extinguishing metaflammation: mechanisms and therapeutic opportunities for immunological control of metabolic dysfunctions

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PART II

IMMUNOMODULATORY (HELMINTH) MOLECULES AND OBESITY-INDUCED METABOLIC DYFSUNCTIONS

CHAPTER 6

Immune regulation of metabolic homeostasis by helminths and their molecules

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Trends in Parasitology. 35(10):795-808. (2019)
PMID: 31492623
doi: 10.1016/j.pt.2019.07.014

Abstract

Since time immemorial, humans have coevolved with a wide variety of parasitic helminths that have contributed to shape their immune system. The recent eradication of helminth infections in modern societies has coincided with a spectacular rise in inflammatory metabolic diseases, such as obesity, nonalcoholic steatohepatitis, and type 2 diabetes. Landmark studies in the emerging field of immunometabolism have highlighted the central role of the immune system in regulating metabolic functions, notably in adipose tissue, liver, and the gut. In this review we discuss how helminths, which are among the strongest natural inducers of type 2 immunity, and some of their unique immunomodulatory molecules, may contribute to the maintenance of tissue-specific and whole-body metabolic homeostasis and protection against obesity-associated meta-inflammation.

Glossary

Alternatively activated macrophage (AAM): an innate immune cell involved in protection against parasites, resolution of inflammation, and tissue repair.

Beiging: occurs when white adipose tissue develops features of thermogenic brown adipose tissue.

Brown adipose tissue (BAT): involved in thermogenesis during cold exposure owing to high expression of mitochondrial uncoupling protein UCP1.

Eosinophil: a short-lived granulocyte induced by helminths and involved in type 2 immune responses.

Extracellular vesicles (EVs): lipid bilayer-delimited particles that are released by most eukaryotic cells and involved in intercellular communication.

Group 2 innate lymphoid cells (ILC2s): one of the cell types involved in the initiation of type 2 immune responses via specific damage signals (alarmins) and cytokines.

Helminths: multicellular parasitic worms, including roundworms, tapeworms, and flukes.

Hepatic steatosis: ectopic accumulation of lipids in hepatocytes, resulting in a fatty liver.

Homeostatic Model Assessment for Insulin Resistance (HOMA-IR): calculated from fasting plasma insulin and glucose levels and used as a proxy for whole-body insulin resistance.

Hygiene hypothesis: proposes that the increases in hyperinflammatory disease prevalence in modern societies result from higher hygiene care and reduced exposure to pathogens and microorganisms, such as early-life parasitic infections.

Insulin resistance: impaired insulin action on its target metabolic organs/cells.

Interleukin-33 (IL-33): an alarmin cytokine released by stromal/epithelial cells that promotes type 2 immune responses

Meta-inflammation: chronic low-grade inflammation occurring in metabolic organs

Microbiota: is composed of a wide array of bacteria, archaea, viruses, fungi, and parasites that reside at mucosal surfaces, notably in the gastrointestinal tract.

Nonalcoholic fatty liver disease (NAFLD): a metabolic disorder characterized by excessive fat accumulation in the liver (see hepatic steatosis).

Nonalcoholic steatohepatitis (NASH): NALFD combined with hepatic inflammation and liver damage.

Regulatory T cell (Treg): a specialized T cell restricting excessive immune responses.

Soluble Egg Antigens (SEA): a mixture of soluble molecules extracted from *Schistosoma mansoni* eggs.

Type 2 diabetes: a metabolic disease characterized by insulin resistance and chronically elevated blood glucose levels.

Type 2 immunity: immune response characterized by increased ILC2s, eosinophils, T_H2 cells, mast cells, basophils, and alternatively-activated macrophages.

Type 2 T helper cell (T_H2): a specialized T helper cell involved in asthma, allergies and immune responses against helminth parasites.

White adipose tissue (WAT): an endocrine organ with a high storage capacity for triglycerides.

Helminths and inflammatory metabolic diseases

Helminth (see Glossary) parasites have a long coevolutionary history with humans, and about one quarter of the world's inhabitants are still infected with a wide variety of these worms (1). In tropical and subtropical areas, where hygiene and sanitation are poor, soil-transmitted helminth infections are highly prevalent, including infections with *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms, together with filarial nematodes and schistosomes such as *Schistosoma mansoni* and *Schistosoma haematobium* (1, 2). By contrast, most Western countries have successfully eradicated helminths and other pathogens during the second half of the last century, contributing to an almost complete disappearance of chronic helminth infections and significant changes in the whole **microbiota** composition, notably in the gastrointestinal tract. However, a concomitant increase in autoimmune and allergic diseases, such as multiple sclerosis, type 1 diabetes, and asthma, has been observed. This suggests that exposure to pathogens and microorganisms, such as parasites, contributes to shaping our immune system and is protective against inflammatory diseases, a general concept known as the '**hygiene hypothesis**' (3, 4).

Importantly, the prevalence of inflammatory metabolic diseases, such as obesity, **nonalcoholic fatty liver disease** (**NAFLD**) and **type 2 diabetes**, is also dramatically increasing in Western societies and some developing countries that have recently experienced a rapid urbanization (5). Among various pathophysiological underlying mechanisms, the obesity-associated chronic low-grade inflammation, also called **meta-inflammation**, contributes to the development of **insulin resistance** and dysregulated glucose/lipid metabolism, ultimately leading to type 2 diabetes, **nonalcoholic steatohepatitis** (**NASH**) and associated cardiovascular diseases (6, 7). During the last decade, landmark studies have highlighted the central role played by the immune system in the regulation of metabolic homeostasis in both rodents and humans. These studies have identified a repertoire of innate and adaptive immune cell subsets that populate metabolic organs and contribute to tissue-specific maintenance of biological functions through complex and yet incompletely understood crosstalk with metabolic cells (8, 9).

Remarkably, chronic infections with various helminths, which result in potent **type 2 immunity** and T cell hyporesponsiveness through induction of a regulatory network (10, 11), were associated with reduced insulin resistance and a lower prevalence of metabolic syndrome and type 2 diabetes in populations living in endemic areas (12-16). This suggests that the modulation of the host immune system by worms may protect against metabolic diseases (17, 18). Similar beneficial effects were reported in preclinical rodent models of type 2 diabetes, where both infection with various helminth species and treatment with helminth-derived molecules promote type 2 immune responses, dampen meta-inflammation, and improve metabolic homeostasis during obesity (19-28).

In this review we discuss our current understanding of the immune-mediated mechanisms by which helminths and some of their unique molecules may contribute to maintenance of tissue-specific and whole-body metabolic homeostasis in their host and prevent obesity-associated meta-inflammation.

(Dys)regulation of metabolic homeostasis by immune cells

The concept of immune cells contributing to metabolic homeostasis is now well recognized and supported by a large body of recent literature. One of the cornerstones that has fueled the development of the immunometabolism field was the discovery that the increase in macrophage-derived proinflammatory cytokine Tumor Necrosis Factor (TNF) in adipose tissue contributes to insulin resistance during obesity (29). In the following section we describe how immune cells control tissue-specific metabolic homeostasis, focusing specifically on macrophages that are considered to play a central role in the etiology of obesity-associated meta-inflammation and regulation of insulin sensitivity in most of the metabolic tissues (9).

White adipose tissue

White adipose tissue (WAT) is by far the metabolic tissue that has been studied most in terms of immune cell phenotyping. During the past decade, a growing body of innate and adaptive immune cells have been shown to be involved in regulating adipose tissue biology (9). In 2011, a landmark study revealed the role of interleukin (IL)-4-producing WAT **eosinophils** in the maintenance of insulin sensitivity (27), establishing the conceptual basis that type 2 immunity could be involved in adipose tissue metabolic homeostasis in mice. In lean WAT, eosinophils are relatively abundant and are maintained through IL-5 and IL-13 produced by tissue-resident **group 2 innate lymphoid cells** (**ILC2s**) and **type 2 T helper** (**T_H2**) cells (30). Moreover, stromal cells expressing **IL-33** and thymic stromal lymphopoietin (TSLP) were recently shown to sustain ILC2s in WAT (31, 32). IL-4 and IL-13, produced locally by eosinophils, T_H2 cells, and/or ILC2s, are thought to promote alternative activation of adipose tissue macrophages (ATMs) (30), which are considered to be the effector cells of an immune cascade that regulates insulin sensitivity. Although the underlying molecular mechanisms remain mostly unknown, these **alternatively-activated macrophages** (**AAMs**) have been suggested to control WAT inflammation through enhanced production of the immunosuppressive cytokine IL-10 (33) and/or induction of **regulatory T cells** (**Tregs**) (34). It has recently been shown that the vast majority of these M2-like AAMs are tissue-resident, tightly associated with WAT vasculature, and displaying high endocytic capacity that might act as a buffer for adipocyte-derived lipids during homeostasis (35).

During obesity, this type 2 immune environment is perturbed. Prolonged caloric excess causes hypertrophic adipocytes to produce chemokines and proinflammatory cytokines. These, in turn, recruit circulating monocytes, which subsequently differentiate into $CD11c^+$ proinflammatory macrophages upon encountering the inflammatory milieu (33). These newly recruited macrophages also produce proinflammatory cytokines and chemokines that directly interfere with canonical insulin signaling and recruit more monocytes, leading to a vicious circle that exacerbates WAT inflammation (9). Due to their expression of some canonical markers, such as inducible nitric oxide synthase (iNOS), and enhanced production of TNF, IL-6, and IL-1β, these macrophages were initially considered to be classically activated, M1-like macrophages (33). However, several reports have recently challenged this concept by providing a more subtle classification and phenotyping of ATMs, along with paradoxical functions (Box 1) (35-39). Taken together, ATMs appear to exist in multiple flavors and are versatile cells with a high degree of plasticity, yielding a spectrum of activation states likely dependent on their changing microenvironment during the development of obesity.

Box 1. The recent evolution of obesity-associated adipose tissue macrophage classification

During the course of obesity, adipose tissue macrophages (ATMs) have long been thought to acquire a proinflammatory phenotype that resembles the M1 polarization state induced by LPS in bone marrow-derived macrophages (BMDMs). However, recent studies have led to an evolution in ATM classification by reporting unexpected new phenotypes and functions. Among the newly identified subsets, ATMs expressing lipid metabolism genes under the control of peroxisome proliferator-activated receptor gamma (PPARγ), and producing TNF and IL-1β, were found to be increased in obese mice and termed 'metabolically-activated macrophages' (MMes). Their transcriptomic signature is similar to in vitro bone marrow-derived macrophages stimulated with high concentrations of glucose, insulin, and palmitate, mimicking the nutrient abundance encountered in the obese WAT microenvironment (36). These MMes potentiate WAT inflammation, but, owing to their lipid metabolism machinery, could also exert protective beneficial action by buffering excess adipocyte lipid release and preventing ectopic lipid deposition, as well as clearing dead adipocytes through lysosomal exocytosis (37). Unbiased single-cell analyses in obese WAT have recently allowed different research groups to characterize ATM subsets more in-depth: (i) a lipid-laden and proinflammatory macrophage population expressing CD9 and exclusively found in crown-like structures surrounding dead adipocytes (38), (ii) a monocyte-derived $CD11c^+CD64^+$ macrophage population expressing CD9 but also an array of anti-inflammatory/detoxifying genes and displaying a low endocytic capacity (35), and (iii) a lipid-associated $CD9^+$ macrophage subset expressing a high level of the lipid receptor Trem2 involved in counteracting inflammation and adipocyte hypertrophy (39). Thus, multiple subtle flavors of obesity-associated ATMs exist, being either protective or detrimental for the regulation of metabolic homeostasis, depending on the adipose tissue micro-environment and diseasåe progression.

Liver

During obesity, an overflow of lipolysis-derived nonesterified free fatty acids (NEFAs) released from insulin-resistant WAT is taken up and stored in ectopic tissues, notably in the liver, where triglyceride accumulation in the form of lipid droplets causes **hepatic steatosis** and NAFLD (40). NAFLD-associated lipotoxicity triggers both oxidative and endoplasmic reticulum stress and hepatic inflammation through various mechanisms that are still incompletely understood, ultimately resulting in NASH and progressive liver damage (40). This inflammatory process is thought to be mediated, at least in part, by liver macrophages that can be divided into two main categories: the liver-resident Kupffer cells (KCs), which are embryonically derived and self-sustained through local proliferation, and newly recruited macrophages which originate from circulating monocytes (41).

Although accumulation of hepatic $CD8^+$ T cells and $CD4^+$ T helper 17 (T_H17) cells during obesity might also contribute (42, 43), many studies have shown that KCs are central players in the initiation of liver inflammation. Once activated, notably by lipid metabolites and/or gut-derived endotoxins, KCs promote the recruitment of circulating monocytes that differentiate into proinflammatory macrophages, driving NAFLD progression and insulin resistance (44-47). In line with this, depletion of KCs protects against hepatic steatosis and insulin resistance (48), as does genetic or therapeutic interference with hepatic recruitment of monocytes through the monocyte chemoattractant protein (MCP)1-CCR2 axis (49, 50). The effects of KCs on hepatic insulin resistance have often been associated with the production of TNF and IL-1β (41), yet, a recent report suggests that hepatic macrophages rather stimulate gluconeogenesis and lipogenesis through the release of insulin-like growth factor-binding protein 7 (IGFBP7), a noninflammatory protein that directly binds to the insulin receptor and triggers activation of extracellular-signal-regulated kinase (ERK) signaling (51). Of note, although AAMs are associated with improved insulin sensitivity in WAT, their function remains unclear in the liver. Furthermore, type 2 immunity was rather shown to promote hepatic fibrosis and progression of NAFLD through IL-4 and IL-13, notably in response to liver injury or *Schistosoma mansoni* infection (52-54).

Other metabolic tissues

The obesity-induced inflammation driven by tissue-specific changes in immune cell composition and/or activation states is also observed in skeletal muscle, pancreas and some hypothalamic areas in the brain and may have profound effects on whole-body metabolic homeostasis (see recent reviews (55-57)).

In skeletal muscle, only a few immune cells can be detected during homeostasis. By contrast, obesity leads to recruitment of monocytes/macrophages and T cells into the

expanding adipose depots surrounding the muscle fibers and their subsequent polarization towards a proinflammatory phenotype (56). The release of cytokines and chemokines from inflamed myocytes further accelerates immune cell recruitment, forming a feed-forward inflammatory loop that negatively regulates skeletal muscle insulin sensitivity and metabolic functions, and contributes to whole-body metabolic dysfunctions (56).

In the pancreas, obesity-associated islet inflammation is involved in the failure of insulin secretion by β-cells during type 2 diabetes progression (57). This is partly mediated by glucose-induced metabolic stress that triggers IL-1β secretion by β-cells, resulting in recruitment of circulating monocytes and polarization towards proinflammatory macrophages, β-cell death, and impaired insulin production (58). In addition, islet-resident macrophages were recently shown to proliferate during obesity and engulf β-cell insulin secretory granules, which may contribute to restricting insulin secretion (59). Recently, it has also been shown that **IL-33-** and ILC2-dependent maintenance of islet-resident retinoic-acid-producing myeloid cells promotes glucose-induced insulin secretion by β-cells in homeostatic conditions, a finely-tuned system that is impaired during obesity (60).

In the brain, microglia are embryonically derived tissue-resident macrophage-like cells that control organ homeostasis, notably through clearance of dead neurons, but they also contribute to the regulation of feeding behavior and whole-body energy expenditure (55, 61). Excess calorie intake activates microglia in the mediobasal hypothalamus (MBH), notably through dietary components like saturated NEFAs and peripheral hormones, leading to secretion of proinflammatory cytokines such as TNF, IL-1β, and IL-6, and chemokines. These microglia-derived signals contribute to recruitment and/or proliferation of monocyte-derived macrophages in both the MBH and arcuate nucleus, thereby amplifying hypothalamic inflammation and leading to central leptin resistance, increased food intake and decreased energy expenditure (62, 63).

Altogether, proinflammatory activation of macrophages in metabolic tissues is associated with impaired insulin sensitivity and metabolic homeostasis, which drives progression towards type 2 diabetes. Repolarizing macrophages to an anti-inflammatory state may therefore contribute to restore tissue homeostasis and limit obesity-induced metabolic dysfunctions.

Helminth immunomodulation and metabolic health

Helminth infection and metabolic homeostasis in humans

A number of epidemiological cross-sectional studies conducted in helminth-endemic areas spread over the entire world have reported inverse correlations between infection with various nematode and trematode species and insulin resistance assessed by **homeostatic**

model assessment for insulin resistance (**HOMA-IR**) or prevalence of metabolic syndrome and type 2 diabetes, in both lean and obese individuals (12-16) (Figure 1). Although the beneficial effects of helminth infection on metabolic homeostasis were usually associated with increased markers of type 2 immunity, such as eosinophilia, IgE, and/or circulating type 2 cytokines, other factors might also be involved, and the observational nature of these studies makes it difficult to draw definitive conclusions about a causal relationship between the two. However, it has recently been reported that deworming individuals infected with soil-transmitted helminths using anthelmintic drugs significantly reduced worm burden and serum markers of type 2 immunity, and resulted in increased HOMA-IR or circulating glucose and insulin levels. These results indicate the worsening of whole-body insulin sensitivity and glucose homeostasis upon deworming (16, 64) and further support the notion that helminth infection protects against metabolic dysfunctions in humans.

Helminths and type 2 immune responses in metabolic organs

Since the seminal preclinical study showing that infection of obese mice with the rodent nematode *Nippostrongylus brasiliensis* resulted in WAT eosinophilia and improved high-fat diet (HFD)-induced metabolic dysfunctions (27), many publications have reported similar effects of helminth infection and/or helminth-derived molecules on metabolic homeostasis, associated or not with induction of a type 2 immune response in metabolic organs (Table 1). *N. brasiliensis* infection also increased WAT YM1-expressing AAMs and had beneficial effects on hepatic steatosis in a signal transducer and activator of transcription 6 (STAT6)-dependent manner (28), establishing a link between helminth-induced type 2 immunity and improved metabolic homeostasis. Infection of genetically or diet-induced obese mice with other nematode and trematode species, including *Heligmosomoides polygyrus*, *Litosomoides sigmodontis, Strongyloides venezuelensis* and *S. mansoni*, also increased eosinophils and various AAM markers in WAT and improved whole-body glucose tolerance and insulin sensitivity (19, 23-25, 28). A key role for these ATMs was established by adoptive transfer of *H. polygyrus*-induced WAT AAMs, which improved whole-body glucose tolerance (26). However, with the exception of the overexpression of a few canonical markers, the identity and functional properties of these AAMs, and the underlying molecular mechanism(s) by which they exert their beneficial metabolic effects, remain unknown.

Importantly, these effects were not merely a result of host parasitism, as treatment of obese mice with *S. mansoni* **soluble egg antigens** (SEA) also increased WAT eosinophils, T_H2 cells and AAMs, and ameliorated hepatic steatosis and whole-body metabolic dysfunctions (20, 23, 65). Similarly, *L. sigmodontis* worm antigens were also able to induce WAT type 2 immunity and improve whole-body glucose tolerance (19). Remarkably, these immunometabolic beneficial effects were also reported with some single molecules.

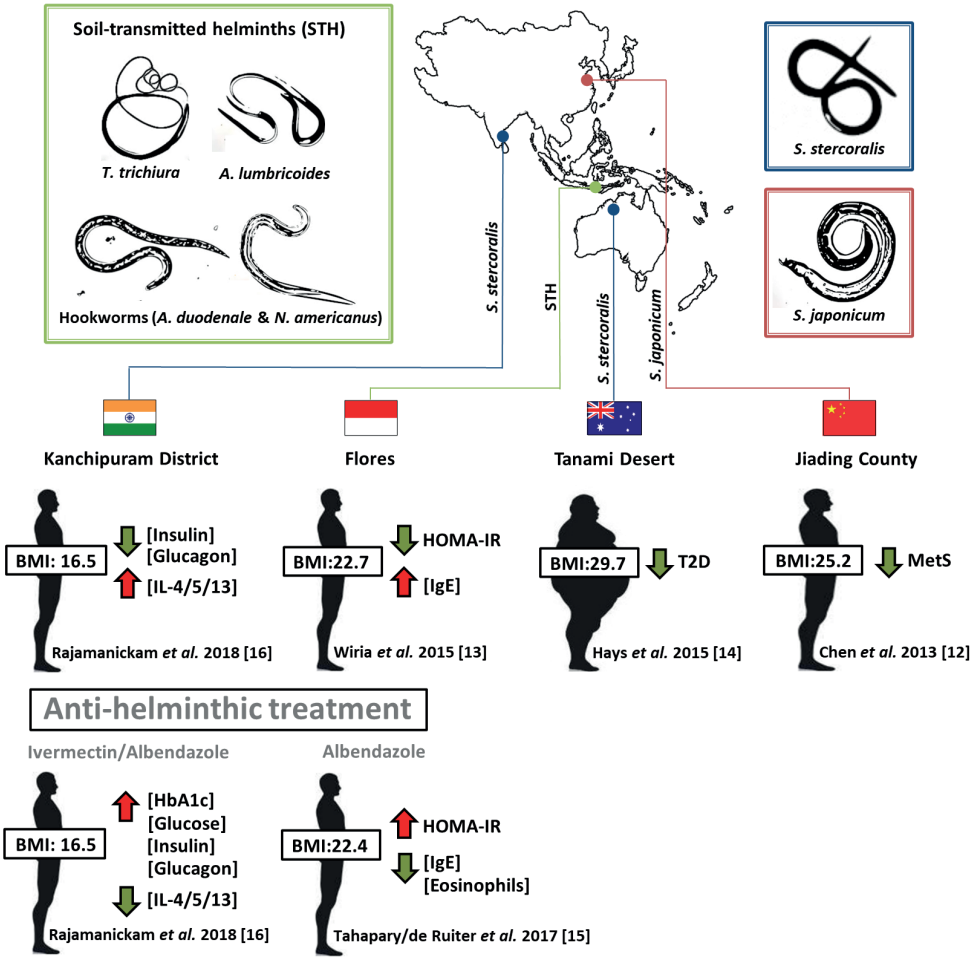

Figure 1. Associations between helminth infection and protection against metabolic diseases. Epidemiological studies conducted in endemic countries have reported an inverse association between infection with the trematode *Schistosoma japonicum* and the nematode *Strongyloides stercoralis* and the prevalence of metabolic syndrome and type 2 diabetes in lean and obese subjects, respectively. Improvements of the homeostatic model assessment for insulin resistance (HOMA-IR), hyperinsulinemia and hyperglucagonemia, hallmarks of whole-body insulin resistance and metabolic dysfunctions, were also observed in rural populations infected with various species of soil-transmitted helminths. These effects were associated with eosinophilia and increased serum levels of total IgE and prototypical type 2 cytokines interleukin (IL)-4, IL-5, and IL-13, suggesting that the helminth-induced type 2 immune response might play a role. Antihelminthic treatment was shown to reduce circulating markers of type 2 immunity and to impair metabolic homeostasis, as characterized by elevated HOMA-IR and hemoglobin A1c (glycated hemoglobin) (HbA1c). Abbreviations: BMI, body mass index; MetS, metabolic syndrome; T2D, type 2 diabetes. Species names: *A. duodenale, Ancylostoma duodenale*; *A. lumbricoides, Ascaris lumbricoides*; *N. americanus, Necator americanus*; *T. trichiura, Trichuris trichiura*. References: (12-16).

For instance, recombinant omega-1, a T_H2-inducing T2-RNAse glycoprotein present in *S. mansoni* egg excretory/secretory products (66), increased WAT type 2 immunity, promoted **WAT beiging**, reduced body weight and improved whole-body glucose tolerance in obese mice by a mannose receptor-, IL-33- and ILC2-dependent mechanism (22).

Furthermore, *Acanthocheilonema viteae*-purified native ES-62 was also recently shown to induce mild WAT eosinophilia and ameliorate HFD-induced liver fibrosis, although the impact on whole-body metabolic homeostasis was rather minimal (21).

Taken together, helminth infection, mixtures of helminth antigens and some helminth-derived single molecules induce type 2 immunity in metabolic tissues, which were either associated with or causal in the improvement of obesity-associated metabolic dysfunctions.

Table 1. Immunometabolic effects of parasite infection or treatment with native and/or recombinant parasite molecules in obese micea

	Mouse models	Immunomodulatory effects	Metabolic effects	Refs
Parasite infection				
Nippostrongylus brasiliensis	DIO male C57BL/6	↑ WAT eosinophils	↓ Fasting blood glucose ↑ Whole-body insulin sensitivity ↑ Whole-body glucose tolerance	(27)
	DIO male C57BL/6 WT, Stat6$^{-/-}$, Il13$^{-/-}$ RIP2-Opa1$^{\downarrow}$KO	↑ WAT YM1 and Arg1 gene expression ↑ YM1^{+} ATMs	↓ Body weight ↓ Hepatic steatosis (STAT6-dependent) ↑ Whole-body glucose tolerance	(28)
Heligmosomoides polygyrus	Chow-fed male KK-Ay/TaJcl	↑ small intestine AAM markers and IL-4, IL-13 and IL-10 genes expression	↓ Hepatic steatosis ↓ HOMA-IR ↑ Whole-body glucose tolerance	(24)
	DIO female C57BL/6	↑ WAT AAM genes expression ↑ AAM markers on ATMs	↓ Body weight gain ↑ Whole-body glucose tolerance ↑ WAT beiging (M2 macrophage dependent)	(26)
	DIO male C57BL/6	N/A	↓ Body weight gain ↑ WAT beiging Altered microbiota composition	(74)
Litosomoides sigmodontis	DIO male BALB/c WT, ΔdblGATA	↑ WAT eosinophils and RELMα+ AAMs ↑ CD4^{+} T cells in WAT	↑ Whole-body glucose tolerance (eosinophil-dependent)	(19)

	Mouse models	Immunomodulatory effects	Metabolic effects	Refs
Strongyloides venezuelensis	DIO male Swiss	↑ MGL1$^+$ ATMs	↑ Whole-body glucose tolerance ↑ Insulin-stimulated glucose uptake Altered microbiota composition	(25)
Schistosoma mansoni	DIO male C57BL/6	↑ WAT eosinophils and YM1$^+$ AAMs	↓ Body weight gain ↓ HOMA-IR ↑ Whole-body insulin sensitivity ↑ Whole-body glucose tolerance	(23)
Parasite antigen mixtures				
L. sigmodontis worm antigens	DIO male C57BL/6 WT and DEREG	↑ WAT RELMα$^+$ AAMs, eosinophils, ILC2s, CD4$^+$ T cells and Foxp3$^+$ Tregs ↓ CD11c$^+$ ATMs	↑ Whole-body glucose tolerance (Treg-independent)	(19)
S. mansoni egg antigens	DIO male C57BL/6	↑ WAT AAM genes expression ↓ WAT CAM genes expression ↑ serum IL-4 and IL-10 levels	↓ HOMA-IR ↑ Whole-body insulin sensitivity (IL-10-dependent) ↑ Whole-body glucose tolerance ↓ Hepatic steatosis	(20)
	DIO male C57BL/6	↑ WAT eosinophils, YM1$^+$ ATMs ↑ IL-4, -5, and -13-expressing T_H2 cells	↓ HOMA-IR ↑ Whole-body insulin sensitivity ↑ Whole-body glucose tolerance	(23)
	DIO male C57BL/6	↑ WAT eosinophils	↔ BAT activation and WAT beiging	(65)
Trichuris suis soluble products	DIO male C57BL/6	↑ WAT eosinophils	↔ BAT activation and WAT beiging	(65)
Parasite single molecules				
HEK-produced recombinant *S. mansoni* omega-1	DIO male C57BL/6 WT, Cd206$^{-/-}$, Rora$^{fl/sg}$ Il7r^{cre}, Il1rl1$^{-/-}$	↑ IL-33 release by adipocytes ↑ WAT T_H2 cells, Tregs, CD206^{hi} AAMs, eosinophils and ILC2s ↓ WAT T_H1 cells and CD206^{low} classically-activated ATMs	↓ Body weight ↓ Fasting blood glucose ↑ Whole-body insulin sensitivity ↑ Whole-body glucose tolerance (CD206-, IL-33- and ILC2-dependent) ↑ WAT beiging	(22)

	Mouse models	Immunomodulatory effects	Metabolic effects	Refs
Acanthocheilonema viteae ES-62	DIO male C57BL/6 DIO female C57BL/6	↑ WAT eosinophils ↑ WAT IL-4 and IL-5 mRNA levels	↓ Liver fibrosis	(21)

[a] AAMs, alternatively-activated macrophages; ATMs, adipose tissue macrophages; BAT, brown adipose tissue; CAMs, classically activated macrophages; DIO, diet-induced obesity; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; IHC, Immunohistochemistry; WAT, white adipose tissue; WT, wild type; DEREG, Tg(Foxp3-DTR/EGFP)

Novel helminth-induced pathways: role in immunometabolic effects?

Modulation of gut microbiota by helminths

The gut microbiota is composed of a large number of microorganisms that are key determinants of both immune and metabolic homeostasis, including type 2 immunity (67). Alterations in their abundance and diversity, the so-called dysbiosis, can affect local and systemic immune responses and contribute to the development of immune and metabolic disorders (68, 69). In recent years, an increasing number of studies have reported the impact of helminth infections, especially those associated with the gastrointestinal tract, on the composition of intestinal microbiota in various mammalian hosts, including in humans (see recent reviews (70-72)). Although the bacterial species found to be affected by helminths vary widely depending on the helminth species, host type, and duration of the infection, those belonging to the family *Lactobacillaceae* are most commonly reported to be affected. Interestingly, these bacteria also contribute to the induction of a regulatory immune response (71, 73).

Dietary imbalance and calorie overload lead to microbiota dysbiosis and increased intestinal permeability, resulting in higher levels of circulating endotoxins such as lipopolysaccharides (LPS) that contribute to meta-inflammation and insulin resistance in peripheral tissues (69). Infection with *S. venezuelensis* has recently been shown to affect microbiota composition in the small intestine of HFD-fed obese mice (Table 1). Specifically, there was a decrease in the phylum *Bacteroidetes* and a concomitant increase in the phylum *Firmicutes*, the latter mostly attributed to a potent increase in *Lactobacillus* species (25). These helminth-induced changes in gut microbiota were associated with improved insulin sensitivity and glucose tolerance that could result, at least in part, from the decrease in gut permeability and plasma LPS levels, the increase in circulating IL-10, and/or the shift of macrophage polarization towards an M2-like phenotype (25). Another recent study has reported that infection with *H. polygyrus* also altered the composition of intestinal microbiota

in HFD-fed obese mice, with increased gut colonization by *Bacillus* and *Escherichia* species (74), which was associated with body weight loss, increased serum levels of norepinephrine (NE) and visceral WAT beiging. The effects were abolished by both antibiotic treatment and pharmacological antagonism of the b3-adrenergic receptor. This suggests that *H. polygyrus* promotes microbiota-derived NE, presumably generated by enriched *Bacillus* and *Escherichia* species, triggering a b3-adrenergic receptor-mediated thermogenic program in adipocyte progenitors and WAT beiging (74). However, the roles of WAT type 2 immune cells and other bacterial metabolites, such as short-chain fatty acids (SCFAs), both previously shown to regulate WAT beiging (75, 76), were not investigated and deserve further investigation. Altogether, it remains an open question whether the changes in gut microbiota, the helminth-induced type 2 immunity in metabolic tissues, or both, are the main driver of the metabolic health improvements. Interestingly, some gut microbiota-derived metabolites have recently been shown to directly regulate WAT inflammation by controlling the expression of a family of miRNAs, leading to the development of obesity and insulin resistance in mice (77). Whether helminth-induced changes in gut microbiota composition observed in obese mice could also affect this gut-WAT axis and significantly contribute to improvements in host metabolic homeostasis remains to be addressed.

Tuft cells

The induction of type 2 immunity by helminths is partly dependent on the epithelial cytokines IL-25, IL-33, and TSLP. Tuft cells, which are rare intestinal taste-chemosensory epithelial cells, have recently been shown to be involved in the initiation of type 2 mucosal immunity to the gastrointestinal helminth *N. brasiliensis* (78-80). Indeed, upon infection, tuft-cell-derived IL-25 activates ILC2s to secrete IL-13, which promotes goblet cell hyperplasia and tuft cell expansion that contribute to epithelial remodeling in the small intestine (79, 80). This circuit was later shown to be dependent on the calcium channel TRPM5 signaling and the succinate receptor SUCNR1 (GPR91) (79, 81, 82). However, both SUCNR1 and helminth-derived SUCNR1-ligand succinate were dispensable for type 2 immunity induction by *N. brasiliensis* (82).

Interestingly, tuft cells and some other chemosensory epithelial cells also link nutritional signals from dietary food in the gastrointestinal tract to afferent neuronal circuits controlling feeding behavior, peripheral nutrient disposal, and energy expenditure (83). While this will require further study, one may speculate that gastrointestinal helminths could affect this gut-brain axis through modulation of tuft cell-mediated chemosensory signaling. This can be either type 2 immunity-dependent or -independent and impact the regulation of food intake and/or peripheral glucose/lipid metabolism during nutritional overload.

Neuromedin U

The gut mucosa is densely innervated, and several immune cells, such as macrophages, mast cells, and ILC2s, have been reported to colocalize and interact with neurons from the enteric nervous system (84). Two landmark studies have recently reported that enteric, cholinergic neurons expressing the neuropeptide neuromedin U (NmU) colocalize with ILC2s that selectively express the NmU receptor 1 (NMUR1) (85, 86). Remarkably, *N. brasiliensis* infection increases NmU expression in enteric neurons of the intestinal lamina propria and triggers NMUR1-dependent ILC2 activation. This leads to the production of prototypical type 2 cytokines, such as IL-5 and IL-13, ultimately resulting in type 2 immunity and accelerated intestinal worm expulsion (85, 86).

Interestingly, NmU was also found to regulate whole-body metabolism, as transgenic mice overexpressing NmU exhibited reduced food intake as well as body weight gain during HFD feeding, and improved whole-body insulin sensitivity and glucose tolerance when compared with wild-type mice (87). Mechanistically, NmU was found to delay gastric emptying by directly triggering contractions of the pylorus and by indirectly activating vagal afferent neurons (88). Whether this NmU-mediated regulation of intestinal motility contributes to the beneficial effect of gastrointestinal nematode infection on glucose tolerance and insulin sensitivity in obese mice remains to be investigated.

Extracellular vesicles

Extracellular vesicles (**EVs**), including microvesicles, exosomes, and apoptotic bodies, are secreted by virtually all eukaryotic cells and have recently emerged as a new mode of intercellular communication by conveying complex mixtures of regulatory factors, such as proteins, lipids, miRNAs, and other structural components (89). Helminths have also been reported to produce EVs and use this mode of communication to manipulate the host immune responses (90, 91). For example, *H. polygyrus*-derived EVs have been shown to be taken up by activated macrophages and to potently suppress IL-33 receptor expression and both effector molecules of type 1 and type 2 immunity *in vitro* (92). But they can also generate strong antibody responses and protective immunity against infection (92) and suppress type 2 inflammation after allergen challenge *in vivo* (93). Of note, the helminth-derived EV composition is generally species- and life-stage-specific and mainly consists of excretory/secretory proteins and small noncoding RNAs, but can also be decorated by membrane-associated glycans (94) that might contribute to their immunomodulatory functions (90).

Interestingly, it has recently been shown that the production rate, size, and cargo composition of WAT-derived EVs, released from both macrophages and adipocytes, are altered

during obesity, contributing to meta-inflammation and insulin resistance through modulation of adipocyte-macrophage crosstalk and/or alteration of communication between WAT and other metabolic organs (95-97). It is therefore tempting to speculate that either worm-derived EVs or EVs released from host immune cells and/or metabolic cells primed by helminth molecules could, directly or indirectly, contribute to the crosstalk between organs that regulate tissue-specific and whole-body metabolic homeostasis. Exploring this hypothesis would be interesting and might result in the identification of specific helminth-derived EVs that could ultimately be used as potential new vectors for modulating meta-inflammation.

Immune-independent effects of helminth molecules on metabolic cells

Due to the plethora of molecules released by helminths inside their host, including some unique glycoproteins, it is finally conceivable that some helminth-derived molecules could also directly manipulate metabolic processes, potentially via glycan-mediated interactions with specific cell-surface receptors on metabolic cells. As such, *S. mansoni* SEA was shown to reduce hepatic steatosis, at least in part by direct inhibition of *de novo* lipogenesis in hepatocytes (20), and *Schistosoma japonicum* SEA was shown to inhibit TNF-induced activation of nuclear factor kappa B (NF-κB) pathway in hepatic stellate cells *in vitro* (98), an effect that might eventually contribute to reduce NASH. Recombinant omega-1 was also shown to promote IL-33 expression and release by adipocytes, presumably through interaction with mannose receptor (22).

Concluding Remarks and Future Directions

During recent years, and in line with results from cross-sectional studies conducted in populations from several endemic regions, it has been shown that infection with various helminth species - but also treatment with helminth-derived SEA mixtures or single molecules from SEA - can improve whole-body metabolic homeostasis in rodent models of obesity and type 2 diabetes. Various mechanisms have been proposed, including induction of type 2 immunity in metabolic organs, promotion of WAT beiging, decrease in hepatic steatosis, and/or changes in gut microbiota composition (Figure 2). However, the exact underlying molecular mechanism(s) by which helminths and their molecules exert their beneficial effects on insulin sensitivity and glucose/lipid homeostasis in obese mice, through either immune-dependent and/or -independent pathways, remain to be fully elucidated (see Outstanding Questions Box). As such, identifying new molecules, but also the exact molecular patterns, targeted receptors, and downstream cellular signaling pathways involved in their immunometabolic effects constitute important future directions.

Finally, whether helminthic therapies and/or development of helminth-derived therapeutics might hold promise for treating metabolic dysfunctions in obese subjects and type 2 diabetic patients is yet to be established. Importantly, controlled human infection (CHI) with *Necator americanus* shows promising results in terms of safety and tolerability (99) while efforts to establish a CHI model with single-sex *S. mansoni* are also ongoing (Trial NCT02755324; (100)). Although currently aimed at developing vaccines, clinical trials using CHI with helminths could be envisaged in the near future for inflammatory metabolic diseases such as obesity, NASH, and type 2 diabetes.

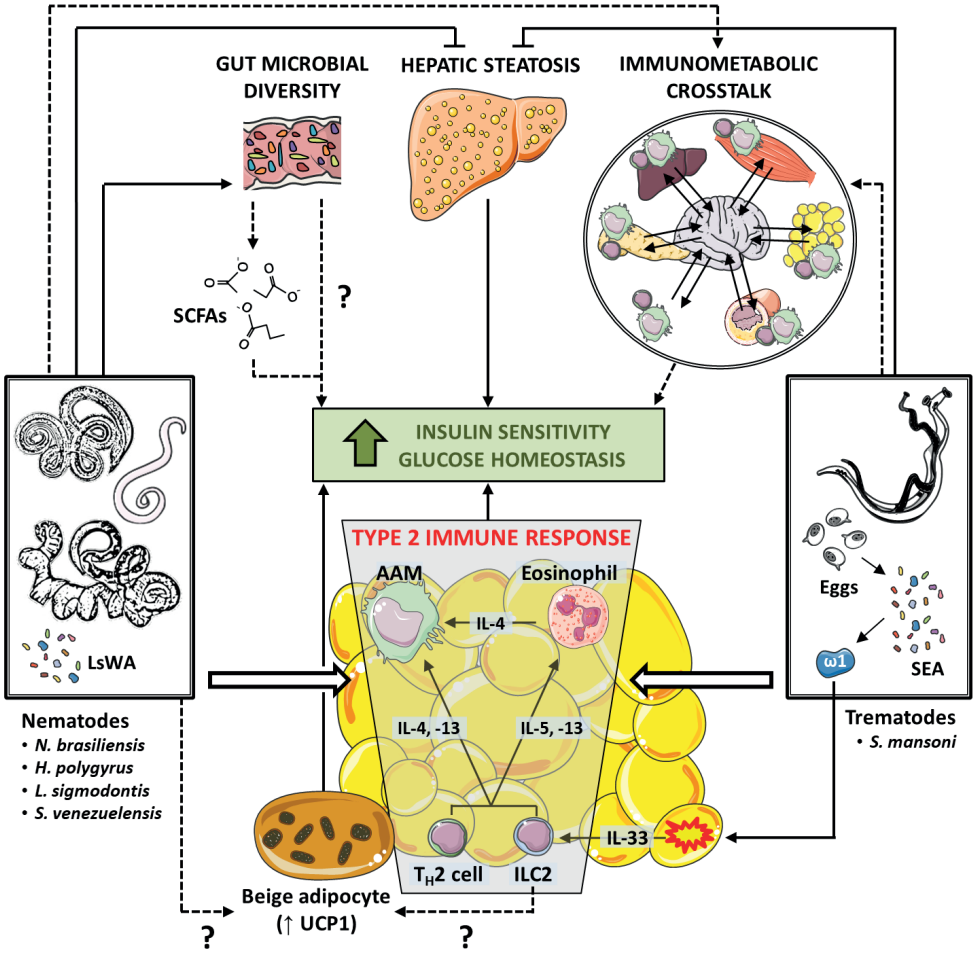

Figure 2. Regulation of metabolic homeostasis by helminths and their molecules. Both nematode and trematode infections were shown to improve whole-body metabolic homeostasis in obese mice through various mechanisms involving induction of type 2 immune responses and alternatively

◀Figure 2. Legend (Continued)

activated macrophages in white adipose tissue (WAT), WAT beiging, and/or reduction in hepatic steatosis. Some gastrointestinal nematodes were reported to change gut microbial composition and diversity, which may contribute to increased WAT beiging, notably through enhanced bacterial production of specific short-chain fatty acids (SCFAs). These SCFAs could improve whole-body metabolic homeostasis directly or indirectly via peripheral and/or brain-mediated mechanisms that are still incompletely understood. Lastly, the type 2 immune response induced by helminths in peripheral tissues may also affect the crosstalk between the brain and metabolic organs (liver, skeletal muscle, WAT, gut, and pancreas) through efferent and afferent pathways and contribute to improved whole-body insulin sensitivity and glucose homeostasis. Treatment with various mixtures of egg antigens and with either native or recombinant single-helminth-derived molecules was also shown to recapitulate some of the beneficial immunometabolic effects observed in helminth-infected obese mice, although the underlying molecular mechanisms proposed might sometimes differ. Abbreviations: AAM, alternatively activated macrophages; IL, interleukin; ILC2, group 2 innate lymphoid cell; LsWA, *L. sigmodontis* worm antigens; SEA, soluble egg antigens; T_H2, type 2 T helper cell; UCP1, uncoupling protein 1. Species names: *H. polygyrus*, *Heligmosomoides polygyrus*; *L. sigmodontis*, *Litosomoides sigmodontis*; *N. brasiliensis*, *Nippostrongylus brasiliensis*; *S. mansoni*, *Schistosoma mansoni*; *S. venezuelensis*, *Strongyloides venezuelensis.*

Outstanding Questions Box

What is the contribution of helminth-induced type 2 immunity in other metabolic organs than adipose tissue, i.e. liver, skeletal muscle, gut and pancreas, on whole-body metabolic homeostasis? What are the immune cells or cell subsets involved?

Does induction of adipose tissue alternatively-activated macrophages in obese mice play a central role in the improvement of insulin sensitivity by helminth molecules? If yes, what are their phenotypical characteristics and by which mechanism(s) do these polarized macrophages modulate adipocyte functions?

Do helminth infections and/or helminth molecules affect WAT beiging and/or BAT activation through type 2 immune response-dependent mechanism(s)?

To what extent do changes in gut microbiota diversity induced by gastrointestinal worms contribute to regulating whole-body metabolic homeostasis?

Do helminth-derived extracellular vesicles play a role in the immune-mediated regulation of tissue-specific insulin sensitivity and glucose/lipid metabolism?

Could direct interactions of helminth molecules with metabolic cells, e.g. adipocytes, hepatocytes, myocytes and/or beta-cells, contribute to the improvement of obesity-associated metabolic dysfunctions?

Is helminth-induced type 2 immunity in metabolic tissues, as observed in rodents, also present in humans, and is it involved in regulating whole-body metabolic homeostasis? If yes, what would have been the evolutionary advantage of this metabolic trade for the worms and their host?

Does helminthic therapy using controlled human infection and/or helminth-derived drugs hold promise as innovative therapeutic approaches for obesity and inflammatory metabolic diseases?

Acknowledgments

The authors want to thank Professor Maria Yazdanbakhsh for her critical reading of the manuscript. This work was supported by funding from the European Federation for the Study of Diabetes (EFSD/Lilly Research Grant Fellowship to B.G.), the Dutch Organization for Scientific Research (ZonMW TOP Grant 91214131 to B.G.), the NWO Graduate School Program 022.006.010 (to H.v.d.Z.), and the Polish Ministry of Science and Higher Education project Mobilność V (DN/MOB/278/V/2017 to A.Z.).

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