

Trichuris muris and comorbidities – within a mouse model context

Kelly S. Hayes  and Richard K. Grencis

Lydia Becker Institute of Immunology and Inflammation, Wellcome Trust Centre for Cell Matrix Research and Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

Review

Cite this article: Hayes KS, Grencis RK (2021). *Trichuris muris* and comorbidities – within a mouse model context. *Parasitology* **148**, 1774–1782. <https://doi.org/10.1017/S0031182021000883>

Received: 8 March 2021
Revised: 22 May 2021
Accepted: 26 May 2021
First published online: 3 June 2021

Key words:

cancer; co-infections; comorbidity; *Trichuris muris*

Author for correspondence:

Kelly S. Hayes,
E-mail: Kelly.hayes@manchester.ac.uk

Abstract

Trichuris muris is a mouse intestinal parasitic nematode that inhabits the large intestine of its host and induces a strong immune response. The effects of this strong anti-parasite response can be found locally within the intestinal niche and also systemically, having effects on multiple organs. Additionally, the anti-parasite response can have multiple effects on infectious organisms and on microbiota that the host is harbouring. It has been shown that Th1 responses induced by *T. muris* can affect progression of bowel inflammation, cause colitic-like intestinal inflammation, reduce barrier function and intestinal mucosal responses. In the brain, *T. muris* can exacerbate stroke outcome and other neurological conditions. In the lung, *T. muris* can suppress airway inflammation and alter immune responses to other parasites. Additionally, *T. muris* induced responses can inhibit anti-tumour immunity. Although this parasite maintains a localized niche in the large intestine, its effects can be far-reaching and substantially impact other infections through modulation of bystander immune responses.

Introduction

Trichuris muris is a mouse intestinal parasitic nematode used as an experimental model for the human counterpart, *T. trichiura*. This nematode is one of the four major soil-transmitted helminths that infect 1.5 billion people worldwide causing significant morbidity (WHO, 2020). These diseases bear a huge impact on the quality of life of infected people and on the economic growth of infected communities (Hotez *et al.*, 2014).

T. muris inhabits the large intestine and caecum of the host, with adult parasites living with their anterior half tunnelled into the host epithelium and their posterior free in the lumen to facilitate egg deposition (Cliffe and Grencis, 2004). The immune response to *T. muris* in mice is very well characterized and there is a distinct polarization of immune response in resistant and susceptible strains of mouse (Else and Grencis, 1991; Else *et al.*, 1992). Resistant animals produce high levels of interleukin 13 (IL-13) and associated T helper type 2 (Th2) cytokines in response to infection (Fig. 1), which are essential for parasite expulsion *via* mechanisms such as epithelial cell turnover and mucin production and muscle contraction (Khan *et al.*, 2003; Cliffe *et al.*, 2005; Hasnain *et al.*, 2010; Chen *et al.*, 2021). In contrast, a susceptible animal produces high amounts of interferon- γ (IFN- γ) and Th1 associated cytokines (Fig. 1) that leads to chronic infection, enabling the parasite to establish to maturity within the large intestine and release eggs into the environment, thereby perpetuating infection. Trickle infections can also be used to more closely mimic a natural infection of repeated low-dose exposures. Weekly trickle infections promote an initial Th1 response but this changes to a dominant Th2 response after 9 weeks (Fig. 1), which prevents any further establishment of worms (Glover *et al.*, 2019). Chronic infection, either in genetically susceptible mice or due to a low-dose infection and its associated Th1 response, are associated with dysregulation within the gut, such as crypt hyperplasia and apoptosis (Cliffe *et al.*, 2007) together with a regulatory response that is required to limit worm-driven pathology (D'Elia *et al.*, 2009; Grencis *et al.*, 2014; Duque-Correa *et al.*, 2019). Interestingly, reducing T regulatory (Treg) cells early on during a low-dose infection does have a small but significant effect on the capacity to expel parasites and subsequently intestinal pathology is reduced, suggesting that this induced Treg response is of benefit to both the host and to the parasite (Sawant *et al.*, 2014). However, this effect on parasite expulsion was lost if Tregs were depleted once infection had become established (Sawant *et al.*, 2014). A key cytokine produced by CD4⁺ T cells IL-10, is critical in host survival during *T. muris* infection (Schopf *et al.*, 2002) although whether Tregs are the major source of IL-10 during *T. muris* infection is unclear. TGF- β is another regulatory cytokine that is produced during *T. muris* infection that can dampen CD4⁺ T cell responses (Li and Flavell, 2008). As with the effects of an early reduction in Tregs, early ablation of TGF- β during a low-dose infection again caused a significant, although partial, reduction in worm numbers (Worthington *et al.*, 2013). When the ability of dendritic cells to induce TGF- β was prevented, mice were able to clear a low-dose infection efficiently although this did not seem to be dependent upon the generation of Tregs (Worthington *et al.*, 2013). Thus, it appears that the regulatory response generated by *T. muris* is complex and involves CD4⁺ T cells, Tregs, IL-10 and TGF- β contributing to the net result of a chronic infection

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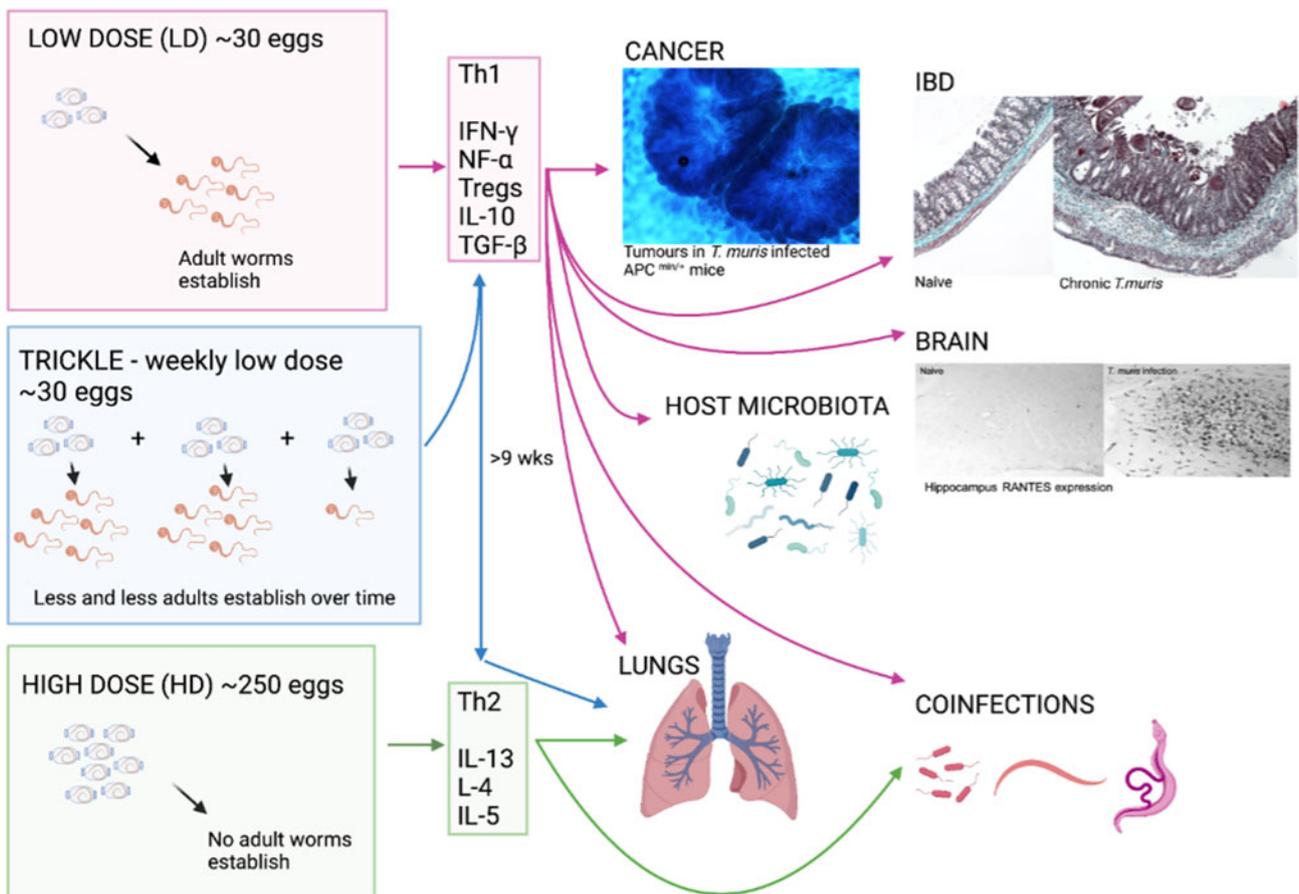


Fig. 1. The whipworm *T. muris*, though caecal dwelling, can affect many other systems in the body. The immune response to *T. muris* is dose-dependent with different cytokines being produced in response to the different doses of eggs given which can lead to chronic infection (Th1) or expulsion (Th2). Each of the immune responses to the differing doses of eggs can impact different systems in the body as depicted by the arrows. As pictured, tumours are increased in size and number in a cancer model with chronic *T. muris*, pathology is increased in chronic infection and shows similarity to IBD, and hippocampus RANTES expression is increased with chronic *T. muris* infection. Changes in microbiota, lung effects and effects on other infections are also apparent with *T. muris* infection. (Created with BioRender.com)

with controlled intestinal inflammation. This review will discuss the differing effects that either low-dose or high-dose intestinal *T. muris* infection can have on both enteral and systemic responses in the host (Fig. 1).

Intestinal inflammation

Inflammatory bowel disease (IBD) in humans represents broadly two distinct immunological conditions; Crohn's disease and ulcerative colitis. Disease onset is prompted in genetically susceptible individuals by atypical responses to microbiota or environmental cues such as diet and stress (Guan, 2019). The influence of human trichuriasis upon IBD has received little attention, with notable exceptions (Broadhurst *et al.*, 2010). This study followed pathological and immunological changes in an individual with ulcerative colitis prior to and following self-treatment with *T. trichiura*. The data supported a modulatory role for whipworm infection upon disease severity with infection associated with disease remission. Due to the intestinal niche that *Trichuris* species inhabit, an effect upon inflammatory disease of the large intestine in the host might be expected. Mechanistically this can be explored in the mouse using *T. muris* together with murine models of IBD. It is plausible that *T. muris* infection may cause IBD symptoms while the host immune response to the parasitic infection could have implications on progression of intestinal inflammation. Specifically, it is known that a low-dose infection of ~20 *T. muris* eggs will proceed to chronicity (Fig. 1), even in

normally resistant strains of mouse, leading to an IFN- γ /Th17-driven disease (Levison *et al.*, 2010) that is controlled by a concomitant IL-10 response (Grencis *et al.*, 2014). Indeed, IL-10 knock-out (KO) and IL-10R KO mice develop severe pathology in response to *T. muris* infection (Schopf *et al.*, 2002; Duque-Correa *et al.*, 2019). This low-dose infection regime can be used to mimic colitis, leading to both phenotypic and transcriptional similarities to other widely used models of IBD (Levison *et al.*, 2010; Foth *et al.*, 2014). Of 32 genes that are known to be transcriptionally different during IBD, 30 are also found to be upregulated in the CD4⁺CD45RB T cell transfer model of colitis (te Velde *et al.*, 2007). Nineteen of these 30 genes, including IFN- γ , were also found to be upregulated in chronic *T. muris* infection (Levison *et al.*, 2010). Indeed, chronic *T. muris* infection shows a degree of similarity to all mouse models of Th1-driven colitis, both phenotypically and transcriptionally, though the degree of similarity does vary from model to model (Levison *et al.*, 2010). Additionally, it has been shown that *T. muris* pathology and Crohn's disease have overlapping QTL regions – overlapping regions of DNA suggesting common genetic parameters (Levison *et al.*, 2013). To exemplify this, the role of two different cytokines have been shown to be important in both *T. muris* and colitis, IL-27 and IL-13. IL-27 is a potent stimulator of Th1 responses (Pflanz *et al.*, 2002) and is more highly expressed in patients with IBD (Nemeth *et al.*, 2017). However, IL-27 is also known to regulate Th17 responses and to stimulate IL-10 production and Treg generation (Awasthi

et al., 2007; Yoshida and Hunter, 2015). Oral delivery of IL-27 recombinant bacteria can ameliorate T cell transfer-induced colitis in mice (Hanson *et al.*, 2014) whilst a *T. muris* infection in an IL-10/IL-27 KO mouse leads to less severe pathology than seen in the IL-10 KO control due to a decreased pro-inflammatory profile (Villarino *et al.*, 2008). Additionally, WSX-1-deficient animals, that lack the functional receptor for IL-27, mount a heightened Th2 response to infection and show an accelerated expulsion of the parasite (Artis *et al.*, 2004; Bancroft *et al.*, 2004). Despite these contrasting results, known IL-27 gene polymorphisms in IBD patients (Li *et al.*, 2009; Wang *et al.*, 2014) make this cytokine an intriguing IBD therapy candidate (Andrews *et al.*, 2016). In contrast, IL-13 is a Th2/Type 2 cytokine (Minty *et al.*, 1993) that is upregulated during an acute resolving *T. muris* infection (Bancroft *et al.*, 1998). IL-13 is a potent suppressor of Th1 responses in humans (de Waal Malefyt *et al.*, 1993; Wynn, 2015), although its role in IBD is complex. Crohn's disease is principally a Th1 and IFN- γ driven condition whilst ulcerative colitis is associated with increased Th2 cytokines such as IL-5 and IL-13 (Fuss *et al.*, 1996, 2004). *T. muris* infection in IL-10/IL-13 α 2 KO mice has been used to highlight the importance of IL-13 in controlling *T. muris*-induced pathology. IL-13 α 2 is the decoy receptor for IL-13 and reduces the bio-availability of IL-13 (Mentink-Kane and Wynn, 2004). When infected with *T. muris*, IL-10/IL-13 α 2 KO mice have a decreased morbidity and mortality as compared to IL10 KO mice (Wilson *et al.*, 2011) demonstrating the protective role of IL-13. In support of this, recent studies have shown that IL-13 acts to mediate recovery and repair in the gut following dextran sulphate sodium (DSS)-induced colitis, which is Th1 driven, as disease was improved in both IL-13 α 2 KO mice and in mice treated with a neutralizing IL-13 α 2 antibody (Karmelet *et al.*, 2019). Additionally, transcripts for IL-13 α 2 have been found to be elevated in human IBD biopsies suggesting a protective role for IL-13 in these patients (Arijs *et al.*, 2009, 2010). Similarly, patients expressing a more active variant of IL-13, with a reduced affinity to the IL-13 α 2 decoy receptor, had a lower risk of developing Crohn's disease (Karmelet *et al.*, 2019).

Although *T. muris* infection can cause varied components of intestinal inflammation, the Treg response (D'Elia *et al.*, 2009; Worthington *et al.*, 2013; Sawant *et al.*, 2014; Duque-Correa *et al.*, 2019) that it also initiates has been taken as a basis for a potential approach to treat IBD. The pig whipworm *T. suis* has been used in human trials for treatment of both Crohn's disease and ulcerative colitis with resulting remission of disease in some patients in small cohort studies (Summers *et al.*, 2005a, 2005b) although no clinical improvement was seen in a larger cohort study (Schölmerich *et al.*, 2017). Although the exact mechanisms of action are unknown, excretory/secretory (E/S) products of *T. suis* on epithelial cells *in vitro* have been shown to elicit IL-6 and IL-10 secretion (Parthasarathy and Mansfield, 2005). Additionally, when *T. suis* E/S products were added to bone-marrow-derived macrophages and dendritic cells, there was a reduction in secretion of pro-inflammatory cytokines and a strong enhancement of IL-10 secretion (Leroux *et al.*, 2018). Remission of ulcerative colitis, following self-infection with *T. trichiura*, was associated with a marked elevation in IL-22 (an IL-10 family member) producing T cells which were hypothesized to promote intestinal repair by increasing goblet cell numbers and mucus production (Broadhurst *et al.*, 2010).

Barrier function in the intestine

During infection, *T. muris* is known to cause epithelial dysregulation in the large intestine (Artis *et al.*, 1999; Cliffe *et al.*, 2007), a process which is also observed in human IBD (Strober *et al.*,

2007). *T. muris* induced TNF- α and IFN- γ production drive apoptosis within the caecal crypts of the large intestine (Artis *et al.*, 1999), which is thought to be in response to IFN- γ -induced epithelial cell hyperproliferation that also occurs (Cliffe *et al.*, 2007) thus leading to a perturbation in intestinal homeostasis. Infection with *T. trichiura*, the human whipworm, may cause trichuris dysentery syndrome (Cooper *et al.*, 1990) in children, which is also associated with an increase in TNF- α production by mucosal macrophages (MacDonald *et al.*, 1994). Increased intestinal apoptosis is also known to lead to a dysregulation of barrier integrity with an associated increase in epithelial permeability in IBD patients (Schulzke *et al.*, 2006; Mankertz and Schulzke, 2007). During acute *T. muris* infection (whereby the worms are expelled before chronicity, Fig. 1), there is an accumulation of epithelial mast cells in the large intestine (Sorobetea *et al.*, 2017). Mast cells produce mast cell protease-1 (MCPt-1) (Metcalfe *et al.*, 1997) and indeed, acute *T. muris* infection is associated with an increase in MCPt-1 both systemically and locally in the large intestine, which is associated with a loss of barrier integrity leading to increased epithelial permeability (Sorobetea *et al.*, 2017). *T. muris* infection in IL-10 KO mice is known to result in marked mortality and morbidity including a loss of Paneth cells and an absence of mucus (Schopf *et al.*, 2002). Pathology in IL-10 KO and IL-10/IL-4 KO mice is also associated with bacterial outgrowth as broad-spectrum antibiotic treatment enhances survival (Schopf *et al.*, 2002). Duque-Correa *et al.* (2019) also showed that IL-10 signalling had a protective effect on loss of barrier integrity leading to bacterial translocation. It is also known that *T. suis* E/S can affect barrier integrity by reducing the expression of tight junction proteins (Hiemstra *et al.*, 2014) although whether this is also a function of *T. muris* E/S is unknown. However, Hasnain *et al.* (2012) showed that adult *T. muris* E/S was able to degrade intestinal mucins and *T. muris*-induced changes in the intestinal mucus barrier have also been demonstrated that may act to increase intestinal permeability (Hasnain *et al.*, 2010, 2011). Infection itself can lead to thickening of the glycocalyx, the glycoprotein and glycolipid covering of the intestinal epithelial cells (Linden *et al.*, 2008) likely due to the increased production of mucin proteins. However, there is also a decreased glycoprotein content within the mucosal barrier during chronic infection that may allow increased contact of the intestinal microbiota with intestinal epithelial cells (Hasnain *et al.*, 2011). Congruous to this, chronic *T. muris* infection can also alter the host intestinal microbiota (Holm *et al.*, 2015; Houlden *et al.*, 2015) and it is known that a modification in the composition and function of the gut microbiota can also change intestinal permeability (Gomaa, 2020).

Microbiota changes in the intestine

Changes in microbiota during a *T. muris* infection are evident from as early as only day 14 post-infection (p.i.). By the time that infection has reached patency (more than day 33 p.i.), there are significant changes in the composition and diversity of the microbiota (Fig. 1) (Holm *et al.*, 2015; Houlden *et al.*, 2015). There was a general shift in the microbiota to a decreased number of bacteria in the Bacteroidetes phyla and an increased number of Gram-positive Lactobacillaceae. Such changes in the microbiota appear to be of benefit to the parasite and changes were transitory and required the presence of the parasite to be maintained (White *et al.*, 2018). In contrast, changes in microbiota composition in an outbred strain of mouse with a chronic *T. muris* infection led to an increase in bacterial invasion of the host intestinal epithelium (Schachter *et al.*, 2020). Interestingly, infection-induced microbiota changes can also promote resistance to damage. In a colitis-susceptible strain of mouse (NOD2 KO), it

has been established that overgrowth of *Bacteroides vulgatus* leads to intestinal abnormalities (Ramanan *et al.*, 2014). However, acute infection with *T. muris*, that drives a Th2 response and a mucus response, led to an increase in *Clostridia* strains of bacteria that inhibited *B. vulgatus* colonization and the resulting *B. vulgatus*-driven abnormalities (Ramanan *et al.*, 2016). The microbiota of the host can also directly influence pathogenesis of *T. muris* as antibiotic treatment of chronically infected IL-10 KO animals, although experiencing similar pathology to control animals, had a significantly reduced mortality (Kopper *et al.*, 2015). Chronic infection induced changes to microflora have also been shown in *T. suis* infected pigs (Li *et al.*, 2012) although there is contrasting evidence as to whether the human whipworm also drives microflora changes (Cooper *et al.*, 2013; Ramanan *et al.*, 2016).

Trichuris effects distal to the site of infection

Despite its intestinal epithelial location, the effects of *T. muris* infection are not only restricted to the site of infection. Chronic *T. muris* infection can modulate responses to chemical skin sensitizers applied to the ear of the mouse. Suppression of local cellular/cytokine Th1/pro-inflammatory responses and ear pathology were observed when using a Th1-promoting compound [2,4-dinitrochlorobenzene (DNCB)] although no depression in IL-13, or ear swelling was noted after sensitizing with the Th2-promoting compound trimellitic anhydride (TMA). Interestingly, the suppression of pathology after DNCB treatment was associated with a reduction in egress of dendritic cells (DCs) from the skin coincident with elevated IL-10 production and a slight increase in CD4⁺FoxP3⁺ cells in the draining lymph node (Grencis *et al.*, 2014). Movement of DCs from the skin to the draining lymph node has been shown to be dependent on local proinflammatory cytokines which can be inhibited by IL-10 production (Cumberbatch *et al.*, 2000).

T. muris effects in the lungs

Chronic *T. muris* infection which drives a strong Th1 response in the intestine, has also been shown to drive the production of IFN- γ (by Th1 cells) and IL-10 (myeloid cells) in the lung of the host (Fig. 1), and so has the potential to suppress the development of Type-2-driven airway inflammation (Chenery *et al.*, 2016). The increased Th1 type response in the lung was able to reduce the lung response to both papain and house-dust mite, together with a reduced eosinophil infiltration and reduced lung mucus production. IL-17 is another cytokine known to be increased in complex asthma and may contribute to disease progression (Doe *et al.*, 2010): additionally, IL-17 is critical for neutrophil expansion and remodelling of lung tissue and may contribute to disease progression in other chronic respiratory conditions (Gurczynski and Moore, 2018). A high-dose infection of *T. muris*, that induces a Th2 response (Fig. 1), can promote a mixed IL-17 and Th2-type immunity to the parasite (Wilson *et al.*, 2011). Induction of Th2 cytokines can also be seen in the host lung following infection with a high dose of *T. muris*, however, this is dependent on IL-17 production and is ablated in an IL-17 KO animal (Ajendra *et al.*, 2020). Interestingly, this IL-17-dependent suppression of IFN- γ , which allowed the promotion of type-2 immune responses, was only apparent in the host lung and was not seen in the intestine. Additionally, a secreted product from *T. muris*, p43, is able to bind to IL-13 *in vitro* and *in vivo* (Bancroft *et al.*, 2019). When given to mice intranasally with IL-13, p43 reduced the percentage of RELM- β positive interstitial lung macrophages as compared to mice treated with IL-13 only. The effects of p43 are further reviewed in this special issue by Bancroft & Grecnis. By-stander effects of *Trichuris* infection in the lung are also seen with other species of *Trichuris*.

T. suis ova treatment in a grass-pollen allergy clinical trial increased Th2 and IL-10 production in patients although this did not affect allergen-specific cytokine responses (Bourke *et al.*, 2012). Interestingly, treatment of ovalbumin-sensitized mice with *T. suis* larval E/S proteins suppressed airway hyperreactivity and bronchiolar inflammation, partially mediated by E/S-induced IL-10 secretion (Ebner *et al.*, 2014). Whether *T. trichiura* has similar abilities to modulate inflammation is uncertain and there are conflicting results in the literature (Rodrigues *et al.*, 2008; Alcântara-Neves *et al.*, 2010; Gonçalves *et al.*, 2020).

T. muris cerebrovascular and neurodegenerative disease

It is well established that infection and systemic inflammation are risk factors for ischaemic brain damage (stroke) and can also affect the progression of some neurodegenerative disorders (He *et al.*, 2020).

Using transient middle cerebral artery occlusion as a model of stroke it was shown that a chronic low-dose *T. muris* infection, which drives a Th1 response (Fig. 1), dramatically exacerbated brain damage caused by experimental stroke (Dénes *et al.*, 2010). Infection led to an increase in pro-inflammatory mediators in the brain and surrounding tissue together with an altered Treg response. Infected mice had elevated Th1-associated cytokines and chemokines after cerebral artery occlusion however, only CCL5 (RANTES) stayed significantly increased after 48 hours post-stroke. Anti-RANTES treatment prevented the infection-driven exacerbation of stroke-induced damage. Analysis of matrix metalloproteinase 9 expression in the brain showed elevated levels after stroke and infection compared to stroke alone indicating augmented vascular injury and blood-brain barrier damage in chronically infected animals. Interestingly, an acute, resolving *T. muris* infection driving a Th2 response had no effect on infarct size demonstrating that it was the Th1 milieu driven by the parasite that was detrimental rather than the parasite itself (Dénes *et al.*, 2010). The detrimental effects of infection are also very much dependent on age as infarct size was found to be significantly increased in chronically infected aged mice as compared to chronically infected young mice (Dhungana *et al.*, 2013). Older mice experienced an increased neutrophil recruitment and upregulation of Th1 cytokines as compared to the younger mice leading to the increased pathology seen.

As well as stroke, it has also been demonstrated that chronic *T. muris* infection can accelerate the onset of experimental clinical prion disease – a chronic, neurodegenerative disease caused by infectious proteins (Donaldson *et al.*, 2020). Mice were infected with a chronic *T. muris* infection after receiving prions, timed so that the peak of parasite-driven inflammation would coincide with known pre-clinical phases of the prion infection. *T. muris* infected mice had a reduced survival time which correlated with increased pro-inflammatory cytokines in the sera and increased numbers of CD8⁺ cells in the brain (Donaldson *et al.*, 2020). *T. muris* infection can also exacerbate neuroinflammation in models of Alzheimer's disease, a chronic neurodegenerative condition (Querfurth and LaFerla, 2010; Montacute *et al.*, 2017). Infection in the Alzheimer's mouse model (3xTg-AD) led to increased levels of inflammation in the brain with increased microglia activation. Interestingly, these transgenic animals were also unable to fully expel a high-dose infection, which is normally acute and resolving (Fig. 1), together with increased Th1 cytokine levels in response to infection in the lymph node draining the large intestine (Montacute *et al.*, 2017). Although not addressed in any *T. muris* infection model, *T. suis* E/S effects in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis (MS), have been assessed (Kuijk *et al.*, 2012; Hansen *et al.*, 2017). Intraperitoneal administration of *T. suis* E/S before

disease onset significantly decreased disease severity and markedly reduced systemic Th1 and Th17 responses (Hansen *et al.*, 2017). However, *T. suis* ova therapy in MS clinical trials have had mixed effects (Voldsgaard *et al.*, 2015; Fleming *et al.*, 2019; Yordanova *et al.*, 2021).

Trichuris and coinfections

Surprisingly little work has been carried on coinfections of *T. muris* and viral or bacterial infections though some work has been done with *Mycobacteria* and *Streptococcus*. Immunity to *Mycobacterium bovis* (*M. bovis*) infection has been shown to be negatively influenced by a *T. muris* coinfection. A high-dose *T. muris* infection, which promotes a Th2 response, down-regulated pulmonary Th1 and Treg cell responses to the bacteria (Fig. 1) (Nel *et al.*, 2014) although this had no effect on bacterial proliferation and dissemination. However, *T. muris* E/S-treated human monocyte-derived macrophages prior to exposure to *M. tuberculosis* led to an M2-type polarization with reduced macrophage phagosome maturation and a resulting increased bacterial burden (Aira *et al.*, 2017). In a *T. muris*-*Streptococcus pneumoniae* coinfection model, nematode infection was associated with an increased carriage of *S. pneumoniae*, though this did not reach significance, with a significant increase in dissemination of the bacteria to the lungs (Law *et al.*, 2021). Anthelmintic treatment led to a smaller, though not significant, load of bacteria. This trend for a higher carriage of bacteria when coinfecting with *Trichuris* was similarly seen in children harbouring *T. trichiura* (Law *et al.*, 2021).

Protozoan infections such as *Plasmodium berghei*, *Trypanosoma brucei* and *Babesia microti* and *B. hylomyisci* will all delay the expulsion of a high dose of *T. muris* infection, particularly at times of high parasitaemia suggesting that at least acute *T. muris* infections do not exert strong immunomodulatory effects on these co-infections (Phillips and Wakelin, 1974; Phillips *et al.*, 1974).

More data are available on the effect of *T. muris* infection on other helminth infections. Experimental infection of *Nematospiroides dubius* [*Heligmosomoides polygyrus* (*bakerii*)], which resides in the small intestine, delayed expulsion of a high dose *T. muris* infection and enhanced survival of a trickle *T. muris* infection (Behnke *et al.*, 1984). The lung, like the gut, is a mucosal surface and many helminth parasites have evolved a migratory phase through the lungs in their life cycle (Craig and Scott, 2014). Cross-talk between the lung and intestinal mucosal surfaces in terms of host immunity is particularly evident during helminth co-infections. *Nippostrongylus brasiliensis* is a rodent small intestinal dwelling parasite that migrates through the host lung before reaching maturity (Bouchery *et al.*, 2017). Intestinal infection with a high dose of *T. muris*, that promotes a Th2 response and is expelled by the host (Fig. 1), reduced the number of *N. brasiliensis* larvae found in the lung at d2 post-infection (Filbey *et al.*, 2019). Interestingly, mice that had been given a trickle infection of *T. muris* (initially driving a Th1 response and then a protective Th2 response) and then a *N. brasiliensis* infection, after the switch to a Th2 dominated response, had an equivalent number of larvae in the lung at d3 post-infection as WT mice (Glover *et al.*, 2019). This suggests either a resolving delay in *N. brasiliensis* migration in the lung as equivalent numbers of adults were found in the intestine (Glover *et al.*, 2019) or a qualitative difference in the Th2 response initiated by a high dose as compared to a trickle infection.

T. muris-induced alteration in the lung cytokine expression has also been demonstrated in co-infection with *Schistosoma mansoni* (Bickle *et al.*, 2008). *S. mansoni* is a trematode that causes chronic infection in mice, causing pathology in the lungs as it migrates (Boros, 1989). Chronic infection with *T. muris* led to a reduced trapping of larvae during their skin-to-lung migration associated with an altered lung cytokine expression.

Interestingly, co-infected lungs had a lower expression of IFN- γ despite the *Trichuris*-driven Th1 response, and it was actually an IL-10-dominated response that appeared to limit antilarval schistosomula immunity (Bickle *et al.*, 2008) and allowed progression of the parasite to the portal system with resulting increased egg burden and pathology in co-infected mice. Conversely, a chronic *T. muris* infection can be resolved by a *Schistosoma* coinfection due to the *S. mansoni* egg-induced Th2 response (Curry *et al.*, 1995). Additionally, *S. mansoni* and *T. muris* coinfecting mice had significantly higher burden of adult *Schistosoma* worms and eggs in the liver (Bickle *et al.*, 2008) thus demonstrating that contrasting effects that the infections can have on one another.

Trichuris and neoplasia

Cancer is a leading cause of death in high-income countries and incidences are increasing in low-income countries. There exists a strong link between inflammation and cancer with chronic infection and the long-term exposure to inflammatory stimuli heightening the risk of neoplastic change (Wang and Wang, 2007).

Chronic *T. muris* infection at day 80 p.i. in a wild-type mouse led to the development of neoplastic change that was similar to that seen in mice that had been treated with the carcinogen azoxymethane (Hayes *et al.*, 2017). Intestinal crypt structure was altered alongside increased incidence of pre-adenomas which were more pronounced (in the case of aberrant crypt foci) in the infected mice as compared to the chemically treated mice. Even though *T. muris* infection can lead to increased epithelial proliferation and apoptosis in the intestine (Artis *et al.*, 1999; Cliffe *et al.*, 2007), both of which can lead to tumour formation (Evan and Vousden, 2001) these intestinal changes were only apparent in the caecum, the parasite niche, rather than throughout the small intestinal tract where neoplastic change was mostly observed (Hayes *et al.*, 2017). Neoplastic change was seen in chronically infected animals even before the peak of parasite-specific cytokine responses was evident in the draining lymph node, although greater significant differences were seen as infection progressed. Infection generated a Th1-predominant response in these animals, however, this was not associated with a reduced neoplasia as might have been expected (Wang *et al.*, 2015).

The APC^{min/+} tumour model in the mouse develops spontaneous adenomas throughout the GI tract (Moser *et al.*, 1990). Chronic infection of APC^{min/+} mice with *T. muris* led to a significant increase in new tumour formation throughout the intestine and not just an increase in tumour size. Blockade of the CD25+ Treg response abrogated this heightened tumour formation demonstrating the role of the *T. muris*-induced Tregs in regulating the anti-tumour response in these animals (Hayes *et al.*, 2017). Tregs have also been characterized within tumour microenvironments that can induce tumour-specific immune tolerance (Wang and Wang, 2007). Clonal expansion of tumour Tregs is thought to occur both locally and systemically and a high proportion of Tregs with the tumour micro-environment is correlative with poor prognosis in many cancer types suggestive of the suppressive role of Tregs on anti-tumour immunity (Mougiakakos, 2011; Fridman *et al.*, 2012; Ahmadzadeh *et al.*, 2019). Interestingly *T. suis* E/S proteins are capable of stimulating the secretion of IL-10 from macrophages though failed to induce CD25⁺Foxp3⁺ T cells unlike *T. muris* E/S which was able to do this (D'Elia *et al.*, 2009; Leroux *et al.*, 2018). Additionally, increased mucosal T cell activation production of IL-10, TGF- β and FoxP3 were found in the colon of an individual with ulcerative colitis who self-infected with *T. trichiura* (Dige *et al.*, 2017). Tregs are known to play a role in both pathology and immunity early on following chronic *T. muris* infection as are TGF- β and IL-10

(D'Elia *et al.*, 2009; Worthington *et al.*, 2013; Sawant *et al.*, 2014; Duque-Correa *et al.*, 2019). It is noteworthy however, that low-dose chronic infection with *T. muris* is associated with a depression in Foxp3⁺CD4⁺T cells in the caecum and colon (Holm *et al.*, 2015; Houlden *et al.*, 2015). Taken together these data suggest that distinct populations of CD4⁺ T cells are involved in regulating tumours at sites away from the parasite niche.

IL-10 and TGF- β are not the only regulatory cytokines associated with a *T. muris* infection and cancer. IL-35 is an immune-suppressive cytokine which belongs to the IL-12 cytokine family and can also act to regulate Th1 immunity (Collison *et al.*, 2007). Chronic *T. muris* infection can drive an inducible cell type (iT(R)35 cells) that exert regulatory effects via IL-35 and are Foxp3 independent (Collison *et al.*, 2010). In a melanoma model of cancer, these *T. muris*-induced cells can be found within the tumour micro-environment (in the skin) and contributed to tumour progression by again regulating the ongoing anti-tumour responses (Collison *et al.*, 2010). In addition, IL-31 is a Th2 T cell cytokine that can suppress type 2 immune responses (Dillon *et al.*, 2004). IL-31 and IL31R play a regulatory role in *T. muris* infection with an induced production of this cytokine in the intestine following infection (Perrigoue *et al.*, 2009). Additionally, infection of IL31R KO mice led to a heightened Th2 cytokine response and enhanced goblet cell hyperplasia with a resulting accelerated expulsion of worms. As this cytokine has also been implicated in cancer progression, it is likely that *T. muris* induced IL-31 production may also enhance tumour progression in a manner similar to IL-35 (He *et al.*, 2020).

Conclusion

T. muris is an intestinal dwelling nematode parasite that can have far-reaching consequences in the host (Fig. 1). Within the intestine itself, chronic *T. muris* in susceptible strains can have pathological consequences that show a degree of similarity to symptoms of IBD. Indeed, several genes upregulated during a chronic *T. muris* infection are also found to be upregulated in IBD patients. Paradoxically, *T. muris* infections can also help modulate IBD symptoms and pathologies due to the parasite-specific Treg response driven by infection. *T. muris* also drives microbiota changes in the host, beneficial to its survival, that have consequences for the host due to the impact that these changes can have on mucus constituents and intestinal permeability. Distal from the site of infection, *T. muris* infections can have an impact on immune responses to chemical sensitizers in the ear. In this case, a chronic *T. muris* driven IL-10 production preventing the egress of DCs from the ear. Chronic *T. muris* infection can also modulate immune responses in the lung to airway allergens which was also associated with an increased IL-10 response. *T. muris* infection can also influence immune responses in the brain and it has been demonstrated that an on-going *T. muris*-driven Th1 response will worsen the damage caused by experimental stroke, a process driven by an elevated and sustained RANTES production. Additionally, *T. muris* can have an effect on other brain inflammations with papers reporting changes in prion diseases and Alzheimer's progression. Although relatively little work has addressed the effects of *T. muris* on other parasite, viral and microbial infections, altered immunity to mycobacteria, pneumococcus, *N. brasiliensis*, *H. bakerii* and *S. mansoni* have been reported. Finally, effects of *T. muris* infection on cancer progression establish that the *T. muris*-driven Treg response plays an important role in inhibiting host immunity to adenoma progression in the intestine leading to development of more tumours. Additionally, two other regulatory cytokines, IL-35 and IL-31, induced by *T. muris* infection are able to modulate tumour immunity. In light of this, the importance of *T. muris* infections

on other diseases and other body systems is profound and warrants further research and investigation, especially considering the widespread nature of this parasite in the human population.

Author contributions. KSH conducted the literature search, designed and wrote the review and figure. RKG provided feedback and comments on the manuscript.

Financial support. The work in RKG's laboratory is funded by Wellcome Trust investigator award (Z10661/Z/18/Z). The Wellcome Trust Centre for Cell Matrix Research, University of Manchester is also supported by centre funding from the Wellcome Trust (088785/Z/09/Z).

Conflict of interest. The authors declare there are no conflicts of interest.

Ethical standards. Not applicable

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