International Journal for Parasitology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Invited Review

Helminth therapies: Translating the unknown unknowns to known knowns

Adnan R. Khan [a,b], Padraic G. Fallon [a,b,c,*]

[a] Trinity Biomedical Sciences Institute, School of Medicine, Trinity College Dublin, Ireland
[b] National Children's Research Centre, Our Lady's Children's Hospital, Dublin, Ireland
[c] Institute of Molecular Medicine, St James's Hospital, Ireland

ARTICLE INFO

Article history:
Received 11 October 2012
Received in revised form 1 December 2012
Accepted 3 December 2012
Available online xxxx

Keywords:
Helminth therapy
Immunome
Autoimmune disease
Atopy

ABSTRACT

The use of live helminth infections is currently in clinical trials as a novel approach for the treatment of a range of allergic and autoimmune diseases. This rapid progression from observational studies some 20 years ago to helminth clinical trials can be attributed to huge advances in not just pre-clinical and clinical evidence, pertaining to the efficacy of these parasites in unrelated diseases, but also a greater understanding of the complex immunological mechanisms that underpin these effects. Helminths have exerted significant evolutionary selective pressures on the host immune genome or "immunome". Studies on helminths were pivotal in a paradigm shift in immunology with recent discoveries of a number of novel immune cell populations. Critically, these new discoveries highlight the need to further understand the underlying mechanism behind the desirable therapeutic effects that helminths offer. With these unknown unknowns there is the distinct possibility that a true, fundamental modus operandi for helminth therapy will arrive long after it has been established in the clinic.

1. Pathogen selection of the immunome of humans today

During the past 40 years, there has been an exponential increase in the incidence of autoimmune and idiopathic inflammatory disorders and atopic disease in 'developed' societies, with a similar pattern emerging in modernized areas of developing countries (Farrokhyar et al., 2001; Moroni et al., 2012). While changes in lifestyle have contributed to the epidemic of inflammatory disorders in modern societies there is a major underlying role for genetic predisposition in the development of aberrant inflammatory responses. This raises the spectra that the origins of many inflammatory diseases today are due to genetic traits selected, subject to environmental stimuli, and retained throughout human evolution. Stemming from Darwin's theory of natural selection is the concept that "infection begets natural selection in *Homo sapiens*" as attributable to the geneticist Haldane (Haldane, 1949). In the immunological context, as a pathogen will kill a host that has no genes that facilitate survival from infection, it follows that such pathogens have exerted significant evolutionary selective pressures on the host immune genome or "immunome". Consequentially, advantageous genetic mutations that confer a resistant phenotype to survive exposure to pathogens have been positively selected during evolution, whilst those genes causing morbidity from infection have undergone negative selection, and were hence removed from the gene pool. Indeed, the widely investigated Toll-like receptor (TLR) family of innate immune receptors may have had an ancestral function in its development and thus, during evolutionary selection, have been co-opted to play a role in immunity to pathogens in multiple species of the animal kingdom (Leulier and Lemaitre, 2008). Therefore it can be suggested that the human immunome of today is the evolutionary consequence of marked and prolonged genetic selective pressure exerted by infectious pathogens (Barreiro and Quintana-Murci, 2010).

Recent socio-economic changes and advances in medical technology in developed societies may have diminished the selection pressure of pathogens at the population level, whilst accentuating the individual genetic mutations that lead to aberrant immune function (Casanova and Abel, 2005). Collectively, the aberrations in immune function that are causal factors in a range of immune-mediated diseases today, such as arthritis, allergy, inflammatory bowel disease (IBD) or multiple sclerosis (MS), may be the ancestral legacy of gene selection in response to infectious pathogens. For example, diversity generated in human leukocyte antigen (HLA) class I genes was significantly influenced by pathogens (Prugnolle et al., 2005). Indeed the advent of genome-wide association studies has identified single nucleotide polymorphisms (SNPs) within the HLA regions associated with a spectrum of non-infectious diseases found in modern humans, such as psychiatric disorders including schizophrenia. Therefore, understanding the host's immunological response to infectious pathogens will inform on mechanisms that differentially activate (induce) or suppress

* Corresponding author at: Trinity Biomedical Sciences Institute, School of Medicine, Trinity College Dublin, Ireland. Tel.: +353 1 896 4409; fax: +353 1 896 4040.
E-mail address: pfallon@tcd.ie (P.G. Fallon).

0020-7519/$36.00 © 2013 Published by Elsevier Ltd. on behalf of Australian Society for Parasitology Inc.
http://dx.doi.org/10.1016/j.ijpara.2012.12.002

(regulate) inflammatory processes at the cellular, tissue and organism levels in individuals predisposed to inflammatory disorders.

2. Helminths: the master selectors of the immunome

Infectious pathogens of humans encompass viruses, bacteria, fungi, prions and parasites (protozoa and helminths). All pathogenic groups have exerted substantial selection pressure on the human immunome. In this review we consider the role of helminths in selection of the immunome and implications for the use of helminths as therapies. Approximately one-third of the world's population is currently infected with helminth parasites, prompting helminth infections of humans to be termed "the great neglected tropical diseases" (Hotez et al., 2008). An indication of the sustained level of helminth parasitism of humans is the comparable estimate that ~30% of humans were infected with helminths some 65 years ago (Stoll, 1947); one assumes this high prevalence of helminth infection in humans was also a feature of the primate ancestors of *Homo sapiens*.

Characteristically, parasitic helminths infect humans early in life and are typified by chronic infections. A consequence of helminths' propensity to infect humans in the pre-reproductive years is that they have exerted significant selective pressure on mutations in genes implicated in immune function, thus ensuring their survival in the host. Hence, advantageous gene mutations preventing helminth-induced fatality in childhood would be passed to the next generation. The hypothesis that helminths act as the major pathogen group exerting selection pressure on immunity was speculative until recently. A systematic approach was used to determine the relative pressure of helminths, viruses or bacteria exerted on a selection of interleukin (IL) genes. Fumagalli et al. (2009) examined 52 globally dispersed human populations, with diverse levels of pathogen richness, for >650,000 SNPs within 91 IL or IL-receptor genes. Helminths were identified as a major selective pressure on a subset of IL genes that, through genome-wide association studies, are associated with human susceptibility to IBD and coeliac disease (Fumagalli et al., 2009).

These recent studies emphasise the role of helminths in the shaping of the human immunome today and, although not validated, it reinforces a role for helminths in the Hygiene Hypothesis. Thus alterations in the levels of microbial factors, in this context helminths, in the environment in westernised or developing societies may contribute to the epidemic of allergic and autoimmune diseases in such societies. Furthermore, using helminth infections as natural inducers of immune responses in experimental settings has delivered novel insights into multiple facets of immune function. Examples of previous discoveries made using helminth models include: the functions of IgE, mechanisms of T helper(h) 2-cell induction, type 2 dendritic cells (DCs), generation and function of eosinophils, a role for IL-13 in fibrosis and characterization of alternatively activated macrophages (Pulendran and Artis, 2012).

3. A new immune paradigm

As alluded to earlier, multiple immune-regulatory mechanisms are underutilised by a variety of helminth infections that may ameliorate unrelated inflammatory conditions or, indeed, exacerbate inflammation. These studies have not only yielded essential information on the clinical tractability of helminth therapy but have provided parasite immunologists with insight into the mechanisms involved in helminth immunity. This led to an immune paradigm on helminth immunity. This was based on the simplified but prevailing paradigm of the 1980–90s, that type 1 immune responses caused disease pathology whereas type 2 responses were protective, for example in the context of schistosomaisis (Fallon et al., 2000). More recently the interplay of T regulatory (Treg) cells (Taylor et al., 2012) and the role of alternatively activated macrophages (Anthony et al., 2007; Kreider et al., 2007) have expanded this paradigm. Thus, the introduction of helminths would lead to modified Th2 responses and induction of regulatory cells with a concurrent subsidence in Th1/17 (autoimmune) or allergic (Th2) responses and suppress diseases and repair/reverse tissue damage (Fig. 1). More recent advances in immunology have seen the discoveries of new immunological mechanisms that appear to have critical roles in helminth immune modulation. These mechanisms indicate key roles for the resident epithelia (Zeigler and Artis, 2010) and innate cells (Oliphant et al., 2011) that are in proximity to the helminth. These 'first responders' are now deemed critical in skewing the adaptive immune response (Fig. 2).

4. The case for helminths as therapies

Whilst the merits of the Hygiene Hypothesis are open to debate (Okada et al., 2010), helminths are widely implicated (Yazdanbakhsh et al., 2002). Thus the absence of helminths in developed societies is argued to contribute to the profound increase in the incidence of allergic and autoimmune diseases observed during the last few decades (Yazdanbakhsh et al., 2002; Dunne and Cooke, 2005; Allen and Maizels, 2011). With respect to allergic diseases, there is considerable overlap between the characteristic, polarised inflammatory type 2 immune response and the regulatory type 2 responses typically evoked by helminth infection (Fallon and Mangan, 2007). However, there is disparity between different helminths and/or epidemiological studies on the relative effects, positive or negative, of helminth infection on allergic phenotypes in humans (Leonardi-Bee et al., 2006). For instance, data from epidemiological studies in helminth endemic populations have shown a negative association between the presence of certain helminths and allergic-like inflammatory responses (see Flohr et al., 2009). Nevertheless, helminth therapies are currently being tested as therapeutic stratagem in patients with a range of inflammatory diseases (Table 1), and the use of *Trichuris suis* ova as an investigational medicinal product (IMP) has been granted by the USA Food and Drug Administration (Elliott and Weinstock, 2009) while *Necator americanus* has been granted an IMP license by the Medicines and Healthcare Regulatory Authority in the UK (Pritchard, 2011).

Separate from epidemiological association studies in human field studies or, more recently, testing live helminth infections in patients, there is a substantial resource of experimental data on helminth modulation of unrelated inflammatory disorders (Table 2). It is relevant to reiterate that not all helminth infections of humans suppress allergic inflammation and may conversely increase inflammatory responses in infected individuals (Leonardi-Bee et al., 2006). Additionally, in mouse helminth models there is evidence that infection can actually exacerbate inflammation, for example, under certain conditions in experimental IBD (Hunter et al., 2007; Smith et al., 2007) and allergic lung inflammation (Mangan et al., 2006; Smits et al., 2007). As the propensity of live helminth infection to regulate immunity is well established, as indeed is the capacity of antigen extracts from helminths to potently stimulate immune activation, a logical option is to identify and isolate the helminth molecules inducing the modulatory activity (Fallon and Alcami, 2006; Harnett and Harnett, 2010a). Indeed, there are a number of molecules from helminths that have been shown to induce regulatory responses, which may have potential as therapeutic moieities (Adisakwattana et al., 2009; Johnston et al., 2009; Harnett et al., 2010a). A molecule from *Acanthocheilonema viteae*, ES-62, is the most investigated helminth-derived therapeutic molecule described to date (Harnett et al., 2010b). It remains to be determined whether therapeutic administration of

Please cite this article in press as: Khan, A.R., Fallon, P.G. Helminth therapies: Translating the unknown unknowns to known knowns. Int. J. Parasitol. (2013), http://dx.doi.org/10.1016/j.ijpara.2012.12.002

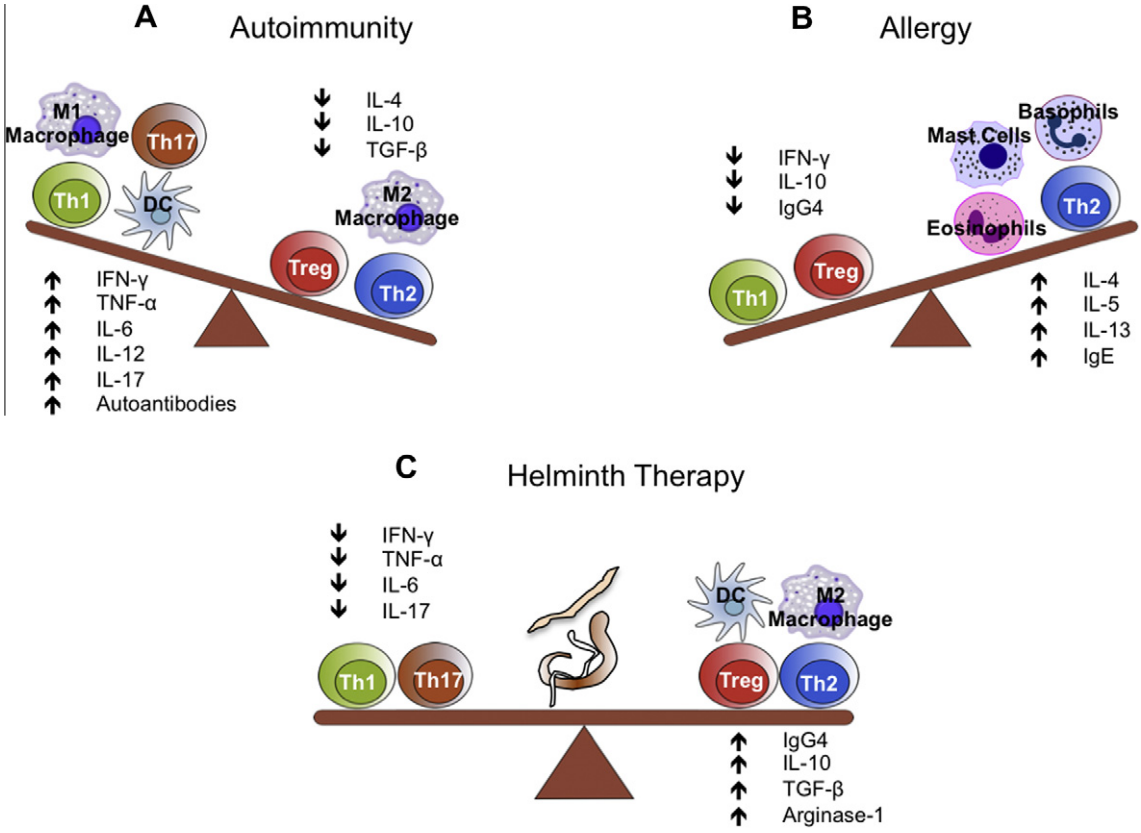

Fig. 1. The classical helminth immunity paradigm. The imbalance in immunity that can lead to (A) autoimmunity is primarily driven by T helper (Th)1 and Th17 cells with macrophages and dendritic cells (DCs) aiding this through an abundance of pro-inflammatory cytokines. In (B) allergic individuals there is a preponderance of Th2 cells, with fewer Th1 cells and a reduction in regulatory T (Treg) cells and other regulatory T cells such as IL-10-producing T cells. Th2 cell secretion of IL-4, IL-5, IL-9 and IL-13 evoke elevated total and allergen-specific IgE, eosinophilia and increases in mast cell and basophil numbers, as well as goblet cell hyperplasia. The use of (C) helminths as therapeutic stratagem will create a hemostatic balance between autoimmune Th1/Th17 and allergic Th2 responses (A and B) through the induction of a regulated environment with managed tissue damage and repair. TGF-β – transforming growth factor-beta.

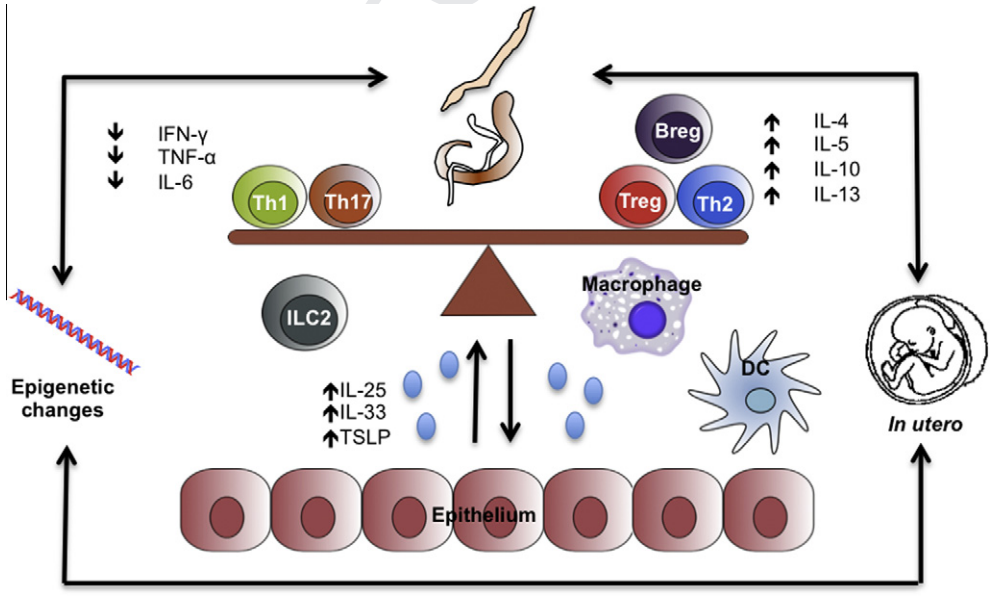

Fig. 2. Emerging new helminth immune regulation paradigm. Enhanced understanding of the mechanisms that underpin inflammatory processes has led to a new paradigm in helminth immunity. Helminths have now been shown to act on epithelia, causing the release of IL-25, IL-33 and thymic stromal lymphopoietin (TSLP). These 'alarmins' induce innate type 2 cells (ILC2) causing the release of IL-4, IL-5 and IL-13, which can then drive T helper (Th)2 responses. Regulatory B (Breg) cells are upregulated in helminth infection, producing IL-10 and can also induce immune suppression through regulatory T (Treg) cells. Together with regulatory-like dendritic cells (DCs) and macrophages these mechanisms depress Th1 and Th17 cells which are involved in the initial inflammatory response. All of the effects of helminth immune-modulation are in the context of genetic predisposition. Through evolutionary regulation of the 'immunome', as well as epigenetic regulation at key developmental stages such as in utero and in early childhood, helminths can alter immune responses.

Please cite this article in press as: Khan, A.R., Fallon, P.G. Helminth therapies: Translating the unknown unknowns to known knowns. Int. J. Parasitol. (2013), http://dx.doi.org/10.1016/j.ijpara.2012.12.002

Table 1
Clinical studies of helminth therapy in human disease.

Disease	Helminth	Outcome	Reference
Multiple Sclerosis	*Trichuris suis*	Five patients with relapsing/remitting MS Fewer neurological and CNS lesions Reoccurrence of symptoms after helminth expulsion	Fleming et al. (2011)
Inflammatory Bowel Disease	*T. suis*	No adverse events observed in CD or UC CD: 12 weeks after single dose of *T. suis ova* 75% remission with a 66% relapse rate UC: 12 weeks after single dose of *T. suis ova* 100% remission with a 33% relapse rate	Summers et al. (2003)
		75.9% of CD patients responded after 12 weeks; 65.5% remitted 79.3% of CD patients responded after 24 weeks; 72% remitted	Summers et al. (2005a)
		43.3% of UC patients responded after 12 weeks compared with 16.7% of placebo Non-significant differences in remission rates observed between treatment groups	Summers et al. (2005b)
		Change in CD activity index 20 weeks p.i. Adverse events recorded include anemia, transient enteropathy and peripheral eosinophilia	Croese et al. (2006)
	Trichuris trichiura	Infection associated with clinical remission and mucosal healing Increased IL-17^{+} and IL-22^{+} cells compared to episodes of colitis	Pullan et al. (1994)
Allergic rhinitis	*T. suis*	No significant change in symptom score, total histamine, grass-specific IgE or change in skin prick test	Bager et al. (2010)
	Necator americanus	No significant reduction in lung function No potentiation of allergen-specific IgE AMP-responsive asthma – no change in airway responsiveness, asthma control or allergen skin test observed	Blount et al. (2009)Feary et al. (2010)
Celiac Disease	*N. americanus*	No significant differences in duodenal pathology found between infected group and placebo Infected subjects reported injection site reactions and transient enteritis	Daveson et al. (2011)

MS, multiple sclerosis; CD, Crohn's disease; UC, ulcerative colitis.

an individual recombinantly engineered helminth molecule can recapitulate the modulatory efficiency of a live infection. One wonders, can a tablet replicate the orchestrated, sustained, site-specific and low-level release of multiple immune modulating molecules that elegantly both evades and modulates immunity, which helminths developed not in a factory, but through evolution?

Collectively, the studies of interactions between helminths and inflammatory disorders reviewed above show variable results, with both positive and negative associations reported in human and animal models. A possible explanation for this heterogeneity in the outcome of these studies can be attributed to the species of helminth, the age when infections were acquired and the intensity of infection. Helminths are often implicitly treated as a homogeneous group, but important inter-species differences exist. The disparity in outcome also applies to the inflammatory disease model employed in a particular study. An example of this is the effect of *Hymenolepis diminuta* reducing disease severity in a dinitrobenzene sulfonic acid (DNBS)-induced model of colitis, yet exacerbating the disease in oxazolone-induced colitis (Hunter et al., 2005; Wang et al., 2010). This suggests a degree of caution must be taken when using data from animal models.

Given the substantive body of work described above there is a significant aura of positivity which surrounds helminth immunity, not only as a useful tool for dissecting immunological mechanisms but also as a potential therapy for a range of inflammatory disorders. The advancements in understanding immune responses to helminth infection have lent themselves to the discovery of new immune populations and a shift in the immune paradigm from a primarily adaptive response to a more balanced innate-driven adaptive response. On encountering helminths, innate cells can be described as sounding the alarm (Fig. 2). However, effective immunity demands activation of the adaptive compartment. To bridge this transition, a plethora of type 2 innate mechanisms are initiated which indicates that helminths utilise several different mechanisms by which to first activate and then escape immune responses.

Recently concluded trials and pre-clinical assessments suggest that more work needs to be conducted in order for a greater clinical consensus to be reached. Murine studies in colitis (Smith et al., 2007) and airway hyperresponsiveness (Mangan et al., 2006; Smits et al., 2007) indicated initial inflammatory events as *S. mansoni* infections underwent acute to chronic phase transition. This raises concerns about the levels of tolerance patients would have, should they experience such effects. To further this argument, a trial of *T. suis* ova in allergic rhinitis demonstrated a lack of efficacy whilst reporting a high prevalence of adverse gastrointestinal reactions (Bager et al., 2011).

Clinical trials of helminths as therapies to date have been on patients from non-endemic countries. It is appropriate to note that helminth therapies will probably be used in developed societies, where the epidemic of inflammatory disorders is most prevalent, and thus in people never previously exposed to helminths. In contrast, the desirable protective effects of helminth infection of humans in field studies have been reported on people in endemic areas (Scrivener et al., 2001). A unique facet of endemicity is that people are infected with helminths early in childhood and may also have been subjected to in utero sensitization, i.e. women can be infected during pregnancy and the fetus exposed to helminth modulation and antigens. Indeed, prenatal exposure to helminths has been shown to alter immunity of neonates and cord blood cellular responses in endemic settings (King et al., 1998). There is a growing recognition that environmental factors during pregnancy and early life may lead to epigenetic changes that can profoundly change gene function and, by extension, the response in a disease setting (Hawrylowicz and Ryanna, 2010). Prominent examples of this are the increased risk of cardiovascular disease and type 2 diabetes in individuals born during the Dutch Famine of 1944–45 (Painter et al., 2005), and those who were young children during the siege of Leningrad in the 1940s (Sparen et al., 2004). It is not known how such early life exposure contributes to any beneficial effect helminth infections have on unrelated inflammation. What is the outcome of a person infected early in life who is subsequently infected with a different helminth as part of a therapy? In this context, one consequence of previous infection is that, as helminth antigens are highly glycosylated, infected individuals can produce antibody responses to glycan structures. This is relevant as some new biological therapies have unexpected side-effects due the presence of pre-existing anti-glycan antibodies that

Please cite this article in press as: Khan, A.R., Fallon, P.G. Helminth therapies: Translating the unknown unknowns to known knowns. Int. J. Parasitol. (2013), http://dx.doi.org/10.1016/j.ijpara.2012.12.002

Table 2
Studies of helminths in modulation of unrelated diseases in experimental disease models.

Model	Helminth	Outcome	Reference
Experimental autoimmune encephalomyelitis	Schistosoma mansoni	Protection against EAE Suppression of IL-12p40, IFN-γ and TNF-α Increase in TGF-β, IL-10 and IL-4	La Flamme et al. (2003) and Sewell et al. (2003)
	Trichinella spiralis	Protection against EAE Suppression of IFN-γ and IL-17. Increase in IL-4 and IL-10 Increase in splenic $CD4^+Foxp3^+$ T cells	Gruden-Movsesijan et al. (2010)
	Fasciola hepatica	Suppression of Th1 and Th17 responses with attenuated symptoms of EAE	Walsh et al. (2009)
Type 1 diabetes	*Heligmosomoides polygyrus* *S. mansoni* *T. spiralis*	Protection from Type I diabetes Inhibition of pancreatic insulitis Increased IL-4, IL-10 and IL-13 in mesenteric and pancreatic lymph nodes Soluble egg antigen prevents diabetes in NOD mice Increase in Foxp3+ T cells and AAM Delayed development of insulitis Splenic IL-4 secretion. No change in IL-10 or IFN-γ	Liu et al. (2009), Zaccone et al. (2010), Saunders et al. (2007) and Saunders et al. (2007)
Rheumatoid arthritis	*H. polgyrus bakeri* *Nippostrongylus brasiliensis*	MRL/lpr miceReduced incidence of arthritis and synovial hyperplasia	Salinas-Carmona et al. (2009)
	S. mansoni	Collagen/CFA sensitization Dose-dependent restriction in polyarticular arthritis Decreased IFN-γ and TNF-α with increased IL-4 and IL-10	Osada et al. (2009)
	Acanthocheilonema viteae *Schistosoma japonicum*	ES-62 active as prophylactic and treatment of arthritis Decreased IFN-γ and TNF-α with increased IL-10 Infection prior to collagen challenge led to upregulation in IL-10 and decrease in IFN-γ, TNF-α, IL-1β and IL-6	McInnes et al. (2003) and Song et al. (2011)
Allergy/asthma	*S. mansoni*	Protection from allergic airway reactivity Decreased allergen-stimulated IL-5. Increased IL-10 and TGF-β production	Mangan et al. (2006)
	S. mansoni	Protection from Airway hyper-responsiveness via $CD19^+IL\text{-}10^+$ Breg Increase in $CD4^+Foxp3^+$ T cells	Amu et al. (2010)
	T. spiralis	Minimal cell infiltration into bronchial tree AHR significantly suppressed Elevated Treg in lung draining lymph nodes	Park et al. (2011)
Inflammatory bowel disease	*S. mansoni* *H. polygyrus bakeri* *T. spiralis* *Hymenolepis diminuta*	Egg exposure attenuated TNBS colitis, diminished IFN-γ and enhanced IL-4 Infected mice refractory to DSS colitis. Protection afforded by $F4/80^+$ macrophages Modulation of intestinal DC function with modulation of IFN-γ and IL-17 response Infection prior to DNBS colitis reduced disease severity and IFN-γ and increased IL-4 and IL-13 Infection prior to DNBS colitis reduced disease severity. IL-10 dependent effect Exacerbation of oxazolone colitis with involvement of IL-5 and eosinophils	Elliott et al. (2003), Smith et al. (2007), Blum et al. (2012), Khan et al. (2002), Hunter et al. (2005) and Wang et al. (2010)
Obesity	*N. brasiliensis*	High fat diet mice demonstrated decreased adipose tissue mass and improved glucose tolerance helminth induced eosinophilia and promotion of alternatively activated macrophages	Wu et al. (2011)

EAE, experimental autoimmune encephalomyelitis; TGF, transforming-growth factor; NOD, non-obese diabetic; AAM, alternatively activated macrophages; Breg, regulatory B cell; AHR, airway hyperresponsiveness; Treg, regulatory T cell; TNBS, trinitrobenzene sulfonic acid; DSS, dextran sulphate sodium; DC, dendritic cell; DNBS, dinitrobenzene sulfonic acid.

cross-react with the drug, for example anaphylactic responses in cancer patients treated with Cetuximab (Chung et al., 2008). This is an issue that requires investigation as helminths may be used therapeutically in naïve (not previously exposed-infected) or sensitised (previously infected) patients.

5. Conclusion

The key to unlocking the potential of helminth therapy is gaining greater understanding of the 'immunome'. Helminths may not be suitable for all patients, however the ability to 'genotype' a cohort for their therapeutic suitability would certainly see efficacy rates improve dramatically. Dissecting and identifying key changes in the immunological genome will occur over time and, armed with this information, a more refined, elegant use for helminths in the clinic could be considered.

At the time of writing at least 16 clinical trials are underway involving helminth therapy in a multitude of diverse conditions including IBD, MS and autism. The juggernaut that is helminth therapy has been gathering pace for some time. It is therefore conceivable that we have reached the 'chicken and the egg' scenario where clinical demand outstrips the rate of mechanistic understanding, even with the variability in efficacy seen from study to study. However, there are precedents for this, as many drugs in clinical use today are effective therapies despite an incomplete understanding of their modes of action, such as sulfasalazine (Cottone et al., 2011). Similarly, helminths are currently under clinical

Please cite this article in press as: Khan, A.R., Fallon, P.G. Helminth therapies: Translating the unknown unknowns to known knowns. Int. J. Parasitol. (2013), http://dx.doi.org/10.1016/j.ijpara.2012.12.002

evaluation despite not knowing their full mechanism of action as therapies. In science, and indeed politics, sometimes we progress despite being unaware of all the outcomes.

> "There are known knowns. These are things we know that we know.
> There are known unknowns. That is to say, there are things that we know we don't know.
> But there are also unknown unknowns. There are things we don't know we don't know."
> Donald Rumsfeld, Defense.gov, 2002

As argued above, helminths have selected the immunome of today, consequentially there are many "unknown unknowns" to emerge as we translate helminths to therapies.

Uncited references

Q4 Mangan et al. (2004) and Rumsfled (2002).

Acknowledgments

We are grateful for members of the Fallon group for comments on the manuscript. The authors are supported by Science Foundation Ireland, Wellcome Trust, UK and National Children's Research Centre, Ireland.

References

Adisakwattana, P., Saunders, S.P., Nel, H.J., Fallon, P.G., 2009. Helminth-derived immunomodulatory molecules. Adv. Exp. Med. Biol. 666, 95–107.

Allen, J.E., Maizels, R.M., 2011. Diversity and dialogue in immunity to helminths. Nat. Rev. Immunol. 11, 375–388.

Amu, S., Saunders, S.P., Kronenberg, M., Mangan, N.E., Atzberger, A., Fallon, P.G., 2010. Regulatory B cells prevent and reverse allergic airway inflammation via FoxP3-positive T regulatory cells in a murine model. J. Allergy Clin. Immunol. 125 (1114–1124), e8.

Bager, P., Arnved, J., Rønborg, S., Wohlfahrt, J., Poulsen, L.K., Westergaard, T., Petersen, H.W., Kritensen, B., Thamsborg, S., Roepstorff, A., Kapel, C., Melbye, M., 2010. *Trichuris suis* ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. J. Allergy Clin. Immunol. 125 (123–30), e1–e3.

Bager, P., Kapel, C., Roepstorff, A., Thamsborg, S., Arnved, J., Rønborg, S., Kritensen, B., Poulsen, L.K., Wohlfahrt, J., Melbye, M., 2011. Symptoms after ingestion of pig whipworm *Trichuris suis* eggs in a randomized placebo-controlled double-blind clinical trial. Ed. T. Mark Doherty. PLoS One 6, e22346.

Barreiro, L.B., Quintana-Murci, L., 2010. From evolutionary genetics to human immunology: how selection shapes host defence genes. Nat. Rev. Genet. 11, 17–30.

Blount, D., Hooi, D., Feary, J., Venn, A., Telford, G., Brown, A., Britton, J., Pritchard, D.I., 2009. Immunologic profiles of persons recruited for a randomized, placebo-controlled clinical trial of hookworm infection. Am. J. Trop. Med. Hyg. 81, 911–916.

Blum, A.M., Hang, L., Setiawan, T., Urban, J.P., Stoyanoff, K.M., Leung, J., Weinstock, J.V., 2012. *Heligmosomoides polygyrus bakeri* induces tolerogenic dendritic cells that block colitis and prevent antigen-specific gut T cell responses. J. Immunol. 189, 2512–2520.

Casanova, J.-L., Abel, L., 2005. Inborn errors of immunity to infection: the rule rather than the exception. J. Exp. Med. 202, 197–201.

Chung, C.H., Mirakhur, B., Chan, E., Le, Q.-T., Berlin, J., Morse, M., Murphy, B.A., Satinover, S.M., Hosen, J., Mauro, D., Slebos, R.J., Zhou, Q., Gold, D., Hatley, T., Hicklin, D.J., Platts-Mills, T.A., 2008. Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. N. Engl. J. Med. 358, 1109–1117.

Cottone, M., Renna, S., Modesto, I., Orlando, A., 2011. Is 5-ASA still the treatment of choice for ulcerative colitis? Curr. Drug Targets 12, 1396–1405.

Croese, J., O'Neil, J., Masson, J., Cooke, S., Melrose, W., Pritchard, D.I., Speare, R., 2006. A proof of concept study establishing *Necator americanus* in Crohn's patients and reservoir donors. Gut 55, 136–137.

Daveson, A.J., Jones, D.M., Gaze, S., McSorley, H., Clouston, A., Pascoe, A., Cooke, S., Macdonald, G.A., Anderson, R., McCarthy, J.S., Loukas, A., Croese, J., 2011. Effect of hookworm infection on wheat challenge in celiac disease – a randomised double-blinded placebo controlled trial. PLoS One 6, e17366.

Dunne, D.W., Cooke, A., 2005. A Worm's eye view of the immune system: consequences for evolution of human autoimmune disease. Nat. Rev. Immunol. 5, 420–426.

Elliott, D.E., Li, J., Blum, A., Metwali, A., Qadir, K., Urban, J.F., Weinstock, J.V., 2003. Exposure to schistosome eggs protects mice from TNBS-induced colitis. Am. J. Physiol. Gastrointest. Liver Physiol. 284, G385–G391.

Elliott, D.E., Weinstock, J.V., 2009. Helminthic therapy: using worms to treat immune-mediated disease. Adv. Exp. Med. Biol. 666, 157–166.

Fallon, P.G., Richardson, E.J., Smith, P., Dunne, D.W., 2000. Elevated type 1, diminished type 2 cytokines and impaired antibody response are associated with hepatotoxicity and mortalities during *Schistosoma mansoni* infection of CD4-depleted mice. Eur. J. Immunol. 30 (2), 470–480.

Fallon, P.G., Alcami, A., 2006. Pathogen-derived immunomodulatory molecules: future immunotherapeutics? Trends Immunol. 27, 470–476.

Fallon, P.G., Mangan, N.E., 2007. Suppression of TH2-type allergic reactions by helminth infection. Nat. Rev. Immunol. 7, 220–230.

Farrokhyar, F., Swarbrick, E.T., Irvine, E.J., 2001. A critical review of epidemiological studies in inflammatory bowel disease. Scand. J. Gastroenterol. 36, 2–15.

Feary, J., Venn, A., Brown, A., Hooi, D., Falcone, F.H., Mortimer, K., Pritchard, D.I., Britton, J., 2010. Experimental hookworm infection: a randomized placebo-controlled trial in asthma. Clin. Exp. Allergy 40, 299–306.

Hawrylowicz, C., Ryanna, K., 2010. Asthma and allergy: the early beginnings. Nat. Med. 16, 274–275.

La Flamme, A.C., Ruddenklau, K., Backstrom, B.T., 2003. Schistosomiasis decreases central nervous system inflammation and alters the progression of experimental autoimmune encephalomyelitis. Infect. Immun. 71, 4996–5004.

Fleming, J.O., Isaak, A., Lee, J.E., Luzzio, C.C., Carrithers, M.D., Cook, T.D., Field, A.S., Boland, J., Fabry, Z., 2011. Probiotic helminth administration in relapsing-remitting multiple sclerosis: a phase 1 study. Mult. Scler. 17, 743–754.

Flohr, C., Quinnell, R.J., Britton, J., 2009. Do helminth parasites protect against atopy and allergic disease? Clin. Exp. Allergy 39, 20–32.

Fumagalli, M., Pozzoli, U., Cagliani, R., Comi, G.P., Riva, S., Clerici, M., Bresolin, N., Sironi, M., 2009. Parasites represent a major selective force for interleukin genes and shape the genetic predisposition to autoimmune conditions. J. Exp. Med. 206, 1395–1408.

Gruden-Movsesijan, A., Ilic, N., Mostarica-Stojkovic, M., Stosic-Grujicic, S., Milic, M., Sofronic-Milosavljevic, L., 2010. Mechanisms of modulation of experimental autoimmune encephalomyelitis by chronic *Trichinella spiralis* infection in dark agouti rats. Parasite Immunol. 32, 450–459.

Haldane, J.B.S., 1949. Infectious disease and evolution. RIC Sci. Suppl. A 19, 68–76.

Harnett, W., Harnett, M.M., 2010. Helminth-derived immunomodulators: can understanding the worm produce the pill? Nat. Rev. Immunol. 10, 278–284.

Harnett, M.M., Melendez, A.J., Harnett, W., 2010. The therapeutic potential of the filarial nematode-derived immunodulator, ES-62 in inflammatory disease. Clin. Exp. Immunol. 159, 256–267.

Hotez, P.J., Brindley, P.J., Bethony, J.M., King, C.H., Pearce, E.J., Jacobson, J., 2008. Helminth infections: the great neglected tropical diseases. J. Clin. Invest. 118, 1311–1321.

Hunter, M.M., Wang, A., Hirota, C.L., McKay, D.M., 2005. Neutralizing anti-IL-10 antibody blocks the protective effect of tapeworm infection in a murine model of chemically induced colitis. J. Immunol. 174, 7368–7375.

Hunter, M.M., Wang, A., Hirota, C.L., McKay, D.M., 2007. helminth infection enhances disease in a murine TH2 model of colitis. Gastroenterology 132, 1320–1330.

Johnston, M.J.G., MacDonald, J.A., McKay, D.M., 2009. Parasitic helminths: a pharmacopeia of anti-inflammatory molecules. Parasitology, 125–147. Q3

Khan, W.I., Blennerhasset, P.A., Varghese, A.K., Chowdhury, S.K., Omsted, P., Deng, Y., Collins, S.M., 2002. Intestinal nematode infection ameliorates experimental colitis in mice. Infect. Immun. 70, 5931–5937.

King, C.L., Malhotra, I., Mungai, P., Wamachi, A., Kioko, J., Ouma, J.H., Kazura, J.W., 1998. B cell sensitization to helminthic infection develops in utero in humans. J. Immunol. 160, 3578–3584.

Kreider, T., Anthony, R.M., Urban Jr., J.F., Gause, W.C., 2007. Alternatively activated macrophages in helminth infections. Curr. Opin. Immunol. 19, 448–453.

Leonardi-Bee, J., Pritchard, D.I., Britton, J., 2006. Asthma and current intestinal parasite infection: systematic review and meta-analysis. Am. J. Respir. Crit. Care Med. 174, 514–523.

Leulier, F., Lemaitre, B., 2008. Toll-like receptors-taking an evolutionary approach. Nat. Rev. Genet. 9, 165–178.

Liu, Q., Sundar, K., Mishra, P.K., Mousavi, G., Liu, Z., Gaydo, A., Alem, F., Lagunoff, D., Bleich, D., Gause, W.C., 2009. Helminth infection can reduce insulitis and type 1 diabetes through CD25- and IL-10-independent mechanisms. Infect. Immun. 77, 5347–5358.

Mangan, N.E., Fallon, R.E., Smith, P., van Rooijen, N., McKenzie, A.N., Fallon, P.G., 2004. Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. J. Immunol. 173, 6346–6356.

Mangan, N.E., van Rooijen, N., McKenzie, A.N., Fallon, P.G., 2006. Helminth-modified pulmonary immune response protects mice from allergen-induced airway hyperresponsiveness. J. Immunol. 176 (1), 138–147.

McInnes, I.B., Leung, B.P., Harnett, M., Gracie, J.A., Liew, F.Y., Harnett, W., 2003. A novel therapeutic approach targeting articular inflammation using the filarial nematode-derived phosphorylcholine-containing glycoprotein ES-62. J. Immunol. 171, 2127–2133.

Moroni, L., Bianchi, I., Lleo, A., 2012. Geoepidemiology, gender and autoimmune disease. Autoimmun. Rev. 11, A386–A392.

Okada, H., Kuhn, C., Feillet, H., Bach, J.-F., 2010. The hygiene hypothesis for autoimmune and allergic diseases: an update. Clin. Exp. Immunol. 160, 1–9.

Oliphant, C.J., Barlow, J.L., McKenzie, A.N.J., 2011. Insights into the initiation of type 2 immune responses. Immunology 134, 378–385.

Osada, Y., Shimizu, S., Kumagai, T., Yamada, S., Kanazawa, T., 2009. *Schistosoma mansoni* infection reduces severity of collagen-induced arthritis via down-regulation of pro-inflammatory mediators. Int. J. Parasitol. 39, 457–464.

Please cite this article in press as: Khan, A.R., Fallon, P.G. Helminth therapies: Translating the unknown unknowns to known knowns. Int. J. Parasitol. (2013), http://dx.doi.org/10.1016/j.ijpara.2012.12.002

Painter, R.C., Roseboom, T.J., Bleker, O.P., 2005. Prenatal exposure to the Dutch famine and disease in later life: an overview. Reprod. Toxicol. 20, 345–352.

Park, H.K., Cho, M.K., Choi, S.H., Kim, Y.S., Yu, H.S., 2011. *Trichinella spiralis*: infection reduces airway allergic inflammation in mice. Exp. Parasitol. 127, 539–544.

Pritchard, D.I., 2011. Worm therapy: for or against? J. Helminthol. 85, 225–227.

Prugnolle, F., Manica, A., Charpentier, M., Guégan, J.F., Guernier, V., Balloux, F., 2005. Pathogen-driven selection and worldwide HLA class I diversity. Curr. Biol. 15, 1022–1027.

Pulendran, B., Artis, D., 2012. New paradigms in type 2 immunity. Science 337, 431–435.

Pullan, R.D., Thomas, G.A., Rhodes, M., Newcombe, R.G., Williams, G.T., Allen, A., Rhodes, J., 1994. Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis. Gut 35, 353–359.

Rumsfled, D., 2002. Defense.gov News Transcript: DoD News Briefing – Secretary Rumsfeld and Gen. Myers, United States Department of Defense. Available from: <http://www.defense.gov/transcripts/transcript.aspx?transcriptid=2636>.

Salinas-Carmona, M.C., de la Cruz-Galicia, G., Pérez-Rivera, I., Solís-Soto, J.M., Segoviano-Ramirez, J.C., Vázquez, A.V., Garza, M.A., 2009. Spontaneous arthritis in MRL/lpr mice is aggravated by *Staphylococcus aureus* and ameliorated by *Nippostrongylus brasiliensis* infections. Autoimmunity 42, 25–32.

Saunders, K.A., Raine, T., Cooke, A., Lawrence, C.E., 2007. Inhibition of autoimmune type 1 diabetes by gastrointestinal helminth infection. Infect. Immunol. 75, 397–407.

Sparen, P., Vagero, D., Shestov, D.B., Plavinskaja, S., Parfenova, N., Hoptiar, V., Paturot, D., Galanti, M.R., 2004. Long term mortality after severe starvation during the siege of Leningrad: prospective cohort study. BMJ 328, 11.

Scrivener, S., Yemaneberhan, H., Zebenigus, M., Tilahun, D., Girma, S., Ali, S., McElroy, P., Custovic, A., Woodcock, A., Pritchard, D., Venn, A., Britton, J., 2001. Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in ethiopia: a nested case-control study. Lancet 358, 1493–1499.

Sewell, D., Qing, Z., Reinke, E., Elliot, D., Weinstock, J., Sandor, M., Fabry, Z., 2003. Immunomodulation of experimental autoimmune encephalomyelitis by helminth ova immunization. Int. Immunol. 15, 59–69.

Smith, P., Mangan, N.E., Walsh, C.M., Fallon, R.E., McKenzie, A.N., van Rooijen, N., Fallon, P.G., 2007. Infection with a helminth parasite prevents experimental colitis via a macrophage-mediated mechanism. J. Immunol. 178, 4557–4566.

Smits, H.H., Hammad, H., van Nimwegen, M., Soullie, T., Willart, M.A., Lievers, E., Kadouch, J., Kool, M., Kos-van Oosterhoud, J., Deelder, A.M., Lambrecht, B.N., Yazdanbakhsh, M., 2007. Protective effect of Schist*osoma mansoni* infection on allergic airway inflammation depends on the intensity and chronicity of infection. J. Allergy Clin. Immunol. 120, 932–940.

Song, X., Shen, J., Wen, H., Zhong, Z., Luo, Q., Chu, D., Qi, Y., Xu, Y., Wei, W., 2011. Impact of *Schistosoma japonicum* infection on collagen-induced Arthritis in DBA/1 mice. a murine model of human rheumatoid arthritis. PLoS One 6, e23453.

Stoll, N.R., 1947. This wormy world. J. Parasitol. 33, 1–18.

Summers, R.W., Elliott, D.E., Qadir, K., Urban, J.F., Thompson, R., Weinstock, J.V., 2003. *Trichuris suis* seems to be safe and possibly effective in the treatment of inflammatory bowel disease. Am. J. Gastroenterol. 98, 2034–2041.

Summers, R.W., Elliott, D.E., Urban, J.F., Thompson, R., Weinstock, J.V., 2005a. *Trichuris suis* therapy in Crohn's disease. Gut 54, 87–90.

Summers, R.W., Elliott, D.E., Urban, J.F., Thompson, R., Weinstock, J.V., 2005b. *Trichuris suis* therapy for active ulcerative colitis: a randomized controlled trial. Gastroenterology 128, 825–832.

Taylor, M.D., van der Werf, N., Maizels, R.M., 2012. T cells in helminth infection: the regulators and the regulated. Trends Immunol. 33, 181–189.

Walsh, K.P., Brady, M.T., Finlay, C.M., Boon, L., Mills, K.H., 2009. Infection with a helminth parasite attenuates autoimmunity through TGF-beta-mediated suppression of Th17 and Th1 responses. J. Immunol. 183, 1577–1586.

Wang, A., Fernando, M., Leung, G., Phan, V., Smyth, D., McKay, D.M., 2010. Exacerbation of oxazolone colitis by infection with the helminth *Hymenolepis diminuta*: involvement of IL-5 and eosinophils. Am. J. Pathol. 177, 2850–2859.

Wu, D., Molofsky, A.B., Liang, H.E., Ricardo-Gonzalez, R.R., Jouihan, H.A., Bando, J.K., Chawla, A., Locksley, R.M., 2011. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science 332, 243–247.

Yazdanbakhsh, M., Kremsner, P.G., van Ree, R., 2002. Allergy, parasites, and the hygiene hypothesis. Science 296, 490–494.

Zaccone, P., Burton, O.T., Gibbs, S., Miller, N., Jones, F.M., Dunne, D.W., Cooke, A., 2010. Immune modulation by *Schistosoma mansoni* antigens in NOD mice. Effects on both innate and adaptive immune systems. J. Biomed. Biotechnol. 2010, 795210.

Zeigler, S.F., Artis, D., 2010. Sensing the outside world: TSLP regulates barrier immunity. Nat. Immunol. 11, 289–293.

Please cite this article in press as: Khan, A.R., Fallon, P.G. Helminth therapies: Translating the unknown unknowns to known knowns. Int. J. Parasitol. (2013), http://dx.doi.org/10.1016/j.ijpara.2012.12.002