Trends in Parasitology

CellPress OPEN ACCESS

Opinion

Conquering rheumatic diseases: are parasitic worms the answer?

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Despite the introduction of novel treatment strategies, management of rheumatic disorders remains associated with substantial unmet clinical need. Of interest therefore, it has recently become apparent that there is a global inverse relationship between the incidence of such conditions and parasitic helminth infection, with striking examples involving rheumatoid arthritis (RA)/systemic lupus erythematosus (SLE) patients and filarial nematode worm infection in studies in India. Such findings reflect that helminths are master manipulators of the immune system, particularly in being able to modulate proinflammatory responses. The aim of this article is thus to consider findings to date on this exciting and intriguing research area to form an opinion on whether parasitic worms may be exploited to generate novel therapies for rheumatic diseases.

Highlights

Autoimmune rheumatic diseases (ARDs), for example, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), are amongst the most common diseases of the developed world and continue to constitute an unmet clinical need.

The global incidence of ARDs shows inverse correlation with parasitic helminth endemicity, and epidemiological data support the helminths protecting against ARD development in humans.

Parasitic helminths and their products have been shown to protect against disease in mouse models of RA and SLE, and such models have been used to elucidate mechanism of action of the helminth products.

Nonimmunogenic, safe, drug-like small-molecule analogues of helminth products have been designed and successfully tested in mouse models of ARDs with a view to ultimately employing such compounds as a novel approach to anti-inflammatory drug treatment in humans.

Rheumatic diseases and problems treating them

Autoimmune rheumatic disease (ARD) is a blanket term encompassing RA, SLE, and a clutch of similar but less common conditions affecting the **musculoskeletal system** (see Glossary) in which pathology is driven by aberrant immune system responses. These chronic inflammatory disorders are distinguished by the location of the inflammatory pathology and the accompanying **autoantibody** profile. Whilst the specific aetiology of ARDs remains largely undefined, and no curative interventions are available, a spectrum of genetic and environmental factors which correlate with disease susceptibility have been proposed [1,2].

From a molecular perspective, ARDs establish when the failure of central and peripheral **tolerance** checkpoints leads to an emergence of auto-reactive B and T cells, autoantibody production, and an eventual irreversible failure in tissue tolerance [3,4]. In RA, for example, the immune milieu follows a **Th1/17 phenotype**, characterised by an increase in interleukin (IL)-1α, IL-1β, IL-2, IL-6, tumour necrosis factor (TNF), interferon-gamma (IFN-γ), and matrix-metalloproteinase (MMP) expression. Pathology is perpetuated by infiltrating $CD4^+$ T cells, macrophages, and **synovial fibroblasts** (SFs) [4–10].

Active disease is generally episodic, with therapeutic strategies aimed at inducing and/or prolonging periods of remission, with disease severity and patient comorbidities heavily influencing the choice of treatment. Pharmaceutical interventions can generally be assigned to three classes: synthetic or **biologic disease-modifying anti-rheumatic drugs (DMARDs)**, analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs), and corticosteroids. Treatments may be delivered exclusively, or in combination.

Conventional DMARD **methotrexate** is considered the gold standard for the treatment of RA and SLE at onset of diagnosis, and may be used in conjunction with sulfasalazine, leflunomide, and/or hydroxychloroquine in mild to moderate disease [11–13]. Following commencement of

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treatment, but prior to symptom resolution, patients will commonly commence NSAIDs or corticosteroids in parallel, as dictated by disease severity, to manage disease activity in the short term [14]. However, as many as 40% of those prescribed methotrexate will not respond to treatment, and with an adverse reaction profile – including gastrointestinal disturbances, nephrotoxicity, myelosuppression, and alopecia – discontinuation of DMARD treatment is common [15–18]. Similarly, whilst largely efficacious in the treatment of inflammation, a detrimental effect of prolonged NSAID usage on gastrointestinal health and bone healing has also been recognised, and corticosteroid usage in RA patients has demonstrated an increased propensity to osteoporosis, fractures, diabetes mellitus, and myocardial infarction [19–22]. Toxicity and adverse event incidence increases with each course of treatment [23].

Emerging in the late 20th century, biologic DMARDs (bDMARDs) inhibit various functional aspects of the immune system like lymphocyte proliferation and inflammatory pathway activation by targeting immune components with high specificity and represent an effective treatment for some patients. Whilst initially dominated by a range of TNF inhibitors (infliximab, adalimumab, certolizumab), the spectrum of bDMARDs has since evolved to include agents which block **T cell costimulation** (abatacept) and antagonise IL-1 (anakinra) [24,25]. **Monoclonal antibodies** against IL-6 (tocilizumab) and CD20 (rituximab), the latter of which induces B cell apoptosis, have also been developed [26,27]. The potent capacity of treatment with bDMARDs to reduce inflammation, however, is offset by the patients' subsequent systemic immunosuppression, leaving them vulnerable to opportunistic bacterial, fungal, and viral infections, reactivation of latent conditions such as tuberculosis and/or hepatitis, and neoplastic development [18,28]. As such, bDMARDs are indicated for use only in cases of moderate-to-severe disease in which conventional treatments have been ineffective.

Whilst numerous treatment options for the induction and maintenance of remission in ARDs exist, inconsistent efficacy and adverse effects constitute significant barriers to their use. Accordingly, the motivation to discover novel therapeutic strategies for inflammatory rheumatic conditions is high.

The link between parasitic helminth infection and rheumatic diseases

Strachan's 'hygiene hypothesis' posits that modern hygiene practices and the associated reduction in transmissible disease would influence immune system cell differentiation towards a Th2 phenotype, with a potential for allergenic hypersensitivity [29]. Reinterpreted by Rook and Brunet in 2005, the 'old friends hypothesis' expands upon this rationale, suggesting that reduced contact with microbial organisms, including parasitic helminths, negatively influenced our ability to regulate immune cell activity [30]. This theory proposes that, throughout mammalian immune system evolution, humans have been continually exposed to a plethora of environmental microbial organisms, to which an ongoing inflammatory response would become deleterious. A heightened proliferation of **regulatory immune system cells** (T cells, B cells, antigen-presenting cells) in response to microbial exposure supresses proinflammatory effector cells, effectively tolerating the foreign microorganism in order to protect the host. However, in the absence of such microbial priming, regulatory cell responses are reduced, and inappropriate immune activation may occur.

Consistent with this, epidemiological studies comparing the geography of human parasitosis to the prevalence of autoimmune inflammatory diseases exposed the inverse correlation between the two; regions where parasitosis is endemic demonstrated a far lower incidence of diseases like inflammatory bowel disease, asthma, and multiple sclerosis [31–33]. Simultaneously, a comprehensive global study conducted by Otón and Carmona [34] indicated significant regional and ethnic variations in the prevalence of RA which followed a similar pattern. Their findings have been

Glossary

Adjuvant-based inducible arthritis: an experimental model in which arthritis can be induced in a laboratory rodent by administration of Freund's complete adjuvant. The mechanism of arthritis induction is not fully understood.
Autoantibodies: antibodies directed against the body's own molecules.
Biologic: see 'Monoclonal antibodies'.
Collagen-induced arthritis (CIA): a mouse model of RA that mimics many features of the human condition and is highly popular in academia and industry.
Cystatins: a large family of cysteine protease-inhibiting enzymes.
Disease-modifying anti-rheumatic drugs (DMARDs): a collection of drugs varying in mechanism of action but sharing a usefulness in treating autoimmune rheumatic diseases like RA.
FoxP3: a transcription factor expressed by regulatory T cells.
Immunogenicity: the ability of a molecule to induce an immune response.
Inflammasome: a protein complex, formed following ligation of intracellular pathogen recognition receptors, that contributes to production of cytokines IL-1 and IL-18.
KEGG: a collection of databases relating to biological pathways.
M1 macrophage: a term used to describe what is considered a 'classic' macrophage, that is, one with proinflammatory properties.
Methotrexate: a drug commonly used in cancer treatment and also used for treating conditions like RA and SLE due to its effects on the immune response.
Microbial dysbiosis: a disruption or imbalance in normal microbial species in organs such as the gut.
Monoclonal antibodies: monospecific reagents that are used as drugs (termed 'biologics' as they are present in Nature) when treating autoimmune rheumatic diseases. In this context, they most commonly target a cytokine or cytokine receptor.
Musculoskeletal system: the organ system that provides support and movement, consisting of components such as bones, cartilage, joints, muscles, and tendons.
Osteoclast: a type of cell involved in maintenance of healthy bone via its controlled degradation but that can assume pathogenic properties to destroy bone in RA. RANK and RANKL

supported by studies further investigating the epidemiology of RA, SLE, and other ARDs, where data confirm these conditions are more frequent in higher income countries, with an increasing incidence in low-to-middle income economies [35–38]. Significantly, prevalence of ARDs amongst global indigenous populations, who have experienced dramatic changes in diet, lifestyle, and sanitation within a generation, is consistently high [34,39,40]. Moreover, particularly striking evidence has emerged inversely correlating filarial nematode infection with the development of ARDs, including RA and SLE, in Indian patients [41]. Thus, although as far as we are aware, this form of epidemiologic study has not been carried out with parasitic worms other than filarial nematodes, there is clear evidence for the prospect of utilising parasitic worms as therapeutics in ARDs. In support of this, pilot studies engaging live helminth therapy conducted across rodent models of rheumatic disease produced encouraging results. Thus, in **adjuvant-based inducible arthritis**, inflammatory pathology was attenuated during infection by the gastrointestinal nematodes *Trichinella spiralis* and *Toxocara canis,* and the liver fluke *Chlonorchis sinensis,* via Th2 polarisation and **FoxP3**$^{+}$ regulatory T cell (Treg) induction [42–45]. In studies utilising the MRL/lpr and NZB/W mouse models of spontaneous SLE, infection with the trematode *Schistosoma mansoni* or the tapeworm *Hymenolepis microstoma* was similarly associated with disease amelioration and regulatory cell induction [46,47]. Overall, we now have significant information on how parasitic worms protect against ARDs (Figure 1, Key figure) and as shown in the next section, this has benefitted greatly from focusing on excretory–secretory (E/S) molecules.

Molecular mechanisms by which parasitic worms provide protection against rheumatic diseases

With proof of concept for helminthic therapies in rheumatic diseases, the effect of individual immunomodulatory helminth-derived E/S molecules could be explored in rheumatic disease models (Figure 2). Indeed, a number of these have been found to be active and they additionally offer an opportunity to explore mechanism of action and hence identify therapeutic targets. The recombinant form of *Necator americanus*-derived **TIMP**-like protein *Na*-AIP-1, for example, has been shown to be a potent immunomodulator during mouse colitis studies incorporating mechanistic analysis [48]. Subsequently, the impact of this molecule was tested in murine **collagen-induced arthritis (CIA)**, a model characterised by Th17-dominant articular inflammation, paw thickening/widening, and erythema which can progress to erosion of the joint and a loss of strength. *Na*-AIP-1 was found to reduce paw width and erythema, and limited cartilage erosion when used both as a monotherapy and in combination with methotrexate [49]. Whilst this particular pilot study was limited by a lack of molecular investigations, previously it has been reported that during colitis *Na*-AIP-1 administration induces transcriptional downregulation of several genes regulating granzyme production, and pathways identified by **KEGG** analysis governing NF-κB, TCR, and TNF signalling – the latter, significantly, in both mouse and human cells.

Likewise, cysteine protease-inhibiting **cystatins** derived from various different helminths have shown anti-inflammatory properties in a number of disorders characterised by immune dysfunction, including colitis [50]. Of relevance to ARDs, prophylactic treatment with recombinant cystatin derived from *S. japonicum* (rSj-Cys) alleviated the clinical pathology associated with CIA [51]. Transcriptional studies confirmed a reduction in IFN-γ, TNF, IL-6, and IL-17 expression, with a concomitant increase in FoxP3^{+} Tregs, IL-4, and IL-10. *In vitro,* rSj-Cys downregulated mRNA associated with NF-κB signalling [52]. Whilst the precise molecular targets of both of these modulatory proteins are not entirely understood, when viewed holistically the data imply that both initiate a polarisation from a Th1/Th17 to a Treg-driven tolerogenic Th2 immune phenotype, which negatively regulates **M1 macrophage** activation – an oft observed mechanism in helminthic immunomodulation.

are molecules involved in osteoclast differentiation/activation.

Regulatory immune system cells: immune system cells that inhibit or control immune responses rather than promote them.

SLE-related atherosclerosis: a cardiovascular disease whose appearance is accelerated and enhanced in severity in SLE.

Synovial fibroblasts: joint stromal cells that can become pathogenic during RA.

T cell costimulation: full activation of T cells requiring ligation of receptors additional to the T cell receptor.

Th1/17 phenotype: T helper cells are divided into subsets such as Th1 and Th17 based on phenotypic markers like transcription factors and secreted cytokines.

TIMPs (tissue inhibitors of metalloproteinases): a family of protease inhibitors.

TLR4/MyD88 signalling: biochemical pathways that are activated following ligation of the pathogen recognition receptor TLR4 and its subsequent interaction with its adaptor protein, MyD88.

Tolerance: failure of the immune system to respond to a molecule. Central tolerance is acquired by immature lymphocytes during their development whereas peripheral tolerance specifies that developed by mature lymphocytes in peripheral tissues.

Key figure

Helminth infection protects against inflammatory arthritis by resetting immune homeostasis and resolving inflammation

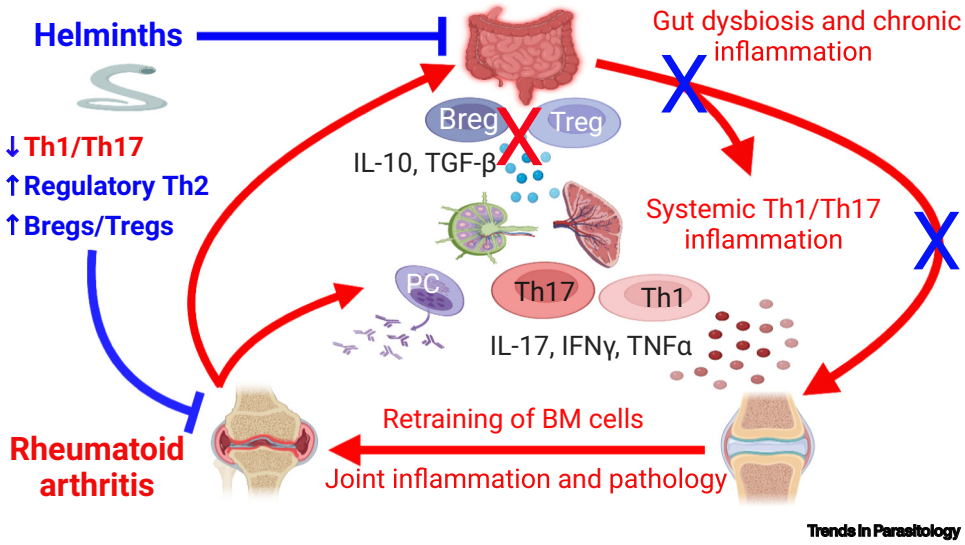

Figure 1. Although the aetiology of rheumatoid arthritis (RA) remains unknown, increasing evidence suggests that chronic inflammation, such as that associated with gut microbiome dysbiosis and loss of intestinal barrier integrity, is a contributing factor to the development and promulgation of the systemic autoimmunity underpinning this disease. Autoimmune inflammation in RA is characterised by a loss of regulatory B (Breg) and T (Treg) cells and increased Th1/Th17 inflammatory responses as well as production of autoantibodies by plasma cells (PCs). Collectively, this results in an inflammatory joint microenvironment that promotes arthritis (cartilage and bone destruction) by driving inflammatory cell infiltration, rewiring of synovial fibroblast function, and osteoclastogenesis. In turn, this environment perpetuates disease by promoting skewing of immune responses towards a more inflammatory phenotype. Infection with helminths acts to disrupt this vicious cycle of chronic inflammation by inducing a modified, regulatory/Th2 (IL-10, TGF-β, and IL-4) response that acts to restore immune homeostasis by counteracting development of Th1 cells, Th17 cells, and IL-17-producing γδ T cells and also, autoantibody-producing PCs whilst restoring Breg and Treg cells. This model was created using Biorender.com where red arrows/cross and blue inhibitory bars/crosses represent promotion and suppression, respectively, of pathological events shown in red font; blue arrows represent increases or decreases in inflammatory (red font) or regulatory (blue font) immune system components. Abbreviations: BM, bone marrow; IFN, interferon; IL, interleukin; TGF, transforming growth factor; TNF, tumour necrosis factor.

The most thoroughly characterised parasite E/S molecule in rheumatic disease models is the *Acanthocheilonema viteae*-derived protein ES-62 and indeed as far as we are aware this molecule represents the first to be tested against disease development in a mouse ARD model. The post-translational addition of multiple phosphorylcholine (PC) moieties to *N*-glycans on this tetrameric molecule assigns a potent immunomodulatory capacity (reviewed by Pineda *et al*. [53]), and to this end ES-62 has displayed dramatic efficacy in murine CIA, as both a prophylactic and a therapeutic intervention [54]. Whilst ES-62 similarly skews from the IL17-dominated milieu to a regulatory environ, it prevents production of this cytokine by both Th17 cells and γδ T cells but not natural killer (NK) or NKT cells, thereby leaving host protective mechanisms against disease-causing organisms in place [53]. Furthermore, this effect is driven by IL10^{+} regulatory B cells (Bregs) rather than Tregs [55] and is associated with disruption of **TLR4/MyD88 signalling** [31,54,55]. Moreover, ES-62 is also able to harness the tissue-repair properties of the cytokine IL-22 to resolve inflammation and counter joint damage during established disease in the CIA

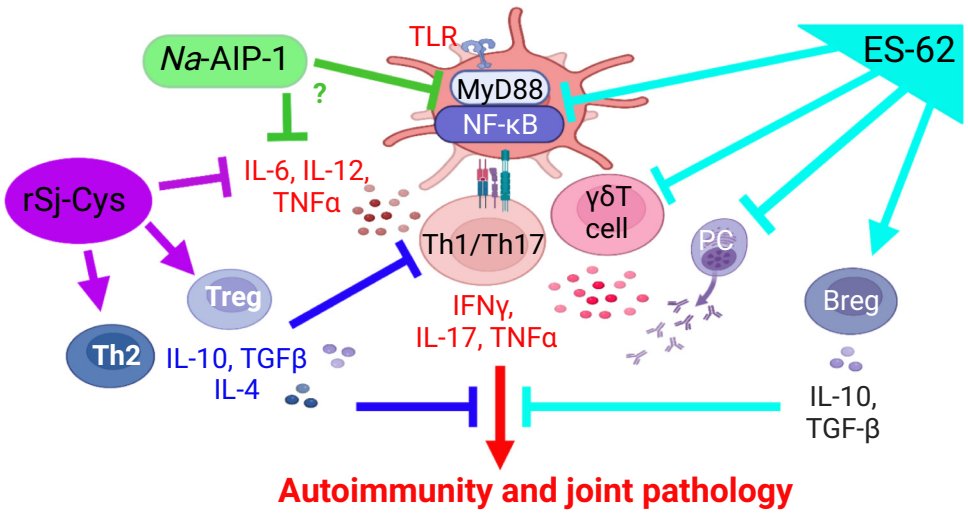

Figure 2. Mechanisms underpinning protection against inflammatory arthritis employed by helminth E/S molecules. The ability of helminths to protect against inflammatory arthritis appears to be the result of their capacity to secrete immunomodulatory E/S molecules such as *Na*-AIP-1, rSj-Cys, and ES-62, evolved to contribute to their survival in the host. At a molecular level, these E/S products appear to target pathogen recognition receptor (PRR) signalling such as the TLR/MyD88-coupled NF-κB activation responsible for the proinflammatory cytokine (e.g., IL-6, IL-12, and TNFα) release that drives pathological Th1/Th17 autoimmune responses. Diagrams were created using Biorender.com, and the actions of Na-AIP-1, rSj-Cys, and ES-62 are represented in green, purple, and turquoise, respectively – arrows represent promotion and bars, suppression. Abbreviations: IFN, interferon; IL, interleukin; PC, plasma cell; TGF, transforming growth factor; TLR, toll-like receptor; TNF, tumour necrosis factor.

model [56]. At the same time, *in vitro* studies have indicated that ES-62 is able to influence haematopoietic cell differentiation towards an anti-inflammatory phenotype [56,57]. Consistent with this, bone marrow (BM) from ES-62-treated CIA-mice displayed reduced proportions of haematopoietic stem cells and **osteoclast** progenitors, with a retardation in the functional maturity of BM-derived osteoclasts and reduced RANK and RANKL mRNA [58]. Remarkably, ES-62 actions are not restricted to haematopoietic cells as it is able to induce epigenetic changes in pathogenic SFs, effectively re-wiring the cell to a novel, hyporesponsive phenotype [59]. These cells, phenotypically distinct from SFs from both the arthritic controls and naive cohorts, display reduced proinflammatory cytokine release and matrix remodelling enzymes in response to stimulation – a phenotype which is retained throughout at least 3–4 weeks of explant culture passage.

The effect of ES-62 in RA is not limited to musculoskeletal pathologies, with ES-62-treated CIA-mice displaying a notable reduction in the intestinal inflammation that precedes and accompanies arthritic pathogenesis [60] despite being ineffective in murine models of inflammatory bowel disease [60,61]. **Microbial dysbiosis** dominated by genera within the *Prevotella, Rickenellaceae, Clostridiaceae* and *Lachnospiraceae* families is associated with the pathogenesis of both human RA and murine CIA, often appearing prior to the development of arthritis, as well as during periods of active disease [62–64]. This imbalance corrupts the delicate effector–regulator homeostasis, instigating IL-17 production and ultimately leading to compromised epithelial barrier integrity, gut inflammation, and the establishment of arthritis. In CIA-mice, ES-62 is able to normalise the gut microbiome and indeed its anti-inflammatory and joint-protective actions dissipate upon the administration of broad-spectrum antibiotics [60]. Interestingly, the regulation of osteoclast-mediated bone remodelling, which ES-62 has also been evidenced to modulate, has also been shown to be influenced by the gut microbiome [58,65].

Importantly, ES-62 has also been found to be active against human cells, including those from RA patients [54,66]. For example, this helminth product can significantly suppress production of TNF by the THP-1 cell line when cultured with peripheral blood T cells from RA patients and both TNF and IL-6 from primary cultures prepared from RA synovial fluid and membranes and exposed to lipopolysaccharides (LPS) [56]. In addition, when peripheral blood mononuclear cells or cells from synovial fluid and membranes from RA patients were cultured with a cocktail of cytokines (IL-12, IL-15, and IL-18) to recapitulate the inflammatory microenvironment of the arthritic joint, pretreatment with ES-62 or ovalbumin-conjugated PC (OVA-PC), but not recombinant ES-62 (lacks PC) or OVA, suppressed the levels of IFN-γ produced. These data again reinforced the important role of the PC moiety in the protective actions of ES-62 [57].

Whilst overall not as heavily investigated to date, there is also significant research supporting the application of helminth molecular therapy in two independent murine models of SLE. In the MRL/Lpr model, which parallels lupus-related renal pathology, ES-62 administration reduced proteinuria and antinuclear (auto)antibody (ANA) production. In keeping with its actions in other inflammatory models, normalisation of splenic and renal $IL10^{+}$ B cells was detected, in association with reversal of enhanced MyD88-dependent signalling [67]. **SLE-related atherosclerosis**, modelled in the spontaneous *gld*.apoE(–/–) murine model, is also substantially impeded by ES-62 administration [68]. Here, treatment with ES-62 was characterised by reduced ANA production, and a dramatic reduction in aortic atherosclerotic lesions, although no statistically significant impact on renal pathology was detected. Of note, ES-62 induces anti-PC antibodies in mice, including in this model and, interestingly, anti-PC antibodies have been shown to be correlated with protection against both cardiovascular disease and SLE (reviewed by Frostegård [69]). However, ES-62 did not induce idiotypes normally associated with protection in *gld*.apoE$^{-/-}$ mice and in addition, the molecule is active in nonimmunogenic small-molecule form in the CIA and MRL/lpr mouse models (see later). Thus, although generation of anti-PC antibodies cannot be ruled out as contributing to ES-62's anti-inflammatory effects *in vivo*, the data obtained to date arguably count against this. Finally, rSj-Cys has also been trialled as an intervention in the *gld*.apoE$^{-/-}$ mouse model of disease, with significantly reduced lesions in both the aorta and renal compartment [70]. This was associated with an inhibition of TLR2 and MyD88 expression in kidney cells.

Generation of therapeutics from parasitic worms for the treatment of rheumatic diseases

In spite of an undeniably more palatable exposure methodology than live helminth therapy, there are still barriers to be overcome before the translation of E/S molecules from bench to clinic. Indeed, whilst numerous studies showcasing the abilities of a range of helminth products in preventing immune-mediated disease pathology have been published, as yet none of the molecules have progressed to Phase 1 clinical trials, let alone to market.

The addition of PC moieties to ES-62, which drive its immunomodulatory capabilities, constitutes an unusual post-translational modification (involving an as-yet unidentified transferase enzyme), making the active molecule unsuitable for recombinant expression in traditional systems [71]. Additionally, the **immunogenicity** of the molecule introduces the potential of a raft of clinical consequences of introducing foreign molecules to the patient – which can range from an impediment to the efficacy of the treatment, to, potentially, anaphylaxis [72]. In order to overcome these issues, a library of nonimmunogenic drug-like small-molecule analogues (SMAs) of ES-62's PC moiety have been synthesised, and as with the parent molecule, these compounds provide protection from inflammatory pathology when utilised prophylactically or as a therapeutic intervention. Of these, SMAs 11a and 12b have been particularly well characterised and shown potency in the CIA model, mimicking the ability of ES-62 to inhibit pathology *in vivo*, and retard the proliferation

and maturation of osteoclasts *in vitro* [57,58,73,74]. SMA 11a also recapitulates the mechanism of action of the native protein by way of downregulating MyD88 and inhibiting Th1/17 responses. Whilst also downregulating MyD88, SMA 12b tends to target IL-17 production less: rather, in CIA it more inhibits the alternative proinflammatory cytokine IL-1β. Moreover, it concomitantly upregulates activation of the transcription factor NRF2, acting to suppress **inflammasome** transcription (and hence further suppression of IL-1β pathology) whilst enhancing the expression of antioxidant genes. In the MRL/lpr SLE model, treatment with SMA 11a and 12b both caused MyD88 downregulation thereby reducing pathogenesis, reflected in a suppression of proteinuria and a reduction in ANA production, although SMA 11a tended to be more effective with respect to the former.

Similarly, PC conjugated to tuftsin has been found to be effective as a treatment when tested in established CIA. This effect was associated with inhibition of pro-inflammatory cytokine production but increased IL-10 and was correlated with heightened expansion of Bregs but, unlike with ES-62, also Tregs [75]. Furthermore, tuftsin-PC has been found to improve kidney disease and increase survival in NZBxW/F1 mice, a model for SLE. Again, this was associated with a decrease in anti-inflammatory cytokines and increased IL-10 and, as with ES-62 treatment in CIA, was linked to changes in the gut microbiome [76].

A synthetic peptide derived from the secretome of the trematode *Fasciola hepatica* has also showed joint-specific efficacy in the CIA model [77]. C-FhHDM-1 is a 34 amino acid-containing molecule which is homologous to the active C-terminal of native protein FhHDM-1, which *in vivo* is cleaved by *Fasciola*-derived cathepsin-L. Treatment commencing 11 days post-arthritis induction dramatically limited clinical indicators of disease, including paw thickness, throughout the experimental period. At termination, joint architecture was retained with minimal infiltration of the synovium. C-FhHDM-1 reduced TNF, IL-17, and IFN-γ in the knee joint, but not systemically, and no increase in $FoxP3^+$ Tregs was detectable. Transcriptional studies revealed modulation of mRNA of molecules such as RANK and RANKL to levels which would inhibit osteoclastogenesis.

Concluding remarks

An increasing understanding of the complex interplay between helminth and host has enabled the discovery of a suite of novel biologics with favourable efficacy profiles in several models of immune-mediated inflammatory diseases (Figure 2) [48,78]. Whilst to date much of the focus has fallen on the resolution of diseases associated with inflammation at mucosal tissue sites, the studies considered within this paper present a strong case for a greater exploration into the potential of these novel compounds in rheumatic disease. By virtue of millennia of coevolution, such helminth-derived molecules come with an enviable tolerability profile – a critical advantage over treatments currently in use. Yet, concerns regarding immunogenicity and protein production present problems which need to be addressed before translation to human studies can be reasonably considered. However, SMAs of these proteins present an exciting new evolution in molecular helminthic therapy which appears to overcome these barriers. Furthermore, the work on ES-62 has shown that it is conceivable to generate SMAs with subtle differences in mechanism of action, raising the possibility of tailoring individual patient treatment strategies or SMA combination treatment. ES-62 SMAs further show a benefit of efficacy in both a prophylactic and a therapeutic administration regime, potentially minimising the need for conventional combination therapies – a decrease in adverse symptomology increasing the likelihood of patient compliance. Further to this, biologics usually have to be administered in a clinical environment, incurring burden to the healthcare system and inconvenience to the patient, whereas, theoretically, SMAs can be produced in pill form for oral administration [79].

Outstanding questions

Are the synthetic compounds active against ARDs in mouse models developed to date of a suitable structure to readily convert to tablet form for testing in mice or is more extensive novel chemistry required?

Assuming translation to tablet form, do we currently have enough mechanistic data on how helminth-derived products, or novel synthetic compounds developed from them, affect the immune system in mouse models of ARDs to justify taking them forward to further ADME studies including in a large nonhuman host such as the dog, and then Phase I clinical trials?

Does the recent observation of some helminths and helminth products modulating the gut microbiome and showing dependence on this for immunomodulatory activity argue for a new microbiome-focused approach to exploitation of helminths for anti-inflammatory drug development?

Does the increasing recent evidence that helminths and their products can affect the properties of haematopoietic stem cells offer any ideas as to how further anti-ARD drug development might be explored with respect to this area?

Does the fact that ES-62 can successfully target a key pathogenic nonimmune system cell in collagen-induced arthritis – the fibroblast – suggest that we should, as a field, extend our range of target cells in ARDs to stromal cells?

Do we have enough information from the ARD world to decide what might make the best helminth-derived treatment in a mechanistic sense, for example, should we be trying to inhibit IL-17 and/or TNF, or increase IL-10, or normalise/increase levels or Bregs and/or Tregs, or all of these things?

Buoyed by the success achieved with mouse models of RA and SLE, will parasitic helminth products show the same potential for treating less well-studied ARDs such as Sjogren's syndrome, systemic scleroderma, and vasculitis, which can likewise cause severe disease and be highly challenging to treat?

The studies reviewed within present favourable examples of disease-limiting SMAs in murine models of RA and SLE. These compounds are easily producible, and at considerably lower cost and higher quantity than current ARD biologics, making commercialisation an attractive prospect for a suitable candidate drug. Nevertheless, although there are no indications of any safety issues with the ES-62 SMAs to date when examined using commercial screens, further safety analysis and in addition absorption, distribution, metabolism, and excretion (ADME) studies (these have been limited as yet – see Outstanding questions) are necessary in determining the translatability of these novel compounds in human disease. Certainly, the potent mechanisms described in ES-62 SMAs 11a and 12b attest to the emphasis that should be placed on continuing to explore the suitability of ES-62 and other helminth-derived immunomodulatory molecules for development as synthetic drugs (see Outstanding questions). Overall, in combination with being easier and more economical to produce, helminth SMAs present as a potential pharmacological treatment for ARDs which may be more tolerable and effective while at the same time more accessible to the patient, overcoming socioeconomic or geographical restriction which currently renders treatment inaccessible for many. To date, as far as we are aware, there has been only one Phase 1 clinical trial (EUCTR2011-006344-71-DE) carried out with respect to helminth therapy and ARDs, involving the use of *Trichuris suis* ova and RA patients and this trial was terminated with the findings unpublished. Our hope is that this situation may ultimately be remedied by the use of helminth products including those yet to be discovered/characterised or their synthetic derivatives and that this use can be extended to less studied but clinically important ARDs (see Outstanding questions).

Given that individual parasitic helminth-derived molecules share similarities but also differences in mechanism of action, what is the sheer breadth of helminth immunomodulation at the mechanistic level, and should we be continuing to explore the secretome of species not characterised to date in the hope of finding new treasures?

Acknowledgments

Funding for research in the Harnett labs was provided by the UK Medical Research Council (award number MR/V000683/1) and Biotechnology and Biological Sciences Research Council (award numbers BB/V000993/1 and BB/V001027/1).

Declaration of interests

The authors declare no competing interests.

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