

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/35155> holds various files of this Leiden University dissertation.

**Author:** Hussaarts, Leonie

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

**Issue Date:** 2015-09-10



SUMMARIZING DISCUSSION

7



## WHAT WAS ALREADY KNOWN ABOUT TH2 POLARIZATION, HELMINTHS, AND METABOLIC DISORDERS?

Helminths are the strongest natural inducers of type 2 immune responses, and many advances in dissecting the mechanisms underlying Th2 polarization have been made using either models of helminth infection or helminth-derived products. It is now recognized that helminth molecules can interact with a variety of receptors on dendritic cells (DCs), including TLRs and CLRs, which either bind or internalize antigens to condition DCs for Th2 skewing via different mechanisms. For example, various reports have indicated that signaling through members of the NF- $\kappa$ B and ERK pathways seem to play a role in Th2 polarization. In addition, filarial-derived cystatins were suggested to regulate immune cell polarization via signaling-independent mechanisms, by interfering with antigen processing. In terms of DC-derived polarizing signals, up- or downregulation of various soluble factors and/or surface molecules was shown to contribute to Th2 skewing. Furthermore, various reports have described a role for T cell-DC interactions and the T cell receptor (TCR) in T cell skewing. Specifically, Th1-inducing antigens were shown to promote stronger T cell-DC interactions than Th2-inducing antigens (reviewed in (1)).

Over the past five years, the mechanisms underlying Th2 polarization have received accelerating interest, since it is now recognized that multiple facets of the Th2-associated immune response are involved in metabolic regulation (2). For example, IL-4 can regulate the balance between fatty acid and glucose oxidation in hepatocytes (3), and a negative association between metabolic syndrome and helminth infection has been reported (reviewed in (4)). Furthermore, using mouse models of *Nippostrongylus brasiliensis* infection, a rodent nematode spontaneously cleared within two weeks, it has been demonstrated that infected mice on a high-fat diet (HFD) are protected from glucose intolerance, associated with increased white adipose tissue (WAT) eosinophilia and the expression of M2-related genes (5;6). In a follow-up

### Summary of what was already known

- Helminth-derived molecules can bind a variety of receptors on DCs, including TLRs and CLRs
- Helminth-derived molecules can modulate DCs for Th2 polarization via signaling-dependent and -independent mechanisms
- Th2 skewing by DCs seems to require upregulation of Th2-polarizing signals and / or downmodulation of Th1-polarizing signals
- Th1-inducing antigens promote stronger T cell-DC interactions than Th2-inducing antigens
- *N. brasiliensis* infection protects mice against HFD-induced insulin resistance and glucose intolerance, increases adipose tissue ILC2s and eosinophils, and promotes the expression of M2-related genes in WAT

study, *N. brasiliensis* was shown to promote accumulation of adipose tissue eosinophils via the induction of group 2 innate lymphoid cells in mice on a chow diet (7). These landmark studies are a major contribution to the field, and highlight the interplay between helminths and metabolic disorders as an exciting area that needs further dissection.

## HOW DID OUR STUDIES ADVANCE THE FIELD?

### **Mechanisms of Th2 polarization: lessons from studying omega-1**

Using *Schistosoma mansoni* soluble egg antigens (SEA), it had been described that schistosome antigens can modulate DCs for Th2 polarization by signaling through p42/p44 MAPK (ERK1/2), which lowers IL-12 production and suppresses Th1 polarization (8). Likewise, it was recently suggested that nuclear accumulation of the atypical NF- $\kappa$ B family member Bcl3 is required for SEA-induced Th2 polarization by downregulating IL-12 mRNA (9). By studying omega-1 in **chapter 2**, we describe a signaling-independent mechanism through which schistosomes can suppress Th1-polarizing signals. Omega-1, a glycosylated T2 ribonuclease (RNase) secreted by *S. mansoni* eggs, was previously identified as the major immunomodulatory component in SEA (10;11). Using recombinant mutants of omega-1 and a co-culture model of human monocyte-derived DCs and allogeneic naïve T cells, we demonstrate that omega-1 requires both its glycosylation and its RNase activity to condition DCs for Th2 polarization. Mechanistically, omega-1 is bound by its glycans and subsequently internalized via the mannose receptor (MR), after which the molecule impairs protein synthesis by degrading both ribosomal and messenger RNA. Interestingly, various Th2-inducing allergens are also RNases (12;13), as well as the endogenous eosinophil-derived neurotoxin that can amplify DC-mediated Th2 polarization (14). Together, these reports suggest that any RNase that ends up in the cytosol of DCs may harbor Th2-priming capacities, through cleavage of ribosomal and/or messenger RNA (1). By doing so, RNases may inhibit the production of IL-12 and other Th1-inducing molecules like Delta-4 (15).

It has been proposed that the mere absence of IL-12 and other Th1-inducing molecules promotes a Th2 response (16), but the results presented in **chapter 3** argue against this hypothesis. In this chapter, we studied the role of the mTOR pathway in T helper polarization by moDCs, building on various reports that suggest involvement of mTOR in T helper cell differentiation (17;18). We show that omega-1 and SEA skew Th2 independent of the mTOR pathway, and additionally demonstrate that blocking the mTOR pathway in LPS-matured moDCs using rapamycin induces a profound Th2 response. These data, together with our finding that co-stimulation of moDCs with rapamycin and helminth antigens results in an additive effect on Th2 cytokine production, led us to conclude that there are mTOR-dependent and -independent mechanisms for Th2 skewing. Interestingly, we also show that rapamycin, unlike SEA and omega-1, skews a very potent Th2 response without affecting LPS-induced IL-12. These data complement early findings showing that mice lacking IL-12 do not develop a Th2 response to microbial pathogens (19), and suggest that there are active signals involved in Th2 differentiation.

In **chapter 4**, we therefore searched for Th2-associated polarizing signals in the DC proteome, since maturation of DCs is largely controlled at the posttranscriptional and posttranslational level (20;21). We employed LC-FTICRMS, a high-throughput gel-free technique for accurate mass measurement and relative quantitation (22), to analyze proteomes of monocyte-derived DCs from nine different donors. We found that SEA and omega-1 strongly increase relative expression of 60S acidic ribosomal protein P2 (RPLP2), which we speculate represent a feedback mechanism secondary to SEA- and omega-1-induced ribosome degradation. In addition, SEA and omega-1 decreased expression of HLA-B, involved in MHC class I-dependent antigen presentation, and CD44, a surface molecule that was previously shown to promote CD4 T cell proliferation by mediation calcium signaling (23). Decreases in HLA-B and CD44 may suggest that Th2-inducing conditions interfere with efficient antigen presentation to T cells. Indeed, both SEA and omega-1 decreased expression of proteins in a protein network enriched for antigen processing and presentation, which supports the hypothesis that weak interaction between T cells and DCs at the level of the immunological synapse may contribute to Th2 polarization by helminth antigens. We also performed GeneMANIA analysis (24), which predicts associations of input genes and related genes, with less stringent thresholds. Analysis of 6h-stimulated samples suggests that SEA and omega-1 may affect cellular glucose metabolism. However, these results are speculative and need experimental confirmation.

Of note, at the start of the LC-FTICRMS project, we hoped to identify novel Th2-associated protein networks. However, we confidently identified only few Th2-associated differentially expressed proteins, which mostly confirmed previously established hypotheses. Because of time and money investments required for an LC-FTICRMS study, we therefore propose that this method is most suitable for screening of samples in which strong effects on proteomes are expected.

In conclusion, our work on DCs and T cell polarization has shed light on the requirements for Th2 skewing by omega-1, which needs both its glycosylation and its RNase activity. We further show that omega-1 does not engage the mTOR pathway to skew T cells, even though blocking mTOR using rapamycin enables DCs to polarize a very potent Th2 response. Lastly, our proteomics study revealed that omega-1 promotes an increase in relative expression of RPLP2, a ribosomal protein, while downregulating proteins involved in antigen presentation. Together, these results highlight that there may be multiple routes for Th2 skewing, involving both signaling-dependent and -independent mechanisms. Follow-up studies will guide design of drugs that reprogram the innate immune system to induce a type 2-associated immune response, which would be desirable in the context of active type 1-mediated metabolic disorders such as obesity and type 2 diabetes.

### **Helminths and high-fat diet-induced metabolic disorders**

**Chapter 5** builds on landmark studies showing that conditions that induce type 2 inflammatory responses improve metabolic disorders. We demonstrate that chronic infection with *S. mansoni* protects against high fat diet-induced weight gain, insulin resistance and glucose intolerance in mice. We further show that infection does not affect energy expenditure, but enhances

peripheral glucose uptake and adipose tissue insulin signaling. These effects were associated with the induction of white adipose tissue (WAT) eosinophilia and M2 polarization. Before our study, the effect of helminths on metabolic homeostasis had only been reported in the context of infection with *N. brasiliensis*, a natural nematode of rodents, and little was studied beyond whole-body glucose tolerance and insulin sensitivity (5;6). Our study on chronic infection with *S. mansoni* thus strengthens and complements these findings, and advances the field by providing data on a wide array of metabolic parameters in the context of a helminth that chronically infects millions of people worldwide (25).

In addition, **chapter 5** describes that the beneficial effect of *S. mansoni* infection can be recapitulated in a pathogen-free setting, through repetitive injections with SEA. Specifically, SEA protected against HFD-induced insulin resistance and glucose intolerance without affecting body weight. Although the beneficial effect of SEA injections on glucose and insulin tolerance had been described previously (26), we are the first to have performed in-depth metabolic characterization of SEA-injected mice. In addition, we studied immune cell polarization at the cellular level, and describe that SEA induces a Th2 response, eosinophilia and M2 polarization in WAT. In liver, SEA also promoted eosinophilia and a Th2 response, which may contribute to glucose homeostasis by directly regulating hepatic insulin sensitivity and glucose production via IL-4 and IL-13, respectively (3;27).

Among the different type-2-associated immune cells, the M2 macrophage probably plays the most central role in the maintenance of glucose homeostasis (2;28). A hallmark of the M2 phenotype is enhanced expression of the MR (29), which we showed in **chapter 2** to be required for internalization of omega-1 and subsequent Th2 polarization. Although the MR is widely used as an M2 marker, its role in metabolic homeostasis was not yet studied. In **chapter 6**, we explored the role the MR in the context of diet-induced obesity using whole-body MR<sup>-/-</sup> mice, and describe that MR deficiency protects against high-fat diet-induced metabolic disorders. Specifically, in mice fed a HFD, lack of MR strongly reduced fat mass gain, adipocyte hyperplasia, glucose intolerance and insulin resistance, and restored locomotor activity and energy expenditure. Thus, despite considerable weight gain, the MR<sup>-/-</sup> mice were more healthy than WT obese mice. In line with the metabolic phenotype, lack of MR protected mice from HFD-induced classical activation of macrophages in adipose tissue and liver.

The protective effect of MR deficiency on HFD-induced metabolic disorders was surprising, given the association between MR and the M2 phenotype (29), and the pivotal role MR plays in the initiation of type 2 immune responses (30-33). Since we also observed low expression of MR by M1 macrophages, we speculate that MR can deliver signals directly into MR-bearing macrophages through interaction with co-receptors, which might result in a pro-inflammatory phenotype under HFD conditions. It is also important to consider that MR<sup>-/-</sup> mice lack whole-body expression of the *Mrc1* gene, and that MR expression is not restricted to leukocytes (34). For example, MR is expressed by microglia and astrocytes in the brain (35). Interestingly, hypothalamic inflammation has been described to contribute to HFD-induced weight gain (36), and activation of the cholinergic anti-inflammatory reflex was recently demonstrated to promote metabolic homeostasis (37). Whether the beneficial effect of MR deficiency on

metabolic homeostasis can be attributed to immune cell signaling, altered neuroinflammation, defects in the anti-inflammatory reflex, or effects beyond the immune system or the brain, remains to be determined.

In sum, our work on helminths and high-fat diet-induced metabolic disorders has provided valuable insights into the effects of helminths and their molecules on metabolic homeostasis. Of particular interest may be the finding that chronic helminth infection enhances peripheral glucose uptake and insulin action in WAT, an effect that may be secondary to the induction of a type 2-associated immune response. Furthermore, our findings on the beneficial effect of SEA on metabolic homeostasis identify helminth molecules as attractive agents for therapeutic manipulation of the immune system in the context of metabolic disorders. Lastly, our data on the unexpected role of MR in the development of metabolic homeostasis provide a novel lead for studying the etiology of diet-induced metabolic disorders.

### Summary of the new findings

In this thesis, we studied the *S. mansoni* egg-derived molecule omega-1, a T2 RNase with strong Th2-polarizing capacities. We investigated how this molecule modulates dendritic cells for Th2 skewing at the molecular level. Furthermore, we analyzed the effect of high-fat diet-feeding in mice chronically infected with *S. mansoni* or chronically exposed to SEA. Lastly, we performed in-depth metabolic and immune cell profiling of MR-deficient mice fed a HFD. The main findings are:

- Omega-1 requires both its glycosylation and its RNase activity to condition DCs for Th2 polarization (**Chapter 2**)
- Omega-1 is bound and internalized via its glycans by the MR, and impairs protein synthesis by degrading both ribosomal and messenger RNA (**Chapter 2**)
- SEA and omega-1 skew Th2 without affecting the mTOR pathway (**Chapter 3**)
- Blocking the mTOR pathway using rapamycin primes DCs for Th2 skewing without affecting IL-12 production (**Chapter 3**)
- SEA and omega-1 alter the DC proteome, with the most pronounced effect on 60S acidic ribosomal protein P2 and proteins involved in antigen presentation (**Chapter 4**)
- Chronic *S. mansoni* infection and SEA treatment protect against metabolic disorders in HFD-induced obesity, and strongly increase adipose tissue eosinophilia and M2 polarization (**Chapter 5**)
- Chronic *S. mansoni* infection reduces adipocyte size and increases peripheral glucose uptake and WAT insulin sensitivity in HFD-fed mice (**Chapter 5**)
- Chronic administration of SEA promotes a Th2 response in WAT and liver of HFD-induced obese mice (**Chapter 5**)
- MR deficiency protects against HFD-induced insulin resistance and glucose intolerance and decreases HFD-induced classical activation of liver and WAT macrophages (**Chapter 6**)



## DIRECTIONS FOR FUTURE RESEARCH<sup>1</sup>

The studies presented in this thesis shed new light on the mechanisms of DC-mediated Th2 polarization by helminth antigens. They further demonstrate that chronic *S. mansoni* infection and SEA administration protect against diet-induced metabolic disorders, and propose that the MR plays an unexpected role in the development of diet-induced insulin resistance. As this thesis progressed, so did the field, and our findings together with several exciting (new) areas of research fuel directions for future studies.

### DC metabolism and Th2 polarization

Recent studies indicate that modulation of metabolic pathways within immune cells can regulate their function and, thereby, the outcome of the immune response (38). For example, BMDCs switch their core metabolism from mitochondrial oxidative phosphorylation to glycolysis upon TLR-ligation, and inhibition of this switch interferes with maturation, IL-12 expression, and the ability to induce CD4<sup>+</sup> T cell proliferation (39;40). The question whether helminths or their products affect glycolytic reprogramming in DCs, and how this relates to Th2 polarization, constitutes an exciting new area of research.

### The T cell receptor

It has been suggested that T cells are polarized towards Th2 if the interaction between DCs and T cells is weak (41-43). Interestingly, others have demonstrated that omega-1 reduces the capacity of BMDCs to form T cell–DC conjugates and diminishes the frequency of CD4<sup>+</sup> T cells progressing through the cell cycle (11). Novel techniques allow for the analysis of DC-T cell interactions *in vivo*, through intravital dynamic 2-photon microscopy (43). Future studies could therefore explore the strength of TCR signaling in the context of helminth-induced Th2 polarization *in vitro* and *in vivo*.

### DCs and the microenvironment

Accumulating evidence points towards a crucial role for the microenvironment in which DCs are primed for T cell polarization. For example, epithelial-derived cytokine alarmins like thymic stromal lymphopoietin (TSLP) and IL-33 condition DCs to skew Th2 (44-46), IL-33 treatment improves Th2 cytokine production and expulsion of *Trichuris muris* (47), and mice deficient for the IL-33 receptor T1/ST2 fail to develop a Th2 response to *S. mansoni* eggs (48). Importantly, T1/ST2 is not only present on DCs but also on lymphocyte subsets including ILC2s, which were shown to potentiate Th2 responses by promoting DC migration and interacting with T cells via MHC-II (49;50). In addition, it has been suggested that DCs require B cells for the initiation of a Th2 response since B cells enable proper localization in the lymph node (51). Together, these studies highlight the importance of studying polarizing alarmins and accessory cells in the search for mechanisms of Th2 polarization.

---

<sup>1</sup> Parts of this section are based on the review "Priming dendritic cells for Th2 polarization: lessons learned from helminths and implications for metabolic disorders" (1).

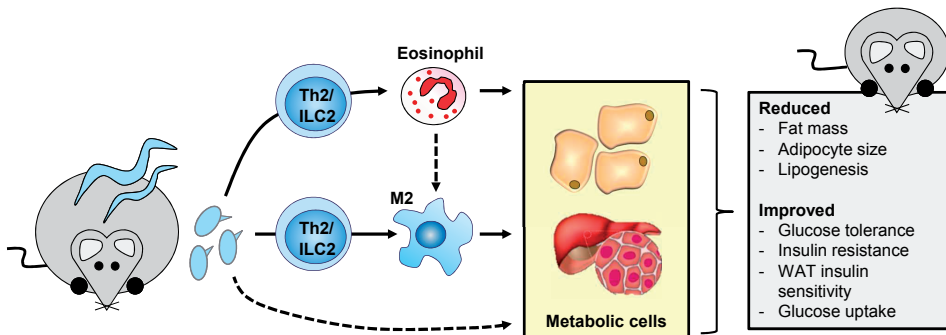
### Helminths, immune cells, and protection against metabolic disorders

It has been demonstrated that maintenance of M2 macrophages in WAT depends on the presence of IL-4-secreting eosinophils (5), which are sustained by IL-5 and IL-13-producing ILC2s (7) in non-infected mice. In our study, we observe an increase in cytokine-producing Th2 cells and eosinophils following chronic exposure to *S. mansoni* or SEA, but the relative contribution of each cell type to the beneficial effect on WAT insulin sensitivity and whole-body metabolic homeostasis remains unknown. Future studies are needed using mice deficient in different immune cell types or their effector cytokines, in order to understand how helminths promote metabolic homeostasis.

Furthermore, in relation to the role of the MR in the development of diet-induced metabolic disorders (chapter 6), the use of conditional knockout mice or adoptive transfer experiments should elucidate whether the protective effect of MR deficiency on high-fat diet-induced insulin resistance can be attributed to MR expression by immune or non-immune cells.

### Helminth molecules and direct interaction with metabolic cells

A recent landmark study demonstrated that SEA can directly suppress lipogenesis in primary hepatocytes (26), which led us to hypothesize that helminth molecules may protect against metabolic disorders by acting as a double-edged sword (Figure 1). Helminth antigens may regulate glucose homeostasis directly by modulating metabolic pathways, or indirectly by polarizing a type 2 immune response. Future studies should therefore focus on the identification of the cellular targets of helminth antigens, as well as the specific helminth-derived molecules involved.



**Figure 1.** *S. mansoni* and protection against metabolic disorders: A double-edged sword? *S. mansoni* egg-derived molecules may protect against metabolic disorders by skewing a type 2 immune response, or through direct interaction with metabolic cells, like adipocytes or hepatocytes.

### Remaining questions

- Do helminth antigens affect DC metabolism and does this contribute to the initiation of a Th2 response?
- Does the strength of TCR signaling play a role in Th2 polarization by helminth antigens?
- What is the role of the microenvironment in DC-mediated for Th2 polarization?
- What is the contribution of different immune cells to the beneficial effect of helminth antigens on diet-induced metabolic disorders, and how do these immune cells interact?
- Do helminth molecules act on non-immune cells to promote metabolic homeostasis?
- Which helminth-derived single molecules contribute to metabolic homeostasis?
- Can the protective effect of MR deficiency on diet-induced obesity be attributed to immune or non-immune cells?

## CONCLUDING REMARKS

To date, the mechanisms that govern Th2 polarization are still not fully understood. Future studies should focus on pinpointing the requirements that qualify DCs for Th2 skewing, bearing in mind that DCs operate in a microenvironment that may influence priming. It is now recognized that type 2 immune responses can also regulate energy metabolism, and studying how helminths generate Th2 responses and contribute to metabolic homeostasis will therefore not only shed light on the mechanisms that promote control of parasite infection, but may provide valuable leads for the development of pharmaceutical agents for the treatment of metabolic disorders.

## REFERENCES

1. Husaarts L, et al. Priming dendritic cells for th2 polarization: lessons learned from helminths and implications for metabolic disorders. *Front Immunol* 2014;5:499.
2. Chawla A, et al. Macrophage-mediated inflammation in metabolic disease. *Nat Rev Immunol* 2011 Nov;11(11):738-49.
3. 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.
4. Wiria AE, et al. Helminth infections, type-2 immune response, and metabolic syndrome. *PLoS Pathog* 2014 Jul;10(7):e1004140.
5. Wu D, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* 2011 Apr 8;332(6026):243-7.
6. 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.
7. Molofsky AB, et al. Innately lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J Exp Med* 2013 Mar 11;210(3):535-49.
8. 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.
9. Gringhuis SI, et al. Fucose-specific DC-SIGN signalling directs T helper cell type-2 responses via I K K epsilon- and CYLD-dependent Bcl3 activation. *Nat Commun* 2014;5:3898.
10. 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.
11. Steinfeldt 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.
12. Bufe A, et al. The major birch pollen allergen, Bet v 1, shows ribonuclease activity. *Planta* 1996;199(3):413-5.
13. Kao R, et al. Mitogillin and related fungal ribotoxins. *Methods Enzymol* 2001;341:324-35.
14. Yang D, et al. Eosinophil-derived neurotoxin acts as an alarmin to activate the TLR2-MyD88 signal pathway in dendritic cells and enhances Th2 immune responses. *J Exp Med* 2008 Jan 21;205(1):79-90.
15. 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.
16. Jankovic D, et al. Th1- and Th2-cell commitment during infectious disease: asymmetry in divergent pathways. *Trends Immunol* 2001 Aug;22(8):450-7.
17. Salmond RJ, et al. The influence of mTOR on T helper cell differentiation and dendritic cell function. *Eur J Immunol* 2011 Aug;41(8):2137-41.
18. Thomson AW, et al. Immunoregulatory functions of mTOR inhibition. *Nat Rev Immunol* 2009 May;9(5):324-37.
19. 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.
20. Le NF, et al. Profiling changes in gene expression during differentiation and maturation of monocyte-derived dendritic cells using both oligonucleotide microarrays and proteomics. *J Biol Chem* 2001 May 25;276(21):17920-31.
21. Richards J, et al. Integrated genomic and proteomic analysis of signaling pathways in dendritic cell differentiation and maturation. *Ann NY Acad Sci* 2002 Dec;975:91-100.
22. Johansson A, et al. Identification of genetic variants influencing the human plasma proteome. *Proc Natl Acad Sci U S A* 2013 Mar 19;110(12):4673-8.
23. Termeer C, et al. Targeting dendritic cells with CD44 monoclonal antibodies selectively inhibits the proliferation of naive CD4+ T-helper cells by induction of FAS-independent T-cell apoptosis. *Immunology* 2003 May;109(1):32-40.
24. Warde-Farley D, et al. The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Res* 2010 Jul;38(Web Server issue):W214-W220.
25. Pearce EJ, et al. The immunobiology of schistosomiasis. *Nat Rev Immunol* 2002 Jul;2(7):499-511.

26. 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.
27. Stanya KJ, et al. Direct control of hepatic glucose production by interleukin-13 in mice. *J Clin Invest* 2013 Jan 2;123(1):261-71.
28. Odegaard JI, et al. Alternative macrophage activation and metabolism. *Annu Rev Pathol* 2011;6:275-97.
29. Stein M, et al. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* 1992 Jul 1;176(1):287-92.
30. Everts B, et al. Schistosome-derived omega-1 drives Th2 polarization by suppressing protein synthesis following internalization by the mannose receptor. *J Exp Med* 2012 Sep 24;209(10):1753-67, S1.
31. Royer PJ, et al. The mannose receptor mediates the uptake of diverse native allergens by dendritic cells and determines allergen-induced T cell polarization through modulation of IDO activity. *J Immunol* 2010 Aug 1;185(3):1522-31.
32. Paveley RA, et al. The Mannose Receptor (CD206) is an important pattern recognition receptor (PRR) in the detection of the infective stage of the helminth *Schistosoma mansoni* and modulates IFN $\gamma$  production. *Int J Parasitol* 2011 Nov;41(13-14):1335-45.
33. Emará M, et al. Recognition of the major cat allergen Fel d 1 through the cysteine-rich domain of the mannose receptor determines its allergenicity. *J Biol Chem* 2011 Apr 15;286(15):13033-40.
34. Martínez-Pomares L. The mannose receptor. *J Leukoc Biol* 2012 Dec;92(6):1177-86.
35. Regnier-Vigouroux A. The mannose receptor in the brain. *Int Rev Cytol* 2003;226:321-42.
36. Thaler JP, et al. Minireview: Inflammation and obesity pathogenesis: the hypothalamus heats up. *Endocrinology* 2010 Sep;151(9):4109-15.
37. Wang L, et al. Pten deletion in RIP-Cre neurons protects against type 2 diabetes by activating the anti-inflammatory reflex. *Nat Med* 2014 May;20(5):484-92.
38. Pearce EL, et al. Metabolic pathways in immune cell activation and quiescence. *Immunity* 2013 Apr 18;38(4):633-43.
39. Krawczyk CM, et al. Toll-like receptor-induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* 2010 Jun 10;115(23):4742-9.
40. Everts B, et al. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKK $\epsilon$  supports the anabolic demands of dendritic cell activation. *Nat Immunol* 2014 Apr;15(4):323-32.
41. 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.
42. 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.
43. 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.
44. Soumelis V, et al. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002 Jul;3(7):673-80.
45. Besnard AG, et al. IL-33-activated dendritic cells are critical for allergic airway inflammation. *Eur J Immunol* 2011 Jun;41(6):1675-86.
46. Eiwegger T, et al. IL-33 links tissue cells, dendritic cells and Th2 cell development in a mouse model of asthma. *Eur J Immunol* 2011 Jun;41(6):1535-8.
47. Humphreys NE, et al. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. *J Immunol* 2008 Feb 15;180(4):2443-9.
48. Townsend MJ, et al. T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses. *J Exp Med* 2000 Mar 20;191(6):1069-76.
49. 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.
50. Oliphant CJ, et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity* 2014 Aug 21;41(2):283-95.
51. 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.



