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Immunosuppressive and transgenerational effects on the development of allergic airway inflammation during *Schistosoma mansoni* infection

Kathrin Straubinger

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Vorsitzender: Univ.-Prof. Dr. Stefan Engelhardt

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1. Univ.-Prof. Dr. Dirk H. Busch

2. Univ.-Prof. Dr. Erika von Mutius, Ludwig-Maximilians-Universität München

3. Priv.-Doz. Dr. Bianca Schaub, Ludwig-Maximilians-Universität München

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Abbreviations

A

AAM	alternatively activated macrophages
ACT	ammoniumchloride-tris
AGM	aorta-gonad-mesonephros
AHR	airway hyperresponsiveness
$Al(OH)_3$	aluminium hydroxide (alum)
APC	antigen presenting cells
Arg1	Arginase 1

B

BAC	bacterial artificial chromosome
BAL	bronchoalveloar lavage
Bcl-2	B cell lymphoma 2
BCG	Bacillus Calmette Guerin
BM	bone-marrow
bp	base pair(s)
Breg	regulatoy B cells
BSA	bovine serum albumin

C

ChaFFs	Chitinase and FIZZ (found in inflammatory zone) family members
CLRs	C-type lectin receptors
CD	cluster of differentiation
CTL	C-type lectin
CTLA	cytotoxic T lymphocyte antigen

D

DC	dendritic cell
DC-SIGN	DC-specific intercellular adhesion molecule-3-grabbing non-integrin
Derp	Dermatophagoides pteronyssinus
DMEM	Dulbecco's modified eagle medium
DMSO	dimethyl sulfoxide
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

hCG	human chorionic gonadotropin
HCl	hydrogen chloride
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	human immunodeficiency virus
HRP	horseradish peroxidase
HSC	hematopoietic stem cell
Hsd3b1	hydroxy-delta-5-steroid dehydrogenase
H_2O	water
H_2SO_4	sulfuric acid

I

iCTB	invasive cytotrophoblasts
IFN	interferon
Ig	immunoglobulin
IGF	insulin-like-growth factor
IL	interleukin
i.p.	intraperitnoneal
IL-1R	IL-1 receptor
IVF	in vitro fertilization

K

kb	kilobase
KCl	potassium chloride
kDa	kilo Dalton
KO	knockout
KOH	potassium hydroxide

L

LLN	lung lymph node
LNP	Lacto-N-fucopentaose
LPS	lipopolysaccharide
LRR	leucine rich repeat
Lypd8	Ly6/PLAUR Domain-Containing Protein 8

M

MHC	major histocompatibility complex
MIH	Institute of medical Mircrobiology, Immunology and Hygiene
MMP	matrix metalloproteinase
MODY	Maturity Onset Diabetes of Young
MR	mannose receptor
MRI	Munich Rechts der Isar
MyD88	myeloid differentiation primary response protein 88

N

NaCl	sodium chloride
NF-κB	*nuclear factor-κB*
NH_4Cl	ammonium chloride
NK	natural killer
NKT	natural killer T cell
NLRP	NOD-like receptor family, pryin domain
NOD	nucleotide oligomerization domain
NOD mice	non-obese diabetic mice

O

OVA	ovalbumin

P

PAMPs	pathogen-associated molecular pattern molecules
PBMCs	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PD-L	programmed death ligand
PE	phycoerythrin
PENK	preproenkephalin
PFA	paraformaldehyde
PGE2	prostaglandin E2
PM	placental malaria
PRR	pattern recognition receptors

R

RAG	recombination activating gene
Reg	regulatory
Relmα	resisitn like molecule α
RNA	ribonucleic acid
Rnf223	ring finger protein 223
ROS	reactive oxygen species
RPMI	Roswell Park Memorial Institute medium
RWE	ragweed extract

S

SCID	severe combined immunodeficiency
SD	standard deviation
SEA	soluble egg antigen
SEM	standard error of the mean
SLPI	secretory leukocyte peptidase inhibitor

S. mansoni	*Schistosoma mansoni*
SpT	spongiotrophoblast
STAT	signal transducer and activator of transcription
Sult1e1	estrogen sulfotransferase
SynT	syncytiotrophoblasts

T

TAE	tris acetat EDTA
TBST	tris-buffered saline and Tween 20
TCR	T cell receptor
TGC	trophoblast giant cells
TGF	transforming growth factor
Th	T helper
TIR	Toll/IL-1 receptor
TIRAP	TIR-containing adapters
TLR	Toll-like receptor
TMB	3,3',5,5'-tetramethylbenzidine
TNF	tumor necrosis factor
Tph1	tryptophan hydroxylase 1
TRAF	tumour-necrosis factor-receptor-associated factor
TRAM	TRIF-related adaptor molecule
TRIF	TIR-domain containing adaptor protein inducing IFN-b
Tris	tris(hydroxymethyl)aminomethane
Treg	regulatory T cells
TSCP	thymic stromal lymphopoietin
TT	tetanut toxoid

V

VDR	vitamin d receptor

v/v	volume per volume

W

WHO	World Health Organization
wt	wildtype
w/v	weight per volume

Y

Ym1	chitinase 3 like protein 3

Abstract

A rapid increase in allergic asthma and other atopic disorders in the industrialized world has been observed over the last decades, whereas both incidence and prevalence of atopy remain much lower in many developing countries. In 1989, the hygiene hypothesis was formulated by the epidemiologist Dr. Strachan, who reported an inverse relationship between family size and development of atopic disorders, and proposed that a lower incidence of infection in early childhood, transmitted by unhygienic contact with older siblings or acquired pre-natally could be a cause of the rise in allergic diseases. Subsequently, the original hypothesis was expandend to include the exposure to a variety of microorganisms and parasites, such as helminths as a protective factor against the increasing incidence of atopy. The helminth *S. mansoni* is one of the major pathogens of schistosomiasis, the second most socioeconomically devastating parasitic disease. This non-transplacental, chronic infection passes through distinct immune responses, ranging from an initial Th1 and a subsequent Th2 phase to an ensuing immunosuppression that is known to dampen allergic reactions. The immunological mechanisms that mediate protection against the development of asthma are not completely understood, although the participation of regulatory immune cells is favored.

In this study the use of a transgenic mouse model allowed the specifc depletion of regulatory T (Treg) cells in a murine model of allergic airway inflammation using ovalbumin or ragweed as model antigens. Thereby, reduction of allergic airway inflammation was observed in *S. mansoni* infected mice, but was completely abrogated due to Treg depletion. Besides effects on asthma susceptibility in the infected host, this study further focused on the question whether predominant exposure to different immune phases during maternal helminth infection could affect the offspring's susceptibility to allergies. Indeed, strong differences ranging from protection to enhancement of allergic disease were found. Phase-specific changes were also observed in placental gene expression, schistosome specific cytokine responses within the placenta and early postnatal immune cell composition in the offspring. Mechanistically, the specific role of maternal Interferon-γ (IFN-γ) was highlighted during the Th1 phase of infection, since maternal IFN-γ deficiency abrogated the previously observed asthma protective effect in the offspring. Furthermore, the placental expression of vitamin D receptor was inversely correlated with asthma protection in the offspring and has experimentally emerged as a potential contributor for the development of allergic asthma pre-natally. In addition, epigenetic inheritance via the gametes as regulating elements for the offsprings' immune phenotype was excluded by in vitro fertilization experiments during the acute phase of infection. These findings revealed for the first time direct effects of a maternal, helminth-driven immunological environment on the progeny's development of allergy susceptibility.

Zusammenfassung

In den letzten Jahrezehnten wird in der industrialisierten Welt ein starker Anstieg von allergischem Asthma und anderen atopischen Erkrankungen beobachtet, während in Entwicklungsländern sowohl die Inzidenz als auch die Prävalenz von Atopien gering sind. Im Jahr 1989 wurde von dem Epidemiologen Dr. Strachan die Hygiene-Hypothese aufgestellt, da dieser eine inverse Beziehung zwischen Familiengröße und Entwicklung atopischer Erkrankungen beobachtet hatte. Als Ursache für den Anstieg atopischer Erkrankungen nahm er daher ein verringertes Auftreten von Infektionen in der frühen Kindheit an, welche durch den Kontakt mit älteren Geschwistern übertragen oder pränatal erworben werden können. Im Laufe der Zeit, wurden eine Vielzahl von Bakterien und Parasiten als potentielle Schutzfaktoren gegen die zunehmende Häufigkeit von Allergien beschrieben und damit diese Hypothese gestützt und erweitert. Der zur Gattung der Saugwürmer gehörende Parasit *S. mansoni* ist einer der wichtigsten Erreger der Bilharziose, der sozioökonomisch zweithäufigsten parasitären Erkrankung nach Malaria in den Tropen. Diese nicht-transplazentare, chronische Infektion führt zu verschiedene Immunreaktionen im Wirt, die von einer anfänglichen Th1- in eine Th2-Phase übergehen und schließlich zur Immunsuppression führen, welche bekanntermaßen allergische Reaktionen unterdrückt. Die immunologischen Mechanismen, die den Schutz gegen die Entwicklung von Asthma vermitteln, sind nicht vollständig verstanden. Es wird jedoch angenommen, dass regulatorische Immunzellen beteiligt sind.

Um den Einfluss sogenannter regulatorischer T-Zellen zu untersuchen, wurde in dieser Studie ein transgener Mausstamm genutzt, welcher die spezifische Depletion dieser Zellen in einem Mausmodell für allergische Entzündungen der Atemwege ermöglicht. Dabei wurde die Protektion gegen allergische Atemwegsentzündungen, die nach Sensibilisierung und Challenge mit Ovalbumin oder Ambrosia als Modell-Antigene in *S. mansoni* infizierten Mäusen beobachtet wurde, vollständig aufgehoben. Neben Auswirkungen auf die Asthma Suszeptibilität im infizierten Wirt, beschäftigt sich die vorliegende Arbeit weiterhin mit der Frage, ob vorherrschende Immunantworten in infizierten Mäusen während der Schwangerschaft die Anfälligkeit für Allergien in der Nachkommenschaft beeinflussen. Tatsächlich wurden in Abhängigkeit der mütterlichen Immunphase zum Zeitpunkt der Verpaarung starke Unterschiede in allergischen Atemwegserkrankungen in den Nachkommen beobachtet. Diese umfassten phasenabhängig sowohl Protektion als auch Verschlechterung der Krankheitsparameter. Weitere Einflüsse der einzelnen mütterlichen Immunphasen wurden auch im Hinblick auf die Genexpression in der Plazenta, der Schistosomen-spezifischen Zytokinproduktion innerhalb der Plazenta und in der frühen postnatalen Immunzellzusammensetzung der

jeweiligen Nachkommen beobachtet. Mechanistisch konnte die spezifische Rolle des mütterlichen Interferon-γ (IFN-γ) während der Th1-Phase der Infektion gezeigt werden, da die Abwesenheit von IFN-γ die zuvor beobachtete Asthma-protektive Wirkung bei den Nachkommen aufgehoben hatte. Darüber hinaus wurde eine inverse Korrelation zwischen der plazentaren Expression des Vitamin D Rezeptors (VDR) und der Asthma Protektion der Nachkommen gefunden. Experimentell konnte die pränatale Rolle des VDR für die Entwicklung von allergischem Asthma bestätigt werden. Darüber hinaus konnte die Vererbung von epigenetischen Veränderungen über die Keimbahn als regulierende Elemente, die den Phänotyp in der Nachkommenschaft prägen, mittels In-vitro-Fertilisation während der akuten Phase der Infektion ausgeschlossen werden. Diese Ergebnisse zeigen zum ersten Mal, dass die mütterliche, Helminthen-geprägte immunologische Umgebung einen direkten Einfluss auf die Entwicklung von allergischen Atemwegserkrankungen in den Nachkommen hat.

1 Introduction

1.1 Biology of *S. mansoni*

1.1.1 Taxonomy, geographical distribution and associated disease

Helminths are complex eukaryotic organisms. Within the phylum *Platyhelminthes,* the class of *Trematoda* contains two groups of parasitic flatworms, *Digenea* and *Aspidogastrea*, commonly referred to as “flukes”. A notable member of *Diginea* is the genus *Schistosoma*, that include twenty one species divided into four groups: *indicum*, *japonicum*, *haematobium* and *mansoni*. These blood-dwelling worms primarily cause the tropical parasitic disease schistosomiasis, also known as bilharzia, which mainly affects either the intestine or the urogenital tract of infected individuals (Table 1). The main human species are *S. mansoni* (occurring in Africa and South America) and *S. japonicum* (occurring in South and East Asia), which cause intestinal and hepatosplenic schistosomiasis, or *S. haematobium* (occurring in Africa), which elicits urinary schistosomiasis [1]. The World Health Organization (WHO) has estimated that more than 230 million people require treatment worldwide and 732 million people are at risk of infection in endemic areas. It is further estimated that sub-sahara Africa accounts for 85-90% of the schistosoma burden [2-4]. The transmission of this prevalent tropical parasitic disease, ranking second only to malaria, has been documented in 77 countries, especially in poor rural communities of the developing world, where individuals have inadequate sanitation and are exposed to water containing the infectious larval form (cercariae). Several million people currently suffer from severe morbidity as a consequence of schistosomiasis. Although some patients present an acute systemic hypersensitivity reaction against migrating schistosomulae (Katayama fever) the renowned chronic pathology and morbidity are primarily instigated by the host’s immune reaction towards schistosome eggs. The eggs are deposited by adult female worms and secrete proteolytic enzymes that provoke typical eosinophilic inflammatory and granulomatous reactions in the tissues [5]. The classical early symptoms of urogenital schistosomiasis include haematuria, dysuria, pollakiuria and proteinuria. Intestinal schistosomiasis has a nonspecific clinical picture of abdominal pain and diarrhea with or without blood in the mucus. Hepatic or hepatosplenic schistosomiasis can be caused by early inflammatory and late fibrotic hepatic disease. The helminth’s survival and transmission is achieved by a combination of its substantial longevity, repeated re-infection and selective immune suppression or deviation of host protective immune responses. Investigating how the

immune system deals with such pathogens is a daunting challenge and has been addressed over the past decade using a wide range of approaches and models.

Manifestation	Species	Geographical distribution
Intestinal schistosmiasis	*Schistosoma mansoni*	Africa, the Middle East, the Caribbean, Brazil, Venezuela, Suriname
	Schistosoma japonicum	China, Indonesia, the Philippines
	Schistosoma mekongi	Several districts of Cambodia and the Lao People's Democratic Republic
	Schistosoma guineensis *Schistosoma intercalatum*	Rain forest areas of central Africa
Urogenital schistosomiasis	*Schistosoma haematobium*	Africa, the Middle East

Table 1: Parasite species and geographical distribution of schistosomiasis [2]

1.1.2 Lifecycle of *S. mansoni*

Schistosomes have developed a complex lifecycle including survival and asexual reproduction in a molluscan intermediate host, two free-swimming larval stages, including a free-living stage in fresh-water and the development into dioecious sexually mature adult worms, with the ability to survive for decades in mammalian definite hosts (Fig. 1-1) [6]. Although the principle lifecycle is similar for all schistosomes, the following section will detail that of *S. mansoni.* Eggs contain a ciliated miracidium larval form, which is released upon contact with water and searches for the intermediate host, *Biomphalaria* snails, guided by light and chemical stimuli. After penetrating the snail, miracidia multiply asexually through two generations of sporocysts and finally develop into cercarial larvae. Triggered by light and temperature infective cercariae are released from the snail 4-6 weeks after infection and actively search for a suitable definite host. Many species of mammals, belonging to the four different orders *primates*, *insectivora*, r*odentia* and *marsupialia* are recognized as natural hosts [7]. During penetration of the host's skin, cercariae lose their forked tails and develop into schistosomulae, which migrate via the lung to the portal vein of the liver and mature into adult worms. The male and female form a reproductive pair, with the female held by the male within a groove called the gynaecophoric channel. The embraced pair migrates to the superior mesenteric veins, where the female starts to produce eggs, which are progressively moved towards the lumen of the intestine, and are excreted via the feces [1, 8-11].

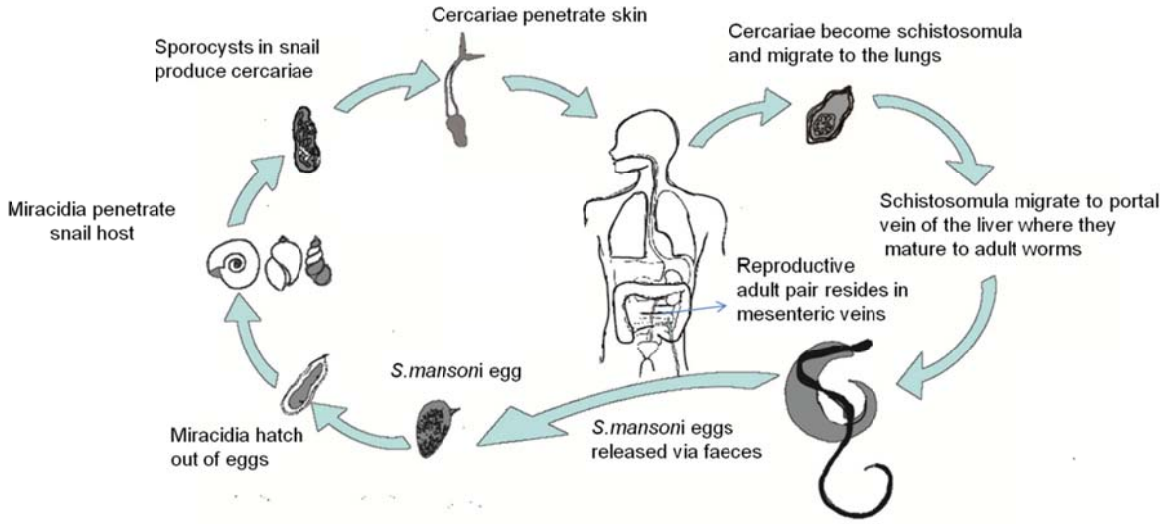


Fig. 1-1.: *S. mansoni* lifecycle.
The complex lifecycle of *S. mansoni* starts with eggs being released from infected individuals via feces into fresh water. The residing miracidium then hatches out of the egg in response to temperature and light and searches for a intermediate snail host (*Biomphalaria glabrata*). The parasite then develops via a mother-sporocyst and daughter-sporocyst generation into cercariae. The cercariae emerge from the snail during daylight and propel themselves in water with the aid of a bifurcated tail, actively seeking for their final host. Penetration of the host's skin into the epidermis is achieved by the use of proteolytic enzymes. During penetration cercariae lose their tail and the head of the cercariae transforms into an endoparasitic larvae, the schistosomule. Each schistosomule spends a few days in the skin and then enters the circulation to migrate via the lung into the portal vein. If it meets a partner of the opposite sex, it develops into a sexually mature adult worm and pairs up. The pair migrates to the mesenteric veins, where the female worm starts to produce eggs, which move into the gut lumen and are partially released into the environment within the feces. Schistosome eggs, which become trapped within the host's tissues are the major cause of pathology in schistosomiasis. They enter the circulation and cause different clinical manifestations such as liver fibrosis, portal hypertension and intestinal bleeding. Adapted from [12].

1.1.3 Immune regulation during infection

1.1.3.1 Innate responses to schistosome-derived components: driving Th1 responses

Due to prolonged dynamic co-evolution between host and parasite, schistosomes are able to live for many years within the potentially hostile environment of the human bloodstream. To protect themselves from host defenses they have adapted by undergoing certain developmental changes which maintain a balanced host-parasite interplay for successful long-term transmission [13]. Such highly effective mechanisms of immune subversion during the course of infection drives the host's immune response progressively through three different phases: An initial Th1 type response gives way to a Th2 type response, which becomes modified to create a regulatory immunosuppressive environment [11]. In detail, acute schistosomiasis is accompanied by the production of Interleukin (IL)-2 and

Interferon-γ (IFN-γ), caused by the infective schistosome before egg production starts [14, 15]. As mentioned above, the initial penetration of cercariae is facilitated by the release of enzymes and immunomodulators [16, 17] into the epidermis. Upon entry, transformation into schistosomulum involves the shedding and replacement of the tegument membranes [18]. Research has shown that such tegument sections upregulate the expression of activation markers such as cluster of differentiation (CD)40 and CD86 and trigger the production of proinflammatory cytokines such as IL-12p40 and TNF-α by dendritic cells (DC). Moreover, using appropriate knockout animals they demonstrated that these effects were dependent on myeloid differentiation primary response protein 88 (MyD88) and Toll like receptor 4 (TLR4) [19]. Furthermore, the surface of cercariae and the newly transformed schistosomula have been shown to activate the complement cascade and thereby elicit proinflammatory responses [20]. A studie with 31 patients suffering from acute schistosomiasis revealed eosionphilia, elevated TNF-α and antigen-specific IgE levels in the serum as well as the production of high amounts of proinflammatory TNF-α, IFN-γ, IL-2, IL-1 and IL-6 by soluble egg antigen (SEA)-restimulated peripheral blood mononuclear cells (PBMCs) [21]. However, pronounced inflammatory response following cercarial penetration is not common in individuals who live in endemic areas, but is predominantely seen in patients, who have not been previously exposed to the parasite antigens [22]. This observation might be explained by *in utero* sensitization and induction of tolerance against helminth antigens due to maternal infection with *S. mansoni* in endemic areas [23] or perhaps by regulatory responses induced by skin-stage schistosomula-derived molecules such as prostaglandin E2 (PGE2), which upregulates IL-10 production during skin penetration [20].

1.1.3.2 Innate responses to schistosome egg antigens: giving rise to Th2 responses

During the course of *S. mansoni* infection, Th2 type inflammation arise from granulomatous, immune-mediated response to eggs that become trapped in the host's liver and intestine. Polarized Th2 immunity comprises the cytokines IL-3, IL-4, IL-5, IL-9, IL-10 and IL-13, antibody isotypes IgG1, IgG4 and IgE and the expansion of eosinophils, basophils, mast cells and alternatively activated macrophages (AAMs) and initially protects both the host and the parasite, against unrestricted Th1 and Th17 cell mediated inflammation [14, 15, 24-26]. Initiation of the Th2 type response begins at the intestinal mucosal epithelial cell barrier where tissue injury, caused by the parasite, as well as parasite derived products, promote the release of alarmins such as IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) [27] [28, 29]. Alarmins, as well as soluble helminth products activate antigen-presenting-cells (APC) such as DCs in mesenteric lymphnodes

and other gut-associated lymphoid tissues, driving Th2 cell mediated IL-4 and IL-13 secretion. In terms of signaling, type 2 initiation by helminth-derived-products does not essentially require TLR-mediated signaling through the Toll-IL-1 receptor (TIR) domain-containing adaptors MyD88 [30] or TIR-domain-containing adaptor protein inducing IFN-β (TRIF) in DCs [31]. However, schistosomal lyso-phosphatidylserine [32], egg derived Lacto-N-fucopentaose III (LNPIII) [33] and egg-derived double stranded RNA [34] have been shown to contribute to Th2 initiation by DC activation through TLR-dependent pathways [19]. Furthermore, there is also evidence that *S. mansoni* SEA interact with C-type lectin receptors (CLRs) such as Dectin-2 on human and mouse DCs. This further activates the NOD-like receptor family, pyrin domain containing 3 (Nlrp3) inflammasome and results in the production of IL-1β. The absence of this pathway skews adaptive immune responses during infection [35] [36]. Several immunomodulatory components within SEA have been identified and intensively studied, up to now. For example, Lewis-X, a glycan moiety present on proteins in SEA has been shown to interact with C-type lectin (CTL) DC-SIGN and can suppress TLR-induced IL-12 production by DCs. The T2 riboneclease Omega-1 on the other hand is thought to be taken up by DCs via its glycans through the CTL mannose receptor (MR). This inhibitis protein synthesis due to the degradation of RNA and generates DCs that preferentially induce a Th2 response [37]. The importance of DCs in the induction of Th2 immune responses was shown by Pythian-Adams et al., who depleted CD11c in vivo, since it resulted in an inhibition of Th2 induction [38]. In summary, during patent infection with schistosomes, DCs undergo a form of maturation, mediated by CD40 ligation that is crucial for the initiation of Th2 responses and is not accompanied by the production of proinflammatory IL-12 [39, 40].

1.1.3.3 Ongoing Th2 response: dampening overshooting Th1 responses

In an ongoing infection, innate effector cell responses are essential for both, activation and maintenance of $CD4^+$Th2 effector cells. In detail, IL-5 production by $CD4^+$T cells triggers eosinophilia and together with IL-4, IL-9 and IL-13 activates mast cells and basophils, on which, antigens cross-link the high affinity Fc epsilon receptor (FcεRI)-bound IgE resulting in the release of soluble mediators [41]. Although eosinophils produce IL-4 and IL-13 primary responses to schistosomes [42] and to the intestinal nematode *Nippostrongylus brasiliensis* [43] were unaffected in studies with eosinophil-deficient mouse models. Nevertheless, IL-4 receptor signaling and CD40-mediated co-stimulation by $CD4^+$ T effector cells further stimulate B cell class switching to IgG1 and IgE, which was correlated with resistance to disease induced by *S. mansoni* infection in human studies [44, 45]. Other studies, using IgE-deficient and IgE-producing mouse models [46],

suggested no essential role for IgE on primary *S. mansoni* infection, which might be explained by low expression levels of the high-affinity IgE receptor on mouse eosinophils [47]. As mentioned above, during *S. mansoni* infection, a pronounced Th2-type response is mandatory to primarily downregulate an otherwise pathological response driven by Th1 and Th17 cells [48], activate wound-healing [49] and mediate anti-helminthic immunity. Although this transition is initially beneficial to the host, persistent Th2 responses might cause liver pathology and hepatosplenic disease [50]. Accordingly, it has been shown that infection of IL-4 deficient mice with *S. mansoni* leads to uncontrolled, TNF-α driven inflammatory responses, majoritively mediated by macrophages that have been classically activated by TLR agonists and additional IFN-γ [37, 51, 52]. These data suggest that IL-4 suppresses the emergence of IFN-γ secreting $CD4^+$ cells and/or plays an important role in limiting access of TLR agonists into diseased tissues [37]. Th2 response driven granulomatous lesions, mainly mediated by IL-13 and TNF, are formed around trapped eggs within the host's tissue and are composed of collagen fibres and cells, including macrophages, eosinophils B cells and $CD4^+$ T cells [52, 53]. The important role of IL-13 became clear in schistosome infected mice, lacking either IL-13 [52], the IL-4 receptor alpha chain (IL4ra) [54] or which are treated with a soluble neutralizing IL-13 receptor (IL-13Ra2-Fc) [55], since non of them develop severe hepatic fibrosis. This was further supported by the finding that mice, lacking soluble IL-13Ra2, which acts as a decoy receptor for IL-13, develop stronger Th2 responses, with exaggerated collagen deposition, portal hypertension and portal systemic shunts and intestinal bleeding. Besides collagen deposition, inflammation and fibrosis is further affected by regulation of extracellular matrix degradation through the expression of matrix metalloproteinases (MMPs) by alternatively activated macrophages (AAMs). In this context, MMP12 contributes to severe disease by influencing the expression of MMP2 and MMP13, which degrade the extracellular matrix [56]. This overshooting pro-inflammatory Th2 cell response needs to be dampened when infection proceeds and gives way to a regulated environment, mainly mediated by the cytokine IL-10, which is produced by modified Th2 cells, macrophages, DCs and regulatory T and B cells.

1.1.3.4 Key mediators for immunosuppression: balancing the host's immune response

Regulatory mechanisms are initiated by the ongoing Th2 type immune response itself, since IL-4 together with IL-13 and IL-21 stimulates AAMs, which are rapidly recruited to sites of infection and in turn produce Chitinase and FIZZ (found in inflammatory zone) family members (ChaFFs) such as arginase-1 (Arg-1), resistin like molecule α (RELMα) and chitinase 3 like protein 3 (Ym1) that have been identified in a variety of Th2-mediated

inflammatory settings and are considered as important effector or wound-repair molecules at the site of parasite infection, with a regulatory function to dampen egg-induced inflammation [57-59]. Here, Arg-1 deficiency in immune cells during *S. mansoni* infection leads to severe intestinal inflammation, liver fibrosis and portal hypertension, since Arg1 expressing AAMs successfully compete with Th2 cells for arginine and thereby limit Th2 cell proliferation. RELMα has been shown to suppress *S. mansoni*-induced Th2 immunity during pulmonary granuloma formation, since both primary and secondary pulmonary granuloma formation were exacerbated in the absence of RELMα. Furthermore, IL-10 production is a hallmark of modified schistosomal egg-induced Th2 response. It leads to an overall loss of proinflammatory T cell proliferation and cytokine production in response to parasite and bystander antigens [60] that gives rise to the expansion of immunoregulatory cells, such as AAMs, regulatory T cells (Treg) and regulatory B cells (Breg) [9, 27, 41, 61], which in turn promote the elevated IL-10 levels. The regulatory role of B cells during *S. mansoni* infection became clear in studies using B-cell deficient mice, in which enhanced Th2 dependent immunopathology has been observed during chronic infection [62]. Despite this complexity of regulatory cell types, $CD4^+CD25^+Foxp3^+$ T cells remain the most prominent regulatory cells during helminth infections. Throughout infection Treg cells arise from developing T cells in the thymus or from naïve $CD4^+$ T cells via expression of the transcription factor forkhead box P3 (FOXP3) along with the cytokines TGF-β and IL-2. Besides quantitative Treg cell expansion, *S. mansoni* infection induces expression of activation and migratory markers such as CD103 [63]. Previous studies demonstrated a major role for expanding Treg cells during schistosomiasis, since absence of these cells enhanced granuloma pathology and disrupted the balance between Th1/Th17 and Th2 response [64-66]. Moreover, SEA was shown to induce Treg cells by acting on DCs, which drive the induction of Foxp3-expressing $CD4^+$ T cells [67]. Of note, expansion of Treg cells during schistosomiasis is TLR2 dependent, since the absence of this pattern recognition receptor leads to severe immunopathological changes and prolonged Th1 response during acute infection [66]. The immunosuppressive capacity of IL-10, which is considered as the key mediator for immunosuppression during *S. mansoni* infection, has been demonstrated in mouse studies using IL-10 deficient infected mice [68]. In these studies, IL-10-deficiency induced an excessive Th1- and Th2-type mixed immune response, which was sustained throughout chronic infection and led to severe granulomatous liver damage and lethal immunopathology [68-71]. Furthermore, in studies using $IL\text{-}4^{-/-}IL\text{-}10^{-/-}$ and $IL\text{-}12^{-/-}IL\text{-}10^{-/-}$ double-knockout mice and $IL\text{-}10^{-/-}RAG^{-/-}$mice, IL-10 was shown to protect schistosome-infected mice from tissue damage and to prolong survival [70] [69, 71]. Accordingly, infection-induced IL-10 suppresses cell activity of macrophages and DCs by inhibiting production of IL-12, IL-18

and TNF [72-74]. Furthermore, DCs in IL-10 deficient *S. mansoni* infected mice showed an increased expression of MHC class II [72, 75], CD40 [72] and co-stimulatory molecules such as CD80 and CD86 [72, 76]. In addition, IL-10 can inhibit effector functions of mast cells and eosinophils and regulate the maintainance of several cells including B cells, NK cells, mast cells and DCs. Furthermore, it modulates IgE:IgG4 ratios, possibly by indirect induction of antibody switch to IgG4 in the B cell progeny, while preventing IgE production [20]. Conclusively, the diverse and complex immune responses during *S. mansoni* infection (Fig. 1-2) result from a finely-tuned host-parasite interplay, where immune evasion strategies ensure parasite survival and transmission and, at the same time, mediate resistance to the parasite, control associated pathologies and induce long-term immunological memory facilitated by a multiple regulatory network.

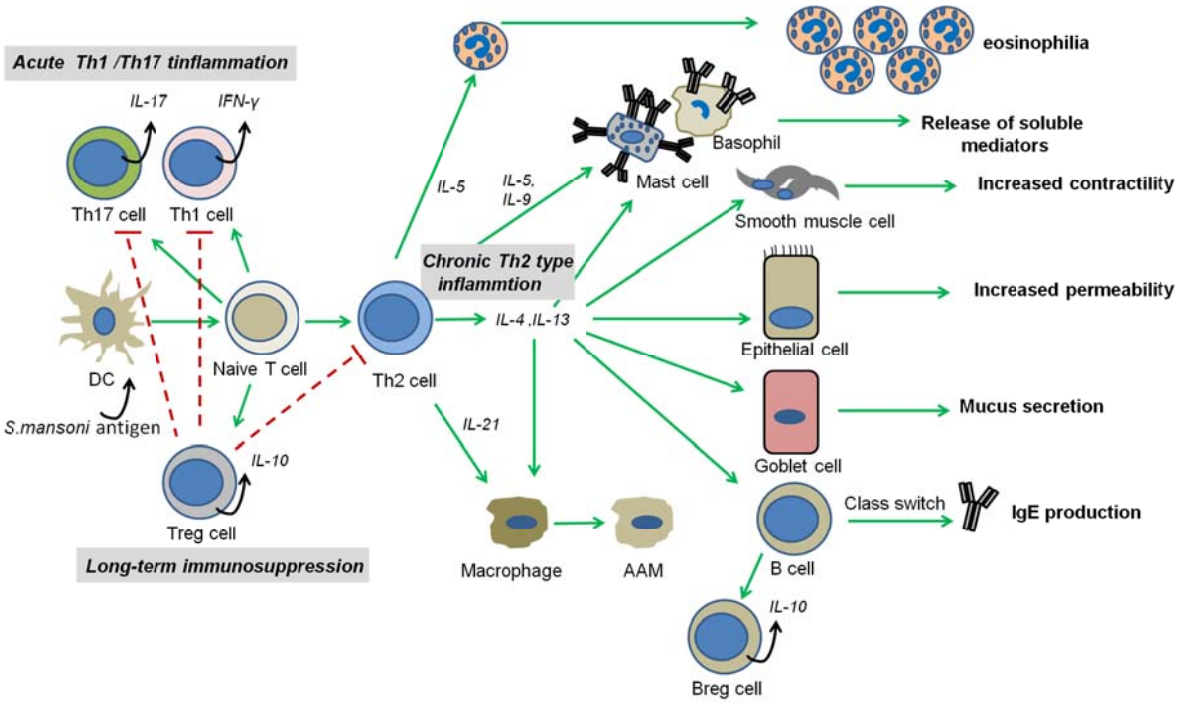


Fig. 1-2: Immune mechanisms and Th2 cell functions during *S.mansoni* infection.
During acute infection, antigen-specific Th1 cell responses are initially stimulated and cells proliferate after DC – T cell interaction in response to parasite antigens. When egg production starts, parasite antigens are presented to $CD4^+$ T cells in mesenteric lymph nodes and other gut-associated lymphoid tissues to drive a primarily protective Th2 type response. Th2 cells exert their effector function through the production of a number of cytokines, including IL-4, IL-13, IL-5 and IL-9 and affect a variety of immune cells, including B cells, macrophages, eosinophils, mast cells, basophils goblet cells as well as smooth muscle cells and epithelial cells. With increasing exposure of the immune system to parasite antigens that are released from metabolically active worms, the immune system becomes hyporesponsive, mainly due to the activation and expansion of regulatory T cells. Adapted and modified from [48].

1.2 *S. mansoni* and allergies

1.2.1 Basic understanding of immunological responses in allergic airway inflammation

During life, the immune system has to distinguish between a dangerous pathogen and ubiquitous environmental allergens, thereby providing defensive responses to the first whilst tolerating the latter. However, many individuals fail to tolerate environmental allergens and develop allergic diseases such as atopic dermatitis, allergic rhinitis and asthma [20]. Allergic asthma is the most common disease in industrialized countries, with a prevalence of up to 29% and the number of reported incidences still increasing [77]. Asthma is characterized by reversible airway obstruction and chronic inflammation of the respiratory tract. These symptoms usually correlate with increased activation and infiltration of immune cells into the pulmonary regions and Th2-type inflammatory responses (Fig. 1-3). In detail, allergens activate epithelial cells to produce thymic stromal lymphopoietein (TSLP), IL-25 and IL-33 which in turn initiate Th2 polarization and increase concentrations of Th2 type inflammatory cytokines; here IL-4 and IL-5 play a major role in the pathogenesis. IL-4, together with IL-13 induces B cells to undergo class switching to produce allergen-specific IgE. Furthermore, IL-4 promotes goblet cell hyperplasia. IL-5 promotes eosinophil differentiation in the bone marrow and recruits eosinophils into the lungs of asthmatics, which is further promoted by natural killer T cells (NKT) as well as $CD8^+$ T cells [78-80]. In the lung, eosinophils proliferate and amplify airway inflammation by the release of mediators such as chemokines and cytokines. Allergen, which is bound to allergen-specific IgE, is connected to the IgE receptor FcεRI on mast cells, basophils and eosinophils, which results in the release of several bronchoconstrictors, including histamine, cysteinyl, leukotrienes, prostaglandin D_2 and the cytokines IL-4, IL-5 and IL-13, which further promote an acute inflammatory response. Besides activation of sensitized mast cells by crosslinking surface-bound IgE molecules, processed peptides from allergens are presented by myeloid DCs, which are conditioned to release chemokines (CCL17, CCL22) to attract Th2 cells. Therefore, DCs are important for the initial sensitization to allergens, but they also maintain the chronic inflammatory response in the lungs. Th1 type responses are reduced in asthmatic patients due to IL-4-induced activation of the transcription factor GATA-binding protein 3 (GATA3) via signal transducer and activator of transcription 6 (STAT 6). Activation of this pathway regulates the expression of IL-4, IL-5 and IL-13 from Th2 cells and also inhibits the expression of Tbet (via STAT4), the crucial transcription factor for Th1 cell differentiation and IFN-γ secretion. Furthermore, IL-33, a recently characterized IL-1 family cytokine, seems to induce Th2 cell differentiation by promoting mast cells to produce several cytokines

including IL-4, IL-5 and IL-6 but not IL-33 [81]. Recently, Th17 cells, another T helper cell subset, have been described to contribute to inflammatory responses during allergic asthma due to the production of IL-4, IL-17, IL-21 and IL-22 [82]. Th17 cells further promote the recruitment and activation of neutrophils and lead to corticosteroid-resistant asthma [83]. IL-9 producing T cell subsets (Th9), which are suggested to derive from Th2 cells under the influence of TGF-β1, are involved in the production of IgE and mast cell recruitment in the lungs [20]. All these pronounced Th responses result in impaired lung function through the induction of bronchial infiltration of eosinophils, activated mucosal mast cells at the airway surface and activated T cells. This leads to remodeling of the airways including collagen deposition under the epithelium, which is described as basement-membrane thickening, as well as thickening of the airway smooth-muscle cell layer as a result of hyperplasia and hypertrophy [84]. Besides counteracting Th1 responses, regulatory $CD4^+$ T cells play an important role in suppressing potentially harmful cytokine production and Th2 cell proliferation and function during allergic airway inflammation. Data derived from murine models of airway inflammation suggested that the adoptive transfer of $CD4^+CD25^+$ regulatory T cells reduces established lung eosinophilia, Th2 cell infiltration into the lung as well as the release of IL-5, IL-13, and TGF-β, mucus hypersecretion and peribronchial collagen deposition [85]. Interestingly, it was found that *Lactobacillus rhamnosus* has a preventative role in asthma development but this modulation requires the presence of Treg cells [86]. There are several systems to deplete Treg cells *in vivo* but one of the most elegant model to date is the DEREG mouse. This bacterial artificial chromosome (BAC)-transgenic Foxp3-DTR (DEREG) allows the selective depletion of $Foxp3^+$ regulatory T cells at any given time-point. The use of this mouse model in asthmatic studies has revealed a major role of Treg cells in preventing allergic airway inflammation during the sensitization phase but not during allergen provocation [87, 88]. Furthermore, immunosuppressive cytokines that are linked to Treg function, such as IL-10 and TGF-β have been implicated in regulating airway inflammation, since co-expression of IL-10 and TGF-β1 by Treg cells completely suppressed airway hyperreactivity [89].

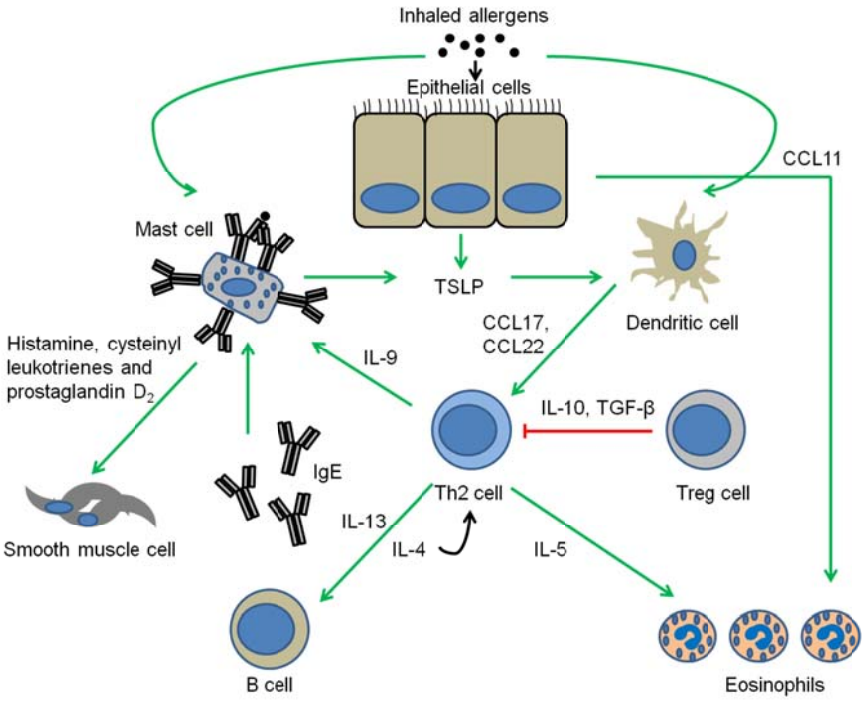


Fig. 1-3: Immune cell responses involved in allergic airway inflammation.
Inhaled allergens crosslink surface-bound IgE on sensitized mast cells, resulting in the release of mediators such as histamine which induce bronchoconstriction. Allergens are processed and peptides are presented on MHC II molecules on antigen presenting cells such as dendritic cells, which are conditioned by thymic stromal lymphopoietin (TSLP). These attract Th2 helper cells that produce IL-4 and IL-13, which stimulate B cells to synthesize allergen-specific IgE. Release of IL-5 from Th2 cells as well as epithelial cell derived CCL11 promotes the recruitment, proliferation and activation of eosinophils. IL-9 on the other hand stimulates mast cell proliferation. Regulation of the inflammatory response might be achieved through IL-10 and TGF-β release from regulatory T cells. Adapted from [84]

1.2.2 Revision of the Hygiene Hypothesis

Chronic helminth infections, such as *S. mansoni*, arise in spite of overwhelming immune responses that could potentially harm both the host and the parasite. This tightly controlled regulation is achieved, in part, by expanding regulatory T cells, IL-10 producing B cells and the immune-dampening properties of helminth-derived antigens that are released into the host's tissue during ongoing infection (1.1.3.4). Epidemiological studies have shown that the presence of autoimmune and allergic disorders are low in countries, where people still suffer from chronic helminth infections. It is now thought that the immunosuppressive effects that rise during helminth infections counteract allergic Th2 responses and might contribute to a lower prevalence of allergic diseases in endemic areas. Hence, despite provoking strong Th2 responses, helminths have become incorporated into an "expanded hygiene hypothesis and complement classical Th1 inducers like bacteria and viruses [90, 91]. Indeed, infection with different helminths has been shown to alter allergic immune responses in humans and mice. For example, clinical

manifestations of asthma is reduced in infected individuals and infection is inversely associated with a positive skin prick test to aeroallergens in schistosome infected children [92]. Experimentally, there is much evidence that helminth infection counterbalances allergic or autoimmune responses. In a murine model of diabetes schistosome infection increased the expression of potentially immunosuppressive TGF-β, integrin beta8 and galectins on destructive $CD4^+$ Th1 cells [67]. This helminth has also been shown to downmodulate unwarranted immune responses in an airway inflammation model using ovalbumin (OVA) as an unrelated antigen [67, 93, 94]. Taken together, a variety of studies contributed to explaining the lower prevalence of allergic diseases in helminth endemic areas and the contrasting rise of these diseases in industrialized western countries [95]. So far, elucidation of the underlying mechanisms by which helminths exert these protective effects in a mature immune system have suggested IgE-blocking as well as the activation of Treg cells and immunosuppressive IL-10 production. IgE-blocking refers to saturation of FcεRI on mast cells and basophils by high levels of nonspecific polyclonal IgE. This was suggested to inhibit degranulation and hypersensitivity reactions caused by allergen-specific IgE. However, there are several recent studies which show that high levels of polyclonal IgE from filarial-and hookworm infected patients do not prevent antigen-induced histamine release [96] [97]. Furthermore, clinical trials with antibodies to IgE have provided direct evidence that the FcεRI numbers on mast cells respond to the concentration of circulating IgE by changing receptor concentration to facilitate additional binding [98]. Another mechanism suggested, is the infection-induced production of IgE which cross-react between helminth antigens and allergens. In this context, structural and immunologic cross-reactivity among filarial tropomyosin and Dermatophagoides pteronyssinus (Derp1) has been reported in monkeys infected with *Loa Loa*. However, field studies could not convincingly confirm the effects of helminth infections on allergen-specific IgE in endemic areas [20]. Another interesting hypothesis refers to the increased production of IgG4 in helminth-infected individuals. The underlying concept is that IgG4 can block IgE-mediated immunity, such as allergies, since IgG4 and IgE subclass antibodies compete for the same epitopes [99]. The importance of IgG4 in allergic scenarios has been demonstrated in a recently published study that associated high IgG4 titers with decreased atopy in children exposed to cat antigens [100]. This is further supported by the finding that elevated levels of the immunosuppressive cytokine IL-10 enhanced IgG4 production [101]. Conclusively, the term “alternative Th2 response” was proposed to describe this modified Th2 response, which is not associated with clinical allergy but accompanied with high levels of IL-10, present in chronically helminth-infected individuals. Accordingly, many studies focus on the role of regulatory T cells and IL-10 producing T and B cells as bystander regulators of allergen-specific immune responses

underlying the observed reduced allergen susceptibility in infected individuals [102]. In this context, a study with *S. mansoni* infected asthmatic patients revealed a higher frequency of $CD4^+$ T cells expressing the tolerance-inducing co-stimulatory factors cytotoxic-T-lymphocyte antigen 4 (CTLA4) and CD40L. Furthermore, monocytes and $CD4^+CD25^+$ T cells of infected individuals expressed higher levels of IL-10 suggesting their role in downmodulation of inflammatory allergic responses in *S. mansoni* infected asthmatics [103].

However, the development and exacerbation of allergic diseases that was described in the previous sections is not limited to the environmental factors that impact the postnatal phase, especially during early life [104-106]. The epidemiologist Dr. Strachan, who originally formulated the “hygiene hypothesis” already proposed that a lower incidence of infection in early childhood, transmitted by unhygienic contact with older siblings or acquired pre-natally could be a cause of the rise in allergic diseases. Over the years, maternofetal transfer of tolerance [107] as well as susceptibility to certain allergens [108] have been reported and mechanistically, prenatal programming by maternal signals have recently been shown to strongly influence the maturation of the offsprings’ immune system *in utero* [109-111]. Therefore, exposure to specific environmental conditions during pregnancy, e.g. allergens or infections needs further investigation with regard to the newborn’s postnatal immune responses and allergy susceptibility.

1.3 Materno-fetal crosstalk and consequences for the immune responses in the offspring

1.3.1 Environment during pregnancy affecting the offsprings’ susceptibility to allergies

Fetal development is characterized by a tightly regulated gene expression profile which dictates cellular proliferation and differentiation. During the second trimester of human pregnancy the immune system starts to mature and becomes especially vulnerable to environmental factors that are transferred by the mother. Thus, it is suggested that an environmental event in one generation could affect the phenotype in subsequent generations and influence early immune system development. Many studies dealing with the effects of feto-maternal transfer of immunity focused on environmental factors that influence the development of allergies and asthma in the offspring. As an example, *in utero* exposure to environmental allergens such as *Der p* or birch pollen was shown to influence the development of subsequent allergic diseases by allergen-specific immune

responses, mediated by fetal T cells [112-114]. A crucial factor for the development of such proliferative immune responses in human is maternal exposure to the antigen during pregnancy, leading to prenatal development of functional T-B cell interactions and production of allergen-specific IgE at birth [115-117]. Other studies revealed that children born and raised in a farming environment were protected from asthma when exposed to a wider range of microbes compared to children from the reference group [118, 119]. These findings were expanded, showing that children born to mothers who were exposed to livestock and consumed non-pasterized farm-produced milk during pregnancy, had a lower risk to develop allergies [105]. In this cohort, Th2 counterbalancing cytokines IFN-γ and TNF-α were shown to be elevated in cord blood samples from the neonates born in this farming environment, as compared to those born to families outside from farming communities [120]. Only recently these findings were confirmed in animal models. Daily exposure of pregnant mice to the non pathogenic bacterium *Acinetobacter lwoffii* protected the offspring against OVA-induced allergic airway inflammation. This bacteria has been identified in rural areas as a protective [121]. Moreover, intact TLR signalling and thereby recognition of the bacterium in the mother was shown to be necessary in order to transfer protection against allergic airway disease in the offspring [122]. Indeed, treatment of pregnant mice with purified LPS conferred protection against allergic airway inflammation in offspring [123, 124]. In line, oral application of the probiotic bacterium *L. rhamnosus* during pregnancy prevented airway eosinophilia and lung inflammation but failed to normalize lung function in offspring [125]. As an underlying mechanism, recently published studies support the idea that asthma protection is mediated epigenetically by maternal exposure to environmental microbes and, in particular that the observed effect of *A. lwoffi* exposure is mediated by histone acetylation of the IFN-γ promoter in $CD4^+$ T cells in the offspring, which was closely associated with IFN-γ expression [126]. The resulting Th1 bias within the offspring might counterbalance Th2 responses later in life [126]. Accordingly, this general hypothesis was supported in a mouse study, where allergy induction by ovalbumin exposure during pregnancy led to reduced frequency of IFN-γ secreting T cells resulting in a lack of inhibition of Th2 type responses in the offspring [124].

1.3.2 Mechanisms of materno-fetal crosstalk via placenta and breastmilk

1.3.2.1 Communication via the placental-uterine axis

Human and murine placentas share considerable molecular and cellular features (Fig. 1-4). In both species, the fully developed placenta is composed of three major layers: the

outer maternal layer, which includes decidual cells of the uterus as well as the maternal vasculature that transfers blood to and from the implantation site; a middle "junctional" region, which attaches the fetal placenta to the uterus and contains fetal trophoblast cells that invade the uterine wall and maternal vessels and finally an inner layer, composed of highly branched villi that are designed for efficient nutrient exchange, called the villous tree in humans and the labyrinth in mice [127]. In humans, maternal blood from decidual spiral arterioles bathe the chorionic villi, a continuous layer of multinucleated syncytiotrophoblasts (SynT), that line the intervillous space (Fig. 1-4a). In mice, maternal decidual spiral arterioles perfuse blood sinuses in the spongiotrophoblast (SpT) layer to reach the labyrinth (Fig. 1-4b). Trophoblast giant cells (TGCs), such as invasive cytotrophoblasts (iCTBs), anchor the placenta to the uterus and invade the spiral arterioles. In mice, maternal blood is in direct contact with a layer of mononuclear trophoblasts that is surrounded by a bilayer of SynTs, which are in close proximity to fetal capillaries [128] (Fig. 1-4b)**.** These structural and morphological features are optimized for feto-maternal exchange and immunological crosstalk. The balance of innate and adaptive immune responses at the maternal-fetal interface promotes survival of the semi-allogeneic embryo and, at the same time, allows effective immune responses to protect the mother from environmental pathogens [129]. This is achieved and/or results in a wide range of mechanisms, including antibody transfer of certain isotypes, transfer of immune complexes, immune cell stimulation and transfer of cytokines and other soluble molecules via the placental membrane, which affects the offspring's as well as the mother's immune system. For example, the trophoblast, an important component of the placenta, has been shown to recognize and respond to microorganisms and their products through the expression of TLRs, thereby affecting the local cytokine milieu [130-132]. In the case of schistosomes, it has been shown that maternal infection with *S. japonica* is associated with increased levels of inflammatory cytokines in maternal peripheral, placental and cord blood. Furthermore, placental IL-1β and TNF-α production by SynTs was observed and this has been associated with decreased birth weight [133]. Other studies revealed a possible transfer of the inflammatory cytokines IL-6, TNF-α and IL-1α across the placenta, which might affect fetal development [134]. Besides the transfer of cytokines and antibodies a recently published study showed alterations in gene expression in murine placentas in an infection-induced intrauterine growth restriction model, induced by maternal challenge with the oral human pathogen *Campylobacter rectus*. In this study, microarray and pathway analyses associated induced growth restriction with downregulated expression of critical growth and developmental related genes, including imprinted genes [135]. This could further impact or result from changes in placental immune cell composition. A wide variety of $CD45^+$ immunocompetent cells, including

uterine natural killer cells, $CD3^+$ T cells and $CD14^+$ mononuclear cells are predominant cell types of the maternal immune system within the decidua [136]. Local immunosuppression established by the interaction between the maternal immune system and fetal cells, which express alloantigens, is neccessary for successful fetal development during pregnancy. It has been shown, that i.p injection of LPS in pregnant mice, which activates TLR-4 mediated cytokine release, leads to maturation of DCs, systemic T and NK cell activation, and infiltration of activated NK cells at the maternal-fetal interface [129]. The importance of placental cell composition has further been demonstrated in a human prospective study, where changes in the balance of decidual leukocyte populations ($CD8^+$ T cells, αβ-T cell receptor positive cells, $CD56^+/CD16^+$ NK cells) created a placental environment, which is associated with preeclampsia [137].

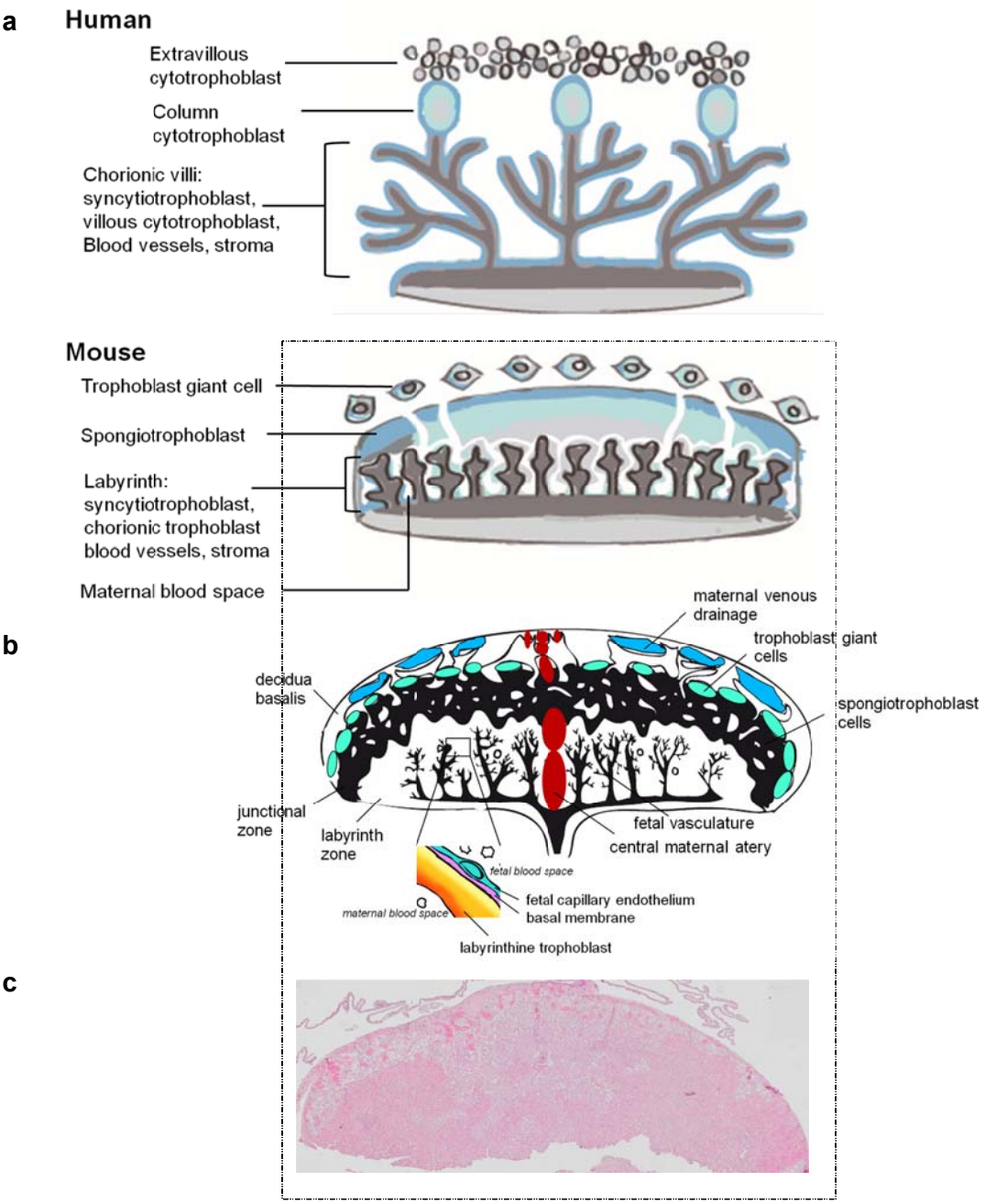


Fig. 1-4: Schematic comparison of mouse and human placenta.
(a) In mice and humans, the fully developed placenta is composed of three major layers: The outer layer of the mouse placenta, which mediates implantation and invasion into the uterus, is composed of trophoblast giant cells. The layer with analogous function in humans is composed of invasive extravillous cytotrophoblast cells. The function of the middle layer of the mouse placenta, the spongiotrophoblast, is largely unknown. However, some of the spongiotrophoblast cells can differentiate into trophoblast giant cells resembling the cytotrophoblast cell columns that anchor the villi of the human placenta. The labyrinth layer of the mouse placenta is comparable in function to the chorionic villi of the human placenta. In both the mouse and human placentas, the labyrinthine and villi, respectively, are covered by syncytiotrophoblasts that lie in direct contact with maternal blood [138]. Adapted from [27]. (b) Schematic representation of a sagittal section of the mouse placenta E15.5: The placenta is oriented with its maternal side at the top and the fetal (flat) side at the bottom. The plane of sectioning is through the center of the placenta and perpendicular to its flat surface. The major placental zones (db, decidua basalis; jz, junctional zone; lz, labyrinth zone) are shown; their constituent cell types are depicted. Magnification of the boxed area, showing in more detail the labyrinthine feto-maternal interface (the zygote-derived tissue between fetal and maternal blood). Adapted and modified from [139]. (c) H&E staining of E18.5 placenta from BALB/c mice.

1.3.2.2 Transmission of immune shaping molecules via breast milk

The interplay between mother and child during pregnancy is further influenced by the introduction of breast milk after birth, which affects the fetal immune system development. Once the umbilical cord has been cut, breast milk offers protection towards infectious diseases by passive transfer of high amounts of microbe-specific immunoglobulins. In addition to IgGs and IgMs, breast milk contains other immune-modulatory compounds such as nucleotides, specific amino acids (taurine, polyamines), polyunsaturated fatty acids (eicosapentaenoic acid, docosahexaenoic acid), monoglycerides, leuric acid, linoleic acid, cytokines and chemokines, isoforms of immunoglobulins (sIgA), soluble receptros (CD14, sTLR2), antibacterial proteins/peptides (lactoferrin, β-lactoglobulin, casein) and intact immune cells [140]. The concept that breast-feeding modulates the immune system despite a genetic predisposition has been supported by studies using $Rag2^{-/-}$ knockout mice, which lack functional T and B cells. $Rag2^{+/-}$ pups fed from $Rag^{-/-}$ mothers, who have very few circulating or milk lymphocytes, showed an impaired immune system measured by antigen-specific immunoglobulins, when compared with those fed from $Rag^{+/+}$ mothers. Those fed by $Rag^{+/-}$ mothers showed similar responses to $Rag^{+/+}$pups [141]. Epidemiological research continues to show that breast feeding provides advantages with regard to general health, growth, and development, while significantly decreasing the risk for a large number of acute and chronic diseases, such as diarrhea [142], lower respiratory infection [143], otits media [144], bacteremia and bacterial meningitis [145], botulism [146] and necrotizing enterocolitis [147]. Moreover, breast milk actively shapes the immune response of the progeny, particularly in the context of allergic disease [148]. Many epidemiologic studies have shown a protective effect of breastfeeding on asthma. Currently discussed underlying mechanisms include the prevention of respiratory infections that predispose to wheezing, promotion of gut colonization by protective bacteria such as *lactobacilli* and *bifidobacteria,* and the presence of the immunosuppressive cytokine TGF-β in breast milk [149]. In mice, breast feeding by OVA-exposed mothers prevented OVA-induced allergic airway inflammation in the progeny independent from the presence of immunoglobulins in breast milk. In this study, protection required the presence of the antigen and TGF-β in breast milk, which induced tolerance mediated by $CD4^+$ Treg cells and TGF-β signaling in T cells. Neutralization of TGF-β in adult offspring before OVA-sensitization did not prevent breastfeeding-induced protection, demonstrating that TGF-β was no longer required once Treg cells have been induced during the neonatal period [149]. In addition, transfer of food antigens via breast milk of allergic mothers has been shown to prevent offspring from developing allergic symptoms in an OVA-induced food allergy model by induction of oral tolerance mechanisms [150]. Nevertheless, the effect of breastfeeding on allergen susceptibility and asthma

transmission remains controversial, particularly in allergen-independent maternal transmission. For example, one study reported that the risk of asthma in children born to asthmatic mothers increased with breastfeeding [151]. The varying effects of breastfeeding on the asthma susceptibility transmission might be explained by variations in the composition of breast milk. Indeed, elevated concentrations of IL-4, IL-5 and IL-13 were detected in the colostrums from allergic mothers compared to non-allergic mothers [152]. However, a recent study showed no association between levels of IL-4, -5, -6, -8, -10, -13, -16, IFN-γ, TGF-β1, -β2, eotaxin or sIgA levels in breast milk with either skin-prick-test-positivity, or development of allergic symptoms during the first two years of life [153]. Thus, there is still controversy concerning the transfer of certain cytokines via breastmilk.

1.3.2.3 Critical windows of vulnerability in fetal development of the immune system

Development of the vertebrate immune system *in utero* and in postnatal life is a dynamic process that requires a sequential series of carefully timed events, including continual differentiation of lineage-restricted stem cells into myeloid or lymphoid leukocytes from a set of pluripotent hematopoietic stem cells (HSC) [154] (Fig. 1-5). With regards to mice these pluripotent stem cells appear in the extraembryonic blood islands within the yolk sac and in intraembryonic splanchnopleuric mesenchyme (AGM) surrounding the heart at approximately day 9 of gestation [155]. Embryonic circulation is established at gestational day 8.5 [154] and from gestation day 10 onwards HSC migrate to the developing fetal liver and differentiate into progenitor cells, which are able to form colonies in response to certain cytokines *in vitro* [156]. The fetal liver is comprised of primary hematopoietic tissue throughout gestation and the rapid expansion of lineage restricted progenitor cells for all types of leukocytes can be detected on day 10 of gestation in mice, which corresponds to about the sixth week in humans [157, 158]. In mice, mature lymphocytes are not found in the developing liver until day 18 of gestation [159]. From day 13 of gestation, the mouse spleen actively contributes to hematopoiesis until several weeks after birth [160]. A rudimentary thymus forms around gestational days 9-10 and is colonized by HSC from the fetal liver to form immature proliferating lymphoid thymocytes which express the T cell specific surface proteins CD3, CD4, CD8 and the T cell receptor [158]. These thymocytes are initially CD4 and CD8 double negative and develop sequentially into $CD4^+CD8^+$ double positive cells and finally mature into separate immunocompetent populations of single positive CD4 and CD8 thymocytes that migrate into the periphery as T helper cell and cytotoxic T cell precursors. Rearrangement of T cell receptor TCR gene segments occurs during this maturation. By day 15 of fetal development cells with the γδ TCR can

be detected in the mouse thymus followed soon after by the appearance of a pre-TCR version of the αβ TCR. At approximately day 17 of fetal mouse development, the TCRαβ-heterodimer is expressed on thymocytes [161, 162]. In humans, pluripotent stem cells enter the developing thymus at week 7 of gestation. The majority of the subsequently differentiating thymocytes express TCR of the γδ type at approximately 9.5 weeks of gestation and of the αβ type at about 10 weeks of gestation [158, 163]. B lymphocyte precursors, pre-B cells, are present among the islands of hematopoietic cells in fetal liver by 8-9 weeks of gestation in humans and 14 days in the mouse. B lymphocytes, which are mainly restricted to IgM expression can be found by days 16-17 in the mouse. IgM synthesis in humans can be detected by approximately week 10-12 of gestation [158]. In mice and humans, pluripotent stem cells migrate from the liver into the bone marrow, where they proliferate by gestational days 17-18 in mice and by week 20 in humans. A limited number of B cell progenitors are detectable in murine bone marrow on day 19 of gestation and T lymphocytes at birth [164]. In contrast, early T cell development in the fetus occurs in the first trimester in humans and B lymphocytes are found in the liver at about 8 weeks of gestation and in the spleen at about 12 weeks [158]. Furthermore, functional NK cells occur at 28 weeks of gestation in the human fetus, whereas NK cell activity in mice is absent at birth and begins 3 weeks later [158]. During the immediate postnatal period, acquired immune function is first detectable in mammals, with functional B and T lymphocyte production in the bone marrow and thymus, respectively [165]. Early postnatal immune system differs in cellular and humoral immune responses from those of adults. During the first month after birth in mice and the first year in human, immaturity of B cells, T cells and macrophages as well as a predominantly IgM-mediated immune response to antigens has been described [165]. Although T cells can be primed shortly after birth, they have been shown to secrete a limited panel of cytokines, but a strong Th1 or Th2 response when re-challenged later in life [163, 166, 167]. Furthermore, the expression of TLR on neonatal macrophages and monocytes is similar in adults, but TLR signaling itself leads to a lower production of the Th1 cytokines TNF-α, IFN, IL-12 and IL-1β compared to production in adults [140]. In summary, distinct windows of vulnerability to environmental exposures are defined during the development of the fetal and early postnatal immune system development. As a consequence, maternal infection might affect the development of different subsets of immune cells, for example T cells, through delayed induction of Th1 responses and subsequent relatively higher Th2 and Th17 activity with loss of Treg cells during maturation and a potential risk for immune-related disorders, such as allergies.

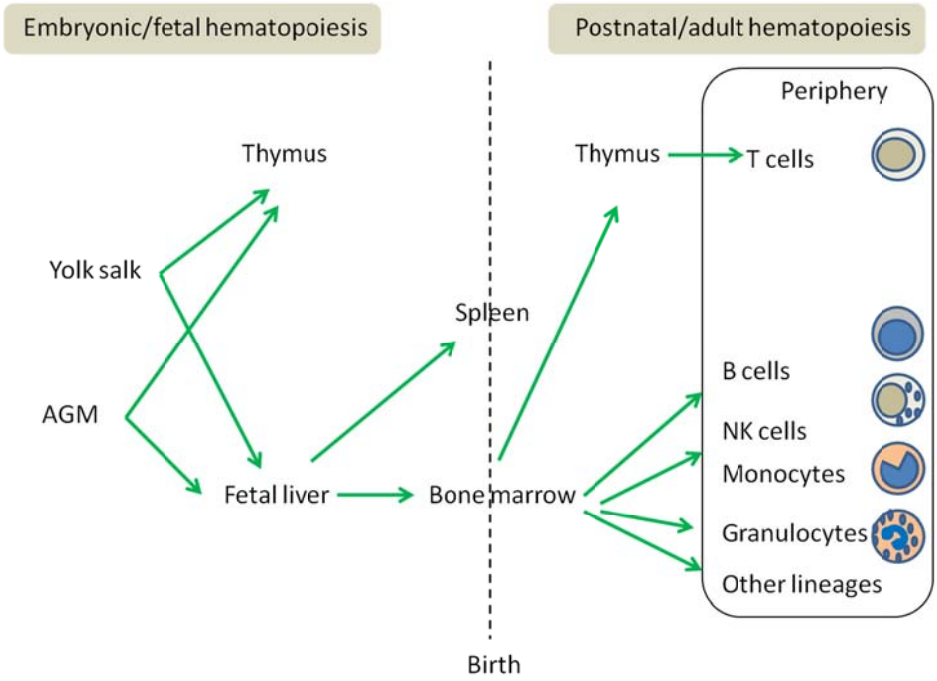


Fig. 1-5: Migration of hematopoietic progenitor cells and mature lymphoid cells.
Migratory pathways of hematopoietic progenitor cells are depicted before and after birth. Hematopoiesis starts during embryogenesis in the yolk sac and aorta-gonad-mesonephros (AGM) region. Hematopoietic progenitor cells then migrate to fetal liver and spleen. Migration to the bone marrow occurs late in fetal development. The bone marrow acts as a site of hematopoieses postnatally and in adult life. Adapted from [168].

1.3.3 *S. mansoni* infection as a model for studying transgenerational effects

The possible mechanisms, mediating effects on fetal immunity during pregnancy and breastfeeding are illustrated in Fig. 1-6, indicative for maternal parasite infection. In general, proposed mechanisms include maternal adaptive immune responses to infectious antigens or allergens, which might cross the placenta or are transferred via breast milk either free or complexed with immunoglobulins. Additionally, maternaly derived cytokines could affect the innate immune responses within the placenta and might result in the modification of the offspings' immune system development. In line, the transfer of maternal cells with either regulatory or inflammatory properties could play an important role. Another possible underlying mechanism that is proposed to drive changes during fetal development by maternal exposure to environmental factors is epigenetic regulation. This is thought to be triggered for example by diet, stress, microbial entities and pharmacological components [169] [126]. Epigenetic modifications are now widely accepted as playing a critical role in the regulation of gene expression and these varations are thought to contribute to the resulting phenotype of an organism throughout its lifetime. These epigentic modifications are referred to as molecular modifications to the DNA, such as the methylation of cysteine residues, or modifications of DNA associated proteins, such

as histone acetylation, which leads to changes in gene expression that occur in the absence of changes in the DNA sequence. Transgenerational epigenetic effects include effects of environmental exposure on adults that alter the phenotype of the developing embryo via the placenta or the newborn via breast milk. Moreover, transgenerational epigenetic inheritance via the gametes has been reported [170]. This process could direct early lineage commitment of immune cells and thereby shape the fetal immune system and potentially affect susceptibility to allergies later in life [171]. Protozoan and helminths have profound effects on the host's immune response, such as impaired responses to immunization and infection [172-174] as well as altered responses against non-parastic antigens or allergens [175], which might also develop *in utero* through the exposure to maternal infection. To investigate the effects of maternal helminth infection during pregnancy and breastfeeding, schistosomiasis comprises an excellent natural infection model, since distinct changes in the mother's immune response occur progressively throughout infection, which offers the possibility to study the effects of a distinct maternal immune phase on the offsprings' immune system development. Another interesting aspect of this infection model is the fact that SEA from hepatointestinal granulomas could circulate to the placenta and cause placental inflammation, probably by stimulating T cell responses. The transplacental transport and immunogenicity of antigens in the fetus have been documented in multiple reports [176-179]. Specifically, placental transfer of maternal SEA has been shown in human pregnancies suggesting a role for this immunomodulatory antigen in maternal cell activation and infiltration within the feto-maternal interface [180] (Fig. 1-6) How antigens cross from the maternal to fetal environment is poorly understood. One hypothesis is that transplacental antigen transfer occurs as immune complexes, via receptor-mediated transport across the syncytiotrophoblastic membrane and endothelium of vessels in fetal villi (Fig. 1-4), with the potential to stimulate an immune response or induce immunoregulatory networks in the fetus affecting susceptibility to infection and disease later in life. Indeed, helminth infection during pregnancy was shown to modulate the offspring's immune system in response to worm antigens [181], as well as unrelated vaccine antigens [182]. In mice, infection with *S. mansoni* during pregnancy and breastfeeding modulates the immunity against homologous antigens in postnatal infection, leading to a reduction in granuloma size and number in the offspring. These findings were associated with early sensitization through transfer of circulating schistosomal antigens and maternal parasite-specific antibodies via the placenta and breast milk [180, 183]. In this regard, oral delivery of a 63-kD *S. mansoni* antigen as well as specific IgG antibodies to this antigen from *S. mansoni* infected mothers to newborns via breast milk has been reported [180]. In addition, a murine study revealed transfer of antibodies to cercariae, adult worms and egg antigens (SEA) from the infected mothers to the offspring, probably

through placenta and milk. Offspring born to infected mothers exhibited much less coagulative hepatic necrosis and showed a lower number of eggs in the small intestine and a less intense and predominant exsudative stage of the hepatic granulomas upon infection [184]. These findings suggest that exposure to *S. mansoni* during breastfeeding modulates the immunity against the helminth antigen in postnatal infection. With regard to allergy risk, a positive correlation between maternal *S. mansoni*-specific IgE level and and gastrointestinal allergy was reported in breast-fed infants from infected mothers [185]. In accordance, a recent study in mice reported that *S. mansoni* infection during pregnancy provided the progeny with an immunosuppressive IL-10 mediated potential, which was abrrogated, when the offspring were born from uninfected but breast-fed by infected mothers [186]. However, in endemic areas anthelminthic treatment of pregnant women with praziquantel is associated with increased risk of infantile eczema [187, 188] suggesting that maternal schistosomiasis during pregnancy, or neonatal life and early breastfeeding may protect against allergy in infancy. In conclusion, the effects of maternal *S. mansoni* infection during pregnancy with the potential to induce systemic immunologic hyporesponsiveness or hyperresponsiveness against homologous or heterologous antigens in the progeny have not been indetified sufficiently up to now and need further investigation.

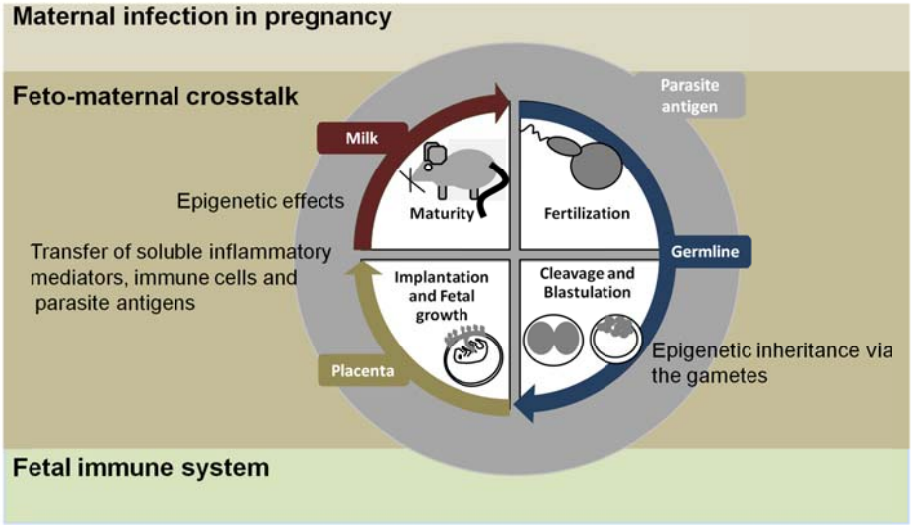


Fig. 1-6: Possible mechanisms mediating the effects of maternal helminth infections on fetal immunity in mice.
Helminths antigens interact differently with the maternal immune system, potentially leading to a variety of modulations in the fetal immune system. The induction of adaptive immune responses might activate maternal immune cells and thereby induce cytokine and immunoglobulin production, as well as epigenetic modifications, depending on the phase of infection. Furthermore, transfer of immunological molecules and immune cells via placenta and breast milk or epigenetic inheritance via the gametes, could differentially shape the immune system development in the offspring and modulate the allergen susceptibility in postnatal life. Adapted from [189].

1.4 Aims of the study

Asthma is one of the most outstanding allergic disease in industrialized countries, whereas in developing countries the frequency is much lower. Protective factors associated with atopy or asthma include poor sanitation, overcrowding and exposure to microbial and other species, such as parasites, in the pre- and postnatal phase of living. The helminth *S. mansoni* is one of the major pathogens of the disease schistosomiasis or bilharzia, which, next to malaria, is the second most socioeconomically devastating parasitic disease. The infection is not transmitted via the placenta and drives the host's immune response progressively from an initial Th1 to a Th2 dominated phase to finally create a regulatory immunosuppressive environment that is known to dampen allergic reactions in the infected host. The immunological mechanisms that mediate protection against the development of asthma are not completely understood, although the participation of regulatory immune cells is proposed. Therefore, the intention of this study was to elucidate the protective role of $Foxp3^+$ Tregs during the development of allergic airway inflammation in *S. mansoni* infected DEREG mice, which allow the specifc depletion of regulatory T cells. In addition, since all of the currently available studies that investigated the effects of schistosome infection on asthma development have used ovalbumin as model allergen, this study addressed the question if the development of allergic airway inflammation in the infected host is also reduced after sensitization and challenge with the clinically highly relevant ragweed allergen. Another yet unsolved question is, whether the observed protection is only mediated by an ongoing infection within the murine host itself or if maternal *S. mansoni* infection during pregnancy alters the allergen susceptibility of the offspring. Thus, another central aim of this study was to investigate the effects of distinct immune phases during maternal schistosomiasis on the development of allergic airway inflammation in the offspring. With the objective to investigate the effects of specific maternal cytokines on the transfer of asthma susceptibility, cytokine knockout strains were used. Additionally, recent findings offered the incentive to analyze the placental milieu (gene expression signature, cytokine production) during the distinct immune phases of schistosome infection, since placental gene expression was shown to be affected by environmental factors, such as infections. Furthermore, this study intented to answer the question, whether epigenetic marks could be inherited via the gametes, thereby affecting the asthma development in the offspring. Finally, 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 the peripheral blood from young offspring.

2 Materials and Methods

2.1 Materials

2.1.1 Equipment

Agarose gel documentation system	Bio-Rad
Agarose gel electrophoresis system (Sub-Cell® GT)	Bio-Rad
Automatic pipettes (2-1000µl)	Gilson®
Balance (440-33N)	Kern
Balance (XB120A)	Precisa
BD FACSCalibur™ flow cytometer	BD
Biological safety cabinet (Hera safe)	Thermo Scientific®
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
CyAn ADP Lx P8	DakoCytomation
Disperser (T10 basic Ultra-Turrax®)	IKA
ELISA microplate reader (Sunrise™)	Tecan
EZ Cytofunnels®	Thermo Scientific®
Fridge	Bosch
Glassware	Schott
Homogeniser (5ml)	B Braun
Incubator (BBD 6220)	Heraeus
LumiNunc™ 96-well plates (white)	Nunc
Microscope (Axioscop)	Zeiss

Microscope (Axiovert)	Zeiss
Milli-Q Integral System	Millipore™
Mini-PROTEAN electrophoresis system	Bio-Rad
Mini-Pump variable flow	Neolab®
MoFlo™ XDP	Beckman Coulter
Multichanel pipettes (Acura® 855; 5-350µl)	Socorex
Multipette® plus	Eppendorf
Orbital shaker (3005)	GFL
OVA-challenge chamber	MRI
Paraffin embedding system (TB 588)	Medite
Paraffin oven	Memmert
Pari Boy® SX nebulizer	Pari
pH-meter (MultiCal®)	WTW
Rotary microtome automatic (RM 2245)	Leica
Rotating mixer (RM 5)	Assistant
Shandon cytoclips	Thermo Scientific®
Shandon Excelsior ES tissue processor	Thermo Scientific®
Stericup® filter units (500ml)	Millipore™
Thermocycler (T3000)	Biometra
Thermomagnetic stirrer (IKAMAG® REO)	IKA
Tissue flotation bath (TFB 35)	Medite
Ultracentrifuge (Optima™ L-100 XP)	Beckman Coulter
Vortex mixer (Reax top)	Heidolph
Water bath	Memmert

2.1.2 Software

CELLQuest Pro™ software (Flow cytometry)	BD
FlowJo (Flow cytometry analysis software)	TreeStar

GraphPad Prism 5 (Biostatistics, curve fitting and scientific graphing programme)	GraphPad Software
IPA	Ingenuity® Systems
Magellan™ (Data analysis software for microplate reader)	Tecan
Nanodrop® 1000 V 3.7.0	Kisker
REST © (Relative Expression Software Tool) V2.013	Quiagen/TUM

2.1.3 Consumables

Blood agar plates	BD
Cell scraper	TPP®
Cell strainer (40-100µm)	BD
Centripreps®	Amicon
Combitips plus® (0.2-2.5ml)	Eppendorf
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
MacConkey agar plates	BD
Parafilm M®	Pechiney
Petri dishes	Greiner
Pipet tips (10-1000µl)	Starlab
Reaction tubes	Zefa-Laborservice
Scalpels	Feather

Shandon filter cards	Thermo Scientific®
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

2.1.4 Reagents

Acetic acid	Roth®
Acetone	Fischer Scientific
Acid fuchsin	Morphisto
Agarose	Sigma®
Aluminium hydroxide ($Al(OH)_3$)	Sigma®
Ammonium chloride (NH_4Cl)	Roth®
Aniline blue	Morphisto
Bovine serum albumin (BSA)	PAA
Bromophenol blue	Roth®, Sigma®
Chloroform/Isoamylalcohol (24:1)	Fluka
Collagenase (from *Clostridium histolyticum*)	Sigma®
Deoxynucleoside triphosphate (dNTPs)	Promega
Deoxyribonuclease I from bovine pancreas (DNAse)	Sigma®
Dimethyl sulfoxide (DMSO)	Sigma®
Diphtheria toxin	Merck
Di-sodium carbonate (Na_2CO_3)	Sigma®
DirectPCR-tail lysis reagent	Peqlab
Dithiothreitol (DTT)	Roth®

Dulbecco`s PBS (Endotoxin-free)	PAA
Ethidium monoazide bromide (EMA)	Invitrogen
Entellan®	Merck
Eosin 1% (v/v)	Morphisto
Ethanol 70%-99.8% (v/v)	MRI Pharmacy
Ethidium bromide	Roth®
Ethylenediaminetetraacetic acid (EDTA)	Roth®
Forene®	Abbott
Formaldehyde solution 37% (v/v)	Sigma®, Merck
Gentamicin (10mg/ml)	PAA
Herculase II	Stratagene
Hydrogen chloride (HCl)	Roth®
4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)	Sigma®
Isopropanol	MRI Pharmacy
Ketanest	Inresa Arzneimittel GmbH
LightCycler® 480 Probes Master	Roche
LPS 0127:B8 (Lipopolysaccharide)	Sigma®
Mayer`s haematoxylin	Morphisto
Metacholin	Sigma®
Methanol	Roth®
β-Mercaptoethanol	Roth®, Sigma®
Sodium acetate	VWR
Narcoren®	Merial
OVA (Albumin, from chicken egg white) grade V and VI	Sigma®
Paraformaldehyde (PFA)	Sigma®
Percoll™	GE Healthcare
Periodic acid	Morphisto
Phenol	Roth®

Phosphate buffered saline (PBS)	MRI
Phosphomolybdic acid	Morphisto
Potassium hydroxide (KOH)	Merck
Powdered PBS (Phosphate buffered saline)	Biochrom
Protease inhibitor cocktail tablets (complete)	Roche
RNAlater®	Ambion®
Rompun	Bayer Health Care
Roti®-Histofix 4% (v/v)	Roth®
Roti®-Safe GelStain	Roth®
Schiff reagent	Morphisto
Sodium chloride (NaCl)	Roth®, Merck
Sodium hydrogen carbonate ($NaHCO_3$)	Merck
Sodium orthovanadate (Na_3VO_4)	Sigma®
Sucrose	Roth®
Sulphuric acid (H_2SO_4)	Merck
3,3',5,5'-Tetramethylbenzidine (TMB) substrate	BD
Tris(hydroxymethyl)aminomethane (Tris)	Merck, Roth®
Trisodium citrate dihydrate	Roth®
Trypan blue solution 0.4% (v/v)	Sigma®
Tween® 20	Sigma®
Vancomycin hydrochloride (VANCO-cell® 500mg)	Cellpharm
Weigert`s haematoxylin	Morphisto

2.1.5 Medium supplements

Fetal calf serum (FCS)	PAA
β-Mercaptoethanol for cell culture	Gibco®
Non-essential amino acids (100x)	PAA®
Penicillin/Streptomycin (100x)	PAA

RPMI 1640 (with L-Glutamine)	PAA
Sodium pyruvate solution (100mM)	PAA

2.1.6 Kit systems

BD OptEIA™ (TMB substrate)	BD
DC protein assay	Bio-Rad
Diff-Quik staining set	Medion Diagnostics
Foxp3 staining buffer set	eBioscience
GoTaq® DNA polymerase (including 5x Green GoTaq® reaction buffer)	Promega
Herculase II reaction buffer	Stratagene
In vivo IL-10 capture assay	BD
Mouse ELISA Kits (Duo Set®; IL-5, IL-10, IL-4)	R&D
Mouse ELISA Kits (Ready-Set-Go) TNF-α, IFN-γ, IL-5, IL-10, IL-13	eBioscience
PeqGOLD Gel Extraction Kit	PEQLAB Biotechnologie
PureLink Quick Plasmid Miniprep Kit	Invitrogen
QuantiTect Reverse Transcription Kit	Quiagen
QIAmp DNA Stool Mini Kit	Quiagen
RNeasy Mini Kit	Quiagen
Taq buffer	Invitrogen
TOPO TA cloning Kit	Invitrogen

2.1.7 Size standards

Gene ruler™ 1kb DNA ladder	Fermentas

2.1.8 Buffers and solutions

2.1.8.1 Buffers and solutions for egg preparation

Vancomycin solution: hydrochloride	500mg	Vancomycin
	10ml	0.9% NaCl (w/v)
Collagenase solution:	500mg	Collagenase
	5ml	Dulbecco`s PBS (1x)
DNase solution:	1g	DNase I
	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	Percoll™
	32ml	0.25M Sucrose

2.1.8.2 Buffers for erythrocyte lysis and bronchoalveloar lavage

ACT buffer:	17mM	Tris
	160mM	NH_4Cl
	pH 7.2	
BAL (Bronchoalveloar lavage) buffer:	50ml	Dulbecco`s PBS (1x)
	1 tablet	Protease inhibitor cocktail

2.1.8.3 Buffers and solutions for FACS

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*

2.1.8.4 Buffers and solutions for ELISA

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	H_2SO_4
Ig-coating buffer:	8.4g/l	$NaHCO_3$
	3.56g/l	Na_2CO_3
Ig-blocking buffer:	50mM	Tris
	3% (w/v)	BSA

2.1.8.5 Buffers for VDR genotyping PCR

Polymerase buffer: buffer	400µl	5x Green GoTaq® reaction
		(including loading buffer)
	40µl	10mM dNTPs
	40µl	1% BSA (w/v)
	1260µl	H_2O
TAE buffer:	40mM	Tris-Acetic acid (pH8.3)
	1mM	EDTA

Tail-digestion solution: reagent	250µl	DirectPCR-tail lysis
	3µl	Proteinase K (20mg/ml)

2.1.9 Cell culture medium

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

2.1.10 Primer sequences

2.1.10.1 VDR genotyping primer

oIMR2060	5'-CAC GAG ACT GAG ACG TG-3'	(mutant)
oIMR5219	5'-CTC CAT CCC CAT GTG TCT TT-3'	(wt)
oIMR5220	5'-TTC TTC AGT GGC CAG CTC TT-3'	(common)

2.1.10.2 Primer for *S. mansoni* specific qPCR

Forward tandem repeat (FP): 5' CAACCGTTCTATGAAAATCGTTGT 3'

Reverse tandem repeat (RP): 5' CCACGCTCTCGCAAATAATCT 3'

Dual labeled probe tandem repeat (labeled probe) with 5' and 3' modifications:

5'
[6FAM]TCCGAAACCACTGGATTTTTATGAT[BHQ1]

2.1.10.3 Primer for RT-qPCR

HPRT (reference 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

Foxp3

Forward primer	5'-TCA GGA GCC CAC CAG TAC A-3'
Reverse primer	5'-TCT GAA GCC CAC CAG TAC A-3'
Dual labeled probe	Universal ProbeLibrary Probe #78, 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

IL-5

Forward primer	5'-ACA TTG ACC GCC AAA AAG AG-3'
Reverse primer	5'-ATC CAG GAA CTG CCT CGT C-3'
Dual labeled probe	Universal ProbeLibrary Probe #91, Roche

IL-10

Forward primer	5'-CAG AGC CAC ATC CTC CTA GA-3'
Reverse primer	5'-GTC CAG CTG GTC CTT TGT TT-3'
Dual labeled probe	Universal ProbeLibrary Probe #41, Roche

Hsd3b1

Forward primer	5'-TGT GAC CAT TTC CTA CAT TCT GA-3'
Reverse primer	5'-CCA GTG ATT GAT AAA CCT TAT GTC C-3'
Dual labeled probe	Universal ProbeLibrary Probe #66, Roche

VDR

Forward primer	5'-CAC CTG GCT GAT CTT GTC AGT-3'
Reverse primer	5'-CTG GTC ATC AGA GGT GAG GTC-3'
Dual labeled probe	Universal ProbeLibrary Probe #89, Roche

2.1.11 Hormones

Chorionic gonadotropin, human (HCG) 10000 I.U. Sigma Aldrich

Dissolve in sterile 0.9% NaCl and store at –80°C for up to 3-4 months

Pregnant-mare-serum-gonadotropin (PMSG), INTERGONAN® Intervet GmbH

Dissolve in sterile 0.9% NaCl and store at –80°C for up to 3-4 months.

2.1.12 Antibodies

Name	***Antigen***	***Con-jugate***	***Species***	***Dilution***	***Producer***	***Method***
α-mouse CD4-APC (Clone GK1.5)	*CD4*	*APC*	*Rat*	*1:1000*	*eBioscience*	*FACS*
α-mouse CD16/32 Fc receptor block (Clone 93)	*CD16/ 32*	-	*Rat*	*1:1000*	*eBioscience*	*FACS*
α-mouse/rat Foxp3-FITC *(Clone FJK-16s)*	*Foxp3*	*FITC*	*Rat*	*1:1000*	*eBioscience*	*FACS*
α-mouse Bcl-2 -PE *(Clone FJK-16s)*	*Bcl-2*	*PE*	*Hamster*	*1:10*	*BD*	*FACS*
α-mouse CD44-FITC (Clone IM-7)	*CD44*	*FITC*	*Rat*	*1:100*	*BD*	*FACS*
α-mouse gdTCR-PE (Clone GL-3)	*gdTCR*	*PE*	*Ar Hamster*	*1:200*	*BD*	*FACS*
α-mouse **LY6C-PerCpCy5.5** (Clone HK1.4)	*LY66C*	*PerCpC y5.5*	*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-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-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 CD3e (Clone 17A2)	*CD3*	-	*Rat*	*1µg/ml*	*eBioscience*	in vitro *stimuli*
α-mouse CD28 (Clone 37.51)	*CD28*	-	*Golden Syrian Hamster*	*1µg/ml*	*eBioscience*	in vitro *stimuli*
α-mouse IL-10 (Clone JES5-2A5)	*IL-10*		*Rat*	*10µg/ml*	*eBioscience*	in vitro IL-10 blocking

α-mouse IgG1-Biotin (Clone RMG1-1)	*IgG1*	*Biotin*	*Rat*	*1:400*	*Biozol*	*ELISA*
Mouse α-ovalbumin IgG1 (Clone 2C6)	*OVA*	-	*Mouse*	*1:1000*	*Biozol*	*ELISA*
Mouse α-ovalbumin IgE (Clone 3G2E1D9)	*OVA*	-	*Mouse*	*1:1000*	*Biozol*	*ELISA*
α-mouse IgE-Biotin (Clone RME-1)	*IgE*	*Biotin*	*Rat*	*1:400*	*Biozol*	*ELISA*
α-mouse IgE (Clone RTK2071)	*IgE*		*Rat*	*1:400*	*Biolegend*	*ELISA*

Table 2: Antibodies used for ELISA, FACS analysis and *in vitro* stimulation

2.2 Methods

2.2.1 Animals

2.2.1.1 Mouse strains and housing

BALB/c, C57BL/6, CBA/J and NMRI mice were purchased from Harlan Winkelmann GmbH (Germany). DEREG C57BL/6 mice were bred and housed at the MIH. BALB/c IFN-[gamma][sup.-/-] mice were supplied by Sabine Specht and originally purchased from The Jackson Laboratory (Bar Harbor, Maine). $VDR^{-/-}$ mice were purchased from The Jackson Laboratory (Bar Harbor, Maine) and fed with a special rescue diet, containing 20% lactose, 2% calcium, 1,25% phosphate (ssniff Spezialdiäten GmbH, Soest). All mouse strains were bred and maintained under specific pathogen-free housing conditions. All animal experiments were performed in accordance with local government regulations (license number for animal testing 2112531 147/08).

2.2.1.2 VDR genotyping PCR

Genotyping primers were suspended in Millipore Q distillated water to a concentration of 100 pmol/µl. A genotyping PCR reaction mix was prepared as follows:

Volume	Component
1µl	Lysate (including mouse DNA)
1µl	Primer (mutant)
1µl	Primer (wt)
1µl	Primer (common)
21µl	Polymerase buffer (see section 2.1.11.1)
0.1µl	Go Taq DNA polymerase

The PCR reactions were performed on a Thermocycler T3000 (Biometra) as follows:

	Temperature	Time	
Initiation	94°C	3min	
Denaturation	94°C	30s	
Annealing	61°C	1min	35 cycles
Elongation	72°C	1min	
Final elongation	72°C	2min	
Storage	10°C	∞	

	mutant allele	wildtype allele
Amplification products	500bp	382bp

DNA-fragments were separated using agarose-gel electrophoresis. Agarose gels (1.5%) were prepared by boiling agarose (Sigma®) in TAE buffer. After cooling to approximately 50°C, a DNA-intercalating agent (ethidium bromide (300 µg/l; Roth®) or Roti®-Safe GelStain (5µl/100ml; Roth®)) was added to the agarose gel solution. The final polymerization was then performed in a horizontal gel chamber (Bio-Rad) at room temperature, in which a comb was placed to form pockets for sample application. Afterwards 15 µl of PCR product (including the synthesized DNA fragments) and 5 µl Gene Ruler™ 1kb DNA ladder (Fermentas) were loaded into the gel pockets. The DNA fragments were separated by applying amperage of 130 mA for 40-60min within a TAE buffer filled agarose gel electrophoresis system (Bio-Rad). Separated DNA fragments

were detected by exciting the intercalating agents with UV light at 254 nm. The emission of the DNA fragments was measured using the agarose gel documentation system (Bio-Rad). The DNA length analysis was performed by comparison of the size standard (Gene Ruler^{TM} 1 kb DNA ladder) with the corresponding DNA fragment, confirming the mouse genotype.

2.2.2 *S. mansoni* lifecycle maintenance

Murine infection of *S. mansoni* reflects both the immunological and parasitological consequences that arise in humans. NMRI mice were used as a mammalian host for *S. mansoni*. To maintain the *S. mansoni* life cycle, NMRI mice were infected i.p. with 140-200 cercariae of a Brazilian strain of *S. mansoni,* which had been released previously from *Biomphalaria glabrata* snails in response to temperature and light. After eight weeks of infection, mice were sacrificed and miracidiae were isolated from the intestine and exposed to *Biomphalaria glabrata* snails to continue the infectious cycle (1.1.2). In addition, livers were used for the preparation of eggs and SEA (2.2.5).

2.2.3 Experimental *S. mansoni* infection

2.2.3.1 Infection protocols

To determine differential cytokine responses during schistosomiasis BALB/c mice were infected with *S. mansoni* by injection of 100 cercariea i.p.. Immune responses were analyzed at week 0, 5, 10, 12, 16 and 29 post infection (3.1.1).
For evaluation of a *S. mansoni* specific qPCR, NMRI mice were infected with 150-300 cercariae i.p.. Post infection, mice were analyzed at weeks 3, 4, 5, 6, 7, 8, 9, and 10 (3.1.2).
For evaluation of protective mechanisms during allergic airway inflammation, 6-8-week-old female BALB/c, C57BL/6 and DEREG C57BL/6 mice were infected by i.p. injection of 120 cercariae. Induction of allergic airway inflammation was performed 7-11 weeks post infection (3.2) .
For offspring studies, BALB/c mice were infected with 100 *S. mansoni* cercariae and mated with uninfected BALB/c males 3.5, 11 and 16 weeks post infection (3.3).
Age-, strain- and sex-matched uninfected mice were used as controls in all experiments.

2.2.3.2 Evaluation of general infection status

The general infection status was scored by the degree of visual infection and organ damage. Specifically, the liver was examined for signs of fibrosis, changes in colour and visible granulomas. The infection status was graded on a scale from 0 to 3 DOI (degree of infection) with 3 denoting the highest level of infection. In addition, total body weight of each individual mouse and organ weight (liver, intestine and spleen) were obtained and compared with uninfected mice.

2.2.3.3 *S. mansoni* egg count analysis from liver and intestine

Liver and intestine samples from individual mice were digested in 5 ml of 5% (w/v) KOH solution under continuous agitation at 37°C for at least 2 hours. After incubation, the released eggs were centrifuged at 400 g for 10 min and 4.5 ml of the supernatant was immediately removed. The remaining eggs (within 500 µl) were then resuspended in 10 µl and counted under the microscope (x10 magnification). The number of eggs in the liver was then calculated as follows:

eggs/mg tissue = (average of counted eggs x 50) / weight of tissue sample (mg)

2.2.4 *S. mansoni* specific qPCR

2.2.4.1 Isolation of parasite DNA from stool, *S. mansoni* eggs and serum

Stool samples from the intestine were collected into 2ml reaction tubes. According to the protocol provided in the QIAmp DNA Stool Mini Kit, DNA was isolated from approximately 100 mg stool. The method was performed as recommended by the manufacturer with little alterations. In brief, the cells in the stool samples were lysed in Stool Lysis Buffer ASL, homogenized by vortexing and incubated at 95°C for 20 min in a shaker in order to lyse stable parasite eggs and release parasite DNA. Afterwards the DNA-containing supernatant was purified from contaminations by adding ½ InhibitEX tablet per sample. For digestion of proteins, proteinase K was added to a final concentration of x in 200 µl buffer AL and samples were incubated for 10 min at 70°C. Samples were loaded onto QIAmp spin columns, followed by two washing steps with buffer AW1 and AW2. Finally, the purified DNA was eluted from the spin column in buffer AE and stored at –20°C for use in qPCR.

DNA from serum samples and purified *S.mansoni* eggs (described in 2.2.5.1) was isolated by phenol-chloroform extraction Therefore, serum samples or egg solutions were mixed with Roti®-phenol at a ratio of 1:1 and centrifuged for 2 min at 16.000 g. The aqueous phase was then transferred into a new tube and 500µl Fluka® chloroform/isoamyl alcohol (ratio 24:1) was added. After centrifugation for 2 min at 16.000g, the supernatant was mixed with 10 µl 3M sodium acetate (pH 5.5) and 1ml ethanol (100 %). After centrifugation for 20 min at 4°C at 16.000 g the supernatant was decanted and the pellet was air dried for about 15 min. The dry pellet was then resuspended in 50 µl DEPC and stored at –20°C for use in qPCR
The yield and purity of DNA was determined using Nanodrop® 1000 (Kisker).

2.2.4.2 qPCR method and running conditions

In order to verify the infection with *S. mansoni*, DNA isolated from stool, serum and *S. mansoni* eggs was amplified by qPCR. Reverse and forward primers and the labeled probe for this experiment were based on findings of Wichmann *et al [190] and lead to the amplification of* a 86 bp fragment of the *S. mansoni* DNA tandem repeat unit as described in section 2.1.10.2

The qPCR reaction mix was prepared as follows:

PCR-Mix	
LightCycler® 480 Probes Master	10µl
forward primer (10µM)	1.0µl
reverse primer (10µM)	1.0µl
Probe	1.0µl
target DNA	2µl
DEPC H_2O	5.0µl

As positive control DNA isolated from stool of infected mice was used and DNA from non-infected mice served as negative control. The PCR was performed using LightCycler®480 (Roche) with the following settings:

	Temperature	Time	
Hot Start	95°C	5min	
Denaturation	95°C	10s	
Annealing/Extension	58°C	30s	45 cycles
Measurement of fluorescence			
Cooling	45°C	15s	

2.2.4.3 Quantification of *S. mansoni* tandem repeat unit

2.2.4.3.1 Amplification of the S. mansoni tandem repeat unit

To generate a plasmid containing the *S. mansoni* tandem repeat unit a PCR was first performed to amplify the target sequence.

The PCR reaction mix was prepared as follows:

PCR-Mix	
5x Herculase buffer	10µl
dNTPs (25mM each)	0.5µl
DMSO	0.5µl
forward primer (10µM)	1.25µl
reverse primer (10µM)	1.25µl
DNA	2µl
Herculase II	0.5µl
DEPC H_2O	36µl

To analyze the resulting PCR products, the samples were loaded on a 2 % garose gel. After electrophoresis the tandem repeat unit DNA with a size of 86 bp was extracted using the peqGOLD Gel Extraction Kit (PEQLAB Biotechnologie GmbH, Erlangen, Germany) according to the manufacturer's instructions.

2.2.4.3.2 Cloning of the S.mansoni tandem repeat unit into the vector pCR 2.1-TOPO

The excised DNA fragment was cloned into the vector pCR 2.1-TOPO with the TOPO TA cloning kit (Invitrogen, Carlsbad, USA). As Herculase II does not add 3' A-overhangs to PCR products the overhangs had to be added post-amplification. Therefore, the following reagent mix was prepared and incubated for 12.5 min at 72°C.

Reagents	volume
10x Taq buffer (200 mM Tris (HCl), 500 mM KCl)	1µl
dNTPs (25mM each)	1µl
DNA (PCR product)	5µl
Taq Polimerase (5U/µl)	1µl
DEPC H_2O	2µl

For ligation into the vector, 0.5 µl pCR 2.1-TOPO, 2 µl of the modified PCR product, 1 µl salt solution and 2.5 µl H_2O were incubated at room temperature for 5 min.

2.2.4.3.3 Transformation of plasmid DNA into chemically competent E.coli

For heat shock transformation of the ligation product into chemically competent E.coli TOP10 bacteria, cells were thawed on ice. After addition of 2µl ligation product the cells were incubated on ice for 10 min followed by a heat shock at 42°C for 30 seconds. 250 µl SOC medium was added to the cells and the mixture was shaked for 1 hour at 37°C. Two different volumes (100 µl and 200 µl) of the cell suspension were plated on prewarmed LB agar plates containing 50 µg/ml kanamycin for antibiotic selection. For blue/white screening, plates were coated with 40 µl of X-Gal (40 mg/ml) prior to plating of bacteria.

2.2.4.3.4 Amplification and isolation of plasmid DNA

White colonies were picked and transferred into 5 ml LB medium for overnight incubation at 37°C on a orbital shaker. On the next day plasmid DNA was extracted using the

PureLink Quick Plasmid Miniprep Kit (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. DNA concentration was determined on a NanoDrop device.

2.2.4.3.5 Determination of a standard curve

The obtained *S. mansoni* plasmid DNA was diluted in three dilution series (1:2, 1:5, 1:10) with 10 dilution steps for each series and PCR was performed using LightCycler®480 (Roche) according to the cycle settings described in section 2.2.4.2 to determine the corresponding C_t-Values for the known plasmid concentrations to calculate a standard curve.

2.2.5 *S. mansoni* egg and SEA preparation

2.2.5.1 Egg preparation from liver tissue

To prepare fresh eggs from liver tissue, NMRI mice were sacrificed eight weeks post infection and livers were isolated using sterile scissors and tweezers. Livers were then washed in pre-cooled 1.2 % (v/v) NaCl solution and bile ducts and gallbladders were removed. Livers were then minced using a scalpel and transferred into a 50 ml tube containing 25 ml liver digestion solution. The tube was then filled up with PBS to 50 ml and incubated under continuous agitation at 37°C overnight using an orbital shaker. Thereafter, digested livers were washed twice by centrifugation at 400 g for 5 min at 4°C. The pellet was resuspended in 25 ml PBS and filtered twice through a 250µm sieve. The filtrate was then layered on Percoll and centrifuged at 800 g for 10 min at 4°C. Due to the density gradient of the Percoll solution, eggs were separated from liver tissue. Eggs were washed three times with 15 ml and 30 ml of 1 mM EDTA solution, respectively, and finally with 30 ml PBS as previously described. After the washing steps, the egg pellet was resuspended in 700µl PBS and 5 µl were microscopically analyzed (x10 magnification) to confirm the purity and to calculate total egg count as follows:

total egg count = (counted eggs x 700 µl) / 5 µl

Finally, isolated eggs were either frozen at -80°C or directly used for SEA preparation or DNA extraction. Contaminations were analyzed by standard microbiological assessments on blood agar and MacConkey agar plates containing 1-2 µl of the egg suspension

(incubation at 37°C for 48 hours). The egg suspension was transferred to a glass homogenizer and pestled for at least 20 min on ice. During this procedure the soluble egg antigens (SEA), a mixture of different (glyco-)proteins and (glyco-)lipids are released. Thereafter, an ultracentrifugation step at 100,000g for 1 hour (4°C) using the Optima™ L-100 XP ultracentrifuge was performed. Afterwards, the supernatant that contains the SEA was transferred to a cryotube, whereas the remaining egg shell pellet was resuspended in 200µl PBS. To detect possible contaminations, 1-2µl of both suspensions were plated on a blood agar and MacConkey agar plate and incubated at 37°C for 48 hours. The protein concentration within the SEA solution and egg shell pellet solution was measured using the DC protein assay kit (Bio-Rad) according to the manufacurer's instructions. Finally, both suspensions were frozen at -80°C.

2.2.6 Induction of allergic airway inflammation

2.2.6.1 Ragweed-induced allergic airway inflammation

2.2.6.1.1 Cultivation of ragweed plants

Seeds from a single plant were applied to standard soil in pots and plants were cultivated in Plexiglass sub-chambers placed within two phytotron walk-in chambers [191]. The light regime, temperature and humidity were adapted to the seasonal cycle, starting with the climate conditions of May 2010. The light period was 14.5 h d^{-1}; day/night temperatures were 20-30°C/ 10-20°C and relative humidities were 80-85%/30-50% (day/night). Watering was done by an automated watering system. Pollen were collected using a modified ARACON system (BETATECH, Ghent, Belgium) that covered the male inflorescenses.

2.2.6.1.2 Preparation of pollen extracts and extract fractions

Aqueous pollen extracts were generated by incubating 10 mg of pollen in 100 µl of sterile PBS for 30 minutes at 37°C with vortexing every ten minutes. After centrifugation for 10 min at 3000 g the supernatant was passed through a 0.2 µm sterile filter, aliquoted and stored at -80°C.

2.2.6.1.3 Sensitization and challenge against ragweed pollen extract (RWE)

BALB/c mice (6-8 weeks) (infected or uninfected) were sensitized to RWE by i.p. injection of 10 µg RWE emulsified in 2 mg $Al(OH)_3$ (Thermo Fisher Scientific) in a total volume of 200 µl PBS on days 51 and 55 post infection. Age matched BALB/c mice received PBS with $Al(OH)_3$. At days 61, 67 and 70 animals were challenged intranasally with 20 µl PBS containing 10 µg RWE per mouse (Fig. 2-1). 48 hours later, mice were terminally anesthetized for further analysis.

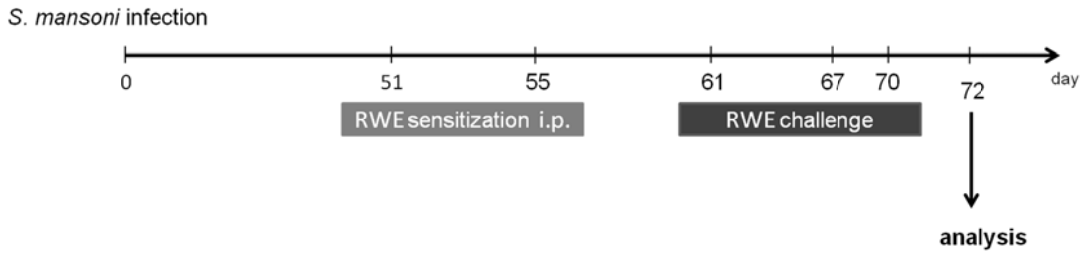


Fig. 2-1: Protocol for ragweed-induced allergic airway inflammation during *S. mansoni* infection

2.2.6.2 OVA-induced allergic airway inflammation

2.2.6.2.1 Sensitization, challenge and Treg depletion

DEREG C57BL/6 mice were sensitized to OVA by i.p injection of 10 µg OVA (grade VI; Sigma-Aldrich) emulsified in 1.5 mg $Al(OH)_3$ in a total volume of 200 µl PBS on days 64 and 71 post infection. Age matched BALB/c mice received PBS with $Al(OH)_3$. Diphtheria toxin (DT) was injected i.p. in DEREG C57BL/6 mice on two consecutive days during sensitization phases. (day 63, 64, 70, 71) in a concentration of 1 µg DT in 200 µl PBS, pH 7.4, per mouse. At days 82, 83 and 84 animals were exposed to aerosolized OVA (grade V; Sigma-Aldrich) (1 % wt/vol diluted in PBS) for 20 min (Fig. 2-2). 48 hours later, mice were terminally anesthetized for further analysis.

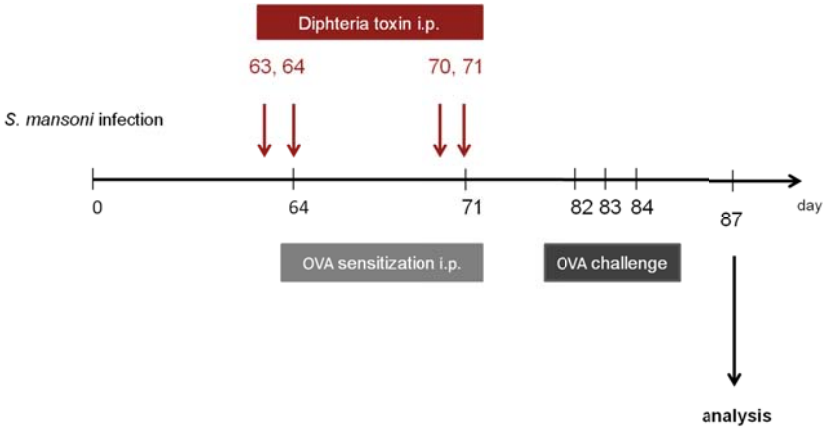


Fig. 2-2: Protocol for OVA-induced allergic airway inflammation and depletion of Treg cells during *S. mansoni* infection.

2.2.6.2.2 Protocol for mating and OVA-induced allergic asthma in offspring studies

Infected BALB/c mice were mated 3.5, 11 or 16 weeks post infection (2.2.3.1). BALB/c Offspring were sensitized to OVA by i.p. injection of 10 µg OVA (grade VI; Sigma-Aldrich) emulsified in 1.5 mg $Al(OH)_3$ (Thermo Fisher Scientific) in a total volume of 200 µl PBS on days 0, 14 and 21 at the age of 6-8 weeks. Age matched control mice received PBS with $Al(OH)_3$. At days 26, 27 and 28 animals were exposed to aerosolized OVA (grade V; Sigma-Aldrich) (1 % wt/vol diluted in PBS) for 20 min (Fig. 2-3). 48 hrs later, mice were terminally anesthetized for further analysis.

Offspring from VDR-/- mice were sensitized with 0.5 mg OVA/kg emulsified in 1.5 mg $Al(OH)_3$ in a total volume of 200 µl PBS and exposed to aerosolized OVA (1 % wt/vol diluted in PBS) for 20 min according to the protocol in Fig. 2-3.

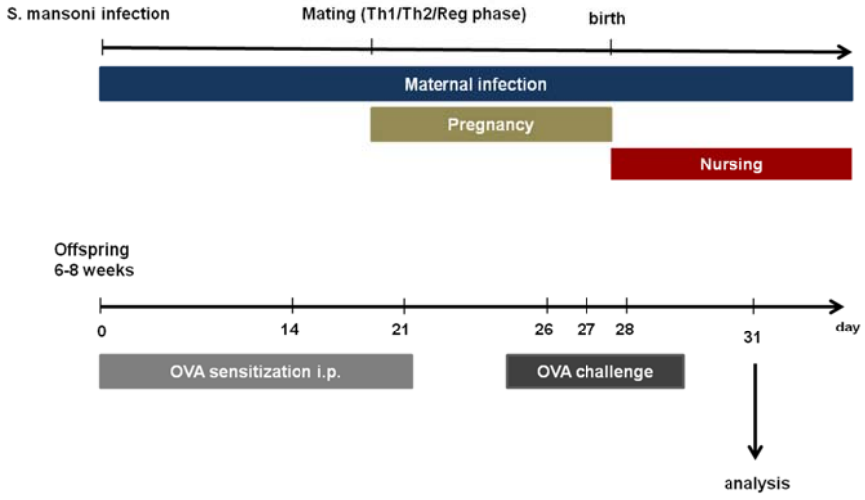


Fig. 2-3: Mating protocol for offspring studies and OVA-induced allergic airway inflammation

2.2.7 Analysis of asthma parameters in the lung

2.2.7.1 Preparation of the bronchoalveolar lavage

To analyse the composition of the infiltrated immune cells and cytokine responses within the bronchoalveolar lavage (BAL), mice were first euthanized with i.p. injection of 50 µl Narcoren. Afterwards, the mouse body was opened using sterile scissors and tweezers. The exposed trachea was disconnected below the larynges using a haemostatic forcep, before the lung was flushed twice with 1 ml BAL buffer using Sterican® hypodermic needles. The obtained BAL was then transferred to 1 ml tubes, weighed and centrifuged at 230 g for 5 min (4°C). Thereafter, the supernatant was frozen at -20°C for cytokine measurement by ELISA. The cell pellet was resuspended in 200 µl FACS buffer, counted and used for cytospin preparation.

2.2.7.2 Cytospin preparation

For microscopical analysis of the composition of immune cells within the BAL, cytospin was performed. In short, glass slides were covered with Shandon filter cards and EZ Cytofunnels® and placed into Shandon cytoclips. 150 µl BAL cell suspension was applied into EZ Cytofunnels. Centrifugation using Shandon Cytospin 3 centrifuge was then performed at 400 g for 5 min. Finally, slide were dried overnight at room temperature and stained with Diff-Quik staining set.

2.2.7.3 Staining and analysis of BAL cells

Cytospins were stained with Diff-Quik staining set according to the manufacturer's instructions. Diff-Quik staining is based on the Romanowski stain [192] and includes aqueous solutions of methylene blue and eosin, dissolved in methanol. This kit allows selective staining of macrophages, eosinophils, neutrophils and lymphocytes. For staining, glass slides were placed consecutively for 10 sec into Diff-Quik Fix (cell fixation), Diff-Quik I (staining of eosinophils) and Diff-Quik II (staining of basophils) solutions. Afterwards, the glass slides were washed in aqua bidest. and dried for 2 hours at room temperature. To differentiate the immune cell populations within the BAL, the Diff-Quik stained sections were microscopically analyzed. At least 100 cells were differentiated into macrophages,

eosinophils, neutrophils and lymphocytes using the Axiovert microscope (x40 magnification) to determine the frequency and percentage of each cell type.

2.2.7.4 Evaluation of lung inflammation and goblet cell formation

For tissue preparation lung samples from individual mice were fixed in Roti®-Histofix (4 %), dehydrated using the Shandon Excelsior ES tissue processor and embedded in paraffin using the TB 588 paraffin embedding system. Sections (3 µm) were cut using the RM 2245 automatic rotary microtome and fixed on glass slides for staining. To evaluate inflammation and goblet cell infiltration within the lung, Periodic acid-Schiff (PAS) staining was performed as depicted in Table 3.

Reagent	Producer	Incubation time	Function
Xylene	Engelbrecht	10min	Dissolving of paraffin
Ethanol (96%)	MRI	2min	Rinsing with mounting water content (watering)
Ethanol (80%)	MRI	2min	
Ethanol (70%)	MRI	2min	
Ethanol (60%)	MRI	2min	
Aqua bidest.	MRI	2min	
Periodic acid (1%)	Morphisto	15min	Oxidation of diol sugar groups leading to aldehyde group formation
Tap water	-	Constant rinsing for 10min	Dissolving of periodic acid
Aqua bidest.	MRI	2min	Watering
Aqua bidest.	MRI	2min	
Schiff reagent	Morphisto	20min	Staining due to the reaction of aldehyde groups with Schiff reagent
Tap water	-	Constant floating for 5min	Dissolving of Schiff reagent
Aqua bidest.	MRI	10s	Rinsing
Mayer`s haematoxylin	Morphisto	6min	Complexation of positive hematoxylin to the negative nucleic acids phosphate groups (nuclear staining)

Tap water	-	Constant floating for 10min	Achievement of deep blue staining by alteration of the pH
Ethanol (96%)	MRI	3min	Rinsing with mounting alcohol content (dehydration)
Ethanol (96%)	MRI	3min	
Isopropanol	MRI	5min	
Xylene	Engelbrecht	10min	Fixation
Entellan®	Merck	-	Fixation and covering with coverslip

Table 3: Periodic-Acid-Schiff (PAS) staining protocol

Paraffin embedded sections (3µm) from the left lungs of individual mice were stained with PAS (Periodic acid-Schiff) and analyzed microscopically for tissue inflammation. Lung tissue inflammation was determined by the degree of cell infiltration around the basal membrane, which was graded on a scale from 0 to 3 (inflammation score). In brief, a value of 0 was assigned when no inflammation was detectable, a value of 1 was assigned for occasional cuffing with inflammatory cells, a value of 2 was assigned for most bronchi or vessels surrounded by a thin layer (one to five cells thick) of inflammatory cells, and a value of 3 was assigned when most bronchi or vessels were surrounded by a layor of more than five inflammatory cells. Per lung section, a mean inflammation score derived from counting of ten bronchi was determined.

2.2.7.5 Airway Hyperresponsiveness (AHR)

Following challenge, airway responsiveness to methacholine (MCh) (Sigma-Aldrich, Taufkirchen, Germany) was determined in individual mice using the Flexivent system (SCIREQ, Montreal, QC, Canada) in the laboratory of Dr. Ali Önder Yildirim (Helmholtz Zentrum München). Following anaesthesia with Ketanest (Inresa Arzneimittel GmbH, Freiburg, Germany) and Rompun (Bayer Health Care, Leverkusen, Germany) mice were paralyzed with Esmeron (N.V. Organon, Oss, Netherlands). The trachea was then intubated with a 1.2 mm tracheal cannula and the lungs were mechanically ventilated at a respiratory frequency of 150 breaths per min, a tidal volume of 10 ml/kg and a positive end-expiratory pressure of 3 ml H_2O. After exposing mice to aerosolized PBS to retrieve the baseline value, bronchoconstriction was induced by increasing the concentration of MCh (1,56, 3,12; 6,25; 12,5 and 25 mgl/ml). Resistance was recorded over 1 minute intervals using a standardized inhalation maneuver.

2.2.8 Preparation and stimulation of spleen and lung lymph node cells

Spleen and lung lymph nodes (LLN) were removed from sacrificed mice using scissors and tweezers under sterile conditions. Thereafter, single cell suspension were prepared and transferred to a 50 ml tube and centrifuged at 230 g for 10 min (4°C). The pellet was then resuspended in 5 ml ACT buffer and incubated for approximately 5 min at room temperature to lyse erythrocytes. Immediately after incubation, the cell suspensions were filtered through a 70 µm cell strainer into a new 50 ml tube and washed with PBS. After centrifugation at 230 g for 10 min (4°C), the supernatant was discarded and the cell pellet dissolved in the appropriate buffer or culture medium. Finally, the cells were counted and used for cell stimulation assays and flow cytometric analysis.

For asthma experiments isolated splenocytes and lung lymphnode cells were seeded in a total volume of 200 µl of complete medium per well of a round-bottom 96-well plate at a concentration of $2x10^5$ cells/ml. Cells were stimulated with OVA (10µg/ml), SEA (20µg/ml) or ragweed (10-20µg/ml). Combined αCD3 (1µg/ml) and αCD28 (1µg/ml) was used as a positive control and cells were incubated for 48 hours at 37°C. Culture supernatant (100-150 µl) was collected and stored at -20°C for cytokine measurement by ELISA (2.2.9.1).

To determine the percentage of change in Bcl-2 expression in the mouse system, $2.5x10^4$ sorted Treg cells ($CD4^+Foxp3^{GFP+}$) from spleens of Balb/c–DEREG mice were co-cultured with $7.5x10^4$ non-Treg cells ($CD4^+Foxp3^{GFP-}$). IL-10 (BD) (400U/ml) was added to the cultures on day 0 and day 3. On day 6 cells were harvested and analyzed for Bcl-2 expression MicroBead coated biotinylated anti-CD3 and anti-CD28 (Miltenyi Biotec, Germany) was added using a cell to bead ratio of 2:1. For *in vitro* IL-10 blocking assays $3x10^5$ splenocytes from female mice were cultured with $6x10^5$ irradiated (30 Gy) male splenocytes. Anti-mouse-IL-10 (10µg/ml) was added on day 0. On day 2 cells were harvested for analysis of Bcl-2 expression.

2.2.9 Analysis of cytokines and immunoglobulins

2.2.9.1 Cytokine detection

Ready-Set-Go® ELISAs (eBioscience) and Duo Set® ELISA kits (R&D) were performed according to the manufacturer's instructions. In brief, 96-well ELISA plates were coated with 50 µl/well capture antibody diluted (as indicated by the manufacturer) in coating buffer and incubated overnight at 4°C. Before blocking with 100 µl blocking buffer for 1 hour at room temperature, plates were washed thrice with washing buffer. After blocking,

plates were washed again three times with washing buffer. Samples and serial standard dilutions were prepared in blocking buffer. The plates were then incubated for at least 2 hours at room temperature. After three additional washing steps, 50 µl/well of biotinylated detection antibody diluted (as indicated by the manufacturer) in blocking buffer was applied and incubated for 1 hour at room temperature. Surplus detection antibodies were then washed away, before adding 50 µl/well of streptavidin-horseradish peroxidase (HRP) conjugate diluted (as indicated by the manufacturer) in blocking buffer. Afterwards, the plates were incubated for approximately 45 min at room temperature in the dark. Following this incubation, plates were washed thrice with washing buffer before adding 50 µl/well of substrate solution (TMB substrate). The reaction was stopped with 50 µl/well of stopping solution. Immediately after stopping the reaction, the plates were measured at 450 nm using the Sunrise^{TM} ELISA microplate reader. The cytokine concentration within the sample was then calculated according to the standard curve.
In vivo IL-10 serum level were measured by the use of the *in vivo IL-10 capture assay* (BD Pharmingen) according to the manufacturer's instruction.

2.2.9.2 Immunglobulin measurement

For measurement of toal IgE as well as OVA-specific IgE and IgG1 levels, blood samples were taken from each individual mouse and centrifuged at 1000g for 20min (4°C). Serum was collected and frozen at -20°C until use for immunoglobulin ELISA analysis. To measure OVA-specific IgE and IgG1 levels, 96-well ELISA plates were coated with 1 µg (IgE measurement) or 0.1 µg (IgG1 measurement) OVA grade V in 100 µl Ig-coating buffer per well. To measure total IgE level, 96-well ELISA plates were coated with 100 µl anti-mouse-IgE antibodie (10 µg/ml) diluted in PBS. Plates were incubated at 4°C overnight, respectively. Plates were then washed five times with washing buffer and unspecific bindings were blocked with 200 µl/well of Ig-blocking buffer for at least 2 hours at room temperature. The plates were again washed five times with washing buffer. Blood serum samples were diluted with Ig-blocking buffer (1:200 to 1:100,000 dilutions) and 50 µl/well were added to the coated plates. In addition, purified mouse IgE, mouse α-OVA IgE or α-OVA IgG1 antibody was used as specific standard and applied in a two-fold serial dilution in Ig-blocking buffer with the highest concentration of 500µg/ml (total IgE), 1000ng/ml (α-OVA IgE) or 250ng/ml (α-OVA IgG1), respectively. The plates were then incubated at 4°C overnight, allowing serum immunoglobulins to bind to coated anti-mouse-IgE or OVA. After 5 additional washing steps with washing buffer 50µl/well of α-mouse IgE-Biotin (1.25 µg/ml) or α-mouse IgG1-Biotin (1,25 µg/ml) was applied and incubated for at least 2 hours at room temperature. Surplus detection antibodies were then washed away, before 50 µl/well streptavidin-horseradish peroxidase (HRP) conjugate

from the Duo Set® ELISA kits was applied. Afterwards, the plates were again incubated for approximately 30 min at room temperature in the dark. Following the incubation, the plates were washed 5 times with washing buffer and 50 µl/well of BD OptEIA™ TMB substrate was added, before further incubating the plates in the dark. The reaction was stopped with 50 µl/well of stopping solution. Immediately after stopping the reaction, the plates were measured at 450 nm using the Sunrise™ ELISA microplate reader. The Ig concentration within the sample was then calculated according to the standard curve.

2.2.10 Flow Cytometry

2.2.10.1 Analysis of regulatory T cells

2.2.10.1.1 Staining of $CD4^+eGFP^+$ regulatory T cells in DEREG mice

As DEREG mice express eGFP under the control of the FoxP3 (Forckhead box 3) promotor, CD4+ regulatory T cells can be detected in those mice by eGFP fluorescence and staining of the CD4 surface molecule. Therefore individual blood samples were collected by cheek puncture for flow cytometric analysis. Fc receptors were blocked in 50 µl Fc block solution for 15-20min at 4°C and samples were subsequently washed by adding 100 µl FACS buffer followed by centrifugation at 230 g for 5 min (4°C). Immediately afterwards, cells were incubated in the dark for 20 min on ice with 50 µl FACS buffer containing flourophore-conjugated monoclonal Ab (Table 2). After incubation, cells were washed in 200 µl FACS buffer and resuspended in 100 µl FACS buffer before analysis on a FACSCalibur™ flow cytometer Data analysis was done with FlowJo (Tristar).

2.2.10.1.2 Staining of $CD4^+Foxp3^+$ regulatory T cells

To stain $CD4^+$ $Foxp3^+$ regulatory T cells in non-transgenic mice, surface CD4 molecules were stained with APC-conjugated anti-CD4 mAB (eBioscience) as described in section 2.2.10.1.1. Afterwards intracellular Foxp3 was stained with the Foxp3 staining buffer set from eBioscience according to the manufacturer's instructions. In brief cells were resuspended in 50 µl fixation/permeabilization working solution and incubated at 4°C overnight in the dark. Cells were then washed twice with permeabilization buffer and either FITC or PE conjugated Foxp3 antibodies (Table 2), diluted in permeabilization

buffer were added. Cells were incubated for 30 min at 4°C in the dark. After incubation, cells were washed twice with permeabilization buffer and resuspended in 100 µl FACS buffer prior to analysis on a FACSCalibur™ flow cytometer. Data analysis was done using FlowJo (Tristar).

2.2.10.1.3 Fluorescence Activated Cell Sorting

For flow cytometric sorting from splenocytes of DEREG Balb/c mice, $CD4^+Foxp3^{GFP+}$ and $CD4^+Foxp3^{GFP-}$ cells were sorted on a FACSAria™ cell sorter. Purity of the sorted populations was ≥ 95%.

2.2.10.2 Analysis of Bcl-2^+ T cells

CD4 surface antibody and intracellular staining for $Foxp3^+$ Treg cells and non-Treg cells was performed as described previously. PE conjugated anti-mouse Bcl-2 and FITC conjugated anti-mouse Foxp3 (Table 2) were applied simultaneously according to the protocol described in 2.2.10.1.2. Ethidium Monoazide Bromide (EMA) was used for dead cell exclusion in fixed mouse samples.

2.2.10.3 Multi-parameter staining panels to analyze peripheral blood leukocytes

The flow cytometric analysis of leukocyte populations in peripheral blood was performed in cooperation with Dr. Thure Adler (Immunology Screen/ German Mouse Clinic/ Helmholtz Zentrum München) based on multi-parameter staining panels, covering markers for B cells (CD19, B220), T cells (CD45, CD4, CD8, CD5, gamma-delta TCR), granulocytes and monocytes (GR-1, CD11b), NK cells (NKp46) and further subsets (CD44, CD25, Ly6C). Two different protocols were used: Peripheral blood underwent either 1) red blood cell lysis (NH4CL-Tris) 2) or not, and was incubated with Fc block and stained with fluorescence-conjugated antibodies (BD Biosciences) and propidium iodide. Cells were acquired from 96 well plates and measured with a three-laser 10-color flow cytometer (LSRII, BD Bioscience). For the analysis, dead cells were eliminated on the basis of their propidium iodide signal and events were gated for $CD45^+$ leukocytes and subsequently analyzed by software analysis (FlowJo) according to the gating strategy illustrated in Fig. 3-17.

2.2.11 Placenta preparation and microarray analysis

2.2.11.1 Cell cycle synchronization

Cell-cycle was synchronized by i.p. injection of PMSG (pregnant-mare-serum-gonadotropin) (5 I.U./mouse) on day 0 and HCG (human chorion gonadotropin) (5 I.U./mouse) on day 2.

2.2.11.2 Placenta isolation and stimulation *in vitro*

Whole placentas were removed from sacrificed mice on day 18 of pregnancy using scissors and tweezers under sterile conditions. Fetal weight and litter size was determined. Placental single cell suspension was prepared and transferred to a 50 ml tube and centrifuged at 230 g for 10 min (4°C). The pellet was then resuspended in 5 ml ACT buffer and incubated for approximately 5 min at room temperature to lyse erythrocytes. Immediately after incubation, the cell suspension was filtered through a 70 µm cell strainer into a new 50 ml tube and washed with PBS. After centrifugation at 230 g for 10 min (4°C), the supernatant was discarded and the cell pellet dissolved in culture medium. For stimulation experiments cells were seeded in a total volume of 200 µl complete medium per well of a round-bottom 96-well plate at a concentration of $1x10^5$ cells/ml. Cells were stimulated with SEA (20µg/ml) and incubated for 48 hours at 37°C. Culture supernatant (100-150 µl) was collected and stored at -20°C for cytokine measurement by ELISA.

2.2.11.3 Placental RNA isolation

Total cellular RNA was isolated from whole RNAlater-stabilized placentas using the RNA isolation kit from Qiagen (Hilden, Germany) according to the manufacturer's instruction. In brief, samples were first lysed and homogenized in 350 µl Buffer RLT using a rotor-stator homogenizer. The lysate was then centrifuged at full speed for 3 min and supernatant was transferred into a new microcentrifuge tube. One volume of 70 % ethanol was added to the cleared lysate to provide ideal binding conditions. The lysate was then loaded onto the RNeasy silica membrane and centrifuged at 8000 g for 15 sec. After discarding the flow-trough 700 µl Buffer RW1 were added to the RNeasy spin column and centrifugation at 8000 g for 15 sec was performed. The flow-trough was discarded and 500 µl Buffer RPE

were added to the RNEasy spin column. Centrifugation was performed at 8000 g for 2 min. Pure, concentrated RNA was eluted in 30 µl RNAse-free water by centrifugation at 8000 g for 1 min. The yield of RNA and its purity was determined using Nanodrop® 1000 (Kisker).

2.2.11.4 Microarray and pathway analyses

Gene expression profiling was performed in cooperation with the Group of Expression Core Facility (Prof. Dr. Thorsten Buch, MIH) on Affymetrix Mouse Gene ST 1.0 high-density oligonucleotide arrays. Total cellular RNA was isolated from whole placentas as decribed in section 2.2.11.3 and further on labeled, fragmented, and hybridized to the arrays using the WT Expression Kit (Affymetrix). Raw data analysis was performed with R and Bioconductor [193]. Arrays were assessed for quality and RMA-normalized [194]. Intensity and variance filter were applied. A linear model was fitted to the expression data for each probe and the estimated coefficients given the set of contrasts were computed using Limma. Genes shown in all venn diagrams were clustered hierarchically and displayed in a heatmap. Pathway analyses were generated through the use of IPA (Ingenuity® Systems, www.ingenuity.com) for species: mouse, tissue: placenta, log ratio Cutoff: 1, p-value Cutoff: 0.06.

2.2.12 Analysis of placental gene expression by RT-qPCR

2.2.12.1 cDNA synthesis

Total cellular RNA was isolated from whole placentas using RNA isolation kit from Qiagen (Hilden, Germany). For cDNA synthesis with removal of genomic DNA contamination the QuantiTect Reverse Transcription Kit from Qiagen (Hilden, Germany) was used according to the manufacturer's recommendation. In brief, for genomic DNA elimination reaction 2 µl gDNA Wipeout Buffer and up to 1 µg template RNA were mixed with RNAse-free water to a final volume of 14 µl per reaction. Reaction components were incubated for 2 min at 42°C. Reverse-transcription reaction components were then added, including 1 µl Quantiscript Reverse Transcriptase, 4 µl Quantiscript RT Buffer and 1 µl RT Primer Mix. Reaction components including RNA template were incubated for 15 min at 42°C. To inactivate Quantiscript Reverse Transcriptase, reaction components were incubated for 3 min at 70°C.

2.2.12.2 RT-qPCR running protocol

A qPCR reaction mix was prepared as follows:

qPCR-Mix	
LightCycler® 480 Probes Master	10µl
forward primer (10µM)	1.0µl
reverse primer (10µM)	1.0µl
Probe	1.0µl
target cDNA	1.0µl
DEPC H_2O	6.0µl

As negative control, 1 µl bidest. water was added instead of target cDNA; The PCR was performed using LightCycler®480 (Roche) with the following cycle settings:

	Temperature	Time	
Hot Start	95°C	5min	
Denaturation	95°C	10s	
Annealing/Extension	58°C	30s	45 cycles
Measurement of fluorescence			
Cooling	45°C	15s	

2.2.13 In vitro fertilization (IVF)

Th IVF core facility of the MIH performed fertilization of mouse oocytes collected from superovulated females, incubated those embryos overnight, and performed embryo transfer surgery of fertilized 2-cell stage embryos into recipient psuedopregnant females. Here, the round of in vitro fertilization involved superovulation with PMSG and HCG (2.2.11.1) of eight acute infected and eight uninfected BALB/c females and fertilizing the collected oocytes with fresh sperm of one uninfected BALB/c male as depicted below. The resulting 2-cell stage embryos were then collected and approximately 80 embryos were transferred per mouse into four uninfected psuedopregnant recipient BALB/c females per group. Offspring from each group were then tested in the model of allergic airway inflammation at the age of 6 weeks (2.2.6.2.2).

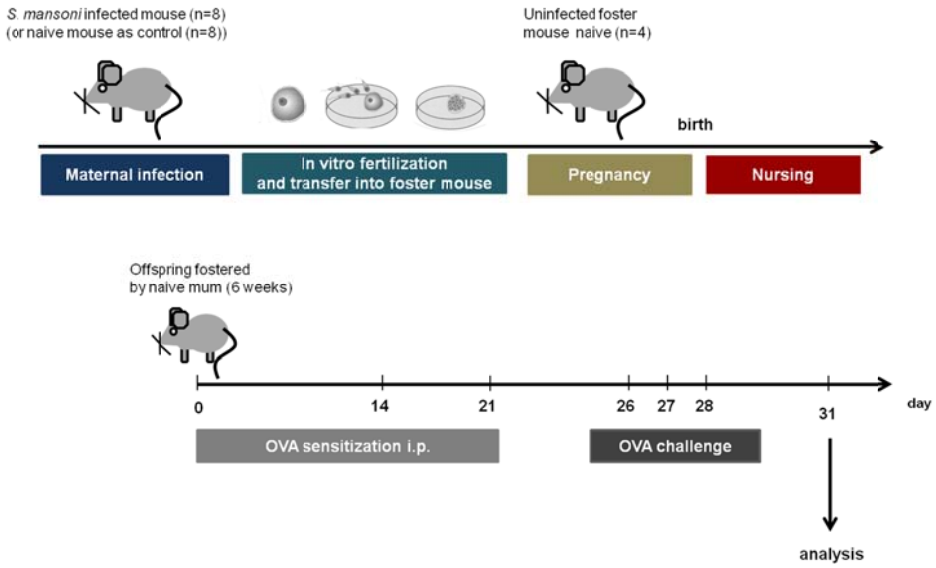


Fig. 2-4: Protocol for in vitro fertilization of eggs derived from *S. mansoni* infected or naïve mice and OVA- induced allergic asthma in offspring

2.2.14 Statistics

Statistical differences were analyzed by either ANOVA or students t-test using GraphPad Prism software (San Diego, CA, USA).

3 Results

3.1 Immune response, detection and monitoring of *S. mansoni* infection

3.1.1 Immune response during *S. mansoni* infection in mice

The dynamics of immune responses in ongoing schistosome infection is well established in the literature [11], but was re-evaluated specifically in BALB/c mice for this study, since differences in mouse and helminth strains as well as animal housing conditions have to be considered. As shown in Fig. 3-1, SEA-specific immune responses can be detected after the first 4 weeks and were determined until the 29th week after infection. Initial IFN-γ release (designated as “Th1 phase”) from SEA-stimulated splenocytes was successively replaced by Th2 cytokines such as IL-4, IL-5 and IL-10 (designated as “Th2 phase”). Following the 16th week of infection IL-10 is the predominant cytokine detected, indicating a strong immunosuppressive phase (designated as “Reg phase”)

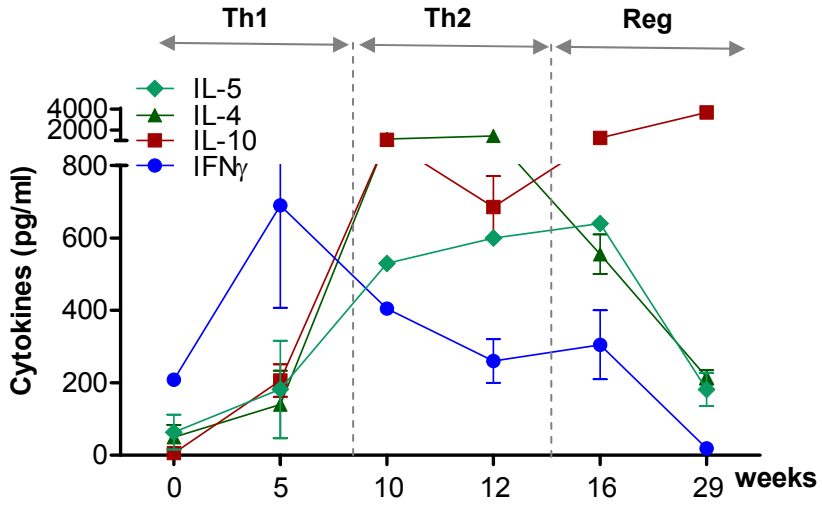


Fig. 3-1: Immune phases during schistosome infection.
Splenocytes from *S. mansoni* infected mice were restimulated with SEA (10 µg/ml) at 5, 10, 12, 16 and 29 weeks post-infection. IL-5, IL-4, IL-10 and IFN-γ were measured in the supernatant 48 hrs after culture by ELISA. A minimum of three mice (pregnant and not pregnant) were analyzed at each time point. Results are shown as mean ± SEM.

3.1.2 Detection and quantification of *S. mansoni* DNA by qPCR in stool and serum

Precise evaluation of *S. mansoni* infection during the experimental phase is mandatory to guarantee robustness and comparability between experiments. Besides the endpoint

analysis of parasitological burden via visual macroscopic and microscopic methods, including degree of infection (DOI), potassium hydroxide (KOH) digestion (2.2.3.3), sodium acetate-acetic acid-formalin (SAF) enrichment, and worm burden, a method, which allows detection, quantification and monitoring of ongoing infections is required. Therefore, the aim was to establish a *S. mansoni*-specific real time Polymerase Chain Reaction (qPCR) as reliable method to detect *S. mansoni* genomic DNA from stool and serum of infected mice for laboratory use. Primers and fluorescently labeled probe were designed to amplify a 86 bp long fragment of the 121 bp tandem repeat unit [190] of *S. mansoni*. qPCR was performed using a Roche LightCycler® 480 and qPCR products were run on an agarose gel. To determine the specificity, sensitivity and stability of the assay DNA was extracted from *S. mansoni* eggs as well as from stool samples and sera from uninfected and infected mice. In addition, DNA was isolated from stool samples that were additionally spiked with 2000 *S. mansoni* eggs. Control samples were included, which either lack *S. mansoni* DNA or the probe. Samples were assigned to the numbers 1-13 as follows:

1: negative contro H_2O
2: stool from uninfected mice without probe
3: stool from uninfected mice with probe
4: serum from uninfected mice without probe
5: serum from uninfected mice with probe
6: *S. mansoni* eggs without probe
7: *S. mansoni* eggs with probe
8: stool from infected mice (validated by DOI and KOH digestion of liver and intestine)
9: stool from infected mice, additionally spiked with *S. mansoni* eggs
10.-13: serum from infected mice (validated by DOI and KOH digestion of liver and intestine)

As a result, the negative control samples 1, 2, 4, and 6 were not amplified during the qPCR, since the samples did not contain the probe (samples 2, 4, 6) or did not contain any DNA (1). DNA from stool and serum from uninfected mice (samples 3, 5) was amplified to a small degree, due to unspecific binding of the probe to the host DNA. Therefore, a cut off at C_t-value 34 was chosen to minimize false-positive results. DNA from *S. mansoni* eggs (sample 7) showed the strongest signal. The sensitivity of this method can be seen by comparison of C_t-values from DNA samples isolated from stool of infected mice, that were either untreated (sample 8) or additionally spiked with 2000 *S. mansoni* eggs (sample 9). However, total DNA that was isolated from the serum of

infected mice could only be detected in one mouse (sample 11) that had a high degree of infection (DOI ≥ 3). Other samples containing serum from infected mice with a DOI below 3 (samples 10,12,13) showed unspecific amplification, with C_t-Values beyond the cut off value. The agarose gel showed bands of the expected size of 86 bp for samples 6, 7, 8, 9, 11 containing DNA from *S.mansoni* eggs, DNA isolated from stool from infected mice, untreated or spiked with eggs, and DNA from serum of the infected mouse with a DOI ≥ 3. In addition, infection of BALB/c mice was monitored weekly by Annika Volmari, a bachelor student in the lab, who isolated DNA from individual stool samples and performed specific qPCR to follow the course of infection and to determine the ideal timepoint for detection in infected mice. As a result, at week 7 after infection, all stool samples of infected mice had a C_t-values below 34, which correlated with egg production and excretion via feces during infection [195].

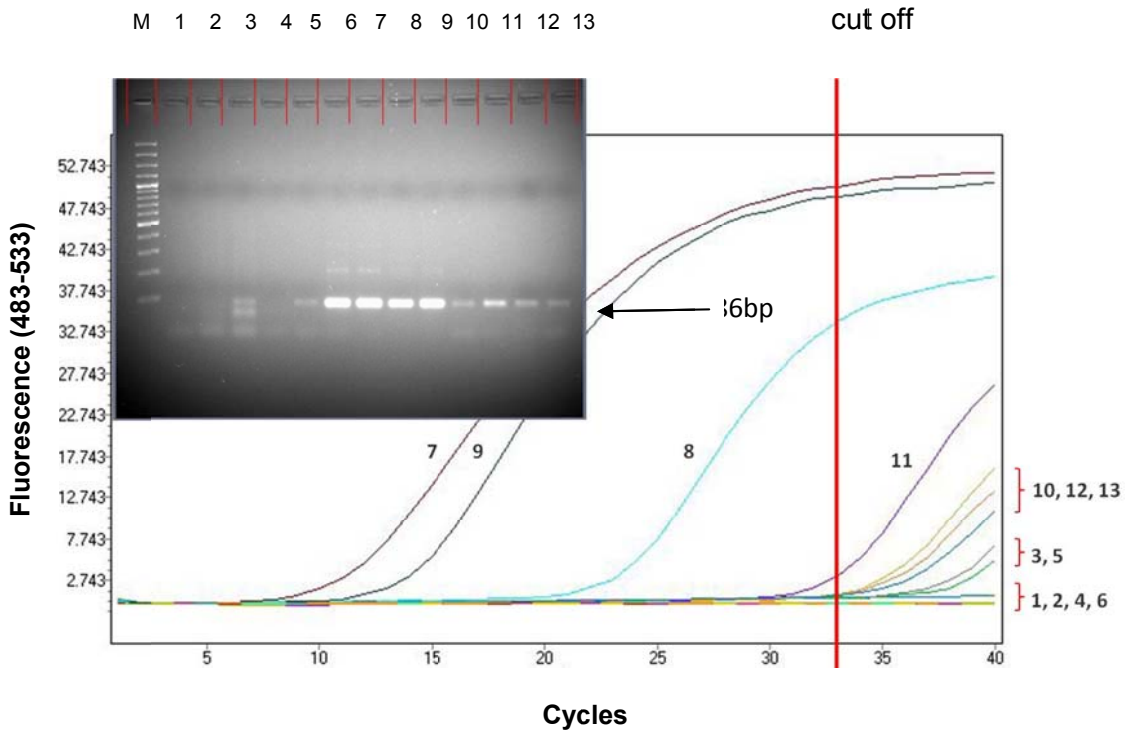


Fig. 3-2: Establishment of *S. mansoni* specific real-time PCR.
DNA was isolated from schistosomal eggs, stool and serum from infected and uninfected mice. *S.mansoni* specific PCR was performed using the following samples: 1. negative H_2O control, 2. stool from uninfected mice without probe, 3. stool from uninfected mice with probe, 4. serum from uninfected mice without probe, 5. serum from uninfected mice with probe, 6. *S. mansoni* eggs without probe, 7. *S. mansoni* eggs with probe, 8. stool from infected mice (validated by DOI and KOH digestion of liver and intestine), 9. stool from infected mice, additionally spiked with *S. mansoni* eggs, 10.-13. serum from infected mice (validated by DOI and KOH digestion of liver and intestine). Results show amplification curves from the light cycler and the corresponding agarose gel of qPCR products (M = 100bp DNA ladder).

In order to reliably determine the degree of infection based on the number of eggs, that are secreted within feces, the *S. mansoni* specific tandem repeat unit was cloned into a plasmid, which serves as a standard. Therefore, the tandem repeat unit was amplified,

purified and cloned as described above (2.2.4.2). The obtained *S. mansoni* plasmid DNA (49.9 ng/µl) was diluted in three dilution series (1:2, 1:5, 1:10) with 10 dilution steps for each series and analyzed by qPCR to determine the corresponding C_t-Values for the known plasmid concentrations (Table 4). The values were correlated to receive a standard curve (Fig. 3-3) for the absolute quantification of the amount of schistosome DNA in stool and serum samples. The exact overlay of the standard curves obtained from the three dilution series indicated the specificity and robustness of this method. Furthermore, DNA could be detected with concentrations as low as 4.49 fg, showing a high sensitivity of the procedure.

DNA concentration [ng/µl]	C_t-Value	DNA concentration [ng/µl]	C_t-Value	DNA concentration [ng/µl]	C_t-Value
1:2 →		1:5 →		1:10	
49.4	8.5	49.9	8.63	49.9	8.49
24.7	9.51	9.88	10.59	4.99	11.81
6.18	11.66	1.98	12.91	0.499	15.78
3.09	10.83	0.4	15.18	0.0499	18.69
1.54	13.64	0.08	17.52	0.00499	22.16
0.77	14.69	0.016	20.28	0.000499	25.56
0.39	15.67	0.0032	22.62	0.0000499	28.9
0.19	16.09	0.00063	24.78	0.00000499	32.04
0.1	17.76	0.00013	27.71	0.000000499	35
0.05	18.89	0.000025	29.88	4.99E-08	35
0.02	19.82	0.0000051	32.73	4.99E-09	35

Table 4: C_t-Values of dilution series of cloned plasmid containing *S. mansoni* DNA.
C_t-Values for three dilution series of *S. mansoni* plasmid DNA (1:2, 1:5, 1:10) were determined by qPCR.

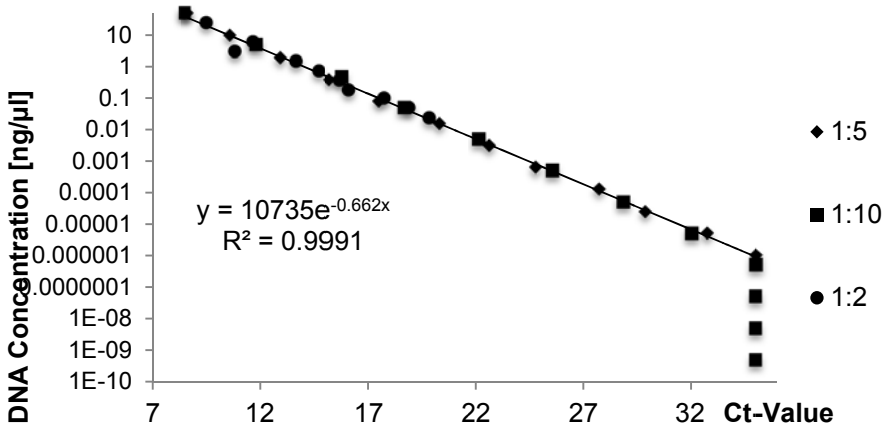


Fig. 3-3: Absolute quantification of *S. mansoni* DNA.
Standard curve, correlating C_t-Values from qPCR and three dilution series (1:2, 1:5, 1:10) of DNA concentrations of a plasmid, containing cloned *S.mansoni* tandem repeat unit.

3.2 Protection against allergic airway inflammation in *S. mansoni* infected mice

3.2.1 Ragweed-induced allergic airway inflammation

3.2.1.1 No changes in serum IgE-level

In the literature, *S. mansoni* infection is closely related to reduced allergic responses in human and mice. In the latter, OVA derived from chicken egg has been used frequently as an unrelated allergen. In this study, a mouse model of ragweed induced allergic airway inflammation has been applied to investigate the response against an environmentally relevant allergen during an ongoing *S. mansoni* infection. Infected and uninfected mice were either treated with PBS (control mice) or ragweed (asthmatic mice) according the protocol for ragweed-induced allergic airway inflammation and asthma parameter including lung inflammation and OVA-specific cytokine responses were analyzed. As shown in Fig. 3-4, ragweed-treated uninfected mice showed elevated serum IgE level after ragweed challenge, whereas in PBS-treated, uninfected mice only low levels of IgE were detectable after aerosolic challenge with ragweed. As expected, infected PBS-treated mice showed significant higher serum IgE levels, compared to uninfected groups, which is explained by the highly polarized Th2 immunity during schistosomiasis. Interestingly, ragweed-sensitization and challenge of infected mice did not lead to additional elevation in serum IgE, compared to PBS-treated, infected mice, suggesting a relative small rise of ragweed-specific IgE.

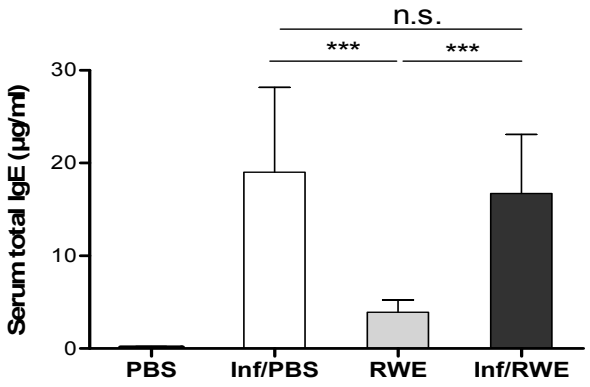


Fig. 3-4: Serum IgE level are not affected by ragweed-sensitization in infected mice.
Infected and uninfected BALB/c mice were sensitized against ragweed or treated with PBS, followed by ragweed challenge according to the protocol for ragweed-induced allergic airway inflammation (uninfected, PBS n=8; infected, PBS n=6, uninfected, ragweed n=7; infected, ragweed n=10). Mice were sacrificed two days after challenge and total serum IgE level were measured by ELISA. Data is shown as mean ± SEM. p-values were determined by Student's *t* test and asterisks show significant differences between groups (***p<0.001, **p<0.01, *p<0.05).

3.2.1.2 Diminished cell infiltration and reduced lung pathology in infected mice

Besides the production of allergen-specific IgE serum level, allergic airway inflammation is further characterized by leukocyte infiltration within the lung. After induction of ragweed-induced allergic airway inflammation, total leukocyte (Fig. 3-5a) and eosinophil (Fig. 3-5b) infiltration into the BAL were significantly reduced in ragweed-treated, *S.mansoni* infected mice compared to ragweed-treated, uninfected mice. Furthermore, total lung inflammation was defined as the average of the peribronchial and perivascular inflammation scores and goblet cell formation has been investigated. As a result, lung inflammation (Fig. 3-5c) and mucus-producing goblet cell formation (Fig. 3-5d) were significantly decreased in ragweed-treated, infected mice compared to ragweed-treated uninfected mice. Fig. 3-5e depicts representative lung cross sections showing reduced lung pathology in infected mice compared to uninfected mice upon asthma induction.

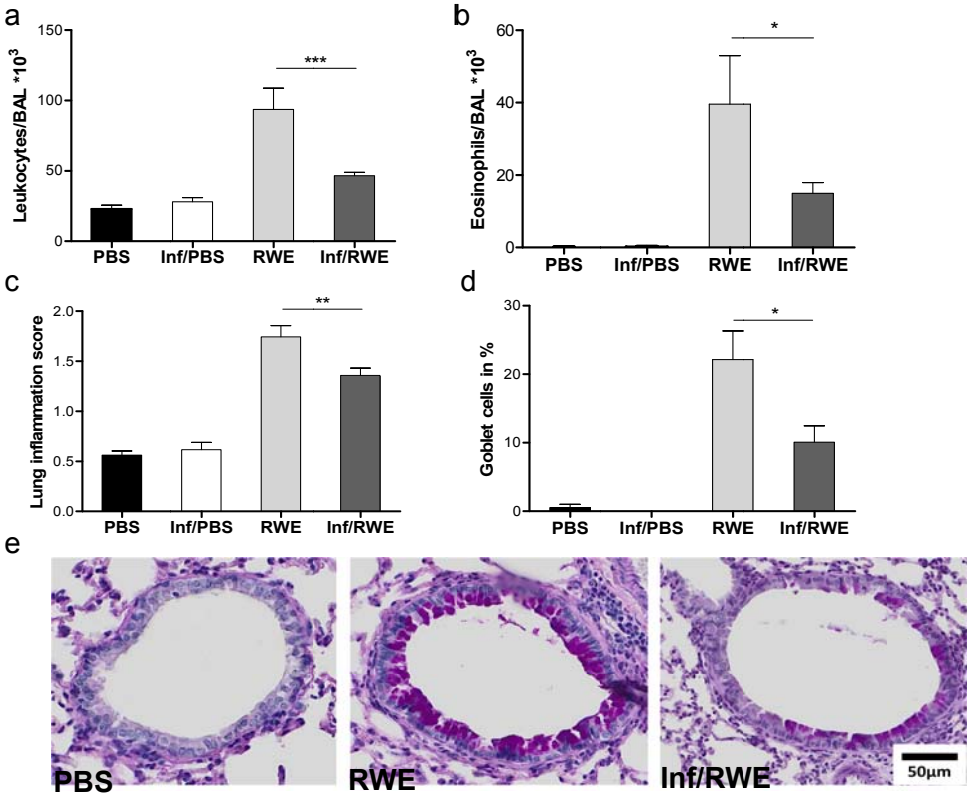


Fig. 3-5: Lung pathology and cell infiltration are reduced in infected mice upon asthma induction
Infected and uninfected BALB/c mice were sensitized against ragweed or treated with PBS, followed by ragweed challenge According to the protocol for ragweed-induced allergic airway inflammation (uninfected, PBS n=8; infected, PBS n=6, uninfected, ragweed n=7; infected, ragweed n=10). Mice were sacrificed on day 20. (a) Total leukocyte number in bronchoalveolar lavage. (b) Total eosinophil number bronchoalveolar lavage. (c) Quantification of lung inflammation. (d) Mucus-producing goblet cells in lung sections. (e) Representative lung tissue cross sections stained with PAS from PBS-treated, as well as infected and uninfected ragweed-treated mice. Data is shown as mean ± SEM. p-values were determined by Student's *t* test. Asterisks show significant differences between groups (***p<0.001, **p<0.01, *p<0.05).

3.2.1.3 Altered cytokine responses during ragweed-induced allergic airway inflammation

Th2 polarization and increased concentrations of Th2 type inflammatory cytokines, such as IL-4 and IL-5 play a major role in the pathogenesis of allergic airway inflammation, whereas Th1 cytokines, such as IFN-γ are decreased in asthmatic patients. To analyze the quality of the cytokine response in goups of infected and uninfected mice, single cell suspension of splenocytes and lung lymph node (LLN) cells were cultured *in vitro* and stimulated with different concentrations of RWE, SEA and αCD3. IFN-γ, IL-4 and IL-10 were measured within culture supernatant by ELISA. With regard to RWE stimulation, IFN-γ production was significantly reduced in splenocytes from infected, ragweed-treated mice (Fig. 3-6a). In contrast, IFN-γ release from LLN cells was significantly elevated in this group (Fig. 3-6b). Comparing ragweed-treated groups, IL-4 production was reduced in splenocytes (Fig. 3-6c), but not in LLN cells (Fig. 3-6d) from infected mice in comparison to uninfected mice upon RWE stimulation. Furthermore, restimulation with RWE led to significant elevated IL-10 level in LLN cells from infected, ragweed-treated mice (Fig. 3-6d). No differences in IL-10 level were measured in splenocytes upon RWE stimulation (Fig. 3-6e). With regard to SEA stimulation, IFN-γ, IL-4 and IL-10 level were significantly elevated in restimulated splenocytes and LLN cells from infected mice compared to uninfected mice, respectively (Fig. 3-6f). With a focus on αCD3 responses, no significant differences were observed between all groups with regard to IFN-γ, IL-4 and IL-10 production.

In summary, these data show that murine infection with *S. mansoni* protects against allergic airway inflammation using ragweed as a clinically relevant allergen. Thereby, allergen-induced Th2 cytokine production was significantly reduced in the spleen of infected animals, whereas counter-balancing IFN-γ and regulatory IL-10 level were elevated in the local lung lymphnodes of these mice.

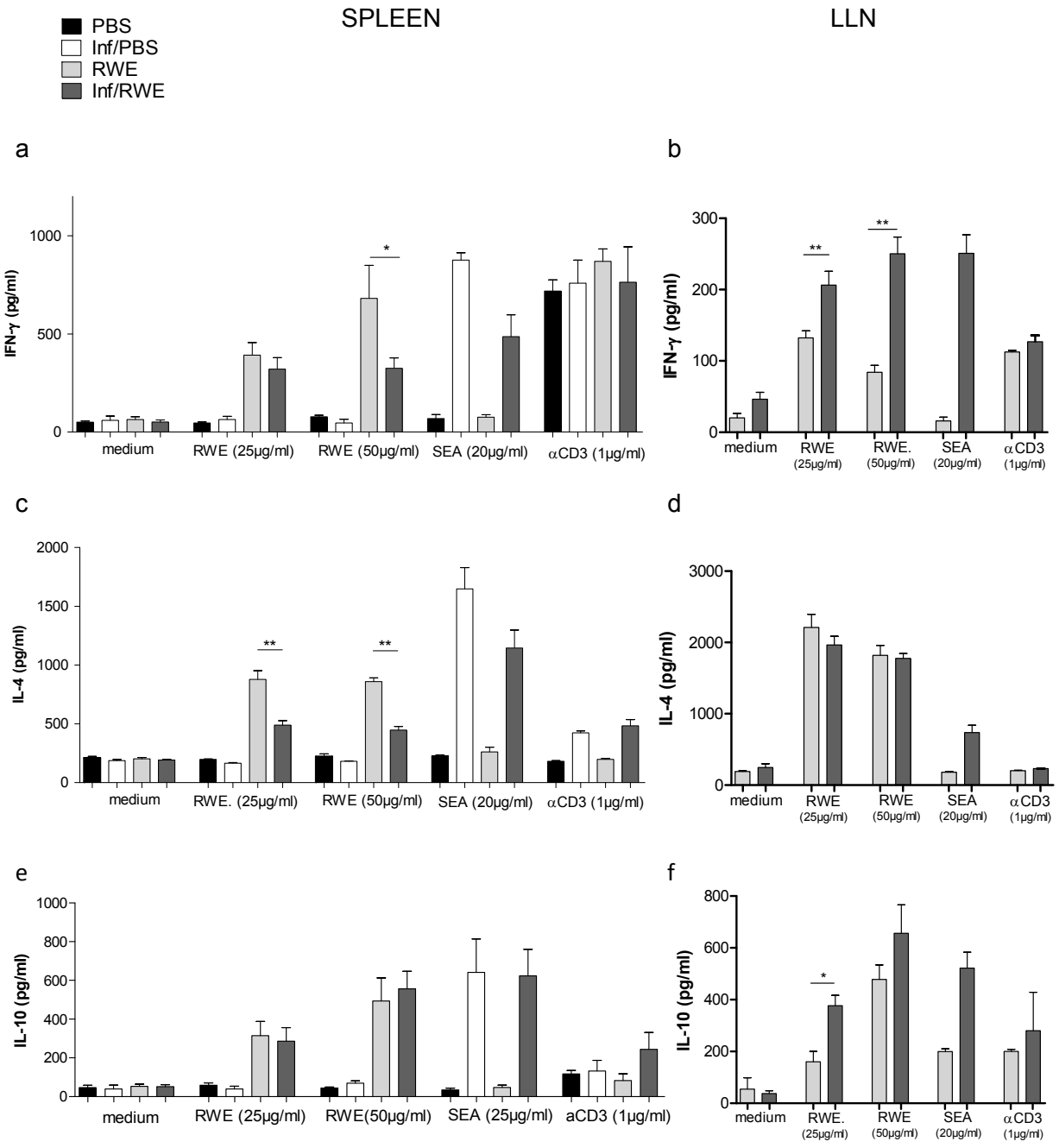


Fig. 3-6: Ragweed-specific cytokine responses are altered in infected mice upon asthma induction.

Infected and uninfected BALB/c mice were sensitized against ragweed or treated with PBS, followed by ragweed challenge According to the protocol for ragweed-induced allergic airway inflammation (uninfected, PBS n=8; infected, PBS n=6, uninfected, ragweed n=7; infected, ragweed n=10). Mice were sacrificed on day 20. Splenocytes and lung lymphnode (LLN) cells were cultivated and stimulated with ragweed or αCD3/αCD28 for 48 hr. IFN-γ, IL-4, and IL-10 were measured in supernatant by ELISA. IFN-γ level in (a) splenocytes and (b) LLN cells. IL-4 level in (c) splenocytes and (d) LLN cells. IL-10 level in (e) splenocytes and (f) LLN cells. Data is shown as mean ± SEM. p-values were determined by Student's *t* test and asterisks show significant differences between ragweed stimulated samples ***p<0.001, **p<0.01, *p<0.05.

3.2.2 Specific role of Treg cells in *S. mansoni*-mediated suppression of asthma

To address whether *S. mansoni* mediated protection of allergic airway inflammation is mediated by Treg cells, DEREG mice, which allow the specific depletion of $egfp^{+}Foxp3^{+}$ cells (Fig. 3-8a) at specified time-points through the administration of diphtheria toxin (DT) were infected with *S.mansoni* cercariae [196]. In initial experiments, the use of DEREG mice on a BALB/c background has emerged as insufficient due to their high sensitivity to DT application and schistosome infection, which resulted in high mortality rates during the experiments. Therefore, the experiments were performed with DEREG mice on a C57BL/6 background. Furthermore, OVA instead of RWE has been used as unrelated allergen to induce a robust, allergic pulmonary inflammation. As depicted in 2.2.6.2.1, Treg cells were depleted in groups of infected and non-infected C57BL/6 DEREG mice during sensitization. As observed before in the ragweed model, infected OVA-treated, not depleted groups (Inf/OVA) had significantly lower levels of leukocyte (Fig. 3-7a) and eosinophil (Fig. 3-7b) infiltration into the lung when compared to uninfected OVA-treated, not depleted groups (OVA). However, depletion of Treg cells in infected groups (Inf/OVA^{DT}) prevented this suppressive effect and levels were higher than in the control OVA groups (Fig. 3-7 a-b). This was also observed when measuring the levels of inflammation in the lung (Fig. 3-7 c-d). In association with the current literature [88], non-infected OVA groups depleted of Treg cells also showed elevated cellular leukocyte and eosinophil infiltration levels (Fig. 3-7a-b). Immunologically, both Treg cell depleted groups showed significantly higher levels of OVA-specific IgE in the sera (Fig. 3-7e) and OVA-specific IL-5 upon recall of splenocytes (Fig. 3-7f). Interestingly, schistosome-specific responses in the infected groups were also significantly increased in the Inf/OVA^{DT} groups (Fig. 3-7g). Moreover, these mice showed lower egg burden in the liver (Fig. 3-7h).

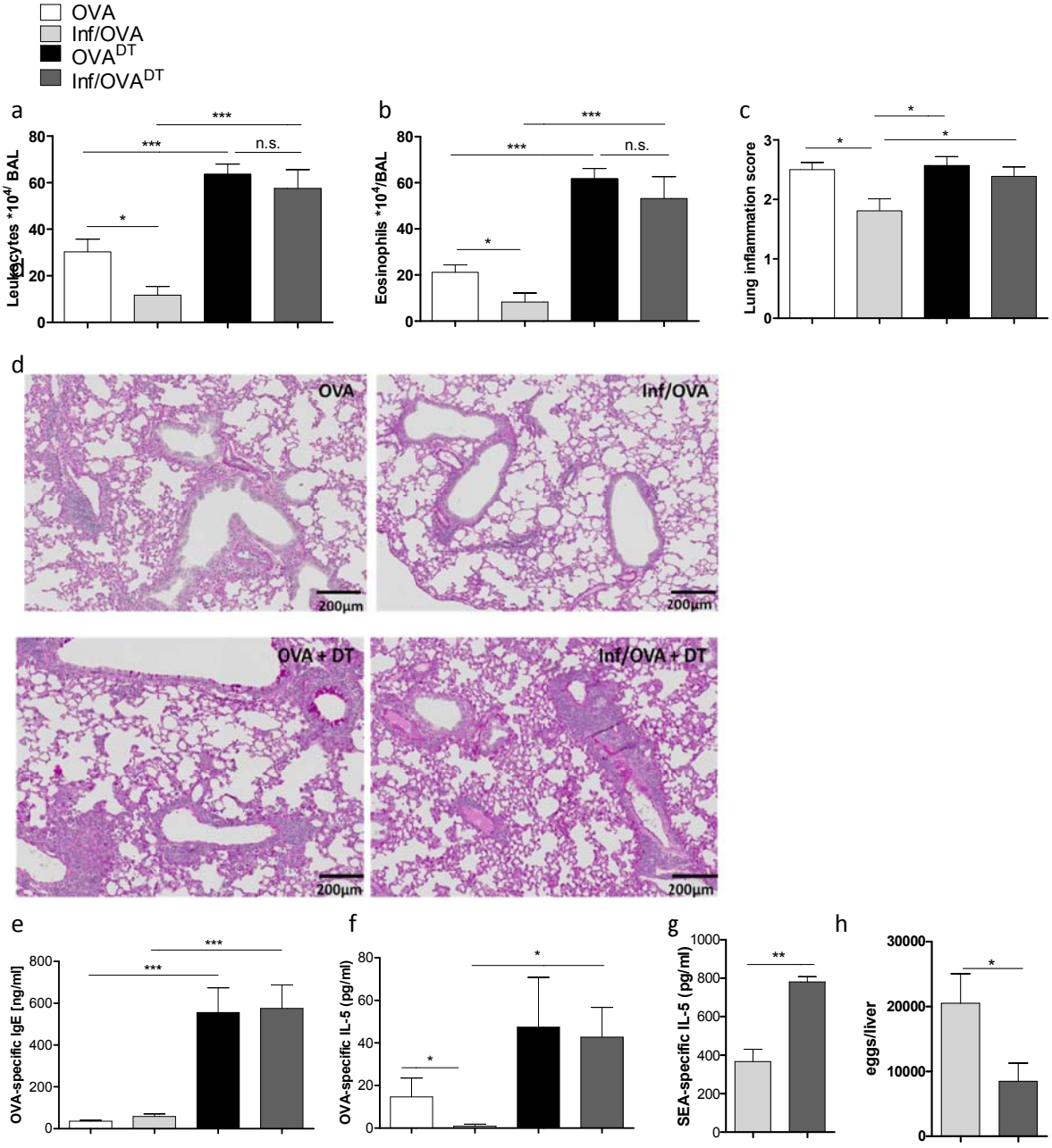


Fig. 3-7: Depletion of Treg cells during sensitization reverts schistosome-mediated dampening of allergic airway inflammation.
As depicted in Figure 2-2, groups of C57BL/6 DEREG mice were infected with *S. mansoni*. On the 9th and 10th week of infection mice were sensitized i.p. with OVA in the presence of alum. Prior to sensitization, Treg were depleted by the administration of DT. During the 12th week of infection, mice were challenged and assessed for the number of leukocytes (a) and eosinophils (b) in the BAL. Levels of inflammation were also scored using lung sections stained with PAS (d). OVA-specific IgE levels were measured in the sera of individual mice via ELISA (e). OVA-specific (f) and SEA (g) IL-5 production was measured following stimulation of spleen cells ($2x10^5$) with 10 µg/ml OVA and 25 µg/ml SEA 72 hours after culture in supernatant by ELISA. Egg burden was measured in livers of individually infected mice (h). Bars show data from one of two similar experiments (OVA, n=7; OVA^{DT} , n=7; Inf/OVA^{DT}, n=5; Inf/OVA, n =8; PBS^{DT}, n=3). Asterisks show statistical differences (Student's t test) between the groups indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).

During the experiments, depletion of Treg cells was controlled by FACS analysis of $CD4^+egfp^+$ T cells in peripheral blood. Fig. 3-8a shows a representative comparison of $CD4^+egfp^+$ T cells in uninfected (left) and infected (right) mice on the day before first depletion and three days thereafter. As shown in the bottom panel of flow cytometry images, no $egfp^+$ cells were visible after depletion. Levels were measured again following asthma induction (Fig. 3-8b). There, the percentage of $Foxp3^+$ Treg cells in infected-depleted mice was approximately 50% lower than in uninfected depleted mice (8.7% vs 3.57%). This was observed in all experimental mice (Fig. 3-8c) and indicates that the re-establishment of Treg cells in infected mice is slower than in non-infected controls and provides a possible explanation as to why infected mice are no longer suppressed from allergic airway inflammation.

In summary, these data show that depletion of Treg cells during OVA-sensitization led to elevated allergic airway inflammation in infected and uninfected animals. Furthermore, a delayed reconstitution of expanding Treg cells was noted in infected OVA-treated mice compared to uninfected mice after Treg cell depletion. In addition, schistosome-specific immune responses were elevated in infected Treg cell depleted mice, whereas egg burden was significantly reduced.

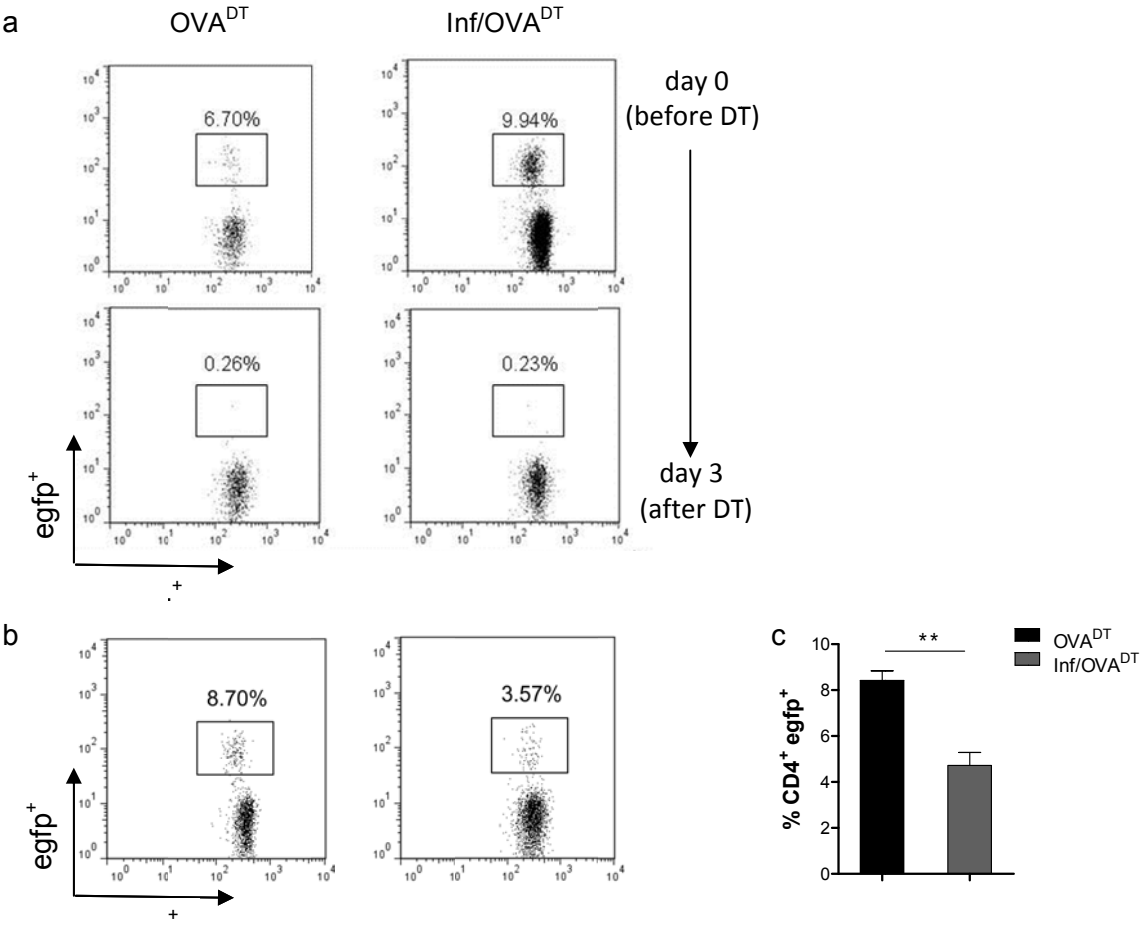


Fig. 3-8: Effective Treg cell depletion but reduced recovery in infected DEREG mice
In (a) the efficacy of Treg depletion was controlled by analyzing the percentage of cells in peripheral blood by flow cytometry. In brief, prior to depletion and three days after DT injections the percentage of $CD4^+egfp^+$ T cells was observed in naive DEREG mice (left) and *S. mansoni* infected DEREG mice (right). Upon asthma induction, the percentage of Treg was determined again in peripheral blood (b). Representative dot plot on the left depicts the levels of $CD4^+egfp^+$ T cells in a OVA^{DT} mouse and the right image those observed in a Inf/OVA^{DT} mouse. Bars represent the mean + SEM of $CD4^+egfp^+$ T cells recovered from 4-5 mice per group (c). Percentages were measured by flow cytometry. Asterisks show statistical differences (Student's t test) between the groups indicated by the brackets (**p<0.01).

3.3 Effects of maternal schistosomiasis

3.3.1 Fetal weight and pregnancy rate

It is known that experimental schistosome infection can impact on maternal and fetal health status during pregnancy and affect abortion rate, litter size and fetus weight [133]. However, there is no study available that focuses on the impact of the distinct maternal immune phases in this regard. Thus, on the basis of the SEA-specific immune responses that were determined as shown in Fig. 3-2, the timepoints of mating were set according to the designated Th1, Th2 and Reg phase, to investigate phase-specific effects on pregnancy outcome. In detail, to analyze effects of maternal Th1 phase females were mated at week 3,5 post infection, for maternal Th2 phase at week 11 post infection and for maternal Reg phase at week 16 post infection. In comparison to uninfected mice, the pregnancy rate of 64.6% was significantly decreased to 42.1% in the Th1 phase of infection, but remained comparable in the Th2 phase with a rate of 64.0% and in the Reg phase with 63.0%. Fetal weight remained comparable between offspring born to uninfected, Th1 and Reg phase mothers but was significantly reduced in offspring from Th2 phase dams (Fig. 3-9a). Litter size was not affected significantly, even though there was a trend towards a decreased number of offspring per litter born from Th1 and Reg phase dams (Fig. 3-9b).

In summary, maternal infection with *S. mansoni* affected phase-dependently pregnancy rate and fetal birth weight, leading to a reduced pregnancy rate in Th1 phase pregnancies and a reduced birth weight in offspring from Th2 phase pregnancies.

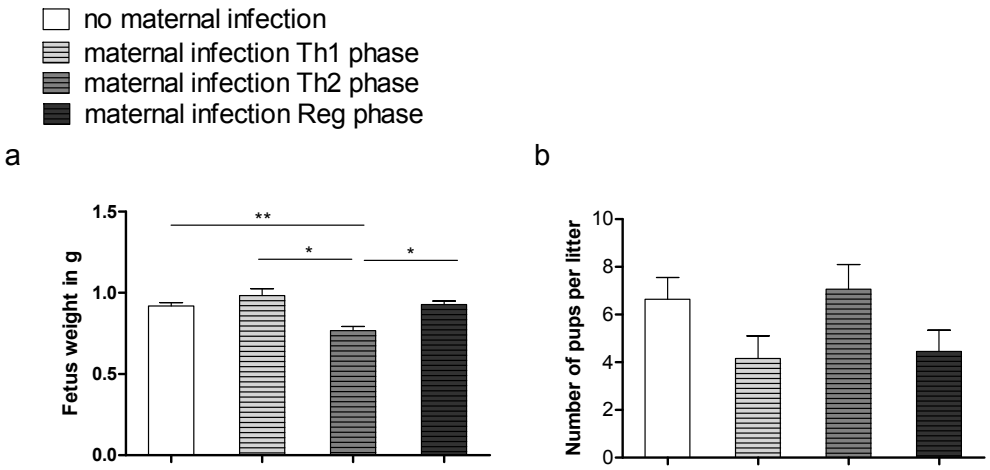


Fig. 3-9: Immune phases of schistosome infection influence pregnancy and fetal weight.
(a) Weight of fetuses from litters with 7-10 offspring at d18 of pregnancy from uninfected mothers (n= 46), mothers mated at week 3,5 (n=19), week 11 (n=46) and week 16 (n=23) of *S. mansoni* infection. (b) Litter size from pregnancies of uninfected mothers (n= 14), mothers mated at week 5 (n=10), week 11 (n=16) and week 16 of *S. mansoni* infection (n=11). Data is shown as mean ± SEM and results are obtained from at least 2 independent experiments. Asterisks show statistical differences (Student's t test) between the groups indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).

3.3.2 Protection and aggravation of allergic asthma in offspring from infected dams

Besides effects on asthma susceptibility in the infected host, this study further focused on the question whether predominant exposure to different immune phases during maternal helminth infection could affect the offspring's susceptibility to allergies. For detailed analysis of the effects of maternal schistosomiasis on the development and outcome of allergic airway inflammation in the progeny, female BALB/c mice were infected with *S.mansoni* cercariae and mated according to the designated Th1, Th2 and Reg phase. Six to eight weeks old offspring were then tested in the model for allergic airway inflammation, using OVA as the allergen (2.2.6.2.2.2). Interestingly, strong differences within the offspring's immune responses, ranging from protection to enhancement of allergic disease, depending on the time of maternal mating were observed. In detail, offspring from mothers mated in the Th1 phase, as well as in the Reg phase of infection showed significantly reduced leukocyte (Fig. 3-10a,c) and eosinophil (Fig. 3-10d,f) numbers in the bronchoalveolar lavage after OVA-treatment, whereas cell infiltration in lungs from offspring from Th2 mated dams was strongly elevated compared to OVA-treated offspring from uninfected mothers, respectively (Fig. 3-10b,e). Local concentration of IL-13, which is known to induce airway eosinophilia and mucus production, was not

affected in the BAL of offspring from Th1 dams (Fig. 3-10g). However, IL-13 was slightly elevated in offspring from Th2 (Fig. 3-10h), but only by trend lower in offspring from mothers mated during the immunosuppressive phase of infection (Fig. 3-10i). Other cytokines (IL-10, IL-5 and IL-4 and IFN-γ) were measured in the BAL but were below the detection limit in all groups.

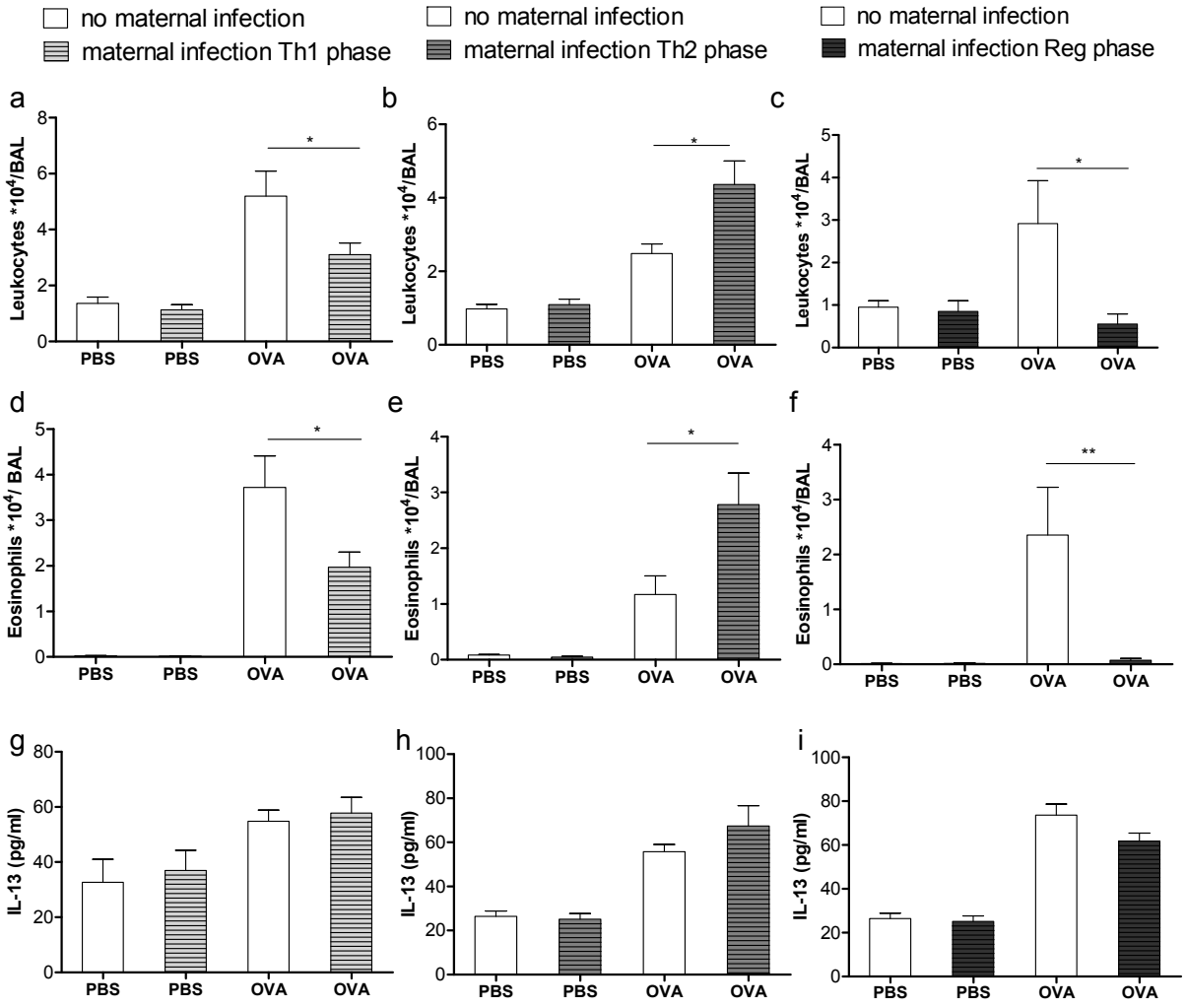


Fig. 3-10: Pregnancy during the Th1 and Reg phase, but not during the Th2 phase of infection leads to reduced cell infiltration into the lungs during allergic airway inflammation. (a-c) Total leukocyte number in BAL. (d-f) Total eosinophil number in BAL (g-i) Local concentration of IL-13 in BAL fluid. Results for offspring of Th1 mated dams represent one out of two independently performed experiments with similar outcomes (offspring from uninfected, PBS-treated n=4; offspring from Th1 infected, PBS-treated n=4; offspring from uninfected, OVA-treated n=6; offspring from Th1 infected, OVA-treated n=10; infected dams n≥4). Data of offspring from Th2 mated mothers represent two independent experiments (offsrpring from uninfected, PBS-treated n=7; offspring from Th2 infected, PBS-treated n=7; offspring from uninfected, OVA-treated n=12; offspring from Th2 infected, OVA-treated n=20; infected dams n≥5). Results for offspring from Reg phase mated dams represent one out of two experiments with similar outcomes (offspring from uninfected, PBS-treated n=4; offspring from Reg infected, PBS-treated n=4; offspring from uninfected, OVA-treated n=6; offspring from Reg infected, OVA-treated n=7; infected dams n≥2). Results are shown as mean ± SEM. Asterisks show statistical differences (Student's t test) between the groups indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).

Partial protection against OVA-induced allergic airway inflammation in offspring from Th1 mothers was also seen by a significant reduction in lung pathology (Fig. 3-11a,g), but not in goblet cell formation (Fig. 3-11d). However, complete protection against OVA-induced lung inflammation (Fig. 3-11c,g) and formation of mucus-producing goblet cells (Fig. 3-11f) in offspring of infected mothers could be mediated by pregnancy during the Reg phase. In strong contrast, lung pathology was significantly aggravated (Fig. 3-11b,g) and the number of goblet cells (Fig. 3-11e) was slightly elevated in offspring from Th2 mated dams compared to naïve OVA-treated offspring.

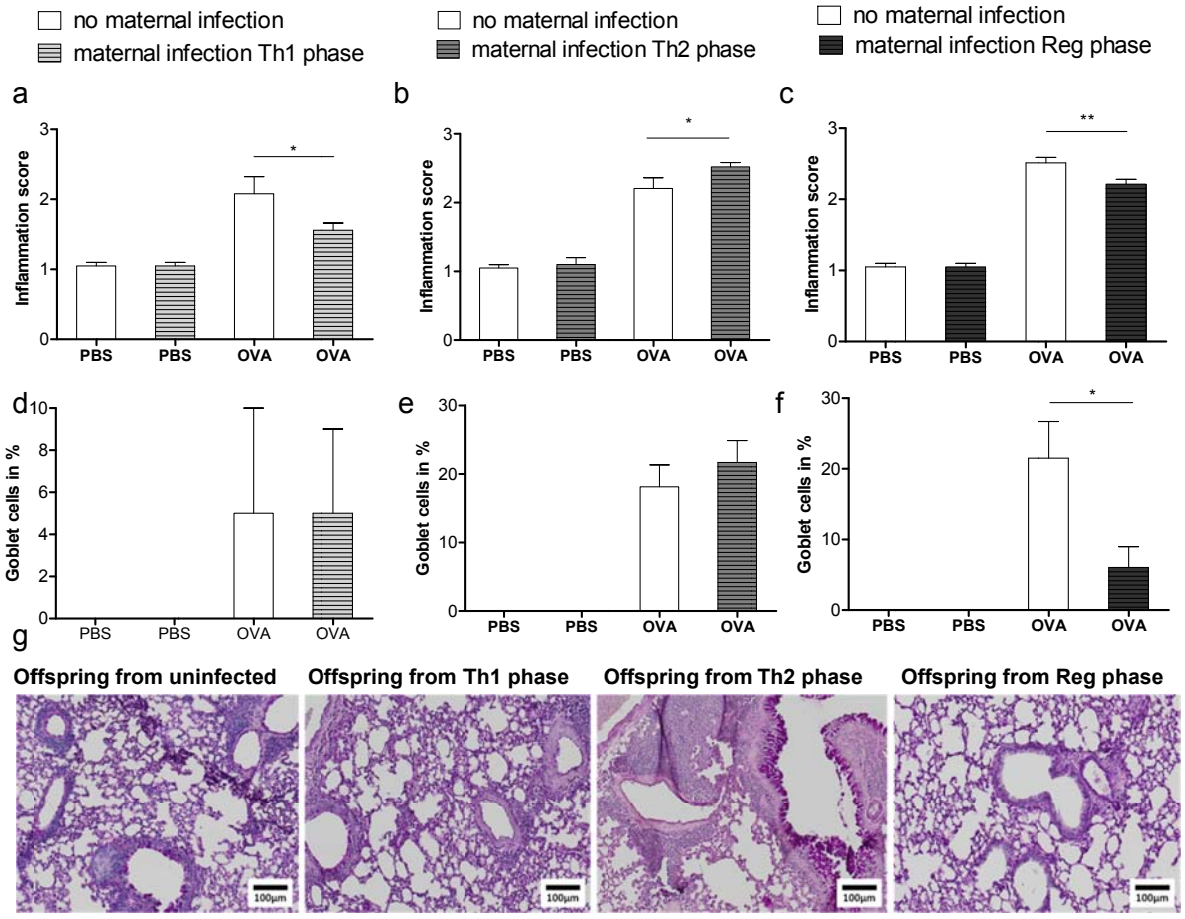


Fig. 3-11: Lung pathology and goblet cell formation range from reduction to aggravation in offspring from infected dams mated during distinct immune phases.
(a-c) Quantification of airway inflammation in offspring. (d-f) Mucus-producing goblet cells in lung sections from offspring. (g) Representative lung tissue cross sections stained with PAS from OVA-treated offspring from uninfected mothers, Th1 mated mothers, Th2 mated mothers and Reg phase mated mothers. Results for offspring of Th1 mated dams represent one out of two independently performed experiments with similar outcomes (offspring from uninfected, PBS-treated n=4; offspring from Th1 infected, PBS-treated n=4; offspring from uninfected, OVA-treated n=6; offspring from Th1 infected, OVA-treated n=10; infected dams n≥4). Data of offspring from Th2 mated mothers represent two independent experiments (offsrpring from uninfected, PBS-treated n=7; offspring from Th2 infected, PBS-treated n=7; offspring from uninfected, OVA-treated n=12; offspring from Th2 infected, OVA-treated n=20; infected dams n≥5). Results for offspring from Reg phase mated dams represent one out of two experiments with similar outcomes (offspring from uninfected, PBS-treated n=4; offspring from Reg infected, PBS-treated n=4; offspring from uninfected, OVA-treated n=6; offspring from Reg infected, OVA-treated n=7; infected dams n≥2).

Results are shown as mean ± SEM. Asterisks show statistical differences (Student's t test) between the groups indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).

Furthermore, pregnancy during Th1 and Reg phase of schistosomiasis, but not during Th2 phase, led to a significant reduction in OVA-specific IgE serum level (Fig. 3-12a-c). Furthermore, OVA-restimulation of splenocytes *in vitro* revealed a significant reduction in the production of the Th2 cytokines IL-5 and IL-10 (Fig. 3-12f,i) in Reg phase offspring. In contrast, no differences were observed in IL-5 and IL-10 production between offspring from naïve and offspring from Th1 (Fig. 3-12d,g) and offspring from Th2 mated dams (Fig. 3-12e,h), respectively. Of note. no differences in the analyzed parameters could be observed between PBS-treated offspring from naïve mothers and from infected mothers in all performed experiments (Fig. 3-10, Fig. 3-11, Fig. 3-12).

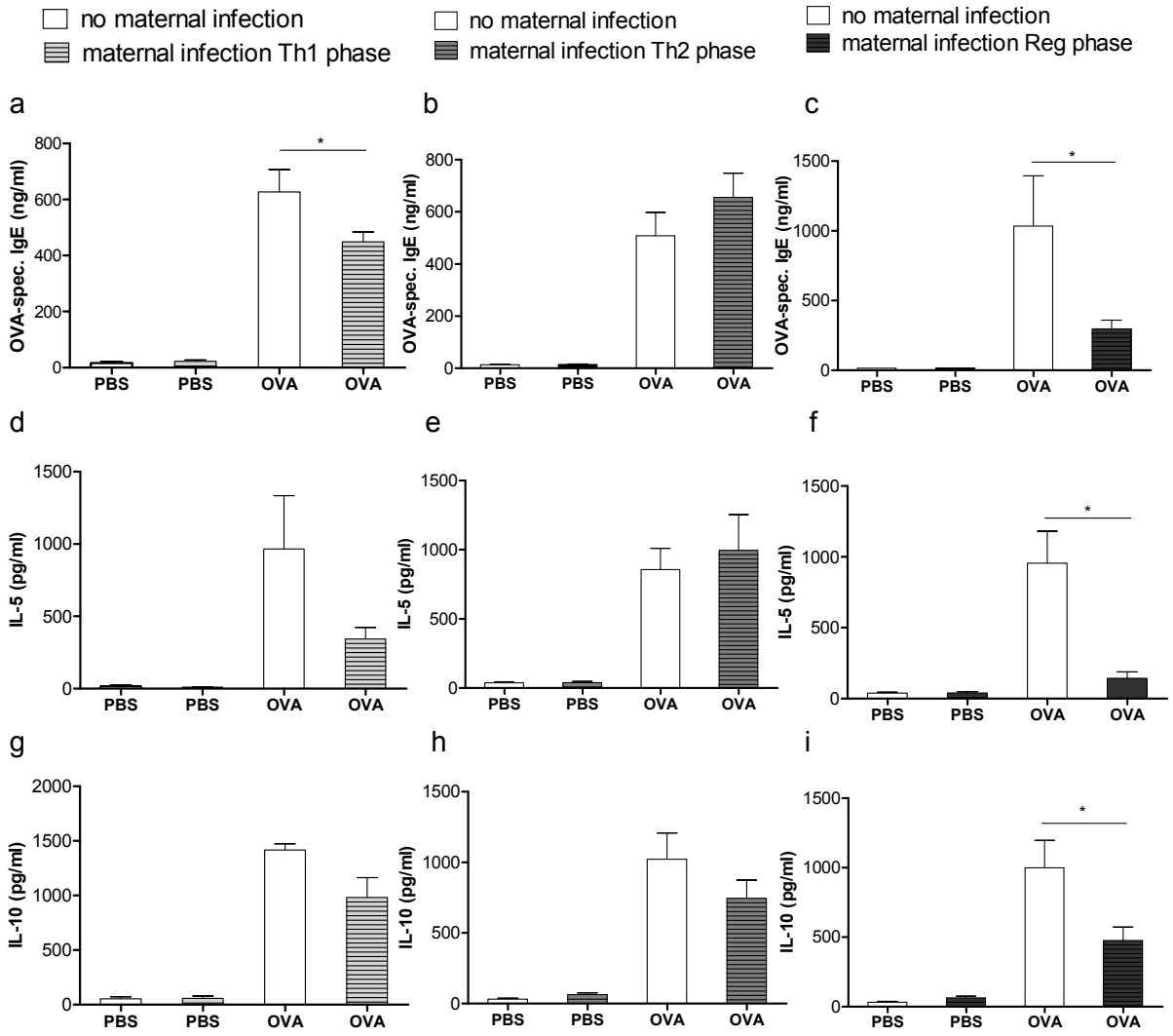


Fig. 3-12: Differential OVA-specific immune responses are observed in offspring from infected dams, mated at distinct phases of infection.
(a-c) OVA-specific serum IgE levels measured by ELISA in sera from offspring. (d-f) IL-5 production in OVA (10 µg/ml) restimulated splenocytes from offspring measured by ELISA 48 hrs

after culture. (g-i) IL-10 response in OVA (10 µg/ml) restimulated splenocytes from offspring measured by ELISA 48 hrs after culture. Results for offspring of Th1 mated dams represent one out of two independently performed experiments with similar outcomes (offspring from uninfected, PBS-treated n=4; offspring from Th1 infected, PBS-treated n=4; offspring from uninfected, OVA-treated n=6; offspring from Th1 infected, OVA-treated n=10; infected dams n≥4). Data of offspring from Th2 mated mothers represent two independent experiments (offsrpring from uninfected, PBS-treated n=7; offspring from Th2 infected, PBS-treated n=7; offspring from uninfected, OVA-treated n=12; offspring from Th2 infected, OVA-treated n=20; infected dams n≥5). Results for offspring from Reg phase mated dams represent one out of two experiments with similar outcomes (offspring from uninfected, PBS-treated n=4; offspring from Reg infected, PBS-treated n=4; offspring from uninfected, OVA-treated n=6; offspring from Reg infected, OVA-treated n=7; infected dams n≥2). Results are shown as mean ± SEM. Asterisks show statistical differences (Student's t test) between the groups indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).

In addition, a functionally important parameter is the airway hyperresponsiveness (AHR) in response to metacholine, which was measured in offspring from Reg phase mated dams compared to offspring from naïve dams upon OVA challenge (Fig. 3-13). Compared to the baseline bronchoconstriction obtained from PBS teated mice, a significant increase in bronchoconstriciton was observed in offspring from uninfected mice upon OVA challenge at a MCh concentration of 1,56 mg/m. However, in this group no differences have been observed for higher concentrations of MCh. Interestingly, OVA-treated offspring from dams mated during the Reg phase of infection did not show any differences in bronchoconstiction compared to PBS-treated mice in this experiment.

In summary, an essential role of the maternal immune phases during *S. mansoni* has been shown with regard to the development of OVA-induced allergic airway inflammation in the offspring. Pregnancies during the Th1 and Reg phase of infection offered protection, whereas Th2 phase pregnancies resulted in aggravation of the disease.

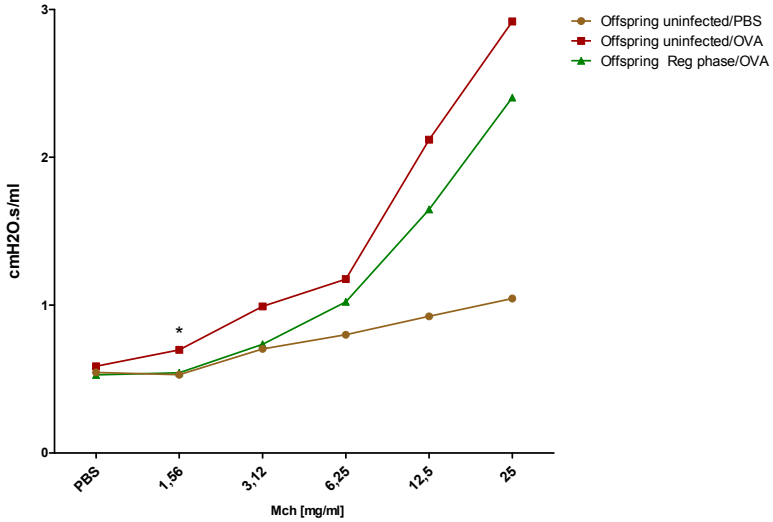


Fig. 3-13: Airway hyperresponsivness in response to metacholine is reduced in offspring from Reg infected dams upon OVA challenge.
Bronchoconstiction of offspring from uninfected (red line) and Reg phase infected (green line) dams measured in response to different concentrations of MCh (0; 1,56, 3,12; 6,25; 12,5 and 25 mgl/ml) upon OVA challenge. Yellow line represents bronchoconstriction in untreated naïve mice. Data are obtained from one experiment (offsrpring from uninfected, PBS-treated n=2; offspring from uninfected, OVA-treated n=8, OVA-treated n=8; infected dams n=3). Asterisks show statistical differences in OVA groups compared to PBS group (Student's t test) (*p<0.05).

3.3.3 Prenatal and early postnatal changes in offspring depending on the maternal immune phase of infection

3.3.3.1 Effects of maternal schistosomiasis on placental gene expression and cytokines

As discussed previously, transmission of immune shaping molecules, such as SEA via placenta has been reported during schistosomiasis and might affect innate and adaptive immune responses at the maternal-fetal interface. To determine SEA-specific production of IFN-γ, IL-4, IL-10 and TNF-α in placentas from Th1, Th2 and Reg phase mated dams, day 18 placentas were isolated and stimulated with SEA (20 µg/ml). Interestingly, proinflammatory TNF-α was detectable in all three phases, but decreased significantly during the course of infection (Fig. 3-14). Furthermore, a rise in IL-4 levels was observed in placentas from Th2 infected dams but was completely absent in Reg phase placentas. IL-10 production was only observed in Reg phase placentas and IFN-γ was not detectable, at all. Of note, no cytokine production was detectable in placentas from uninfected dams.

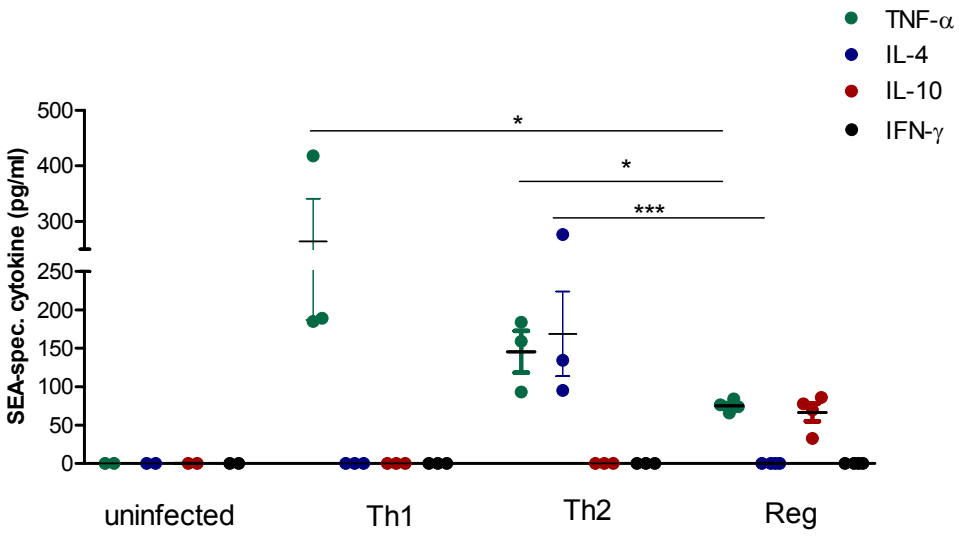


Fig. 3-14: SEA-specific cytokine production within the placenta depends on the course of infection.
TNF-α, IL-4 and IL-10 production measured by ELISA in day 18 placentas of uninfected, Th1, Th2 and Reg phase mated dams after restimulation with 20 µg/ml SEA 48 hrs after culture. Asterisks show statistical differences (Student's t test) between the groups (uninfected n=2; Th1 infected n=3; Th2 infected n=3; Reg infected n=4) indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).

The next aim was to investigate whether *S. mansoni* infection, which was shown to affect SEA-specific adaptive immune responses within the fetal-maternal interface (Fig. 3-14)

differentially regulates placental gene-expression. Therefore, RNA was isolated from day 18 placentas from uninfected, Th1 phase, Th2 phase and Reg phase mothers to perform microarray analysis using Affymetrix Gene Chips. After filtering according to the p-value (≤0.06) and logFC value (≥1), a list of differentially expressed genes was obtained for each phase (Table 5). In placentas from Th1 mothers 84 differentially expressed genes were found, with 83 genes being exclusively downregulated. In contrast, all 54 differentially expressed genes from placentas of Th2 dams were upregulated and furthermore did not overlap with genes regulated in the Th1 phase. Finally, 18 genes were differentially expressed in placentas from Reg dams, with 14 genes being downregulated and 4 genes being upregulated.

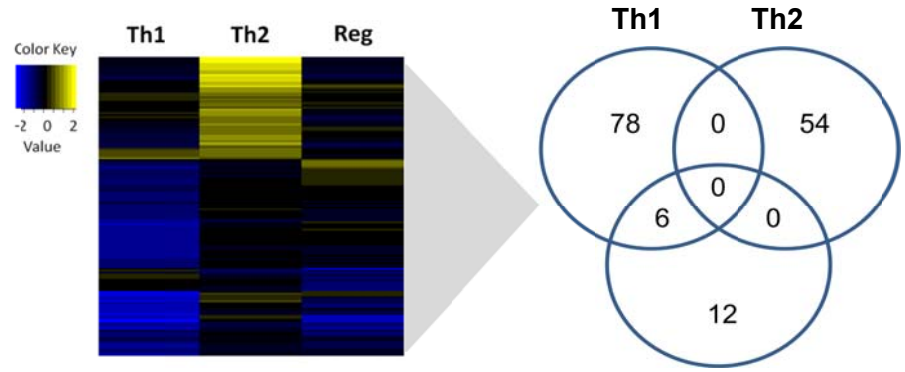
Th1		Th2		Reg	
Gene	**logFC**	**Gene**	**logFC**	**Gene**	**logFC**
Srd5a1	1.10	*Penk*	2.98	*1600015I10Rik*	1.16
Hsd3b1	-2.37	*Kap*	2.96	*Hsd3b1*	0.94
Cyp21a1	-2.11	*Sult1d1*	2.77	*2210415F13Rik*	0.88
Saa3	-2.07	*Cuzd1*	2.73	*Tph1*	0.81
2210415F13Rik	-2.06	*Akr1c18*	2.65	*Lyz2*	-1.59
Sprr2a1	-2.06	*Akr1b7*	2.52	*Anpep*	-1.20
Alb	-1.98	*Napsa*	2.50	*Sod3*	-1.11
Sprr2a1	-1.96	*5330417C22Rik*	2.41	*Psg22*	-1.09
Tph1	-1.87	*Fam13a*	2.41	*Pla2g7*	-1.01
Fgg	-1.87	*Dpt*	2.39	*Apob*	-0.99
Vdr	-1.83	*Ly6f*	2.30	*Htr1d*	-0.99
Rbp2	-1.76	*Prap1*	2.24	*Ceacam15*	-0.95
Fga	-1.72	*Havcr1*	2.20	*Ly6g6c*	-0.90
Spink3	-1.71	*Tmprss4*	2.20	*Prl5a1*	-0.90
Cfi	-1.60	*Ccl21a*	2.10	*Slc6a4*	-0.88
Serpina1e	-1.57	*Guca2b*	2.07	*Vdr*	-0.85
Apom	-1.56	*Hsd11b2*	2.03	*9930005F22Rik*	-0.82
Spp2	-1.55	*Aqp5*	1.88	*Sult1e1*	-0.81
Itih3	-1.54	*2310033E01Rik*	1.86		
Serpina1a	-1.53	*Cfh*	1.82		
Fgb	-1.52	*Cnn1*	1.81		
Slc27a2	-1.49	*C3*	1.80		
Cpn1	-1.46	*Aldh1a2*	1.76		
Sult1e1	-1.44	*Lcn2*	1.64		
Entpd2	-1.43	*Abcb1b*	1.62		
5033414D02Rik	-1.42	*Nptx1*	1.61		

Slc3a1	-1.42	*Slc28a3*	1.49
Cdhr2	-1.41	*Mal2*	1.49
Slc7a9	-1.40	*Csf1*	1.47
NA	-1.39	*Fgl2*	1.43
Maob	-1.36	*Olfm1*	1.33
Mgst1	-1.35	*Ctsg*	1.26
Fcgr3	-1.34	*Otub2*	1.24
Apoc2	-1.34	*Mboat2*	1.20
1300017J02Rik	-1.33	*Tgfb2*	1.20
Gc	-1.31	*Fut2*	1.17
Agt	-1.31	*Tmem20*	1.17
Serpind1	-1.30	*Gzmb*	1.16
Kng1	-1.27	*Prps2*	1.16
Pklr	-1.27	*Angptl7*	1.15
Bex4	-1.25	*Olfml3*	1.13
Rab17	-1.24	*Prf1*	1.11
Apoc1	-1.24	*Lbp*	1.10
Ttr	-1.23	*Tagln*	1.09
Pdzk1ip1	-1.23	*Gzmf*	1.07
F2	-1.22	*Gzmc*	1.07
Eps8l3	-1.19	*Wfdc2*	1.05
Npl	-1.19	*Postn*	1.03
Serpina1b	-1.17	*Figf*	1.02
Fmo1	-1.17	*Gzmg*	0.99
Atp6v0a4	-1.15	*Gzme*	0.98
Ctsh	-1.15	*Crispld2*	0.96
Fcgrt	-1.07	*Gzmd*	0.95
H2-t9	-1.06		
Gipc2	-1.05		
Rbp4	-1.05		
Dab2	-1.04		
9930005F22Rik	-1.04		
Cldn2	-1.04		
Nnat	-1.04		
Hbb-b1	-1.02		
Apoa4	-1.00		

Table 5: List of differentially expressed genes in day 18 placentas of Th1, Th2 and Reg phase mated dams relative to uninfected.
Down-regulated genes relative to uninfected placentas are indicated in green and upregulated genes in red. (p-value≤0.06, logFC≥1).

The heatmap depicted in Fig. 3-15a shows phase-dependent changes in placental gene expression profiles of placentas from Th1, Th2 and Reg phase infected dams in comparison to uninfected. Interestingly, the Venn-diagram revealed an overlap of six differentially expressed genes between the Th1 and the Reg phase (Fig. 3-15a), namely hydroxy-delta-5-steroid dehydrogenase (Hsd3b1), the vitamin D receptor (VDR), tryptophan hydroxylase 1 (Tph1), estrogen sulfotransferase (Sult1e1), Ly6/PLAUR Domain-Containing Protein 8 (Lypd8) and ring finger protein 223 (Rnf223). No overlap was observed between the Th2 phase and the Th1 or Reg phase, respectively. In addition, RT-PCR analysis was perfomed to confirm some of the results from the microarray analysis (IFN-γ, IL-5, IL-10, Hsd3b1, Foxp3, VDR). No significant differences were observed in the relative expression of IFN-γ, IL-5, IL-10 and Foxp3 from Th1, Th2 and Reg phase placentas compared to uninfected placentas, respectively. However, a significant reduction in the gene expression of VDR and Hsd3b1 was detected in placentas from Th1 and Reg phase dams, whereas the expression of these genes was not affected in Th2 placentas (Fig. 3-15).

a



b

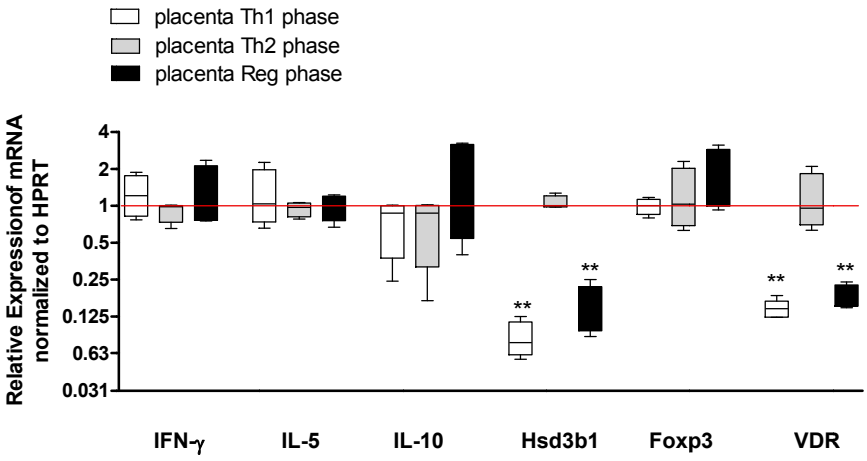


Fig. 3-15: Maternal schistosomiasis affects phase dependently placenta environment

(a) Heatmap of differentially expressed genes in placentas from dams infected in the Th1, Th2 and Reg phase of infection at day 18 of pregnancy compared to day 18 placentas of uninfected mice, respectively; significantly downregulated genes in placentas from infected dams are indicated in yellow, significantly upregulated genes in blue. The Venn-diagram shows the numbers of differentially expressed genes overlapping between Th1, Th2 and Reg-phase placentas (infected mothers n ≥ 2 per group, uninfected mothers ≥ 3 per group, placentas ≥ 5 per group). (b) Relative expression of mRNA of IFN-γ, IL-5, IL-10, Hsd3b1, Foxp3, VDR normalized to HPRT from day 18 placentas of Th1 (n=4), Th2 (n=4) and Reg (n=4) phase infected dams compared to uninfected dams (n=5). Significant differences between infected and uninfected were calculated by relative expression software tool (REST). Asterisks show statistical differences (Student's t test) in comparison to uninfected (*p<0.05, **p<0.01, ***p<0.001).

In order to structure the gene data, reduce complexity and gain further insight into the underlying biology differentially expressed gene pathway analysis was performed by the use of IPA (Ingenuity® Systems, www.ingenuity.com) specified for placental tissue. The comparison of the top 15 canonical pathways affected in the Th1 and Reg phase revealed an overlap in the following pathways: serotonin and melatonin biosynthesis, mineralcorticoid biosynthesis, glucocorticoid biosynthesis, Maturity Onset Diabetes of Young (MODY) signaling, and glycolysis I. In contrast, pathway analysis of genomic data from Th2 placentas revealed the upregulation of the pathways for thyroid hormone metabolism and the biosynthesis of androgen, which was also affected in the Reg phase. An overview of all differentially regulated pathways of Th1, Th2 and Reg phase placentas is depicted in Fig. 3-16.

In summary, microarray and pathway analyses revealed a number of genes and pathways to be differentially expressed within the placenta of *S. mansoni* infected dams depending on the phase of maternal infection. Thereof, six genes overlapped between the protective Th1 and Reg phases, including the VDR, Hsd3b1 and Tph1. Furthermore, phase-specific placental cytokine production was observed upon SEA-restimulation.

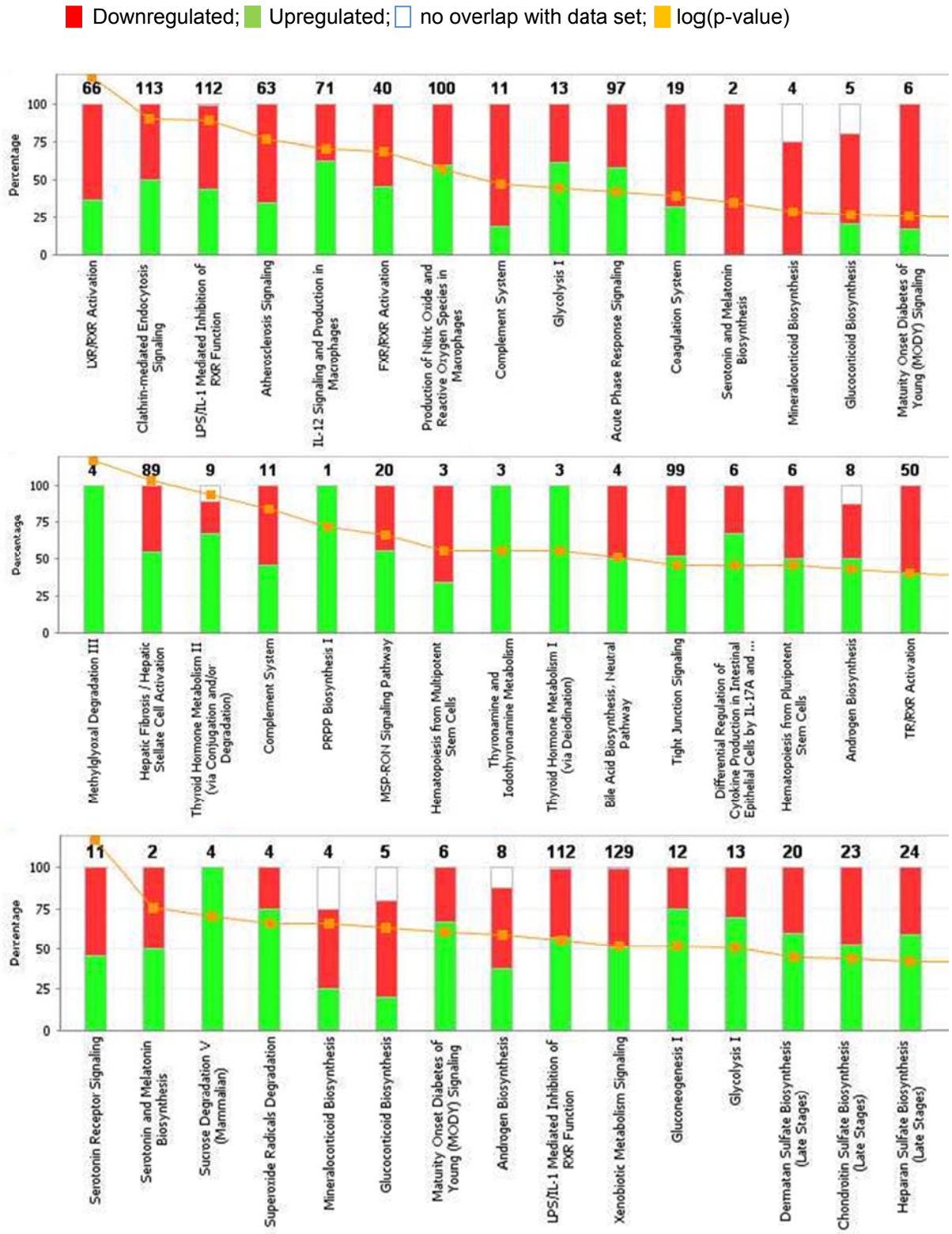


Fig. 3-16: Top 15 canonical pathways differentially regulated within placentas from Th1, Th2 and Reg phase infected dams.
Top 15 differentially regulated pathways are depicted from Th1 **(up)**, Th2 **(middle)** and Reg **(down)** phase placentas. Total number of genes that contribute to a certain pathway are depicted above the bars. Red colour represents percentage of downregulated genes (Cutoff logFC ≥1) within the pathway and green colour shows percentage of upregulate genes (Cutoff logFC ≥1). Yellow line depicts decreasing p-value with a Cutoff at 0.06. Pathway analysis was assessed by IPA (Ingenuity® Systems).

3.3.3.2 Changes in early postnatal immune cell composition

Since placental microarray analysis revealed differentially expressed genes within the placenta of infected mice, which might affect the development of the fetal immune system we tested for changes within the offsprings' early immune cell formation. Therefore, flow cytometric analyses of peripheral blood leukocytes from 3-week-old offspring was performed using classical immune cell markers. The portion of the different leukocyte subtypes was evaluated according to the gating strategy in Fig. 3-17 and revealed significant differences in early immune cell composition between offspring from dams mated during the Th1, Th2 and Reg phase of infection (Fig. 3-18). Compared to uninfected offspring, offspring from Th1 mated dams showed significantly reduced B cells, but highly elevated numbers of granulocytes. In contrast, percentage of B-cells was not affected in blood from offspring from Th2 mated dams, but granulocyte counts were significantly decreased. Furthermore, the proportion of $CD4^+$ and $CD8^+$ T cells was significantly higher. Offspring from Reg phase mated dams showed a significantly elevated frequency of B cells, whereas the percentage of NK cells and $CD4^+CD44^+$ T cells was significantly reduced compared to age matched offspring from uninfected dams.

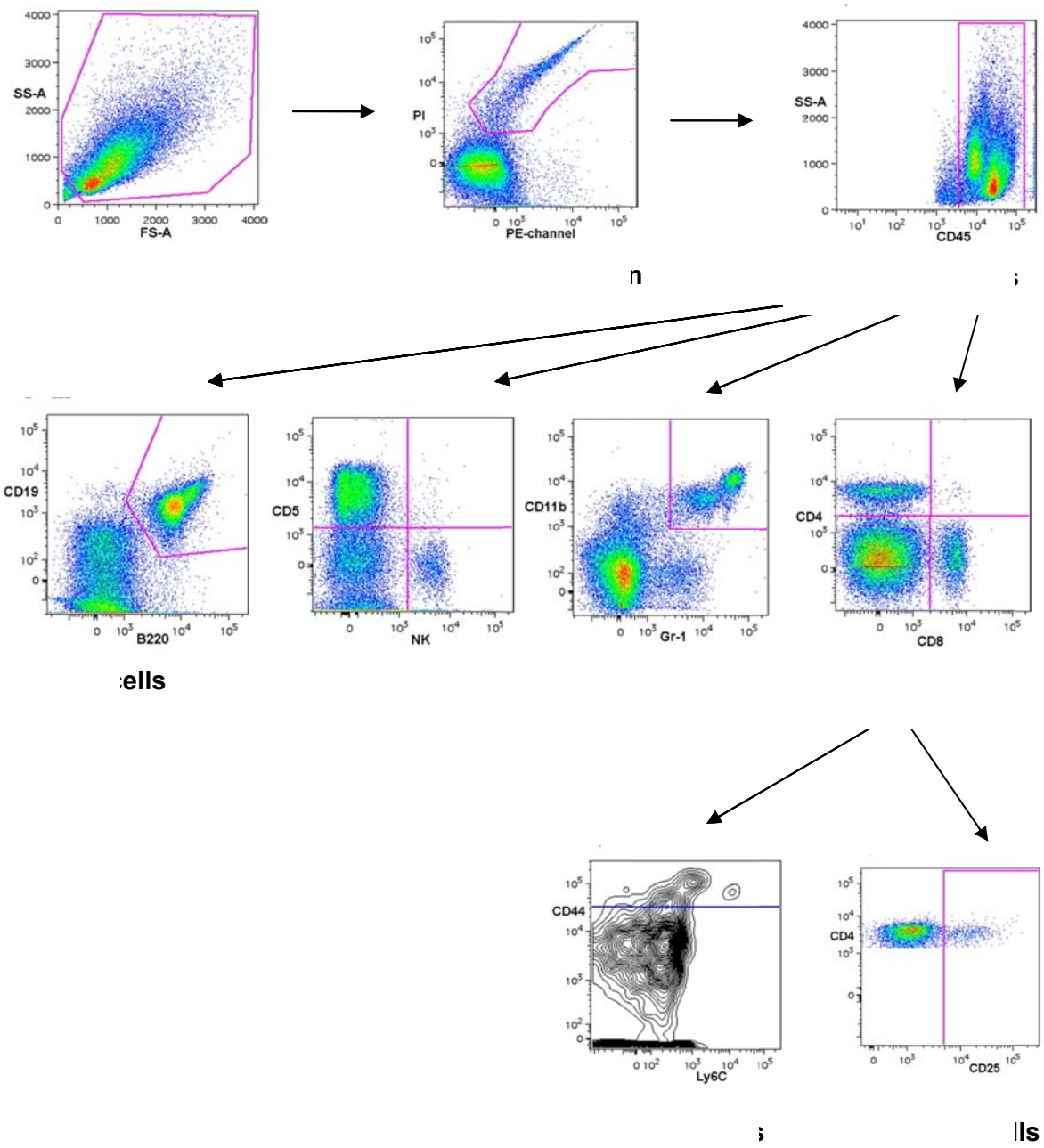


Fig. 3-17: Gating strategy for flow cytometric analysis of leukocyte populations in peripheral blood based on multi-parameter staining panels.
Analysis covered markers for B cells (CD19, B220), T cells (CD45, CD4, CD8, CD5), granulocytes and monocytes (GR-1, CD11b), NK cells (NKp46) and further subsets (CD44, CD25, Ly6C).

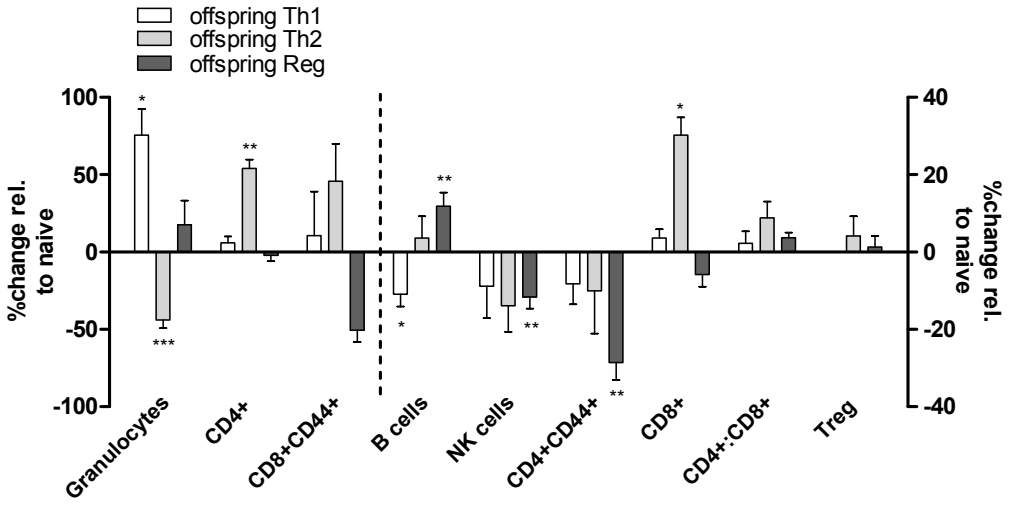


Fig. 3-18: Maternal schistosomiasis affects phase dependently early immune cell composition.
Percentage of change in immune cell populations isolated from blood of three weeks old offspring from Th1, Th2 or Reg infected dams compared to age-matched uninfected offspring, respectively. (offspring from uninfected ≥ 7 per analysis, offspring from Th1 infected n=14, offspring from Th2 infected n=22; offspring from Reg infected n=12; infected mothers ≥ 3 per group). Data in bar graphs represent mean ± SEM. Asterisks show statistical differences (Student's t test) in comparison to offspring from uninfected (*p<0.05, **p<0.01, ***p<0.001).

3.3.4 Partial protection is lost in offspring from acutely infected IFN-γ knockout mice

Since phase-dependent differences have been observed with regard to gene expression and adaptive immune responses at the maternal-fetal interface of *S. mansoni* infected dams, the role of maternally-derived cytokines was analyzed, with respect to the offspring's allergen susceptibility. IFN-γ is the predominant cytokine in the Th1 phase and its role during acute maternal schistosomiasis can be assessed by the use of single knockout mice. Importantly, it has been reported that mice lacking the IFN-γ receptor (IFN-γR$^{-/-}$) run in similar dynamics pertaining to *S. mansoni* worm development and egg excretion compared to wildtype mice [197]. Thus, by mating infected IFN-γ$^{-/-}$ BALB/c females within the designated timeframe of the Th1 phase of infection one can determine if the previously observed asthma protective effects in the offspring from Th1 mated dams (Fig. 3-10a,d; Fig. 3-11a; Fig. 3-12a) are mediated by the maternal IFN-γ production, since all other features of schistosomiasis, such as circulating parasite antigens are not affected in this knockout strain. Offspring from infected and uninfected IFN-γ$^{-/-}$ BALB/c and from uninfected BALB/c wildtype mice were tested in the model of allergic airway inflammation as depicted in 2.2.6.2.2. As a result, analysis of asthma parameters in offspring from acute infected and uninfected IFN-γ$^{-/-}$ revealed that suppression of allergic responses was abrogated due to the lack of maternal IFN-γ. In fact, offspring from

infected IFN-γ$^{-/-}$ females showed elevated leukocyte (Fig. 3-19a) and eosinophil (Fig. 3-19b) infiltraton into the lung, as well as significantly impaired lung pathology (Fig. 3-19d,g) and mucus-producing goblet cell formation (Fig. 3-19e,g). Furthermore, IL-5 (Fig. 3-19f) production was elevated in OVA-restimulated splenocytes *in vitro*. No differences in OVA-specific serum IgE levels were detectable between OVA-treated groups (Fig. 3-19c).

In summary, these data show that a lack of maternal IFN-γ during the acute phase of infection led to enhanced allergic airway inflammation in the offspring in comparison to offspring from uninfected wildtype or IFN-γ$^{-/-}$ females.

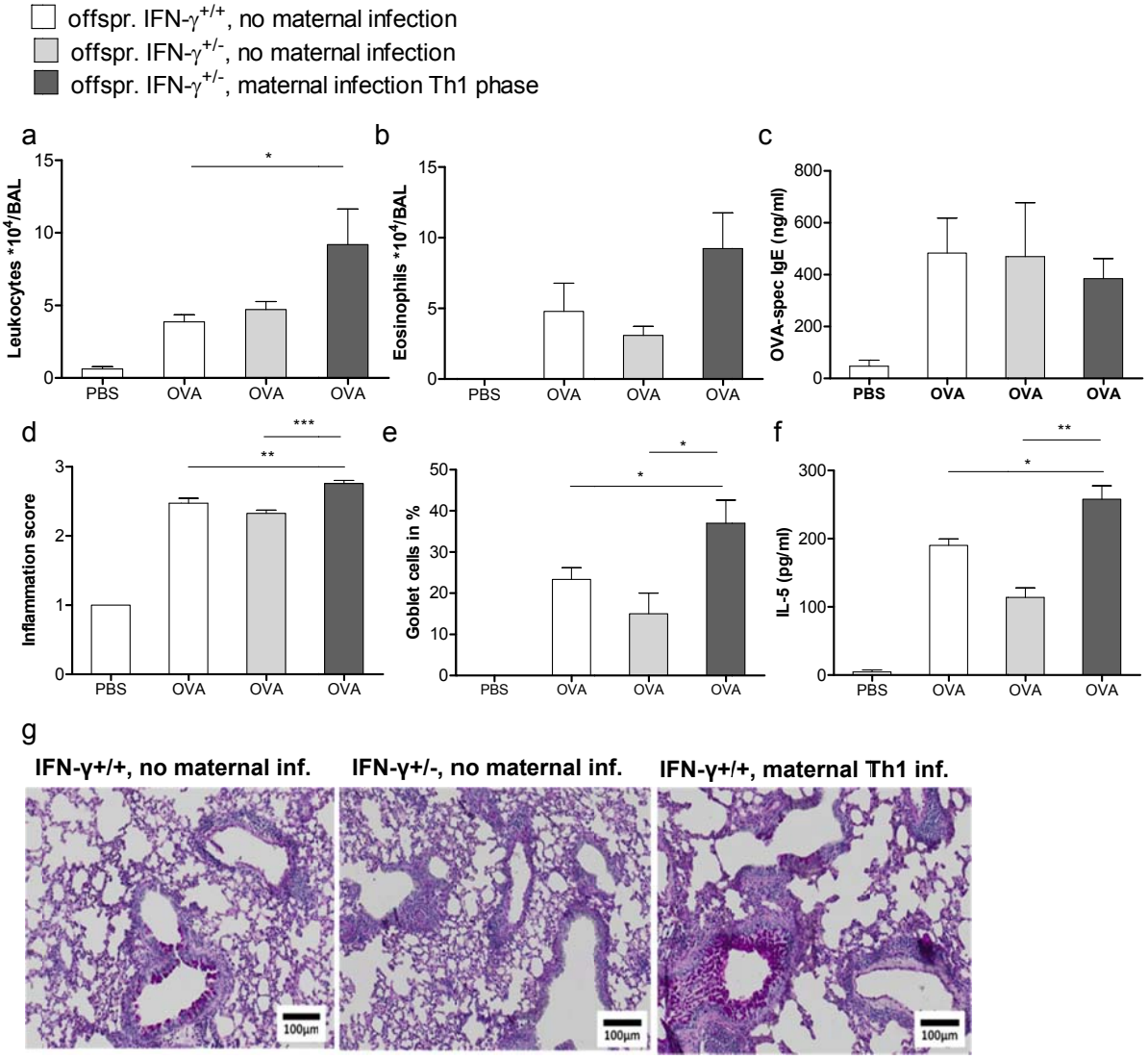


Fig. 3-19: Partial protection is lost in offspring from acutely infected IFN-γ knockout mice.
(a) Total leukocyte number in BAL. (b) Total eosinophil number in BAL. (c) OVA-specific serum IgE levels measured by ELISA. (d) Quantification of lung inflammation. (e) Mucus-producing goblet cells in lungs from offspring. (f) IL-5 levels in OVA (10 µg/ml) restimulated splenocytes measured by ELISA 48 hrs after culture. (g) Representative lung tissue cross sections from offspring stained with PAS from OVA-treated animals. Results represent one out of two independently performed experiments with similar outcomes (offspring from uninfected wt, PBS-treated n=7; offspring from

uninfected wt, OVA-treated n=13; IFN-$\gamma^{+/-}$ offspring from uninfected, PBS-treated n=5; IFN-$\gamma^{+/-}$ offspring from Th1 infected, OVA-treated n=9; infected mothers ≥ 2). Data is shown as mean ± SEM. Asterisks show statistical differences (Student's t test) between the groups indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).

3.3.5 No transfer of protection against allergic airway inflammation via the germline

Another potential mechanism by which distinct, unbalanced immune responses due to maternal schistosome infection might influence the offsprings' immunological development is defined as transgenerational epigenetic inheritance via the gametes. To test for this mechanism we analyzed the Th1 phase of infection, representatively. Eggs derived from Th1 phase infected BALB/c females, or uninfected females as controls, were fertilized *in vitro* with sperm from uninfected BALB/c males. Blastocysts were transferred into uteri of uninfected foster mice and 6-8 weeks old offspring from both groups were subjected to the OVA induced model for allergic airway inflammation (2.2.6.2.2). No differences could be observed between OVA-treated groups in total leukocyte (Fig. 3-20a) and eosinophil (Fig. 3-20b) number in the BAL. In addition, OVA-specific serum IgE levels were comparable between OVA-treated groups (Fig. 3-20c). Furthermore, upon asthma induction no differences in lung pathology (Fig. 3-20d,f) and goblet cell formation (Fig. 3-20e,f) were observed. Overall, the partial protection against OVA-induced allergic airway inflammation, which has been seen before in offspring from Th1 mated mothers, was lost in offspring derived from *in vitro* fertilized eggs from Th1 phase females, when fostered by uninfected mothers.

In summary, no differences in the development of OVA-induced allergic airway inflammation have been observed in offspring derived from *in vitro* fertilized eggs from Th1 infected females and fostered by uninfected dams, compared to offspring derived from uninfected egg donors.

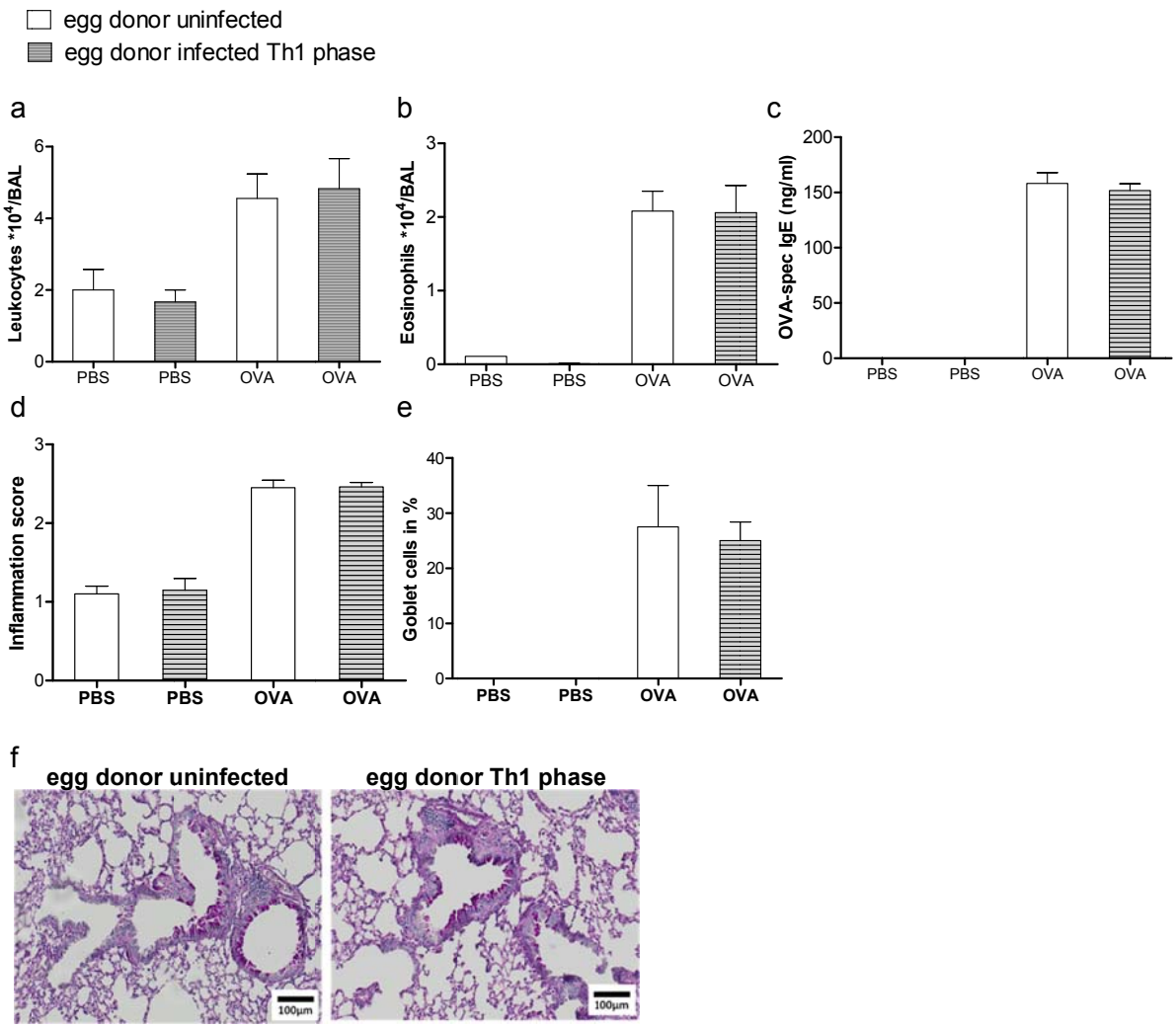


Fig. 3-20: Partial protection against allergic airway inflammation in offspring is not transferred via the germline.
Offspring were fostered by uninfected mice after *in virto-fertilization* and transfer of eggs from Th1 infected or control mice. (a) Total leukocyte number in BAL. (b) Total eosinophil number in BAL. (c) OVA-specific serum IgE levels measured by ELISA (d) Quantification of lung inflammation. (e) Mucus-producing goblet cells in lungs from offspring. (f) Representative lung tissue cross sections from OVA-treated offspring stained with PAS. Results represent one experiment (offspring from uninfected egg donor, PBS-treated n=4; offspring from Th1 infected egg donor, PBS-treated n=4; offspring from uninfected egg donor, OVA-treated n=6; offspring from Th1 infected egg donor, OVA-treated n=7; Infected mothers ≥ 8). Data is shown as mean ± SEM. p-values were determined by Student's *t* test.

3.4 Effects of the maternal VDR on allergen susceptibility in the progeny

3.4.1 Evaluation of mating combination and pregnancy related immune regulation

It has been reported, that the VDR plays a role during the development of allergic airway inflammation, since $VDR^{-/-}$ mice failed to develop experimental allergic asthma [198].

However, the influence of maternal VDR expression on asthma susceptibility in the offspring has not been investigated. Since the VDR was found to be downregulated in placentas from Th1 and Reg phase dams in the current study, its specific role during pregnancy with regard to the development of allergic airway inflammation in the offspring was analyzed. As a first step, an appropriate mating combination was evaluated, since the activation of the maternal immune system in response to fetal cells is an anticipated requirement to determine the effects of VDR on maternal immune cells within the placenta. Therefore, the frequency of maternal Foxp3$^+$ Treg cells was determined on day 11 of pregnancy in the spleen of females from C57BL/6xC57BL/6 and CBA/JxCBA/J syngeneic matings and C57BL/6xBALB/c and CBA/JxBALB/c allogeneic matings (Fig. 3-21a). This resulted in an increase of Foxp3$^+$ Treg cells during pregnancy paralleled by increased serum IL-10 level (Fig. 3-21b) as measured by in vivo capture assay in allogeneic, but not syngeneic matings. Moreover, splenocytes of pregnant C57BL/6 and CBA/J females from allogeneic matings were stimulated *in vitro* with irradiated lymphocytes from syngeneic and allogeneic males to determine maternal IL-10 and IFN-γ production. Again, induction of maternal immune responses with regard to IL-10 and IFN-γ production was only observed after allogeneic, but not syngeneic stimulation (Fig. 3-21b-c). Thus, the allogeneic mating combination C57BL/6xBALB/c was chosen for further experiments (3.4.2).

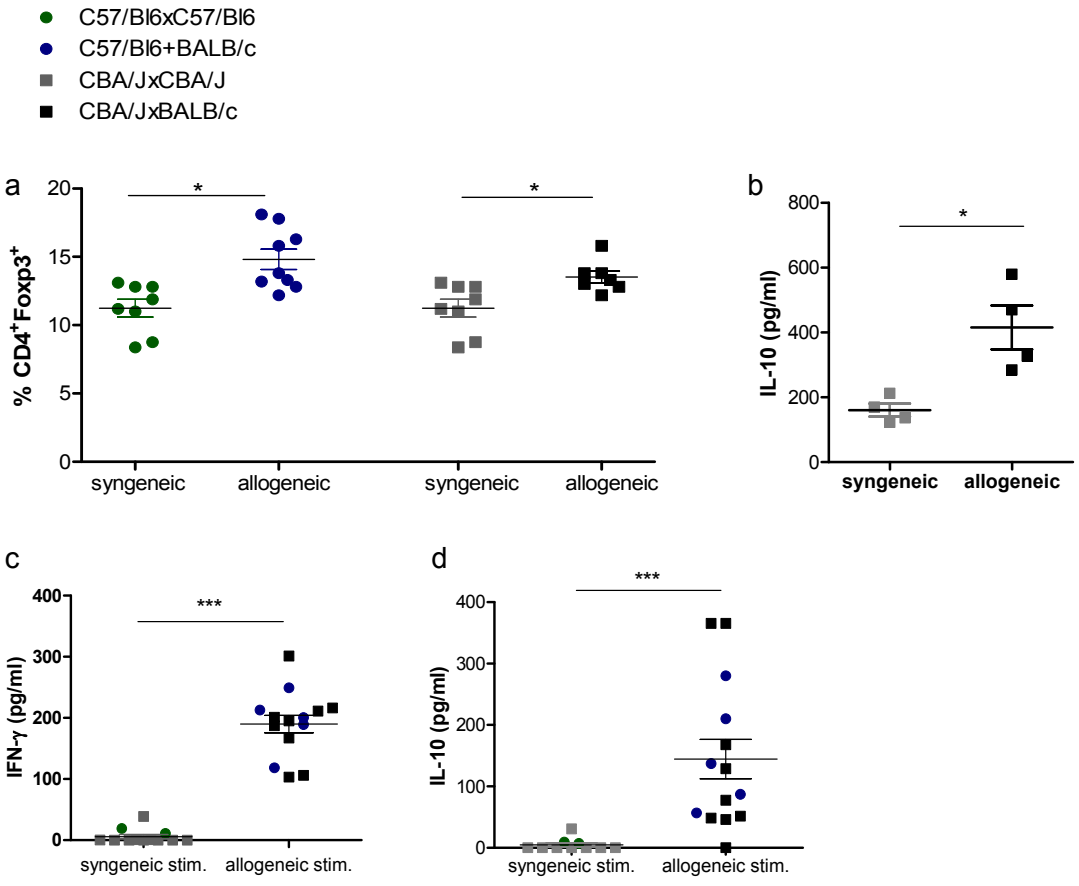


Fig. 3-21: Maternal immune response is induced in allogeneic matings.
(a) Percentage of Foxp3$^+$ Treg cells in spleens of C57BL/6 and CBA/J females from syngeneic (C57BL/6xC57BL/6 and CBA/JxCBA/J) or allogeneic (C57BL/6xBALB/c and CBA/JxBALB/c) mating combinations determined by flow cytometry on day 11 of pregnancy. (b) IL-10 levels (pg/ml) in serum from syngeneic-pregnant (CBA/JxCBA/J, n=4) or allogeneic pregnant (CBA/JxBALB/c, n=4) animals on day 8 of pregnancy. IL-10 (c) and IFN-γ (d) release from splenocytes ($3x10^5$ cells) of pregnant C57BL/6 or CBA/J females on day 11 of allogeneic pregnancies stimulated with irradiated (30Gy) splenocytes ($6x10^5$ cells) of syngeneic or allogeneic males measured in cell culture supernatant by ELISA. Data is shown as mean ± SEM. Asterisks show statistical differences (Student's t test) between the groups (syngeneic mating combination n=8; allogeneic mating combination n=9) indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).

With regard to the observed rise in Treg cells and maternal IL-10 serum level during pregnancy, our collaboration partner Prof. Dr. Ralph Nanan (Sydney Medical School Nepean, The University of Sydney) made the surprising observation of a strong correlation of peripheral blood Treg cells between the mother and the fetus in human pregnancies. Gene microarray analysis of Treg cells identified a typical IL-10-dependent signature in maternal and fetal Treg cells. Furthermore, Treg cells in pregnant women, but not in non-pregnant women were shown to possess higher expression of IL-10 receptor alpha which directly correlated with the expression of the anti-apoptotic Bcl-2. In addition, they demonstrated by FACS analysis that Bcl-2 is upregulated in Treg cells in human pregnancy and is tightly regulated between the mother and the baby (*Fetal-maternal alignment of regulatory T cells correlates with Interleukin 10 and Bcl-2 upregulation in pregnancy* Brigitte Santner-Nanan and Kathrin Straubinger et al, JI, accepted 2013). In mice, using the allogeneic CBA/J x Balb/c pregnancy model that is known to present normal pregnancies, we confirmed these findings, since pregnant CBA/J mice had significantly higher Treg cell frequency (Fig. 3-21a), paralleled by increased serum IL-10 level (Fig. 3-21b) and more Bcl-2$^+$ cells within the expanding Treg cell population (Fig. 3-22a,b). Since these data implicate an important role of IL-10 on Bcl-2 expression in Treg cells we tested this *in vitro* by co-culturing GFP$^+$ Treg with non-Treg cells isolated from DEREG Balb/c mice and found upregulation of Bcl-2 expression in Treg cells in the presence of IL-10 (Fig. 3-22c,d). To test whether endogenous IL-10 has similar effects on Bcl-2 expression on Treg cells *in vitro*, female CBA/J splenocytes were cultured with irradiated male BALB/c splenocytes in order to simulate the allogeneic constellation during normal pregnancies or with irradiated male CBA/J splenocytes as control. IL-10 production was strongly induced in splenocytes from female CBA/J mice when stimulated with allogeneic Balb/c male lymphocytes (Fig. 3-21d). In these cultures, blocking of endogenous IL-10 production led to a selective decrease in Bcl-2 expression in Treg cells but not in non-Treg cells (Fig. 3-22e).

In summary, these results show that maternal immune responses are induced during allogenic pregnancies. Furthermore, Bcl-2 expression is upregulated in Treg cells during allogeneic pregnancies. The *in vitro* data further support the interpretation of the *in vivo* data that IL-10 contributes to upregulation of Bcl-2 expression in Treg cells.

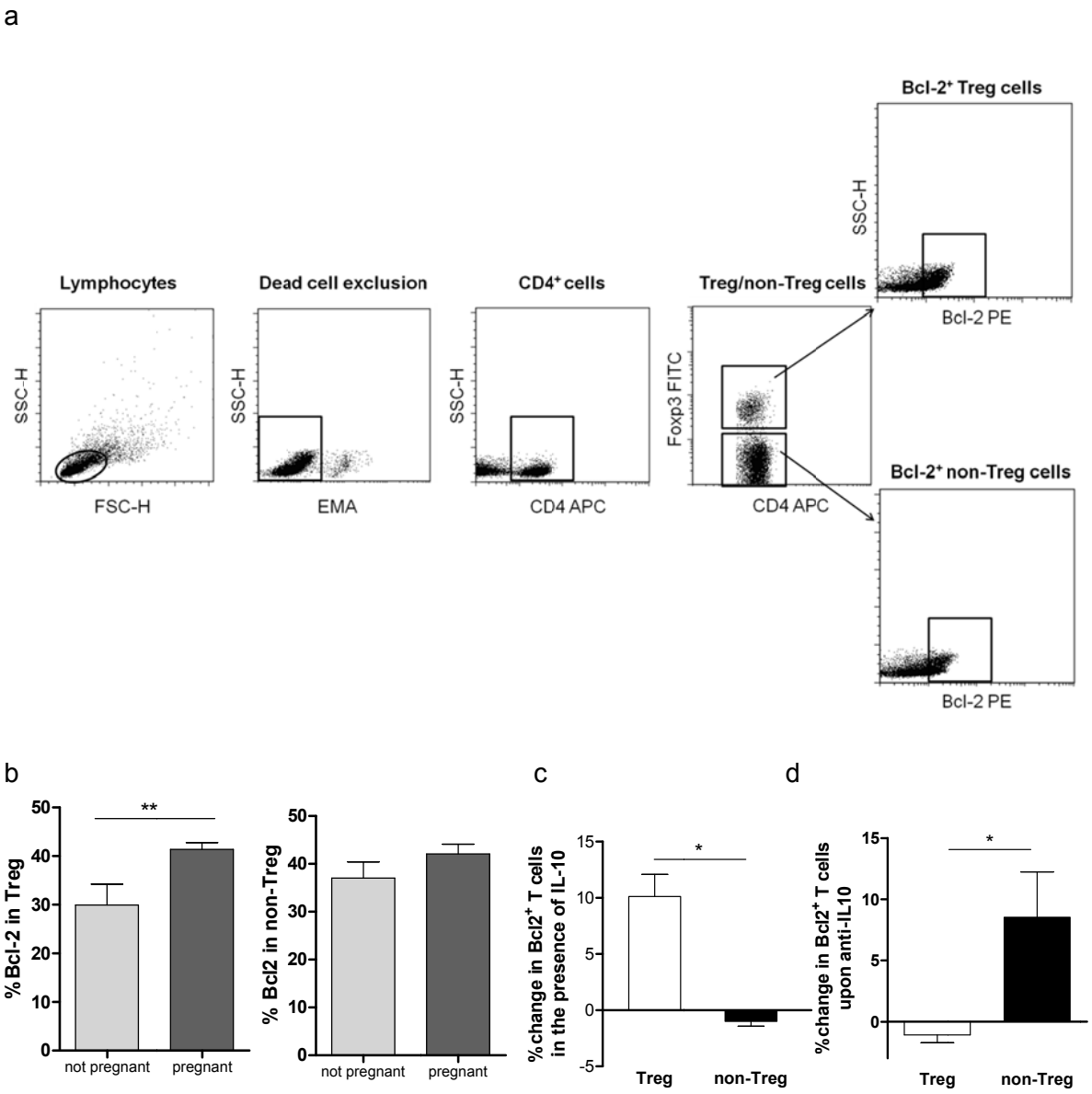


Fig. 3-22: Significantly higher percentages of Bcl-2$^+$ cells within the expanding Treg cell population in pregnant CBA/J mice and differential effects of IL-10 on Bcl-2 expression in T cells *in vitro*. (a) Representative dot plots of the Bcl-2 gating strategy in Treg and non-Treg cells from pregnant and non-pregnant CBA/J females. (b) Summary bar graph (mean ± SEM) of CD4$^+$Foxp3$^+$Bcl-2$^+$ cells (left) or CD4$^+$Foxp3$^-$Bcl-2$^+$ cells (right) in pregnant (n=7) vs non-pregnant (n=7) mice. (c) Summary bar graphs (mean ± SEM) showing percentage of change in Bcl-2$^+$ cells in co-cultured CD4$^+$GFP$^+$Treg cells and CD4$^+$GFP$^-$ non-Treg cells isolated from splenocytes of DEREG BALB/c mice upon stimulation with 400IU/ml of IL-10 plus anti-CD3/CD28. Data represent 2 individual experiments. (d) Summary bar graph (mean ± SEM) showing percentage of change in Bcl-2$^+$ cells upon anti-IL-10 treatment in splenocytes from female CBA/J mice (n=11), stimulated with irradiated allogeneic male BALB/c lymphocytes. Asterisks show statistical differences (Student's t test) between the groups indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).

3.4.2 Reduction of allergic asthma in offspring from VDR$^{-/-}$ dams

In order to determine the role of the maternal VDR on asthma susceptibility in the offspring, VDR$^{-/-}$ females on a C57BL/6 background were mated with wildtype BALB/c mice to induce maternal immune responses during pregnancy (3.4.1). To control for heterozygous effects of the VDR in asthma development in the progeny, offspring from BALB/c females mated with VDR$^{-/-}$ males on a C57BL/6 background were also analyzed. As a first step, differences in early postnatal growth were evaluated. Therefore, the weight of the offspring was determined at different timepoints between three and nine weeks after birth. Interestingly, offspring from VDR$^{-/-}$ females had significantly reduced weight compared to offspring from VDR$^{-/-}$ males throughout the entire period of time (Fig. 3-23a). In order to investigate further differences with regard to asthma development, offspring from the two groups were sensitized and challenged with OVA according to the protocol depicted in Fig. 2-3. Thereby, OVA dose was adjusted to the weight of the mice as described in section 2.2.6.2.2 in order to avoid a bias. The analysis of the asthma parameters revealed a significant recuction of leukocyte (Fig. 3-23b) and eosinophil (Fig. 3-23c) infiltration into the lungs of offspring from VDR$^{-/-}$ dams. Furthermore, goblet cell formation (Fig. 3-23e), but not lung inflammation (Fig. 3-23d) was significantly reduced in this group. Interestingly, OVA-restimulation of splenocytes led to increased production of IFN-γ in offspring from VDR$^{-/-}$ dams, whereas IL-5 and IL-10 production was not different to those observed in offspring from VDR$^{-/-}$ males (Fig. 3-23f). No differences were observed in OVA-specific IgE (Fig. 3-23g) and IgG1 (Fig. 3-23h) serum levels.

In summary, offspring born to VDR$^{-/-}$ females showed reduced allergic airway inflammation. Thereby, no differences between OVA-specific IL-5 and IL-10 production have been observed *in vitro*. However, IFN-γ production was significantly elevated in these mice upon OVA-restimulation.

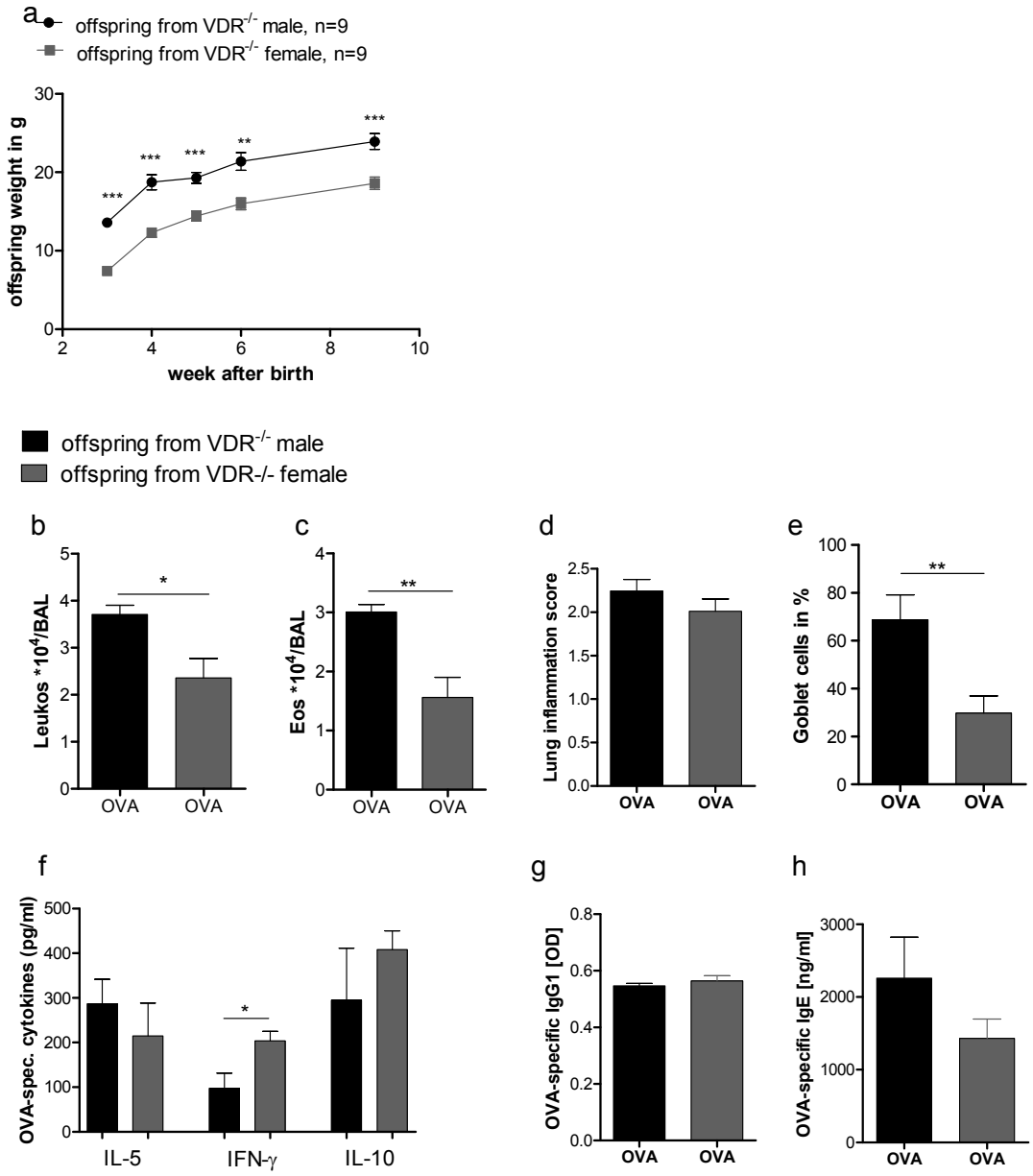


Fig. 3-23: Offspring from VDR$^{-/-}$ dams show reduced allergic airway inflammation.
(a) Weight of offspring from VDR$^{-/-}$ females and VDR$^{-/-}$ males. (b) Total leukocyte number and (c) total eosinophil number in BAL. (d) Quantification of lung inflammation. (e) Quantification of lung goblet cell formation. (f) OVA-specific cytokine level measured by ELISA 48 hrs after culture. (g) OVA-specific serum IgE (h) and IgG1 levels. Results represent one experiment. Data is shown as mean ± SEM. Asterisks show statistical differences (Student's t test) between the groups (offspring from VDR$^{-/-}$ females n=9; offspring from VDR$^{-/-}$ males n=9; dams n =1 per group) indicated by the brackets (*p<0.05, **p<0.01, ***p<0.001).

4 Discussion

4.1 Protection against allergic asthma during chronic schistosomiasis and the role for regultaroy T cells

Schistosome infections result in immune responses that are tightly controlled by various host mechanisms including the activation and expansion of different cell types, such as regulatory T and B cells [66, 199] or the release of immunosuppressive schistosomal antigens [200]. These are considered to dampen bystander immune responses against unrelated antigens or allergens. Although humans are the main definite host of *S. mansoni* in many regions, this helminth also infects rodents. Moreover, immunological and pathological responses observed in humans are mirrored in mice. Worms require five to six weeks to mature and enter patency which is classified as the production of eggs by fecund females [10]. In many studies this murine model has been used to investigate the role of helminth infections during allergic diseases induced by the classical ovalbumin model of allergic airway inflammation, where both protective and aggravated responses have been noted [93, 201, 202]. For example, studies from Pacifico *et al.* reported a protective effect in BALB/c mice, starting 9 weeks post infection [202]. However, Smits *et al*. showed that only chronically infected C57BL/6 mice (12 to 16 weeks) were protected from allergic airway inflammation and that this protection was dependent on the amount of released eggs [93]. This is in contrast to Mangan *et al*., who reported aggravated airway hyperresponsiveness and enhanced OVA-specific Th2 responses in acute and chronically schistosome-infected BALB/c mice [201]. Interestingly, they used double the amount of OVA and adjuvant compared to other studies which might perhaps elicit such strong immunization responses that can no longer be counterbalanced by a schistosome-induced regulatory environment. These opposing studies clearly highlight that the nature of the acute inflammatory model is influenced by the choice of mouse strain. The most commonly used strain for antigen challenge models is BALB/c as they develop a strong Th2 biased immunological response, however, other strains have been used successfully [203-205]. In the current study, both BALB/c and C57BL/6 mice were used to investigate the effects of *S.mansoni* infection on the development of allergic airway inflammation. Besides the mouse strain, the model allergen as well as the sensitization and challenge protocol influence the development of allergic airway inflammation and have to be considered when interpreting and comparing the results [206, 207]. The protein ovalbumin derived from chicken egg is frequently used as an allergen in murine experimental asthma, since it mimics the three main parameter of human asthma defined by inflammatory cell recruitment, lung function impairment and airway remodeling. Since it is

rarely implicated in human asthma, alternative allergens with greater clinical relevance, for example house dust mite (HDM) [208] and ragweed extract [209] have been applied. However, literature research revealed that the effects of schistosome infection on the development of allergic airway inflammation have not been tested in response to other allergens than OVA. Furthermore, most of the currently used protocols for allergic airway inflammation require multiple systemic administration of the allergen in the presence of an adjuvant such as aluminium hydroxide ($AlOH_3$) that is known to promote the development of the Th2 phenotype [210]. In the current study, both OVA and ragweed were used as model allergens. Therefore, after the sensitization period in the presence of $AlOH_3$ mice were challenged with the respective allergen via the airway over a period of three days. As a result, schistosome-induced protection against OVA-induced allergic airway inflammation was observed in C57BL/6 mice 12 weeks post infection (section 3.2.2), supporting the findings of Smits et al [93], who suggested that the induction of suppressive mechanisms regulating the immune reactions to inhaled allergens appear during chronic, but not acute helminth infection. With regard to the “Th2 prone” BALB/c strain, protection against allergic airway inflammation was observed using ragweed as model allergen already at nine weeks post infection (section 3.2.1). Experiments with infected BALB/c mice required the induction of allergic airway inflammation at this time-point since in contrast to C57BL/6 mice, schistosome disease progression is accelerated resulting in high mortality rates. Mechanistically, an elevated release of IFN-γ and IL-10 has been observed in lung lymphnode cells from infected mice upon ragweed restimulation *in vitro*, which might counterbalance local allergic Th2 responses. Conclusively, *S. mansoni* infected mice (BALB/c and C57BL/6) had reduced allergic airway inflammation when sensitized and challenged during patent infection suggesting the requirement of continous egg deposition as a prerequisite for protection. This concept was further supported by a recent study from our group showing that infected mice had dampened allergic responses when OVA challenge but not sensitization occurred in the patent phase. In contrast, no asthma protection was observed in infected mice when both sensitization and challenge were performed in the prepatent phase or six weeks after anti-hemlinthic treatment with praziquantel (Layland and Straubinger et al., PLoS NTD revision submitted). With regard to the underlying mechanisms of helminth-mediated protection against allergies, the expansion and activation of regulatory T cells that are able to downregulate allergen-induced responses are suggested. In this context, Wilson et al. reported suppression of allergic airway inflammation in chronic *H. polygyrus* infected mice, which was reversed by treatment with antibodies to CD25 [175]. With regard to *S. mansoni* it has been reported by our group that $Foxp3^+$ Treg cells expand homeostatically during the course of infection and that this phenomenon already started around the fifth

week of infection when the first SEA-specific Th1 responses were detected [66]. Upon induction of allergic airway inflammation significantly elevated numbers of $Foxp3^+$ Treg cells were detected in the lung lymphnodes of infected groups compared to uninfected groups of mice. However, within the lung tissues of infected mice only a small number of $CD3^+Foxp3^+$ Treg cells was present in comparison to uninfected groups. Thus, the conclusion was that during schistosome infection the infiltration of inflammatory cells within the lung is regulated at the site of the draining lung lymphnodes and not within the lung tissue itself upon allergen challenge. In this regard, it will be interesting to decipher how Treg in infected OVA-treated mice are retained in the lung lymphnodes and locally suppress OVA-specific immune responses. Furthermore, besides the expansion of Treg cells during the course of infection, their stronger suppressive capacity might account for the observed dampened reactions against bystander antigens. In previous studies it has been shown that infection-induced Treg but not Treg from naive mice suppressed SEA-specific $CD4^+$ T cell effector responses and developed a unique gene expression profile including upregulation of granzyme B and anti-inflammatory molecules such as secretory leukocyte peptidase inhibitor (SLPI) [63]. This change of phenotype could account for their pronounced suppressive activity since it was shown, for example, that activated Treg cells can actually kill B cells in a granzyme-B-dependent manner [211]. It is now discussed that Treg adapt their mode of immune suppression according to the altered requirements found under inflammatory conditions in comparison to those in the steady-state [212]. The role of these molecules in Treg-mediated suppression of allergies *in vivo* will be an interesting field of future research. In the current study, the specific, asthma-protective role of $Foxp3^+$ Treg cells during infection was assessed by the use of the DEREG mouse model, which allows depletion of Treg cells at the experimenter's desired time-points [196, 213]. This model ensures that all $Foxp3^+$ T cells are depleted regardless of their CD25 co-expression and also circumvents the criticized application of anti-CD25 antibodies in which recently activated effector T cells are targeted as well [214]. Here, Treg depletion during OVA-sensitization in C57BL/6 DEREG mice completely abrogated protection against allergic airway inflammation in *S. mansoni* infected mice (section 3.2.2). In addition, within the lung, cellular infiltration and pathology scores were higher in Treg depleted groups when compared to infected and uninfected OVA-treated control groups. Furthermore, levels of allergic asthma were comparable between infected and uninfected Treg depleted groups. These findings confirm other studies showing the general requirement for Treg cells in allergic airway inflammation during the sensitization phase [88] and downmodulation of IgE level and airway eosinophilia, but not airway hyperresponsiveness in a model of SIT-induced tolerance [214]. In accordance, in the current study OVA-specific IgE levels were enhanced in both depleted groups indicating a

possible role of Treg in initiating Th2 responses. In addition, schistosome-specific immune responses were elevated in infected Treg depleted groups of mice. Furthermore, in these groups egg burden was significantly reduced, which confirms previous findings in schistosome-infected mice treated with anti-CD25 antibody [66]. A delayed reconstitution of expanding Treg cells was noted in infected OVA-treated mice compared to uninfected OVA-treated mice after Treg depletion. Thus, the removal of Treg cells during a chronic infection, in contrast to the steady-state or immunization situation, probably gives rise to more pronounced and quicker Th responses against schistosome antigens. This might favor immune reactions against OVA and eventually results in decelerated or unbalanced Treg cell reconstitution. Collectively, by comparative experiments using different protocols for allergic airway inflammation the present data support recent findings that schistosome infection can indeed suppress allergic airway responses. These findings were expanded by the introduction of ragweed as a naturally occurring model allergen, which further emphasizes the clinical relevance for human asthma. Furthermore, the findings of this study support the idea that schistosome-mediated suppression requires patency and highlight the specific role of expanding $Foxp3^+$ Treg cells that dampen the development of allergic airway inflammation during the sensitization phase. Future work will focus on mechanisms by which helminth-induced Treg cells suppress the sensitization phase, including their effects on T-cell stimulatory activity of DCs upon antigen presentation.

4.2 Helminth infection during pregnancy

4.2.1 Effects on pregnancy rate and success

As mentioned in the chapters before almost one–third of the world's population is infected with at least one helminth and infection during pregnancy is therefore common with prevalence rates from 10-70% in endemic countries [215-217]. Thereof, an estimated 40 million women of child-bearing age are infected with schistosomes, however, accurate numbers of infection rates during pregnancy are not known. It is clear that a poor maternal health status indicated by poor nutritional status or anemia contributes to higher maternal and infant mortality and low birth weight [218] especially in developing countries. Helminth infections can cause both, malnutrition and anemia [219]. Furthermore, most helminth infections induce immunosuppression within the infected host at some stage of disease, which has effects on bystander immune responses such as reactivity to vaccines and allergens but also on the progression of co-infections such as HIV and malaria (4.2.6). Therefore, adverse effects of maternal helminth infection on the course and outcome of

pregnancy are anticipated. However, studies investigating the effect of helminth infection in combination with pregnancy did not confirm these apprehension. Rather, it seems that pregnancy is in itself is an optimally controlled and protected state that primarily hinders overwhelming responses to fetal antigens whilst allowing those necessary to control infections [220]. Nevertheless, large epidemiological or experimental studies are missing to evaluate the consequences of maternal helminth infection during pregnancy with regard to maternal health and fetal development. Furthermore, it is necessary to segregate the findings for each helminth species independently as it is becoming clear that different helminth species exert different effects. For example, a recent systematic review of 19 reports on the impact of hookworm infection on pronounced maternal anemia during pregnancy revealed that infection intensity might be associated with lower hemoglobin levels [221]. Up to now, this relationship has not been found for other helminths. Concerning fetal development and health, different parameters such as abortion rates and birth weight are usually evaluated and have revealed inconclusive results. For example, in a Kenyan study, birth weight remained uninfluenced by maternal helminth infection [222] but was decreased in infants within a crossectional study conducted in Ghana [223]. Besides epidemiological studies in endemic areas, effects of helminth infections on the outcome of pregnancies have been addressed in mouse studies. In the current study, maternal infection with *S. mansoni* revealed that depending on the course of infection, differences occurred in both pregnancy rate and birth weight. In comparison to uninfected mice, the pregnancy rate of 64.6% was significantly decreased to 42.1% in the Th1 phase of infection, but remained comparable in the Th2 phase with a rate of 64.0% and in the Reg phase with 63.0%. This could be explained by the strong Th1 response, elicited during the first weeks of infection, which might disrupt the balance of innate and adaptive immune responses at the maternal-fetal interface and promote recognition and rejection of the developing embryo. Furthermore, the increase of IL-10 levels during the course of *S. mansoni* infection might contribute to the transplacental immune regulation, which is mandatory for a successful pregnancy. *In vitro* and *ex vivo* human and mouse data implicated a role for IL-10 and its effect on Bcl-2 in the fetal-maternal alignment of Treg cells. These findings highlight an important link between the maternal and the fetal immune system during pregnancy and a previously unrecognized role of IL-10 in Treg cell physiology, which might also apply in pregnancies of chronically schistosome infected mothers (section 3.4.1). Interestingly, fetal weight remained comparable between offspring from uninfected, Th1 and Reg phase dams but was significantly reduced in offspring from Th2 phase dams (section 3.3.1). This phenomenon might be associated with the general health status of the female during pregnancy, since infected BALB/c mice showed high morbidity and mortality rates during the designated Th2 phase of infection, which could

have an impact on the offspring's nutrition and pregnancy maintenance. Moreover, many studies investigated a possible association between birth weight and asthma risk. However, low as well as high birth weight have been associated with asthma and atopy and some studies did not find a correlation at all. These controversies were also observed within the current study: low-birth-weight offspring from Th2 infected wildtype mice showed elevated levels of allergic airway inflammation (section 3.3.1), whereas low-birth-weight offspring from VDR knockout mice were protected in this model (section 3.4.2). Conclusively, it is to note that the distinct immune responses during helminth infections affect both, the host and the developing fetus with consequences for pregnancy rate, birth weight and maternal health status.

4.2.2 Effects on fetal responsiveness to homologous and heterologous antigens

Results from a variety of human and mouse studies cleary highlight the possibility that the quality of immune responses against homologous [183, 224] and heterologous [225] antigens might already develop *in utero* or short after birth via breastmilk, through the exposure to maternal helminth infections. These immune responses have been investigated with regard to immunization against worm antigens as well as unrelated vaccine antigens such as Bacillus Calmette-Guerin (BCG) or tetanus toxoid (TT) and allergens. In this regard, Hepatitis B vaccine efficacy in infants born to schistosome infected mothers in Egypt was reduced in one study [226, 227], however no influence was detectable in another study conducted [228] (scheme 1). In line, within a birth cohort in Uganda, responses to Mycobacterium tuberculosis (cCFP) and TT were analyzed in whole blood from one-year-olds. Maternal *Mansonella perstans* infection altered the offsprings' immune responses in association with pronounced IL-10 production to mycobacterial antigens and TT, whereas IFN-γ, IL-5 and IL-13 responses remained unaltered. Other maternal helminth infections showed little effects [229]. In the same cohort deworming with albendazole and praziquantel during pregnancy had no effects on the childrens' responses to BCG and tetanus immunization. Only in infants of hookworm infected mothers albendazole treatment led to a reduced IL-5 and IL-13 production to TT [230]. Further insights are awaited from prospective studies such as the ECUAVIDA birth cohort study in tropical Ecuador, that will assess the impact of geohelminths during the last trimester of pregnancy and in early life on the developing immune responses to heterologous antigens. There, immunity to common vaccines like hepatitis B, tetanus and Haemophilus influenza will be investigated B during the first 5 years of life. Furthermore the development of eczema, allergen skin reactivity and asthma will be followed up [231].

With regard to homologous worm antingens, *in utero* sensitization to schistosome antigens has been detected in offspring from *S. mansoni* and *S. haematobium* infected mothers as measured by immediate and delayed skin responses to parasite antigens [232] or proliferation [233] and cytokine responses [181] of cord-blood mononuclear cells stimulated with schistosome antigens (scheme 1). Antigens from *S. mansoni* have been detected in cord blood, breast milk and urine of infants up to 28 days after delivery [180]. However, the effects of maternal *S. mansoni* infection on the immune responses to heterologous antigens, which might affect allergen susceptibility in the offspring are inconclusive until now. In a recent human study it was demonstrated that children born from schistosome-infected mothers, who were treated with the antihelminthic praziquantel during pregnancy, showed a higher incidence of eczema in infancy, indicating transgenerational allergy-preventing effects of maternal *S. mansoni* infection [187]. The current study expands on these findings, since an essential role for maternal, helminth-driven distinct immune responses that shape the development of allergy susceptibility in the progeny was shown in a natural infection model with the human parasite *S.mansoni* (section 3.3.2). Partial protection against OVA-induced allergic airway inflammation was offered by pregnancy during the Th1 phase and complete protection was provided by pregnancy during the Reg phase of infection. In contrast, pregnancy during the Th2 phase aggravated allergic airway inflammation in the progeny. Interestingly, leukocyte and eosinophil infiltration into the lung, as well as lung pathology score after asthma induction were affected in Th1, Th2 and Reg phase offspring, respectively. In addition, in offspring from mothers mated during the Th1 and Reg phase not only the effector phase of immune responses was suppressed, but also primary sensitization as these mice had reduced OVA-specific IgE antibodies. Explanations for reduced class-switching could comprise a reduced capacity of antigen presentation by APC and/or defective T and B cell interaction which needs to be investigated in depth in the future. These findings could contribute to explain the contradictory results from the previously mentioned epidemiological studies on hepatitis B vaccine responsiveness in offspring from schistosome-infected mothers in Egypt [227, 228], since they hightlight the necessity to consider and evaluate the maternal immune status beforehand.

In the context of maternal influence on the offsprings immune reactivity an interesting aspect is the general role of maternal transfer of parasite antigens which has been found either as immune-complexes via the FcyR at the placental-blood barrier or directly via the mother milk and by enteric resorption. One can assume that parasite antigens, which enter the offsprings' circulation affect the developing immune system in many ways: antigen presentation within the thymus might trigger clonal elimination of T cells, whereas extra-thymical presentation in the secondary lymphoid organs in the absence of

secondary costimulatory signals might favor either T cell anergy or, upon engagement of PD-L1 or CTLA-4 might lead to the induction of peripheral tolerance and regulatory T cells [211]. Furthermore, helminth antigens possess immunomodulatory properties, which were shown to suppress innate immune reponses to TLR ligands *in vitro* [35, 234] and to induce the expansion of Treg cells *in vitro* and *in vivo* [60, 66, 235]. Accordingly, innate immune responses were partly reduced in PBMCs from children in helminth endemic areas [236]. Furthermore, raised levels of proinflammatory cytokines like TNF-α and IL-1β were detected in maternal and cord blood samples as well as in situ in the placentas of women infected with *S. japonicum* during pregnancy [133]. Again, the question whether and how *S. mansoni* antigens, which are transported from infected mothers to the fetus could affect the offsprings' innate immune system in a similar manner remains unanswered. In the current study, placental cytokine production was evaluated upon SEA-restimulation of whole placenta cells in mothers during the Th1, Th2 and Reg phase of infection (section 3.3.3.1). Interestingly, the phase specific peripheral maternal cytokine profile was reflected within the placenta, showing a predominant production of TNF-α in placentas from Th1 mothers, that is counterbalanced by the production of IL-4 during the Th2 phase and completely suppressed during the Reg phase, where IL-10 is the dominant cytokine. Of note, cytokine production was only detectable after schistosome restimulation and not within the steady state. Furthermore, the quality of immune responses differed between the Th2 and Reg phase, although in both phases, schistosome eggs are produced by fecund female worms. These findings point towards a specific role of helminth-induced maternal cells with either inflammatory or immunosuppressive capacity that migrate into the feto-maternal interface. The importance of maternally-derived factors, such as immune cells, cytokines and chemokines or SEA (scheme 1), shown to be transferred via the placenta and breast milk in human and murine pregnancies [180, 184] was therefore investigated by the use of helminth-infected IFN-γ knockout mice, which run in a similar dynamics pertaining to worm development and egg excretion when compared to wildtype mice [197] (section 3.3.4). Acute infection of these mice with *S. mansoni* provided the opportunity to segregate between the role of transferred parasite antigens and maternal IFN-γ release in the transfer of partial protection that has been observed in Th1 offspring. Interestingly, allergic airway inflammation in offspring from infected IFN-γ knockout dams, mated during the acute phase of infection were even enhanced and the level of allergic asthma in these offspring was comparable to offspring from Th2 infected wildtype mice. This leads to the assumption that the immunological switch from Th1 to Th2 response occurs earlier in infected mice, that lack IFN-γ, which is the predominant cytokine during the first weeks of infection. Therefore, the quality of maternal immune responses during pregnancy, rather than the transfer of parasite antigens might shape the

“Th1 offsprings’” susceptibility to allergies. Furthermore, the significance of feto-maternal SEA transfer during the Th2 and Reg phase of infection might be neglected, since “Th2-“ and “Reg-phase offspring” showed different phenotypes in the model of allergic airway inflammation as well as different placental cytokine profiles, even though fecund female worms have been producing eggs from the 5th week of infection onwards. Since none of the currently available knockout strains is specifically deficient for the multiple Th2 cytokines that are present during the Th2 phase of schistosome infection, such as IL-4, IL-5 and IL-13, it was not possible to test directly for the effects of maternally-derived Th2 immune responses on the aggravation of allergic asthma in the offspring. Furthermore, with regard to the Reg phase the use of a IL-10 knockout strain would most likely lead to high morbidity and mortality rates in infected females due to the excessive Th1-and Th1-type immune response, which is sustained during chronic infection leading to lethal immunopathology [68]. In surviving animals, these strong T effector responses might lead to inflammation-induced fetal demice during pregnancy. In this regard, Murphy et al. showed that fetal resorption in IL-10$^{-/-}$ mice was associated with a significant increase in uterine NK cell cytotoxic activation and invasion into the placenta in response to very low doses of LPS [237].

With regard to the development and the regulation of the fetal immune system T and B lymphocytes as well as APCs develop by the end of the first trimester of gestation and the latter can already prime antigen-specific T cells. The program initiated within these cells, however, is not as diverse as within the fully developed adult immune system and differentiation towards a regulatory and Th17 phenotype rather than a Th1 phenotype is favoured [238]. In accordance, differences in proportions of immune cells and innate immune responses within PBMCs were observed between infants born in developed and infants born in developing countries [236]. In our studies we could show that maternal schistosomiasis led to phase-dependent changes within early immune cell composition, including B-, T-, NK-cells and granulocytes with regard to frequency and activation status, which might direct allergic immune responses (scheme 1) (section 3.3.3.2). In this regard, the elevated frequencies of CD4$^+$ and CD8$^+$ T cells found in blood from Th2 offspring might result from a high thymic output und could contribute to the obsereved allergen reactivity in the model of allergic airway inflammation. Thus, analysis of thymus size and T cell development within the thymus might be of interest for further studies. In contrast, the reduced frequency of activated CD4$^+$CD44$^+$ T cells in offspring from Reg phase dams might reflect the reduced allergen-reactivity in the asthma model. However, further experiments are required to determine a potential Th1 or Th2 bias in these offspring by analyzing the capacitiy of naïve T cells to differentiate into Th1 or Th2 effector cells or suppressive Treg cells upon adequate stimulation *in vitro*.

So far, only one other experimental study in mice investigated the influence of maternal *S. mansoni* infection during pregnancy and breastfeeding on the immunity in adult offspring [225]. In this study, Swiss Webster mice were infected with a low dose of 20 cercariae and mated during the 9th week of infection. To determine the role of breastmilk, male offspring from infected or uninfected mice were divided into subgroups and were either suckled by infected or uninfected fosters, resulting in four experimental groups. At the age of six weeks, male offspring were immunized with OVA and alum and hypersensitivity reactions were determined eight days later by footpad swelling after OVA injection. As a result *S. mansoni* infection during pregnancy led to increased immunosuppressive IL-10 mediated potential, which was not observed in offspring that were born from uninfected, but breast-fed by infected mothers. Furthermore, comparison of the two subgroups of offspring that were born from infected mothers and either suckled by infected or uninfected mothers revealed a significant reduction of IL-10 release from OVA restimulated splenocytes of offspring suckled by infected mothers. The authors suggested that suckling by infected mothers induced great responsiveness to an unrelated antigen and abrogated the inhibitory potential. However, no clear results were obtained with regard to immediate and delayed type hyperreactivity. On the basis of the findings of the current study, the different effects that were observed between offspring from infected dams that were either suckled by infected or by uninfected fosters could be explained by the timepoint of mating at the 9th week post infectcion. Since the immune phases have not been determined in infected Swiss Webster mice a switch in the schistosome-induced maternal immune phase could have taken place between experimental pregnancy or/and breastfeeding. Therefore, a detailed analysis of the maternal immune responses in these mice could help to explain and to interprete the results, since our findings suggest that the maternal immune status during schistosomiasis directs the progeny's immune responses to heterologous antigens.

4.2.3 Placental gene expression during infection

Placental gene expression needs to be tightly controlled, since it was shown to influence fetal development for example in a bacterial infection model, where altered gene expression was correlated with intrauterine growth restriction in the progeny [239]. This indicated that changes within placental gene expression due to maternal infection could regulate the development of the fetus (scheme 1). To gain further insights into the effects of schistosome infection during pregnancy, placental gene expression signature was analyzed in response to maternal *S. mansoni* infection (section 3.3.3.1). In our studies microarray analysis revealed a number of genes to be differentially expressed within the placenta due to maternal *S. mansoni* infection. Interestingly, almost all differentially

expressed genes were downregulated in placentas from Th1 and Reg phase dams, whereas the majority of genes was upregulated in Th2 placentas compared to uninfected. Furthermore, there was no overlap in differentially expressed genes between placentas from Th2 mothers and Th1 or Reg phase mothers, respectively. However six genes overlapped between the protective phases (Th1, Reg), namely hydroxy-delta-5-steroid dehydrogenase (Hsd3b1), the vitamin D receptor (VDR), tryptophan hydroxylase 1 (Tph1), estrogen sulfotransferase (Sult1e1), Ly6/PLAUR Domain-Containing Protein 8 (Lypd8) and ring finger protein 223 (Rnf223). With regard to the function of these genes it is suggested that Tph1 catalyzes the synthesis of serotonin in response to placental lactogen and prolactin. Serotonin occurs in a subpopulation of mouse islet beta cells and triggers the production of insulin that is needed to support growth and energy homeostasis of mother and fetus [240]. With regard to Sult1e1, it was shown in a study by Tong et al. [241] that ablation of murine Sult1e1, which catalyzes the inactivation of estrogens, caused placental thrombosis and spontaneous fetal loss. This phenotype was associated with elevated free estrogen levels systemically and in the amniotic fluid, increased tissue factor expression in the placenta and heightened platelet sensitivity to agonist-induced activation ex vivo [242]. In addition, Hsd3b1 immunoreactivity was detected in intermediate trophoblast and syncytiotrophoblast in early placentas and, similar to the VDR, was shown to play an important role in maintaining pregnancy and tolerance against fetal antigens within the placenta [243]. The specific role of Vitamin D in the regulation of placental inflammation and its effect on the development of allergic airway inflammation will be discussed in the next section (4.2.4). With regard to differentially expressed genes within Th2 placentas, the opioid precursor proenkephalin (PENK) should be highlighted, since it was shown in different *in vitro* systems that placental opioid receptors regulate the secretion of acetylcholine [244] and hCG [245] and the release of human placental lactogen [246] from trophoblast tissue. In addition, both PENK gene expression [247] and opioid receptor binding sites [248] change during rodent pregnancy, leading to the suggestion that the opioid system is involved in maternal adaptation to pregnancy and uterine motility in many species including humans. Interestingly, Martinez et al. reported a significant correlation between plasma met-enkephalin levels in newborns and birth weight [249], which was significantly reduced in Th2 offspring in the current study. Furthermore, penk gene expression was shown to be stress-induced [250] and might therefore indicate perinatal stress, which has been associated with reduced birth weight [251] and an increased risk for childhood asthma [252].

In order to build a more complete picture and interprete the genomic data, pathway analyses using Ingenuity software were performed. When comparing the top 15 canonical pathways between Th1, Th2 and Reg phase placentas it became clear that again, there

was an overlap between the protective Th1 and Reg phase. The overlapping pathways, that are rather downregulated, include the serotonin and melatonin biosynthesis, mineralcorticoid biosynthesis, glucocorticoid biosynthesis, Maturity Onset Diabetes of Young (MODY) signaling, and glycolysis I. Most of these pathways contribute to glucocorticoid synthesis and metabolism and the production of sex hormones, such as progestogens and estrogens, which may significantly influence the maintenance of pregnancy and might contribute to the lower pregnancy rate that has been observed in Th1 infected dams. Furthermore, progesterone was shown to favor the development of human Th2 cytokine producing cells as well as the production of IL-4 in established Th1 cell clones [253]. This switch from the Th1 to the Th2 cytokine profile at the maternal-fetal interface is a crucial step that is involved in the maintenance of successful pregnancy [254]. Furthermore, it is known that events that influence glucocorticoid secretion during gestation, such as maternal physical or psychological stress, or maternal under-nutrition or infections, can have significant effects on fetal growth and development which ultimately affects the health and wellbeing of the offspring [255-257]. In this regard, Joachim et al. [260] showed that prenatal stress increased airway hyperresponsiveness, lung eosinophilia and serum IgE level in murine adult offspring. In addition, glucocorticoids may have Th2 promoting effects, since their ability to directly act on Th2 cells by upregulating their IL-4, IL-10 and IL-13 production has been observed and might lead to elevated susceptibility to asthma and allergies [259]. Pathways contributing to sex-hormone synthesis were also affected in Th2 placentas revealing the upregulation of the thyroid hormone metabolism and the biosynthesis of androgen, which is the precursor of estrogens [261]. In contrast, the pathway of androgen biosynthesis was rather downregulated in placentas from the protective Reg phase. However, further studies are needed to investigate the relationship between glucocorticoid or sex hormone synthesis and pregnancy outcome during maternal helminth infection.

Interestingly, in Th2 placentas the pathway analysis revealed further differences in hematopoiesis from multipotent and pluripotent stem cells when compared to naïve placentas. Since hematopoiesis determines the development of the fetal and early postnatal immune system maternal infection might influence the formation of different immune cell subsets, for example T cells with a potential risk for immune-related disorders, such as allergies. Another hint for changes in the hematopoietic system came from the analysis of blood from three weeks old offspring from the Th1, Th2 and Reg phase, which revealed differences within the immune cell composition. Thus, further studies are needed to analyze hematopoietic stem cells during maternal *S.mansoni* infection within the placenta at different timepoints during pregnancy, as well as within the fetal liver and bone marrow.

4.2.4 Specific role of the vitamin D receptor

One of the most interesting candidates that has been tested in this study is the VDR, which was found to be downregulated in placentas from Th1 and Reg phase dams. The biologically active form of Vitamin D is produced in the proximal tubule of the kidney by hydroxylation of calcidiol ($25(OH)D_3$) at position 1 to calcitriol (1,25 dihydroxy-vitamin D ($1,25(OH)_2D_3$)). In addition, the placenta was identified as major site for the conversion into the active form of vitamin D, with both maternal decidua and fetal trophoblast showing activity of the obligatory enzyme 1α-hydroxylase (CYP27B1) [262, 263]. The hormonal activity of calcitriol is mediated by binding to the steroid hormone receptor VDR, which undergoes conformational changes leading to heterodimerization with the retinoid X receptor (RXR) and the interaction of the dimeric complex with vitamin D response elements (VDREs) located in promoter regions. Thereby, the transcription of target genes, involved in calcium metabolism and in the immune system is controlled [264]. The VDR is expressed by many types of immune cells including circulating monocytes [265], macrophages [266], DCs [267], B cells [268] and activated T cells. In T cells it has been shown that calcitriol stimulates Th2 responses by increased transcription of IL-5 and IL-4. In contrast, Th1 responses were decreased. Furthermore, calcitriol was associated with the development of IL-10 producing Treg cells and a decreased Th17 differentiation. Within the placenta vitamin D plays an important role in controlling fetal-placental immune responses during pregnancy. Collectively, autocrine metabolism of calcidiol into the active form calcitriol promotes antibacterial and anti-inflammatory responses in maternal decidua [271] and fetal trophoblast [272, 273], whereas dysregulated vitamin D metabolism (CYP27B1 knockout) or function (VDR knockout) in fetal cells from the placenta promotes inflammatory responses to immune challenge. According to the physiological demand $1,25(OH)_2D_3$ downregulates its own biosynthesis by repressing the 25-hydroxyvitamin D-1α-hydroxylase gene [274, 275] and increases its metabolism by upregulating the 24-hydroxylase gene through VDR-dependent actions [276, 277]. Treatment *in vitro* with $1,25(OH)_2D_3$ was shown to suppress IFN-γ production by primary murine $CD4^+$ T cells [278]. Thus, downregulation of the VDR on different immune cells, including T cells may lead to overabundance of proinflammatory Th1 cytokines and a decrease in Th2 responses. The resulting Th1 bias within the offspring might counterbalance Th2 responses such as allergies and asthma later in life. In mice, no experimental studies are available investigating the effects of maternal VDR deficiency and allergen susceptibility in the offspring. However, asthma-induced VDR knockout mice failed to develop airway inflammation, eosinophilia or airway hyperresponsiveness, despite high levels of IgE and Th2 cytokines [198]. To test for the exclusive effect of maternal VDR on the allergen

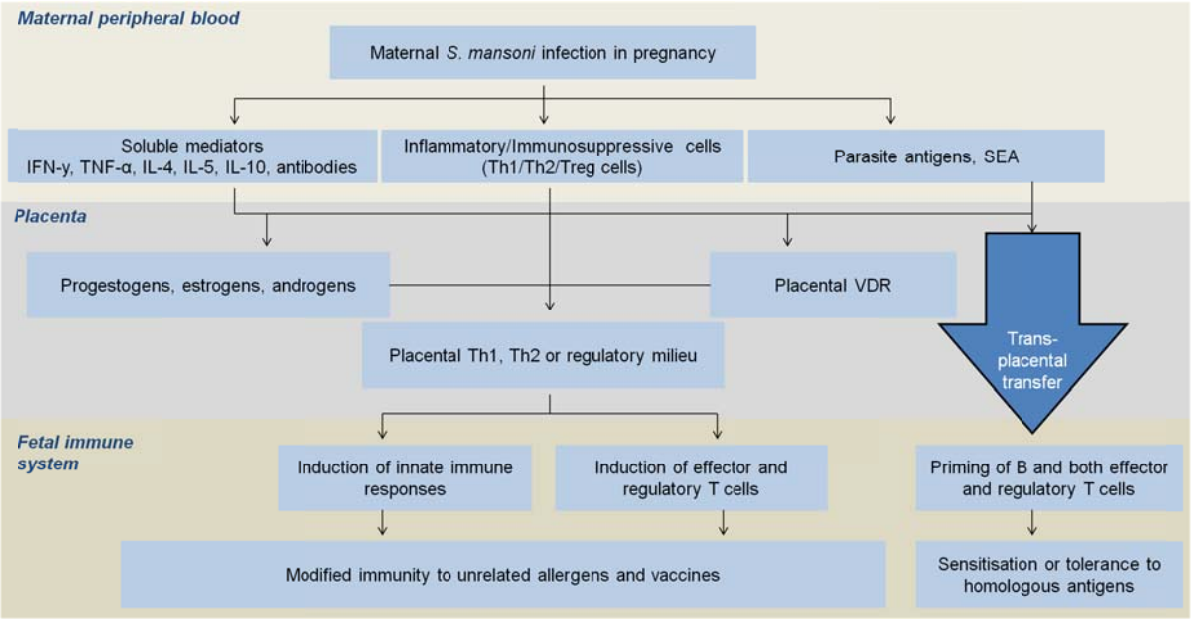
susceptibility in the offspring, $VDR^{-/-}$ females on the C57BL/6 background were mated with wildtype C57BL/6 males or vice versa to generate $VDR^{+/-}$ offspring (section 3.4). The experiment in the current study resulted in decreased leukocyte and eosinophil infiltration into the lung as well as reduced goblet cell formation in lungs of offspring from $VDR^{-/-}$ females upon induction of OVA-induced allergic airway inflammation. Furthermore, cytokine production of splenocytes upon stimulation with OVA *in vitro* revealed no differences between IL-5 and IL-10, but elevated IFN-γ levels, confirming the suggested Th1 bias due to the lack of maternal VDR. However, despite the special rescue diet, which is provided for $VDR^{-/-}$ mice, general health condition as well as pregnancy rate and success is extremely low and further experiments are currently in progress to confirm these interesting results. Furthermore, it is to note that the mating combination is a crucial factor when interpreting pregnancy-related results from murine experimental studies, since syngeneic matings do not trigger maternal alloreactive lymphocytes, which might lead to misevaluation and underestimation of investigated factors with regard to the immune system during pregnancy. Therefore, we used the allogeneic mating combination C57BL/6xBALBc since it promoted recognition of the developing fetal cells by the maternal immune system during pregnancy, in contrast to syngeneic pregnancies [279].
Interestingly, conflicting results are obtained from human studies investigating the effects of vitamin D supplementation during pregnancy and the allergy development in the offspring. A birth cohort study that investgated the effects of maternal diet showed an inverse correlation between maternal vitamin D intake during pregnancy and the development of asthma and allergic rhinitis in offsrpring at the age of 5 years [269]. However, a cohort study from the United Kingdom showed that maternal exposure to higher concentrations of vitamin D during pregnancy (>75 nmol/l) was associated with an increased risk of asthma in children at 9 years of age [270]. When interpreting these results on the basis of the findings presented in the current study, one could speculate that the dose of vitamin D, supplemented during pregnancy, might differentially affect VDR expression on placental immune cells, with the potential to induce either Th1 or Th2 immune responses, that might affect allergen susceptibility in the offspring.

4.2.5 Transgenerational epigenetic inheritance

When evaluating the effects of environmental factors, such as maternal helminth infection during pregnancy, one has to consider transgenerational epigenetic effects, which are either mediated by environmental exposure that alter the offspring's phenotype via the placenta or via breast milk or the induction of epigenetic marks of the mature gametes

with the potential to contribute to the phenotype of the progeny. Per definition, the latter refers to effects on phenotype that are passed from one generation to the next by molecules in germ cells that cannot be explained by changes to the primary DNA sequence [170]. First evidence for epigenetic marks that escape reprogramming in early developing embryos came from the discovery of parental imprinting of the mouse insulin-like growth factor II (IGF2) gene [280] and the mouse H19 gene [281]. The monoallelic expression of these genes is dependent on the parent-of-origin of the allele, since either the maternal allele is expressed exclusively because the paternal allele is imprinted (repressed) or vice versa. The process begins during gamete formation when in males certain genes are imprinted in the developing sperm and in females, other genes are imprinted in the developing egg. The maternal and paternal alleles of these genes are in different transcriptional states in the cells of the adult. This implies that different epigenetic states are established in the germline of the parents, and are inherited via the gametes [170]. In addition, first evidence for epigenetic inheritance via the gametes across more than one generation was based on studies that reported that the transgenerational activity of some transgenes was variable among inbred littermates and the likelyhood of activity was inheritable to some extent [282-284]. One example for an endogenous allele at which transgenerational inheritance via the germline has been shown in mice is agouti viable yellow (A^{vy}), which determines the portion of yellow colour in the fur. Interestingly, inbred mice, that carry this allele show differences in fur colour [285] ranging from yellow to mottled and pseudoagouti (brown). This was dependent on environmental factors (maternal methyl-rich diet [286]) that influence DNA methylation state of the promoter elements [287] which control transcription of the *agouti* coding sequence in the early post-implantation embryo [288]. To discriminate between gametic and non-gametic inheritance down the maternal line, cross-foster experiments of embryo transfer to non-exposed dams is required. Focusing on the Th1 phase of maternal helminth infection we performed *in vitro* fertilization of eggs from Th1 mated mothers, fostered by uninfected BALB/c females to test for a germline-based inheritance of partial protection. As a result, partial protection against OVA-induced allergic airway inflammation, which had been observed in offspring born and fostered by Th1 mothers, was lost in offspring derived from *in vitro* fertilized eggs from Th1 infected females, when fostered by uninfected mothers. These findings indicate that partial protection against allergic airway inflammation in “Th1 offspring” is not transferred via the germline. They further strengthen the previous findings that the quality of the maternal immune response during pregnancy and breastfeeding directs allergen susceptibility in the offspring, which might induce epigenetic effects, that alter the offspring’s phenotyp via the placenta or breast milk. Recently published studies support the idea that asthma protection could be mediated epigenetically by maternal exposure to

environmental microbes since the asthma-protective effect of *A. lwoffi* exposure during pregnancy was correlated to increased histone acetylation of the IFN-γ promoter in the $CD4^+$ T cell compartment of the offspring [126]. However, these effects were transient and clear evidence for a causal relationship is still missing. In this regard, further studies are required to test for schistosome-induced epigentic marks in the offspring transferred via placenta and breast milk and the stability of this phenotype across more than one generation.



Scheme 1: Epidemiologic and experimental evidence for modified immunity to unrelated allergens and vaccines and to homologous antigens in offspring from schistosome infected mothers.

4.2.6 Perspectives in deworming policies in the context of co-infections

Experimental *S. mansoni* infection of mice is widely used to model the pathophysiologic features of the infection and the elicited immune responses in the host. However, when interpreting results obtained from experimental studies, several aspects have to be considered such as the high intensity of experimental schistosome infection and the single exposure of cercariae in mice compared to the recurrent exposure that is observed in humans in endemic areas. Nevertheless, some results from this study are closely related to the human condition in endemic areas: protection against allergic airway inflammation was most pronounced in offspring from dams mated during the regulatory phase of infection and was mediated by *S. mansoni*-induced maternal immune responses. Similarly, the majority of women in endemic areas will be chronically schistosome infected at child-bearing age, which might contribute to the local low prevalence of asthma and allergies in children. Accordingly, a higher risk for infantile eczema was reported after

antihelminthic treatment during pregnancy. However, in the context of deworming policies in endmic areas possible effects of maternal helminth infections on the course and intensity of co-infections such as HIV transmission (MTCT) and placental malaria need to be considered. In a retrospective study of pregnant Kenyan women, investigators found an increased risk for mother-to-child-transmission of HIV if women were infected with one or more helminths. The helminths detected were lymphatic filariasis, schistosomiasis and geohelminthes. The increased MTCT risk also correlated with IL-5 and IL-13 production from helminth-antigen stimulated cord blood cells [289]. Mechanstically, *in utero* lymphocyte activation e.g. by transplacental helminth antigens and upregulation of HIV co-receptors on $CD4^+$ T cells might propagate transmission by enhanced uptake in the fetus as it has been shown before in adults [290]. In helminth endemic areas co-infection rate with malaria is high and placental malaria (PM) occurs in up to 25% of pregnant women in areas of stable transmission, causing up to 200,000 disease-related infant deaths. PM leads to global reduction of antibody transfer especially that of the IgG1 isotype [291-293]. This has been shown specifically for antibodies against RSV, Varicella, HSV and vaccine-associated antibodies against measles and tetanus toxoid. The underlying mechanisms have not been elucidated but potentially include downregulation of FcyR expression in the inflamed placenta or competition of high titers of specific IgG at the feto-placental interface [294]. Whether or not schistosome-mediated placental infection aggravates these findings remains to be determined. Furthermore, as already mentioned, helminth infections during pregnancy are known to be associated with adverse outcomes, including maternal anemia, low birth weight, and perinatal mortality. Deworming during pregnancy has therefore been strongly advocated and in 2002 the World Health Organization recommended the treatment with praziquantel during pregnancies in areas, where schistosomiasis is endemic. Nevertheless, its benefits and possible disadvantages for mother and child have not been rigorously evaluated up to now.

5 Literature

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Publications

Fetal-maternal alignment of regulatory T cells correlates with Interleukin 10 and Bcl-2 upregulation in pregnancy

Brigitte Santner-Nanan[*,1], **Kathrin Straubinger**[†,1], **Peter Hsu**[*,1], Grant Parnell[*], Ben Tang[*], Bei Xu[‡], Angela Makris[‡], Annemarie Hennessy[‡,§], Michael J. Peek[*], Dirk H. Busch[†,¶], Clarissa Prazeres da Costa[†] and Ralph Nanan[*,2]

[1]these authors contributed equally to this work

[*]Sydney Medical School Nepean, The University of Sydney, Kingswood 2751, New South Wales, Australia,[†]Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich 81675, Germany,[‡]Heart Research Institute, The University of Sydney, Newtown, New South Wales, Australia,[§]University of Western Sydney, School of Medicine, Campbelltown 2560, New South Wales, Australia,[¶]Clinical Cooperation Groups ''Antigen-specific Immunotherapy'' and "Immune Monitoring", Helmholtz Center Munich (Neuherberg) and Technische Universität München, Munich 81675, Germany

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Schistosoma mansoni-mediated Suppression of Allergic Airway Inflammation Requires Patency and Foxp3$^+$ Treg Cells

Laura E. Layland[1,2,§], **Kathrin Straubinger**[1,§], Manuel Ritter[1], Eva Loffredo-Verde[1], Holger Garn[3], Tim Sparwasser[4], Clarissa Prazeres da Costa*[1]

[§] - shared first authorship

[1]Institute of Medical Microbiology, Immunology and Hygiene (MIH), Technische Universität München, Munich, Germany; [2]Institute of Medical Microbiology, Immunology and Parasitology (IMMIP), University Clinic Bonn, Bonn, Germany; [3]Institute of Laboratory Medicine and Pathobiochemistry, Medical Faculty, Philipps-University Marburg, Marburg, Germany; [4]Institut für Infektionsimmunologie TWINCORE - Zentrum für Experimentelle und Klinische Infektionsforschung GmbH, Hannover, Germany.

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Different immune phases of maternal helminth infection have distinct effects on allergic immune responses in the offspring

Kathrin Straubinger[1], Manuel Ritter[1], Olivia Prazeres da Costa[1], Thorsten Buch[1], Dirk H. Busch[1], Laura E. Layland[3*] and Clarissa U. Prazeres da Costa[1*].

[1]Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Munich, Germany;[2] Institute for Medical Microbiology, Immunology and Parasitology (IMMIP), University Clinic Bonn, Bonn, Germany

Manuscript in preparation

Impact of Helminth Infections on our Immune System edited by William Horsnell.
Book chapter entitled **"Maternal Helminth Infections"**

Kathrin Straubinger[1] and Clarissa Prazeres da Costa[1]

[1]Institute for Medical Microbiology, Immunology and Hygiene, Technische Universität München, Trogerstrasse 30, 81675 München

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Poster presentation at the 40th Annual Meeting of the German Society for Immunology (DGfI), September 22-25, 2012, Leipzig, Germany

Oral presentation at "Doktorandenretreat Angerer Alm", SFB/Tr22, February 2010 and 2012, St. Johan in Tirol, Austria

Oral presentation at the 15th Symposium "Infektion und Immunabwehr" oft he FG Infektionsimmunologie der DGHM and AK Infektionsimmunologie der DGfI, March 10-13, 2011, Burg Rothenfels, Germany

Oral presentation at the European respiratory society (ERS) research seminar "Developmental origins of chronic lung disease, February 24-26 2012, Spitzingsee, Germany

Oral presentation at the joint International Congress of the American Society for Reproductive Immunology (ASRI) and the European Society for Reproductive Immunology (ESRI), 31 May – 2 June 2012, Hamburg, Germany, Journal of Reproductive Immunology 94 (2012) 1-4

Oral presentation at the Munich Minisymposia in Immunology, February 21st 2013, Munich, Germany

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