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. heamatobium* 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. heamatobium*), 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-κB signaling pathway also seems involved in Th2 polarization, as SEA- or LNFPIII-stimulated BMDCs from NF-κB1 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-κB 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-κB 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-1β 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-κB 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**).

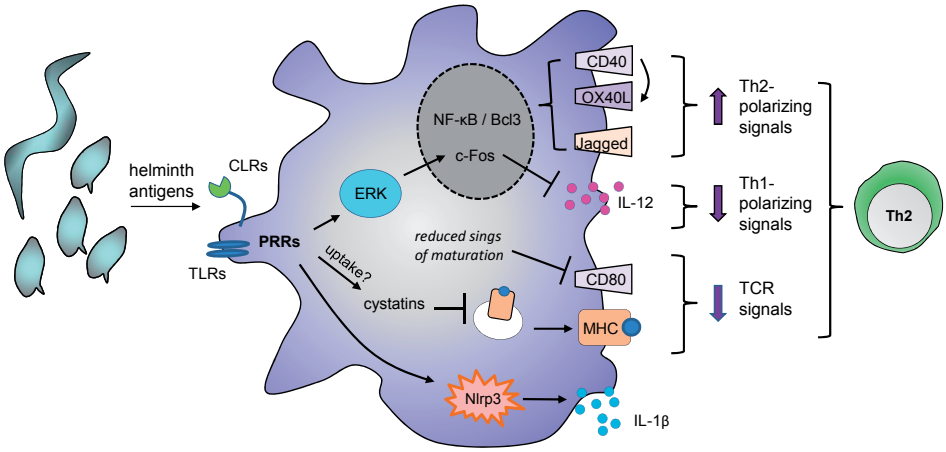

Figure 1. Possible mechanisms by which helminth molecules modulate DCs for Th2 polarization. Helminth antigens are recognized by DCs through ligation of pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and C-type lectin receptors (CLRs). Depending on the antigen, binding promotes 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 RELMα, 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 Ca^{2+} 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)-1β 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

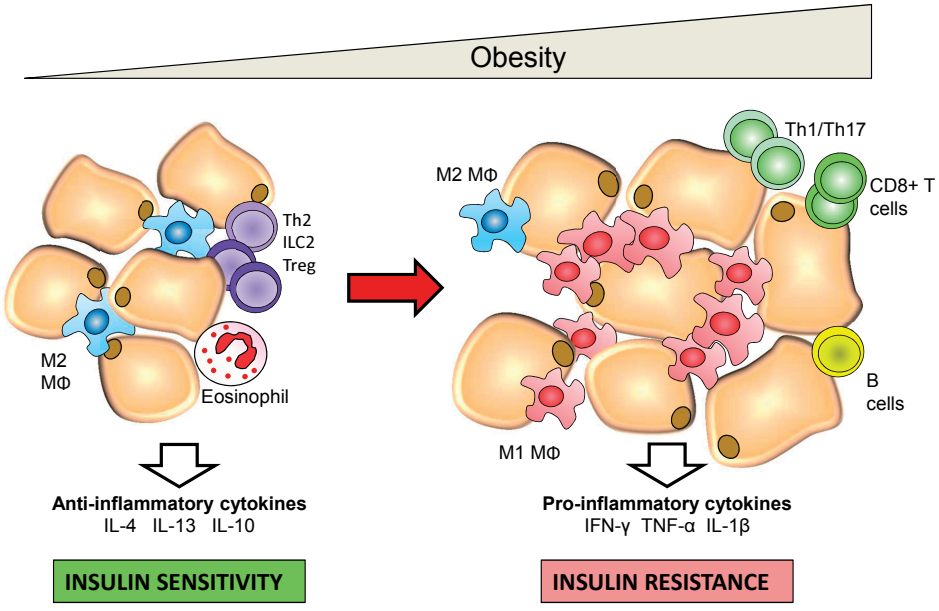

Figure 2. Obesity and inflammation of adipose tissue. As obesity develops, the expanding adipose tissue promotes the transition from an anti-inflammatory to a pro-inflammatory state.

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.

REFERENCES

1. Spellberg B, et al. Type 1/Type 2 immunity in infectious diseases. *Clin Infect Dis* 2001 Jan;32(1):76-102.
2. Gause WC, et al. Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. *Nat Rev Immunol* 2013 Aug;13(8):607-14.
3. Tong ZH, et al. Subpopulations of helper T lymphocytes in tuberculous pleurisy. *Tuberculosis (Edinb)* 2013 May;93(3):279-84.
4. Chen F, et al. An essential role for TH2-type responses in limiting acute tissue damage during experimental helminth infection. *Nat Med* 2012 Feb;18(2):260-6.
5. Monticelli LA, et al. Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus. *Nat Immunol* 2011 Nov;12(11):1045-54.
6. Herbert DR, et al. Arginase I suppresses IL-12/IL-23p40-driven intestinal inflammation during acute schistosomiasis. *J Immunol* 2010 Jun 1;184(11):6438-46.
7. Pesce JT, et al. Arginase-1-expressing macrophages suppress Th2 cytokine-driven inflammation and fibrosis. *PLoS Pathog* 2009 Apr;5(4):e1000371.
8. Ricardo-Gonzalez RR, et al. IL-4/STAT6 immune axis regulates peripheral nutrient metabolism and insulin sensitivity. *Proc Natl Acad Sci U S A* 2010 Dec 28;107(52):22617-22.
9. Donath MY, et al. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol* 2011 Feb;11(2):98-107.
10. Chawla A, et al. Macrophage-mediated inflammation in metabolic disease. *Nat Rev Immunol* 2011 Nov;11(11):738-49.
11. Pearce EJ, et al. The immunobiology of schistosomiasis. *Nat Rev Immunol* 2002 Jul;2(7):499-511.
12. Wilson MS, et al. Immunopathology of schistosomiasis. *Immunol Cell Biol* 2007 Feb;85(2):148-54.
13. Brunet LR, et al. IL-4 protects against TNF-alpha-mediated cachexia and death during acute schistosomiasis. *J Immunol* 1997 Jul 15;159(2):777-85.
14. Hoffmann KF, et al. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. *J Immunol* 2000 Jun 15;164(12):6406-16.
15. Wynn TA. Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases. *J Clin Invest* 2007 Mar;117(3):524-9.
16. Chiaramonte MG, et al. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J Clin Invest* 1999 Sep;104(6):777-85.
17. Fallon PG, et al. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J Immunol* 2000 Mar 1;164(5):2585-91.
18. Jankovic D, et al. Schistosome-infected IL-4 receptor knockout (KO) mice, in contrast to IL-4 KO mice, fail to develop granulomatous pathology while maintaining the same lymphokine expression profile. *J Immunol* 1999 Jul 1;163(1):337-42.
19. Baumgart M, et al. Naturally occurring CD4+Foxp3+ regulatory T cells are an essential, IL-10-independent part of the immunoregulatory network in Schistosoma mansoni egg-induced inflammation. *J Immunol* 2006 May 1;176(9):5374-87.
20. Hesse M, et al. The pathogenesis of schistosomiasis is controlled by cooperating IL-10-producing innate effector and regulatory T cells. *J Immunol* 2004 Mar 1;172(5):3157-66.
21. Maizels RM, et al. Regulation of pathogenesis and immunity in helminth infections. *J Exp Med* 2009 Sep 28;206(10):2059-66.
22. Hussaarts L, et al. Regulatory B-cell induction by helminths: implications for allergic disease. *J Allergy Clin Immunol* 2011 Oct;128(4):733-9.
23. Steinman RM, et al. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* 1973 May 1;137(5):1142-62.
24. Pulendran B, et al. Programming dendritic cells to induce T(H)2 and tolerogenic responses. *Nat Immunol* 2010 Aug;11(8):647-55.
25. Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol* 2003 Dec;3(12):984-93.
26. Palucka K, et al. Cancer immunotherapy via dendritic cells. *Nat Rev Cancer* 2012 Apr;12(4):265-77.

27. Phythian-Adams AT, et al. CD11c depletion severely disrupts Th2 induction and development in vivo. *J Exp Med* 2010 Sep 27;207(10):2089-96.
28. Smith KA, et al. Type 2 innate immunity in helminth infection is induced redundantly and acts autonomously following CD11c(+) cell depletion. *Infect Immun* 2012 Oct;80(10):3481-9.
29. Smith KA, et al. Chronic helminth infection promotes immune regulation in vivo through dominance of CD11cloC. *J Immunol* 2011 Jun 15;186(12):7098-109.
30. Briseno CG, et al. Complementary diversification of dendritic cells and innate lymphoid cells. *Curr Opin Immunol* 2014 Aug;29:69-78.
31. Kumamoto Y, et al. CD301b(+) dermal dendritic cells drive T helper 2 cell-mediated immunity. *Immunity* 2013 Oct 17;39(4):733-43.
32. Gao Y, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity* 2013 Oct 17;39(4):722-32.
33. Connor LM, et al. Helminth-Conditioned Dendritic Cells Prime CD4+ T Cells to IL-4 Production In Vivo. *J Immunol* 2014 Sep 15;193(6):2709-17.
34. Leon B, et al. Regulation of T(H)2 development by CXCR5+ dendritic cells and lymphotoxin-expressing B cells. *Nat Immunol* 2012 Jul;13(7):681-90.
35. Tang H, et al. The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling. *Nat Immunol* 2010 Jul;11(7):608-17.
36. Halim TY, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity* 2014 Mar 20;40(3):425-35.
37. Kapsenberg ML. Dendritic-cell control of pathogen-driven T-cell polarization. *Nat Rev Immunol* 2003 Dec;3(12):984-93.
38. Iwasaki A, et al. Regulation of adaptive immunity by the innate immune system. *Science* 2010 Jan 15;327(5963):291-5.
39. Aksoy E, et al. Double-stranded RNAs from the helminth parasite Schistosoma activate TLR3 in dendritic cells. *J Biol Chem* 2005 Jan 7;280(1):277-83.
40. van der Kleij D, et al. A novel host-parasite lipid cross-talk. Schistosomal lyso-phosphatidylserine activates toll-like receptor 2 and affects immune polarization. *J Biol Chem* 2002 Dec 13;277(50):48122-9.
41. Goodridge HS, et al. Immunomodulation via novel use of TLR4 by the filarial nematode phosphorylcholine-containing secreted product, ES-62. *J Immunol* 2005 Jan 1;174(1):284-93.
42. Correale J, et al. Helminth antigens modulate immune responses in cells from multiple sclerosis patients through TLR2-dependent mechanisms. *J Immunol* 2009 Nov 1;183(9):5999-6012.
43. Thomas PG, et al. Maturation of dendritic cell 2 phenotype by a helminth glycan uses a Toll-like receptor 4-dependent mechanism. *J Immunol* 2003 Dec 1;171(11):5837-41.
44. Kane CM, et al. Schistosoma mansoni egg antigen-mediated modulation of Toll-like receptor (TLR)-induced activation occurs independently of TLR2, TLR4, and MyD88. *Infect Immun* 2008 Dec;76(12):5754-9.
45. Jankovic D, et al. Parasite-induced Th2 polarization is associated with down-regulated dendritic cell responsiveness to Th1 stimuli and a transient delay in T lymphocyte cycling. *J Immunol* 2004 Aug 15;173(4):2419-27.
46. van Liempt E, et al. Schistosoma mansoni soluble egg antigens are internalized by human dendritic cells through multiple C-type lectins and suppress TLR-induced dendritic cell activation. *Mol Immunol* 2007 Apr;44(10):2605-15.
47. Ritter M, et al. Schistosoma mansoni triggers Dectin-2, which activates the Nlrp3 inflammasome and alters adaptive immune responses. *Proc Natl Acad Sci U S A* 2010 Nov 23;107(47):20459-64.
48. van Die I, et al. The dendritic cell-specific C-type lectin DC-SIGN is a receptor for Schistosoma mansoni egg antigens and recognizes the glycan antigen Lewis x. *Glycobiology* 2003 Jun;13(6):471-8.
49. Gringhuis SI, et al. Fucose-specific DC-SIGN signaling directs T helper cell type-2 responses via IKKepsilon- and CYLD-dependent Bcl3 activation. *Nat Commun* 2014;5:3898.
50. Terrazas CA, et al. Helminth-excreted/secreted products are recognized by multiple receptors on DCs to block the TLR response and bias Th2 polarization in a cRAF dependent pathway. *FASEB J* 2013 Nov;27(11):4547-60.
51. Terrazas CA, et al. Impaired pro-inflammatory cytokine production and increased Th2-

biasing ability of dendritic cells exposed to Taenia excreted/secreted antigens: A critical role for carbohydrates but not for STAT6 signaling. *Int J Parasitol* 2010 Aug 1;40(9):1051-62.

52. Arthur JS, et al. Mitogen-activated protein kinases in innate immunity. *Nat Rev Immunol* 2013 Sep;13(9):679-92.
53. Kane CM, et al. Helminth antigens modulate TLR-initiated dendritic cell activation. *J Immunol* 2004 Dec 15;173(12):7454-61.
54. Cervi L, et al. Cutting edge: dendritic cells copulsed with microbial and helminth antigens undergo modified maturation, segregate the antigens to distinct intracellular compartments, and concurrently induce microbe-specific Th1 and helminth-specific Th2 responses. *J Immunol* 2004 Feb 15;172(4):2016-20.
55. Everts B, et al. Omega-1, a glycoprotein secreted by Schistosoma mansoni eggs, drives Th2 responses. *J Exp Med* 2009 Aug 3;206(8):1673-80.
56. Rigano R, et al. Echinococcus granulosus antigen B impairs human dendritic cell differentiation and polarizes immature dendritic cell maturation towards a Th2 cell response. *Infect Immun* 2007 Apr;75(4):1667-78.
57. Balic A, et al. Selective maturation of dendritic cells by Nippostrongylus brasiliensis-secreted proteins drives Th2 immune responses. *Eur J Immunol* 2004 Nov;34(11):3047-59.
58. Segura M, et al. Impairment of dendritic cell function by excretory-secretory products: a potential mechanism for nematode-induced immunosuppression. *Eur J Immunol* 2007 Jul;37(7):1887-904.
59. Brannstrom K, et al. The Schistosoma mansoni protein Sm16/SmSLP/SmSPO-1 assembles into a nine-subunit oligomer with potential To inhibit Toll-like receptor signaling. *Infect Immun* 2009 Mar;77(3):1144-54.
60. Everts B, et al. Helminths and dendritic cells: sensing and regulating via pattern recognition receptors, Th2 and Treg responses. *Eur J Immunol* 2010 Jun;40(6):1525-37.
61. Agrawal S, et al. Cutting edge: different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J Immunol* 2003 Nov 15;171(10):4984-9.
62. Arima K, et al. Distinct signal codes generate dendritic cell functional plasticity. *Sci Signal* 2010;3(105):ra4.
63. Soumelis V, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002 Jul;3(7):673-80.
64. Ito T, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. *J Exp Med* 2005 Nov 7;202(9):1213-23.
65. Artis D, et al. Dendritic cell-intrinsic expression of NF-kappa B1 is required to promote optimal Th2 cell differentiation. *J Immunol* 2005 Jun 1;174(11):7154-9.
66. Thomas PG, et al. A helminth glycan induces APC maturation via alternative NF-kappa B activation independent of I kappa B alpha degradation. *J Immunol* 2005 Aug 15;175(4):2082-90.
67. Bell BD, et al. The transcription factor STAT5 is critical in dendritic cells for the development of TH2 but not TH1 responses. *Nat Immunol* 2013 Apr;14(4):364-71.
68. Hartmann S, et al. Modulation of host immune responses by nematode cystatins. *Int J Parasitol* 2003 Sep 30;33(11):1291-302.
69. Dong C, et al. Cell fate decision: T-helper 1 and 2 subsets in immune responses. *Arthritis Res* 2000;2(3):179-88.
70. Jankovic D, et al. Mechanisms underlying helminth- induced Th2 polarization: default, negative or positive pathways? *Chem Immunol Allergy* 2006;90:65-81.
71. Oswald IP, et al. IL-12 inhibits Th2 cytokine responses induced by eggs of Schistosoma mansoni. *J Immunol* 1994 Aug 15;153(4):1707-13.
72. Jankovic D, et al. In the absence of IL-12, CD4(+) T cell responses to intracellular pathogens fail to default to a Th2 pattern and are host protective in an IL-10(-/-) setting. *Immunity* 2002 Mar;16(3):429-39.
73. Hussaarts L, et al. Rapamycin and omega-1: mTOR-dependent and -independent Th2 skewing by human dendritic cells. *Immunol Cell Biol* 2013 Aug;91(7):486-9.
74. Cook PC, et al. Alternatively activated dendritic cells regulate CD4+ T-cell polarization in vitro and in vivo. *Proc Natl Acad Sci U S A* 2012 Jun 19;109(25):9977-82.
75. de Jong EC, et al. Microbial compounds selectively induce Th1 cell-promoting or Th2

cell-promoting dendritic cells in vitro with diverse th cell-polarizing signals. *J Immunol* 2002 Feb 15;168(4):1704-9.

76. Steinfelder S, et al. The major component in schistosome eggs responsible for conditioning dendritic cells for Th2 polarization is a T2 ribonuclease (omega-1). *J Exp Med* 2009 Aug 3;206(8):1681-90.
77. Amsen D, et al. Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell* 2004 May 14;117(4):515-26.
78. Krawczyk CM, et al. Th2 differentiation is unaffected by Jagged2 expression on dendritic cells. *J Immunol* 2008 Jun 15;180(12):7931-7.
79. Worsley AG, et al. Dendritic cell expression of the Notch ligand jagged2 is not essential for Th2 response induction in vivo. *Eur J Immunol* 2008 Apr;38(4):1043-9.
80. van Riet E, et al. Combined TLR2 and TLR4 ligation in the context of bacterial or helminth extracts in human monocyte derived dendritic cells: molecular correlates for Th1/Th2 polarization. *BMC Immunol* 2009;10:9.
81. MacDonald AS, et al. Cutting edge: Th2 response induction by dendritic cells: a role for CD40. *J Immunol* 2002 Jan 15;168(2):537-40.
82. MacDonald AS, et al. Impaired Th2 development and increased mortality during Schistosoma mansoni infection in the absence of CD40/CD154 interaction. *J Immunol* 2002 May 1;168(9):4643-9.
83. Jenkins SJ, et al. Dendritic cell expression of OX40 ligand acts as a costimulatory, not polarizing, signal for optimal Th2 priming and memory induction in vivo. *J Immunol* 2007 Sep 15;179(6):3515-23.
84. Connor LM, et al. Helminth-Conditioned Dendritic Cells Prime CD4+ T Cells to IL-4 Production In Vivo. *J Immunol* 2014 Aug 8;
85. Constant S, et al. Extent of T cell receptor ligation can determine the functional differentiation of naive CD4+ T cells. *J Exp Med* 1995 Nov 1;182(5):1591-6.
86. Hosken NA, et al. The effect of antigen dose on CD4+ T helper cell phenotype development in a T cell receptor-alpha beta-transgenic model. *J Exp Med* 1995 Nov 1;182(5):1579-84.
87. Boonstra A, et al. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. *J Exp Med* 2003 Jan 6;197(1):101-9.
88. van Panhuys N, et al. T-Cell-Receptor-Dependent Signal Intensity Dominantly Controls CD4(+) T Cell Polarization In Vivo. *Immunity* 2014 Jul 17;41(1):63-74.
89. Schipper HS, et al. Adipose tissue-resident immune cells: key players in immunometabolism. *Trends Endocrinol Metab* 2012 Aug;23(8):407-15.
90. Weisberg SP, et al. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003 Dec;112(12):1796-808.
91. Xu H, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 2003 Dec;112(12):1821-30.
92. Hotamisligil GS, et al. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 1996 Feb 2;271(5249):665-8.
93. Hotamisligil GS, et al. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993 Jan 1;259(5091):87-91.
94. Feingold KR, et al. Stimulation of lipolysis in cultured fat cells by tumor necrosis factor, interleukin-1, and the interferons is blocked by inhibition of prostaglandin synthesis. *Endocrinology* 1992 Jan;130(1):10-6.
95. Green A, et al. Tumor necrosis factor increases the rate of lipolysis in primary cultures of adipocytes without altering levels of hormone-sensitive lipase. *Endocrinology* 1994 Jun;134(6):2581-8.
96. Rosen ED, et al. Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* 2006 Dec 14;444(7121):847-53.
97. Mraz M, et al. The role of adipose tissue immune cells in obesity and low-grade inflammation. *J Endocrinol* 2014 Sep;222(3):R113-R127.
98. Lumeng CN, et al. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 2007 Jan;117(1):175-84.
99. Wu D, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* 2011 Apr 8;332(6026):243-7.

100. Molofsky AB, et al. Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J Exp Med* 2013 Mar 11;210(3):535-49.

101. Yang Z, et al. Parasitic nematode-induced modulation of body weight and associated metabolic dysfunction in mouse models of obesity. *Infect Immun* 2013 Jun;81(6):1905-14.

102. Bhargava P, et al. Immunomodulatory glycan LNFPIII alleviates hepatosteatosis and insulin resistance through direct and indirect control of metabolic pathways. *Nat Med* 2012 Nov;18(11):1665-72.

103. Doenhoff MJ, et al. An anti-atherogenic effect of Schistosoma mansoni infections in mice associated with a parasite-induced lowering of blood total cholesterol. *Parasitology* 2002 Nov;125(Pt 5):415-21.

104. Wolfs IM, et al. Reprogramming macrophages to an anti-inflammatory phenotype by helminth antigens reduces murine atherosclerosis. *FASEB J* 2014 Jan;28(1):288-99.

105. Winer S, et al. Normalization of obesity-associated insulin resistance through immunotherapy. *Nat Med* 2009 Aug;15(8):921-9.

106. Hams E, et al. Cutting edge: IL-25 elicits innate lymphoid type 2 and type II NKT cells that regulate obesity in mice. *J Immunol* 2013 Dec 1;191(11):5349-53.

107. Aravindhan V, et al. Decreased prevalence of lymphatic filariasis among diabetic subjects associated with a diminished pro-inflammatory cytokine response (CURES 83). *PLoS Negl Trop Dis* 2010;4(6):e707.

108. Chen Y, et al. Association of previous schistosome infection with diabetes and metabolic syndrome: a cross-sectional study in rural China. *J Clin Endocrinol Metab* 2013 Feb;98(2):E283-E287.