differentiated and lineage-restricted stem cells. This step is followed by differentiation of more mature progenitor cells that are able to form colonies in response to specific cytokines stimulation (Mebius et al., 2001). At day 14 in murine and week 8-9 in human pregnancy B lymphocyte precursors, pre-B cells, are detectable in the fetal liver, whereas B lymphocytes expressing IgM can be found at day 16-17 in mice and week 10-12 in human development of the immune

system (Landreth, 2002). The immunoglobulin gene rearrangement leads to the expression of a mature B cell receptor (BCR) that can bind an antigen. These immature B cells express a complete IgM molecule on their surface ($B220^{low}IgM^{+}$) (Eibel, 2015). The fetal liver continues to be the primary haemato-lymphoetic organ until near the end of gestation. The expansion of lineage-restricted progenitor cells for all types of leukocytes are first detectable on day 11 in murine and week 6 in human pregnancy (Landreth, 2002; Owen et al., 1977). At day 13 of gestation HSC are found in the developing spleen (Landreth, 1993). Even though the expansion of hematolymphoid cells in the splenic organ never reaches the amount found in the fetal liver, the spleen retains this low production also in postnatal life. On day 11 of gestation the organogenesis of the thymus is initiated, and shortly after colonized by immigrant HSCs from the liver (Rodewald et al., 1998; Shortman et al., 1998). The hematopoiesis in the thymic organ is basically restricted to the production of lymphoid thymocytes, since the thymic hematopoietic cells express surface markers including Thy-1, $CD3^{+}$, $CD4^{+}$, $CD8^{+}$, and TLR (Rodewald et al., 1998). Interestingly, it was found that immature proliferating thymocytes generate colonies *in vitro* when exposed to the proliferative cytokine IL-7 and IL-7R signaling, which probably have major influence during *in utero* development (Moore et al., 2017). At day 17 of fetal immune system development, after the toll-like-receptor (TLR) selection, cell surface TLR-αβ-heterodimers are expressed on thymocytes. These immature thymocytes start off with being CD4 and CD8 double negative at day 13, followed by a $CD4^{+}$ and $CD8^{+}$ double positive phase at day 15.5, and finally become single positive $CD4^{+}$ and $CD8^{+}$ thymocytes, which are released to the peripheral blood as T helper cell and cytotoxic T cell precursors approximately at day 18 of gestation (Pardoll et al., 1987). On day 17.5 the cavity of the bone marrow is formed and immediately colonized by HSC from AGM (not yolk sac HSC), where they proliferate. The bone marrow replaces the hematopoiesis in the fetal liver at day 18 in mice and continues as the primary organ of the haemato-lymphopoiesis in postnatal life (Rolink et al., 1993). However, regarding maternal helminth infections, their impact on the fetal development of the immune system is still poorly defined. So far, Labuda and his colleagues revealed that the infection with *S. haematobium* leads to significant alterations within the B cell compartment observed by the increased percentage of memory B cells (MBC) and reduced numbers of naïve B cells (Labuda et al., 2013). In conclusion, during the development of the fetal and new-born

immune system five critical phases of vulnerability occur, of which the different developmental stages of immune cells are interesting regarding the constant *in utero* exposure to helminth derived antigens.

1.6 Aim of the study

The prevalence of allergic diseases, such as asthma, in populations living in helminth-endemic areas remains uncommon (Hartgers et al., 2008), while the rate of allergies still rises in developed countries. Protective factors associated with allergies are, besides poor sanitation and overcrowding, the exposure to microbial and other species, such as parasites, during pre- and postnatal life. The helminth *Schistosoma mansoni* causes the disease schistosomiasis or also called bilharzia and drives the host's immune response from an initial T_H1 response to an egg-induced T_H2 phase to finally result in an immunosuppressive phase, which protects the host and parasite from overwhelming immune responses and is furthermore known to dampen allergic reactions in the infected host. One reason for the observed reduction of allergies might be that chronic helminth infections induce an immunosuppressive environment within the host's immune system and therefore counterbalance allergic T_H2 responses. Interestingly, the allergen-preventing effect does not only occur within the schistosome-infected host, but also influences the offspring's susceptibility to allergies. Previously described by our group was the protection against T-cell driven asthma in offspring born to mothers during the T_H1 and immunosuppressive phase of infection. Since the protective effect was strongest during the regulatory phase of infection and most humans are chronically infected with schistosomiasis, we focused particularly on the regulatory phase of schistosoma infection. In this regard, the immunological mechanisms that mediate protection against the onset of asthma in offspring of schistosome-infected mothers are still unclear. Therefore, the intention of this study was to reveal distinct effects of the maternal parasite infection during pregnancy and breastfeeding on the development of the offspring's immune system, contributing to the allergy-preventing effect in the progeny. In this context, one major goal was to examine the *in vitro* T cell differentiation into T_H1 and T_H2 cells in offspring of chronic schistosome-infected mothers in a murine mouse model, since AAI is mainly T cell driven and depends on the activation of T_H2 cells. Secondly, to solve the potential underlying mechanism for differences in T cell differentiation, epigenetic modifications were assessed by the analysis of histone acetylation patterns of naïve $CD4^+CD62L^+$ T cells. Since altered responses

to allergens in the offspring might be explained by changes within the early immune system, one further aim was to define the immune cell composition in peripheral blood and splenocytes in offspring of infected mothers in comparison to naïve offspring. With the objective to investigate the effects of maternal infection on the offspring's lymphopoiesis, the early developmental stages of B and T cells in thymus and bone marrow were examined. Finally, since there is increasing evidence that prenatal T-cell priming of the fetal immune system can occur via trans-placental exposure to helminth derived antigens (Pit et al., 2000), this study intended to answer the question, whether the primary immune sensitization to maternally-derived parasite antigens results in the formation of memory T cells in the progeny.

2 Materials and Methods

2.1 Materials

2.1.1 **Equipment**

Table 1: Equipment

Name	**Company**
Automatic pipettes (2-1000µl)	Gilson®
Balance (440-33N)	Kern
Balance (XB120A)	Precisa
BD FACSCalibur™ flow cytometer	BD
Biological safety cabinet (Hera safe)	Thermo Scientific®
Power-up Bioruptor	Diagenode
Centrifuge (Biofuge fresco)	Heraeus
Centrifuge (Eppendorf 5424)	Eppendorf
Centrifuge (Megafuge 3.0R)	Thermo Scientific®
Centrifuge (Shandon Cytospin 3)	Thermo Scientific®
Cooling plate (COP 30)	Medite
CyAn ADP Lx P6	DakoCytomation
Disperser (T10 basic Ultra-Turrax®)	IKA
ELISA microplate reader (Sunrise™)	Tecan
Fridge	Bosch
Glassware	Schott
Homogenizer (5ml)	B Braun
Incubator (BBD 6220)	Heraeus
LumiNunc™ 96-well plates (Daxinger et al., 2012)	Nunc
Microscope (Axioscop)	Zeiss
Microscope (Axiovert)	Zeiss
MoFlo™ XDP	Beckman Coulter

Multichanel pipettes (Acura® 855; 5-350µl)	Socorex
Multipette® plus	Eppendorf
Orbital shaker (3005)	GFL
pH-meter (MultiCal®)	WTW
Rotating mixer (RM 5)	Assistant
Stericup® filter units (500ml)	Millipore™
Thermocycler (T3000)	Biometra
Thermomagnetic stirrer (IKAMAG® REO)	IKA
Vortex mixer (Reax top)	Heidolph
Water bath	Memmert

2.1.2 **Reagents**

Table 2: Reagents

Name	**Company**
Acetic acid	Roth®
Acetone	Thermo Fisher Scientific
Ammonium chloride solution	Stem cell Technologies
Aniline blue	Morphisto
Bovine serum albumin (BSA)	PAA
1-Bromo-3-Chloro-Propan	Sigma-Aldrich
Bromophenol blue	Roth®, Sigma®
Chloroform/Isoamylalcohol (24:1)	Fluka
Collagenase (from Clostridium histolyticum)	Sigma®
Deoxynucleoside triphosphate (dNTPs)	Sigma®
Deoxyribonuclease I from bovine pancreas (DNAse)	Sigma®
Dimethyl sulfoxide (DMSO)	Sigma®

Di-sodium carbonate (Na_2CO_3)	Sigma®
Dithiothreitol (DTT)	Roth®
Dulbecco's PBS (Endotoxin-free)	PAA
Ethidium monoazide bromide (EMA)	Invitrogen
Ethanol 70%-99.8% (v/v)	MRI Pharmacy
Ethidium bromide	Roth®
Ethylenediaminetetraacetic acid (EDTA)	Roth®
Formaldehyde solution 37% (v/v)	Sigma®, Merck
Gentamicin (10mg/ml)	PAA
Acetylated histone 4 antibodies (H4ac)	Milipore
Acetylated histone 3 antibodies (H3ac)	Milipore
Hank's balanced salt solution (HF2+)	Thermo Fisher Scientific
Histone deactylases (HDAC)	Enzo Lifescience
Ionomycin	Sigma®
Isopropanol	MRI Pharmacy
LightCycler® 480 Probes Master	Roche
Lithium chloride (LiCl)	Jena Bioscience
mProtein A sepharose beads	GE Healthcare, US
nProtein G sepharose beads	GE Healthcare, US
Octylphenoxy poly(ethyleneoxy)ethanol (IGEPAL® CA-630)	Sigma-Aldrich
OVA (Albumin, from chicken egg white) grade V and VI	Sigma®
Paraformaldehyde (PFA)	Sigma®
Percoll™	GE Healthcare
Periodic acid	Morphisto
1,4-Piperazinediethanesulfonic acid (PIPES)	Sigma-Aldrich
Phenol	Roth®

Phorbol 12-myristate 13-acetate	Sigma Aldrich
Phosphate buffered saline (PBS)	MRI
Potassium chloride (KCl)	Sigma Aldrich
Propidium iodide	R&D
Protease inhibitor cocktail tablets (complete)	Roche
Proteinase K (20mg/ ml)	Sigma-Aldrich
RNAlater®	Ambion®
RNase A (10mg/ ml)	Sigma-Aldrich
Schiff reagent	Morphisto
Sodium acetate	VWR
Sodium chloride (NaCl)	Roth®, Merck
Sodium dodecyl sulfate (SDS)	Roth®, Merck
Sodium deoxycholate (DOC)	Sigma-Aldrich
Sodium hydrogen carbonate ($NaHCO_3$)	Merck
Sucrose	Roth®
Sulphuric acid (H_2SO_4)	Merck
Sybr Green	Quiagen
3,3',5,5'-Tetramethylbenzidine (TMB) substrate	BD
Tetramethylbutylphenyl-polyethylene glycol solution (Triton X100)	Roche
Tris(hydroxymethyl)aminomethane (Tris)	Merck, Roth®
TRI Reagent	Sigma-Aldrich
Trypan blue solution 0.4% (v/v)	Sigma®
Tween® 20	Sigma®
Vancomycin hydrochloride (VANCOcell® 500mg)	Cellpharm

2.1.3 **Kit Systems**

Table 3: Kit systems

Name	**Company**
AffinityScript One-Step RT-PCR Kit	Agilent
CD4+CD62L+ T Cell Isolation Kit II, mouse	Miltenyi Biotec
Cytofix/Cytoperm™	BD
Foxp3 staining buffer set	eBioscience
Mouse ELISA Kits (IL-10)	R&D
Mouse ELISA Kits (Ready-Set-Go) IFN-γ, IL-4, IL-5, IL-13	eBioscience
PeqGOLD Gel Extraction Kit PEQLAB	Biotechnologie
QuantiTect Reverse Transcription Kit	Qiagen
QIAmp Fast DNA Stool Mini Kit	Qiagen
QIAquick PCR purification kit	Qiagen
RNeasy Mini Kit	Qiagen

2.1.4 **Software**

Table 4: Software

Name	Company
CELLQuest ProTM software (Flow cytometry)	BD
FlowJo (Flow cytometry analysis software)	TreeStar
GraphPad Prism 7	GraphPad Software
Nanodrop® 1000 V 3.7.0	Kisker
REST © (Relative Expression Software Tool) V2.013	Quiagen/TUM

2.1.5 **Consumables**

Table 5: Consumables

Name	**Company**
Blood agar plates	BD
Cell strainer (40-100µm)	BD
Cover slips	Roth®
Cryo vials	Alpha Laboratories
Culture plates (6-, 12-, 24-, 96-well)	BD
ELISA plates (96 well)	Nunc
Eppendorf tubes (0.5-2ml)	Eppendorf
FACS tubes (Microtubes, 1.2ml)	Alpha Laboratories
Fast-Read 102®	Biosigma
Glass slide	Langenbrinck
Hypodermic needles (Sterican®)	B Braun
LS Columns	Miltenyi Biotec
MS Columns	Miltenyi Biotec
QIAquick columns	Qiagen
Parafilm M®	Pechiney
Petri dishes	Greiner
Pipet tips (10-1000µl)	Starlab
Reaction tubes	Zefa-Laborservice
Scalpels	Feather
Sterile filters (0.22µm, 0.4µm)	VWR Lab Shop
Serological pipettes (5-50ml)	Greiner
Sub-Q syringes (1ml)	BD
Syringes (1-25ml)	B Braun
Syringe filter (0.2µm, 0.45µm)	Sartorius
Tissue culture flask (50-500ml)	BD

Tubes (15ml, 50ml)	Greiner

2.1.6 **Medium supplements**

Table 6: Medium supplements

Name	**Company**
Fetal calf serum (FCS)	PAA
HEPES	Thermo Fisher Scientific
β-Mercaptoethanol for cell culture	Gibco®
Non-essential amino acids (100x)	PAA
RPMI 1640 (with L-Glutamine)	PAA
Penicillin/Streptomycin (100x)	PAA
Sodium pyruvate solution (100mM)	PAA

2.1.7 **Buffers and solutions**

2.1.7.1 Buffers and solutions for egg preparation

Table 7: Buffers and solutions for egg preparation

Solution	**Amount**	**Substance**
Vancomycin solution:	500mg	Vancomycin hydrochloride
	10ml	0.9% NaCl (w/v)
Collagenase solution:	500mg	Collagenase
	5ml	Dulbecco's PBS (1x)
DNase solution:	1g	DNase I
	146ml	146ml Dulbecco's PBS (1x)
Egg-PBS solution:	1x	Dulbecco's PBS (1x)
	0.1% (v/v)	Vancomycin solution
	0.5% (v/v)	Gentamicin
Liver digestion solution:	25ml	Egg-PBS solution
	1ml	Collagenase solution
	3ml	DNase solution
	500µl	Penicillin/Streptomycin
Percoll solution:	8ml	PercollTM
	32ml	0.25M Sucrose

2.1.7.2 Buffers for erythrocyte lysis

Table 8: Buffers for erythrocyte lysis

solution	**amount**	**substance**
ACT buffer:	17mM	Tris
	160mM	NH4Cl
	pH 7.2	

2.1.7.3 Buffers and solutions for FACS

Table 9: Buffers and solutions for FACS

solution	**amount**	**substance**
FACS buffer:	1x	PBS (pH 7.2-7.4)
	2% (v/v)	FCS
Fc block solution:	1x	FACS buffer
	0.1% (v/v)	α-mouse CD16/32
HF2+ buffer	2%	FCS
	10 mM	HEPES buffer
		Antibiotics

2.1.7.4 Buffers and solutions for ELISA

Table 10: Buffers and solutions for ELISA

solution	**amount**	**substance**
Reagent diluents:	1x	PBS (pH 7.2-7.4)
	1% (w/v)	BSA
Washing buffer:	1x	PBS (pH 7.2-7.4)
	0.05% (v/v)	Tween® 20
Stopping solution:	2M	H2SO4
Ig-coating buffer:	8.4g/l	NaHCO3
	3.56g/l	Na2CO3
Ig-blocking buffer:	50mM	Tris
	3% (w/v)	BSA

2.1.7.5 Buffers used throughout chromatin immunoprecipitation procedure

Table 11: Buffers used throughout chromatin immunoprecipitation

solution	**amount**	**substance**
Lysis buffer I	5 mM	PIPES pH8

	85 mM	KCl
	0,5%	NP40 (Igepal-CA630)
	1 per 50 ml	Protease inhibitor cocktail tablets
Lysis Buffer II	10 mM	Tris-HCl pH 7,5
	150 mM	NaCl
	1%	NP40 (Igepal-CA630)
	1%	DOC (Natriumdeoxycho-late)
	0,1%	SDS
	1mM	EDTA
	1 tablet for 50 mL	Protease inhibitor cocktail tablets
Wash buffer I	20mM	Tris-HCl pH 8
	150mM	NaCl
	2mM	EDTA
	0,1%	SDS
	1%	Triton X100
Wash buffer II	20mM	Tris-HCl pH 8
	500mM	NaCl
	2mM	EDTA
	0,1%	SDS
	1%	Triton X100
Wash buffer III	10mM	Tris-HCl pH 8
	1%	NP40 (Igepal-CA630)
	1%	DOC
	1mM	EDTA
	0,25 M	LiCl
1x TE	10 mM	Tris-HCl pH 8
	1 mM	EDTA

Elution Buffer	1%	SDS
	0,1 M	$NaHCO_3$

2.1.8 **Cell culture medium**

Table 12: Cell culture medium

	amount	**substance**
Complete medium:	1x	RPMI 1640
	10% (v/v)	FCS
	1% (v/v)	Penicillin/Streptomycin
	1% (v/v)	Non-essential amino acids
	1% (v/v)	Sodium pyruvate solution
	0.1% (v/v)	β-Mercaptoethanol for cell culture

2.1.9 **Primer sequences**

2.1.9.1 Primer for *S. mansoni* specific RT-PCR

Table 13. Primer sequence for *S. mansoni* specific RT-PCR

Primer	**Sequence**
Forward tandem repeat (FP):	5'CAACCGTTC-TATGAAAATCGTTGT 3'
Reverse tandem repeat (RP):	5' CCACGCTCTCGCAAATAATCT 3'
Dual labelled probe tandem repeat (labelled probe) with 5' and 3' modifications:	5'[6FAM]TCCGAAACCACTGGAT-TTTTATGAT[BHQ1]

2.1.9.2 Primer for qRT-PCR of T_H1, T_H2 differentiated and undiff/ T_H0 cells

Table 14: Primer sequences for qRT-PCR of T_H1, T_H2 differentiated and undiff/ T_H0 cells

Primer	**Sequence**
HPRT (housekeeping gene)	
Forward primer	5'-TCC TCC TCA GAC CGC TTT T-3'

Reverse primer	5’-CCT GGT TCA TCA TCG CTA ATC-3’
Dual labeled probe	Universal ProbeLibrary Probe #95, Roche
t-bet	
Forward primer	5‘- CAA CCA GCA CCA GAC AGA GA -3’
Reverse primer	5‘- ACA AAC ATC CTG TAA TGG CTT -3’
Dual labelled probe	Universal ProbeLibrary Probe #19, Roche
IFN-γ	
Forward primer	5’-ATC TGG AGG AAC TGG CAA AA-3’
Reverse primer	5’-TTC AAG ACT TCA AAG AGT CTG AGG TA-3’
Dual labeled probe	Universal ProbeLibrary Probe #21, Roche
GATA-3	
Forward primer	5‘- TTA TCA AGC CCA AGC GAA G -3’
Reverse primer	5‘- TGG TGG TGG TCT GAC AGT TC -3’
Dual labeled probe	Universal ProbeLibrary Probe #108, Roche
IL-4	
Forward primer	5’- TCT GCC TCC ATC ATC CTT CT-3’
Reverse primer	5’- ACA CCA TAA TCG GCC TTT CA-3’
Dual labeled probe	Universal ProbeLibrary Probe #2, Roche

2.1.9.3 Primer for qRT-PCR of histone acetylation studies

Table 15: Primer sequences for qRT-PCR of histone acetylation studies

Primer	**Sequence**
RPL32 (housekeeping gene)	
Forward primer	5’- TCA TTT CTC AGG CAC ATC TT-3’
Reverse primer	5’- ACT CAC CGTAAA ACA GAT GG-3’
IFN-γ	
Forward primer	5’- CAT ACC CTT TCC TTG CTT TTC -3’
Reverse primer	5’- TTG TGG GAT TCT CTG AAA GCA -3’
IL-4	

Forward primer	5’- TCT GCC TCC ATC ATC CTT CT-3’
Reverse primer	5’- ACA CCA TAA TCG GCC TTT CA-3’
IL-5	
Forward primer	5’- ACC CTG AGT TTC AGG ACT CG-3’
Reverse primer	5’-TCC CCA AGC AAT TTA TTC TCT C -3’
IL-17A	
Forward primer	5’- AAT TTC TGC CCT TCC CAT TT - 3’
Reverse primer	5’- CCC AGG AGT CAT CGT TGT TT - 3’
FoxP3	
Forward primer	5’- ATC GTG AGG ATG GAT GCA TTA ATA - 3’
Reverse primer	5’- CCA CTG GGA AGG TCC CTA GC - 3’
RORγT	
Forward primer	5’- TCT CCC CTA TGC CTG TCA CCT G - 3’
Reverse primer	5’- TGA TTT TGC CCA AGG ACT CAC AC

2.1.10 **Antibodies**

Table 16: Antibodies

Name	**Anti-gen**	**Conjugate**	**Spe-cies**	**Dilu-tion**	**Producer**	**Method**
α-mouse-CD4-APC (Clone GK1.5)	CD4	APC	Rat	1:1000	eBioscience	FACS
α-mouse-CD4-FITC (Clone RM4-5)	CD4	FITC	Rat	1:1000	eBioscience	FACS
α-mouse-CD4-PE (Clone RM4-5)	CD4	PE	Rat	1:1000	eBioscience	FACS
α-mouse-CD62L-FITC (MEL-14)	CD62L	FITC	Rat	1:100	eBioscience	FACS

α-mouse/rat Foxp3-FITC (Clone FJK-16s)	Foxp3	FITC	Rat	1:1000	eBioscience	FACS
α-mouse-CD44-FITC (Clone IM-7)	CD44	FITC	Rat	1:100	BD	FACS
α-mouse-LY6C-PerCpCy5.5 (Clone HK1.4)	LY66C	PerCpCy5.5	Rat	1:400	eBioscience	FACS
α-mouse-CD62L-PEcy7 (Clone MEL-14)	CD62L	PEcy7	Rat	1:2000	eBioscience	FACS
α-mouse-CD45-A700 (Clone 30-F11)	CD45	A700	Rat	1:1000	BioLegend	FACS
α-mouse-CD25-APC (Clone PC61)	CD25	APC	Rat	1:100	BD	FACS
α-mouse-t-bet-APC (Clone eBio4B10)	t-bet	APC	Mouse	1:100	eBioscience	FACS
α-mouse-IFN-γ-PE (Clone XMG1.2)	IFN-γ	PE	Rat	1:100	eBioscience	FACS
α-mouse-GATA-3-PE (Clone TWAJ)	GATA-3	PE	Rat	1:100	eBioscience	FACS

α-mouse-IL-4-APC (Clone 11B11)	IL-4	APC	Rat	1:100	eBioscience	FACS
α-mouse-CD8a-APCA750 (Clone 5H10)	CD8a	APC A750	Rat	1:400	Invitrogen	FACS
α-mouse-CD3-eF450 (Clone 17A2)	CD3	eF450	Rat	1:100	eBioscience	FACS
α-mouse-CD4-PO (Clone RMA-5)	CD4	Pacific Orange	Rat	1:400	Invitrogen	FACS
α-mouse-IgD-FITC (Clone 11-26c.2a)	IgD	FITC	Rat	1:1000	BD	FACS
α-mouse-NKp46-PE (Clone 29A1.4)	NKp46	PE	Rat	1:200	eBioscience	FACS
α-mouse-MHCII-PerCpCy5.5 (Clone M5/114.15.2)	MHC-II	PerCpCy5	Rat	1:1000	Biolegend	FACS
α-mouse-CD19-PECy7 (Clone 1D3)	CD19	PECy7	Rat	1:1000	BD	FACS
α-mouse-CD5-APC (Clone PC61)	CD5	APC	Rat	1:2000	BD	FACS

α-mouse-B220-APCA750 (Clone RA3-6B2)	B220	APC A750	Rat	1:100	Invitrogen	FACS
α-mouse-B220-PB (Clone RA3-6B2)	B220	Pacific blue	Rat	1:100	eBioscience	FACS
α-mouse-CD11b-PB (Clone M1/70.15)	CD11	Pacific blue	Rat	1:800	Invitrogen	FACS
α-mouse-GR1-PO (Clone RB6-8C5)	GR1	Pacific Orange	Rat	1:1000	Invitrogen	FACS
α-mouse-CD43-FITC (Clone eBioR2/60)	CD43	FITC	Rat	1:100	eBioscience	FACS
α-mouse-IgM-PE (Clone II/41)	IgM	PE	Rat	1:100	eBioscience	FACS
α-mouse-CD4-PECy5 (Clone RM4-5)	CD4	PECy5	Rat	1:100	eBioscience	FACS
α-mouse-CD8a-PECy7 (Clone 53-6.7)	CD8a	PECy7	Rat	1:100	eBioscience	FACS
α-mouse CD3e (Clone 17A2)	CD3	-	Rat	1µg/ml	eBioscience	in vitro stimuli

α-mouse CD28 (Clone 37.51)	CD28	-	Golden Syrian Ham-ster	1µg/ml	eBioscience	in vitro stimuli
α-mouse IgG (Clone RM104)	IgG	-	Rabbit	0,5 µg	Abcam	ChIP
α-mouse IL-4-Biotin	IL-4	Biotin	Mouse	1:250	eBioscience	ELISA
α-mouse IFN-γ-Biotin	**IFN-γ**	Biotin	Mouse	1:1000	eBioscience	ELISA
α-mouse IL-10-Biotin	**IL-10**	Biotin	Mouse	1:250	R&D	ELISA

Table 17: Supplements for T cell differentiation assays

Name	**Interleukin**	**Isotype**	**Concentra-tion**	**Company**	**Method**
Mouse-IL-2	Recombi-nant IL-2	Mouse	100 µg/ml	BioLegend	In vitro
Mouse-IL-4	Recombi-nant IL-4	Mouse	100 µg/ml	BioLegend	In vitro
Mouse-IFN-γ	Recombi-nant IFN-γ	Mouse	100 µg/ml	BioLegend	In vitro
Mouse-IL-12	Recombi-nant IL-12	Mouse	200 µg/ml	BioLegend	In vitro
α-mouse-IL-4 (Clone 11B11)	-	Rat	0,5 mg/ml	BioLegend	In vitro
α-mouse-IFN-γ (Clone XMG1.2)	-	Rat	0,5 mg/ml	BioLegend	In vitro
α-mouse-IL-12 (Clone C18.2)	-	Rat	0,5 mg/ml	BioLegend	In vitro

2.2 Methods

2.2.1 **Mouse strains**

BALB/c mice were purchased from Harlan Winkelmann (GmbH). All mice were kept under specific pathogen-free (SPF) conditions at MIH. Animal experiments were performed in accordance with local government authorities Bezirksregierung Oberbayern (license number for animal testing AZ. 55.2.1.54-2532-147-08).

2.2.2 ***Schistosoma mansoni*** **life cycle maintenance**

Six-to-eight-week-old NMRI mice were intraperitoneally infected with 140 – 200 cercariae of *S. mansoni*. After eight weeks of infection the NMRI mice were killed and miracidiae were isolated from their intestines. The miracidiae hatched in water and penetrated the skin of Biomphalaria glabrata snails. After six weeks cercariae were released in water and were used to uphold the infection cycle.

2.2.3 **Infection with *Schistosoma mansoni* and mating protocol**

2.2.3.1 Infection and mating protocol

Six-to-eight-week-old female BALB/c mice were intraperitoneally infected with an injection dose of 100 cercariae of *S. mansoni*. After 8 weeks of infection a specific *S. mansoni* RT-PCR (as described in section 2.2.4.) was performed to identify successfully infected mice. Only infected mice were kept and mated during the chronic phase of infection in week 16 with uninfected, 8-week-old, male BALB/c mice. In all experiments age-matched uninfected female BALB/c mice were used as controls. After 3 weeks of pregnancy offspring of *S. mansoni* infected and naïve mice were born and raised by their mothers. At the age of 8 weeks analyses of offspring of schistosome-infected and naïve mothers were performed.

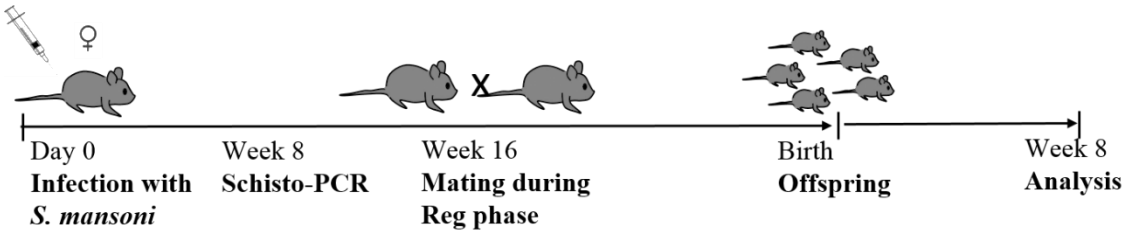

Figure 5: **Mating protocol**

Adult female BALB/c mice were intraperitoneally infected with 100 cercariae of S. *mansoni*. After 8 weeks of infection the *S. mansoni*-specific RT-PCR was performed to evaluate the infection status. Only infected mice were kept and mated with uninfected BALB/c males in week 16 week during the chronic phase of infection. After three weeks of pregnancy offspring were born and raised. At the age of 8 weeks the progeny was used for further experimental studies.

2.2.3.2 Egg count analysis from the liver

To determine the infection status of the mother, female BALB/c mice were sacrificed after having raised their offspring. Livers were isolated under sterile conditions, gallbladders and bile ducts were removed. After weighing the whole liver, a small sample was used for the egg count analysis. Samples were digested in 5 ml of 5% KOH in a shaker for 2 hours at 37°C. After digestion, the probes were centrifugated at 400 g for 10 minutes. 4.5 ml of the supernatant were eliminated. The remaining 500 µl were resuspended and 10 µl were examined under the microscope. The eggs were counted, and an average was built. The amount of eggs was calculated as follows:

$$\frac{\text{eggs}}{\text{mg tissue}} = \text{average of counted eggs x } 50 / \text{weight of tissue sample (mg)}$$

Only offspring of mothers over 10.000 counted eggs were used for the experiments.

2.2.4 ***S. mansoni* specific RT-PCR**

Before the mating of female BALB/c mice with uninfected males the *S. mansoni*-specific RT-PCR was performed in week 8 after infection to identify successfully infected female BALB/c mice.

2.2.4.1 DNA-isolation of the parasite from stool probes

To collect specific stool samples mice were separated individually overnight. Six stool samples were collected and placed in a 2 ml tube on ice. DNA of the parasites were isolated from the stool probes according to the manufacturer's protocol in the QIAamp Fast

DNA Stool Mini Kit (Qiagen). InhibitEX Buffer was added to each stool sample and incubated at 95°C in a shaker for 20 minutes to lyse the stool probes and to purify the DNA from contaminations. The samples were centrifugated at 24,000 g for 1 minute to pellet stool particles. Proteinase K and buffer AL were added to the supernatant to digest proteins and incubated for 10 minutes at 70°C. After adding ethanol (96-100%), the lysate was applied to QIAamp spin columns, centrifugated and placed in a new collection tube. Two wash buffers AW1 and AW2 were used and the samples were centrifugated again. To release the DNA buffer ATE was pipetted directly onto the QIAamp membrane and centrifugated. The purified DNA of the parasite were collected in 2 ml collection tube and stored at -20°C. The yield and purity of the DNA was measured at Nanodrop®.

2.2.4.2 qRT-PCR method

To test the infection status the purified DNA samples from stool probes were further amplified by a *S. mansoni* specific RT-PCR. Primer sequences are shown in section 2.1.9.1. The PCR-mix consisted of 10µl LightCycler® 480 Probes Master, 1 µl forward primer, 1 µl reverse primer, 1 µl dual labelled probe, 2 µl DNA and 5,0 µl DEPC H20 as described in 2.1.9.1. The qRT-PCR was started with one cycle at 95°C for 5 minutes (hot start), followed by 45 amplification cycles for 10 s at 95°C and for 30 s at 58°C. The cooling was finally performed with one cycle at 45°C for 15 s. As a negative control DNA of naïve age-matched female BALB/c mice were used and as a positive control DNA of infected BALB/c female mice (counted eggs > 10.000), respectively. The cut-off was set at 35 amplification cycles. All DNA samples with 35 or less cycles were considered as infected, the infected ones were kept and mated with naïve BALB/c males during the chronic phase of infection in week 16.

2.2.5 ***S. mansoni* egg and SEA preparation**

2.2.5.1 *S. mansoni* egg preparation

After approximately 7 weeks of infection eggs of the parasite *S. mansoni* were laid in the livers of the mice. According to that eight weeks schistosome-infected NRMI mice were sacrificed and their livers were isolated. After washing the livers in pre-cooled 1.2% (v/v) NaCl solution and removing the bile ducts and gallbladders, livers were cut into small pieces and transferred into a 50 ml tube containing 25 ml liver digestion solution. The samples were filled up with PBS to 50 ml and incubated under continuous agitation at 37°C overnight. The livers were washed again twice by centrifugation at 400 g for 5 min

at 4°C, then cell pellets were resuspended in 25 ml PBS and filtered through a 250 µm sieve. The cell suspension was then applied on Percoll and centrifugated at 800 g for 10 min at 4°C. Since the density gradient of eggs and liver tissue varies, eggs were separated from liver tissue. After washing the eggs with 15 ml, 30 ml of 1 mM EDTA solution and finally with 30 ml PBS solution, the egg pellet was resuspended in 700 µl PBS. To count the eggs 5 µl of the lysate were examined under the microscope (x10).

$$\text{total egg count} = \text{counted eggs x } 700\ \mu l/5\ \mu l$$

To detect possible contaminations 2 µl of the egg suspension were plated on blood agar and MacConkey agar plates and incubated for 48 hours at 37°C.

2.2.5.2 SEA preparation

Isolated eggs from infected NMRI mice were used for soluble egg antigen (SEA) preparation. The suspension containing the eggs was filled in a glass homogenizer. While mincing the suspension for 20 min on ice the soluble egg antigen was released. After an ultracentrifugation step at 100,000 g for 1 hour at 4°C using the Optima^{TM} L-100 XP ultracentrifuge, the supernatant containing the SEA was transferred to a cryotube. The remaining egg shell pellet was resuspended and used for contamination control. Contaminations were analyzed by plating 2 µl of egg shell and soluble egg antigen suspension on blood agar and MacConkey agar plates (incubation for 48 hours at 37°C). The protein concentration of the SEA solution was measured using the DC protein assay kit (Bio-Rad) according to the manufacturer's protocol.

2.2.6 **Preparation of splenocytes with erythrocyte lysis**

Offspring of schistosome-infected and naïve mice were sacrificed at the age of 8 weeks. Spleens were isolated under sterile conditions and softly mashed with the plunger of the syringe in a 6 well plate half filled with PBS. The samples were directly pipetted onto a 70 µm cell strainer and collected with a 50 ml Falcon tube. The solution was centrifugated at 400 g for 10 minutes at 4°C and the supernatant was immediately removed. For the erythrocyte lysis 3 ml ACT buffer was added for 3 to 5 minutes on ice. After the centrifugation at 400 g at 4°C for 10 minutes the supernatant was carefully removed. To count the splenocytes the probes were resuspended in 10 ml. 10 µl of the probes were mixed with 90µl Trypan blue solution 0.4% (v/v), living cells were counted under the microscope with the Neubauer chamber and calculated as follows:

$$\text{cell number/ml} = \text{mean x dilution factor (10) x } 10^4$$

2.2.7 Stimulation of splenocytes and mesenteric lymph node cells with SEA antigen

Spleens and mesenteric lymph nodes were carefully removed from sacrificed BALB/c mice under sterile conditions. Single cell suspensions were prepared and counted as previously described in 2.2.6. $2x10^5$ spleen and mesenteric lymph node cells were resuspended in a total volume of 200 µl complete medium and plated on a round-bottom 96 well plate. Cells were stimulated with 20 µg/ ml SEA and incubated for 48 hours at 37°C. As positive controls, cells were stimulated with combined αCD3 (1µg/ml) and αCD28 (1µg/ml). A negative control without stimulation (unstimulated) was also prepared. After the incubation for 48 hours cell suspensions were centrifugated at 400 g for 10 minutes. The supernatant (100 – 150 µl) was carefully removed and frozen at 20°C for further cytokine measurement by ELISA (2.2.11).

2.2.8 Sorting of naïve $CD4^+CD62L^+$ and $CD4^+CD62L^+CD44^{+/-}$ T cells

2.2.8.1 Sorting naïve T cells with magnetic activated cell sorting

Splenocytes of schistosome-infected and naïve offspring were isolated with the T Cell Isolation Kit II (Miltenyi Biotec) to gain the population of $CD4^+CD62L^+$ naïve T cells. According to the manufacturer's protocol the procedure consisted of two steps: the depletion of non-$CD4^+$ T cells by indirect magnetic labelling, followed by a positive selection of $CD4^+CD62L^+$ T cells by direct labelling with CD62L MicroBeads. In brief, the splenocyte cell pellet was resuspended in 400 µl buffer and labelled with 100 µl of CD4+ T cell Biotin-Antibody Cocktail II. The biotin-conjugated antibodies were directed against CD8a (Ly-2), CD45R (B220), CD49b (DX5), CD11b (Mac-1), Ter-119, CD25 and TCRγ/δ. The samples were mixed and refrigerated for 10 minutes. 300 µl buffer and 200 µl Anti-Biotin MicroBeads were added to catch the biotin-conjugated antibodies. After washing, cell suspensions were resuspended in 500 µl buffer and loaded onto LS columns. The columns were washed three times with 3 ml buffer. The effluent which contained the unlabeled $CD4^+$ cell fraction was collected. To isolate the $CD4^+CD62L^+$ T cells the pre-enriched $CD4^+$ T cell suspension was mixed with 200 µl CD62L MicroBeads. Cell suspension was applied to the MS column and washed three times. Afterwards the MS column was removed and placed on a 5 ml collection tube. To flush out the magnetically labeled $CD4^+CD62L^+$ cells, 1 ml of buffer was added onto the column and the plunger was firmly pushed down the column.

2.2.8.2 Efficiency and purity of MACS sorted naïve T cells

The efficiency and purity of magnetic labeled cell sorting was examined by using flow cytometric analysis. The collected cell suspension was stained with 50 µl Fc block and Ethidium monoazide bromide (EMA) for 20 minutes at 4°C. After a washing step with 100 µl FACS buffer the surface staining was performed with APC-conjugated anti-CD4 (diluted 1:200) and FITC-conjugated anti-CD62L (diluted 1:100) to detect the living naïve $CD4^+CD62L^+$ T cells. Cell suspension were incubated for 25 minutes at 4°C. Afterwards cell suspensions were washed 100 µl FACS buffer and centrifugated at 230 g for 10 minutes. The pelleted cells were resuspended in 100 µl FACS buffer and directly applied to the Calibur II Cytometer (BD Biosciences). The data analysis was performed with FlowJo (Tristar). The portion of naïve $CD4^+CD62L^+$ T cells was compared to unsorted splenocytes as shown in Fig. 10B.

2.2.8.3 Sorting naïve T cells by flow cytometry

By using flow cytometry, a second method was used to sort $CD4^+CD62L^+$ naïve T cells. Splenocytes were prepared as described in section 2.2.6. Splenocyte cell suspensions were resuspended in 50 µl Fc block and Ethidium monoazide bromide solution (EMA) for 20 minutes at 4°C. Afterwards a washing step with 100 µl FACS buffer was applied and the cells were centrifugated at 230 g for 5 min at 4°C. The surface staining included the following marker: APC-conjugated anti-CD4 (diluted 1:200) and FITC-conjugated anti-CD62L (diluted 1:100). The stained cell suspension was incubated for 25 min at 4°C, then washed with 100 µl FACS buffer and centrifugated at 230 g for 10 min. The cell acquisition was performed with the CyAn ADP Lx P8 cytometer (BD Biosciences). Gating strategy is shown in Fig. 15B. The effluent containing the population of $CD4^+CD62L^+$ cells was collected in FACS tubes and used for histone acetylation studies (section 2.2.14).

2.2.8.4 Sorting $CD4^+CD62L^+CD44^{+/-}$ T cells by flow cytometry

Splenocytes were prepared as described in section 2.2.6. Cell suspensions were resuspended in 50 µl Fc block and Ethidium monoazide bromide solution (EMA) for 20 minutes at 4°C. After a washing step with 100 µl FACS buffer, cells were centrifugated at 230 g for 5 min at 4°C. The surface staining included the following marker: APC-conjugated anti-CD4 (diluted 1:200), FITC-conjugated anti-CD62L (diluted 1:100), and PE-conjugated-CD44 (diluted 1:100). Cell suspensions were incubated for 25 min at 4°C,

then washed with 100 µl FACS buffer and centrifugated at 230 g for 10 min. The cell acquisition was performed with the CyAn ADP Lx P8 cytometer (BD Biosciences). $CD4^+CD62L^+CD44^{pos}$ and $CD4^+CD62L^+CD44^{neg}$ cells were collected separately and used for further T cell studies as described in section 2.2.9.3. Gating strategy is shown in Fig 15C.

2.2.9 **In vitro differentiation of naïve T cells**

The protocol of the *in vitro* differentiation of naïve $CD4^+CD62L^+$ T cells into T_H1 and T_H2 cells was adapted from Current Protocols in Immunology (Fitch et al., 2006)

2.2.9.1 T_H1 cell differentiation protocol

For T_H1 *in vitro* differentiation, 10 ng/ml IL-2, 10 µg/ml α-IL-4, 20 ng/ml IL-12 and 20 ng/ml IFN-γ were added to $2x10^6$ $CD4^+CD62L^+$ MACS sorted naïve T cells (for flow cytometry, RT-PCR/ ELISA analyses) and stimulated with αCD3 and αCD28 T cell beads (ratio 1:2). The cell suspension was incubated for 3 days in 750µl RPMI complete medium in a 48 well plate. Each mouse sample was stimulated separately for flow cytometry and RT-PCR/ ELISA. As negative control, a T_H0/undiff condition was set up with the identical number of cells and stimulated with 10 ng/ml IL-2, 10µg/ml α-IFN-γ and 10µg/ml α-IL-4 for 3 days, respectively. On day 3 T_H1 differentiated cells were stimulated with 750 ng/ml ionomycin, 50 ng/ml phorbol 12-myristate 13-acetate in the presence of 10 µg/ml Brefeldin A for 4.5 hours and subsequently stained for flow cytometry analysis as described in section 2.2.10. To determine cytokines in the supernatant and transcription factor mRNA expression levels, T_H1 differentiated T cells were stimulated with αCD3/ αCD28 T cell beads (ratio 1:2) for 6 hours.

2.2.9.2 T_H2 cell differentiation protocol

For T_H2 *in vitro* differentiation, 10 ng/ml IL-2, 10 µg/ml α-IFN-γ, 10 µg/ml α-IL-12 and 25 ng/ml IL-4 were added to $3x10^6$ $CD4^+CD62L^+$ MACS sorted naïve T cells (for flow cytometry) or $1,5x10^6$ cells (for RT-PCR/ ELISA) and stimulated with αCD3/ αCD28 T cell beads (ratio 1:2). The cell suspension was incubated for 3 days in 750µl RPMI complete medium in a 48 well plate. On day 3 cells were split in two 48 well plates, filled up with 375µl RPMI complete medium, stimulated with 10ng/ ml IL-2 and incubated another 3 days. As negative control a T_H0/ undiff condition with $3x10^6$ cells for flow cytometry and $1,5x10^6$ cells for RT-PCR/ ELISA was stimulated with 10 ng/ml IL-2, 10µg/ml α-

IFN-γ and 10µg/ml α-IL-4, respectively, and treated equally. On day 6 the T_H2 differentiated cells were stimulated for flow cytometry and for cytokine detection and RT-PCR as described in section 2.2.9.1.

2.2.9.3 T_H0/ undiff condition of $CD4^+CD62L^+$ naïve and $CD4^+CD62L^+CD44^{+/-}$ T cells

$1{,}5x10^6$ cells of MACS sorted $CD4^+CD62L^+$ naïve or MoFlow sorted $CD4^+CD62L^+CD44^{+/-}$ T cells were stimulated with 10 ng/ml IL-2, 10µg/ml α-IFN-γ, 10µg/ml α-IL-4, and αCD3αCD28 T cell beads (ratio 1:2) in 750µl RPMI complete medium in a 48 well plate. On day 3 cells were split in two 48 well plates, filled up with 375µl RPMI complete medium and stimulated with 10ng/ ml IL-2 for another 3 days. On day 6 cells were stimulated with αCD3/ αCD28 T cell beads (ratio 1:2) for 6 hours to quantify cytokines in the supernatant by ELISA as described in section 2.2.11.

2.2.10 **Flow cytometry**

2.2.10.1 Staining protocol of T_H0/undiff, T_H1 and T_H2 differentiated cells

To determine intracellular cytokine and transcription factor expression T_H0/undiff, T_H1 and T_H2 differentiated cells were stained with CD4 surface molecule, anti-IFN-γ and anti-t-bet for T_H1 and anti-IL-4 and anti-GATA-3-antibodies for T_H2 differentiated cells. Cell suspension with differentiated cells were centrifugated and supernatants removed. To block Fc receptors and to exclude dead cells, the cell pellet was resuspended in 50 µl Fc block and Ethidium monoazide bromide (EMA) solution for 20 minutes at 4°C. After a washing step with 100 µl FACS buffer and centrifugation at 230 g for 5 min at 4°C the surface staining was performed with FITC-conjugated anti-CD4 antibodies (diluted 1:200) for TH0/undiff, T_H1 and T_H2 differentiated cells for 25 minutes at 4°C. Subsequently cells were washed with FACS buffer and centrifugated at 230 g for 10 minutes. To stain cells intracellularly the Cytofix/Cytoperm Kit from BD was used according to the manufacturer's protocol. In brief, the cell suspension was resuspended with 200 µl Cytofix/Cytoperm for 30 minutes at 4°C, washed twice with 200µl Perm/Wash buffer (diluted with VE water 1:10), and the supernatant was removed. After permeabilization T_H1 differentiated cells were intracellularly stained with PE-conjugated anti-IFN-γ antibodies (1:200) and T_H2 differentiated cells with APC-conjugated anti-IL-4 antibodies (1:200) from eBioscience. For staining cell suspension were incubated for 40 minutes at 4°C. The stained cells were washed twice by 200 µl Perm/Wash buffer and resuspended in 200 µl FACS buffer for the analysis on the CyAn ADP Lx P8 or Calibur II Cytometers

(BD Biosciences). To stain cells intranuclearly the Foxp3 staining buffer set from eBioscience was used according to the manufacturer's instructions. In brief, 300 µl FixPerm was added for 30 minutes at 4°C to permeabilize cell walls and nuclei. Two washing steps with permeabilization buffer were performed. APC-conjugated anti-t-bet antibodies (diluted 1:100) in permeabilization buffer were added to T_H1 differentiated cells and PE-conjugated anti-GATA-3 (1:100) antibodies to T_H2 differentiated cells. Cell suspensions were incubated for 40 minutes in the dark at 4°C. After incubation, cells were washed twice with 300µl permeabilization buffer and cell pellets resuspended in 200 µl FACS buffer for the analysis. Cell acquisition was performed with CyAn ADP Lx P8 or Calibur II Cytometers (BD Biosciences). The data analysis was performed with FlowJo (Tristar).

2.2.10.2 Gating strategy of T_H1, T_H2 and T_H0/ undiff cells

T_H1 and T_H2 differentiated cells were analyzed by the software FlowJo according to the gating strategy illustrated in Fig. 11. For the analysis, dead cells were excluded on their Ethidium monoazide bromide (EMA) signal and events were gated for CD4 positive T cells. Subsequently $CD4^+$ cells were analyzed for IFN-γ^+ and t-bet^+ for T_H1 and IL-4^+ and GATA-3^+ signals for T_H2 differentiated cells. To increase the accuracy undifferentiated T cells (T_H0/ undiff cells) were used to be the cut off. All events above the signal of T_H0/ undiff cells were assumed positive.

2.2.10.3 Staining protocol of leukocyte main lineages in peripheral blood and spleen

In cooperation with Dr. Thure Adler, Helmholtz Zentrum München, Neuherberg, the population of leukocytes in peripheral blood was analyzed by flow cytometry. The multiparameter staining panel included markers for B cells (CD19, B220), T cells (CD3, CD5), granulocytes (CD11b, Ly6G or GR-1), NK cells (NK1.1, NKp46) and further subsets (CD44, CD25, CD62L) (BD Biosciences). Peripheral blood was either lysed by red blood cell lysis (NH4CL-Tris) or not. Propidium iodide was added to exclude dead cells. Cells were measured with a three-laser 10-color flow cytometer (LSRII, BD Bioscience). The data analysis was performed with FlowJo (Tristar) according to the gating strategy shown in Fig. 7. The method paper by Sunderkötter and his colleagues, 2014, was used as template.

2.2.11 **Cytokine detection**

To determine cytokines in the supernatant, Ready-Set-Go® ELISAs (eBioscience) and Duo Set® ELISA kits (R&D) were used according to the manufacturer's protocol. In

brief, 96 well plates were coated with 50 µl/ well capture antibodies diluted according to the manufacturer's instruction and kept overnight at 4°C. Subsequently, plates were washed three times with ELISA washing buffer and blocked with 150 µl/ well blocking buffer for 1 hour at room temperature. After blocking, plates were washed again three times with ELISA washing buffer. Probes were prepared in blocking buffer and added in the dilution of 1:2. At the same time, the serial standard was prepared in blocking buffer and applied to the plates. Plates were incubated for 2 hours at room temperature. Before adding 50 µl/ well detection antibodies diluted in blocking buffer for 1 hour at room temperature, plates were washed thrice with ELISA washing buffer. After the incubation with detection antibodies plates were washed again three times with ELISA washing buffer. Afterwards 50 µl/ well streptavidin-horseradish peroxidase (HRP) conjugate diluted in blocking buffer according to the manufacturer's instructions were added and incubated in the dark for 30 minutes. Before adding 50 µl/ well of substrate solution (TMB substrate), plates were washed with ELISA washing buffer three times. To stop the reaction with the substrate 50 µl/ well of stopping solution was added. The plates were immediately measured at 450 nm by using the Sunrise^{TM} ELISA microplate reader. The cytokine concentration was calculated according to the standard curve.

2.2.12 **Analysis of gene expression in T_H1 and T_H2 differentiated cells**

2.2.12.1 RNA isolation

To analyze mRNA expression levels by qPCR, RNA was isolated from T_H1 and T_H2 differentiated cell suspensions. In brief, 40µl 1-Bromo-3Chloro-Propan were added to lyse cells. Solutions were homogenized by vortexing and incubated at room temperature for 15 minutes. While centrifuging the samples at 240 g for 15 minutes at 4°C, 100 µl isopropanol was filled in new 2 ml collection tubes. The upper phase containing the RNA was added to isopropanol and left for 10 minutes at room temperature. After samples were centrifugated at 240 g for 20 minutes at 4°C, supernatant was removed. 500µl of 75% ethanol was then added to the cell pellet to clear the lysate and centrifugated at 240 g at 4°C for 5 minutes. After removing the supernatant completely, the cell pellet was centrifugated again at 240 g at 4°C for 5 minutes. The pelleted cells were now dried for 5 minutes at room temperature. After a sufficient drying time the cell pellet was resuspended in 20 µl DEPC water. The yield and purity of the gained RNA was measured by using Nanodrop® 1000 (Kisker).

2.2.12.2 cDNA synthesis

To process the gained RNA into cDNA the AffinityScript One-Step RT-PCR Kit from Agilent was used according to the manufacturer's instructions. In brief, 800 ng/ µl RNA in a total volume of 13 µl DEPC water was resuspended in a 2 ml tube. After adding 1µl oligo(dT) Primer Sigma (500ng/µl) the samples were incubated for 5 minutes at 65°C and for 10 minutes at room temperature. The following reaction components were added to the samples: 2µl Affinity Script Buffer, 2µl dNTPs (10mM Fermentas) and 1µl Affinity Script RT/RNAse Block. To synthesize cDNA samples were incubated for 1 hour at 47,5°C and to inactivate Affinity Script Reverse Transcriptase reaction components were incubated for 15 minutes at 70°C. The purified cDNA was stored at -80°C.

2.2.12.3 RT-PCR for T_H0/undiff, T_H1 and T_H2 differentiated cells

Primes and probes for RT-PCR of t-bet, IFN-γ, IL-4 and GATA-3 were designed using Roche Universal Probe Library software. Primer sequences are shown in section 2.1.9.2. The PCR-mix consisted of 10µl LightCycler® 480 Probes Master, 1µl forward primer, 1 µl reverse primer, 1 µl probe, 1 µl targeted cDNA and 6,0 µl DEPC H20. A negative control was prepared with 1 µl of diethyl pyrocarbonate (DEPC) water. The qPCR was started at 95°C for 5 minutes (hot start), followed by 45 amplification cycles at 95°C, 60°C and 72°C, when the denaturation, annealing/ extension and measurement of fluorescence occurred. The cooling was finally performed with one cycle at 45°C for 45 s. Statistical differences were acquired by using the software REST© V2.013 (Qiagen/TUM).

2.2.13 **Analysis of B and T cells from thymus and bone marrow**

2.2.13.1 Preparation of thymus and bone marrow

The following experiments were performed in cooperation with Dr. Rouzanna Istvanffy, PhD, III. Medizinische Klinik und Poliklinik, Klinikum Rechts der Isar, Technische Universität München. To prepare thymocytes and bone marrow cells, offspring of schistosome-infected mice were sacrificed at the age of eight weeks. Thymuses, tibias and femurs were isolated using sterile scissors and tweezers. Thymuses were gently minced in a 6 well plate using the plunger of a syringe and pipetted onto 70 µm nylon strainers. The effluent containing the thymocytes were collected in a falcon tube, and a single cell suspension was prepared. Bone marrow cells were flushed from femurs and tibias with HF2+ buffer (Hank's balanced salt solution, supplemented with 2% FCS, 10 mM HEPES buffer

and antibiotics). After erylysis with ammonium chloride solution (Stemcell Technologies) leukocytes were counted using trypan blue (Invitrogen) in a Neubauer hemocytometer (Renström et al., 2009).

2.2.13.2 Staining protocol of B and T cells in thymus and bone marrow cells

After preparing single cell suspensions, thymocytes and bone marrow cells were centrifugated and supernatants removed. Before staining, cells were washed with HF2+ buffer and centrifugated. Propidium iodide was added to exclude dead cells. To separate hematopoietic populations the following surface molecules were used: PB-conjugated anti-B220, FITC-conjugated anti-CD43, PE-conjugated anti-IgM, PECy5-conjugated anti-CD4 and PECy7-conjugated anti-CD8a (eBioscience^{TM}, Affymetrix^{TM}). In HF2+ buffer stained cell suspensions were incubated in the dark for 15 minutes at 4°C, and then again washed with HF2+ buffer (Renström et al., 2009). After resuspending cell pellets in FACS buffer, cell acquisition was performed using the Cyan ADP Lx P8 Cytometer. Data analysis was performed using the software FlowJo (Tristar).

2.2.13.3 Gating strategy of B and T cells within bone marrow and thymus

Bone marrow cells and thymocytes from offspring with naïve and Reg-phase infected mothers were analyzed by flow cytometry. B cell populations in bone marrow were defined as immature B ($B220^{low}IgM^{+}$), mature B cells ($B220^{high}IgM^{+}$), pro- and pre–B cells ($B220^{+}IgM^{-}$), pro-B cells ($B220^{+}CD43^{+}IgM^{-}$) and pre-B cells ($B220^{+}CD43^{-}IgM^{-}$) (Fig. 9C). T cell progenitors were defined as double negative DN ($CD4^{-}CD8^{-}$) cells. Cells in the intermediate developmental stage were defined as double positive DP ($CD4^{+}CD8^{+}$), and mature cells as single positive SP $CD4^{+}$ and SP $CD8a^{+}$ (Fig. 9D).

2.2.14 **Histone acetylation studies of $CD4^{+}$ $CD62L^{+}$ naive T cells**

2.2.14.1 Freezing of living naive T cells

To isolate $CD4^{+}$ $CD62L^{+}$ naïve T cells from splenocytes of 8-week-old offspring of uninfected or schistosome-infected mothers, two different protocols were used: splenocytes were either separated by magnetic activated cell sorting using the T Cell Isolation Kit II (Miltenyi Biotec) or by flow cytometry (FACS) as described in section 2.2.8. $CD4^{+}$ $CD62L^{+}$ naïve T cells were frozen with 10% dimethyl sulphoxide in heat-inactivated fetal

calf serum to prevent crystallizations that will eventually harm cells during cryopreservation. Cell suspensions were stored at -80°C until the chromatin-immunoprecipitation (ChIP) analysis was performed.

2.2.14.2 Preparation of $CD4^+$ $CD62L^+$ naïve T cells for chromatin-immunoprecipitation

The chromatin-immunoprecipitation (ChIP) of $CD4^+$ $CD62L^+$ naïve T cells was performed in cooperation with Hani Harb, Institute of Laboratory Medicine, Pathobiochemistry and Molecular Diagnostics, Philipps University Marburg, Marburg, Germany. According to the method paper 'Epigenetic Regulation in Early Childhood: A Miniaturized and Validated Method to Assess Histone Acetylation' by Harb et al. the ChIP analysis was performed (Harb et al, 2015). In brief, DNA-proteins in isolated naïve T cells were cross-linked with histones in the presence of 1% formaldehyde for 10 minutes at room temperature. Chromatin was treated with 5 M sodium butyrate to inhibit several histone deacetylases (HDAC) that potentially harm the chromatin. After centrifugation at 8,000 g for 5 min at room temperature lysis buffer I were applied and the suspension containing the chromatin was incubated for 20 min at room temperature. After a second centrifugation step the chromatin was incubated with lysis buffer II containing 1% sodium dodecyl sulfate (SDS) for 5 minutes at room temperature and 3 minutes on ice. Afterwards the chromatin was transferred into the power-up Bioruptor (Diagenode) and underwent a sonication with 30 high power cycles. Since each cycle included 30 s with power and 30 s without power the chromatin was cut into individual pieces. After a centrifugation step for 15 min at 13,000 g the supernatant was collected and placed in a collection tube diluted with 0,1% SDS.

2.2.14.3 Chromatin-Immunoprecipitation (ChIP)

For the chromatin-immunoprecipitation, sepharose beads were washed once with lysis buffer II and centrifugated at 1,800 g for 2 min. After the supernatant had been removed, the sepharose beads were blocked with 1 mg/ml BSA and 40 µg salmon sperm DNA at 4°C overnight. The beads were centrifugated at 1,800 g for 5 min and washed with 5 ml lysis buffer II. Subsequently, two preclearing steps with sepharose- and IgG-coupled beads were applied to the chromatin to remove unspecifically to sepharose beads (preclearing I) and IgG antibodies (preclearing II) bound chromatin. The purified chromatin was placed in a new tube and one tenth of the chromatin volume was removed as input control. Antibodies against acetylated histone H4 (H4ac; Milipore) and acetylated histone H3 (H3ac, Milipore) were added and incubated overnight at 4°C. As negative control, an

IgG sample (0.5 µg; Abcam) was prepared. 30 µl of sepharose beads were added to each sample and incubated for 2 hours at 4°C. After centrifugation at 8,000 g for 2 minutes, seven washing steps and adding TE buffer, the supernatant was discarded and 500 µl of the elution buffer was added to the sepharose beads. The solution was vortexed, incubated for 30 min at room temperature and centrifugated again at 8,000 g for 2 min. The supernatant containing the precipitated chromatin was placed in a new collection tube. To undo the cross-linking of DNA and histones the following mixture was applied to the suspension: 20 µl of 5 M NaCl, 10 µl of 0.5 M EDTA (pH 8.0), 20 µl of 1 M Tris (pH 7.2), 1 µl of proteinase K (20 mg/ml; Sigma-Aldrich) and 1 µl of RNase A (10 mg/ml; Sigma-Aldrich). After overnight incubation, the DNA was purified using the QIAquick PCR purification kit (Qiagen) as recommended by the manufacturer.

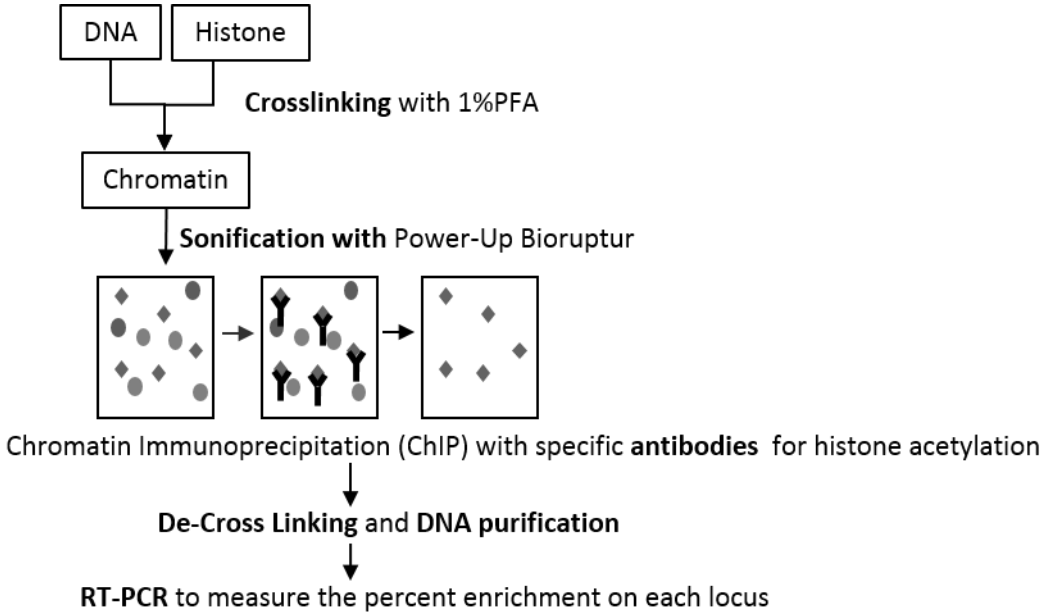

Figure 6: **Chromatin-Immunoprecipitation (ChIP) of $CD4^+CD62L^+$ naïve T cells**

After the crosslinking of DNA-proteins in isolated $CD4^+CD62L^+$ T cells with histones in presence of 1% PFA, chromatin was crushed in individual pieces by sonification with the power-up bioruptur (Diagenode). Antibodies against both histones H3 and H4 were added, and the supernatant containing the precipitated chromatin was collected. The DNA were isolated from the histones and purified using the QIAquick PCR purification kit (Qiagen). Further RT-PCR analyses were performed to analyze the percent enrichment of histone acetylation on different promoter regions.

2.2.14.4 qRT-PCR of histone acetylation

The purified DNA was amplified by using quantitative RT-PCR to measure the percentage of enrichment of histone acetylation on the following promoter regions: IL-4, IL-5, IFN-γ, IL-17, FoxP3 and RORγT. Primer sequences are shown in section 2.1.9.3. All

amplifications were performed in duplicates by using 2 µl of DNA per reaction. To calculate the exact enrichment of the samples the input control (10%) was subtracted first. In particular, the dilution factor of 10 (log_2 of 10 = 3,3) was subtracted from the cycle threshold (Ct) value of the diluted input control. Subsequently, the percentage of enrichment of the negative control (IgG) was subtracted from this value.

$$\% \text{ enrichment} = 100 \times 2^{(CTinput-3,3)-CTsample}$$

2.2.14.5 ΔΔCT-method to analyze the percent enrichment of histone acetylation on each promoter region

The delta-delta-CT method is a convenient method to analyze the relative changes in gene expressions. The negative control and samples were normalized to the housekeeping gene (RPL32). Since housekeeping genes are expressed constantly in all cells of an organism, they are applied to minimize the variations caused by sample handling. The normalization of the samples was performed according to the following formula (Livak et al., 2001):

$$\text{normalized enrichment to gene of interest} = \frac{\% \text{ enrichment to gene of interest}}{\% \text{ enrichment to HKG}}$$

2.2.15 **Statistics**

Statistical differences were analyzed by using GraphPad Prism software 5 (San Diego, CA, USA). One-Way ANOVA or Student's t-test were used when data were normally distributed, or elsewise the Mann-Whitney test when data were not normally distributed, respectively. Normal distribution was assessed by d'Agostino and Pearson omnibus normality test.

3 Results

3.1 Influence of maternal schistosomiasis on the offspring's immune cell composition

The concept that schistosomiasis during pregnancy may alter the offspring's immune status has been studied over a long period of time (Lewert et al., 1969; Zhao et al., 2013). There is evidence that the fetal immune system is already *in utero* imprinted by the maternal helminth infection. Epidemiological studies have revealed that newborn offspring born to mothers with schistosomiasis expressed schistosome specific IgM and IgE antibodies and increased percentages of CD5 B cells in their cord-blood cells (Novato-Silva et al., 1992; Seydel et al., 2012). In this study, the composition of several immune cell subsets to both innate and adaptive immune responses following allergic respiratory inflammation and chronic schistosomiasis were examined in naïve offspring and offspring born to schistosome infected mothers. In addition to the examined frequency of immune cells in peripheral blood and spleen cells, different developmental stages of B and T cells were investigated in thymus and bone marrow to get an overview of possible alterations within the development of immune cells.

3.1.1 **Changes in innate and adaptive immune cell composition**

The maternal infection with schistosomiasis during pregnancy may lead to an altered frequency of different immune cell subsets and/ or altered functionality. In cooperation with Dr. Thure Adler, Helmholtz Zentrum Munich, the percentage of granulocytes, NK cells as well as T and B cells were examined. Granulocytes were studied due to their crucial part in inflammation and essential role in allergies, natural killer cells due to their role as mediators of innate responses and their contribution to the development of adaptive immunity. Additionally, T and B cells were examined as main mediators of cellular and humoral immune responses in allergies and schistosomiasis, with a specific focus on T cell subsets as a critical predictor for the severity of allergic responses: inflammatory cytokine secreting $CD4^+$ effector T cells activated by allergens, and $CD8^+$ effector T cells playing a causative role in IgE-mediated allergic inflammation. It is widely accepted that the frequency of precursor cells is a critical parameter for the magnitude of a T cell response (Obst, 2015); therefore, different T cell subsets such as naïve, effector, memory and regulatory T cells were examined. Naïve T cells phenotyped by their surface markers

$CD4^+/CD62L^+$ or $CD8^+/CD62L^+$ were examined due to their major role in antigen recognition and subsequent differentiation in effector T cells ($CD4^+/CD44^+$ or $CD8^+/CD44^+$) by upregulating the expression of CD44, the classical marker of activation. Upon activation of effector T cells, antigen-experienced T cells are established as memory cells characterized by the surface expression of $CD4^+CD62L^+CD44^+$ or $CD8^+CD62L^+CD44^+$. Regulatory T cells ($CD4^+/CD25^+$ or $CD8^+/CD25^+$ T cells) were examined due to their role of suppressing overwhelming immune responses in schistosomiasis and their 'dual' functionality in allergic diseases: Treg cell are essential to initiating tolerance to allergen peptides and on the other hand their proallergic phenotype resulting in aggravated allergic disease (Noval Rivas et al., 2016). In detail, the subpopulations of leukocytes from spleen and peripheral blood were analyzed by flow cytometry based on multi-parameter staining panels. Cell populations were detected in accordance with the gating strategy shown in Fig. 7 and included markers for B cells (CD19, B220), T cells (CD3, CD5), granulocytes (CD11b, Ly6G or GR-1 high), NK cells (NK1.1, NKp46), and the following T cell subsets: naïve T cells ($CD4^+/CD62L^+$ or $CD8^+/CD62L^+$), effector T cells ($CD4^+/CD44^+$ or $CD8^+/CD44^+$), memory T cells ($CD4^+CD62L^+/CD44^{++}$ or $CD8^+CD62L^+/CD44^{++}$) and regulatory T cells ($CD4^+/CD25^+$ or $CD8^+/CD25^+$ T cells). As a result, offspring of *S. mansoni* infected mothers showed distinct differences in the innate immune cell composition with an increased percentage of granulocytes in peripheral blood (Fig. 8A) and decreased numbers of NK cells in splenocytes (Fig. 8B). However, the percentages of granulocytes in spleen and NK cells in peripheral blood showed no differences. Interestingly, the percentage of B (CD19, B220) and T cells (CD3, CD5) was not altered (Fig. 8A, B). Regarding the adaptive immunity, naïve T cells ($CD8^+/CD62L^+$ and $CD4^+/CD62L^+$) showed higher frequencies in peripheral blood in offspring born to schistosome infected mothers compared to naïve offspring (Fig. 8C, E). Regulatory T cells as important negative regulators of adaptive immunity, effector T cells and memory T cells showed no differences in numbers in spleen and peripheral blood. Also, the ratio between T helper cells ($CD4^+$) and cytotoxic T cells ($CD8^+$) showed no alterations. The normal ratio in humans and mice is 2:1, and an altered ratio can indicate immunodeficiency or autoimmunity. Taken together, the data indicated that the perinatal exposure to *Schistosoma mansoni* altered the immune cell composition in offspring by variations of frequencies within the immune cell composition: offspring of schistosome infected mothers clearly showed alterations within the numbers of innate and adaptive immune cells by

increased levels of granulocytes (Fig. 8A), decreased numbers of NK cells (Fig. 8B) and higher percentages of naïve T cells (Fig. 8C, E).

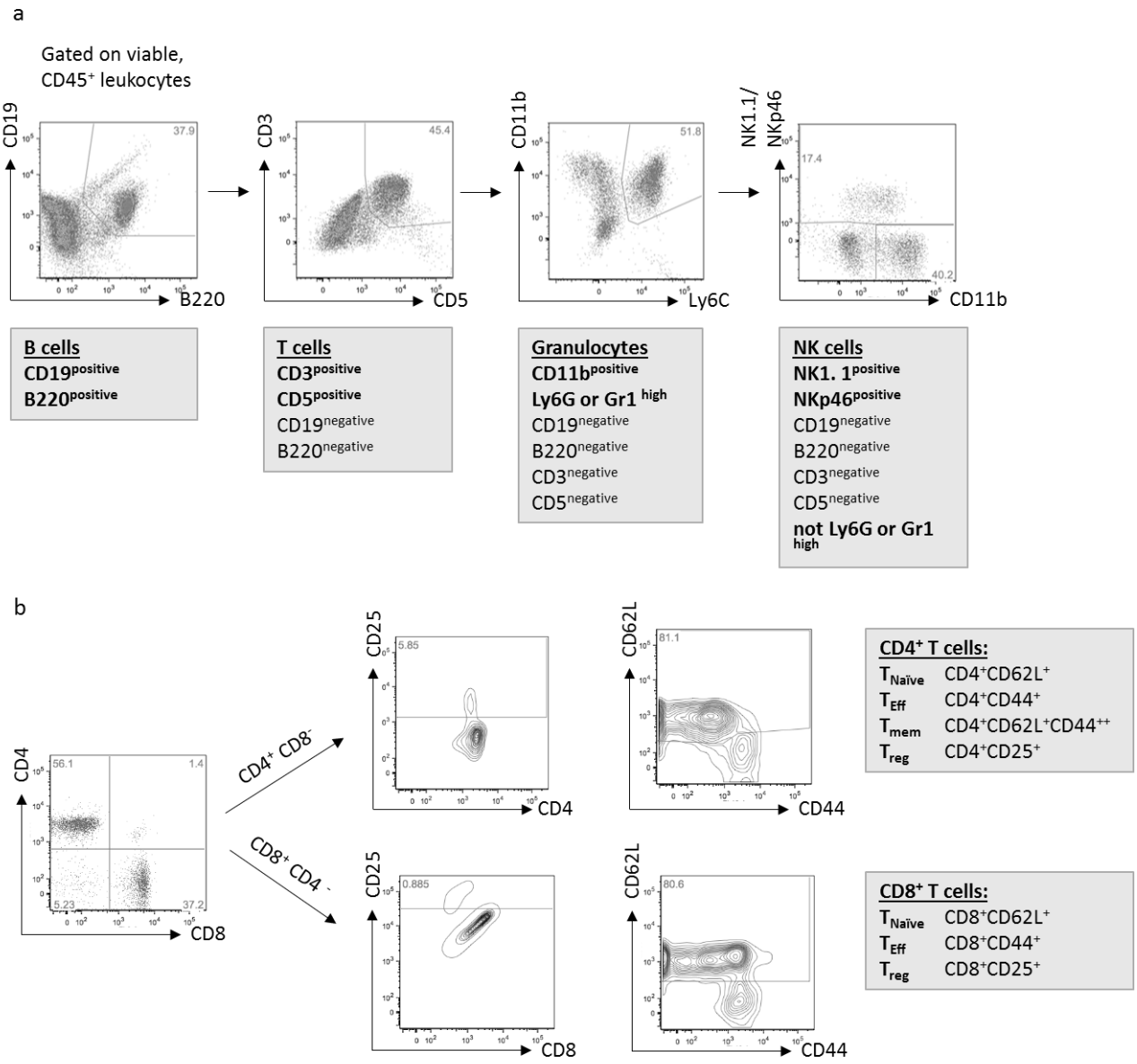

Figure 7: **Determination of frequencies of main leukocytes and T cell subsets by flow cytometry**

The multi-parameter staining panel included markers for B cells (CD19, B220), T cells (CD3, CD5), granulocytes (CD11b, Ly6G or GR-1 high) and NK cells (NK1.1, NKp46) shown in Fig. 7A. Further T cell subsets were divided in CD4+CD8- and $CD8^+CD4^-$ cells. T cell subsets were defined as followed: naïve T cells ($CD4^+/CD62L^+$ or $CD8^+/CD62L^+$), effector T cells ($CD4^+/CD44^+$ or $CD8^+/CD44^+$), memory T cells ($CD4^+CD62L^+/CD44^{++}$ or $CD8^+CD62L^+/CD44^{++}$) and regulatory T cells ($CD4^+/CD25^+$ or $CD8^+/CD25^+$) shown in Fig. 7B.

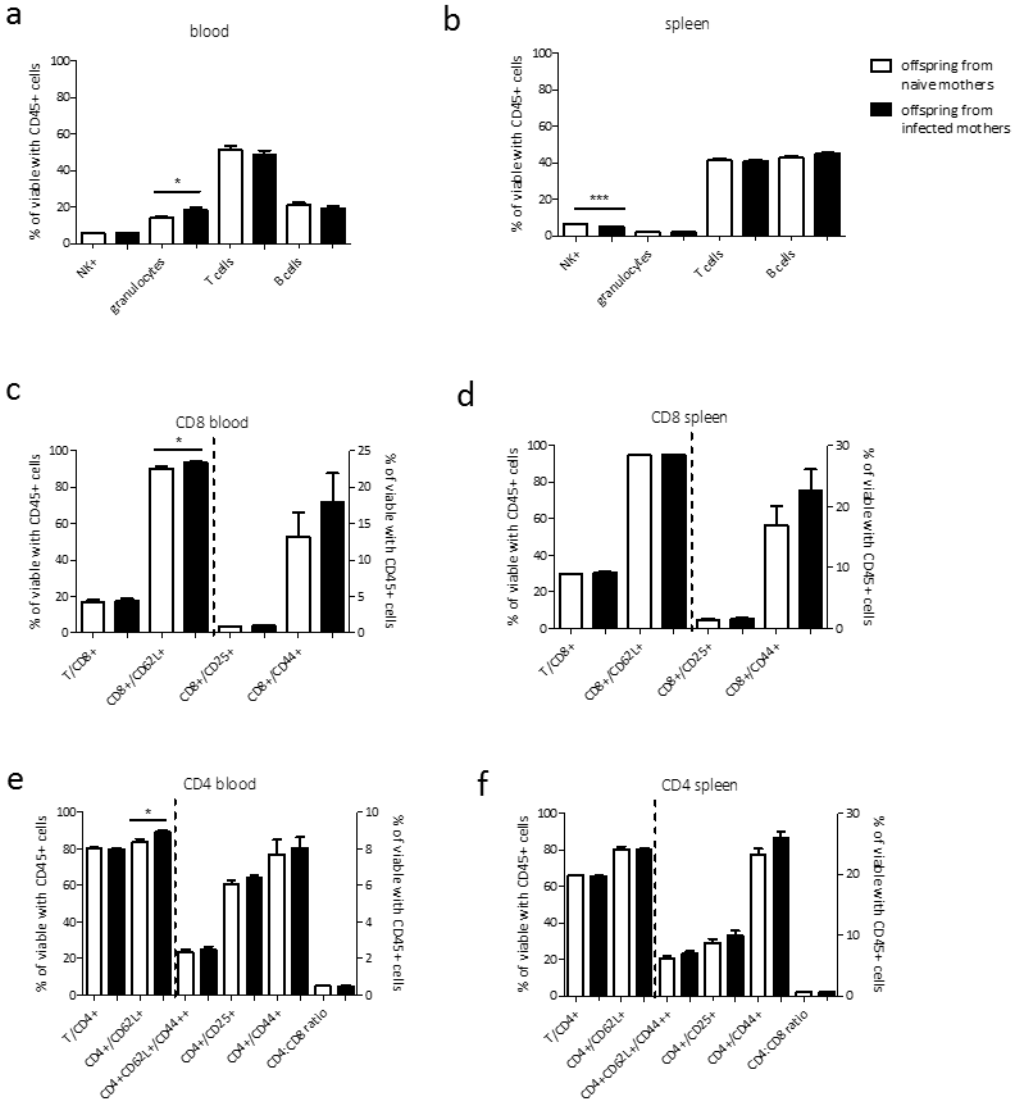

Figure 8: **Maternal infection with *S. mansoni* leads to an altered immune cell composition in peripheral blood and spleen.**

Main leukocyte subsets of adult offspring born to naïve and infected mothers were analyzed by flow cytometry based on multi-parameter staining panels in peripheral blood (Fig. 8A) and spleen (Fig. 8B). In peripheral blood, T cells were further divided into $CD8^+$ (Fig. 8C) and $CD4^+$ (Fig. 8E) T cell subsets. Splenic T cell samples were further separated into $CD8^+$ (Fig. 8D) and $CD4^+$ (Fig. 8F) T cells. Naïve T cells were defined as $CD4^+/CD62L^+$ or $CD8^+/CD62L^+$, effector T cells as $CD4^+/CD44^+$ or $CD8^+/CD44^+$, memory T cells as $CD4^+CD62L^+/CD44^{++}$ or $CD8^+CD62L^+/CD44^{++}$, and regulatory T cells as $CD4^+/CD25^+$ or $CD8^+/CD25^+$ T cells. Data are pooled from three independent experiments and shown as mean ± SEM with $n \geq 4$ offspring per experiment (offspring: n = 16 with naïve versus 20 with infected mothers, mothers: n = 9 naïve versus 7 infected mothers). Asterisks show statistical differences indicated by brackets. (* $p<0.05$, *** $p<0.001$). Statistical differences were obtained after using the parametric Student's t test in case of normal distribution and the Mann-Whitney test when data were not normally distributed, respectively.

3.1.2 **Unmodified development of B and T cells from thymus and bone marrow**

The distinct differences within the composition of mature immune cells (shown in Fig. 8A, C, E) in offspring of schistosome infected mothers have led to the assumption that the early development of B and T cell might also be affected by the *in utero* exposure to maternal schistosomiasis. Therefore, the basic developmental stages of B and T cells in adult offspring of naïve and Reg-phase infected mice were analyzed. Interestingly, the total numbers of peripheral T and B cells remain lifelong constant in mammalian organisms (Reed et al., 2005), in case of T cells due to their thymic production (Berzins et al., 1998) and self-renewal (Sprent et al., 2003), and, in case of B cells due to their proliferation from hematopoietic stem cells (HSC) in the bone marrow (Carsetti, 2000). This stability of precursor cells implicates a constant proportion of differently staged cells in the thymus for T cells and in the bone marrow for B cells which can be examined by flow cytometry. In the thymus, T cell progenitors, defined as CD4 and CD8 double negative cells DN, differentiate to immature $CD4^+$ and $CD8^+$ double positive (DP) thymocytes. The lineage-specific differentiation of immature double positive cells into single positive (SP) $CD4^+$ or $CD8^+$ thymocytes is regulated by somatically generated T cell receptors (TCRs). Mature cells are finally released to peripheral blood as T helper cell or cytotoxic T cell precursors (Pardoll et al., 1987). Importantly, the frequency of DP capable of expressing TCR clonotypes dictates the steady-state size of the peripheral CD4 cell compartment and its potential for homeostatic proliferation (Reed et al., 2005). B cell precursors pass through defined stages of development. RAG-dependent rearrangement of V, D, and J heavy-chain gene segments starts in the pro-B cell stage, which can be identified by the surface marker of B220 and CD43, as well as IgM negativity ($B220^+CD43^+IgM^-$). After the rearrangement of immunoglobulins, µ-heavy chains are expressed, and the B cell precursors develop further in pre-B cells which are CD43 negative ($B220^+CD43^-IgM^-$). The immunoglobulin gene rearrangement leads to the expression of a mature B cell receptor (BCR) that can bind an antigen. These immature B cells express a complete IgM molecule on their surface ($B220^{low}IgM^+$). After the loss of self-reactivity of the B cell receptor, IgM^+ immature B cells leave the bone marrow and become mature follicular B cells ($B220^{high}IgM^+$) (Eibel, 2015). One can assume that the slightest shift in the developmental stages of B and T cells might lead to unbalancing the peripheral CD4 and CD8 T as well as B cell composition. The following experiments were performed in cooperation with Dr. Rouzanna Istvanffy, III. Medizinische Klinik und Poliklinik, Klini-

kum Rechts der Isar, Technische Universität München. In brief, thymocytes were prepared from isolated thymuses, while bone marrow cells were flushed from tibias and femurs of eight-week-old offspring with naïve and schistosome-infected mothers. To separate hematopoietic populations, a flow cytometric analysis with the following markers for the T cell development was performed: T cell progenitors are defined as DN ($CD4^{-}CD8^{-}$) cells, cells in the intermediate developmental stage are defined as DP ($CD4^{+}CD8^{+}$) and mature cells are defined as single positive SP $CD4^{+}$ and SP $CD8a^{+}$. The different developmental stages of B cell are defined as immature B ($B220lowIgM^{+}$), mature B cells ($B220highIgM^{+}$), pro- and pre–B cells ($B220^{+}IgM^{-}$) which were further divided into pro-B cells ($B220^{+}CD43^{+}IgM^{-}$) and pre-B cells ($B220^{+}CD43^{-}IgM^{-}$). Cell acquisitions were detected in accordance with gating strategy shown in Fig 9C, D. Our data showed no alterations in the frequency of different developmental subsets within the T cell compartment in the thymus (Fig. 9A) nor within the B cell compartment in the bone marrow (Fig. 9B) in offspring with maternal schistosomiasis compared to naïve offspring (Klar, Perchermeier et al., 2017). Taken together, despite the findings of the altered frequencies within the immune cell composition (Fig. 8A, C, E), the percentages of B and T cell progenitor cells were not changed (Fig. 9A, C).

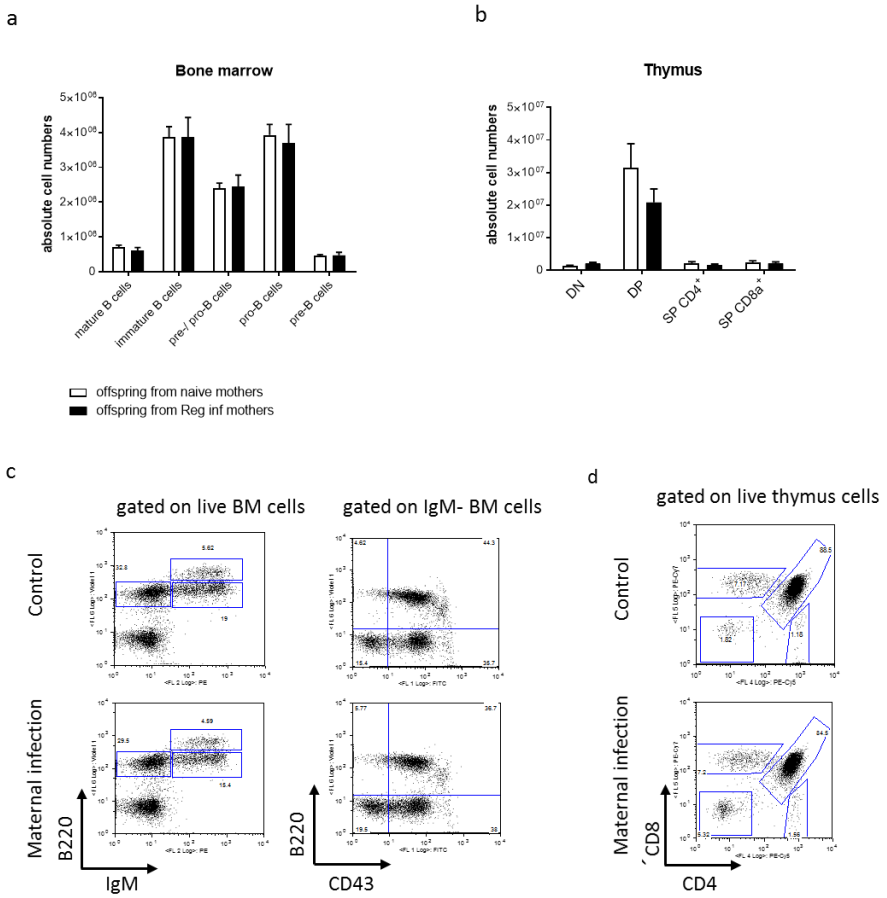

Figure 9: **Chronic maternal *S. mansoni* infection does not alter the composition of offspring's B-and T-cell compartment.**

Bone marrow and thymic cells of offspring of BALB/c mice infected with *S. mansoni* or naïve mothers were analyzed by flow cytometry. B cell subsets in the bone marrow were defined as followed: immature B (B220lowIgM$^+$); mature B cells (B220highIgM$^+$); pro- and pre–B cells (B220$^+$IgM$^-$); pro-B cells (B220$^+$CD43$^+$IgM$^-$) and pre-B cells (B220$^+$CD43$^-$IgM$^-$) (Fig. 9A). Gating strategy is shown in Fig. 9C. T cell progenitors were defined as DN (CD4$^-$CD8$^-$) cells, cells in the intermediate developmental stage as DP (CD4$^+$CD8$^+$) and, mature cells as SP CD4+ and SP CD8a+ (Fig. 9B). Gating strategy of T cell precursor cells shown in Fig. 9D. Representative results of one of three independent experiments were shown as mean ± SEM with n ≥ 4 offspring per experiment (offspring: n = 15 of naïve vs. 19 of infected mothers, mothers: n = 5 naïve vs. 4 infected mothers).

3.2 Impact of maternal schistosomiasis on offspring's T cell differentiation capacity

Chronic allergic airway inflammation is often considered a result of abnormal activation of T_H2 cells towards harmless environmental allergens (Thiriou et al., 2017). The dis-

turbed balance between type 1 and type 2 inflammation is one of the tenets in the development of allergic diseases, since dominant T_H2 cells activate basophils, eosinophils, B cells and mast cells which are responsible for the typical asthmatic symptoms (Woodfolk, 2007). One can assume that the differentiation capacity of naïve T cells into T_H1 and T_H2 cells might be the decisive mechanism why offspring of chronic schistosome infected mothers showed decreased symptoms of allergies (Straubinger et al., 2014). Maternal stress has been identified as a contributing factor to alter the T cell differentiation and be a potential risk factor of postnatal allergic disease (von Hertzen, 2002). There is also increasing evidence that prenatal T-cell priming of the fetal immune system can occur via trans-placental exposure to the helminth derived antigens and such primary immune sensitization can affect the appropriate maturation of the postnatal immune responses (LaBeaud et al., 2009). Thus, besides the increased frequencies of naïve $CD4^+$ and $CD8^+$ T cells in offspring born to schistosome infected mothers (shown in Fig. 8C, E), their capacity to differentiate into T_H1 or T_H2 cells might also be affected.

3.2.1 **Efficiency and purity of $CD4^+CD62L^+$ MACS sorted naïve T cells**

Purity of $CD4^+CD62L^+$ naïve T cells, isolated from splenocytes by magnetic activated cell sorting (MACS), was determined by flow cytometry, to ensure comparable portions. After cell acquisition with the Calibur II Cytometer (BD Biosciences) cell populations were detected in accordance with the gating strategy shown in Fig. 10A. In comparison to unsorted splenocytes, the portion of $CD4^+CD62L^+$ T cells could be increased from 12,6% to 86,4% by MACS (Fig. 10B).

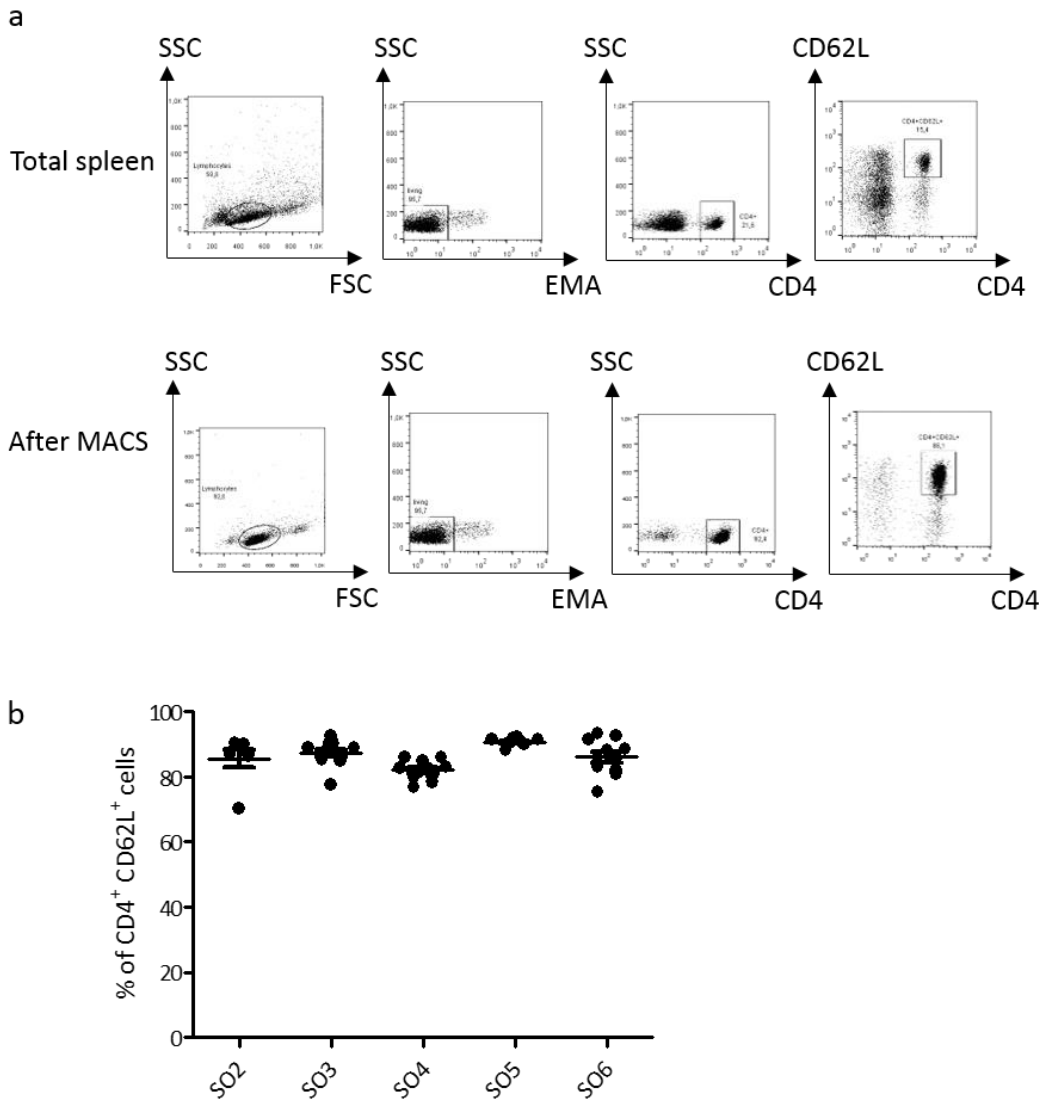

Figure 10: **Gating strategy for flow cytometric analysis and efficiency analysis of MACS sorted CD4$^+$CD62L$^+$ T cells**

To evaluate the efficiency of magnetic activated cell sorting, MACS sorted naïve CD4$^+$CD62L$^+$ T cells and splenocytes were analyzed by flow cytometry. The analysis covered markers for living CD4$^+$ and CD62L$^+$ cells as shown in Fig. 10A. To examine the purity of the CD4$^+$CD62L$^+$ sorted T cell population five independent experiences (SO2-SO6) were performed and shown as mean ± SEM with n ≥ 7 mice per experiment (Fig. 10B).

3.2.2 **Impaired T_H2 cell differentiation capacity**

To test the possible influence of maternal schistosomiasis on the offspring's T cell differentiation, CD4$^+$CD62L$^+$ naïve T cells from splenocytes of offspring of infected and naïve mothers were differentiated *in vitro* into T_H1 and T_H2 cells. Expression and protein levels

of cytokines and transcription factors were measured by RT-PCR, ELISA and intranuclear and intracellular staining (ICS). The gating strategy for the transcription factor GATA-3 and T_H2-related cytokine IL-4 is shown in Fig. 11B. To differentiate T_H2 cells, naïve T cells were incubated with IL-2, α-IFN-γ, α-IL-12, IL-4 and αCD3/αCD28 T cells beads for 7 days. T_H2 differentiated cells of offspring of infected mothers showed reduced expression of the T_H2-related transcription factor GATA-3 measured by RT-PCR (Fig. 12A) in comparison to T_H2 differentiated cells of naïve offspring. In accordance, T_H2 differentiated T cells of offspring of infected mothers showed a significantly lower release of IL-4 cytokine measured by ELISA (Fig. 12B). This was also reproduced by a short-term PMA/Ionomycin stimulation and measured by intracellular FACS staining. Here, IL-4 producing cells were significantly reduced in offspring with maternal infection, whereas the transcription factor GATA-3 showed no differences (Fig. 12C). (Klar, Perchermeier et al., 2017)

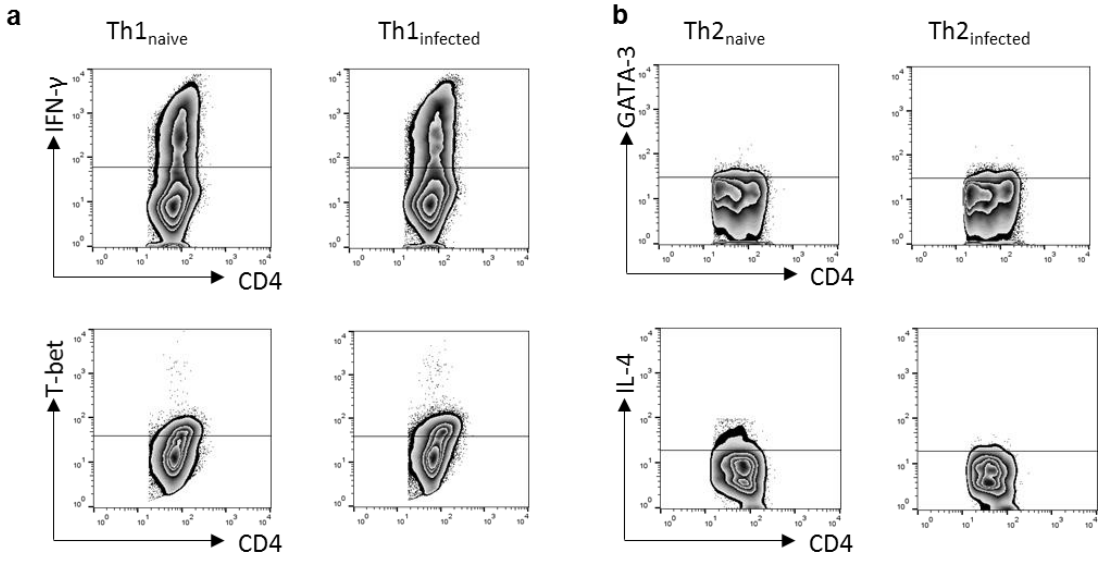

Figure 11: **Gating strategy and representative FACS plots for CD4$^+$ T_H1 and T_H2 differentiated cells.**

Representative contour plots of T_H1 and T_H2 differentiated CD4$^+$CD62L$^+$ T cells of offspring of infected and naïve mothers gated on the expression of T_H1-related cytokine IFN-γ and transcription factor t-bet (Fig. 11A) and T_H2-related cytokine IL-4 and transcription factor GATA-3 (Fig. 11B) after stimulation with 750ng/ml Ionomycin, 50ng/ml PMA and 10µg/ml BFA for 4,5h before staining.

3.2.3 **Stronger T_H1 cell differentiation capacity**

For the *in vitro* differentiation of T_H1 cells, naïve T cells were incubated with IL-2, α-IL-4, IL-12, IFN-γ and αCD3/αCD28 T cells beads for 3 days. T_H1 differentiated cells showed a significantly higher expression of the transcription factor t-bet observed by RT-

PCR (Fig. 12D) in offspring of infected mothers compared to naïve offspring. In accordance, stimulation of these T_H1 cells with PMA/Ionomycin revealed a stronger ability to produce the transcription factor t-bet (Fig. 12F). In addition, the T_H1 differentiation capacity was further tested by the release of IFN-γ by ELISA and intracellular staining. Here, IFN-γ was significantly stronger in offspring with maternal infection measured by ELISA (Fig. 12E), whereas the expression of IFN-γ after PMA/ Ionomycin stimulation was not affected (Fig. 12F). In summary, offspring of *S. mansoni* infected mothers clearly showed a stronger capacity to differentiate into T_H1 cells, while the differentiation into T_H2 cells was impaired in comparison to offspring of naïve mothers. (Klar, Perchermeier et al., 2017)

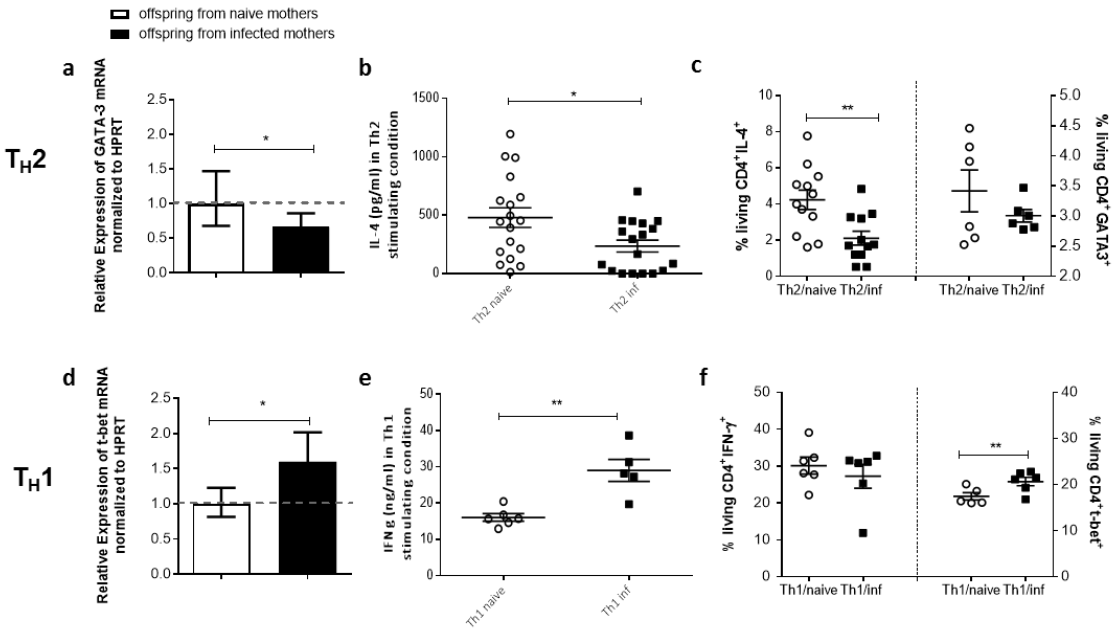

Figure 12: **Maternal *S. mansoni* infection leads to stronger T_H1 and impaired T_H2 cell differentiation of $CD4^+CD62L^+$ T cells.**

MACS-sorted naïve $CD4^+CD62L^+$ T cells from the spleen of offspring of naïve and chronic schistosome infected mothers were differentiated into T_H1 and T_H2 cells. Expression and protein levels of cytokines and transcription factors were measured by RT-PCR, ELISA, intranuclear, and intracellular staining (ICS). Shown is the gene expression of transcription factor t-bet (T_H1) and GATA-3 (T_H2) relative to the naïve offspring. Statistical differences were obtained after using REST software (Fig. 12A, D). Cytokine levels of IFN-γ (Fig. 12E) and IL-4 (Fig. 12B) under T_H1 and T_H2 differentiating conditions are detected by ELISA and shown after subtracting the corresponding cytokine levels of undifferentiated T cells. Frequencies of $CD4^+$ effector cells were analyzed by ICS for IFN-γ, t-bet (T_H1) (Fig. 12F), IL-4 and GATA-3 (T_H2) expression levels (Fig. 12C). Asterisks show statistical differences indicated by brackets. (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). Statistical differences were obtained after using the parametric Student's t test in case of normal distribution and the Mann-Whitney test when data were not normally distributed. Data shown as mean ± SEM depicting one of two representative experiments (Fig. 12A, E, F) or pooled data from two experiments with similar outcomes (Fig. 12B, C, D). Each experiment was conducted with offspring: $n \geq 5$, mothers: $n \geq 3$ per group.

3.3 Altered histone acetylation pattern of cytokine promoter regions within the $CD4^+CD62L^+$ naïve T cell compartment

As a potential underlying mechanism for the observed differences in T cell differentiation, epigenetic modifications such as DNA methylation and histone acetylation were worth considering. The accessibility of chromatin strongly influences the transcription

rate and thus the possible pathways of differentiation (Avni et al., 2002; Fields et al., 2002). It is widely accepted that the commitment toward an allergic phenotype is tightly regulated by DNA methylation and histone acetylation at the T_H2 locus control region (Bégin et al., 2014). In this study, histone acetylation pattern of naïve $CD4^+CD62L^+$ T cells on six different promoter regions, which are involved in the development of allergic airway inflammation, were assessed in naïve offspring and in progeny with maternal infection. The promoter region of IL-4 and IL-5 were examined due to their important role in stimulating eosinophils and B cells, IFN-γ as counterpart to the genesis of allergic airway inflammation, and IL-17 and the T_H17-related transcription factor RORγT due to their determination of the severity in asthma. Moreover, the transcription factor of regulatory T cells FoxP3 were examined due to their role of immunomodulatory effects. The chromatin-immunoprecipitation (ChIP) of $CD4^+CD62L^+$ naive T cells was performed in cooperation with Dr. Hani Harb, Institute of Laboratory Medicine, Pathobiochemistry and Molecular Diagnostics, Philipps University Marburg, Marburg, Germany. In detail, after the accomplishment of the ChIP assay of naïve $CD4^+CD62L^+$ T cells, RT-PCRs of histone H3 and H4 acetylation were performed to measure the percentage of enrichment of acetylation on each promoter region. The data gained showed a significant decrease of histone H4 acetylation at the promoter region of IL-4 (Fig. 13A) and IL-5 (Fig. 13B), and, in contrast, an increase of histone H4 acetylation at the promoter region of FoxP3 (Fig. 13E) and RORγT (Fig. 13F) in offspring of infected mothers in comparison to naïve offspring. However, no differences of the acetylation status on histone 3 were detectable. Additionally, H3 and H4 acetylation were not altered on the promoter regions of IFN-γ (Fig. 13C) nor IL-17 (Fig. 13D). The data clearly revealed that maternal schistosomiasis modified the acetylation patterns of histone H4 at the promoter regions of IL-4 and IL-5 by reducing acetyl groups, and at FoxP3 and RORγT by increasing them. (Klar, Perchermeier et al., 2017).

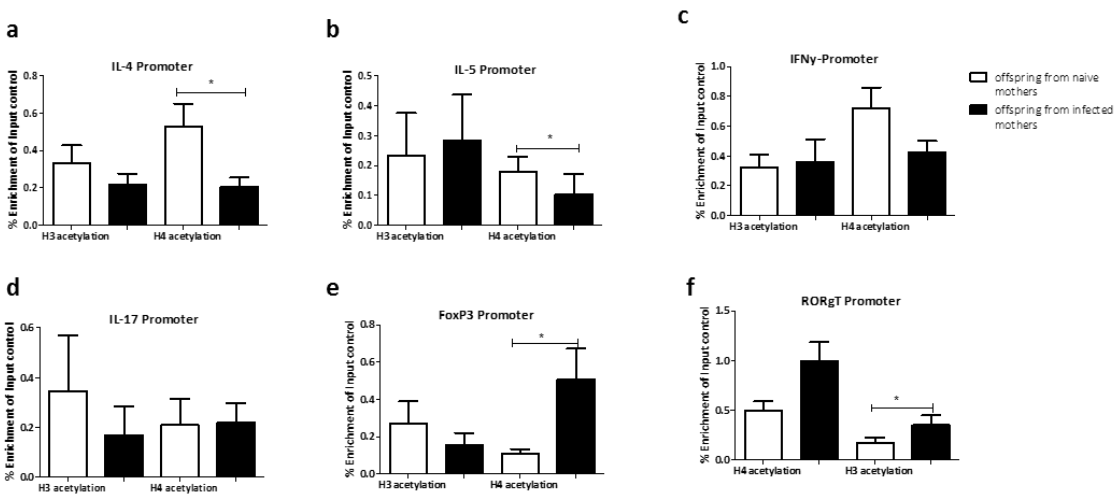

Figure 13: **Maternal *S. mansoni* infection influences the offspring's histone acetylation within the naïve T cell compartment.**

$CD4^+CD62L^+$ MACS or MoFlow sorted splenocytes from offspring with maternal infection and naïve mothers were analyzed by ChIP assay. Histone H3 and H4 acetylation and RT-PCR were performed to assess the percent enrichment at the promoter regions of IL-4 (Fig. 13A), IL-5 (Fig. 13B), IFN-γ (Fig. 13C), IL-17 (Fig. 13D), FoxP3 (Fig. 13E) and RORγT (Fig. 13F). Results were pooled from three independent experiments and depicted as mean ±SEM. Statistical significances between the indicated groups (offspring: n = 16 of naïve vs. 16 of infected mothers, mothers: n = 9 naïve vs. 6 infected mothers) were obtained after using the parametric Student's t test in case of normal distribution and the Mann-Whitney test when data were not normally distributed (IL-5, FoxP3). (* $p<0.05$).

3.4 Detection of spontaneous IL-4 production within the central memory T cell compartment

3.4.1 No functional impairment of antigen-dependent and independent reactivity

Besides the effects on early immune cell composition and the diverse differentiation ability of naïve T cells into T_H1 or T_H2 cells, the exposure to schistosoma antigens during late pregnancy or of the newborn through breast feeding might influence the cytokine secretion of T cells, the T helper profile and the intensity of respective immune responses in the offspring of infected mothers. To test the hypothesis that offspring of schistosome infected mothers show T_H1 or T_H2 biased immune responses under nonspecific or parasite-specific stimulation, cytokine production of splenocytes and mesenteric lymph node

cells were analyzed *ex vivo*. For this, cells were either incubated in complete medium for 48h without any stimulation to examine the spontaneous cytokine production *ex vivo* or stimulated with αCD3/αCD28 T cell beads to characterize their T cell driven immune response by antigen-independent activation. Also, due to the possible mother-to-child transfer of parasite specific antibodies via the placenta-blood barrier and breast milk (Attallah et al., 2003), cells were incubated with schistosome specific antigen, the so-called soluble egg antigen (SEA), to detect schistosome-antigen specific cells in the progeny and their individual cytokine response under specific antigen stimulation. In detail, $4x10^5$ splenocytes or mesenteric lymph node cells from Reg phase offspring and naïve mice were stimulated with culture medium only (unstimulated), SEA (antigen-dependent stimulation) or αCD3/αCD28 T cell beads (antigen-independent stimulation) for 48 hours. Cytokine levels of IFN-γ (Fig. 14A), IL-4 (Fig. 14B), IL-13 (Fig. 14C), IL-5, and IL-10 (not detectable, data not shown) were quantified by ELISA. Here, offspring of infected mothers showed a significantly higher, spontaneous IL-4 production *ex vivo* under unstimulated conditions compared to naïve offspring; however, on a very low level (Fig. 14B). In contrast, the stimulation with αCD3/αCD28 T cell beads significantly increased the splenic IL-4 production in naïve mice compared to unstimulated cells, while the additional stimulation with αCD3/αCD28 T cell beads in offspring with maternal schistosomiasis did not lead to an increase in IL-4 secretion (Fig. 14B). Cytokine levels of IFN-γ and IL-13 were not altered at all (Fig. 14A, C). Interestingly, naïve mice and offspring with maternal infection did not produce different cytokine levels under stimulation with schistosome-specific antigens (SEA) in splenocytes and mesenteric lymph nodes (Fig. 14A-E). Of note, no differences in IFN-γ (Fig. 14D) and IL-4 (Fig. 14E) cytokine levels of mesenteric lymph node cells were detectable. In summary, offspring of *S. mansoni* infected mothers showed an increase in splenic IL-4 production *ex vivo*, while cytokine production by splenic and mesenteric lymph node cells was not elevated in response to antigen-dependent nor antigen-independent stimulation, whereas cells of offspring of naïve mothers showed their potential to produce IL-4 upon T cell activation.

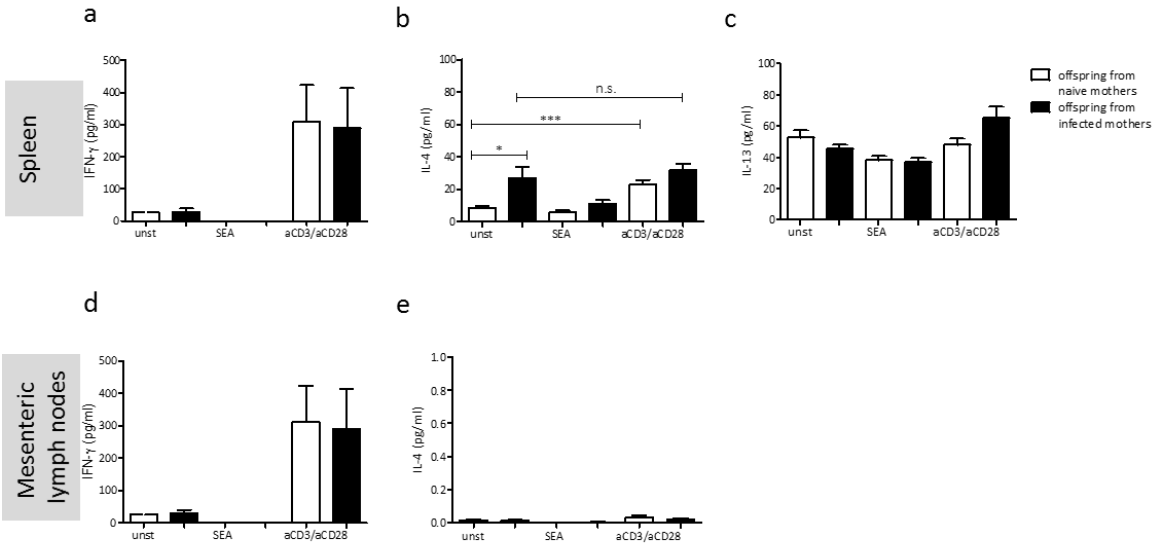

Figure 14: **Splenocytes and mesenteric lymph node cells of offspring with maternal infection show no functional impairment of antigen-dependent and –independent reactivity, but an increased spontaneous IL-4 production *ex vivo*.**

Splenocytes and mesenteric lymph node cells were incubated with culture medium only (unst.), stimulated with 20µg/ml soluble egg antigen (SEA) or 1µg/ ml αCD3/αCD28 T cell beads (αCD3/αCD28). Cytokine levels of IFN-γ (Fig. 14A), IL-4 (Fig. 14B) and IL-13 (Fig. 14C) were quantified in splenocytes by ELISA. Cytokine levels of IFN-γ (Fig. 14D) and IL-4 (Fig. 14E) were quantified in mesenteric lymph node cells by ELISA. Results were pooled from three independent experiments and shown as mean ± SEM (offspring: $n \geq 15$ with naive vs. $n \geq 15$ with infected mothers, mothers: $n \geq 5$ naïve vs. 4 infected mothers.) Asterisks show statistical differences indicated by brackets. (* $p<0.05$, *** $p<0.001$). Statistical differences were obtained after using the parametric Student‘s t test in case of normal distribution and the Mann-Whitney test when data were not normally distributed.

3.4.2 Detection of spontaneous IL-4 production within the $CD4^+CD62L^+CD44^+$ memory T cell compartment

The observed spontaneous IL-4 production (Fig. 14B) in offspring of infected mothers was only detectable on a very low cytokine level, using the current protocol. In order to tap and detect the full cytokine-producing potential, a new T cell assay with a longer incubation period (up to 7 days) has been established to obtain optimal cytokine levels and to specify the spontaneous IL-4 production. In detail, $1{,}5x10^6$ MACS sorted $CD4^+CD62L^+$ naïve T cells were cultured with αCD3/ αCD28 T cell beads, IL-2, α-IFN-γ, and α-IL-4 to inhibit the de novo differentiation of naïve T cells. On day 6, cells were stimulated with αCD3/ αCD28 T cell beads for 6 hours and cytokine levels of IFN-γ and IL-4 were quantified by ELISA. Cytokine levels of IFN-γ were measured to exclude more

accurately the possibility that T cells of offspring of mothers with schistosomiasis have a T_H1 biased cytokine profile. Here, data showed no spontaneous IFN-γ production in both groups (Fig. 16B). Interestingly, the IL-4 production was significantly higher in offspring with maternal infection compared to naïve offspring (Fig. 16A). Even though a variety of cells (including non-naïve CD4+ T cells i.e. cytotoxic T cells, regulatory T cells, activated T cells, $CD8^+$ T cells, B cells, NK cells, macrophages, granulocytes, endothelial cells and erythroid cells) were depleted via negative selection by indirect magnetic labelling within the used MACS separation kit, $CD44^+$ cells were maintained. Since memory T cells were not depleted within the $CD4^+CD62L^+$ naïve T cells and CD44 expression usually distinguished murine naïve from memory T cells, the T cell assay was repeated comparing $CD4^+CD62L^+CD44^+$ central memory T cells and $CD4^+CD62L^+CD44^-$ naïve T cells. In detail, $CD4^+CD62L^+$ were separated into $CD44^{pos}$ and $CD44^{neg}$ cells by using flow cytometry according to the gating strategy shown in Fig. 15. $CD4^+CD62L^+CD44^{+/-}$ were cultured with IL-2, α-IFN-γ and α-IL-4 for 7 days. Cytokine levels of IFN-γ and IL-4 were quantified by ELISA. The spontaneous IL-4 production could not be observed in CD44 depleted cells, whereas $CD4^+CD62L^+CD44^+$ central memory T cells of offspring with maternal infection produced significantly higher amounts of IL-4 in comparison to naïve offspring (Fig. 16C). Again, IFN-γ was not detectable in both groups (Fig. 16D). In summary, splenocytes of offspring of schistosomiasis infected mothers showed a spontaneous IL-4 production *ex vivo* (Fig. 16A) compared to offspring of uninfected mothers, which could be further allocated within the central memory T cell compartment (Fig. 16C).

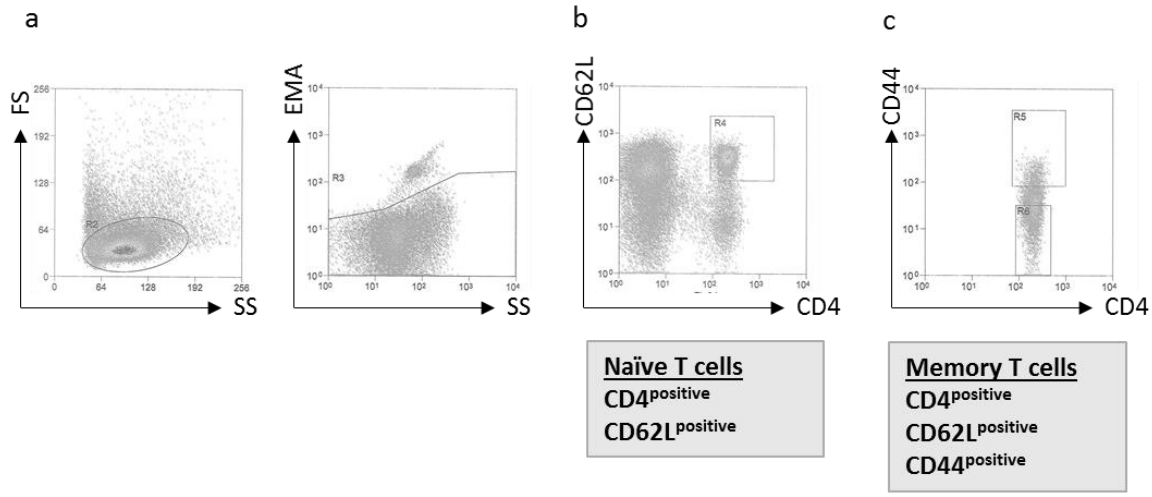

Figure 15: **Gating strategy for flow cytometric sorted $CD4^+CD62L^+$ naïve T cells and $CD4^+CD62L^+CD44^+$ memory T cells.**

Splenocytes of offspring of naïve and infected mothers were sorted by flow cytometry. The staining panel for naïve T cells included the following markers: APC-conjugated anti-CD4 and FITC-conjugated anti-CD62L. Cells were gated on viable lymphocytes (Fig. 15A) and $CD4^+CD62L^+$ sorted naïve T cells were collected (Fig. 15B). To separate CD44 positive and CD44 negative cells, PE-conjugated anti-CD44 antibodies were added (Fig. 15C). $CD4^+CD62L^+CD44^+$ central memory T cells and $CD4^+CD62L^+CD44^-$ naïve T cells were both collected.

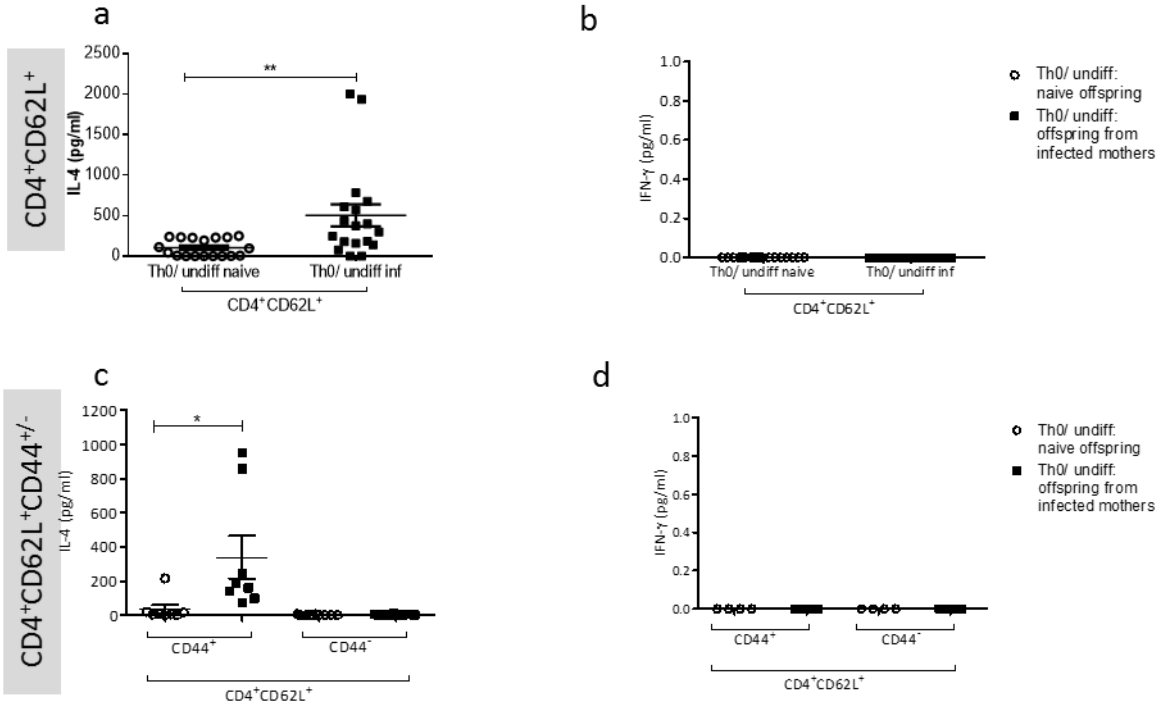

Figure 16: **Spontaneous IL-4 production is comprised within the CD4$^+$CD62L$^+$CD44$^+$ memory T cell compartment.**

CD4$^+$CD62L$^+$MACS sorted splenocytes were stimulated with IL-2, α-IFN-γ, α-IL-4 and αCD3/αCD28 T cell beads for 7 days. After restimulation with αCD3/αCD28 T cell beads for 6 hours, cytokine levels of IL-4 (Fig. 16A) and IFN-γ (Fig. 16B) were measured by ELISA. Results are shown as mean ± SEM and demonstrate pooled data of three independent experiments (offspring: n = 18 of naïve and infected mothers, mothers: n = 11 naïve vs. 8 infected mothers). CD4$^+$CD62L$^+$CD44 ^{positive} or CD44^{depleted} MoFlow sorted cells were stimulated as described above and cytokine levels of IL-4 (Fig. 16C) and IFN-γ (Fig. 16D) were quantified by ELISA. Results demonstrated pooled data of two independent experiments with offspring: n ≥ 7 with naïve and infected mothers, mothers: n = 4 naïve vs. 2 infected mothers. Asterisks show statistical differences indicated by brackets (* $p<0.05$, ** $p<0.01$). Statistical differences were obtained after using the parametric Student‘s t test in case of normal distribution and the Mann-Whitney test when data are not normally distributed.

4 Discussion

4.1 Effects of maternal *S. mansoni* infection on the offspring's immune system

4.1.1 Altered T_H1/ T_H2 cell differentiation by epigenetic modifications

The differentiation and activity of naïve T cells depends, amongst other things, on antigen-presentation, cytokines in the environment and epigenetic modifications. Histone post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination and ADP-ribosylation, are fundamental for the differentiation of naïve T cells into T_H1 or T_H2 cells by modulating the accessibility of DNA and chromatin to the transcriptional machinery (reviewed in Rando et al., 2009). During the differentiation of T_H1 and T_H2 cells the T_H1-related transcription factor T-bet and the T_H2-related transcription factor GATA-3 control lineage-specific histone acetylation of IFN-γ and IL-4 gene loci (Fields et al., 2002). This histone acetylation specifically weakens the interaction between histones and the negatively charged DNA, promoting the transcription rate, thus, the differentiation of naïve T cells towards T_H1 and T_H2 cells, respectively. In contrast, however, the deacetylation of histones catalyzed by histone deacetylases (HDACs) leads to an increased stability of the histone-DNA-complex and, thus, represses transcription. In T_H1 cells IFN-γ promoter regions are hyperacetylated, while in T_H2 cells IL-4 promoter regions are hyperacetylated (Fields et al., 2002). Interestingly, methylation is associated with both: increased and repressed transcription, depending on the methylated loci (Barski et al., 2007; Wang et al., 2008). In previous studies by Straubinger et al. the offspring of chronic *S. mansoni* infected mothers showed protection against OVA-induced allergic airway inflammation (Straubinger et al., 2014). In our study, we hypothesize that this phenotype depends on the (in-)ability of naïve T cells to differentiate into T_H1 and T_H2 cells, respectively. Naïve $CD4^+CD62L^+$ T cells of offspring with schistosome-infected mothers and naïve offspring were cultured under T_H1 and T_H2 conditions (as described in section 2.2.9). Based on the T cell differentiation protocol of Current Protocols in Immunology (Fitch et al., 2006) naïve $CD4^+CD62L^+$ T cells were incubated for 7 days with IL-2, α-IFN-γ, α-IL-12, IL-4 and αCD3/αCD28 for T_H2 cell differentiation and for 3 days with IL-2, α-IL-4, IL-12, IFN-γ and αCD3/αCD28 for T_H1 cell differentiation. Interestingly, in other T cell differentiation protocols (Flaherty et al., 2015), the supply with recombinant IFN-γ for the T_H1 cell differentiation is omitted from naïve T cells. However,

in our studies recombinant IFN-γ was supplied, since it is fundamental to emerge a dominant T_H1 phenotype to detect the subtle differences between the differentiation of naïve T cells of offspring of schistosome-infected and naïve mice. In some protocols the blocking of IL-12 is omitted during the differentiation of T_H2 cells (Flaherty et., al, 2015). In our protocol the use of α-IFN-γ and α-IL-12 was provided to suppress the phenotype of T_H1 cells *completely*. In our studies, offspring of *S. mansoni* infected mothers showed a significantly impaired differentiation of naïve $CD4^+CD62L^+$ T cells into T_H2 cells shown by the reduced production of IL-4 expressing $CD4^+$ T cells (Fig. 12C) and the decreased expression of T_H2 related transcription factor GATA-3 (Fig. 12A). Moreover, the stimulation of T_H2 differentiated cells revealed a reduced production of intracellular IL-4, measured by ELISA (Fig. 12B). In sharp contrast, the maternal infection with *S. mansoni* led to a stronger T_H1 cell differentiation shown by a significantly higher expression of the T_H1 related transcription factor t-bet (Fig. 12D, F) and higher levels of the intracellular cytokine IFN-γ, measured by ELISA (Fig. 12E). However, intracellular IFN-γ staining of $CD4^+$ T cell did not show significant differences (Fig. 12F), possibly due to methodological and technical procedures: the characterization of T_H1 differentiated cells by ELISA required a long-term stimulation with αCD3/αCD28 T beads for 48 hours, whereas cells for the analysis with flow-cytometry were stimulated with PMA/Ionomycin for a short-term only (4.5 hours). Interestingly, the results observed in our T cell differentiation experiments correlated with the epigenetic findings. The histone H4 acetylation at IL-4 and IL-5 promoter regions were significantly decreased (Fig. 13A, B). Decreased histone acetylation leads to repressed chromatin and makes the promoter regions of IL-4 and IL-5 less accessible for the transcriptional machinery, which possibly leads to the observed reduced protein levels of IL-4 shown in Fig. 12B and C, when $CD4^+CD62L^+$ naïve T cells were restimulated under T_H2 conditions. However, no differences in histone H3 and H4 acetylation at the IFN-γ promoter regions were observed, despite the observed increased protein levels of IFN-γ (Fig. 12E, F) and increased transcription factor t-bet (Fig. 12D). Besides histone acetylation, other epigenetic modifications such as DNA methylation might be the dominant mechanism that control T_H1 differentiation gene loci in these $CD4^+CD62L^+$ naïve T cells (Tumes et al., 2017). The observed pronounced T_H1 cell and impaired T_H2 cell differentiation is particularly interesting in the context of allergies. The stronger ability of naïve T cells to differentiate into T_H1 cells in offspring with maternal *S. mansoni* infection compared to naïve offspring (Fig. 12D-F) might counterbalance OVA-triggered T_H2 responses potentially contributing to the observed suppression of

OVA-induced allergic airway inflammation in offspring with maternal infection (Straubinger et al., 2014). Accordingly, cord blood cells of newborns who developed allergies later in life are shown to have a reduced ability to produce IFN-γ and consequently a weaker T_H1 cell response (Tang et al., 1994). Similarly, patients with autoimmune disorders such as multiple sclerosis which is marked with an overproduction of T_H1-related cytokines in myelin-specific T cells and increased frequencies of IL-12 producing monocytes, shows reduced susceptibility to allergies (Oro et al., 1996; Tang et al., 1998). The stronger capacity of naïve T cells of offspring with maternal *S. mansoni* infection to differentiate to T_H1 cells might be one reason for the observed reduction of allergic airway inflammation in the offspring. Along with this, several studies have demonstrated that atopic diseases are associated with epigenetic modifications in immune cells, which correlate with the severity of the disease (Su et al., 2009). Especially during pregnancy, the exposure to different infection-related factors ranging from lipopolysaccharide (LPS) to the non-pathogen *Acinetobacter lwoffii* F78 alters the risk of developing allergies later in life possibly by involving epigenetic modifications (Blümer et al., 2005; Brand et al., 2011). Interestingly, repeated findings that the maternal exposure to LPS is associated with the smaller risk to develop allergies in offspring mice (Blümer et al., 2005; Debarry et al., 2010) may relate to the ability of endotoxins to bind histones, thereby disrupting chromatin remodeling and resulting in specific gene silencing (Miller et al., 2008). In addition, Brand et al. have revealed that the prenatal exposure to *Acinetobacter lwoffii* reduced the susceptibility to allergies (Brand et al., 2011). This effect is caused by a T_H1 bias of $CD4^+$ cells, shown by increased levels of histone H4 acetylation at IFN-γ promoter regions in $CD4^+$ T cells. Interestingly, in the study presented here, offspring of *S. mansoni* infected mothers showed decreased histone H4 acetylation at IL-4 and IL-5 promoter regions (Fig. 13A, B), which *epigenetically* regulates the gene expression within the T_H2 loci and thus might contribute to the observed decreased ability of naïve T cells to differentiate into T_H2 cells (Fig. 12B, C). The inability of naïve $CD4^+CD62L^+$ T cells of offspring of helminth-infected mother to differentiate into T_H2 as properly as T cells of naïve offspring can be considered as one possible explanation for the observed allergy preventing effect in offspring with maternal helminth infection, since T_H2 cells are the key player along the allergic cascade. Additionally, since IL-5 along with NK cells recruit eosinophils into the lung (Clutterbuck et al., 1989; Lloyd et al., 2010), the repressed chromatin within the IL-5 promoter region shown by the decreased histone acetylation (Fig. 13B)

might reduce the recruitment of eosinophils from the bone marrow and thus the eosinophilic inflammation in the lung. Our findings strengthen the possibility that epigenetic modifications triggered by the maternal infection with helminths mediate the susceptibility to asthma by influencing the differentiation of $CD4^+CD62L^+$ naïve T cells. A better understanding of the role of epigenetics in these processes is crucial to understand the pathogenesis of chronic inflammatory conditions of mothers as well as their influence on the fetal development. Although it is still poorly defined how the maternal environment influences the fetal epigenetics, it is tempting to speculate that the fetal immune system interacts with the cytokine milieu caused by the chronic schistosomiasis of the mother and, thus induce the observed epigenetic modifications. Since the hematopoiesis of precursor T cells (double positive and double negative) takes place during day 11 and 17 of murine pregnancy and ends with the release of single positive CD4 and CD8 thymocytes to the peripheral blood (Pardoll, 1987), these precursor T helper cells might be especially vulnerable to epigenetic modifications induced by chronic *S. mansoni* infection of the mothers during this timepoint. To deepen our knowledge about epigenetic modifications, besides histone acetylation, the methylation status of naïve T cells of offspring born to schistosome infected mothers is worth being considered in future experiments. Our findings support 'the expanded hygiene concept' and indicate that non-transplacental helminth infections during pregnancy alter the T cell differentiation capacity of the offspring by epigenetic mechanisms.

4.1.2 **Role of FoxP3 gene and Treg cells**

Besides the unequal balance of T_H1 and T_H2 cell differentiation, insufficient Treg function and activation are associated with allergic airway inflammation. Within the study presented here, the acetylation status of FoxP3 promoter regions of naïve $CD4^+CD62L^+$ T cells was investigated. Interestingly, the histone H4 at FoxP3 promoter regions was hyperacetylated in offspring with maternal *S. mansoni* infection compared to naïve offspring (Fig. 13E). The increased acetylation status makes the chromatin less compact and more accessible to the transcriptional machinery. According to the literature, Tregs play a major role in preventing allergic airway inflammation. One can assume that Foxp3 promoter regions of naïve T cells transcriptionally more active in offspring of *S. mansoni* infected mothers may lead to an immunosuppressive environment with allergy-preventing effects in the offspring if challenged with OVA. It is hypothesized that the transcription factor FoxP3 is crucial for the balance of the immune system and is responsible for

the suppression of T_H2 responses following exposure to allergens (Pellerin et al., 2014). Schaub et al. have demonstrated that maternal farm exposure to and consumption of unpasteurized milk relates to increased Treg cells with demethylated (transcriptionally active) FoxP3 gene expression in neonatal cord blood and is associated with tolerance induction of allergies (Schaub et al., 2009). Interestingly, FoxP3 protein expression within $CD4^+CD25^{high}$ T cells is significantly reduced in asthma patients, which may cause a Treg deficiency to suppress T_H2 proliferation effectively (Provoost et al., 2009). In contrast to that a recent study by Harb et al. have shown that childhood allergic asthma is associated with increased levels of histone H3 acetylation at FoxP3 promoter regions compared to healthy controls (Harb et al., 2015). Additionally, FoxP3 gene expression is significantly increased in asthma patients in comparison to healthy individuals, and the Treg cell suppressive capacity was observed in both groups (Raedler et al., 2015). FoxP3 levels in asthma patients are still controversially discussed. These opposing studies clearly highlight the importance of FoxP3 protein expression in the context of allergies. Besides the increased acetylation status at FoxP3 promoter regions, the percentage of viable $CD4^+CD25^+$ cells (Fig. 8E, F) and $CD8^+CD25^+$ cells (Fig. 8C, D) analyzed by flow cytometry was not altered in offspring born to *S. mansoni* infected mothers in comparison to naïve offspring. Equal numbers of peripheral Treg cells are also found in allergy studies. In 2009, Provoost et al. have demonstrated that the frequencies of $CD4^+CD25^+$ and $CD4^+CD25^+FoxP3^+$ remained similar in asthmatics compared to healthy individuals (Provoost et al., 2009). According to the current opinion that allergic diseases are characterized by a relative deficiency in Treg cells, allowing T_H2 cells to expand, several studies have shown that allergic patients, including asthmatics, had an insufficient $CD4^+CD25^+$ Treg cell response in both the bronchoalveolar lavage and peripheral blood monocytes cells (PBMCs) compared with healthy subjects (Wang et al., 2009). Shown in mouse models $CD4^+CD25^+$ Tregs inhibit the differentiation of naïve CD4 T into T_H2 cells (Xu et al., 2003). Interestingly, the adoptive transfer of $CD4^+CD25^+$ Treg cells before allergen challenge significantly reduces airway hyperresponsiveness and inflammation (Kearley et al, 2005). This suppression was IL-10 dependent and was reversed by anti-IL-10R antibody, suggesting that IL-10 dampens the activity of T_H2 cells (Ling, 2004). However, other authors have demonstrated that patients with atopic asthma have increased levels of Treg in peripheral blood cells in comparison to healthy subjects, but not non-atopic asthmatic individuals (Raedler et al., 2015). Some researchers have hypothesized that the genetic variations and epigenetic modifications that affect molecules in regulatory T cells

such as the FoxP3 gene can cause dysfunction of regulatory T cells and can thus influence the development of immune-mediated diseases (Marques et al., 2015). However, the observed increased numbers of Treg cells in asthmatic patients, particularly patients with atopic asthma, may indicate a counter-regulatory mechanism that is not yet sufficient to control allergic inflammation (Marques et al., 2015). Enhancing the transcription rate of FoxP3 has also been used to treat and prevent allergic diseases. The main therapy to control allergy and asthma are corticosteroids, either ingested or inhaled, both of which are associated with an enhanced $FoxP3^+$ expression and increased suppressor function (Karagiannidis et al., 2004). Interestingly, corticosteroids induce their anti-inflammatory potential, at least in parts, through histone acetylation. The transcription rate of anti-inflammatory genes (e.g. mitogen-activated protein kinase phosphatase-1) is increased, whereas transcription rates of pro-inflammatory genes (e.g. NF-κB) were inhibited by inducing deacetylation (Ito et al., 2006). Taken together, the controversial results of FoxP3 genes and Treg cells regarding allergies illustrate the complexity and current relevance of the topic and need further investigation. Regarding our findings, we hypothesize that maternal helminth infection induces an immunosuppressive environment in the progeny by epigenetic modifications at FoxP3 promoter regions of naïve T cells, and might, thus, reduce the risk of developing allergies later in life. Namely, the increased histone H4 acetylation at FoxP3 promoter regions of naïve T cells might facilitate the differentiation of naïve T cells into regulatory T cells, which are known to dampen pro-inflammatory T_H2 responses. Our data were collected before challenging the immune system of the offspring of *S. mansoni* infected mothers with allergens, such as OVA. It would be interesting to see, how the acetylation status at FoxP3 promoter regions and numbers of Treg cells changes during sensitization and challenge with OVA in offspring of schistosome-infected mothers in comparison to naïve offspring.

4.1.3 **Function of NK cells**

Besides regulatory T cells, innate immune cells are increasingly recognized as key players in the initiation and perpetuation of allergic responses. In this study presented here, the composition of immune cells in peripheral blood and spleen were examined. Interestingly, the number of NK cells ($NK1.1^+$, $NKp46^+$) in offspring of *S. mansoni* infected mothers in comparison to naïve offspring were decreased (Fig. 8B). Clinical studies have revealed that NK activity was increased in peripheral blood of asthmatic individuals (Timonen et al., 1985) but decreased immediately following antigen challenge (Jira et al.,

1988). It has been postulated that this decrease of NK cell activity is caused by a migration of NK cells from the peripheral blood to lymphoid compartments and the lung. More recently, Lin et al. have revealed that NK cell frequency was increased in peripheral blood of asthmatics during acute exacerbation in comparison to stable asthmatic children (Lin et al., 2003). In our study, the decreased frequency of NK cells in offspring with *S. mansoni* infected mothers might be one component of allergy-preventing effects when sensitized and challenged with OVA. Clearly, further studies are required to characterize the phenotype and function of NK cells in allergic individuals. Studies by Ple et al. revealed that NK cells in the lung lymph nodes showed increased levels of CD86, suggesting that cross-talk between these cells and CD4 T cells might be important, potentially increasing T_H2 cytokine production. The depletion of NK cells following OVA challenge led to a significant decrease of eosinophils to the airway response to antigen challenge (Ple et al., 2010). However, scientists hypothesize that NK cells influence multiple pathways during the development of asthma (Haworth et al., 2011). There is good evidence that NK cells are pro-inflammatory and promote asthma progression (Arase et al., 2003). NK cells can be activated by IgE through FcγRIII resulting in the release of several cytokines, which allows the assumption that NK cells may contribute to IgE-mediated allergic response (Karimi et al., 2013). Regarding the controversial role of NK cells with promotion and inhibition of allergic lung inflammation, a better understanding of the underlying mechanisms is needed. Our experiments revealed a decreased frequency of NK cells in offspring of *S. mansoni* infected mothers, which might lead to a reduced cross-talk with $CD4^+$ T cells and thus decreased T_H2 cytokine production.

4.2 Influence of maternal *S. mansoni* infection on immune cell composition of the offspring

There are only few studies in which the correlation of maternal infection and peripheral immune cell composition in the progeny has been examined. We hypothesize that circulating parasite antigens may enter the offspring's circulation by placental transfer of helminth-antigens or directly by breastfeeding and enteric resorption (Rosario et al., 2008; Scott et al., 2004; Smithers et al., 1967; Urdahl et al., 2011) and may induce an altered immune cell composition in the offspring. However, the way of the antigen transport from the infected mother to the fetus still remains unclear. Several studies have demonstrated that the sensitization with schistosome antigens occurs already *in utero* in offspring of *S. mansoni* and *S. haematobium* infected mothers shown by higher proliferating cord blood

mononuclear cells (CBMC) (restimulated with schistosomal antigens) of neonates (Novato-Silva et al., 1992) and cytokine pattern of IL-4, IL-5 and IL-10 similar to that observed in adults (King, 1993; King, 1996; Novato-Silva et al., 1992). Additionally, van der Kleij and colleagues have revealed that cytokine responses to schistosomal stimulus of PBMCs in infants born in *S. haematobium* endemic areas were lower, compared to infants born in developed countries, indicating that schistosomal lipids activate the innate immune system, as shown by differences in proportions of immune cells and lower level of cytokine responses to TLR-2 and TLR-4 (van der Kleij et al., 2004). In our study, we observed differences in the peripheral T cell composition in peripheral blood of offspring of *S. mansoni*-infected mothers without re-stimulation with schistosomal antigens. In detail, offspring with maternal infection showed increased numbers of $CD8^+/CD62L^+$ and $CD4^+/CD62L^+$ cells in comparison to offspring with naïve mothers (Fig. 8C, E). The elevated levels of naïve $CD4^+$ and $CD8^+$ T cells might indicate a greater amount of naïve T cells in the progeny induced by the maternal infection with schistosomiasis. Interestingly, our findings are limited to peripheral blood, since no differences in $CD4^+$ and $CD8^+$ splenocytes between naïve offspring and offspring born to infected mothers were detectable (Fig. 8D, F). This might be caused by a lymphocyte migration from the spleen to the peripheral blood as reported by others (Helmby et al., 2000; Kumararatne et al., 1987). Additionally, the percentage of viable $CD4^+$, $CD8^+$, effector ($CD4^+/CD44^+$ or $CD8^+/CD44^+$) T cells and regulatory ($CD4^+/CD25^+$ or $CD8^+/CD25^+$) T cells as well as the ratio of CD4/CD8 was not altered (Fig. 8C-F). Moreover, the total percentage of B and T cells was similar in naïve offspring and offspring with maternal infection (Fig. 8A, B). This consistency of equal B and T cell numbers was also detectable in percentages of immature, pre- and pro-B cells in bone marrow (Fig. 9A) as well as in precursor T cells in the thymus (Fig. 9B). Major differences in the early B and T cell development might be detectable during earlier periods: during the fetal period, directly after birth, or in infants. Although the absolute numbers of (precursor) B and T cells were comparable in our experiments, the functionality of these cells may be different. Functionality tests will be considered in future experiments. Besides B and T cells, the antigen-presenting cells are essential for the effective adaptive immune response. Accordingly, Santos et al. have revealed that previous breast-feeding by *S. mansoni*-infected mothers improved antigen-presentation by B cells in adult offspring born to naïve mothers and subsequently suckled by *S. mansoni* infected mothers (shown by the increased frequency of $CD40^+CD80^+$ B cells). In contrast to that, the gestation in infected mothers and breast feeding by naïve

mice imprinted offspring with weak antigen presentation. In detail, during the response to the non-related antigen OVA the frequency of $CD86^+CD11c^+$ dendritic and $CD40^+CD80^+CD86^+$ cells was drastically decreased in offspring born to *S. mansoni* infected mothers and breastfed by naïve mothers (Santos et al., 2014). It may be possible that the acquisition of these antibodies in offspring suckled by mothers infected with *S. mansoni* leads to a pre-activation of B cells, enhancing the production of antibodies in mice during their adulthood, which emphasizes the role of breastfeeding as a stimulant of the offspring immunity in the long term (Santos et al., 2014). In contrast to our results, Santos' findings are only detectable after sensitization with the heterologous antigen OVA. However, in our study the animals were not sensitized *in vivo*. Additionally, Santos et al. mated their schistosome-infected mothers during the 9^{th} week of infection (on day 60). This mating time point is clearly within the T_H2 phase of infection. According to the results of Straubinger et al., offspring of mothers mated during the T_H2 phase of *Schistosomiasis* show aggravated allergy symptoms (Straubinger et al., 2014). Taken together, the non-transplacental infection with helminths during pregnancy expands the amount of naïve $CD4^+$ and $CD8^+$ T cell in peripheral blood of offspring of *S. mansoni* infected mothers and might represent an ideal starting point to prevent future homologous and heterologous infections.

4.3 Altered central memory T cell compartment in the progeny by maternal *S. mansoni* infection

Besides the alterations of T cell differentiation, epigenetics and immune cell composition, the fetal exposure to maternal *S. mansoni* infection might lifelong modulate the offspring's immune system. The priming of naïve T cells consists of the primary recognition of specific peptide-MHC complexes, followed by their activation, and leads to expanded populations of differentiated effector cells, some of which advance to lifelong memory T cells. Regarding our experiments, we detected that splenocytes of offspring with maternal *S. mansoni* infection produced a basic level of IL-4 under unstimulated conditions. Concomitant immunity is thought to play a major role in infections with malaria, leishmaniasis, tuberculosis and helminths infections (Rosario et al., 2008; Scott et al., 2004; Smithers et al., 1967; Urdahl et al., 2011). The immune response against re-infection in these settings is designated as a memory response. So far, several epidemiological studies have revealed that the majority of people exposed to schistosomiasis stay asymptomatic

in endemic areas, while humans living in non-endemic areas develop more severe symptoms (Nash et al., 1982), possibly caused by the exposure to schistosomiasis during pregnancy (da Paz et al., 2017). Passive transfer of schistosome-antibodies or the transmission of parasite antigens via FcγR at the placental-blood barrier or directly via mother milk are potential mechanism for the observed immunity of the offspring (Attallah et al., 2003; Charnaud et al., 2016; Gill et al., 1983). Recently, Darby et al. have shown that the maternal cellular immunity against the infection with the nematode *Nippostrongylus brasiliensis* can be transferred to the progeny via breastfeeding, which is mainly mediated by maternally derived pathogen-experienced T_H2 cells (Darby et al., 2019). Regarding our studies, $CD4^+CD62L^+$ T cells in offspring of *S. mansoni* infected mothers produced higher amounts of IL-4 compared to naïve offspring (Fig. 16A). The production of IL-4 was further limited to $CD4^+CD62L^+CD44^+$ and could not be observed in CD44-depleted cells (Fig. 16C). Further experiments are needed, if these memory T cells are SEA-specific. However, splenocytes of offspring born to *S. mansoni* infected mothers showed no enhanced response to soluble egg antigen (SEA) in our studies (Fig. 14A-C). Further experiments will clarify the specificity against other defined schistosome products such as worm or egg antigens to investigate further the hypothesis that the *in-utero* exposure to schistosomiasis significantly alters the offspring's central memory T cell compartment with possible schistosome-specificity, which might eventually be one of the reasons explaining that the majority of people living in schistosome endemic areas suffer from mild symptoms, if at all.

4.4 Future directions: vaccine efficacy and perinatal helminth infections

The maternal helminth infection enables distinct epigenetic alterations within different promoter regions (Fig. 13A, B, E, F), enhances the T_H1 (Fig. 12D, E) and impairs the T_H2 cell differentiation capacity of naïve T cells in the progeny (Fig. 12A-C). These modifications may further influence other T cell driven immune responses such as autoimmune diseases, bacterial, and viral infections or vaccinations. It would be intriguing to see whether the observed enhanced T_H1 differentiation has a clinical impact on vaccine efficacy. So far, epidemiological vaccine studies have shown that children in sub-Saharan Africa, where helminth infections are endemic, respond less well to vaccines against tuberculosis (BCG), typhoid fever, and measles (LaBeaud et al., 2009). On the contrary, the infection of the mother with hookworm during pregnancy, significantly increases the

response to the vaccine BCG in their children within the first year of life (Elliott et al., 2005). Indeed, there is evidence that the perinatal exposure to parasite infections during pregnancy are thought to prime or tolerize the fetal immune system by the transplacental transfer of parasite antigens (Krämer et al., 1999; Novato-Silva et al., 1992; Renaud et al., 1971). Regarding helminth infections, one should mention that parasites show species-specific differences in immunomodulatory effects and vaccine-related immunity. This has led to the recommendation of antiparasite treatment which may prevent immunomodulation by parasite infections and improve vaccine efficacy (LaBeaud et al., 2009). However, whether maternal helminth infections influence the ability to induce protective antibody responses to vaccines is still controversially discussed, since clinical data differ. For example, no effects on vaccination efficacy of hepatitis B immunization are observed in Egyptian infants born to schistosome-infected mothers when anti-HBs titers were measured at 9 months of age (Bassily et al., 1997). On the contrary, a different study reveals that anti-HBs antibody development in offspring born to *S.mansoni*-HBsAg-positive mothers was significantly delayed (Ghaffar et al., 1989). Additionally, the immune cells of infants exposed to filarial nematode *Mansonella perstans* during pregnancy were associated with distinct changes including increased IL-10 production to mycobacterial antigens and tetanus toxoid, but unaltered IFN-γ, IL-5 and IL-13 levels (Elliott et al., 2010). Since an optimal vaccine should elicit a distinct T_H1 response to eliminate infected cells and induce antibodies that can prevent infections, the experimentally enhanced T_H1 cell differentiation of naïve T cells in offspring born to schistosome-infected mothers in our study (Fig. 12D, E) might help to understand the differences in vaccination efficacy of progeny born to helminth-infected mothers. In addition, future research should highlight the varied effects of different parasites (alone and combined) on the human vaccine-related immunity. Current vaccine strategies might need to be revisited in the light of the effects of concurrent as well as maternal helminth infections. The *in utero* exposure to maternal helminths clearly alters vaccine efficacy and immunity of the offspring to homologous and heterologous antigens later in life. One of the underlying mechanisms for this observed altered immunity in offspring with *Schistosomiasis* infected mothers may the epigenetically enhanced T_H1 and weakened T_H2 cell differentiation.

5 Publications and presentations

5.1 Publication

Chronic schistosomiasis during pregnancy epigenetically reprograms T cell differentiation in offspring of infected mothers

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European Journal of Immunology, accepted April 2017

5.2 Presentations

Poster presentation at the ‘Gemeinsame Jahrestagung der Deutschen Gesellschaft für Infektiologie (DGI) und des Deutschen Zentrums für Infektionsforschung (DZIF), November 19 – 21, 2015, Munich, Germany

Oral presentation at the 13th Winter School in Allergy and Clinical Immunology, of the European Academy of Allergy and Clinical Immunology (EAACI), February 5 – 8, 2015, Les Arcs 1800, France

Oral presentation at the 18th Symposium ‘Infektion und Immunabwehr der FG Infektionsimmunologie der DGHM und AK Infektionsimmunologie der DGfI’, March 12 – 14, 2014, Burg Rothenfels, Germany

6 Literature

Adams-Waldorf, K. M., & McAdams, R. M. (2013). Influence of Infection During Pregnancy on Fetal Development. *Reproduction (Cambridge, England), 146*(5), p. 151-162. Retrieved from http://doi.org/10.1530/REP-13-0232

Adegnika, A., Ramharter, M., Agnandji, S. T., Ateba Ngoa, U., Issifou, S., Yazdanbahksh, M., & Kremsner, P. G. (2010). Epidemiology of parasitic co-infections during pregnancy in Lambaréné, Gabon. *Tropical Medicine & International Health, 15*(10), p. 1204-1209. Retrieved from http://doi.org/10.1111/j.1365-3156.2010.02598.x

Aït-Khaled, N., Pearce, N., Anderson, H. R., Ellwood, P., Montefort, S., Shah, J. (2009). Global map of the prevalence of symptoms of rhinoconjunctivitis in children: The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three. *Allergy, 64*(1), p. 123-148. Retrieved from http://doi.org/10.1111/j.1398-9995.2008.01884.x

Ajanga, A., Lwambo, N. J., Blair, L., Nyandindi, U., Fenwick, A., & Brooker, S. (2006). Schistosoma mansoni in pregnancy and associations with anaemia in northwest Tanzania. *Trans R Soc Trop Med Hyg, 100*(1), p. 59-63. Retrieved from http://doi.org/10.1016/j.trstmh.2005.06.024

Akdis, C. A., & Akdis, M. (2009). Mechanisms and treatment of allergic disease in the big picture of regulatory T cells. *J Allergy Clin Immunol, 123*(4), p. 735-746. Retrieved from http://doi.org/10.1016/j.jaci.2009.02.030

Alaskhar Alhamwe, B., Khalaila, R., Wolf, J., von Bülow, V., Harb, H., Alhamdan, F., Potaczek, D. P. (2018). Histone modifications and their role in epigenetics of atopy and allergic diseases. *Allergy, Asthma, and Clinical Immunology : Official Journal of the Canadian Society of Allergy and Clinical Immunology, 14*, p. 39. Retrieved from http://doi.org/10.1186/s13223-018-0259-4

Allakhverdi, Z., Comeau, M. R., Jessup, H. K., Yoon, B.-R. P., Brewer, A., Chartier, S., Delespesse, G. (2007). Thymic stromal lymphopoietin is released by human epithelial cells in response to microbes, trauma, or inflammation and potently activates mast cells. *The Journal of Experimental Medicine, 204*(2), p. 253-258. Retrieved from http://doi.org/10.1084/jem.20062211

Allan, R. S., Zueva, E., Cammas, F., Schreiber, H. A., Masson, V., Belz, G. T., Amigorena, S. (2012). An epigenetic silencing pathway controlling T helper 2 cell lineage commitment. *Nature, 487*(7406), p. 249-253. Retrieved from https://doi.org/10.1038/nature11173

Amu, S., Saunders, S. P., Kronenberg, M., Mangan, N. E., Atzberger, A., & Fallon, P. G. (2010). Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. *Journal of Allergy and Clinical Immunology, 125*(5), p. 1114-1124. Retrieved from http://doi.org/10.1016/j.jaci.2010.01.018

Angeli, V., Faveeuw, C., Delerive, P., Fontaine, J., Barriera, Y., Franchimont, N., Trottein, F. (2001). Schistosoma mansoni induces the synthesis of IL-6 in pulmonary microvascular endothelial cells: Role of IL-6 in the control of lung eosinophilia during infection, *European Journal of Immunology, 31*(9). Retrieved from http://doi.org/10.1002/1521-4141(200109)31:9<2751::AID-IMMU2751>3.0.CO;2-4

Arase, N., Arase, H., Hirano, S., Yokosuka, T., Sakurai, D., & Saito, T. (2003). IgE-mediated activation of NK cells through Fc gamma RIII. *J Immunol, 170*(6), p. 3054-3058. Retrieved from http://doi.org/10.4049/jimmunol.170.6.3054

Asher, M. I., Montefort, S., Björkstén, B., Lai, C. K. W., Strachan, D. P., Weiland, S. K., & Williams, H. (2006). Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *The Lancet, 368*(9537), p. 733-743. Retrieved from http://doi.org/10.1016/S0140-6736(06)69283-0

Attallah, A. M., Abbas, A. T., Dessouky, M. I., El-emshaty, H. M., & Elsheikha, H. M. (2006). Susceptibility of neonate mice born to Schistosoma mansoni-infected and noninfected mothers to subsequent S. mansoni infection. *Parasitology Research, 99*(2), p. 137-145. Retrieved from http://doi.org/10.1007/s00436-006-0127-x

Attallah, A. M., Ghanem, G. E., Ismail, H., & El Waseef, A. M. (2003). Placental and oral delivery of Schistosoma mansoni antigen from infected mothers to their newborns and children. *Am J Trop Med Hyg, 68*(6), p. 647-651. Retrieved from https://doi.org/10.4269/ajtmh.2003.68.647

Avni, O., Lee, D., Macian, F., Szabo, S. J., Glimcher, L. H., & Rao, A. (2002). T(H) cell differentiation is accompanied by dynamic changes in histone acetylation of cytokine genes. *Nat Immunol, 3*(7), p. 643-651. Retrieved from http://doi.org/10.1038/ni808

Bach , J.-F. (2002). The Effect of Infections on Susceptibility to Autoimmune and Allergic Diseases. *New England Journal of Medicine, 347*(12), p. 911-920. Retrieved from http://doi.org/10.1056/NEJMra020100

Barnes, P. J. (2008). The cytokine network in asthma and chronic obstructive pulmonary disease. *The Journal of Clinical Investigation, 118*(11), p. 3546-3556. Retrieved from http://doi.org/10.1172/JCI36130

Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Schones, D. E., Wang, Z., Zhao, K. (2007). High-resolution profiling of histone methylations in the human genome. *Cell, 129*(4), 823-837. Retrieved from http://doi.org/10.1016/j.cell.2007.05.009

Baru, A. M., Hartl, A., Lahl, K., Krishnaswamy, J. K., Fehrenbach, H., Yildirim, A. Ö., Sparwasser, T. (2010). Selective depletion of Foxp3+ Treg during sensitization phase aggravates experimental allergic airway inflammation. *European journal of immunology, 40*(8), p. 2259-2266. Retrieved from http://doi.org/10.1002/eji.200939972

Bassily, S., Kotkat, A., Hyams, K. C., Youssef, F. G., El-Masry, N. A., Arthur, R., Brown, F. M. (1997). Immunogenicity of Recombinant Hepatitis B Vaccine Among Infants of Mothers with Active Schistosomiasis. *The American Journal of Tropical Medicine and Hygiene, 57*(2), p. 197-199. Retrieved from https://doi.org/10.4269/ajtmh.1997.57.197

Bégin, P., & Nadeau, K. C. (2014). Epigenetic regulation of asthma and allergic disease. *Allergy, Asthma, and Clinical Immunology : Official Journal of the Canadian Society of Allergy and Clinical Immunology, 10*(1), p. 27-27. Retrieved from http://doi.org/10.1186/1710-1492-10-27

Berzins, S. P., Boyd, R. L., & Miller, J. F. (1998). The role of the thymus and recent thymic migrants in the maintenance of the adult peripheral lymphocyte pool. *J Exp Med, 187*(11), p. 1839-1848. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2212318/

Bettelli, E., Carrier, Y., Gao, W., Korn, T., Strom, T. B., Oukka, M., Kuchroo, V. K. (2006). Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature, 441*(7090), p. 235-238. Retrieved from http://doi.org/0.1038/nature04753

Blackburn, C. C., & Selkirk, M. E. (1992). Inactivation of platelet activating factor by a putative acetylhydrolase from the gastrointestinal nematode parasite Nippostrongylus brasiliensis. *Immunol., 75*(1), p. 41-46.

Blackwell, A. D. (2016). Helminth infection during pregnancy: insights from evolutionary ecology. *International Journal of Women's Health, 8*, p. 651-661. Retrieved from http://doi.org/10.2147/IJWH.S103529

Blümer, N., Herz, U., Wegmann, M., & Renz, H. (2005). Prenatal lipopolysaccharide-exposure prevents allergic sensitization and airway inflammation, but not airway responsiveness in a murine model of experimental asthma. *Clinical & Experimental Allergy, 35*(3), p. 397-402. Retrieved from http://doi.org/10.1111/j.1365-2222.2005.02184.x

Brand, S., Kesper, D. A., Teich, R., Kilic-Niebergall, E., Pinkenburg, O., Bothur, E., Renz, H. (2012). DNA methylation of TH1/TH2 cytokine genes affects sensitization and progress of experimental asthma. *Journal of Allergy and Clinical Immunology, 129*(6), p. 1602-1610. Retrieved from https://doi.org/10.1016/j.jaci.2011.12.963

Brand, S., Teich, R., Dicke, T., Harb, H., Yildirim, A. Ö., Tost, J., Renz, H. (2011). Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. *Journal of Allergy and Clinical Immunology, 128*(3), 618-625.e617. Retrieved from http://dx.doi.org/10.1016/j.jaci.2011.04.035

Bredo, G., Storie, J., Shrestha Palikhe, N., Davidson, C., Adams, A., Vliagoftis, H., & Cameron, L. (2015). Interleukin-25 initiates Th2 differentiation of human CD4(+) T cells and influences expression of its own receptor. *Immun Inflamm Dis, 3*(4), p. 455-468. Retrieved from http://doi.org/10.1002/iid3.87

Broide, D. H. (2001). Molecular and cellular mechanisms of allergic disease. *Journal of Allergy and Clinical Immunology, 108*(2), p. 65-71. Retrieved from http://dx.doi.org/10.1067/mai.2001.116436

Brooker, S., Hotez, P. J., & Bundy, D. A. P. (2008). Hookworm-Related Anaemia among Pregnant Women: A Systematic Review. *PLoS Neglected Tropical Diseases, 2*(9), p. 291. Retrieved from http://doi.org/10.1371/journal.pntd.0000291

Brune, K., Frank, J., Schwingshackl, A., Finigan, J., & Sidhaye, V. K. (2015). Pulmonary epithelial barrier function: some new players and mechanisms. *American Journal of Physiology - Lung Cellular and Molecular Physiology, 308*(8), p. 731-745. Retrieved from http://doi.org/10.1152/ajplung.00309.2014

Campbell, J. J., Bowman, E. P., Murphy, K., Youngman, K. R., Siani, M. A., Thompson, D. A., Butcher, E. C. (1998). 6-C-kine (SLC), a Lymphocyte

Adhesion-triggering Chemokine Expressed by High Endothelium, Is an Agonist for the MIP-3β Receptor CCR7. *The Journal of Cell Biology, 141*(4), p.1053-1059.

Cardoso, L. S., Oliveira, S. C., Góes, A. M., Oliveira, R. R., Pacífico, L. G., Marinho, F. V., Araujo, M. I. (2010). Schistosoma mansoni antigens modulate the allergic response in a murine model of ovalbumin-induced airway inflammation. *Clinical and Experimental Immunology, 160*(2), p. 266-274. Retrieved from http://doi.org/10.1111/j.1365-2249.2009.04084.x

Carsetti, R. (2000). The Development of B Cells in the Bone Marrow Is Controlled by the Balance between Cell-Autonomous Mechanisms and Signals from the Microenvironment. *The Journal of Experimental Medicine, 191*(1),p. 5-8.

Cembrzynska-Nowak, M., Szklarz, E., Inglot, A. D., & Teodorczyk-Injeyan, J. A. (1993). Elevated release of tumor necrosis factor-alpha and interferon-gamma by bronchoalveolar leukocytes from patients with bronchial asthma. *Am Rev Respir Dis, 147*(2), p. 291-295. Retrieved from http://doi.org/10.1164/ajrccm/147.2.291

Chandran, S. S., Verhoeven, D., Teijaro, J. R., Fenton, M. J., & Farber, D. L. (2009). TLR2 engagement on dendritic cells promotes high frequency effector and memory CD4 T cell responses. *J Immunol, 183*(12), p. 7832-7841. Retrieved from http://doi.org/10.4049/jimmunol.0901683

Charnaud, S. C., McGready, R., Herten-Crabb, A., Powell, R., Guy, A., Langer, C., Fowkes, F. J. I. (2016). Maternal-foetal transfer of Plasmodium falciparum and Plasmodium vivax antibodies in a low transmission setting. *Scientific Reports, 6*, 20859. Retrieved from http://doi.org/10.1038/srep20859

Chen, W., Jin, W., Hardegen, N., Lei, K. J., Li, L., Marinos, N., Wahl, S. M. (2003). Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med, 198*(12), p. 1875-1886. Retrieved from http://doi.org/10.1084/jem.20030152

Chensue, S. W., Warmington, K., Ruth, J. H., Lukacs, N., & Kunkel, S. L. (1997). Mycobacterial and schistosomal antigen-elicited granuloma formation in IFN-gamma and IL-4 knockout mice: analysis of local and regional cytokine and chemokine networks. *J Immunol, 159*(7), p. 3565-3573.

Chiavarini, M., Bragetti, P., Sensini, A., Cenci, E., Castronari, R., Rossi, M. J., Minelli, L. (2011). Breastfeeding and transmission of cytomegalovirus to preterm infants. Case report and kinetic of CMV-DNA in breast milk. *Italian Journal of Pediatrics, 37*, p. 6. Retrieved from http://doi.org/10.1186/1824-7288-37-6

Christian, P., Khatry, S., & West, J. K. (2004). Antenatal anthelmintic treatment, birthweight, and infant survival in rural Nepal. *Lancet* (364), p. 981-983. Retrieved from http://doi.org/10.1016/S0140-6736(04)17023-2

Clutterbuck, E., Hirst, E., & Sanderson, C. (1989). Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6, and GMCSF. *Blood, 73*(6), p. 1504-1512.

Conrad, M. L., Ferstl, R., Teich, R., Brand, S., Blumer, N., Yildirim, A. O., Renz, H. (2009). Maternal TLR signaling is required for prenatal asthma protection by the nonpathogenic microbe Acinetobacter lwoffii F78. *J Exp Med, 206*(13), p. 2869-2877. Retrieved from http://doi.org/10.1084/jem.20090845

Constant, S., Pfeiffer, C., Woodard, A., Pasqualini, T., & Bottomly, K. (1995). Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. *The Journal of Experimental Medicine, 182*(5), p. 1591-1596. Retrieved from http://doi.org/10.1084/jem.182.5.1591

Cook, P. C., Aynsley, S. A., Turner, J. D., Jenkins, G. R., Van Rooijen, N., Leeto, M., Mountford, A. P. (2011). Multiple Helminth Infection of the Skin Causes Lymphocyte Hypo-Responsiveness Mediated by Th2 Conditioning of Dermal Myeloid Cells. *PLOS Pathogens, 7*(3), p. 1001323. Retrieved from http://doi.org/10.1371/journal.ppat.1001323

Cooper, P. J. (2009). Interactions between helminth parasites and allergy. *Current Opinion in Allergy and Clinical Immunology, 9*(1), p. 29-37. Retrieved from http://doi.org/10.1097/ACI.0b013e32831f44a6

Corrêa, C. L., Lisboa, P. C., Oliveira, E. d., Moura, E. G. d., Oliveira, R. M. F. d., Gomes, A. C., & Machado-Silva, J. R. (2011). The outcome of acute schistosomiasis infection in adult mice with postnatal exposure to maternal malnutrition. *Memórias do Instituto Oswaldo Cruz, 106*, p. 584-593. Retrieved from http://doi.org/10.1590/s0074-02762011000500011

Cumano, A., & Godin, I. (2001). Pluripotent hematopoietic stem cell development during embryogenesis. *Curr Opin Immunol, 13*(2), p. 166-171.

da Paz, V. R. F., Sequeira, D., & Pyrrho, A. (2017). Infection by Schistosoma mansoni during pregnancy: Effects on offspring immunity. *Life Sciences, 185*, p. 46-52. Retrieved from http://dx.doi.org/10.1016/j.lfs.2017.07.021

Daniłowicz-Luebert, E., O'Regan, N., Steinfelder, S., & Hartmann, S. (2011). Modulation of Specific and Allergy-Related Immune Responses by Helminths. *Journal of Biomedicine and Biotechnology, 2011*, 18. Retrieved from http://doi.org/10.1155/2011/821578

Darby, M. G., Chetty, A., Mrjden, D., Rolot, M., Smith, K., Mackowiak, C., Horsnell, W. G. C. (2019). Pre-conception maternal helminth infection transfers via nursing long-lasting cellular immunity against helminths to offspring. *Science Advances, 5*(5), 3058. Retrieved from http://doi.org/10.1126/sciadv.aav3058

Das, P. K., Sirvidya, A., Vanamail, P., Ramaiah, K. D., Pani, S. P., Michael, E., & Bundy, D. A. (1997). Wuchereria bancrofti microfilaraemia in children in relation to parental infection status. *Trans R Soc Trop Med Hyg, 91*(6), p. 677-679. Retrieved from http://doi.org/10.1016/s0035-9203(97)90521-6

Daxinger, L., & Whitelaw, E. (2012). Understanding transgenerational epigenetic inheritance via the gametes in mammals. *Nat Rev Genet, 13*(3), p. 153-162. Retrieved from http://doi.org/10.1038/nrg3188

de Jesus, A. R., Silva, A., Santana, L. B., Magalhães, A., de Jesus, A. A., de Almeida, R. P., Carvalho, E. M. (2002). Clinical and Immunologic Evaluation of 31 Patients with Acute Schistosomiasis mansoni. *The Journal of Infectious Diseases, 185*(1), 98-105. Retrieved from http://doi.org/10.1086/324668

Debarry, J., Hanuszkiewicz, A., Stein, K., Holst, O., & Heine, H. (2010). The allergy-protective properties of Acinetobacter lwoffii F78 are imparted by its lipopolysaccharide. *Allergy, 65*(6), 690-697. Retrieved from http://doi.org/10.1111/j.1398-9995.2009.02253.x

Del Prete, G., Maggi, E., Parronchi, P., Chretien, I., Tiri, A., Macchia, D., Romagnani, S. (1988). IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J Immunol, 140*(12), p. 4193-4198.

Dietert, R. R., Etzel, R. A., Chen, D., Halonen, M., Holladay, S. D., Jarabek, A. M., Zoetis, T. (2000). Workshop to identify critical windows of exposure for children's health: immune and respiratory systems work group summary. *Environmental Health Perspectives, 108*(Suppl 3), p. 483-490. Retrieved from http://doi.org/10.1289/ehp.00108s3483

Eberhard, M. L., Hitch, W. L., McNeeley, D. F., & Lammie, P. J. (1993). Transplacental Transmission of Wuchereria bancrofti in Haitian Women. *The Journal of Parasitology, 79*(1), p. 62-66. Retrieved from http://doi.org/10.2307/3283278

Eibel, H. (2015). Early B Cell Development. In A. Plebani & V. Lougaris (Eds.), *Agammaglobulinemia.* p.. 1-17. Cham: Springer International Publishing. Retrieved from http://doi.org/10.1007/978-3-319-22714-6_1

Elliott, A. M., Mawa, P. A., Webb, E. L., Nampijja, M., Lyadda, N., Bukusuba, J., Whitworth, J. A. G. (2010). Effects of maternal and infant co-infections, and of maternal immunisation, on the infant response to BCG and tetanus immunisation. *Vaccine, 29*(2-2), 247-255. Retrieved from http://doi.org/10.1016/j.vaccine.2010.10.047

Elliott, A. M., Namujju, P. B., Mawa, P. A., Quigley, M. A., Nampijja, M., Nkurunziza, P. M., Whitworth, J. A. (2005). A randomised controlled trial of the effects of albendazole in pregnancy on maternal responses to mycobacterial antigens and infant responses to Bacille Calmette-Guerin (BCG) immunisation *BMC Infect Dis, 5*, p. 115. Retrieved from http://doi.org/10.1186/1471-2334-5-115

Elson, L. H., Days, A., Calvopiña, M., Paredes, W., Araujo, E., Guderian, R. H., Nutman, T. B. (1996). In utero exposure to Onchocerca volvulus: relationship to subsequent infection intensity and cellular immune responsiveness. *Infection and Immunity, 64*(12), p. 5061-5065.

Engels, D., Chitsulo, L., Montresor, A., & Savioli, L. (2002). The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Tropica, 82*(2), p. 139-146. Retrieved from http://dx.doi.org/10.1016/S0001-706X(02)00045-1

Fairley, C. K., Miller, E., Smoleniec, J. S., & Caul, O. E. (1995). Observational study of effect of intrauterine transfusions on outcome of fetal hydrops after parvovirus B19 infection. *The Lancet, 346*(8986), p. 1335-1337. Retrieved from https://doi.org/10.1016/S0140-6736(95)92346-2

Fallon, P. G., Ballantyne, S. J., Mangan, N. E., Barlow, J. L., Dasvarma, A., Hewett, D. R., McKenzie, A. N. J. (2006). Identification of an interleukin (IL)-25–dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *The Journal of Experimental Medicine, 203*(4), p. 1105-1116. Retrieved from https://doi.org/10.1084/jem.20051615

Fehling, H. J., Lacaud, G., Kubo, A., Kennedy, M., Robertson, S., Keller, G., & Kouskoff, V. (2003). Tracking mesoderm induction and its specification to the hemangioblast during embryonic stem cell differentiation. *Development, 130*(17), p. 4217-4227. Retrieved from https://doi.org/10.1242/dev.00589

Fenwick, A. (2012). The global burden of neglected tropical diseases. *Public Health, 126*(3), p. 233-236. Retrieved from https://doi.org/10.1016/j.puhe.2011.11.015

Fields, P. E., Kim, S. T., & Flavell, R. A. (2002). Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation. *J Immunol, 169*(2), p. 647-650. Retrieved from https://doi.org/10.4049/jimmunol.169.2.647

Fitch, F.W., Gajewski, T.F. and Hu-Li, J. (2006), Production of T_H1 and T_H2 Cell Lines and Clones. *Current Protocols in Immunology,* 72: 3.13.1-3.13.15. Retrieved from https://doi.org/10.1002/0471142735.im0313s72

Flaherty, S., & Reynolds, J. M. (2015). Mouse Naïve CD4+ T Cell Isolation and In vitro Differentiation into T Cell Subsets. *Journal of visualized experiments : JoVE,* (98), 52739. Retrieved from https://doi.org/10.3791/52739

Fontenot, J. D., Gavin, M. A., & Rudensky, A. Y. (2003). Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat Immunol, 4*(4), p. 986-992. Retrieved from https://doi.org/330-336. doi:10.1038/ni904

Forster, R., Schubel, A., Breitfeld, D., Kremmer, E., Renner-Muller, I., Wolf, E., & Lipp, M. (1999). CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell, 99*(1), p. 23-33.

Freitas do Rosario, A. P., Muxel, S. M., Rodriguez-Malaga, S. M., Sardinha, L. R., Zago, C. A., Castillo-Mendez, S. I., D'Imperio Lima, M. R. (2008). Gradual decline in malaria-specific memory T cell responses leads to failure to maintain long-term protective immunity to Plasmodium chabaudi AS despite persistence of B cell memory and circulating antibody. *J Immunol, 181*(12), p. 8344-8355. Retrieved from https://doi.org/10.4049/jimmunol.181.12.8344

Gallagher, M., Malhotra, I., Mungai P. L., Wamachi A. N., Kioko J. M., Ouma J. H., Muchiri E., King C. L. (2005). The effects of maternal heminth and malaria infections on mother-to-child HIV transmission. *AIDS, 19*(16), p. 1849-55. Retrieved from https://doi.org/10.1097/01.aids.0000189846.90946.5d

Gause, W. C., Halvorson, M. J., Lu, P., Greenwald, R., Linsley, P., Urban, J. F., & Finkelman, F. D. (1997). The function of costimulatory molecules and the development of IL-4-producing T cells. *Immunol Today, 18*(3), p. 115-120.

Gebreegziabiher, D., Desta, K., Desalegn, G., Howe, R., & Abebe, M. (2014). The Effect of Maternal Helminth Infection on Maternal and Neonatal Immune Function and Immunity to Tuberculosis. *PLoS ONE, 9*(4), p. 93429. Retrieved from https://doi.org/10.1371/journal.pone.0093429

Germain, R. N. (2002). T-cell development and the CD4-CD8 lineage decision. *Nat Rev Immunol, 2*(5), p. 309-322. Retrieved from https://doi.org/10.1038/nri798

Ghaffar YA, K. M., El-Sobky M, Bahnasy R, Strickland GT. (1989). Response to hepatitis B vaccine in infants born to mothers with schistosomiasis. *Lancet 334*(8657) *p. 272*. Retrieved from https://doi.org/10.1016/s0140-6736(89)90453-4

Gibson, D. I. (2010). Nature and Classification of Parasitic Helminths *Topley & Wilson's Microbiology and Microbial Infections*: John Wiley & Sons, Ltd. Retrieved from https://doi.org/10.1002/9780470688618.taw0192

Gill, T. J., Repetti, C. F., Metlay, L. A., Rabin, B. S., Taylor, F. H., Thompson, D. S., & Cortese, A. L. (1983). Transplacental immunization of the human fetus to tetanus by immunization of the mother. *Journal of Clinical Investigation, 72*(3), p. 987-996.

Grausenburger, R., Bilic, I., Boucheron, N., Zupkovitz, G., El-Housseiny, L., Tschismarov, R., Ellmeier, W. (2010). Conditional Deletion of Histone Deacetylase 1 in T Cells Leads to Enhanced Airway Inflammation and Increased Th2 Cytokine Production. *The Journal of Immunology, 185*(6), p. 3489-3497. Retrieved from https://doi.org/10.4049/jimmunol.0903610

Gryseels, B. (2012). Schistosomiasis. *Infectious Disease Clinics of North America, 26*(2), p. 383-397. Retrieved from https://doi.org/10.1016/j.idc.2012.03.004

Grzych, J. M., Pearce, E., Cheever, A., Caulada, Z. A., Caspar, P., Heiny, S., Sher, A. (1991). Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. *The Journal of Immunology, 146*(4), p. 1322-1327.

Guadalupe, I., Mitre, E., Benitez, S., Chico, Martha E., Nutman, Thomas B., & Cooper, Philip J. (2009). Evidence for In Utero Sensitization to Ascaris lumbricoides in Newborns of Mothers with Ascariasis. *The Journal of Infectious Diseases, 199*(12), p. 1846-1850. Retrieved from https://doi.org/10.1086/599214

Gupta, R., Sheikh, A., Strachan, D. P., & Anderson, H. R. (2004). Burden of allergic disease in the UK: secondary analyses of national databases. *Clinical & Experimental Allergy, 34*(4), p. 520-526. Retrieved from 1 https://doi.org/0.1111/j.1365-2222.2004.1935.x

Haque, A., & Capron, A. (1982). Transplacental transfer of rodent microfilariae induces antigen-specific tolerance in rats. *Nature, 299*(5881), p. 361-363.

Harb H, A. M., Ashley S, Tulic KM, Pfefferle PI. Potaczek DP, Martino D et al. . Epigenetic regulation in early childhood: a miniaturized and validated method to assess histone acetylation. *Int. Arch. Allergy. Immunol.* 168(3), p. 173-181. Retrieved from https://doi.org/10.1159/000442158

Harb, H., Raedler, D., Ballenberger, N., Bock, A., Kesper, D. A., Renz, H., & Schaub, B. (2015). Childhood allergic asthma is associated with increased IL-13 and FOXP3 histone acetylation. *J Allergy Clin Immunol, 136*(1), p. 200-202. Retrieved from https://doi.org/10.1016/j.jaci.2015.01.027

Harding, F. A., McArthur, J. G., Gross, J. A., Raulet, D. H., & Allison, J. P. (1992). CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature, 356*(6370), p. 607-609. Retrieved from https://doi.org/10.1038/356607a0

Harrington, L. E., Hatton, R. D., Mangan, P. R., Turner, H., Murphy, T. L., Murphy, K. M., & Weaver, C. T. (2005). Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol, 6*(11), p. 1123-1132. Retrieved from https://doi.org/10.1038/ni1254

Harris, N., & Gause, W. C. (2011). B cell function in the immune response to helminths. *Trends in immunology, 32*(2), p. 80-88. Retrieved from https://doi.org/10.1016/j.it.2010.11.005

Hartgers, F. C., Obeng, B. B., Kruize, Y. C. M., Duijvestein, M., de Breij, A., Amoah, A., Yazdanbakhsh, M. (2008). Lower Expression of TLR2 and SOCS-3 Is

Associated with Schistosoma haematobium Infection and with Lower Risk for Allergic Reactivity in Children Living in a Rural Area in Ghana. *PLoS Neglected Tropical Diseases, 2*(4), p. 227. Retrieved from https://doi.org/10.1371/journal.pntd.0000227

Hartl, D., Koller, B., Mehlhorn, A. T., Reinhardt, D., Nicolai, T., Schendel, D. J., Krauss-Etschmann, S. (2007). Quantitative and functional impairment of pulmonary CD4+CD25hi regulatory T cells in pediatric asthma. *J Allergy Clin Immunol, 119*(5), p. 1258-1266. Retrieved from https://doi.org/10.1016/j.jaci.2007.02.023

Haworth, O., Cernadas, M., & Levy, B. D. (2011). NK cells are effectors for resolvin E1 in the timely resolution of allergic airway inflammation. *J Immunol, 186*(11), p. 6129-6135. Retrieved from https://doi.org/10.4049/jimmunol.1004007

Helmby, H., Jonsson, G., & Troye-Blomberg, M. (2000). Cellular changes and apoptosis in the spleens and peripheral blood of mice infected with blood-stage Plasmodium chabaudi chabaudi AS. *Infection and Immunity, 68*(3), p. 1485-1490. Retrieved from https://doi.org/10.1128/iai.68.3.1485-1490.2000

Hesse, M., Piccirillo, C. A., Belkaid, Y., Prufer, J., Mentink-Kane, M., Leusink, M., Wynn, T. A. (2004). The Pathogenesis of Schistosomiasis Is Controlled by Cooperating IL-10-Producing Innate Effector and Regulatory T Cells. *The Journal of Immunology, 172*(5), p. 3157-3166. Retrieved from https://doi.org/10.4049/jimmunol.172.5.3157

Horn, L.-C., Langner, A., Stiehl, P., Wittekind, C., & Faber, R. (2004). Identification of the causes of intrauterine death during 310 consecutive autopsies. *European Journal of Obstetrics and Gynecology and Reproductive Biology, 113*(2), p. 134-138. Retrieved from https://doi.org/10.1016/S0301-2115(03)00371-3

Hotez , P. J., Molyneux , D. H., Fenwick , A., Kumaresan , J., Sachs , S. E., Sachs , J. D., & Savioli , L. (2007). Control of Neglected Tropical Diseases. *New England Journal of Medicine, 357*(10), p. 1018-1027. Retrieved from https://doi.org/10.1056/NEJMra064142

Hotez, P. J., Molyneux, D. H., Fenwick, A., Ottesen, E., Ehrlich Sachs, S., & Sachs, J. D. (2006). Incorporating a Rapid-Impact Package for Neglected Tropical Diseases with Programs for HIV/AIDS, Tuberculosis, and Malaria. *PLOS Medicine, 3*(5), p. 102. Retrieved from https://doi.org/10.1371/journal.pmed.0030102

Infante-Duarte, C., Horton, H. F., Byrne, M. C., & Kamradt, T. (2000). Microbial lipopeptides induce the production of IL-17 in Th cells. *J Immunol, 165*(11), p. 6107-6115. Retrieved from https://doi.org/10.4049/jimmunol.165.11.6107

Ito, K., Yamamura, S., Essilfie-Quaye, S., Cosio, B., Ito, M., Barnes, P. J., & Adcock, I. M. (2006). Histone deacetylase 2-mediated deacetylation of the glucocorticoid receptor enables NF-kappaB suppression. *J Exp Med, 203*(1), p. 7-13. Retrieved from https://doi.org/10.1084/jem.20050466

Jameson, S. C., Lee, Y. J., & Hogquist, K. A. (2015). Innate Memory T cells. *Adv Immunol, 126*, p. 173-213. Retrieved from https://doi.org/10.1016/bs.ai.2014.12.001

Janeway CA Jr, Travers P., Walport M. (2001). Immunobiology: The Immune System in Health and Disease. The mucosal immune system. *in Garland Science, New York*. 5th edition.

Jenkins, M. K., Chen, C. A., Jung, G., Mueller, D. L., & Schwartz, R. H. (1990). Inhibition of antigen-specific proliferation of type 1 murine T cell clones after stimulation with immobilized anti-CD3 monoclonal antibody. *J Immunol, 144*(1), p. 16-22.

Jenkins, S. J., Hewitson, J. P., Ferret-Bernard, S., & Mountford, A. P. (2005). Schistosome larvae stimulate macrophage cytokine production through TLR4-dependent and -independent pathways. *International immunology, 17*(11), p. 1409-1418. Retrieved from https://doi.org/10.1093/intimm/dxh319

Jira, M., Antosova, E., Vondra, V., Strejcek, J., Mazakova, H., & Prazakova, J. (1988). Natural killer and interleukin-2 induced cytotoxicity in asthmatics. *Allergy, 43*(4), p. 294-298. Retrieved from https://doi.org/10.1111/j.1398-9995.1988.tb00903.x

Kaisho, T., & Akira, S. (2002). Toll-like receptors as adjuvant receptors. *Biochim Biophys Acta, 1589*(1), p. 1-13. Retrieved from https://doi.org/10.1016/s0167-4889(01)00182-3

Karagiannidis, C., Akdis, M., Holopainen, P., Woolley, N. J., Hense, G., Ruckert, B., Schmidt-Weber, C. B. (2004). Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. *J Allergy Clin Immunol, 114*(6), p. 1425-1433. Retrieved from https://doi.org/10.1016/j.jaci.2004.07.014

Karimi, K., & Forsythe, P. (2013). Natural killer cells in asthma. *Front Immunol, 4*, (159), p. 1-8. Retrieved from https://doi.org/10.3389/fimmu.2013.00159

Kearley, J., Barker, J. E., Robinson, D. S., & Lloyd, C. M. (2005). Resolution of airway inflammation and hyperreactivity after in vivo transfer of CD4+CD25+ regulatory T cells is interleukin 10 dependent. *J Exp Med, 202*(11), 1539-1547. Retrieved from http://doi.org/10.1084/jem.20051166

King, C. H., Dickman, K., & Tisch, D. J. (2005). Reassessment of the cost of chronic helmintic infection: a meta-analysis of disability-related outcomes in endemic schistosomiasis. *The Lancet, 365*(9470), p. 1561-1569. Retrieved from http://dx.doi.org/10.1016/S0140-6736(05)66457-4

King, C. L. (1993). Cytokine control of parasite-specific anergy in human lymphatic filariasis. Preferential induction of a regulatory T helper type 2 lymphocyte subset. *J. Clin. Invest., 92*, p. 1667–1673.

King, C. L. (1996). Cytokine control of parasite parasite-specific anergy in human urinary schistosomiasis: IL-10 modulates lymphocyte reactivity. *J. Immunol., 156*, p. 4715–4721.

King, C. L., Malhotra, I., Mungai, P., Wamachi, A., Kioko, J., Ouma, J. H., & Kazura, J. W. (1998). B cell sensitization to helminthic infection develops in utero in humans. *J Immunol, 160*(7), p. 3578-3584.

Klar, K., Perchermeier, S., Bhattacharjee, S., Harb, H., Adler, T., Istvanffy, R., Loffredo-Verde E, Oostendorp RA, Renz H, Prazeres da Costa, C. (2017). Chronic schistosomiasis during pregnancy epigenetically reprograms T-cell differentiation in offspring of infected mothers. *European journal of*

immunology, 47(5), p. 841-847. Retrieved from http://dx.doi.org/10.1002/eji.201646836

Koch, S., Larbi, A., Derhovanessian, E., Özcelik, D., Naumova, E., & Pawelec, G. (2008). Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. *Immunity & Ageing, 5*(1), p. 6. Retrieved from http://dx.doi.org/10.1186/1742-4933-5-6

Kramer, M. S. (2003). The epidemiology of adverse pregnancy outcomes: an overview. *J Nutr, 133*(5 Suppl 2), p. 1592-1596. Retrieved from http://doi.org/10.1093/jn/133.5.1592S

Krämer, U., Heinrich, J., Wjst, M., & Wichmann, H. E. (1999). Age of entry to day nursery and allergy in later childhood. *The Lancet, 353*(9151), p. 450-454. Retrieved from https://doi.org/10.1016/S0140-6736(98)06329-6

Kumararatne, D. S., Phillips, R. S., Sinclair, D., Parrott, M. V., & Forrester, J. B. (1987). Lymphocyte migration in murine malaria during the primary patent parasitaemia of Plasmodium chabaudi infections. *Clin Exp Immunol, 68*(1), p. 65-77.

Kurtis, J. D., Higashi, A., Wu, H.-W., Gundogan, F., McDonald, E. A., Sharma, S., Friedman, J. F. (2011). Maternal Schistosomiasis Japonica Is Associated with Maternal, Placental, and Fetal Inflammation. *Infection and Immunity, 79*(3), p. 1254-1261. Retrieved from https://doi.org/10.1128/IAI.01072-10

LaBeaud, A. D., Malhotra, I., King, M. J., King, C. L., & King, C. H. (2009). Do Antenatal Parasite Infections Devalue Childhood Vaccination? *PLoS Neglected Tropical Diseases, 3*(5), p. 442. Retrieved from https://doi.org/10.1371/journal.pntd.0000442

Labuda, L. A., Ateba-Ngoa, U., Feugap, E. N., Heeringa, J. J., van der Vlugt, L. E. P. M., Pires, R. B. A., Smits, H. H. (2013). Alterations in Peripheral Blood B Cell Subsets and Dynamics of B Cell Responses during Human Schistosomiasis. *PLoS Neglected Tropical Diseases, 7*(3), p. 2094. Retrieved from https://doi.org/10.1371/journal.pntd.0002094

Lambertucci, J. R. (2010). Acute schistosomiasis mansoni: revisited and reconsidered. *Memórias do Instituto Oswaldo Cruz, 105*(4), p. 422-435. Retrieved from https://doi.org/10.1590/s0074-02762010000400012

Landreth, K. S. (1993). B lymphocyte development as a developmental process *Developmental Immunology*, p. 238-273.

Landreth, K. S. (2002). Critical windows in development of the rodent immune system. *Human & Experimental Toxicology, 21*(9-10), 493-498. doi:10.1191/0960327102ht287oa

Layland, L. E., Straubinger, K., Ritter, M., Loffredo-Verde, E., Garn, H., Sparwasser, T., & Prazeres da Costa, C. (2013). Schistosoma mansoni-Mediated Suppression of Allergic Airway Inflammation Requires Patency and Foxp3+ Treg Cells. *PLoS Neglected Tropical Diseases, 7*(8), p. 2379. Retrieved from https://doi.org/10.1371/journal.pntd.0002379

Lenzi, J. A., Sobral, A. C., Araripe, J. R., Grimaldi Filho, G., & Lenzi, H. L. (1987). Congenital and nursing effects on the evolution of Schistosoma mansoni infection in mice. *Mem Inst Oswaldo Cruz, 82 Suppl 4*, p. 257-267.

Lewert, R. M., & Mandlowitz, S. (1969). Schistosomiasis: prenatal induction of tolerance to antigens. *Nature, 224*(5223), p. 1029-1030.

Liang, B., Workman, C., Lee, J., Chew, C., Dale, B. M., Colonna, L., Clynes, R. (2008). Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. *J Immunol, 180*(9), p. 5916-5926. Retrieved from https://doi.org/10.4049/jimmunol.180.9.5916

Lin, S. J., Chang, L. Y., Yan, D. C., Huang, Y. J., Lin, T. J., & Lin, T. Y. (2003). Decreased intercellular adhesion molecule-1 (CD54) and L-selectin (CD62L) expression on peripheral blood natural killer cells in asthmatic children with acute exacerbation. *Allergy, 58*(1), p. 67-71.

Ling, E. M., Smith, T., Nguyen, X. D., Pridgeon, C., Dallman, M., Arbery, J., Robinson, D. S. (2004). Relation of CD4+CD25+ regulatory T-cell suppression of allergen-driven T-cell activation to atopic status and expression of allergic disease. *Lancet, 363*(9409), 608-615. Retrieved from https://doi.org/10.1016/s0140-6736(04)15592-x

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods, 25*(4), p. 402-408. Retrieved from http://dx.doi.org/10.1006/meth.2001.1262

Lloyd, C. M., & Hessel, E. M. (2010). Functions of T cells in asthma: more than just T(H)2 cells. *Nature reviews. Immunology, 10*(12),p. 838-848. Retrieved from https://doi.org/10.1038/nri2870

Lucas, A., Brooke, O. G., Morley, R., Cole, T. J., & Bamford, M. F. (1990). Early diet of preterm infants and development of allergic or atopic disease: randomised prospective study. *BMJ, 300*(6728), p. 837-840.

Maizels, R. M., & Yazdanbakhsh, M. (2003). Immune Regulation by helminth parasites: cellular and molecular mechanisms. *Nat Rev Immunol, 3*(9), p. 733-744. Retrieved from https://doi.org/10.1038/nri1183

Malhotra, I., Ouma, J., Wamachi, A., Kioko, J., Mungai, P., Omollo, A., King, C. L. (1997). In utero exposure to helminth and mycobacterial antigens generates cytokine responses similar to that observed in adults. *J Clin Invest, 99*(7), p. 1759-1766. Retrieved from https://doi.org/10.1172/JCI119340

Mangan, N. E., Fallon, R. E., Smith, P., van Rooijen, N., McKenzie, A. N., & Fallon, P. G. (2004). Helminth Infection Protects Mice from Anaphylaxis via IL-10-Producing B Cells. *The Journal of Immunology, 173*(10), p. 6346-6356. Retrieved from https://doi.org/10.4049/jimmunol.173.10.6346

Marques, A. H., O'Connor, T. G., Roth, C., Susser, E., & Bjørke-Monsen, A.-L. (2013). The influence of maternal prenatal and early childhood nutrition and maternal prenatal stress on offspring immune system development and neurodevelopmental disorders. *Frontiers in Neuroscience, 7*(120). Retrieved from https://doi.org/10.3389/fnins.2013.00120

Marques, C. R., Costa, R. S., Costa, G. N. d. O., da Silva, T. M., Teixeira, T. O., de Andrade, E. M. M., Figueiredo, C. A. (2015). Genetic and epigenetic studies of FOXP3 in asthma and allergy. *Asthma research and practice, 1*(10). Retrieved from https://doi.org/10.1186/s40733-015-0012-4

Matricardi, P. M., Rosmini, F., Riondino, S., Fortini, M., Ferrigno, L., Rapicetta, M., & Bonini, S. (2000). Exposure to foodborne and orofecal microbes versus airborne

viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ : British Medical Journal, 320*(7232), p. 412-417. Retrieved from https://doi.org/10.1136/bmj.320.7232.412

McLane, M. P., Haczku, A., van de Rijn, M., Weiss, C., Ferrante, V., MacDonald, D., Levitt, R. C. (1998). Interleukin-9 promotes allergen-induced eosinophilic inflammation and airway hyperresponsiveness in transgenic mice. *Am J Respir Cell Mol Biol, 19*(5), p. 713-720. Retrieved from https://doi.org/10.1165/ajrcmb.19.5.3457

Mebius, R. E., Miyamoto, T., Christensen, J., Domen, J., Cupedo, T., Weissman, I. L., & Akashi, K. (2001). The fetal liver counterpart of adult common lymphoid progenitors gives rise to all lymphoid lineages, CD45+CD4+CD3- cells, as well as macrophages. *J Immunol, 166*(11), p. 6593-6601. Retrieved from https://doi.org/10.4049/jimmunol.166.11.6593

Medeiros, M., Jr., Figueiredo, J. P., Almeida, M. C., Matos, M. A., Araújo, M. I., Cruz, A. A., Carvalho, E. M. (2003). Schistosoma mansoni infection is associated with a reduced course of asthma. *Journal of Allergy and Clinical Immunology, 111*(5), p. 947-951. Retrieved from https://doi.org/10.1067/mai.2003.1381

Miller, R. L., & Ho, S.-m. (2008). Environmental Epigenetics and Asthma: Current Concepts and Call for Studies. *American Journal of Respiratory and Critical Care Medicine, 177*(6), p. 567-573. Retrieved from https://doi.org/10.1164/rccm.200710-1511PP

Money, D., & Allen, V. M. (2016). The Prevention of Early-Onset Neonatal Group B Streptococcal Disease. *Journal of Obstetrics and Gynaecology Canada, 38*(12),p. 326-335. Retrieved from https://doi.org/10.1016/j.jogc.2016.09.042

Monks, C. R., Freiberg, B. A., Kupfer, H., Sciaky, N., & Kupfer, A. (1998). Three-dimensional segregation of supramolecular activation clusters in T cells. *Nature, 395*(6697), p. 82-86. Retrieved from https://doi.org/10.1038/25764

Moore, A. J., In, T. S., Trotman-Grant, A., Yoganathan, K., Montpellier, B., Guidos, C. J., Anderson, M. K. (2017). A key role for IL-7R in the generation of microenvironments required for thymic dendritic cells. *Immunol Cell Biol*. *95*(10), p. 933-942. Retrieved from https://doi.org/10.1038/icb.2017.74

Mor, G., Romero, R., Aldo, P. B., & Abrahams, V. M. (2005). Is the trophoblast an immune regulator? The role of Toll-like receptors during pregnancy. *Crit Rev Immunol, 25*(5), p. 375-388.

Mpairwe, H., Webb, E. L., Muhangi, L., Ndibazza, J., Akishule, D., Nampijja, M., Elliott, A. M. (2011). Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. *Pediatric Allergy and Immunology, 22*(3), p. 305-312. Retrieved from https://doi.org/10.1111/j.1399-3038.2010.01122.x

Nagler-Anderson., C. (2006). Helminth-Induced Immunoregulation of an Allergic Response to Food.,in *Parasites and Allergy*. Basel. F. T. Monique Capron (Ed.). Retrieved from https://doi.org/10.1159/000088876

Nakamura, K., Kitani, A., Fuss, I., Pedersen, A., Harada, N., Nawata, H., & Strober, W. (2004). TGF-beta 1 plays an important role in the mechanism of CD4+CD25+ regulatory T cell activity in both humans and mice. *J Immunol, 172*(2), p. 834-842. Retrieved from https://doi.org/10.4049/jimmunol.172.2.834

Nash, T. E., Cheever, A. W., Ottesen, E. A., & Cook, J. A. (1982). Schistosome infections in humans: Perspectives and recent findings. *Annals of Internal Medicine, 97*(5), p. 740-754. https://doi.org/10.7326/0003-4819-97-5-740

Nausch, N., Bourke, C. D., Appleby, L. J., Rujeni, N., Lantz, O., Trottein, F., Mutapi, F. (2012). Proportions of CD4+ memory T cells are altered in individuals chronically infected with Schistosoma haematobium. *Scientific Reports, 2*, p. 472. https://doi.org/10.1038/srep00472

Nograles, K. E., Zaba, L. C., Shemer, A., Fuentes-Duculan, J., Cardinale, I., Kikuchi, T., Guttman-Yassky, E. (2009). IL-22-producing "T22" T cells account for upregulated IL-22 in atopic dermatitis despite reduced IL-17-producing TH17 T cells. *J Allergy Clin Immunol, 123*(6), p. 1244-1252. Retrieved from https://doi.org/10.1016/j.jaci.2009.03.041

Noval Rivas, M., & Chatila, T. A. (2016). Regulatory T cells in allergic diseases. *J Allergy Clin Immunol, 138*(3), p. 639-652. Retrieved from https://doi.org/10.1016/j.jaci.2016.06.003

Novato-Silva, E., Gazzinelli, G., & Colley, D. G. (1992). Immune Responses During Human Schistosomiasis Mansoni. XVIII. Immunologic Status of Pregnant Women and Their Neonates. *Scandinavian Journal of Immunology, 35*(4), 429-437. Retrieved from https://doi.org/10.1111/j.1365-3083.1992.tb02878.x

O'Garra, A., & Murphy, K. (1996). Role of cytokines in development of Th1 and Th2 cells. *Chem Immunol, 63*, p. 1-13.

Obst, R. (2015). The Timing of T Cell Priming and Cycling. *Front Immunol, 6*(563). Retrieved from https://doi.org/10.3389/fimmu.2015.00563

Oro, A. S., Guarino, T. J., Driver, R., Steinman, L., & Umetsu, D. T. (1996). Regulation of disease susceptibility: decreased prevalence of IgE-mediated allergic disease in patients with multiple sclerosis. *J Allergy Clin Immunol, 97*(6), p. 1402-1408.

Othman, A. A., Shoheib, Z. S., Saied, E. M., & Soliman, R. H. (2010). Congenital exposure to Schistosoma mansoni infection: Impact on the future immune response and the disease outcome. *Immunobiology, 215*(2), p.101-112. Retrieved from http://dx.doi.org/10.1016/j.imbio.2009.04.004

Ouyang, W., Lohning, M., Gao, Z., Assenmacher, M., Ranganath, S., Radbruch, A., & Murphy, K. M. (2000). Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity, 12*(1), p. 27-37.

Ouyang, W., Ranganath, S. H., Weindel, K., Bhattacharya, D., Murphy, T. L., Sha, W. C., & Murphy, K. M. (1998). Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity, 9*(5), p. 745-755.

Owen, J. J., Wright, D. E., Habu, S., Raff, M. C., & Cooper, M. D. (1977). Studies on the generation of B lymphocytes in fetal liver and bone marrow. *J Immunol, 118*(6), p. 2067-2072.

Pardoll, D. M., Fowlkes, B. J., Bluestone, J. A., Kruisbeek, A., Maloy, W. L., Coligan, J. E., & Schwartz, R. H. (1987). Differential expression of two distinct T-cell receptors during thymocyte development. *Nature, 326*(6108), p. 79-81.

Paveley, R. A., Aynsley, S. A., Turner, J. D., Bourke, C. D., Jenkins, S. J., Cook, P. C., Mountford, A. P. (2011). The Mannose Receptor (CD206) is an important pattern recognition receptor (PRR) in the detection of the infective stage of the

helminth Schistosoma mansoni and modulates IFNγ production. *International Journal for Parasitology, 41*(13), p. 1335-1345. Retrieved from http://dx.doi.org/10.1016/j.ijpara.2011.08.005

Pawankar, R. (2014). Allergic diseases and asthma: a global public health concern and a call to action. *World Allergy Organization Journal, 7*(1), p. 12. Retrieved from https://doi.org/10.1186/1939-4551-7-12

Pawankar R, Canonica. G. W., Holgate S. T., Lockey R. F., Blaiss M. (2013). WAO White Book on Allergy 2011-2012 Executive Summary, Chapter 5, p. 145-150. *World Health Organization.*

Pearce, E. J., Caspar, P., Grzych, J. M., Lewis, F. A., & Sher, A. (1991). Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, Schistosoma mansoni. *The Journal of Experimental Medicine, 173*(1), p. 159-166. Retrieved from https://doi.org/10.1084/jem.173.1.159

Pellerin, L., Jenks, J. A., Begin, P., Bacchetta, R., & Nadeau, K. C. (2014). Regulatory T cells and their roles in immune dysregulation and allergy. *Immunol Res, 58*(2-3), p. 358-368. Retrieved from https://doi.org/10.1007/s12026-014-8512-5

Pennock, N. D., White, J. T., Cross, E. W., Cheney, E. E., Tamburini, B. A., & Kedl, R. M. (2013). T cell responses: naïve to memory and everything in between. *Advances in Physiology Education, 37*(4), p. 273-283. Retrieved from https://doi.org/10.1152/advan.00066.2013

Peters, N. C., Pagán, A. J., Lawyer, P. G., Hand, T. W., Henrique Roma, E., Stamper, L. W., Sacks, D. L. (2014). Chronic Parasitic Infection Maintains High Frequencies of Short-Lived Ly6C(+)CD4(+) Effector T Cells That Are Required for Protection against Re-infection. *PLOS Pathogens, 10*(12), e1004538. Retrieved from https://doi.org/10.1371/journal.ppat.1004538

Pinot de Moira, A., Sousa-Figueiredo, J. C., Jones, F. M., Fitzsimmons, C. M., Betson, M., Kabatereine, N. B., Dunne, D. W. (2013). Schistosoma mansoni Infection in Preschool-Aged Children: Development of Immunoglobulin E and Immunoglobulin G(4) Responses to Parasite Allergen-Like Proteins. *The Journal of Infectious Diseases, 207*(2), p. 362-366. Retrieved from https://doi.org/10.1093/infdis/jis676

Ple, C., Barrier, M., Amniai, L., Marquillies, P., Bertout, J., Tsicopoulos, A., Duez, C. (2010). Natural killer cells accumulate in lung-draining lymph nodes and regulate airway eosinophilia in a murine model of asthma. *Scand J Immunol, 72*(2), p. 118-127. Retrieved from https://doi.org/10.1111/j.1365-3083.2010.02419.x

Prescott, S. L., & Clifton, V. (2009). Asthma and pregnancy: emerging evidence of epigenetic interactions in utero. *Curr Opin Allergy Clin Immunol, 9*(5), p. 417-426. Retrieved from https://doi.org/10.1097/ACI.0b013e328330634f

Presser, K., Schwinge, D., Wegmann, M., Huber, S., Schmitt, S., Quaas, A., Schramm, C. (2008). Coexpression of TGF-β1 and IL-10 Enables Regulatory T Cells to Completely Suppress Airway Hyperreactivity. *The Journal of Immunology, 181*(11), p. 7751-7758. Retrieved from https://doi.org/10.4049/jimmunol.181.11.7751

Provoost, S., Maes, T., van Durme, Y. M., Gevaert, P., Bachert, C., Schmidt-Weber, C. B., Tournoy, K. G. (2009). Decreased FOXP3 protein expression in patients with asthma. *Allergy, 64*(10), p. 1539-1546. Retrieved from https://doi.org/10.1111/j.1398-9995.2009.02056.x

Raedler, D., Ballenberger, N., Klucker, E., Bock, A., Otto, R., Prazeres da Costa, O., Schaub, B. (2015). Identification of novel immune phenotypes for allergic and nonallergic childhood asthma. *J Allergy Clin Immunol, 135*(1), p. 81-91. Retrieved from https://doi.org/10.1016/j.jaci.2014.07.046

Ramaswamy, K., Kumar, P., & He, Y.-X. (2000). A Role for Parasite-Induced PGE2 in IL-10-Mediated Host Immunoregulation by Skin Stage Schistosomula of Schistosoma mansoni. *The Journal of Immunology, 165*(8), p. 4567-4574. Retrieved from https://doi.org/10.4049/jimmunol.165.8.4567

Rando, O. J., & Chang, H. Y. (2009). Genome-Wide Views of Chromatin Structure. *Annual review of biochemistry, 78*, p. 245-271. Retrieved from https://doi.org/10.1146/annurev.biochem.78.071107.134639

Reed, A. J., Zarrabi, Y., Perate, A. L., Jeganathan, A., Naji, A., & Noorchashm, H. (2005). The frequency of double-positive thymocytes expressing an αβ TCR clonotype regulates peripheral CD4 T cell compartment homeostasis. *Immunology, 116*(3), p. 400-407. Retrieved from https://doi.org/10.1111/j.1365-2567.2005.02240.x

Reinhardt, R. L., Khoruts, A., Merica, R., Zell, T., & Jenkins, M. K. (2001). Visualizing the generation of memory CD4 T cells in the whole body. *Nature, 410*(6824), p. 101-105. Retrieved from https://doi.org/10.1038/35065111

Renaud, R., Brettes, P., Carrie, J., Castanier, C., Renaud, L., Chesnet, Y., Loubière, R. (1971). Bilharziasis of the internal female genital organs. *Revue francaise de gynecologie et d'obstetrique, 66*(1), p.1-18.

Renaud, R., P. Brettes, C. Castanier, and R. Loubiere. (1972). Placental bilharziasis. *Int. J. Gynaecol. Obstet., 10*(1), p. 24-30. Retrieved from https://doi.org/10.1002/j.1879-3479.1972.tb00812.x

Renström, J., Istvanffy, R., Gauthier, K., Shimono, A., Mages, J., Jardon-Alvarez, A., Oostendorp, R. A. J. (2009). Secreted Frizzled-Related Protein 1 Extrinsically Regulates Cycling Activity and Maintenance of Hematopoietic Stem Cells. *Cell Stem Cell, 5*(2), p. 157-167. Retrieved from https://doi.org/10.1016/j.stem.2009.05.020

Riedler, J., Braun-Fahrländer, C., Eder, W., Schreuer, M., Waser, M., Maisch, S., von Mutius, E. (2001). Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *The Lancet, 358*(9288), p. 1129-1133. Retrieved from https://doi.org/10.1016/S0140-6736(01)06252-3

Rodewald, H. R., & Fehling, H. J. (1998). Molecular and cellular events in early thymocyte development. *Adv Immunol, 69*, p. 1-112.

Rogers, M. F., Taylor, A. W., & Nesheim, S. R. (2010). Preventing perinatal transmission of HIV: the national perspective. *J Public Health Manag Pract, 16*(6), p. 505-508. Retrieved from https://doi.org/10.1097/PHH.0b013e3181ef1964

Rolink, A., & Melchers, F. (1993). B lymphopoiesis in the mouse. *Adv Immunol, 53*, p. 123-156.

Romagnani, S. (2006). Regulation of the T cell response. *Clin Exp Allergy, 36*(11), p. 1357-1366. Retrieved from https://doi.org/10.1111/j.1365-2222.2006.02606.x

Rujeni, N., Taylor, D. W., & Mutapi, F. (2012). Human Schistosome Infection and Allergic Sensitisation. *Journal of Parasitology Research, 2012*, 154743. Retrieved from https://doi.org/10.1155/2012/154743

Pit, DS., Polderman, AM., Schulz-Key, H., & Soboslay, P. (2000). Prenatal immune priming with helminth infections: Parasite-specific cellular reactivity and Th1 and Th2 cytokine responses in neonates. *Allergy 55*(8), p. 732-739.

Samelson, L. E., Patel, M. D., Weissman, A. M., Harford, J. B., & Klausner, R. D. (1986). Antigen activation of murine T cells induces tyrosine phosphorylation of a polypeptide associated with the T cell antigen receptor. *Cell, 46*(7), p. 1083-1090.

Santis, M. D., Cavaliere, A. F., Straface, G., & Caruso, A. (2006). Rubella infection in pregnancy. *Reproductive Toxicology, 21*(4), p. 390-398. Retrieved from https://doi.org/10.1016/j.reprotox.2005.01.014

Santos, P. d. E. A., Lorena, V. M. B., Fernandes, É., Sales, I. R. F., Albuquerque, M. C. P., Gomes, Y., Souza, V. M. O. (2014). Maternal schistosomiasis alters costimulatory molecules expression in antigen-presenting cells from adult offspring mice. *Experimental Parasitology, 141*, p. 62-67. Retrieved from https://doi.org/10.1016/j.exppara.2014.03.017

Santos, P. d. E. A., Sales, I. R. F., Schirato, G. V., Costa, V. M. A., Albuquerque, M. C. P. A., Souza, V. M. O., & Malagueño, E. (2010). Influence of maternal schistosomiasis on the immunity of adult offspring mice. *Parasitology Research, 107*(1), p. 95-102. Retrieved from 10.1007/s00436-010-1839-5

Savioli, L., Engels, D., & Endo, H. (2005). Extending the benefits of deworming for development. *The Lancet, 365*(9470), p. 1520-1521. Retrieved from https://doi.org/10.1016/S0140-6736(05)66433-1

Schaub, B., Liu, J., Höppler, S., Schleich, I., Huehn, J., Olek, S., von Mutius, E. (2009). Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. *Journal of Allergy and Clinical Immunology, 123*(4), p. 774-782. Retrieved from https://doi.org/10.1016/j.jaci.2009.01.056

Schmidt-Weber, C. B. (2008). Th17 and treg cells innovate the TH1/TH2 concept and allergy research. *Chem Immunol Allergy, 94*, p. 1-7. Retrieved from https://doi.org/10.1159/000154844

Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T. K., Kastelein, R. A. (2005). IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity, 23*(5), p. 479-490. Retrieved from https://doi.org/10.1016/j.immuni.2005.09.015

Scott, P., Artis, D., Uzonna, J., & Zaph, C. (2004). The development of effector and memory T cells in cutaneous leishmaniasis: the implications for vaccine development. *Immunol Rev, 201*, p. 318-338. Retrieved from https://doi.org/10.1111/j.0105-2896.2004.00198.x

Seydel, L. S., Petelski, A., van Dam, G. J., van der Kleij, D., Kruize-Hoeksma, Y. C., Luty, A. J., Kremsner, P. G. (2012). Association of in utero sensitization to Schistosoma haematobium with enhanced cord blood IgE and increased

frequencies of CD5- B cells in African newborns. *Am J Trop Med Hyg, 86*(4), 613-619. Retrieved from https://doi.org/10.4269/ajtmh.2012.11-0463

Shaheen, S. O., Barker, D. J. P., Heyes, C. B., Shiell, A. W., Aaby, P., Hall, A. J., & Goudiaby, A. (1996). Measles and atopy in Guinea-Bissau. *The Lancet, 347*(9018), p. 1792-1796. Retrieved from http://dx.doi.org/10.1016/S0140-6736(96)91617-7

Shortman, K., Vremec, D., Corcoran, L. M., Georgopoulos, K., Lucas, K., & Wu, L. (1998). The linkage between T-cell and dendritic cell development in the mouse thymus. *Immunol Rev, 165*, p. 39-46.

Siegrist, D., & Siegrist-Obimpeh, P. (1992). Schistosoma haematobium infection in pregnancy. *Acta Trop, 50*(4), p. 317-321.

Sleigh, A., Li, X., Jackson, S., & Huang, K. (1998). Eradication of schistosomiasis in Guangxi, China. Part 3. Community diagnosis of the worst-affected areas and maintenance strategies for the future. *Bulletin of the World Health Organization, 76*(6), p. 581-590.

Smith, P., Fallon, R. E., Mangan, N. E., Walsh, C. M., Saraiva, M., Sayers, J. R., Fallon, P. G. (2005). Schistosoma mansoni secretes a chemokine binding protein with antiinflammatory activity. *The Journal of Experimental Medicine, 202*(10), p. 1319-1325. Retrieved from https://doi.org/10.1084/jem.20050955

Smithers, S. R., & Terry, R. J. (1967). Resistance to experimental infection with Schistosoma mansoni in rhesus monkeys induced by the transfer of adult worms. *Trans R Soc Trop Med Hyg, 61*(4), p. 517-533.

Smits, H. H., Hammad, H., van Nimwegen, M., Soullie, T., Willart, M. A., Lievers, E., Yazdanbakhsh, M. (2007). Protective effect of Schistosoma mansoni infection on allergic airway inflammation depends on the intensity and chronicity of infection. *The Journal of allergy and clinical immunology, 120*(4), p. 932-940. Retrieved from https://doi.org/10.1016/j.jaci.2007.06.009

Sprent, J., & Surh, C. D. (2003). Cytokines and T cell homeostasis. Review. *Immunol Lett, 85*(2), p. 145-149. Retrieved from https://doi.org/10.1016/S0165-2478(02)00221-3

Steel, C., Guinea, A., McCarthy, J. S., & Ottesen, E. A. (1994). Long-term effect of prenatal exposure to maternal microfilaraemia on immune responsiveness to filarial antigens. *Lancet, 343*, p. 890-893.

Steketee, R., Nahlen, B., Parise, M., & Menendez, C. (2001). The burden of malaria in pregnancy in malaria-endemic areas. *The American Journal of Tropical Medicine and Hygiene, 64*(1), p. 28-35. Retrieved from https://doi.org/10.4269/ajtmh.2001.64.28

Stensgaard, A.-S., Utzinger, J., Vounatsou, P., Hürlimann, E., Schur, N., Saarnak, C. F. L., Kristensen, T. K. (2013). Large-scale determinants of intestinal schistosomiasis and intermediate host snail distribution across Africa: does climate matter? *Acta Tropica, 128*(2), p. 378-390. Retrieved from http://dx.doi.org/10.1016/j.actatropica.2011.11.010

Stock, P., Akbari, O., Berry, G., Freeman, G. J., Dekruyff, R. H., & Umetsu, D. T. (2004). Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. *Nat Immunol, 5*(11), p. 1149-1156. Retrieved from https://doi.org/10.1038/ni1122

Strachan, D. P. (1989). Hay fever, hygiene, and household size. *BMJ : British Medical Journal, 299*(6710), p. 1259-1260.

Straubinger, K., Paul, S., Prazeres da Costa, O., Ritter, M., Buch, T., Busch, D. H., Layland, LE, Prazeres da Costa, C. U. (2014). Maternal immune response to helminth infection during pregnancy determines offspring susceptibility to allergic airway inflammation. *Journal of Allergy and Clinical Immunology, 134*(6), p. 1271-1279.e1210. Retrieved from http://dx.doi.org/10.1016/j.jaci.2014.05.034

Su, R. C., Becker, A. B., Kozyrskyj, A. L., & Hayglass, K. T. (2009). Altered epigenetic regulation and increasing severity of bronchial hyperresponsiveness in atopic asthmatic children. *J Allergy Clin Immunol, 124*(5), p. 1116-1118. Retrieved from https://doi.org/10.1016/j.jaci.2009.08.033

Sugiyama, M., Arakawa, H., Ozawa, K., Mizuno, T., Mochizuki, H., Tokuyama, K., & Morikawa, A. (2007). Early-life risk factors for occurrence of atopic dermatitis during the first year. *Pediatrics, 119*(3), p. 716-723. Retrieved from https://doi.org/10.1542/peds.2006-0893

Sunderkötter, C., Nikolic, T., Dillon, M. J., van Rooijen, N., Stehling, M., Drevets, D. A., & Leenen, P. J. M. (2004). Subpopulations of Mouse Blood Monocytes Differ in Maturation Stage and Inflammatory Response. *The Journal of Immunology, 172*(7), p. 4410-4417. Retrieved from https://doi.org/10.4049/jimmunol.172.7.4410

Szabo, S. J., Dighe, A. S., Gubler, U., & Murphy, K. M. (1997). Regulation of the Interleukin (IL)-12R β2 Subunit Expression in Developing T Helper 1 (Th1) and Th2 Cells. *The Journal of Experimental Medicine, 185*(5), p. 817-824. Retrieved from https://doi.org/10.1084/jem.185.5.817

Tang, L., Benjaponpitak, S., DeKruyff, R. H., & Umetsu, D. T. (1998). Reduced prevalence of allergic disease in patients with multiple sclerosis is associated with enhanced IL-12 production. *J Allergy Clin Immunol, 102*(3), p. 428-435. Retrieved from https://doi.org/10.1016/s0091-6749(98)70131-9

Tang, M. L., Kemp, A. S., Thorburn, J., & Hill, D. J. (1994). Reduced interferon-gamma secretion in neonates and subsequent atopy. *Lancet, 344*(8928), p. 983-985. Retrieved from https://doi.org/10.1016/s0140-6736(94)90338-7

Thiriou, D., Morianos, I., Xanthou, G., & Samitas, K. (2017). Innate immunity as the orchestrator of allergic airway inflammation and resolution in asthma. *Int Immunopharmacol, 48*(Supplement C), p. 43-54. Retrieved from https://doi.org/10.1016/j.intimp.2017.04.027

Thomas, S. L., Griffiths, C., Smeeth, L., Rooney, C., & Hall, A. J. (2010). Burden of Mortality Associated With Autoimmune Diseases Among Females in the United Kingdom. *American Journal of Public Health, 100*(11), p. 2279-2287. Retrieved from https://doi.org/10.2105/AJPH.2009.180273

Thornton, A. M., & Shevach, E. M. (1998). CD4(+)CD25(+) Immunoregulatory T Cells Suppress Polyclonal T Cell Activation In Vitro by Inhibiting Interleukin 2 Production. *The Journal of Experimental Medicine, 188*(2), p. 287-296. Retrieved from https://doi.org/10.1084/jem.188.2.287

Timonen, T., & Stenius-Aarniala, B. (1985). Natural killer cell activity in asthma. *Clinical and Experimental Immunology, 59*(1), p. 85-90.

Tumes, D. J., Papadopoulos, M., Endo, Y., Onodera, A., Hirahara, K., & Nakayama, T. (2017). Epigenetic regulation of T-helper cell differentiation, memory, and plasticity in allergic asthma. *Immunological Reviews, 278*(1), 8-19. Retrieved from https://doi.org/10.1111/imr.12560

Tweyongyere, R., Mawa, P. A., Kihembo, M., Jones, F. M., Webb, E. L., Cose, S., Elliott, A. M. (2011). Effect of praziquantel treatment of Schistosoma mansoni during pregnancy on immune responses to schistosome antigens among the offspring: results of a randomised, placebo-controlled trial. *BMC Infectious Diseases, 11*(1), p. 234. Retrieved from https://doi.org/10.1186/1471-2334-11-234

Urdahl, K. B., Shafiani, S., & Ernst, J. D. (2011). Initiation and regulation of T-cell responses in tuberculosis. *Mucosal Immunol, 4*(3), p. 288-293. Retrieved from https://doi.org/10.1038/mi.2011.10

van der Kleij, D., van den Biggelaar, A. H., Kruize, Y. C., Retra, K., Fillie, Y., Schmitz, M., Yazdanbakhsh, M. (2004). Responses to Toll-like receptor ligands in children living in areas where schistosome infections are endemic. *J Infect Dis, 189*(6), p. 1044-1051. Retrieved from https://doi.org/10.1086/382089

von Hertzen, L. C. (2002). Maternal stress and T-cell differentiation of the developing immune system: possible implications for the development of asthma and atopy. *J Allergy Clin Immunol, 109*(6), p. 923-928. Retrieved from https://doi.org/10.1067/mai.2002.124776

Wang, L. H., Lin, Y. H., Yang, J., & Guo, W. (2009). Insufficient increment of CD4+CD25+ regulatory T cells after stimulation in vitro with allergen in allergic asthma. *Int Arch Allergy Immunol, 148*(3), p. 199-210. Retrieved from https://doi.org/10.1159/000161580

Wang, Z., Zang, C., Rosenfeld, J. A., Schones, D. E., Barski, A., Cuddapah, S., Zhao, K. (2008). Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet, 40*(7), 897-903. Retrieved from https://doi.org/10.1038/ng.154

Watanabe, Y., Onodera, A., Kanai, U., Ichikawa, T., Obata-Ninomiya, K., Wada, T., Nakayama, T. (2014). Trithorax complex component Menin controls differentiation and maintenance of T helper 17 cells. *Proceedings of the National Academy of Sciences of the United States of America, 111*(35), p. 12829-12834. Retrieved from https://doi.org/10.1073/pnas.1321245111

Weissman, I. L. (2000). Stem cells: units of development, units of regeneration, and units in evolution. *Cell, 100*(1), p. 157-168. Retrieved from https://doi.org/10.1016/s0092-8674(00)81692-x

WHO. (2017). Schistosomiasis: WHO reports substantial treatment progress for school-age children. Retrieved from http://www.who.int/neglected_diseases/news/WHO_schistosomiasis_reports_substantial_treatment_progress_sac/en/ (cited 18/05/19)

Williams, H., Robertson, C., Stewart, A., Aït-Khaled, N., Anabwani, G., Anderson, R., Weiland, S. K. (1999). Worldwide variations in the prevalence of symptoms of atopic eczema in the international study of asthma and allergies in childhood. *Journal of Allergy and Clinical Immunology, 103*(1), p. 125-138. Retrieved from https://doi.org/10.1016/S0091-6749(99)70536-1

Williams, M. A., Tyznik, A. J., & Bevan, M. J. (2006). Interleukin-2 signals during priming are required for secondary expansion of CD8+ memory T cells. *Nature, 441*, 890-893. Retrieved from https://doi.org/10.1038/nature04790

Wilson, C. B., Rowell, E., & Sekimata, M. (2009). Epigenetic control of T-helper-cell differentiation. *Nat Rev Immunol, 9*(2), p. 91-105. Retrieved from https://doi.org/10.1038/nri2487

Woodburn, P. W., Muhangi, L., Hillier, S., Ndibazza, J., Namujju, P. B., Kizza, M., Elliott, A. M. (2009). Risk Factors for Helminth, Malaria, and HIV Infection in Pregnancy in Entebbe, Uganda. *PLoS Neglected Tropical Diseases, 3*(6), e473. Retrieved from https://doi.org/10.1371/journal.pntd.0000473

Woodfolk, J. A. (2007). T-cell responses to allergens. *Journal of Allergy and Clinical Immunology, 119*(2), p. 280-294. Retrieved from https://doi.org/10.1016/j.jaci.2006.11.008

World Health Organization. (2002). Prevention and control of schistosomiasis and soil-transmitted helminthiasis: WHO Technical Report Series N° 912. Report of a WHO expert committee. Retrieved from http://www.who.int/intestinal_worms/resources/who_trs_912/en/ (cited 17/09/27)

Wynn, T. A. (2007). Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *Journal of Clinical Investigation, 117*(3), p. 524-529. Retrieved from https://doi.org/10.1172/JCI31487

Wynn, T. A., & Cheever, A. W. (1995). Cytokine regulation of granuloma formation in schistosomiasis. *Current Opinion in Immunology, 7*(4), p. 505-511. Retrieved from http://dx.doi.org/10.1016/0952-7915(95)80095-6

Wynn, T. A., Eltoum, I., Cheever, A. W., Lewis, F. A., Gause, W. C., & Sher, A. (1993). Analysis of cytokine mRNA expression during primary granuloma formation induced by eggs of Schistosoma mansoni. *The Journal of Immunology, 151*(3), p. 1430-1440.

Xu, D., Liu, H., Komai-Koma, M., Campbell, C., McSharry, C., Alexander, J., & Liew, F. Y. (2003). CD4+CD25+ regulatory T cells suppress differentiation and functions of Th1 and Th2 cells, Leishmania major infection, and colitis in mice. *J Immunol, 170*(1), 394-399. Retrieved from http://dx.doi.org/10.4049/jimmunol.170.1.394.

Yang, X. O., Pappu, B. P., Nurieva, R., Akimzhanov, A., Kang, H. S., Chung, Y., Dong, C. (2008). T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity, 28*(1), p. 29-39. Retrieved from https://doi.org/10.1016/j.immuni.2007.11.016

Yatich, N. J., Jolly, P. E., Funkhouser, E., Agbenyega, T., Rayner, J. C., Ehiri, J. E., Williams, J. H. (2010). The Effect of Malaria and Intestinal Helminth Coinfection on Birth Outcomes in Kumasi, Ghana. *The American Journal of Tropical Medicine and Hygiene, 82*(1), p. 28-34. Retrieved from https://doi.org/10.4269/ajtmh.2010.09-0165

Yazdanbakhsh, M., Kremsner, P. G., & van Ree, R. (2002). Allergy, parasites, and the hygiene hypothesis. *Science, 296*, p. 490-494. Retrieved from https://doi.org/10.1126/science.296.5567.490

Yazdanbakhsh, M., & Sacks, D. L. (2010). Why does immunity to parasites take so long to develop? *Nature reviews. Immunology, 10*(2), p. 80-81. Retrieved from https://doi.org/10.1038/nri2673

Yoder, M. C. (2002). Embryonic hematopoiesis in mice and humans. *Acta Pædiatrica, 91*, p. 5-8. Retrieved from https://doi.org/10.1111/j.1651-2227.2002.tb02899.x

Zhao, F., Huang, X., Hou, X., Deng, Y., Wu, M., Guan, F., Lei, J. (2013). Schistosoma japonicum: susceptibility of neonate mice born to infected and noninfected mothers following subsequent challenge. *Parasite Immunol, 35*(5-6), p. 157-163. Retrieved from https://doi.org/10.1111/pim.12027

Zheng, W., & Flavell, R. A. (1997). The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. *Cell, 89*(4), p. 587-596. Retrieved from https://doi.org/10.1016/s0092-8674(00)80240-8