Factors affecting disease manifestation of toxocarosis in humans: Genetics and environment

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**ABSTRACT**

Toxocara canis is regarded as the main cause of human toxocarosis but the relative contribution of T. cati is probably underestimated; serological and other diagnostic methods used in most studies of this zoonotic disease do not distinguish between the two parasites. The definitive hosts for T. canis are canidiia. Pups generally have higher infection rates than adult animals and are a major source of eggs in the environment. Humans usually acquire T. canis infection by accidental ingestion of embryonated eggs or encapsulated larvae from the environment or contaminated food, such infections may lead to visceral larva migrans (VLM), ocular larva migrans (OLM) or covert toxocarosis (CT). Although a mixed Th1- and Th2-mediated immunological response, particularly with high levels of IgE and eosinophilia is observed, the underlying mechanisms of molecular and immunopathogenesis for the development of the symptomatic syndromes of VLM, OLM, or of asymptomatic CT are largely unclear. Studies have indicated that immunological defences against various infectious diseases may be highly influenced by complex interactions of environmental and host genetic factors e.g. MHC class I and II, also known as human leucocyte antigen (HLA). Toxocara spp. infections are associated with a polarized CD4+ Th2 response and high IgE levels and eosinophilia, mediated mainly by HLA class II molecules. Associations have been made between HLA class II and pathological severity and host genetic effects on exposure to infection. Recent research suggests Foxp3+ CD4+CD25+expressing Regulatory T cells play a role in regulation of the immunopathology of granulomas in experimental toxocarial granulomatous hepatitis and in enhanced expression of TGF-β1, which is an important factor for the local survival and function of Treg observed during T. canis invasion in the mouse small intestine, liver, muscle, and brain. Since the potential susceptibility loci HLA class II molecules, are considered involved in the regulation of a Th2-dominant immunity which is highly controlled by Foxp3+ CD4+CD25+ Treg cells by stimulation through TGF-β1, which thus provides a beneficial environment to T. canis larvae but severe injuries to local organs. However, TGF-β1 variant Leu10Pro known to be involved in disease severity warrants further elucidation as this too may have a role in the severity of human toxocarosis. Exploration of TGF-β1 polymorphism, Foxp3+ CD4+CD25+ Treg cells, and MHC polymorphisms may allow insight into the contribution made by environmental and genetic factors in influencing disease syndrome type and severity in humans with toxocarosis.

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1. Life cycle of *Toxocara* spp.

The genus *Toxocara* belongs to class Nematoda, order Ascaroidea, and Superfamily Ascaridoidea and comprises 21 species. *Toxocara canis* and *T. cati* are the most commonly
involved species in human toxocarosis (Despommier, 2003). Nevertheless, the ascariids responsible for causing toxocarosis in the human host are mainly *T. canis* and probably to a lesser extent, *T. cati*. The definitive hosts of *T. canis* and *T. cati* are the dog and cat respectively, inhabiting the lumen of the small intestine. Worldwide surveys of *T. canis* occurrence showed that the prevalence ranged from 86 to 100% in pups and 1 to 45% in adult dogs (Barriga, 1988; Habluetzel et al., 2003; Sowemimo, 2007; Batchelor et al., 2008; Dai et al., 2009; Rohen, 2009; Soriano et al., 2010). The proportion of cats infected with *T. cati* appears relatively high for example 79% of stray cats in a survey conducted in Denmark, and 91% of feral farm cats in the UK were found to be infected (Fisher, 2003). Following infection of definitive hosts, larvae may penetrate through the small intestine then enter the circulation and the juvenile parasites may go on to complete the life cycle. Transplacental infection is common in dogs but in contrast, lactogenic transmission of *T. cati* is the most significant route of infection to kittens (Overgaauw, 1997). Worms develop to the adult stage in the small intestine about 4–5 weeks after the larval hatch. Fertilized female worms may release several hundred thousand non-embryonated eggs per day, contributing to high levels of environmental contamination via the faeces. Embryonation occurs in the soil within two to five weeks, depending on the temperature and humidity (Despommier, 2003). At 12–18 °C this requires 54 days for the eggs to become infective whereas at 25–30 °C the time was shortened to 14 days. However, infective eggs have been shown to survive under optimal circumstances for at least 1 year (Lloyd, 1993). The importance of *T. cati* as a cause of human toxocarosis has yet to be fully evaluated; as several studies have implicated the parasite as the causative agents of ocular larva migrans (OLM) in patients and a definitive investigation on the impact on human health is required (Fisher, 2003).

2. Human toxocarosis and clinical spectrum

*T. canis* is the main cause of human toxocarosis which constitutes a serious epidemiological issue in many countries. Humans are one of many paratenic hosts, becoming infected mainly by ingestion of parasite eggs, or to a lesser extent by the consumption of chicken or cow livers (Despommier, 2003; Morimatsu et al., 2006; Yoshikawa et al., 2008; Choi et al., 2012).

Although, many infections caused by *Toxocara* spp. are asymptomatic, larval migration into internal organs via the blood can lead to a variety of clinical syndromes e.g. visceral larva migrans (VLM) and ocular larva migrans (OLM) (Glickman, 1993; Fan et al., 2003).

The manifestation of symptoms in human toxocarosis depends on many factors, including which organs are affected and the magnitude of the infection. However, toxocarosis is usually a non-fatal disease. Varying degrees of inflammatory injuries in cases of paediatric or adult toxocarosis associated with granulomatous hepatitis, asthma, endomyocarditis, generalized lymphadenopathy, endophthalmitis, cutaneous manifestation and meningencephalitis have been uncommonly reported (Chan et al., 2001; Vidal et al., 2003; Gavignet et al., 2008; López Mde et al., 2010; Salvador et al., 2010; Stoicescu et al., 2011; Caldera et al., 2012; Damasceno and Damasceno, 2012). Additionally, there is now considerable interest in the role of *T. canis* in epilepsy, particularly partial epilepsy (Bächli et al., 2004; Nicoletti et al., 2007; Quattrocchi et al., 2012). Also, it is reported that cerebral toxocarosis in a woman from Germany progressed to cognitive defects i.e. dementia (Richartz and Buchkremer, 2002). Because of the variability of the signs and symptoms of the disease, toxocarosis was divided into two main forms in 1988: VLM and ocular toxocarosis (OT) (Taylor et al., 1988). Between 1992 and 1993, a third clinical form called covert toxocarosis was described in seropositive patients, with gastrointestinal disturbances, weakness, and lethargy (Nathwani et al., 1992; Rasmussen et al., 1993). Currently, human toxocarosis is clinically classified into four major types (Smith et al., 2009).

2.1. Visceral larva migrans (VLM)

In general, high parasitic loads or repeated infection can lead to VLM (Kayes, 1997). Typical patients are children aged between 1 and 7 years, but infection is common in adults. Potential symptoms and other clinical findings include fever, hepatomegaly, abdominal pain, vomiting, diarrhoea, coughing/wheezing, asthma, inappetence, weight loss, fatigue and headache. Occasionally in toxocaral hepatitis, multiple nodules are detected as low-density lesions on computed tomography in the liver (Stoicescu et al., 2011).

2.2. Ocular larva migrans (OLM)

OLM usually occurs in children aged between 5 and 10 years and typical syndrome presents as unilateral vision impairment accompanied by occasional strabismus. Larval invasion of the retina usually leads to granuloma formation, which occurs typically peripherally or in the posterior pole. These granulomas drag the retina and create a distortion, heterotopia, or detachment of the macula, commonly resulting in blindness; although visual acuity impairment depends on the specific area involved (Gillespie et al., 1993; Woodhall et al., 2012). OLM can also cause diffuse endophthalmitis or papillitis; secondary glaucoma can follow. Long-term infection with *Toxocara* spp. sometimes leads to a choroidal neovascular membrane formed after presenting earlier as choriorretinitis (Despommier, 2003). The risk factors identified for patients who have OT include geophagia, convulsions, prior dog ownership, and ingestion of raw meats and snails (Lee et al., 2010).

2.3. Neurotoxocarosis (NT)

The site of the CNS invasion includes the brain and spinal cord and is referred to as neurotoxocarosis (NT) which depends on multiple factors e.g. the number of ingested larvae, genetic factors of the host, and previous exposure may all contribute to complicated pathogenesis of the NT (Xinou et al., 2003). Although the frequency and localization of *Toxocara* spp. larvae in the CNS in humans is unknown, cases of CNS involvement comprising meningitis, encephalitis, myelitis, cerebral vasculitis,
seizures or optic neuritis have been uncommonly reported. NT is likely to be related to the number of larvae entering the brain or spinal cord, the severity of subsequent CNS damage and inflammation. Following these manifestations, affected patients complain about headache, fever, oversensitivity to light, weakness, dorsalgia, confusion, tiredness and visual impairment (Xinou et al., 2003; Caldera et al., 2012).

2.4. Covert or common toxocarosis (CT)

Most human infections with Toxocara spp. present far less severe systemic manifestations, if any. In general, the seropositive subjects detected in population-based surveys are asymptomatic or only have non-specific or mild symptoms. The results of two case–control studies in Ireland and France led to the description of a new clinical entity in seropositive children and adults that was named ‘covert or common toxocarosis’, respectively (Taylor et al., 1987, 1988; Magnaval et al., 1994). Covert toxocarosis was characterized clinically in children by fever, anorexia, headache, abdominal pain, nausea, vomiting, lethargy, sleep and behavioural disorders, pharyngitis, pneumonia, cough, wheezing, limb pains, cervical lymphadenitis and hepatomegaly. Common toxocarosis in adults was characterized clinically by weakness, pruritus, rash, difficult breathing, and abdominal pain (Magnaval et al., 1994).

It is likely that many people with these clinical forms of Toxocara spp. infection often go undiagnosed because these clinical manifestations are non-specific.

3. Diagnostics in clinical practice

Given multiple sections and considerable expertise, the examination of a biopsy of the affected tissue can provide a definitive diagnosis but such biopsies are rarely available. At the moment, in epidemiology, supportive and confirmative diagnosis of human toxocarosis has to be assessed largely by immunodiagnostic or molecular techniques. An ELISA based on T. canis larval excretory–secretory (TES) antigens, for example, offers a sensitive and specific method for the detection of T. canis infections in humans (Fan et al., 2004a; Jones et al., 2008; Smith et al., 2009) but the usefulness of such assays is reduced by the problem of antigenic cross-reactivity, especially in underdeveloped countries where polyparasitism is common (Smith et al., 2009). Western blotting based on the fractionated, native, excretory–secretory antigens of T. canis larvae (TES-WB) can give better specificity, with reactivity to bands of low molecular weight (24–32 kDa) proven to be specific for T. canis infection (Smith et al., 2009). However, western blotting is more expensive and labour-intensive than ELISA; thus alternatively, detection of reactive IgG by using recombinant T. canis antigens (Yamasaki et al., 2000; Wickramasinghe et al., 2008; Mohamad et al., 2009) as well as IgE antibodies in ELISA may conveniently obtain convincing results (Norhaida et al., 2008). Moreover, detection of IgG subclasses seems also valid in immunodiagnosis of Toxocara spp. infection and among the four human IgG subclasses, specific IgG2 antibodies to glycan of TES antigens yield the highest sensitivity but suffer from reduced specificity in ELISA (Watthanakulpanich et al., 2008), while the finding of reactive IgG4 antibodies probably against protein contributes to increased specificity (Noordin et al., 2005). However, it is also possible to detect Toxocara spp. DNA from tissues in suspected clinical toxocarosis by PCR (Caldera et al., 2012).

4. Global seroprevalence

Globally epidemiological studies indicate that the seroprevalence of T. canis infection in people living in developed countries was in general relatively low, e.g. 13.9% in USA, 2.4% in Denmark, 1.6% in Japan, 0.7% in New Zealand and 7.5% in Australia; in contrast, higher seroprevalences are found in developing countries e.g. 93% in La Reunion, 81% in Nepal, 63.2% in Indonesia, 58% in Malaysia, 44.6% in Swaziland, 36% in Brazil and 30% in Nigeria (Smith and Noordin, 2006; Liao et al., 2010). Recent seroprevalence studies of T. canis infection among schoolchildren in African Swaziland and Sao Tomes and Principe conducted in 2010 appear very high, reaching 86.4% (n = 360) and 98.8% (n = 255), respectively as assessed by TES-WB (Fan et al., unpublished data). However, a valid comparison of seroprevalence data between countries is hampered by the variation in detection methods (ELISA or western blots) and cut-off titres and by the general difficulty in exploring the relationships between titres, infection and clinical disease (Alderete et al., 2003; Smith et al., 2009). Altogether, seroepidemiological studies of Toxocara spp. infection indicate that T. canis larvae impose a severe impact on worldwide human health and cannot be ignored.

5. Immunopathogenesis of toxocarosis

In the paratenic hosts like mice and humans, T. canis L3 larvae neither moult, grow nor replicate (Smith et al., 2009). Although studies concerning humoral immune response e.g. IgG subclass response and cellular immune response e.g. cytokine expression from clinical toxocarosis patients are limited, Obwaller et al. (1998) reported that the predominant IgG subclass in toxocarosis patients with VLM, OLM or asymptomatic individuals was IgG1 followed by IgG2, IgG4 and IgG3 by using a TES ELISA. Subclasses IgG1, 2 and 4 showed significant differences between the sera of VLM patients and asymptomatics, but not between OLM patients and asymptomatics, while Malla et al. (2002) indicated that significantly higher IL-5 expression was found in human toxocarosis as compared to normal healthy subjects in India. Since IgG4 reflects prolonged antigenic stimulation (Obwaller et al., 1998), it seems an ideal diagnostic marker to monitor whether any viable larvae reside in the body with toxocarosis before or after treatment. The pathogenesis of toxocarosis in mice resembles that in humans, mice are easy to handle and experimentally infect with easily controlled inoculation of eggs to experimental animals, hence mice are a good model for human infection allowing the study of the immune response and associated immunopathological injuries in the murine hosts. This approach has been exploited by many laboratories (Smith and Noordin, 2006).
The immune response may include both humoral and tissue factors. Although mouse isotypes (IgG1, IgG2a, IgG2b and IgG3) are different from human (IgG1, IgG2, IgG3 and IgG4); in a study in mice using a TES ELISA, the time and in inoculum-dependent increases in serum IgG1 were found to be greater than for IgG2a (Pinelli et al., 2007). Fan et al. (2003) found that although IgG1 antibody is the primary responder it did not seem to have a larvicidal effect with no correlation being observed between the level of IgG1 and the decrease in mean total larval recovery in mice; on the contrary, the murine IgG1 antibodies may cause pathogenesis related to immediate-type hypersensitivity thus leading to tissue damage instead of killing the T. canis larvae in the tissues. Murine model studies also indicated that granulomatous reactions may occur after frequent exposure. Pathological studies indicated that granulomatous inflammation characterized by aggregates of eosinophils, neutrophils, and some monocytes, and the larvae are partially surrounded by an abundant collagen capsule. The larvae are often gradually encapsulated by mature granulomas with the central portion formed by mononuclear cells or leucocytes (Fan et al., 2003; Lin et al., 2008; Wu et al., 2008). Substantial studies have indicated that TES is able to trigger the inflammatory response, so that is the reason why the larvae are not found in many granulomas and, even if found, they are intact and presumably viable (Kayes, 1997; Fan et al., 2003; Lin et al., 2008; Wu et al., 2008). Moreover, the pathological consequences mainly depend on the death of the larvae of T. canis; although viable larvae may cause ocular pathology found in OT. Larval death triggers the beginning of early and delayed hypersensitivity responses (Despommier, 2003). The formation of granulomas seems complicated and the event usually constitutes a mixed Th1- and Th2-mediated immunological response resulting in a manifestation of delayed hypersensitivity (Th1) coincided with IgE and eosinophilia which are typical of Th2-mediated responses (Kayes, 1997).

The reason for the development of the symptomatic syndrome of VLM is not completely clear but the incriminating factors are believed to be related to the types of immune response involved. The major host responses to the larval antigens include marked eosinophilia and hypergammaglobulinemia e.g. IgE and IgG antibodies and eosinophils are manifestations of Th2 type cells and of the cytokines e.g., IL-4, IL-5, and IL-13 secreted by these cells. Also, there is reason to believe that the antigens released from the T. canis larvae prompt the induction of this population of cells. There is much evidence that the persistent release of parasite antigens may continuously stimulate host immune system with concomitant production of eosinophils and IgE antibodies and thus causing systemic complications. The primary role of eosinophils is to eliminate antibody-bound parasites through the release of cytotoxic granule proteins (Giembycz and LSD, 1999). IL-5 is a major regulator of eosinophil accumulation in inflamed tissues and can modulate eosinophil behaviour at every stage from maturation to survival. IL-4 induces B-cell class switching to IgE and IgG4, and up-regulates major histocompatibility complex (MHC) class II production; in contrast, IL-4 decreases the production of Th1 cells, macrophages, IFN-γ, and IL-12 expression of dendritic cells (Hosoyama et al., 2011). Tissue macrophages play an important role in chronic inflammation and wound repair. The presence of IL-4 in extravascular tissues promotes macrophages alternatively activated in vitro (M2 cells) and inhibits classical activation of myeloid precursor cell line into M1 cells which differentiate in response to cytokines and expresses many characteristics of tissue macrophages (Jelachich et al., 1999). An increase in repair macrophages (M2) is coupled with secretion of IL-10 and transforming growth factor beta (TGF-β) that result in a diminished pathological inflammation thus leading to further development of wound repair and/or fibrosis (Luo et al., 2012). However, both eosinophilia and hyperglobulinaemia, are apparently ineffective at ridding the body of infective larvae.

Substantial evidence has indicated that TGF-β1 played an important role in maintenance of immunological balance in various parasitic infections e.g., mice expressing a T-cell-specific dominant-negative TGF-β receptor II show dampened Th2 immunity and diminished resistance to Heligmosomoides polygyrus infection (Reynolds and Maizels, 2012), as well as the production of anti-inflammatory cytokines derived from Th2 cells stimulation by TGF-β1 are necessary to down-regulate proinflammatory cytokines in murine schistosomiasis (Brunet et al., 1998), suggesting that TGF-β1 might be important in maintaining the balance between protection and pathology in parasitic infections (Omer et al., 2000).

There is much interest in the role of TGF-β1 in immunopathogenesis of toxocarosis. Recent studies have indicated that during T. canis larval penetration through the small intestine or invasion of the liver or brain, enhanced TGF-β1 expressions were observed mainly in infiltrated leucocytes surrounding the granulomatous inflammatory sites in the small intestine (Fan et al., 2004b), musculature (Fan, unpublished data), and liver (Wu et al., 2008) or astrocytes in the injured brain (Liao et al., 2008). Nevertheless, until now, the underlying molecular and immunological mechanisms associated with TGF-β1/Th2 responses that may lead to protection against or resistance to Toxocara spp. larval invasion as well as the associated manifestation caused by immunopathological changes are largely unclear and require further elucidation.

Substantial evidence has indicated that exposure to a pathogen is regulated by ecological and behavioural factors through genetic control (Quinnell, 2003; Dold and Holland, 2011). Since the ability of hosts to regulate parasites and thus control resistance or susceptibility at an individual and population level, through either innate or acquired immune responses, is a very important determinant of host variation in susceptibility to helminth infection which are ultimately determined by host genetics (Quinnell, 2003); therefore, recent emphasis has been placed on investigating various immunological parameters and genetic factors as possible contributors to the predisposition to helminth infections (Dold and Holland, 2011); as whether this issue is also the same to Toxocara spp. infection warrants further exploration. Immunologically, helminth infections are associated with polarized CD4+ Th2 responses and with high IgE levels. Moreover, substantial studies have indicated that the immune defence against various infectious
of genetic factors, whether this issue is also the same to *Toxocara* spp. infection warrants further exploration.

However, some studies investigating behavioural-mediated acquisition as well as disease severity of *Toxocara* spp. infection indicated that they may include e.g. number of larvae ingested, frequency of infective eggs intake, host immune response to invading larvae as well as larval secreted and/or excreted products, and inflammatory reactions elicited by the larvae in the tissues (Glickman and Schantz, 1981; Carvalho and Rocha, 2011). In a murine model study, Epe et al. (1994) examined the behaviour and pathogenicity of *T. canis* larvae in different mouse strains with different MHC haplotype including inbred strains BALB/c (H-2d), C3H/He (H-2b), C57BL/6j (H-2b), and DAB/2j (H-2d) and the outbred strain NMRl. The highest larval counts in the brain of all strains were found in BALB/c mice. The percentage of eosinophils in the blood of BALB/c mice increased after the 8th week p.i., whereas it decreased in the other strains, with histological and pathophysiological changes of a lesser extent in BALB/c strain than in the others. In mice of the strains C3H/He and C57BL/6j, deaths occurred from the 4th week p.i. onward, and the number of larvae found in their brains was lower than those observed in BALB/c. Koizumi and Hayakawa (1984) showed that higher number of larvae recovered from the liver of BALB/c than that in C57BL/6j and C3H/He mice infected by *T. canis*. These findings did not seem to be explained sufficiently by MHC related immunity, although comparison is difficult unless MHC-congenic strains are compared. There is no evidence that mechanical damage caused by migrating larvae in the brain tissue is mainly responsible for symptoms of central nervous toxocarosis. Thus, the assumption that the MHC is involved in the allergic-inflammatory response in the brain could not be proven because infected mice of the BALB/c and DAB/2j strains reacted completely differently, although both are equipped with the same MHC haplotype. As whether MHC polymorphism is possibly involved in determination of susceptible or resistant to *T. canis* infection and associated manifestation severity in human toxocarosis remains largely unknown.

### 6. Environmental factors contributing to manifestation severity of human toxocarosis

The effects of host genetics are usually considered to be on resistance to infection, through effects on innate or acquired immunity. Thus, there may be genetic control of parasite survival, growth or fecundity. Accordingly, susceptibility/resistance to parasitic infections should be highly related to host polygenic factors instead of monogenic factors. However, there could also be genetic effects on exposure to infection through, for example, environmental and/or behavioural genes affecting hygiene behaviour or geophagy. This hypothesis is supported by the observation that at least a proportion of the 3–14% variance of worm burden is explained by environmental effects (Quinnell, 2003). However, variation in exposure between hosts is likely to be large, reflecting the aggregated distributions of infective stages in the environment e.g. hookworm larvae or *Ascaris* eggs in soil and contacts with the environment.
such as geophagy and schistosome infective water contact (Quinnell, 2003).

The physical environment plays a crucial role in maintaining and distributing the infective *T. canis* eggs provided that there are suitable conditions. Infective *T. canis* eggs can last for months to years outside the host under optimal conditions because of a resistant outer acellular shell which, in the laboratory, has been shown to protect against various harsh chemicals e.g. formalin, various inorganic acids, extreme temperature changes and various degrees of moisture. Practical assessment of frequency of exposure to environmental embryonated eggs or encapsulated larvae for individual toxocarosis patients is difficult, including correlating exposure to soils contaminated by *T. canis* infective eggs geophagic behaviour, or potential risk factors for exposure such as socioeconomic status, education or access to sanitation, but may be useful in future genetic studies. Analysis of the clinical toxocarosis cases from PubMedline from 1990 to 2012, show a total of 290 papers with 368 reported cases. Interestingly, Europe has the most cases, 169, followed by Asia with 104 cases and then North America with 62. In Latin America 27 cases were reported; in Australia, 5 cases. Only one case is reported from Tunisia (Fig. 1). By country distribution, France, Japan, USA, and Brazil reported the highest number of cases (49 (29%), 31 (30%), 61 (98%), and 20 (74%)) respectively. It is noteworthy that most of the reported cases are of OLM with symptoms (Fig. 2).

In clinical toxocarosis cases, the pathogenic mechanisms of VLM and OLM are different. Smaller amounts of *Toxocara* spp. larvae infections seem to favour the development of OLM than VLM, reflecting why antibody titres of *Toxocara* spp. are generally lower in cases of OLM than in cases of VLM. Biopsied materials taken from clinical cases and experimentally infected animals indicated that various tissue invasion by larvae may elicit a Th2-dominant granulomatous inflammation which may lead to frequent encapsulation of the *T. canis* larva in the host tissue, alternatively it can be considered a reaction that promotes long stay and prolonged infectivity of the larvae in hosts (Glickman and Schantz, 1981; Fan et al., 2003). According to the report from Pena et al. (2011) there is a high genetic similarity between Brazilians and Europeans, which may explain why disease development is similar in the Brazilian and French populations acquiring *Toxocara* spp. infection. Despite different genetic backgrounds, living conditions and environmental exposure factors e.g. soil in parks contaminated with embryonated eggs, OLM predominates in human toxocarosis cases in Japan, America and France. It has been reported that if people accidentally ingest small numbers of *T. canis* ova through constant exposure to contaminated environments, this may lead to OLM. *Toxocara* spp. eggs containing urban public parks is very common in most countries, regardless of whether the country is developed or developing (Jarosz et al., 2010; Manini et al., 2012). Since human seropositivity can be observed in areas where the soil is contaminated by eggs of *Toxocara* spp., the risk increases according to the degree of environmental contamination (Won et al., 2008); however, risk factors may differ among regions (Andrade et al., 2001). The growing number of pet animals, mainly in large urban centres, has led to closer contact of these animals with humans, increasing the degree of exposure (Gennari et al., 1999). Nevertheless, the exploration of different MHC polymorphism between those patients from these countries assessed by using single-nucleotide polymorphisms (SNPs) should be anticipated in the future to examine the discrepancy in disease development and severity between different ethnic groups with *Toxocara* spp. infection.

7. TGF-β1 may play a pivotal role in manifestation severity in toxocarosis by regulation of CD4+ T-cell subsets through modulation of Foxp3 expression: promising prospects?

The larvae of *Toxocara* spp. in the tissues induce granulomatous inflammation whose eosinophilic component is prominent. Most of the pathology in this infection results from tissue damage by the inflammatory responses to the presence of larvae and the activity of TcnES
antigens produced by the larvae (Kayes, 1997; Magnaval et al., 2001). T. canis infection elicits both Th1 and Th2 responses with predominance of the second. Granuloma formation is considered a manifestation of Th1-mediated delayed-hypersensitivity, whereas high levels of IgE and eosinophilia are predominantly mediated by Th2 responses (Kayes, 1997; Kuroda et al., 2001).

T regulatory (Treg) cells have been recently described as an essential component of the immune system for the maintenance of T-cell homeostasis. These cells have been characterized by constitutive expression of CD4+ and CD25+ (IL-2 receptor a chain) on the cell surface and by the presence of Forkhead box p3 (Foxp3) (Sakaguchi, 2005). Treg cells are anergic and do not produce IL-2. Instead, on stimulation, they suppress the proliferation and cytokine production of conventional CD4+ T-cells, as well as that of CD8+ T-cells and established Th1 and Th2 cells (Piccirillo and Shevach, 2004; Chatila, 2005). Moreover, these cells play an important role in chronic infections, particularly parasitic diseases (Belkaid et al., 2002). The CD4+CD25+ Treg subset, which spontaneously arises in the thymus, can also be peripherally induced by antigen (Zhang et al., 2001). Foxp3-deficient (−/−) mice and patients were found to be suffering from immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, where mutations or deletions in the foxp3 gene halts the development of Treg cells and consequentially, leads to a fatal autoimmune and inflammatory disease (Bennett et al., 2001; Fontenot et al., 2003).

Foxp3-expressing cells have recently been recognized as a cornerstone for the homeostasis of the immune system, and as key cells in many infectious diseases. Moreover, they have been found to contribute to the regulation of parasite-induced immunopathology in many parasitic infections (Maizels and Smith, 2011). Chronic infection with S. mansonii, which creates extensive damage of liver, illustrates such control. Only tight control of the egg-induced immune response allows survival of the infected host (Hoffmann et al., 2000). However, it was shown that immunosuppressive CD4+CD25+ Treg isolated from hepatic granulomas and from lymphoid tissues are a main producer of IL-10 in schistosome–infected mice (McKee and Pearce, 2004).

On the other hand, when natural Treg successfully preserves host homeostasis by controlling excessive immune responses, one consequence of such control is enhanced pathogen survival and, in some cases, long-term pathogen persistence. Parasite persistence due to immune suppression by natural Treg also provides a major benefit to the host by maintaining life-long immunity to reinfection. However, in some cases, regulatory control is excessive; this allows the parasite to expand in an uncontrolled manner and thus fails to secure host survival e.g. depletion of natural Treg protected mice from death caused by the lethal strain of Plasmodium yoelli by restoring a vigorous effector immune response, which eradicated the parasites (Hisaeda et al., 2004). Because Treg offer an opportunity for parasites to generate favourable conditions for persistence, it is conceivable that their induction and survival can also be manipulated by parasites. The anti-inflammatory cytokine TGF-β1, which is produced at high levels during chronic parasitic infections, is also an important factor for the local survival and function of Treg (Green et al., 2003). It has been confirmed that TGF-β1 is able to promote nTreg cell expansion as well as generation of Foxp3* inducible Treg cells from CD4+CD25− T-cells as manipulation through IL-10 expression (Pyzik and Piccirillo, 2007).

Interestingly, Othman et al. (2011) recently found a progressive increase in Foxp3-expressing cell counts in the liver starting 5 weeks p.i., these cells were detected...
within and around Toxocara spp.-induced granulomas as well as in isolated inflammatory foci in the portal tracts or within the hepatic parenchyma. Likewise, expression of Foxp3 mRNA in the spleen significantly increased at 5 and 16 weeks p.i. Furthermore, immunization of mice with TES antigen prior to experimental infection caused earlier mobilization and recruitment of Foxp3+ cells to the liver and enhanced splenic expression of Foxp3 transcripts. These results suggest a potential role of Foxp3-expressing regulatory cells in the evolution of the immunopathological events during infection by T. canis. This may be considered alongside the findings of Wu et al. (2008) who demonstrated enhanced expression of TGF-β1 in inflammatory cells as early as 4 weeks p.i. in experimental granulomatous hepatitis caused by T. canis; thereby providing indirect evidence for the presence of Treg, although TGF-β1 is also produced by many other cell types. Further studies may also indirectly support TGF-β1 playing an important role in the regulation of Treg as evidenced by the fact that during T. canis larval penetration through the small intestine or when invading the liver or brain, enhanced TGF-β1 expressions were observed mainly in infiltrated leucocytes surrounding the granulomatous inflammatory sites in the small intestine (Fig. 3), liver (Fig. 4), and musculature (Fig. 5) or by astrocytes in the injured brain. Interestingly, partial inhibition of TGF-β1 expression may lessen the pulmonary injury caused by experimental toxocarial pneumonia by reducing some chemokine expression e.g. eotaxin (Fan et al., unpublished data) as well as injury associated biomarkers expressions e.g. GFAP in the experimental neurotoxocoris (Fan et al., unpublished data).

Recent evidence indicates that there is a substantial genetic component to the control of TGF-β1 concentration in blood circulation (Grainger et al., 1999). Several studies have shown associations between certain SNPs, such as +869T-C and Leu10Pro, and increased serum concentration of TGF-β1 in human beings (Grainger et al., 1999). It has been suggested that the Leu10Pro polymorphism may account for some of the reported associations of TGF-β1 with these diseases e.g. cancer (Colletta et al., 1991), systemic sclerosis, pulmonary fibrosis, osteoporosis, and atherogenesis (Grainger et al., 1995). This is based on several studies (Grainger et al., 1999; Dunning et al., 2003) that have shown that the Pro10Pro genotype produces more serum TGF-β1 while the Leu10Leu genotype produces less. Recently, an association was found between the TGF-β1 Leu10Pro variant and lack of Mf in the blood. The latent (cir-culating filarial antigen (CFA)+; microfilaria (Mf)−) group had a higher frequency of the Leu10Leu genotype, which produces less TGF-β1, than Mf+ patients (Debrah et al., 2011). Whether this is also true in toxocarosis patients with Pro10Pro genotype who have apparent symptoms due to severe pathological injury, while patients with Leu10Leu genotype do not develop into severe pathological syndromes warrants further elucidation in the future.
8. Conclusion

Epidemiological study has indicated that if children under 5 years old acquire *T. canis* infection, they will usually develop covert toxocariasis or VLM if they develop a symptomatic syndrome, while infected adolescents or adults with overt symptoms tend to develop OLM, although both children and adults can develop covert toxocariasis or one of the overt syndromes. Immunological studies indicated that a mixed type of Th1 (minor) and Th2 (predominant) mediated immunopathological changes emerged in the local organs invaded by *T. canis* larvae although it depends how many larvae are ingested, the frequency of intake of infective eggs, the host immune response to invading larvae as well as larval secretory and/or excretory products and inflammatory reactions elicited by the larvae in the tissues. Genetically, *Toxocara* spp. infections are associated with polarized CD4+ Th2 responses with high IgE levels and eosinophilia. HLA class II molecules, potential susceptibility loci, are considered involved in the disease development and severity of experimental toxocariasis. The regulation of such Th2-dominant immunity is highly controlled by Foxp3+ CD4+CD25+ Treg cells as stimulated through TGF-β1 thus providing a beneficial environment to *T. canis* larvae but severe injuries to local organs. However, whether TGF-β1 variant Leu10Pro is involved in the disease severity of human toxocariasis warrants further elucidation. Therefore, exploration of the expressions of Th1-, Th2-mediated cytokines, TGF-β1 polymorphism, Foxp3+ CD4+CD25+ Treg cells, and MHC polymorphisms from clinical toxocariasis patients may allow insight into complicated interactions between environmental and genetic factors contribution to disease type and the severity of human toxocariasis in the future.

Conflict of interest statement

None.

References


