Can parasitic worms cure the modern world's ills?

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Abstract

There has been increasing recognition that the alarming surge in allergy and autoimmunity in the industrialised and developing worlds, shadows the rapid eradication of pathogens, such as parasitic helminths. Appreciation of this has fuelled an explosion in research investigating the therapeutic potential of these worms. This review considers the current state-of-play with a particular focus on exciting recent advances in the identification of potential novel targets for immunomodulation that can be exploited therapeutically. Furthermore, we contemplate the prospects for designing worm-derived immunotherapies for an ever-widening range of inflammatory diseases, including, for example, obesity, cardiovascular disease and ageing as well as brain developmental disorders like autism.

Worms really may be good for you, after all

An evolutionary viewpoint of the development, regulation and function of the immune system is gaining increasing traction. This reflects awareness that, in addition to shaping metabolism and the function of major organs [1, 2], co-evolution with commensal and environmental microbes and “Old Friend” infections has generated a mammalian immune system that optimises the symbiotic survival of bacteria, fungi, latent viruses and parasites (macrobiota), whilst minimising pathological consequences for the host [3-6]. Specifically, exposure to such persistent, tolerated infections rather than the more recently evolved “crowd infections” (e.g. childhood viruses, like measles) that diametrically either kill or induce protective immunity, appears to be required early in life for induction of the regulatory networks that prevent autoimmunity and allergic inflammatory responses to harmless agents, and also for the homeostatic resolution of infection-fighting inflammation, once the pathogen is cleared [3-6]. Recognition of this balanced education of the immune system has revolutionised our understanding of how its dysregulation provides a unifying mechanism for the development of allergic and autoimmune inflammatory disorders, metabolic syndrome, and the chronic low-grade inflammation that characterises ageing. The alarming rise in each of these appears to shadow the dysbiosis of the macrobiota resulting from the sudden and swift eradication of organisms like helminths in rapidly developing societies (Fig. 1). Likewise, it accounts for the epidemiological evidence that, rather than protect against allergic and autoimmune inflammatory disorders, ‘crowd infections’ may even trigger them, reconciling findings that previously were perceived as flaws in the Hygiene Hypothesis [3].

Despite the fact that “therapeutic” infection with parasitic worms may not always result in improved clinical outcome and may even exacerbate the target pathology [7], there has been intense focus on exploiting the immunoregulatory actions of helminths to develop novel therapies to treat disease. Extensive studies (reviewed [8-10]) have strongly evidenced the potential of worm therapy in animal models and although less consistent, particularly with respect to allergic responses, this has been supported by epidemiological data from endemic regions. Indeed, there are striking inverse incidences between infection with filarial nematode worms and both rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [11-13], as well as a lower prevalence of filarial infection in type 2 diabetes patients, in India. Likewise, lower body mass index and fasting blood glucose levels have been reported in those with a history of schistosomiasis in rural China [14]. Further support is provided by health initiatives such as deworming being associated with increases in the prevalence of atopy in endemic areas, an outcome that is now questioning the wisdom of current mass eradication programmes [10]. Collectively, these observations have driven clinical trials of therapeutic infection with pig whipworm (*Trichuris suis*) and human hookworm (*Necator americanus*) in a range of allergic and autoimmune inflammatory diseases as well as autism (reviewed in [9]). However, despite the reported success of helminth self-infection in combating inflammatory bowel diseases (IBD) like ulcerative colitis [15] and other autoimmune and allergic inflammatory disorders and their neuropsychiatric comorbidities such as anxiety and autism [16], the ability of worm therapy to consistently improve clinical outcome, apart from some promising results in coeliac disease [17, 18], remains disappointingly unproven to date [9, 10, 19]. Nevertheless, and because it is not ideal that patients are treated with live pathogens, great strides have been made in

recent years in identifying the roles and targets of molecules excreted-secreted (ES) by helminths (secretome; [20-23]) to modulate the immune response and promote tissue repair (Box 1): this raises the exciting possibility that ES can potentially be exploited as biologics and/or form the basis for the development of small molecule drugs [19-26]. We shall therefore discuss the current focus areas of helminth-based therapy that are advancing our understanding of development of the immune response and its dysfunction in disease, concentrating on the identification of novel and safe targets of potential therapeutic intervention.

Infection with parasitic worms helps identify key immunoregulatory mechanisms

Type 2 (Th2) immune responses are induced to clear parasitic helminth infections, but the worms have evolved a variety of mechanisms to elicit regulatory responses that promote their survival and counter the Th1/Th17-mediated pathology that would likely arise in the absence of such a modified Th2 response [8]. These regulatory responses notably involve regulatory B- and T-cells and macrophages (often termed M2 or alternatively-activated [AAM] macrophages) and the production of a range of cytokines, particularly IL-10, IL-35 and TGF-β, and AAM products like RELMα, Arginase and Ym1 [27]. Such regulatory responses have the serendipitous side-effect of also alleviating aberrant inflammation irrespective of its phenotype, explaining the ability of helminths to target both Th2-driven allergic and Th1/Th17-driven autoimmune inflammation [8].

In addition to highlighting their therapeutic potential, studies investigating immunomodulation by helminths have identified and validated key regulators of

disease pathogenesis. For example, regulatory T cell responses (Treg, Tr1 and iTR35 cells) are induced to limit inflammation in chronic helminth infection [8, 28] and protection against allergic and autoimmune disease by helminths is increasingly recognised as involving the mobilisation of a variety of regulatory B cells (Bregs) which, in some cases, appear responsible for consequent induction of protective regulatory T cell responses [29-32]. Thus, in models of acute asthma, whilst transfer of splenic and lung-derived Marginal Zone (MZ)-like Breg populations, induced upon infection with *Schistosoma mansoni,* conferred protection against ovalbumin (OVA)-induced airway hyper-responsiveness (AHR) in an IL-10-dependent but Treg-independent manner [29], a splenic T2-MZP subset of Bregs afforded protection in an IL-10- and regulatory T cell-dependent manner [29-31]. Likewise, a $CD19^{+}CD23^{hi}$ population of B cells induced by *Heligmosomoides polygyrus* reduced development of house dust mite (HDM)-induced airway inflammation and also experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (MS) in mice. However, in the case of EAE, protection did not appear to be dependent on IL-10 production by such B cells [33], despite this being the case for MS patients in endemic areas [32, 34].

More recently, the use of helminth infection models has identified novel roles for eosinophils and basophils [35-37] in regulating Th2-, innate lymphoid cell (ILC)-, and AMM-mediated immunity and thus in fighting infection, maintaining metabolic homeostasis in adipose tissue and promoting tissue repair [38-40]. Moreover, these models have highlighted the role of tuft cells, specialised epithelial cells that are found in low numbers in healthy gut tissue but are expanded in response to a range of helminth infections and initiate ILC-2-mediated inflammatory and goblet cell

responses that promote worm expulsion [41-43]. Similar epithelial effects may also pertain in the lungs [43, 44], where ILC2 cells are recruited not only to mediate helminth-induced lung inflammation [45] but also to promote tissue repair [46]. Furthermore, IL-22 which can be produced by ILC3s to inhibit systemic inflammation by combating bacterial infection and promoting gut barrier integrity [47], similarly promotes expulsion of both *Nippostrongylus brasiliensis* and *Trichuris muris* by stimulating goblet cell responses [48]. Such IL-22-driven effects are reminiscent of those observed in protection against ulcerative colitis following self-infection with *Trichuris trichiura* where the accumulation of Th22 cells in the mucosa was also associated with goblet cell hyperplasia and mucus production [15].

Collectively these recent studies investigating immune responses to helminths have advanced our fundamental understanding of the molecular and cellular mechanisms underpinning type-2 immunity and have highlighted not only previously unsuspected interactions between cells of the innate and adaptive immune systems but also amongst these cells and their stromal environments (Fig. 2).

Helminth-based therapies to reset immune homeostasis

Although immunomodulation arising from helminth infection may be a result of the induction of regulatory T cell responses [8], the therapeutic effects of this may be a fortuitous consequence of bystander suppression. Consistent with this, although ES products can induce IL-10 regulatory responses [22], there is less evidence that they induce Tregs. Nevertheless, ω1, an ES product from *S. mansoni* induces Tregs in NOD mice [49] and a TGFβ mimic in *H. polygyrus* ES was found to drive their differentiation *in vitro* [50]. However, none of the protection afforded by ES-62, a

particularly well-characterised secreted product of *Acanthocheilonema viteae* that protects against both allergic and autoimmune inflammation is achieved in this way [22, 25, 26]. Indeed, helminths or their products appear able to dampen inflammation in Th1/Th2/Th17-associated pathologies by modulating various distinct targets that may differ depending on the model investigated.

Thus, an inflammatory disease-centric approach may have obscured an intrinsic mode of action of helminth ES products, namely to restore immune homeostasis irrespective of the phenotype of aberrant inflammation. This may explain the findings that ES-62 can both reinstate the Th1/Th2 balance away from Th2 in airway inflammation by inducing IFNγ whilst inhibiting Th1/Th17 responses in collagen-induced arthritis (CIA) [22, 25, 26]. However, ES-62 does not achieve the latter by invoking a compensatory induction of Th2 cytokines, but rather, by harnessing the inflammation-resolving, tissue repair properties of IL-22 in the arthritic joint [51, 52]. By contrast, ES-62 exhibits inverse effects on the IL-17/IL-22 inflammatory axis whilst preventing disease development in the MRL/Lpr mouse model of SLE [53]. Rationalising this, whilst Th17 cells can act as master regulators of Th1 and Th2 responses, IL-17 and IL-22 can exhibit dual pathogenic and inflammation-resolving properties even within the same disease model depending on the stage of pathology and inflammatory microenvironment [52, 53]. The homeostatic effects of ES-62 can also be explained in terms of its ability to reset inflammatory MyD88 signalling to normal levels and, in doing so, generate IL-10-producing Bregs that are associated with its protection against CIA, lupus-like nephritis and chronic airway inflammation and remodelling [53-55]. Consistent with this, transfer of splenic B cells from ES-62-conditioned mice is sufficient to prevent development of autoantibody production and

pathogenic Th22 responses as well as reset the protective M2:M1 cell balance in the kidney in recipient lupus-prone mice [53]. Such a key role for MyD88 downregulation in preventing disease is supported by lineage-specific deficiency in the adaptor molecule being sufficient to abrogate lupus-like (B cells) [56, 57] and high fat diet (HFD)-induced cardiovascular (myeloid and endothelial cells) [58] pathologies in inflammatory mouse models. The mechanisms exploited by ES-62 remain to be fully delineated but involve the homeostatic induction of selective autophagy, which normally acts to limit TLR-driven inflammation, to degrade MyD88 and downstream effectors [59, 60].

By contrast, Wmhsp60 of *Wolbachia* (an endosymbiont of many filarial nematode species), which inhibits autophagy via stimulating counter-regulatory mTOR signalling, drives TLR4-MyD88-mediated inflammation that results in a senescent, inflamm-ageing-like phenotype of monocytes [61]. *Wolbachia* appears to be important for embryogenesis, growth and survival of many filarial parasites and there is increasing evidence that the inflammatory responses it elicits contribute to the (potentially catastrophic) pathology resulting from parasite death and loss of helminth immunomodulation. As normal monocyte function can be restored and inflammation limited by the use of the mTOR inhibitor, rapamycin, these findings provide a potential breakthrough on how to prevent the severe adverse effects associated with anti-filarial chemotherapy [61].

Helminth infections modulate the epigenetic landscape of inflammatory cells

The macrobiota can shape immune responses by modulating hematopoiesis ("Trained Immunity") via processes that can become dysfunctional in inflammatory

disease, cancer and ageing [62], with many of these defects in the regenerative potential of haemopoietic stem cells reflecting changes in their epigenetic landscape that are potentially therapeutically reversible. Interestingly, helminth induction of AAMs involves epigenetic modifications to drive the required transcriptional program [63]. Specifically, an H3K27 demethylase termed Jumonji domain containing-3 (Jmjd3) has been shown to be essential for macrophage differentiation, activation and helminth-induced M2 polarisation [64]. Moreover, Histone Deacetylase-3 (HDAC3)-deficient macrophages exhibit a phenotype similar to IL-4-induced AAMs [65] and consistent with this, pulmonary inflammation resulting from exposure to *S. mansoni* eggs, which is limited by AAMs, was found to be reduced in mice with HDAC3-deficient macrophages [63]. Further support that helminths and their products can impact on epigenetic regulation of macrophage responses was provided by the imprinting of M2 polarisation and associated IL-10 responses by *T. suis* soluble products being reversed by HDAC inhibitors that resulted in reduced histone acetylation of the TNF-α and IL-6 promoters [66]. Moreover, suppression of M1 responses in a *Mesocestoides corti* model of neurocysticercosis is associated with reductions in activating H3K4Me3 and H3K9/14Ac marks at the promoters of TNF$-\alpha$, IL-6, NOS2, MHC-II and CIITA [67].

Helminths can likewise impact on DNA methylation and relating to this, *S. mansoni* infection drives induction of a functionally plastic population of $CD4^+$ T cells that can be distinguished by its unique DNA methylation signature, relative to classical Th1 and Th2 phenotypes [68]. Furthermore, studies exploiting dendritic cells (DCs) that lack the methyl-CpG-binding protein, Mbd2, showed that such cells were defective in their ability to induce Th2 responses to helminths and allergens and this was

associated with multiple changes in their transcriptional programme associated with H3K9/K14 acetylation marks and consequently, regulation of chromatin structure [69]. Intriguingly, analysis of priming of Th2 responses by skin DCs reflected induction of quite distinct transcriptional profiles in response to *N. brasiliensis* versus the contact sensitiser, dibutyl phthalate (DBP) as exemplified by a rather surprising type-I IFN signature induced by the helminth [70].

Given that epigenetic (i.e. reversible) modification of gene expression can be transgenerational [71], the effect of helminth infection during pregnancy is an area of interest. Of note in the context of education of (neonate) immune responses and its dysfunction in inflammatory disease, chronic *S. mansoni* infection during pregnancy was reflected by reduced airway hyper-responsiveness in the children. This was associated with decreased polarisation towards Th2 responses in infants that corresponded with reduced levels of histone acetylation in the IL-4 promoter regions in naive T cells [72] suggesting that maternal infection may impact on induction of immunoregulatory networks and their pathogenic dysfunction during childhood by transgenerational changes to the epigenetic landscape of immune system cells.

Targeting integrated inflammatory and metabolic pathways to increase health and life span

Increasing evidence suggests that chronic low-grade inflammation (driven by IL-6, IL-1β, IL-18, TNFα) generated by the rapid eradication of “old friends” like helminths, allied to widespread adoption of a HFD and sedentary lifestyle, promotes the dsyregulation of (mTOR-regulated) metabolic pathways underpinning the ageing process as well as its comorbidities, T2D, obesity and cardiovascular disease

(metabolic syndrome). Thus, interest is beginning to focus on whether exploiting the anti-inflammatory and tissue repair properties of helminths can improve life-span or at least improve the well-being and health of our ageing populations. Evidence from diet-induced obesity and atherosclerosis models, as well as epidemiological studies of filarial infection and schistosomiasis, support the hypothesis that helminths can protect against metabolic syndrome [14, 26]. Such protection has been related to reduced type-1 inflammation and/or resetting of metabolism (e.g. improved glucose tolerance and insulin sensitivity, browning of adipose tissue), reflecting the roles of ILC2s, eosinophils and AAMs in supporting metabolic homeostasis in adipose tissue [37, 39, 40, 73, 74] and the upregulation of genes controlling glucose and lipid metabolism by STAT6 [75]. Moreover, as helminths can utilise blood lipids and glucose and alter lipid metabolism, in conjunction with the homeostatic effects of Th-2 polarisation on metabolism, infection can lower blood cholesterol and increase insulin sensitivity to protect against obesity and associated cardiovascular disease [76].

Treatment with helminth-derived molecules can likewise protect against metabolic syndrome [26]. For example, schistosome soluble egg antigen (SEA) and LNFP111 improved insulin sensitivity and reduced type-1 inflammation [77]. SEA also restored the type 2 response in adipose tissue through eosinophil recruitment [78] and consequent macrophage polarisation towards an M2 phenotype, and increased the numbers of IL-4$^+$, IL-5$^+$ and IL-13$^+$ CD4$^+$ T cells [79]. Likewise, schistosome egg-derived ω1 improves metabolic status by stimulating the IL-33-mediated recruitment of ILC2s, eosinophils, and AAMs in the adipose tissue [80], whilst protection by

Litomosoides sigmodontis antigen (LsAg) also involves the recruitment of eosinophils [81].

Some of these effects likely reflect the ability of helminths to directly target the key regulatory nodes, MyD88 and mTOR, that interact to integrate inflammatory and metabolic pathways. For example, recent studies suggest mTORC2 signalling appears essential and specific to AAM-M2 differentiation and its deficiency in macrophages renders mice incapable of clearing pulmonary infection with *N. brasiliensis* and prevents their ability to regulate metabolic control of thermogenesis [82]. Moreover, as mTOR and autophagy are counter-regulatory [60], this provides another point of potential intervention by helminths: consistent with its induction of autophagy to limit TLR-mediated inflammation, ES-62 suppresses activation of PI3K/AKT, upstream regulators of mTOR [59, 83], whilst *Brugia malayi* microfilariae inhibit the mTOR pathway and induce autophagy in human DCs [84]. However, the ability of helminths to induce autophagy is not always good news, as the chronic oxidative stress underpinning transformation in hepatocellular carcinoma by the fluke *Dicrocoelium dendriticum* is associated with induction of autophagic vesicles by its somatic antigens [85].

Perhaps more directly pertinent to their ability to modulate host metabolism and immune responses, parasite-glycolytic enzymes can be secreted, either free-form or in exosomes. In addition, as with their mammalian counterparts, there is increasing recognition that they exhibit multifunctional properties, allowing them, when attached to the parasite surface to play roles in mediating adherence and invasion, as well as in modulating immune responses (reviewed in [86]). Thus, GAPDH from

Haemonchus contortus appears to have C3-binding activity, enolase from *Steinernema glaseri* suppresses immune responses in insects and glucose-6-phosphate isomerase from *Echinoccus multicularis* is thought to promote angiogenesis around the metacestode and promote its development and acquisition of nutrients [86].

Collectively, therefore, these findings suggest potential in exploiting helminth products to modulate host metabolism in the context of inflammation to improve health- and lifespan.

The microbiome and therapy - it takes two to tango!

Neonates and animals raised under germ-free (GF) conditions have an "incomplete" immune system that is somewhat Th2-polarised and exhibits reduced levels of B and T cells and also, gut-associated immune responses [87, 88]. "Education" of the full immune response requires instruction by particular components of the macrobiome with, broadly speaking, type 1 responses elicited by pathogenic bacteria, systemic commensal bacteria and viruses, type 2/regulatory phenotypes by helminths, *Clostridiales* and *Bacteroides fragilis*, and type 17 by segmented filamentous bacteria (SFB) and fungi [6, 87, 88]. This interdependence places increasing importance in analysing helminth-induced immunomodulation in the context of the larger macrobiome and co-infection status to determine how the resulting crosstalk balances induction of appropriate responses without pathology [18, 89]. For example, it has recently been reported that microbiota-induced Tregs express the nuclear hormone receptor RORγT and can therefore also differentiate into Th17 cells [90]. In the absence of such induced Tregs, Th2-immunity to helminths is improved

but pathology associated with type-2 inflammation is exacerbated. Thus, the plasticity of this lineage allows the microbiota to balance appropriate immune responses at mucosal surfaces [90]. Reciprocally, Th2 responses elicited by enteric *N. brasiliensis* infection were found to reduce abundance of SFB, which have been implicated in the induction of pathological Th17 responses in inflammatory disorders [91].

The fundamental interdependence of this co-evolution is illustrated by the ability of intestinal helminths to detect commensal bacterial cues that inform them that they have reached an appropriate microenvironment for their development where in turn, they can promote expansion of bacteria that induce regulatory responses to foster their survival [6, 92]. Much of the interest in helminth-endemic regions to date has focused on the interactions between GI-tract located worms and the bacterial microbiome, concentrating on *T. trichiura* but with inconsistent results, either indicating no effect or enrichment of bacterial diversity in infected children [18]. However, in experimental animal models, worm infections appear generally to be associated with a decrease in bacterial diversity [93]. Nevertheless, cross-sectional analysis of persistent infection with the related *T. muris* was associated with enrichment of *Lactobacillus* that was accompanied by a shift from regulatory to inflammatory immune responses [94]. By contrast, a more longitudinal analysis showed that *T. muris* infection mainly modulated *Bacteroidetes*, in particular by reducing diversity and abundance of *Prevotella* and *Parabacteroides* species and that such perturbation of the microbiota was transitory, essentially returning to normal upon clearance of the parasite [93, 95]. However, not all key immune system cell populations associated with chronic *T. muris* infection recovered [95], suggesting

that whilst perturbation of the microbiota due to helminth infection is reversible, some immunoregulatory networks appeared more stably modulated with implications for future pathophysiology.

Reflecting this, as with other immunomodulatory interventions, helminth therapy has the potential for compromising immune responses to infections as evidenced, for example, by the impaired anti-viral immunity to murine norovirus resulting from experimental enteric coinfection with *Trichinella spiralis.* Although this co-infection impacted on the wider macrobiome, helminth-induced impairment of antiviral immunity was evident in GF mice, seemingly as a result of the induction of AAMs [96]. Moreover, acute helminth infection with *H. polygyrus* or *S. mansoni* resulted in Th2-driven, IL-4/STAT6-mediated reactivation of murine gamma-herpes virus infection *in vivo* [97]. By contrast, enteric infection with *H. polygyrus* was found to reduce viral load and lung inflammation following respiratory syncytial virus (RSV) infection in a mouse model by induction of a microbiota-dependent type I IFN response in both the duodenum and the lungs [98].

Dysbiosis of the microbiome has been implicated in the pathogenesis of a wide range of allergic and autoimmune inflammatory diseases as well as in metabolic syndrome and ageing [6, 99, 100]. Thus, investigation is underway to determine whether the protective actions of worms are direct and/or involve perturbation of microbiome-driven immunregulatory networks. Early studies focusing on GI helminth modulation of IBD [9, 101] showed that infection with *H. polygyrus* induced a type 2 response and drove changes in the composition of the microbiota (reduced *Bacteroides vulgatus* burden and expansion of *Clostridiales)* that protected mice

from disease [6, 102]. Validating the relevance of these findings, individuals from endemic areas exhibited a similar protective microbiota that was reversed on deworming [103]. Interestingly from a therapeutic standpoint, analysis of patients with stable coeliac disease exposed to escalating doses of gluten showed that protection exhibited by those experimentally infected with hookworm was associated with an increasing enrichment of bacterial diversity over the course of the trial [104], specifically with respect to an increased abundance of *Bacteroides* species at the site of helminth infection in the duodenum [17].

With respect to interactions at other sites, which can harbour their own microbiota, a recent report showed that helminth infection and commensal bacteria interact to induce non-canonical regulatory T cells that act to maintain skin barrier function in the context of repeated challenge by pathogens [105], findings with potentially important implications for skin pathologies [106]. Most strikingly, however, the ability of *H. polygyrus* to ameliorate allergic asthma was abolished in mice treated with antibiotics. Chronic infection with the helminth resulted in increased abundance of gut *Clostridiales* species and consequently enhanced short-chain fatty acid production, both locally and systemically, that was responsible for the helminth-induced regulatory T cell activity that conferred protection. Indeed, transfer of the worm-modified microbiota was sufficient to mimic the protection against allergic asthma. Similar effects were observed in pigs infected with *Ascaris suum* and humans with *N. americanus*, indicating a conserved immunoregulatory network [107].

Finally, and reminiscent of studies showing that microbial and metabolic alterations in early infancy affect risk of childhood asthma [108], an emerging area of interest is how helminth infection might modulate the gut-brain axis and consequently brain development during pregnancy, a dynamic period for the macrobiome [2, 109]. Impact on the macrobiota could potentially underpin the beneficial effects associated with helminth infection in neuroinflammatory and cognitive disorders, like autism [110].

Concluding Remarks and Future Perspectives

To date, trials of helminth therapies have generally proved disappointing [9, 111]: the reasons for this are not clear but presumably reflect a complicated mix of factors (e.g. age, gender, diet, health and infection history, exercise, environment, inappropriate helminth species and site of parasitism) that impact on the interplay between the immune system and the macrobiome. Nevertheless, the potential of the approach has generated enormous interest in the immunomodulatory agents secreted by the parasites [20] that may allow more targeted therapies tailored for particular actions and (treatment of) disorders, rather than the use of helminths *per se*. Similarly, interest has also begun to focus on the potential of helminth and/or helminth product-conditioned effector cell transfer [112], the use of recombinant antigens as a new class of biologics [20, 111] or faecal macrobiota transplantation (FMT) from helminth-treated animals as evidenced by the ability of such FMT to mimic the protection against asthma afforded by live infection [107]. Finally, there is scope for more conventional drug discovery directed at targets identified by helminth action or mimicking the active moieties of helminth ES products. For example, small molecule analogues (SMAs) of the active phosphorylcholine-moiety of ES-62 exhibit

(differential) efficacy in a range of models of allergic and autoimmune inflammatory disorders [54, 113-115] that might lead to the generation of combination drug therapies aimed at targeting particular defects in immunoregulation underpinning the lifestyle and age-associated comorbidities currently plaguing our societies.

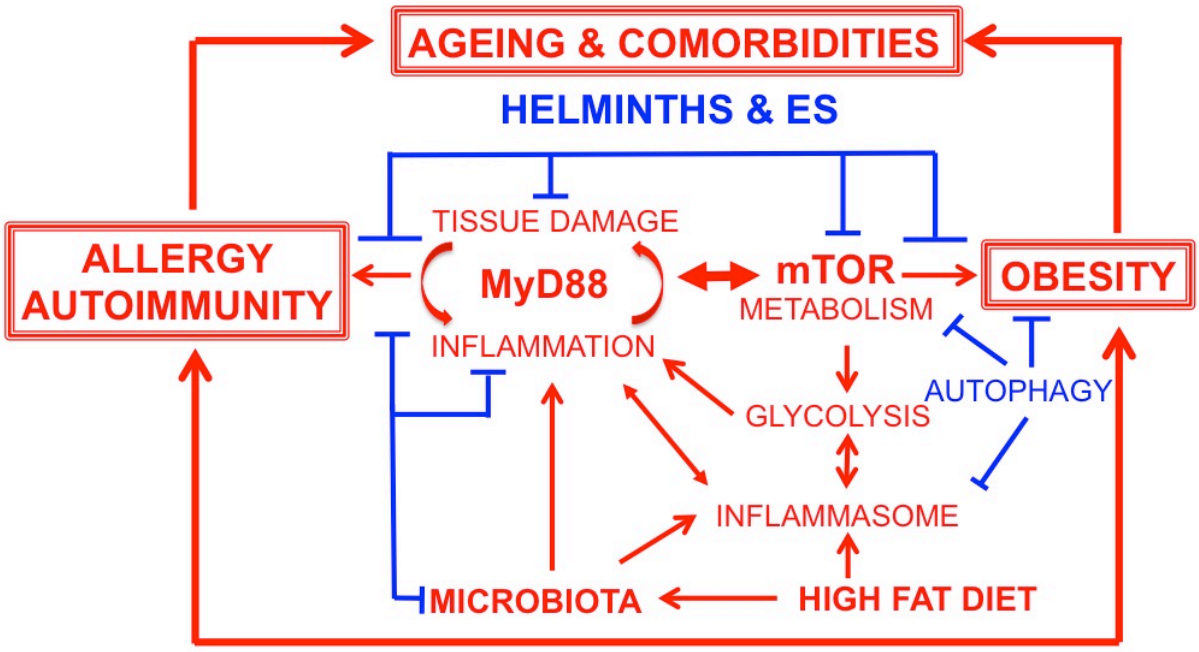

Figure 1. Helminths and their excreted/secreted (ES) products reset MyD88-dependent inflammatory and mTOR-regulated metabolic pathways.

Dysregulation of these pathways may generate triggers for the development of allergic and autoimmune disorders and metabolic syndrome, important comorbidities of ageing. Helminth-based therapies can potentially normalise aberrant signaling (represented in red) and/or induce counter-regulatory pathways (represented in blue) to restore homeostatic regulation of this network. This can be achieved by resetting the balance of effector:regulatory B and T cells, M1:M2 macrophages and their cytokines and immunoregulatory products to resolve inflammation and promote tissue repair.

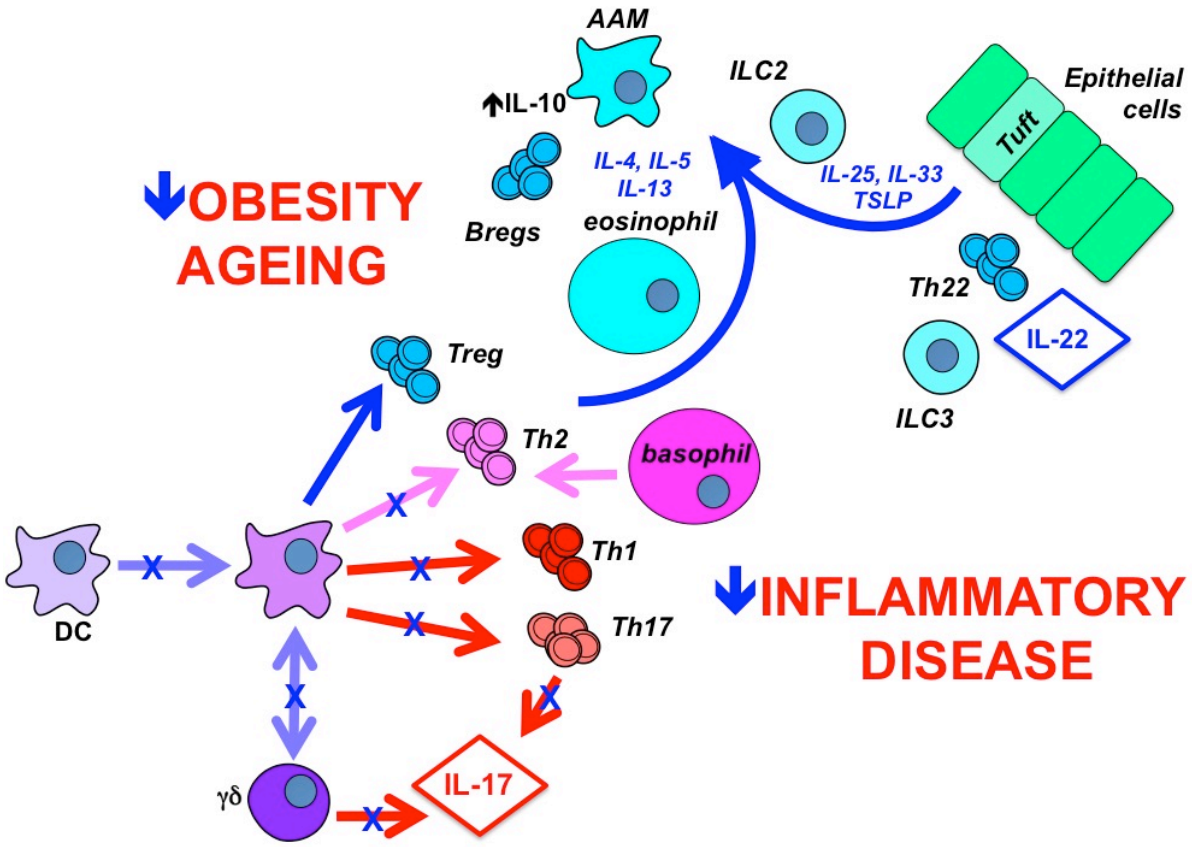

Figure 2. Helminths exhibit therapeutic potential in inflammatory disease by targeting integrated inflammatory and stromal cell networks. Helminths can interact with a range of innate and adaptive immune system cells to disrupt pathogenic regulatory networks promoted by stromal cells in particular microenvironmental niches: protective responses (blue arrows) promoted and pathogenic mediators (red arrows) suppressed (blue crosses) by helminths to effect immunoregulation are shown.

Trends Box

The rapid eradication of parasitic worms in the last 50 years has been shadowed by the rise in allergy, autoimmunity and more recently, by their reciprocal risk factor, obesity in the industrialised and developing worlds

- Consideration of the Hygiene Hypothesis has identified that eradication of worms may have left an unbalanced, hyperactive immune system and suggested the therapeutic potential of parasitic worms in inflammatory disease
- Clinical trials employing live worms to date have generally proved somewhat disappointing and so the focus has shifted to exploitation of individual worm-derived immunomodulators as therapeutics or consideration of developing drugs based on their structure and/or targets of action
- Exciting recent advances in our understanding of how worms subvert immune responses have highlighted potential new therapeutic targets for exploration
- The range of diseases for which helminths have therapeutic potential now extends from allergy and autoimmunity to cardiovascular disease, metabolic syndrome and autism

Box 1. Useful helminth ES products

The disappointing outcome of worm therapy trials allied to reservations about interventions using live pathogens has generated intense focus on identifying and characterising the ES molecules produced by helminths (secretome) as a first step to producing a new class of "biologics" with evolutionarily selected safety profiles (reviewed in [22-24, 116, 117]). As an example, ES-62, a phosphorylcholine (PC)-containing glycoprotein secreted by *Acanthocheilonema viteae* is amongst the best characterised of helminth ES products and has demonstrated therapeutic promise in certain allergic and autoimmune conditions (reviewed in [22, 24-26]). However, the advent of more sensitive technologies allowing characterization of expressed sequence tags and of late, proteomic analysis has identified large numbers of ES from an ever-increasing range of parasitic helminth species [23, 118] and, in the case of *Brugia malayi*, the secretome has been evaluated at different parasite stages [23, 119-121].

Currently, there is much interest in exploiting ES to treat autoimmune and allergic disease by the therapeutic transfer of "conditioned" myeloid cells (reviewed [112])*:* the potential of this approach is evidenced in animal models by the ability of DCs exposed to *F. hepatica* total extract to suppress CIA [122] or in the case of *Trichinella spiralis- or Hymenolepis diminuta* antigen, experimental allergic encephalomyelitis (EAE) [123] and colitis, respectively [124]. Likewise, the generation of AAM-like macrophages by *A. viteae* cystatin also indicates their potential in treatment of allergic airway inflammation and experimental colitis [112, 125].

Notably, not all ES-products are proteins. Rather they comprise a wide range of biologically active molecules including glycans [20] and microRNAs [20, 21, 126, 127] as well as small molecule metabolites, exemplified by the immunoregulatory actions of fluke peptides, eicosanoids (*Schistosoma mansoni*), ascaroside lipids and short chain fatty acids (SCFA) generated by *Toxocara canis, Ostertagia circumcincta* and *Haemonchus contortus* [20]. Although many of these agents are released in free-form, the discovery of helminth-derived exosomes/extracellular vesicles containing varied cargo mixes [21, 126-128] has generated much interest in their function. Those containing immunoregulatory microRNAs have been identified from *Fasciola hepatica*, *Heligomosomides polygyrus, Litomosoides sigmodontis, Dicrocoelieum dendriticum* and *Dirofilaria immitis*, which following dissection of their roles could be exploited therapeutically [21].

Outstanding Questions Box

How can we exploit the emerging new concepts in helminth immunomodulation to inform on development of the immune response and pathogenesis in inflammatory-based disease and consequently, to design novel, safe immunotherapies? In particular:

- how do parasitic worms and their ES products harness host homeostatic mechanisms to reset the effector:regulatory balance (Tregs:Teffs; Bregs:Beffs; M1:M2 macrophages) and limit/resolve inflammation and promote tissue repair e.g. by stimulating autophagy?
- what are the helminth-induced changes in the epigenetic landscape of progenitor and/or effector cells that result in “training” of host immune responses?
- can we mimic helminth-mediated modulation of host metabolomics to reset immunophenotypes (e.g. Th subsets and memory responses) and inflammation (glycolytic and oxidative phosphorylation metabolism)?
- how can we therapeutically reproduce the effects of helminths on macrobiome/host metabolome to subvert inflammation and protect against disease?
- does chronic infection, particularly during pregnancy, have beneficial transgenerational (epigenetic/macrobiome) effects?

Why is lack of protection against inflammatory disease the most frequent outcome of clinical trials with live infections? In particular:

- what is the impact of the macrobiome, co-infections and differential inflammatory microenvironments on both parasite infection and target pathological disorder?
- what are the best helminth-based therapies - worm-derived biologics, conditioned cell transfer or drugs designed to mimic their active moieties or mode of action or alternatively, (probiotic) dietary supplements?

Do we need to reconsider our programs of mass eradication/vaccination against parasitic helminths in the developing world?

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