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Current Opinion

Worming our way closer to the clinic

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ABSTRACT

In a recent issue of "The International Journal for Parasitology: Drugs and Drug Resistance" Prof. David Pritchard from the University of Nottingham offers his intriguing opinion on the current status of "worm therapy" and outlines future research priorities aimed at bringing this research area closer to the clinic. In this response article we discuss various aspects of the current state of the research field and offer some alternative viewpoints regarding the future of "worm therapy".

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1. Introduction

The opinion article from Prof. Pritchard casts a much needed critical-eye over the current status and future of clinical "worm therapy" (Pritchard, 2012). The use of experimental helminth infections or helminth-derived products as therapeutics is a promising and expanding field of research, with significant potential for the treatment of inflammatory, allergic and autoimmune disorders. Chronic helminth infections, particularly those of the gastrointestinal tract, are increasingly accepted as part of the natural flora with which humans have co-evolved over millennia. Thus, helminth infections are inherently associated with the so-called "hygiene hypothesis" that underlies much of the current etiological thinking regarding the increased prevalence of allergy and autoimmunity in the western world. This theory has been strengthened by a range of epidemiological studies demonstrating a link between the loss of helminth infections and the rise of allergic and inflammatory diseases in developing countries, as well as by a multitude of laboratory studies in murine models and to a lesser extent in humans. These studies demonstrate the efficacy of live helminth infections, administration of secreted helminth products or of defined, recombinantly produced helminth molecules in the suppression of a wide range of inflammatory diseases.

Given the abundance of data already accrued it now seems appropriate that the scientific community reflects upon the advances made in recent years and considers how best to proceed in order to reach the ultimate goal of bringing "worm therapy" into the clinics, and to the patients who could potentially benefit from it.

2. Where do we go from here?

For "worm therapy" to be successfully transitioned into the clinics it is necessary to consider short-term benefits as well as long-term priorities. In the short term it may indeed be beneficial, as suggested by Prof. Pritchard, to focus on the administration of live helminths. There are a significant number of patients suffering from autoimmune, inflammatory and allergic diseases for whom conventional therapies have been unsuccessful. As such many patients are willing to try experimental treatments, including "worm therapy". There are even documented cases of patients who have sought to infect themselves with gastrointestinal nematodes in order to treat symptoms of severe inflammatory disease (Broadhurst et al., 2010). In the short-term validated safe worm therapies such as *Trichuris suis* ova (TSO) should be investigated on a much larger scale in the clinics to determine efficacy, therapeutic longevity and safety. Indeed the results from initial trials with TSO in patients with inflammatory bowel disease (Summers et al., 2005a,b), coupled with recent smaller scale trials in multiple sclerosis patients (Benzel et al., 2011; Fleming et al., 2011) suggest TSO may be viable as a treatment for inflammatory disease. However, it should be noted that a study by Bager et al. in allergic rhinitis patients failed to show any significant improvement in disease symptoms following TSO therapy (Bager et al., 2010). The outcomes of a number of ongoing clinical trials currently investigating TSO treatment are eagerly anticipated.

Nonetheless, it is our opinion that although developing live worm therapy may be immediately attractive, the characterisation of defined and targeted therapies based on helminth-derived molecules should prove more beneficial and preferable in the long term. Application of live parasites has several caveats, not least inevitable problems with patient compliance. Parasitic helminths

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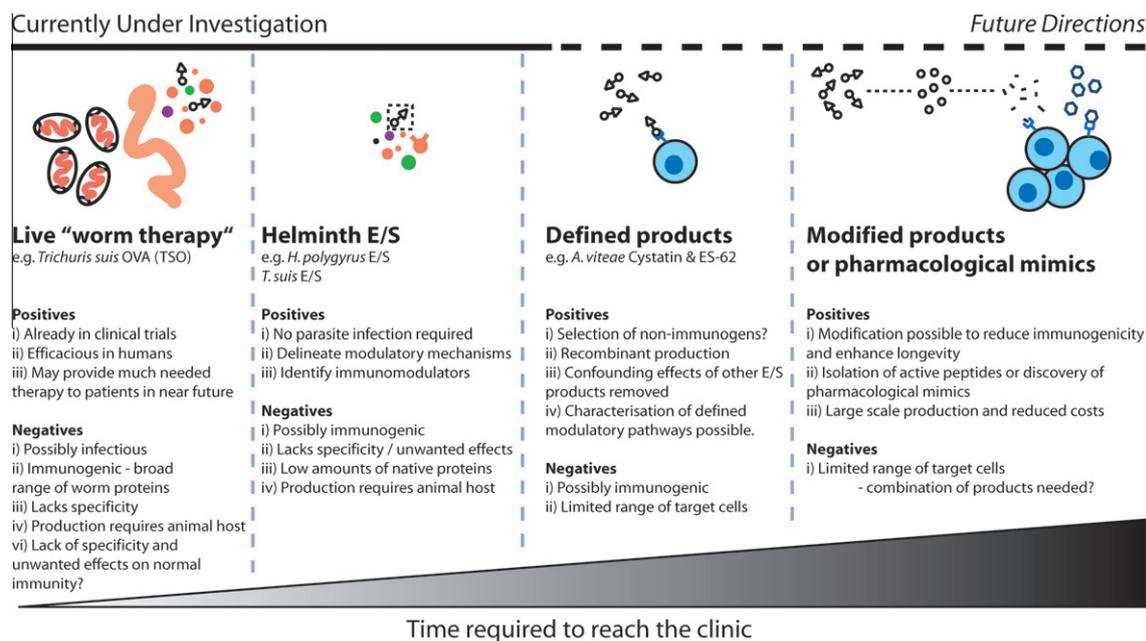


Fig. 1. Strategy for the development of helminth-derived therapies for clinical application.

are by their very nature pathogens and exposure to infectious helminths is likely to result in significant side effects associated with tissue damage and inflammation. Indeed, Bager et al. have reported significant side effects in patients receiving TSO therapy – including diarrhoea and abdominal pain (Bager et al., 2011). Although unlikely, it is also still unclear whether *T. suis* may establish and persist in the human host (Kradin et al., 2006).

In a limited number of cases the specific immunomodulatory protein that mediates immunosuppression during helminth infections has been identified – such as the filarial immunomodulators Av17 and ES-62 (Harnett and Harnett, 2009; Klotz et al., 2011). In addition, the recent publication of the transcriptomes and secretomes of several commonly studied helminths will no doubt lead to the identification of a number of new immunomodulatory products (Hewitson et al., 2008, 2011b; Cantacessi et al., 2011a,b). Thus, single defined helminth products can be characterised in order to elucidate their mechanisms of immunosuppression and the best candidates developed for therapeutic use (Fig. 1).

Helminth infections induce a wide range of immune regulatory mechanisms and have been shown to inhibit a variety of inflammatory and allergic diseases via the induction of CD4+ regulatory T cells (Grainger et al., 2010), CD8+ regulatory cells (Metwali et al., 2006), regulatory B cells (Wilson et al., 2010) and the modulation of a wide range of innate cell types including macrophages (Schnoeller et al., 2008; Klotz et al., 2011), dendritic cells (Segura et al., 2007; Hang et al., 2010) and mast cells (Melendez et al., 2007), amongst others. The modulatory activity of any helminth-derived therapy will have to be balanced against unwanted suppressive effects on protective immune responses, vaccination and anti-tumour immunity. Indeed, regulatory T cells induced by helminth infection have been shown to suppress the response to BCG vaccination and to concurrent malaria infection in children in endemic regions (Wammes et al., 2010). Helminth infections have also been shown to interfere with concurrent immunity to pathogenic bacterial species by modulation of the immune system (Weng et al., 2007). In addition, more focus should be placed on developing a better understanding of the interactions of gastrointestinal helminth infections with the intestinal microbiota with which they share the intestinal niche. Maintaining the balance of intestinal flora is central to the prevention of intestinal inflammatory

disorders and for the regulation and maintenance of immune homeostasis. Indeed helminth infection has been shown to result in changes in the composition of intestinal bacteria including increased frequency of *Lactobacillaceae* (Walk et al., 2010) and *Enterobacteriaceae* species (Rausch and Hartmann, unpublished data). Indeed, *T. suis* infection induces significant changes in the host microbiota, which are likely to be of clinical importance (Wu et al., 2012; Li et al., 2012). Thus, priority needs to be given to developing therapies that modulate only the unwanted inflammatory response (i.e. allergic or autoimmune response) while maintaining otherwise normal immunity in the same patient.

3. Can defined helminth immunomodulators be effective as therapeutics?

Central to Prof. Pritchard's critique is the assumption that the use of defined helminth derived immunomodulatory products as therapeutics is doomed to failure as such products will be neutralised and rendered ineffective by the host immune response. In contrast, Prof. Pritchard argues that live worm therapies should be given priority in the short term as they have shown some promise in the clinics and hopefully, can be made available to patients in the near future. However, one problem with this approach is that live parasite infections result in tissue damage and the induction of danger signals and pro-inflammatory stimuli thus, leading to inflammation. Furthermore, the host is exposed to the full spectrum of helminth-derived products including potent antigens, inflammatory stimuli and potentially disease causing allergens in addition to the desired helminth immunomodulators. It is widely acknowledged that the ability of helminths to modulate the immune system and suppress bystander inflammatory diseases is due to their production of immunomodulatory products that induce a variety of suppressive cell types in the host that maintain immune suppression and prevent parasite eradication. Thus, if Prof. Pritchard's argument that current candidate helminth products would be neutralised by antibodies in the host is correct then it would also be expected that live “worm therapy” in patients would also be ineffective as the very same helminth derived immunomodulators that mediate immunosuppression would also be neutralised during infection and become ineffective.

In contrast, the persistence of chronic gastrointestinal helminth infections or filarial infections in both humans and rodents has been demonstrated to be due to the effects of immunomodulatory products that occur in the presence of a full host adaptive immune response and the production of antibodies. Indeed IgG and IgE subclass antibodies were produced against *T. suis* derived products by patients during clinical trials with TSO (Bager et al., 2010, 2011), but nonetheless the effect of TSO therapy has been shown to persist and maintain remission in treated patients long after the production of host antibodies (Summers et al., 2005a,b). Thus, this suggests that either the activity of helminth immunomodulators can persist even in the presence of antibodies that recognise them, or that non-immunogenic products may be present that mediate immunosuppression. In line with this Grainger et al. found that helminth derived TGF- β activity was still required to maintain immunosuppression even during the late chronic stages of gastrointestinal helminth infection. Blockade of TGF- β signalling, but not neutralisation of host derived TGF- β cytokine, during chronic infection resulted in a restoration of Th2 cytokine production and a reduced parasite burden (Grainger et al., 2010). Similarly, abrogation of long-term filarial infection by drug treatment results in increased allergic responses and immunoreactivity in de-wormed patients suggesting a persistent and active immunosuppression of anti-helminth responses during chronic filarial infections (van den Biggelaar et al., 2004; Flohr et al., 2009). Thus, helminth derived immunomodulatory activity appears to be evident long after the development of host adaptive immune responses suggesting the activity of key worm derived immunomodulators may not be neutralised by the host immune system. Indeed, Prof. Pritchard acknowledges that the effects of live worm therapy seem to be long lasting and therefore, that non-immunogenic or non-neutralised helminth derived products may exist and priority should be placed on identifying these products.

Many helminth derived products are relatively poor antigens in terms of their ability to activate antigen presenting cells and in fact can down modulate the expression of co-stimulatory markers induced by other stimuli such as TLR-ligands (Reviewed by (Carvalho et al., 2009)). Thus, the activation and presentation of helminth-derived antigens by dendritic cells is largely considered to be muted, although robust Th2 responses may still be raised during infection. Moreover, several studies have suggested the presence of non-immunogenic helminth derived products. For example, the specific *Heligmosomoides polygyrus* products that are recognised by the host antibody response have recently been reported to be limited and transfer of immune sera against these recognised proteins was not sufficient to alter the persistence of infection, suggesting that the immunomodulators that maintain chronic infection may be distinct from the dominant immunogenic proteins recognised by the host immune system (Hewitson et al., 2011a). Furthermore, antibody responses raised against *H. polygyrus* proteins were found to be predominantly directed against glycan motifs. Therefore, a further possibility is that potent helminth derived immunomodulators that are recognised by the host immune response can be modified in order to remove the immunogenic epitopes and thus, enhance their longevity as therapeutics. It is not known to date whether many of the current candidate helminth immunomodulators are neutralised following repeat treatments in the host and it is clear that focused studies are required in order to determine whether currently researched candidates maintain their efficacy after long term application in chronic and recurring inflammatory diseases.

4. Why it is too early to discount mouse models

Current research into helminth immunomodulation tends to be focused mostly on murine systems and in his opinion article

Prof. Pritchard calls for a reduction in the use of mouse disease models in order to investigate the mechanisms of helminth immunomodulation. Although mouse models have taught immunologists a lot about the pathogenesis of human disease it is clear that many murine disease models represent an optimised system that may apply to only a narrow range of human patients. Indeed it is important to begin to transfer our knowledge of helminth immunomodulation from experimental mouse models into human systems and to test candidate products on human material whenever possible. However, the latter comes with considerable limitations. Firstly, application of live parasite products into human patients requires a significant investment of time and money in order to carry out safe and ethical clinical trials. Secondly, immunological analyses with human samples are largely limited to assays based around peripheral blood mononuclear cells (PBMCs) derived from donor blood, as tissue biopsies are often very difficult to obtain in sufficient numbers. Investigation of helminth derived products in PBMC based assays will go a long way to determining whether such therapies may be efficacious in human subjects, but such systems are also not a good indicator of local tissue responses and key inflammatory cell types such as macrophages, which are not present outside of the tissues. In contrast, murine systems give researchers access to unparalleled tools and yield information that is simply unobtainable in their absence. For example, work with transgenic mice or cytokine reporter mice can provide invaluable information regarding the mechanisms by which the immune system of the host is stimulated and modulated by helminths and their products. In this respect, it is important to note that since the first trials reporting efficacy of *T. suis* ova therapy in colitis and Crohn's disease patients, little new information has become available regarding the basic biology or immunological mechanisms through which this therapy functions despite Phase II clinical trials being already underway. Critically, a better understanding of the cellular networks and mechanisms through which immunomodulation by helminth products works will allow for the refinement and progression of treatments – in particular, the ability to generate pharmacological mimics or drugs that stimulate the same identified pathways to induce suppression. This approach would also ultimately reduce the need for exposure to any parasite material and thus, negate any fears about immunogenicity and side effects. Thus, mouse models are still needed to provide important information about the underlying mechanisms of therapeutic helminth infection and provide a research and development tool with which to screen for potential problems. Nonetheless, it is clear that work in mouse models can only go so far as to predict possible mechanisms of action and may not highlight side effects that could occur in other species. Studies in large animal models with a more similar biology to humans, such as pigs and non-human primates, will therefore be necessary in transitioning any findings from the lab and in developing candidate helminth derived therapies.

5. Moving forward

To further develop live-helminth therapy, or helminth-derived product based therapies, an increased dialogue between academic researchers and the pharmaceutical industry is needed in order to maximise the chances of developing successful treatments. The development of defined helminth-derived products seems to be advantageous in the long term as it allows for the production of high quantities of safe, consistent product that can be produced without the need for intermediate animal hosts and which may ultimately be used in combination should such an approach prove more efficacious than administration of single helminth-derived products. The pharmaceutical industry will play an essential role in testing any candidate proteins for safety and efficacy in humans

and large animal systems and ultimately, will be responsible for the clinical trials and investments necessary for making such therapy a reality. As such, basic laboratory studies will need to focus on proving efficacy and modes of action where possible, while also cooperating with potential investors and developers to ensure the candidate is a financially viable and unique product in addition to being an efficacious biological. Increased interactions and joint initiatives between academic researchers, clinicians and pharmaceutical companies may help to accelerate these processes and establish priorities and should be established as soon as possible to build on the potential of current candidates.

6. Summary

Recent advances in the field of parasite immunology have led to a better understanding of the mechanisms by which helminth infections modulate the immune system of their hosts in order to facilitate their long term survival. The resulting immunomodulation of bystander immune responses has strong potential for exploitation in a therapeutic setting. Recent increases in transcriptomic and proteomic information will likely identify further promising candidates and thus, in order to proceed researchers must consider which factors and criteria are most important in order to yield successful treatments that can be adapted for the clinics. A better understanding of the specific target cells and cellular pathways addressed by helminth derived immunomodulators and the longevity of therapeutic effects will likely lead to new therapeutic approaches.

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