Received Date : 30-Sep-2016

Revised Date : 20-Dec-2016

Accepted Date : 05-Jan-2017

Article type : Commissioned Review or Article

Parasite excretory-secretory products and their effects on metabolic syndrome

Jenny Crowe[1*], Felicity E. Lumb[2*], Margaret M. Harnett[1], William Harnett[2+]

[1]Institute of Infection, Immunity and Inflammation, Glasgow Biomedical Research Centre, University of Glasgow, G128TA, U.K. [2]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, U.K.

*These authors contributed jointly to this paper

+ Corresponding author: w.harnett@strath.ac.uk

Disclosures: None

Abstract

Obesity, one of the main causes of metabolic syndrome (MetS), is an increasingly common health and economic problem worldwide, and one of the major risk factors for developing type 2 diabetes and cardiovascular disease. Chronic, low-grade inflammation is associated with MetS and obesity. A dominant type 2/anti-inflammatory response is required for metabolic homeostasis within adipose tissue: during obesity, this response is replaced by

infiltrating, inflammatory macrophages and T cells. Helminths and certain protozoan parasites are able to manipulate the host immune response towards a TH2 immune phenotype that is beneficial for their survival and there is emerging data that there is an inverse correlation between the incidence of MetS and helminth infections, suggesting that, as with autoimmune and allergic diseases, helminths may play a protective role against MetS disease. Within this review, we will focus primarily on the excretory-secretory products that the parasites produce to modulate the immune system and discuss their potential use as therapeutics against MetS and its associated pathologies.

Introduction

Metabolic syndrome (MetS), which presents as a cluster of conditions such as hypertension, abdominal obesity, high fasting plasma glucose and dyslipidemia, is associated with a greater risk of developing type 2 diabetes (T2D) and cardiovascular diseases (CVD) such as atherosclerosis, the leading causes of mortality worldwide. The underlying mechanisms of MetS are still not fully understood but it is notable that the majority of patients with the syndrome exhibit some degree of insulin resistance (IR). There are many factors that contribute to the development of IR, including obesity, physical inactivity, age, diet and genetic factors, with obesity and physical inactivity being the main driving force in most cases. Thus, the mechanisms by which obesity may contribute to metabolic dysfunction have been under intense investigation in recent years. Hotamisligil *et al* were the first to observe a significant increase in the levels of the pro-inflammatory cytokine TNF-α in obese mice, thereby linking inflammatory responses to obesity[1]. Chronic inflammation has since been found to be strongly associated with obesity and MetS - obese people with MetS have an increased level of circulating inflammatory markers such as C-reactive protein[2] and there is

an increased incidence of cardiovascular diseases in patients with inflammatory diseases such as rheumatoid arthritis (RA)[3]. Under normal physiological circumstances, excess nutrients are processed and stored by professional metabolic tissues – the liver, white adipose tissue (WAT) and skeletal muscle. In obese individuals, these tissues can become overloaded, resulting in an increase in free fatty acids in the tissues and circulation, leading to cellular metabolic dysregulation manifesting as mitochondrial dysfunction, oxidative stress and an increase in intracellular lipids. Obesity-induced cellular dysfunction results in the activation of a number of intracellular signalling pathways such as those involving mTOR, JNK and IKKβ. In turn, these pathways converge and inhibit insulin signalling, primarily via serine phosphorylation of insulin receptor substrate (IRS) proteins, blunting insulin action in these tissues and leading to IR[4].

Intracellular signalling pathways involved in obesity and MetS

The signalling cascade of PI3K/AKT/mTOR has a profound influence on cell survival[5], regulation of insulin sensitivity/resistance and cell metabolism[6]. Specifically, the recently demonstrated ability of the mTOR inhibitor, rapamycin, to prevent insulin resistance in humans[7] has highlighted this pathway as a point of intervention for MetS. The PI3K/AKT signalling cascade is activated by ligation of a range of receptors including G protein coupled receptors, B and T cell receptors and tyrosine kinase receptors such as the insulin and insulin like-growth factor 1 (IGF-1) receptor (Figure 1)[8]. The last two receptors stimulate IRS1 and 2 to activate PI3K, which in turn results in the phosphorylation and activation of Akt[9,10]. In addition, the activation of the IIS (insulin/insulin like growth signalling) pathway leads to the up-regulation of mTOR complex 2 (mTORC2) and PDK1 activity, which are both required for the complete phosphorylation of Akt[11]. To date, Akt has been demonstrated to have

multiple roles: regulating mTORC1 activation, mediating cell survival via the suppression of apoptosis-associated proteins such as Bad[12], inhibiting Fox0 transcription[13], and regulation of NF-κB activity[14]. Reflecting this, in the context of insulin signalling, the activation of Akt is essential for the upregulation of mTORC1 activity (via the inactivation of tuberous sclerosis complex 1 and 2 (TSC1/2) and activation of S6 kinase (S6K))[11]. The mTOR complex is primarily conserved for nutrient sensing: thus, activation of mTOR can be mediated by environmental amino acids, fatty acids, glucose and hormones[15], an important factor when one considers the mechanisms of insulin resistance (IR) in terms of MetS. Indeed, recent findings suggest that nutrition can impact on mTOR activation, demonstrating that calorie restriction can reduce activation of mTOR and prevent IR[16]. By contrast, a number of studies have demonstrated that over-activation of mTOR results in the formation of a negative feedback loop, whereby IRS-1 and IRS-2 are downregulated by S6K signalling[17], thereby impairing insulin sensing. Thus, it is evident that mTOR deactivation is key to the control of IR and that nutrient/calorie restriction may have a role in regulating this.

The stress-activated c-Jun amino-terminal kinase (JNK) and the inhibitor of κ kinase (IKK) are proposed to be central mediators of obesity-associated inflammation and stress responses (reviewed by[18]). Certainly, expression of both of these kinases is increased in the liver, skeletal muscle and adipose tissue of obese mice and genetic ablation of JNK1 renders mice resistant to weight gain and metabolic pathologies[19]. Additionally, mice that are heterozygous for IKKβ, an upstream activator of NF-κB, are protected from insulin resistance in both diet-induced and genetic obesity[20]. These kinases are potentially activated by a number of pathways during obesity. For example, signalling via the Toll-like receptors (TLRs) of the innate immune system potently activates JNK and IKK and TLRs have also been shown to be upregulated in adipose tissues during obesity. Indeed, macrophages and adipocytes can be

activated to produce pro-inflammatory cytokines IL-6 and TNF-α after stimulation with saturated fatty acids (FAs) such as palmitate in a TLR4-dependent manner and knock-down of TLR4 reduces the activation of NF-κB in adipose tissue of obese mice[21]. Moreover, MyD88 is a key signalling adaptor molecule required by most TLRs that has also been shown to play a role in dysregulated signalling during obesity, as evidenced by targeted depletion of MyD88 in the CNS of mice protecting them from weight gain and leptin resistance[22]. Highlighting the role of TNF–α downstream of TLR signalling, Hotamisligil and colleagues[1] have directly linked increased levels of TNF-α with insulin resistance and obesity whilst others[23] have demonstrated that blockade of TNF-α signalling, via the genetic deletion of TNF-α and its two receptors TNFR1 and 2, reduces the incidence of IR in HFD-fed mice. This reflects that ligation of TNFR1 and 2 receptors can trigger the activation of MAPK and NFκB signalling[25], thereby promoting pro-inflammatory cytokine release[24] and indicating that, either via direct activity or synergistic mechanisms, the TLR pathway is likely to contribute to the pathogenesis associated with in metabolic syndrome.

Other innate immunity sensors like the NLRP3 (nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3) inflammasome, activation of which leads to the cleavage of pro-caspase-1 and results in the production of mature IL-1β and IL-18, have also been demonstrated to play a critical role in the development of insulin resistance[26]. Thus, monocyte-derived macrophages from newly diagnosed, untreated type 2 diabetic patients show elevated expression of components of this inflammasome compared to healthy controls[27]. Moreover, a high-fat diet was found to induce caspase-1 activation in the AT of mice[28], whilst ablation of NLRP3 improves insulin signalling in diet-induced obese mice and this is associated with a decrease in IL-1β in AT and reduced circulating IL-18[29,30]. IL-1β is one of the main cytokines to be implicated in the development of insulin resistance and

accordingly it has been shown that IL-1β$^{-/-}$ mice on a HFD do not exhibit IR[30]. Furthermore, saturated FAs such as palmitate and stearate, were shown to activate NLRP3 in macrophages in a ROS-dependent manner[30]. Collectively, this led to the proposal that the increased levels of glucose and fatty acids in the organs and circulation of obese individuals are recognised by the NLRP3 inflammasome as metabolic danger signals[31]. As the inflammasome can also be activated by molecules such as ceramides, ATP, oxidised LDL, uric acids and cholesterol crystals, all of which, along with FAs, are elevated in obesity and can increase ROS production, a prerequistite for NLRP3 signalling[32], the NLRP3 inflammasome may be activated by a range of substances during obesity. Demonstrating the importance of NLRP3 activation in obesity, Dalmas and colleagues[33] found that IL-1β derived from adipose tissue macrophages (ATMs) from obese type 2 diabetic patients induces the expression of IL-22 and IL-17 by adipose-resident CD4^{+} T cells. These cytokines, in turn, can further stimulate the production of pro-IL-1β in macrophages[33] highlighting the interplay between tissue resistant cells and indicating that the local cytokine milieu should be considered during the development of inflammasome-centric treatments. Nonetheless, these findings strongly support the NLRP3 inflammasome as an attractive therapeutic target against obesity and MetS, and indeed treatment with the IL-1 inhibitor Anakinra, has been shown to improve β-cell secretory functions and reduce systemic inflammatory markers CRP and IL-6 in T2D patients[34]. Together, these studies demonstrate that there are multiple signalling pathways that are activated in times of nutrient excess that contribute to the inflammatory response in obesity and that targeting of these pathways can be beneficial when it comes to novel treatments for patients with MetS.

Cellular composition of adipose tissue during obesity

Activation of these inflammatory pathways and consequent recruitment of infiltrating cells has a profound effect on the cellular composition of white adipose tissues (WAT). For example, in lean mice adipose ATMs are predominantly of an Alternatively Activated Macrophage (AAM)/M2-like tissue protective phenotype characterised by expression of Arginase 1, chitinase (Ym1) and IL-10 production. Whilst the transcription factor peroxisome proliferator activator receptor-γ (PPARγ) is required for maturation[35] IRF5 appears to act to suppress accumulation of AAM in AT[36] Inflammation during diet-induced obesity triggers adipocytes to secrete pro-inflammatory mediators such as IL-6, IL-1β, TNF-α, MIPs and CCL-2 that recruit circulating macrophages into the tissue[37,38], such that the number of macrophages in WAT positively correlates with increasing adiposity[39]. ATMs are known to play a key role in the inflammation associated with obesity. Although initially thought to be akin to M1-like inflammatory macrophages, a new subset of macrophages that lack expression of markers like CD38 and exhibit a distinct transcription profile, have recently been identified in adipose tissue and termed metabolically activated macrophages (MMe)[40]. These macrophages can be differentiated *in vitro* via stimulation with insulin, glucose and palmitate, stimuli mimicking the conditions present during obesity. MMes express ABCA1 and CD36 and produce a host of pro-inflammatory cytokines and thus are phenotypically similar to ATMs found in human and murine adipose tissue[40]. Eosinophils have been demonstrated to be the major IL-4-producing cells in WAT and are essential for the maintenance of the AAM population in health[41]. In obesity, these cells are replaced by neutrophils and mast cells that help to generate the pro-inflammatory conditions in the WAT that are thought to contribute to IR. Additionally, it has recently been shown that the associated decrease in eosinophils in WAT is associated with a loss of group 2 innate

lymphoid cells (ILC2s), that have been reported to be important for the production of the IL-5 and IL-13 that is required to sustain eosinophils and AAMs respectively in the WAT[42]. ILC2s have been detected in murine epididymal WAT and human subcutaneous fat and are decreased in both obese human donor adipose tissue and in the adipose tissue of mice fed a HFD[43]. By contrast, diet-induced obesity promotes the expansion of ILC1 cells that produce IFN-γ, driving differentiation of pro-inflammatory macrophages[44]. Other lymphocyte populations undergo similar shifts in phenotype: thus, whilst in healthy adipose tissue IL-4-producing TH2 cells and adipose-specific $CD4^+$ $FOXP3^+$ T regulatory cells are essential for metabolic homeostasis, during diet-induced obesity these cells are replaced with infiltrating inflammatory TH1 cells[45,46]. Similarly, infiltrating cytotoxic $CD8^+$T cells have been demonstrated to arrive prior to M1 macrophages in adipose tissue and depletion of these cells results in reduced adipose inflammation and an improvement in glucose tolerance suggesting they may be pivotal in the initiation of inflammatory responses in obesity[47]. Studies utilising MHC I^{null} and MHC II^{null} mice revealed that B cells are crucial for the activation of $CD4^+$ and $CD8^+$ T cells in adipose tissue, and corresponding with this, B $cell^{null}$ mice have improved insulin sensitivity when fed HFD[48]. However, adipose-specific IL-10-secreting B regulatory cells, recently identified by Nishimura *et al*[49], have been found to reduce inflammation within adipose tissues of obese mice. Consistent with this, depletion of IL-10 from B cells increased the infiltration of M1 cells and $CD8^+$ T cells in adipose tissue and transfer of IL-10 Bregs, but not splenic B cells, from lean mice to obese B cell KO mice decreases the secretion of IFN-γ from $CD8^+$ T cells[49]. These studies suggest that, as with macrophages, there is a delicate balance of effector B and T cell subsets present in the AT that exert positive and negative effects on the immune response. Overall, it can be stated that a TH2-biased, anti-inflammatory immune profile promotes metabolic homeostasis in AT.

Parasites and associated immune responses

Helminths, and the products they produce, are the most potent natural inducers of a type-2 immune response. They typically prime T cells towards a TH2 phenotype characterised by the production of IL-4, IL-5 and IL-13, increase tissue eosinophil numbers, and prime B cells to generate high levels of IgE. In addition, helminths bias macrophages towards an AAM/M2 phenotype and prime dendritic cells to induce TH2 responses. Helminths have co-evolved with humans for millennia and some are able to reside in individuals for decades without causing any severe pathology, which is beneficial not only to the parasite but also to the host. This is a result of their ability to modify the typical TH2 response with a regulatory component characterised by the presence of Breg and/or Treg cells, AAM/M2-like macrophages and the production of IL-10 and TGF-β cytokines and IgG4 antibodies[50]. One of the key mechanisms utilised by helminths to induce this immune phenotype is the production of excretory-secretory (ES) products which interact with and influence their host's immune system[51,52]. However, in the past fifty years, due to increased hygiene and advancement of medicines we have drastically reduced the rate of infectious diseases such as those associated with helminths in the developed world. By contrast, during this time, the rate of allergic, autoimmune and inflammatory conditions such as asthma, rheumatoid arthritis and MetS-associated diseases has increased. In attempting to link these observations together it is pertinent that helminth infections and their products can be utilised in murine model systems to treat allergic and autoimmune diseases and currently there are number of clinical trials in progress, or being planned, that utilise helminths in the treatment of a range of allergic and autoimmune diseases (reviewed by[53,54]).

Helminths are not alone in their ability to modulate the host immune response to create an optimum environment – protozoan parasites, such as *Leishmania* and *Toxoplasma*, are able to impair the host's initial TH1 response and bias it towards a TH2 phenotype to benefit their own survival. Specifically, *L. mexicana*, which causes the development of non-healing cutaneous lesions during infection, can suppress TH1-associated production of IL-12[55–57] by macrophages and DCs, and consequently can inhibit IFN-γ production[58]. Further promoting this TH1-TH2 switch, products from the parasite can also significantly enhance IL-4 production in the draining LN[59]. In addition, there is growing evidence to suggest that other intracellular protozoans such as *Toxoplasma, Plasmodium* and *Trypanosoma* species may also be capable of skewing host production of TH1/TH2 associated cytokines. Reflecting this, a number of studies have demonstrated that infection with *P. chabaudi* or *T. cruzi* have protective effects in the murine model of multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE), during which both parasites trigger the upregulation of IL-27 production, promoting a protective TH2 type phenotype[60,61]. To date, many of the mechanisms underpinning how these parasites manipulate the host cell machinery are yet to be defined. It is clear, however, that much like helminths, these intracellular parasites have evolved methods of steering the host immune response, possibly via the production of surface bound and/or excretory-secretory molecules.

As inflammation has now been established as a significant underlying mechanism of MetS-associated diseases it has recently been speculated that parasites may protect against MetS and this is supported by emerging evidence of an inverse correlation between helminth infections and incidence of MetS[62]. For example, Aravindhan *et al* examined the prevalence of filarial infection among diabetic, pre-diabetic and non-diabetic subjects in a cross-sectional study in India and reported a significant decrease in the incidence of filarial infection in

diabetic patients compared to non-diabetic subjects[63]. Consistent with this, helminth infection has been shown to be effective at reducing weight gain and improving glucose tolerance in obese mice. For example, Yang *et al* used both a genetic deficiency and a diet-induced model of obesity to demonstrate that infection with the gastrointestinal nematode *Nippostronglyus brasiliensis* caused less weight gain than that observed in wild-type littermates or uninfected mice on high-fat diet (HFD) respectively[64]. Likewise, chronic infection with the trematode, *Schistosoma mansoni*, decreased fat mass and adipocyte hypertrophy in HFD-fed mice, which were accompanied by improvements in whole-body glucose tolerance and insulin sensitivity[65]. Recently, it has additionally been demonstrated that infection with the filarial nematode *Litomosoides sigmodontis* improves glucose tolerance in diet-induced obese mice, and this is associated with an increase in eosinophils, AAM and $CD4^+$ T cells in the epididymal AT[66]. Furthermore, *S. mansoni* infection has been demonstrated to reduce atherosclerotic lesion development in $ApoE^{-/-}$ mice[67,68], a well-established model for the study of atherosclerosic lesion formation[69], and this is consistent with a reduced frequency of atherosclerosis in schistosomiasis patients[67].

Perhaps providing a molecular rationale for this, it has also recently been demonstrated that parasites can directly affect the mTOR signalling pathway: Narasimhan *et al* found exposure of human monocyte-derived dendritic cells to *Brugia malayi* microfilarie (MF) to have a similar effect as treatment with Rapamycin in that it resulted in downregulation of phosphorylation of mTOR, p70S6K1 and 4EBP1 while also inducing autophagy in these cells, as evidenced by upregulation of phosphorylation of Beclin-1, induction of LC3II and degradation of p62[70]. Similarly, it has been demonstrated that *L. major* promastigotes express a surface metalloprotease, GP63 that is important for mediating parasite engulfment[71], and which influences a number of key signalling molecules[72] in the intracellular environment of

the macrophage. Most notably GP63 can inhibit mTORC1, consequently resulting in a blockade in phosphorylation of translational initiation factor 4E-BP1[73]. This dephosphorylated state of 4E-BP1 causes suppression of macrophage protein synthesis[74] and has been shown to be important for *L. major* parasite survival and replication. Moreover, it has been demonstrated that following infection, *L. donovani*-parasitised macrophages express lower levels of AKT[75] which will have a profound downstream effect on mTORC1 activity. Indeed, the importance of mTOR activity in macrophages has recently been investigated, utilising macrophage specific mTOR null mice to demonstrate that in the absence of mTOR activity, HFD-fed mice exhibit a reduced liver and adipose inflammatory gene expression profile[76]. These studies are of particular interest as very recent work has demonstrated that elevated dephosphorylated 4E-BP1 levels can protect against diet-induced obesity, insulin resistance and associated MetS[77] while autophagy is known to play a key role in the suppression of production of inflammasome-associated cytokines IL-1β and IL-18 by stabilising mitochondria and preventing release of mitochondrial DNA into the cytoplasm[78]. As inhibition of the inflammasome or IL-1β has been demonstrated to prevent IR, targeting a regulator could represent a potential future therapy against diabetes and MetS.

Currently, there is a great deal of ongoing work examining the effect of helminth infection on metabolic syndrome: however, the present review will examine in detail the potential of parasite products, in particular, excretory secretory products, to influence the immune system, and specifically, their effect on the metabolic syndrome.

Excretory-secretory products of parasites

Helminths are able to modulate the host immune response to ensure their own survival. One of the main mechanisms employed by these pathogens is the release of "excretory-secretory" (ES) products that actively dampen the host immune response to the parasite. These products are a diverse mix of proteins, glycans, lipids and nucleic acids and while they may utilise different mechanisms, in general they induce a type 2/regulatory phenotype in the host (see Table 1). ES-62, a 62kDa glycoprotein, is the major ES protein of the rodent filarial nematode *Acanthocheilonema viteae* and is perhaps the best characterised of all the secreted helminth products. ES-62 contains the unusual post-translational modification of phosphorylcholine (PC) moieties attached via an *N*-linked glycan[79,80], and this feature appears to be responsible for the majority of the anti-inflammatory effects of ES-62[81,82]. These effects include priming DCs towards a TH2 phenotype; inhibiting macrophage and mast cell activation; promoting induction of B regulatory cells and inhibiting TH1 and TH17 polarisation (reviewed in[83]). Similarly, *S. mansoni* soluble egg antigen (SEA) which contains ES products, skews the host immune response to the worm from an inflammatory TH1 to a TH2 phenotype. DCs treated with SEA *in vitro* are polarised to prime TH2 responses and are refractory to TLR stimulation[84] while macrophages treated with one of the components of SEA, LNFPIII, a trisaccharide LewisX-containing glycan, differentiate fully into an alternatively activated phenotype with upregulated expression of CD301, Ym1 and Arg1 and produce IL-10[85]. *In vivo* administration of ES products is sufficient to induce a strong TH2 response – for example, ES from adult *N. brasiliensis* (NES) induces a strong TH2 response, even in the presence of the TH1/TH17 polarising agent, complete Freund's adjuvant[86]. Helminth ES products from a variety of species have also been found to be therapeutic in multiple mouse models of inflammatory disease including collagen-induced arthritis (CIA),

type 1 diabetes (T1D), experimental autoimmune encephalomyelitis (EAE), colitis, and asthma and other allergies (reviewed in[52]). As discussed, MetS is associated with chronic inflammation and thus, given their potent anti-inflammatory effects, and therapeutic potential in inflammatory diseases it seems likely that helminth ES products could have a significant impact on MetS.

Protozoan parasites also produce a range of secreted products that influence the host to ensure their own survival. One of the best studied molecules constitutes a group of cysteine proteases (CPs) produced by *L. mexicana*[87]. It has been demonstrated that this highly active group of Cathepsin-L like proteases are primarily produced by the amastigote (intracellular form of parasite)[88] and their role as a key virulence factor during infection has been confirmed in a number of studies through the use of a variety of CP mutant promastigotes[55]. Further evidence suggests that the most abundantly expressed form of *L. mexicana* specific CP, CPB2.8 can drive a TH2 response *in vivo.* Pollock *et al* have demonstrated that administration of purified recombinant CPB2.8 can stimulate both IL-4 and IL-5 production in the draining lymph node and can enhance circulating IgE titres[59]. The mechanisms underpinning these findings remain unclear, however it should be considered that, the CPs are similar in structure to other allergy-inducing proteases such as dust mite derived DerpI, a potent inducer of IL-4 and IgE[89]. Thus, it is perhaps unsurprising that CPB2.8 is so effective in polarising the immune response from a TH1 dominated healing response, to a TH2 dominated chronic phentotype. Hence, these *Leishmania* secreted CPs represent a group of potentially novel immunomodulatory molecules.

Helminth ES and MetS

The strong TH2 response generated in response to SEA and LNFPIII led Bhargava *et al* to investigate whether these products have any effect on chronic inflammation with respect to obesity and subsequently improve metabolic function in HFD-fed mice. Injection of either for 4-6 weeks after the onset of obesity in HFD-mice augmented the production of IL-10, as well as increasing insulin sensitivity[85]. Unlike with live helminth infection[65], neither treatment had any effect on body weight or circulating lipid or adiponectin concentrations[85]. However, both LNFPIII and SEA had significant effects on the cell composition/interactions in the WAT – there was a decrease in the number of observed crown-like structures (CLS), and the gene expression of inflammatory genes such as *TNF-α, Casp1, nlrp3, il18* and *il1β* was reduced with a corresponding increase in *il10* and the M2 genes *Arg1* and *MgL1*[85]. It has recently been demonstrated that SEA mediates this improvement in metabolic homeostasis by restoring the type 2 response in the WAT through the induction of eosinophil recruitment[65], which has previously been shown to be crucial in promoting the presence of M2 macrophages[41]. Correspondingly, SEA shifts the M1/M2 ratio towards an M2 phenotype, and increased the numbers of IL-4$^+$, IL-5$^+$ and IL-13$^+$ CD4$^+$ T cells in gonadal WAT[65]. In keeping with improved insulin sensitivity there was also an increase in insulin receptor B, insulin receptor substrate 2, C/ebp-α and glucose transporter 4 gene expression in the WAT of LNFPIII-treated mice. These effects are not due to direct effects of LNFPIII on adipocytes: they appear to be mediated indirectly via production of IL-10 by macrophages as conditioned medium from LNFPIII-primed macrophages from WT but not IL-10$^{-/-}$ mice improved insulin responsiveness in 3T3-l1 adipocytes[85]. SEA and LNFPIII were also demonstrated to have a strong protective effect on diet-induced hepatic steatosis, with treated HFD-mice exhibiting

reduced serum levels of the triglycerides, AST and ALT as well as decreases in genes associated with lipogenesis such as *Srebp*, in the liver[85].

Of interest, while not a secreted helminth product, *L. sigmodontis* antigen (LsAg) has also been demonstrated to mediate some protection against MetS, as therapeutic administration of LsAg for a two-week period improves glucose tolerance in diet-induced obese mice. This protection was found to require eosinophils but to be independent of $CD4^+FOXP3^+$ T cells[66]. Treatment of the pre-adipocyte cell line (3T3-L1) with LsAg was found to inhibit their differentiation into mature adipocytes suggesting LsAg may also be able to suppress adipogenesis[66]. It will therefore be interesting to see whether Ls ES products have similar properties.

Helminth ES and Atherosclerosis

Atherosclerosis is a lipid-driven disease of the arteries, caused by lipid deposition and intimal thickening of the aorta and larger arteries, characterised by sustained inflammatory responses and specifically, the chronic activation of macrophages[90,91]. MetS significantly increases the risk of atherosclerosis, which is one of the main underlying pathologies for cardiovascular diseases such as stroke and myocardial infarctions, as alluded to earlier, the leading cause of death in the Western world[92]. Wolfs *et al* demonstrated that weekly treatment with SEA resulted in a 44% reduction in atherosclerotic plaque size in a cholesterol-induced murine model of atherosclerosis[91]. This was associated with a significant decrease in circulating inflammatory monocytes, as well as reduced plaque necrosis and inflammation, and reduced gene expression of CD68, TNF-α and MCP-1 in the aortic arch[91]. Chronic exposure to SEA has also been shown to cause a 30% reduction in plasma serum cholesterol and LDL levels in $ApoE^{-/-}$ mice fed a high-fat diet[93], however there was no effect on lesion size or

inflammation, a perhaps surprising observation which the authors speculated could be a result of using dead eggs and heat-treated SEA in this study[93].

ES-62's anti-inflammatory properties dictate that it can protect against inflammatory diseases in models such as collagen-induced arthritis and the MRL/lpr model of systemic lupus erythematosus[94–96]. Patients with these diseases are at greater risk of developing atherosclerosis and thus the protective effect of ES-62 was investigated in gld.ApoE$^{-/-}$ mice, that are commonly utilised as a model for the study of the accelerated cardiovascular disease that can occur in some lupus patients[97]. When treated with ES-62, via osmotic pumps for 12 weeks to release ES-62 at a steady rate and mimic natural infection, these mice demonstrated reduced atherosclerotic lesion area of nearly 60% compared to PBS-treated mice, with reduced numbers of macrophages and collagen at the lesion site. Similar to studies in the MRL/lpr mouse, they also had some evidence of reduced renal disease as measured by decreased proteinuria, as well as decreased levels of the anti-nuclear antibodies (ANA) that contribute to kidney disease[98].

Can other parasite molecules influence the outcome of MetS?

The seminal work carried out by Bhargava (2012) has shown that modulation of inflammatory responses by a parasite-derived product can significantly impact on the outcome of MetS in obese mice[85]. Specifically, it has been shown that suppression of M1 inflammatory responses and polarization towards an M2 phenotype are advantageous in the fight against metabolic disease, therefore suggesting the parasite products that influence M1/M2 polarisation during chronic parasite infection may provide a potential novel therapy for treatment or prevention of MetS. It is interesting to speculate therefore that other parasite

products with the ability to influence the differentiation of macrophages may also have the potential to be therapeutic in MetS. With this is mind, it is of particular interest that the intracellular protozoan *T. gondii*, has been shown to utilise some of its key proteins conserved for cellular invasion to do just that. Rhoptry proteins (ROPs) are secreted from the apical complex during the process of host cell entry and are either released into the host cytosol or retained at the parasitophorous vacuole membrane (PVM). Recently, a number of studies have demonstrated how ROPs, specifically ROP16 (in Type I/III strains of *T. gondii*) can target host transcription and cause prolonged phosphorylation of STAT3 and STAT6, resulting in the suppression of IL-12 production[99,100]. Further to this, ROP16-mediated STAT6 activation has also been associated with enhanced levels of arginase-1 production, in both macrophages and fibroblasts. Thus, the secreted ROP16 appears to be instrumental in ablating TH2 suppressive cytokine production and also promoting M2 macrophage polarization[101]. Moreover, ROPs do not appear to be the only protozoan products to exhibit this effect: for example, a *T. gondii*-specific peroxiredoxin (Prx) known as rTgPRx[102], which has recently been shown to be a potent anti-inflammatory molecule[103], appears to promote development of an AAM phenotype via STAT6-dependent and -independent enhancement of arginase-1 and YM1 expression. In addition, the recombinant protein has been shown to stimulate the expression of IL-10 to further reinforce the regulatory/TH2 type response.

These marked effects of the protozoan Prx may not be so surprising as a number of studies had previously demonstrated the effectiveness of Prxs derived from the helminths *S. mansoni* and *Fasciola hepatica*, in similarly driving alternative activation of macrophages *in vivo*. Thus, treatment of BALB/c mice with recombinant *F. hepatica* Prx, induces Ym1 expression in peritoneal macrophages, and increases circulating titres of IL-4[104,105]. Additionally, as mentioned previously, various ES products from several helminth species stimulate alternatively activated macrophages: thus, ES-62-treated macrophages are refractory to LPS-

induced pro-inflammatory cytokine production[106] and treatment of macrophages with SEA induces an anti-inflammatory profile with decreased levels of LPS-induced IL-12 and TNF-α and increased IL-10 production[91].

Targeting other relevant inflammatory mediators

It has recently been demonstrated that ES-62 interacts, via its PC moieties, with CRP in human serum[107]. This interaction does not result in activation of the complement cascade because the ES-62-CRP-C1q complex generated appears to be unable to efficiently cleave C2. In this way ES-62 secreted by the parasite during infection can provide some protection against the activation of complement, thus preventing parasite opsonisation. However, it also further demonstrates the ability of ES-62 to dampen inflammatory responses, in this case CRP-dependent that may play an important role in conditions that represent medical emergencies such as myocardial infarction and stroke. Furthermore, PC binding to CRP has recently been demonstrated to bias migrating monocytes and T cells towards an M2 and TH2 phenotype respectively in an *in vitro* model[108] and therefore it is possible that ES-62 by binding to CRP may also have this capability.

We have previously discussed the importance of NLRP3 inflammasome activation in driving inflammation and disease during MetS and therefore, molecules with the ability to suppress such responses could provide a potential therapeutic for the syndrome. For example, treatment of human macrophages with *F. hepatica* helminth defence molecule 1 (FhHDM-1), a cathelicidin-like molecule secreted by the parasite, significantly reduces their production of IL-1β in response to phagocytosis of Alum particles[109] while a small molecule analogue (SMA) of ES-62 (based on its PC moiety), SMA 12b, has been found to mediate its

protective effects in the CIA model via suppression of NLRP3 activation and decreased IL-1β production[110]. The protozoan product rTfPRx suppresses macrophage ROS production via modulation of NLRP3 inflammasome activation and is capable of inhibiting both caspase-1 and IL-1β production following LPS-stimulation of the inflammasome[103]. Finally, *Leishmania*-derived, GP63 also appears to modulate activation of the NLRP3 inflammasome, suppressing the production of IL-1β, both during infection and, importantly, in macrophages treated with purified GP63[111]. These modulators all exhibit potential as modifiers of the NLRP3 inflammasome pathway and as such are exciting candidates for the basis of future therapeutics.

As mentioned earlier in the review, MyD88 appears to be a crucial integrator of TLR and inflammasome signals impacting on obesity and MetS[112], and one of the key mechanisms of ES-62 in its protection in CIA and lupus models is the degradation of MyD88[95,96]. Interestingly, ES-62 has also been demonstrated to strongly suppress the basal levels of active Akt in DCs *in vitro* in a TLR4-dependent manner[81] thus suggesting that it can target multiple layers of the pathways necessary to reduce the damaging pro-inflammatory immune response generated during MetS.

Conclusions

Inflammation associated with MetS has now been established to be central to the development of insulin resistance and cardiovascular disease. During obesity, the cellular composition of adipose tissue changes from an anti-inflammatory/TH2 environment characterised by M2, eosinophils, TH2 $CD4^+$ T cells and Bregs to a pro-inflammatory environment characterised by higher numbers of M1, neutrophils, mast cells, TH1 and

cytotoxic $CD8^+$ T cells. Throughout this review we have discussed the host immune regulation that helminths and protozoans can elicit during infection and the secretory molecules that these parasites use to exert these effects, particularly their ability to drive a TH2 response while suppressing an opposing inflammatory phenotype. Therefore, it is possible that helminth- and protozoan-derived molecules that enhance protective anti-inflammatory responses, such as Type 2 immunity, alternative activation of macrophages and modulation of mTOR signalling could be beneficial in the treatment of metabolic disorders (Figure 2).

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Figure Legends

Figure 1: The role of PI3K/Akt, mTORC1, mTORC2, and the NLRP3 inflammasome in the development of metabolic syndrome. A schematic detailing the key signalling molecules and pathways activated/supressed during nutrient excess, insulin resistance and associated chronic inflammation.

Figure 2: Parasitic ES products are able to influence a range of signalling pathways such as PI3K/Akt and the inflammasome, as well as biasing immune system cells towards a type 2 phenotype, both of which suggest they may be able to provide protection against MetS.

Table 1: The mechanisms of action of some of the best characterised parasite-derived excretory-secretory products on the immune system and in murine disease models is summarised.

Table 1

Parasite excretory-secretory product	Immune responses affected	Animal disease model	References
A. viteae ES-62	Downregulates MyD88 to subvert TLR-mediated activation of macrophages, DCs and mast cells in a TLR4-dependent manner; resets macrophage and TH1/TH2/TH17 homeostasis; inhibits B2 cell activation and induces IL-10-secreting B1 cells and various Breg populations	Collagen-induced arthritis (CIA); ovalbumin-induced airway hypersensitivity; oxazolone-induced contact sensitivity, MRL/lpr model of SLE, *Gld*.ApoE$^{-/-}$ model of lupus-associated accelerated atherosclerosis	12,83,95,96,98,113, 131,14 ,141,15
S. mansoni SEA	Priming of DCs towards a TH2 phenotype, induction of TH2 cells *in vivo*, induction of Tregs	Experimental Autoimmune Encephalomyelitis (EAE); Type 1 Diabetes (T1D) in non-obese diabetic (NOD) mice; CIA; glucose tolerance in HFD-fed mice; cholesterol-driven model of atherosclerosis	18,85,116–119
S. mansoni LNFPIII	Induction of AAM phenotype; priming of DCs towards a TH2 phenotype	glucose tolerance in HFD-fed mice; psoriasis; T1D; EAE	20,85,116,120,121
F. hepatica ES	Priming of DCs to induce TH2 and Treg responses; induction of AAMs; inhibition of TH17 responses in vivo	T1D in NOD mice; CIA	24,122–125
F. hepatica HDM-1	induction of AAM; inhibition of NLRP3 inflammasome activation in macrophages	murine models of T1D and multiple sclerosis	25,124,126
F. hepatica peroxiredoxin	Induction of AAM	not tested	04105
N. brasiliensis ES	Induction of TH2 cells *in vivo* via DC priming	allergic lung inflammation	27,86,127,128
H. polygyrus ES	Inhibition of TH1 responses; inhibition of macrophage NO production; inhibition of PAMP-mediated DC responses	not tested	50,51
L. mexicana CPB2.8	Induction of TH2 response in vivo, induction of IgE	not tested	58,59
T. gondii Rhoptry proteins	Suppression of IL-1; enhanced arginase-1 in macrophages and fibroblasts	not tested	100,101
T. gondii peroxiredoxin	Induction of AAM; stimulation of IL-10; inhibition of macrophage ROS and IL-1β production via inhibition of NLRP3 inflammasome activation	not tested	102,103