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Immune modulation by schistosomes : mechanisms of T helper 2 polarization and implications for metabolic disorders

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GENERAL INTRODUCTION

Based on:
Priming dendritic cells for Th2 polarization:
lessons learned from helminths and
implications for metabolic disorders

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1. IMMUNE RESPONSES

Mammalian immune responses are a result of interplay between the non-specific innate immune system and the adaptive immune system. The innate immune system provides a first line of defense, while the adaptive immune system is antigen-specific and required for long-term protection against infections. CD4⁺ T helper (Th) are members of the adaptive immune system that regulate immunity and inflammation through the secretion of specific cytokines. Different classes of pathogens require activation of specific Th cell subsets, and immune responses are therefore often classified based on the central Th cell subsets involved.

In case of rapidly replicating microorganisms, such as bacteria and viruses, an antimicrobial type 1 immune response is invoked. The principal regulators of the type 1 immune response are Th1 cells, which secrete the pro-inflammatory cytokine interferon- γ (IFN- γ). IFN- γ stimulates macrophage activation, antigen uptake and presentation, and intracellular killing of microbes. The type 2 immune response, on the other hand, encompasses the host response to parasitic worms (also known as helminths), and is characterized by the presence of Th2 cells, which secrete interleukin-4 (IL-4), IL-5 and IL-13 to mediate B cell activation and IgE antibody production. Furthermore, type 2 immune responses are characterized by an expanding group of innate immune cells, including eosinophils, mast cells, group 2 innate lymphoid cells, and alternatively activated macrophages. Together, these cells control infection and/or mediate parasite expulsion through smooth muscle contraction and mucus production (reviewed in (1;2)). In addition to Th1 and Th2 cells, different T helper cell subsets have been discovered over the past ten years, including Th17 which protect against protect against extracellular bacteria and fungi, and Th22 and Th9 cells, which have a range of functional activities (3).

Aside from anti-helminth immunity, it has become increasingly clear that type 2 immune responses have additional functions. For example, literature has described a close association between type 2 immune responses and wound repair (4-7). Strikingly, recent evidence indicates that multiple facets of the type 2 immune response can also regulate metabolism and protect against insulin resistance. For just one example, the prototypical Th2 cytokine IL-4 can regulate the balance between fatty acid and glucose oxidation in hepatocytes (8). Conversely, pro-inflammatory immune responses have been shown to participate in the pathogenesis of diet-induced diabetes (9;10). By studying the molecular mechanisms that govern helminth-induced Th2 polarization, we may therefore learn valuable lessons for both the protection against helminth infection and pathways involved in metabolic regulation.

2. AN INTRODUCTION TO SCHISTOSOMES

Schistosomes are helminths frequently used to study T helper 2 polarization, not only because of their prevalence, but also because of their ability to infect both mice and humans. Schistosomes are the causative agent of schistosomiasis and chronically infect over 200 million people worldwide (11). There are five species of schistosomes, of which *Schistosoma japonicum*, *S. mansoni*, and *S. haematobium* are the most common. The life cycles of these three

schistosomes are largely comparable. Briefly, when infected individuals urinate or defecate in water, eggs are excreted that hatch to release miracidiae, which can penetrate the fresh water snail. In the snail, the miracidiae develop into sporocysts, which generate cercariae that can infect the human host. Upon infection, the cercariae lose their tail and become schistosomulae, which enter circulation and migrate through several tissues to the hepatic portal vein, where the mature male and female worms form pairs. Schistosome pairs then migrate to the mesenteric veins (*S. japonicum* and *S. mansoni*), or to the venous plexus of the bladder (*S. haematobium*), where the female starts to produce hundreds of eggs per day. The eggs penetrate through the tissues to the intestine or the bladder, and are again released with feces or urine. Not all eggs go through this process though. Depending on the species, the blood flow carries many eggs to the liver, where they induce granuloma formation, or to the bladder, where they can promote bladder cancer (11).

Since mice are highly susceptible to *S. mansoni* infection, the host immune response to this species has been the most widely studied (12). The first weeks after infection, during schistosomulae migration, are characterized by a T helper 1 (Th1) response. After 5-6 weeks post infection, with the onset of egg deposition in the liver and intestines, the immune response changes. The Th1 component decreases and a strong egg-specific Th2 response, characterized by IL-4, IL-5 and IL-13 cytokine expression, develops (11). The development of the Th2 response is essential for reducing morbidity of the host by excessive Th1-like inflammatory reactions: wild type (C57BL/6) mice enter a chronic phase of egg accumulation 8 weeks post infection, however IL-4-deficient mice die of uncontrolled Th1-associated inflammatory reactions to parasites (13).

Although it is clear that the induction of Th2 responses serves an important host-protective role during the initial stage of infection, persistent type 2 cytokine responses may also result in immunopathology and morbidity (14;15). Egg-induced granuloma formation is detrimental to the host as it is associated with the induction of IL-13-dependent liver fibrosis. As such, schistosome-infected mice in which IL-13 is blocked fail to develop liver fibrosis, which leads to prolonged survival of these mice (16-18). Therefore, to prevent Th2-associated damage to the host, controlling the Th2 response is at least equally important as its generation. Th2 cell control becomes visibly active around week 12 post infection, when the chronic phase of infection emerges. Egg production continues but the Th2 response diminishes and newly formed liver granulomas have a smaller size than those formed at earlier times during infection (11). At this stage, control of the Th2 response is provided by regulatory T cells (Treg cells) (19;20), alternatively activated macrophages (7;21), and regulatory B cells (22).

3. DENDRITIC CELLS AND T HELPER 2 POLARIZATION

The mechanisms that initiate the Th2 response in helminth infection are still not fully understood, although it is clear that dendritic cells (DCs) (23), the most efficient antigen-presenting cells (APCs) in the immune system, play a crucial role (24). DCs are located in peripheral tissues, where they continuously sample the environment to capture antigens from invading microbes. Upon recognition of pathogen-associated molecular patterns (PAMPs), DCs

undergo phenotypic changes that allow them to migrate to the lymph nodes and to provide the signals required for the activation of T cells (25;26). The importance of DCs in Th2 skewing is highlighted by studies showing that depletion of CD11c⁺ DCs interferes with the induction of a Th2 response to *S. mansoni* and *Helimosomoides polygyrus* (27-29). Interestingly, it has become increasingly clear that distinct DC subsets induce different Th responses (reviewed in (24;30)), and in the last few years, several studies analyzed the role of DC subsets in the initiation of Th2 responses to helminth infection.

3.1 Dendritic cell subsets associated with Th2 polarization

Two independent groups recently showed that the development of a Th2 response to *Nippostrongylus brasiliensis* depends on dermal CD301b⁺ DCs (31;32). Specifically, depletion of CD301b⁺ DCs prior to infection reduces IL-4 production by CD4⁺ T cells, without affecting the percentage of T follicular helper (Tfh) cells or germinal center B cells (31). Mechanistically, Th2-inducing PDL2⁺CD301b⁺ DCs were shown to depend on DC-specific expression of the transcription factor interferon regulatory factor 4 (IRF4) (32). In line with these findings, CD11c^{int}MHCII^{hi} dermal DCs expressing PDL2 and CD301b were also identified as a Th2-priming DC subset in *N. brasiliensis* infection (33). Of note, CD301b⁺ DCs alone are insufficient to generate a Th2 response *in vitro* (32) or *in vivo* (31), suggesting that additional requirements exist. For example, optimal localization of DCs within the lymph node may play a crucial role. In *H. polygyrus* infection, CXCR5-expressing CD11c⁺ DCs migrate to the lymph node and localize adjacent to B cell follicles (34). Depletion of CXCR5 or B cell-derived lymphotoxin alters the localization of the DCs and, as a consequence, impairs the development of Tfh and Th2 cells (34). In addition, it has been suggested that DCs require signals from basophils (35) and group 2 innate lymphoid cells (ILC2s) (36) to prime Th2 responses to allergens. Together, these studies suggest that specific DC subsets, as well as the microenvironment in which these subsets encounter CD4⁺ T cells, are important for Th2 development *in vivo*.

3.2 Sensing helminth-derived antigens

DCs are equipped with pattern recognition receptors (PRRs) that recognize a wide array of PAMPs. The classical paradigm describes that triggering of PRRs, including the Toll-like receptors (TLRs), RIG-I-like receptors, NOD-like receptors, scavenger receptors, and C-type lectin receptors (CLRs), induces DC maturation and subsequent antigen-specific activation of T helper cells (37).

While signaling through most TLRs induces Th1/Th17 responses (38), Th2-inducing helminth-derived molecules have also been described to interact with DCs through TLR2, 3 and 4 (39-42). Although the schistosome-related glycan LNFPIII, which contains Lewis X (Le^x) trisaccharides, requires TLR4 for Th2 skewing (43), various studies suggest that TLRs are dispensable for Th2 polarization by helminth antigens. For example, bone marrow-derived DCs (BMDCs) from TLR2- and TLR4-knockout mice can still skew Th2 when pulsed with *S. mansoni* soluble egg antigens (SEA) (44), and the TLR adaptor protein MyD88 is not required for Th2 skewing by SEA-stimulated splenic DCs (45). Interestingly, human monocyte-derived dendritic cells (moDCs) stimulated

with phosphatidylserine lipids from schistosomes induce IL-10-producing T cells through TLR2 (40). Therefore, helminth products may employ TLRs for the induction of regulatory responses, but it seems that other PRRs are required for the initiation of a Th2 response.

Indeed, CLRs that sense helminth glycans play an important role in Th2 skewing. For example, SEA is internalized by moDCs through DC-specific ICAM-3-grabbing nonintegrin (DC-SIGN), macrophage galactose-type lectin (MGL) and mannose receptor (MR) (46), and binds to Dectin-2 on BMDCs (47). Binding of SEA to DC-SIGN was shown to depend on Le^x (48), and a recent study showed that blocking DC-SIGN-associated signaling inhibits Th2 skewing (49). Likewise, excretory/secretory products from the tapeworm *Taenia crassiceps* (TcES) bind MR and MGL on BMDCs (50), and the Th2-skewing capacity of TcES is glycan-dependent (51). In sum, these studies indicate that helminth-derived antigen preparations can bind a variety of PRRs, which may induce distinct intracellular events that promote Th2 polarization.

3.3 Intracellular mechanisms associated with Th2 polarization

PRR-mediated signaling classically induces DC maturation via mitogen-activated protein kinases (MAPK) (52). However, in contrast to microbial ligands, helminth products often fail to induce classical signs of maturation and are well-known to downregulate TLR-mediated maturation (46;53-59). Indeed, unlike many TLR ligands, Th2-inducing compounds fail to phosphorylate p38 MAPK but instead promote phosphorylation of p42/p44 MAPK (ERK1/2) (reviewed in (60)). ERK1/2 stabilizes c-Fos, and inhibiting either c-FOS or ERK1/2 enhances IL-12 production by moDCs (61), suggesting that activation of this pathway suppresses Th1-polarizing cytokines. Likewise, TSLP promotes ERK1/2 phosphorylation (62) and fails to induce IL-12 production by myeloid DCs (63;64).

It was noted that the NF- κ B signaling pathway also seems involved in Th2 polarization, as SEA- or LNFPIII-stimulated BMDCs from NF- κ B1 knockout mice fail to prime a Th2 response (65;66). Furthermore, it was recently demonstrated that Le^x residues, via DC-SIGN, activate LSP1 in moDCs, leading to nuclear accumulation of the atypical NF- κ B family member Bcl3 and downregulation of IL-12 mRNA. These events also seem required for SEA-induced T cell polarization, since silencing either LSP1 or Bcl3 interferes with Th2 skewing (49). Similarly, the Th2-inducing capacity of TSLP was shown to involve activation of NF- κ B and STAT5 (62;67).

Lastly, SEA can signal through spleen tyrosine kinase (Syk) downstream of Dectin-2, activating the Nlrp3 inflammasome and increasing TLR-triggered release of IL-1 β by BMDCs. However, infection of various inflammasome-deficient mice with *S. mansoni* demonstrated that activation of this pathway does not seem to favor any particular Th response (47). Thus, helminth antigens can activate signaling, and certain members of the NF- κ B and ERK pathways in particular seem to play a role in Th2 polarization.

In addition to signaling-dependent mechanisms, a number of studies identified a role for cysteine protease inhibitors secreted by filarial nematodes (cystatins) in regulating host immune responses by interfering with antigen processing (reviewed in (68)). Therefore, helminths may employ both signaling-dependent and independent mechanisms to condition DCs for Th2 skewing (**figure 1**).

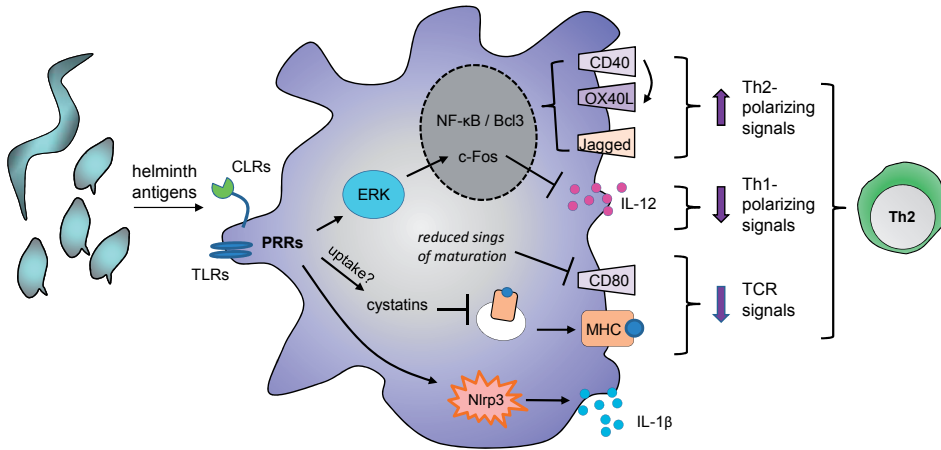


Figure 1. Possible mechanisms by which helminth molecules modulate DCs for Th2 polarization. Helminth antigens are recognized by DCs through ligation of pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and C-type lectin receptors (CLRs). Depending on the antigen, binding promotes phosphorylation of ERK1/2, nuclear accumulation of NF-κB or Bcl3, and/or activation of the Nlrp3 inflammasome which mediates IL-1β secretion. Phosphorylation of ERK1/2 stabilizes c-Fos, leading to downregulation of IL-12 expression. In addition, DCs can upregulate expression of Th2-associated CD40 and Jagged, which are under the control of NF-κB and ERK1/2, respectively (74;75). Upon encounter of T cells expressing CD40L, signaling through CD40 promotes OX40L expression in an autocrine manner. Alternatively, PRRs may mediate uptake of antigens that interfere with antigen presentation on MHCs, such as cystatins. In addition, helminth products fail to upregulate classical markers of maturation. Altogether, these events favor DC-mediated Th2 polarization.

3.4 Primed DCs and initiation of T cell polarization

A major difference between Th1 and Th2 development is that a Th1 response requires persistent production of Th1-polarizing cytokines, like IL-12, which are exclusively produced by APCs. By contrast, once primed DCs induce IL-4 production by a few activated T helper cells, the Th2 response is self-sustained through autocrine production of IL-4 (69;70). Therefore, in order to understand mechanisms of Th2 polarization, it is critical to identify the DC-associated polarizing signals that control early IL-4 production by activated T cells.

3.4.1 Soluble factors and surface molecules

As discussed above, DCs stimulated with helminth molecules or TSLP fail to express IL-12. Moreover, injection of IL-12 can block the development of a Th2 response to *S. mansoni* eggs (71). These findings led to the so-called 'default concept', which states that Th2 differentiation spontaneously occurs in the absence of a Th1-priming signal like IL-12. However, mice lacking IL-12 do not develop a Th2 response to microbial pathogens (72), and blocking the mTOR pathway in moDCs skews a potent Th2 response without affecting IL-12 (73), suggesting that there are active signals involved in Th2 differentiation.

Such a signal may be provided by a soluble factor secreted by DCs, like RELM α , which was shown to promote IL-10 and IL-13 secretion by lymph node cells following adoptive transfer of SEA-stimulated BMDCs (74). However, supernatants from SEA-primed moDCs do not skew towards Th2 (75), and SEA-stimulated BMDCs do not induce Th2 when separated from CD4⁺ T cells in transwells (76), indicating that an active polarizing signal in these studies is likely provided by surface molecules. Indeed, the Notch ligands Delta-4 and Jagged-2 have been linked to Th1 and Th2 polarization, respectively (77), and helminth antigens were shown to upregulate Jagged-2 on BMDCs (78;79) and to suppress Delta-4 expression in moDCs (80). However, Jagged-2-deficient BMDCs can still skew Th2 when challenged with SEA (78;79), suggesting that other molecules may be involved. For example, CD40 has been suggested to provide a polarizing signal, as its expression on SEA-stimulated BMDCs is required for the induction of a Th2 response (81), and mice lacking CD40 ligand suffer from impaired Th2 development during *S. mansoni* infection (82). Mechanistically, signaling through CD40 promotes OX40L expression, which is essential for optimal Th2 skewing by SEA-conditioned BMDCs (83) and moDCs (75), as well as TSLP-conditioned myeloid DCs (64). However, treatment with anti-OX40L does not significantly affect the Th2 response to *N. brasiliensis* infection (84), and it has been suggested that OX40L acts as a costimulatory molecule rather than a polarizing signal, since SEA-treated OX40L-knockout DCs induce Th2 cells, but fail to stimulate appropriate T cell expansion (83). Altogether, these studies suggest that there may not be one specific DC-associated molecule required for Th2 polarization, but rather a combination of signals that mediate both optimal T cell priming and expansion.

3.4.2 A role for the T cell receptor

Early reports have described that the antigen dose can determine the outcome of Th differentiation, with a high dose generally favoring Th1 development (85-87). These findings were confirmed in a recent report, which also indicated that Th1-inducing adjuvants promote a higher Ca²⁺ flux (representing T cell receptor (TCR)-signaling strength), and induce larger synapse size, than Th2-promoting molecules (88). In addition, it has been suggested that T cells activated by Th2-inducing ligands are less proliferative, as priming of splenic DCs with SEA reduces the frequency of CD4⁺ T cells progressing through the cell cycle, and drug-induced arrest of cell cycle progression promotes Th2 polarization (45). Together, these observations suggest that helminth molecules may reduce TCR triggering, impairing T cell proliferation in favor of Th2 differentiation. Indeed, treatment of splenic DCs with SEA results in shorter T cell-DC interaction times and lower TCR signaling when compared to a Th1-inducing adjuvant (88). Omega-1, a glycosylated identified as the major component in SEA (55), was also shown to reduce the capacity of BMDCs to form T cell-DC conjugates and to diminish the frequency of CD4⁺ T cells progressing through the cell cycle (76). Mechanistically, interaction between T cells and DCs was shown to depend at least in part on the co-stimulatory molecule CD80 (88). As discussed above, helminth products fail to induce upregulation of costimulatory molecules, which may also explain why DCs treated with helminth molecules are less capable of forming stable interactions with T cells.

4. IMPLICATIONS FOR METABOLIC DISORDERS

A growing body of literature indicates that obesity is associated with chronic low-grade inflammation of metabolic organs, in particular adipose tissue. In healthy adipose tissue, a wide variety of immune cell types play an important role in housekeeping, removal of apoptotic cells, and maintenance of homeostasis (89). However, fat accumulation results in chemokine secretion by adipocytes, attracting classically activated M1 macrophages that secrete pro-inflammatory cytokines like interleukin (IL)-1 β and tumor necrosis factor- α (TNF- α) (10;90;91). These cytokines interfere with insulin signaling (92;93) and induce lipolysis (94;95), thereby increasing circulating free fatty acids which promote peripheral insulin resistance (96). In addition to M1 macrophages, other pro-inflammatory immune cells have been associated with insulin resistance, including Th1 cells, Th17 cells, CD8⁺T cells and B lymphocytes (97) (**figure 2**).

By contrast, alternatively activated macrophages, also called M2 macrophages, prevail in lean white adipose tissue (WAT) and are involved in the maintenance of adipose tissue insulin sensitivity, partly through secretion of the anti-inflammatory cytokine IL-10 (10;98). The M2 phenotype is promoted by Th2-type cytokines IL-4, IL-5 and IL-13, which were shown to be secreted by WAT eosinophils (99) and ILC2s (100). In addition, various reports have shown that Th2-inducing conditions, such as *N. brasiliensis* infection (99;101), allergic inflammation (8), or SEA administration (102), improve insulin sensitivity and glucose tolerance in diet-induced obese mice. Furthermore, both *S. mansoni* infection (103) and SEA administration (104) reduce

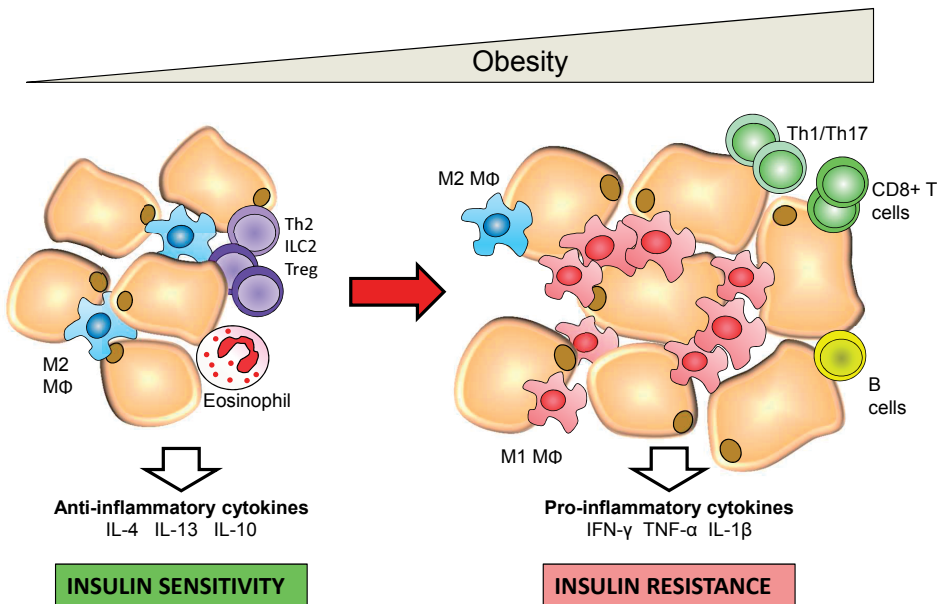


Figure 2. Obesity and inflammation of adipose tissue. As obesity develops, the expanding adipose tissue promotes the transition from an anti-inflammatory to a pro-inflammatory state.

the development of atherosclerotic lesions in mice, and adoptive transfer of CD4⁺T cells (mostly via Th2 cells) and IL-4 treatment can protect against diet-induced insulin resistance (8;105). Lastly, type 2-associated ILC2s (100;106) and eosinophils (99) were shown to play a crucial role in maintenance of whole-body metabolic homeostasis by sustaining adipose tissue alternatively activated M2 macrophages. These findings are in line with epidemiological studies indicating that infection with helminths inversely correlates with metabolic syndrome (107;108). These landmark studies identify the interplay between helminths and energy metabolism as an exciting new area that needs further dissection.

5. SCOPE OF THE THESIS

Over the last few decades, a wide array of studies have shed light on the possible mechanisms by which helminth molecules condition DCs for Th2 skewing. Nevertheless, due to the complex nature of many helminth-derived antigen preparations, it proved difficult to pinpoint specific receptors and/or mechanism involved. Therefore, the identification of omega-1, a glycosylated RNase, as the major immunomodulatory component in SEA has provided us with a powerful tool to further dissect the molecular mechanisms underlying Th2 polarization (55;76). The first part of this thesis centers on the following question:

How does omega-1 modulate dendritic cells for T helper 2 polarization?

Chapter 2 studies the molecule omega-1, and analyzes the requirement of glycosylation and RNase activity in the modulation of DCs for Th2 polarization, *in vitro* and *in vivo*.

In **chapter 3** we study the role of the mTOR pathway in the induction of Th2 responses by moDCs stimulated with SEA and omega-1.

Chapter 4 further characterizes moDCs primed for Th2 polarization by SEA or omega-1 using a mass spectrometry-based approach.

The second part of this thesis builds on landmark studies showing that type 2 inflammatory responses protect against metabolic disorders. These studies have identified the interplay between helminths and metabolic homeostasis as an exciting new area that needs further dissection. We focus on the following question:

*What are the effects of chronic *S. mansoni* infection and SEA administration on metabolic homeostasis?*

In **chapter 5**, we study the effects of chronic *Schistosoma mansoni* infection and SEA treatment on whole-body glucose homeostasis and insulin sensitivity in a mouse model of diet-induced

obesity. We perform in-depth metabolic profiling and analyze the immune cell composition of metabolic organs.

The MR is a marker for M2 macrophages, and was identified in Chapter 2 as the main receptor responsible for internalization of omega-1 by moDCs. In **chapter 6**, we therefore study the effect of HFD-feeding on metabolic homeostasis and immune cell polarization in mice deficient for the MR.

Chapter 7 summarizes the importance of our findings within the larger body of literature, and provides directions for future research towards understanding the link between schistosomes, Th2 polarization and metabolic disorders.

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