

Toxocara infection and its Association with Allergic Manifestations

Elena Pinelli* and Carmen Aranzamendi

Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, Centre for Infectious Disease Control Netherlands. National Institute for Public Health and the Environment (RIVM), The Netherlands

Abstract: *Toxocara canis* and *Toxocara cati* are roundworms of dogs and cats that can also infect humans worldwide. Although these parasites do not reach the adult stage in the human host the larvae migrate to different organs and can persist for many years. Migration of larvae through the lungs may result in respiratory distress such as wheezing, coughs, mucous production and hyper-reactivity of the airways. Epidemiological and experimental studies suggest that infection with this helminth contributes to the development of allergic manifestations, including asthma. These findings are however conflicting since in others studies no association between these two immunopathologies has been found. This article reviews information on *Toxocara* spp. and findings from epidemiological and experimental studies on the association between *Toxocara* infection and allergic manifestations. In addition, the immunological mechanisms and the factors involved in the helminth allergy-association are discussed.

Keywords: Allergy, asthma, helminths, immune responses, toxocara.

INTRODUCTION

Human toxocariasis is a zoonotic infection caused by *Toxocara canis* and *T. cati*, the roundworms of dogs and cats respectively. These helminths have a cosmopolitan distribution and seroprevalence studies indicate that this is one of the most common helminthic infections in humans worldwide [1]. Evidence from epidemiological studies [2-4] and experimental models [5] suggests that infection with *Toxocara* worms contributes to the development of allergic diseases, including asthma which is prevalent worldwide [6]. A common immunological feature in allergic asthma and toxocariasis is the induction of a Th2 type of immune response characterized by the production of high levels of IgE and eosinophilia. Infection with *Toxocara* spp. shares in addition common clinical features with allergic asthma such as wheezing, coughs, mucus hyper-secretion and bronchial hyper-reactivity. Although few epidemiological studies have suggested no association between *Toxocara* infections and asthma [7-9] no studies so far have reported on an inverse association. The hygiene hypothesis proposes that infections with different pathogens including helminths, confer protection against allergies [10, 11]. *Toxocara* infections however, do not appear to protect against allergy but on the contrary, it may contribute to the development of this immunopathology. In this review the different epidemiological studies and findings from experimental models in addition to the immunological mechanisms and factors involved in this association are discussed.

TOXOCARA: TRANSMISSION, EPIDEMIOLOGY, CLINICAL DISEASE AND DIAGNOSIS

The Parasite

Toxocara canis and *Toxocara cati* are roundworms of dogs and cats respectively that can also infect humans

worldwide. These worms occupy the lumen of the small intestine of these animals. Female worms can produce more than 200,000 eggs per day which are passed together with the faeces of the infected animals into the environment. Playgrounds, backyards and sand-boxes are common places where dogs and cats defecate and where *Toxocara* eggs are present. The eggs embryonate within 2 to 6 weeks and ingestion of these eggs containing an infectious larva (Fig. 1) will result in infection [12]. After ingestion by the definitive hosts, the eggs hatch and the freed larvae penetrate the small intestine and enter the general circulation and migrate to different organs. The larvae migrate first to the liver where they moult to the third stage, re-enter the general circulation and are carried to the lungs. In the lungs the larvae penetrate the alveolar space, crawl up the bronchioles into the trachea, bypass the epiglottis and are swallowed. In the small intestine, larvae moult for a fourth time, transforming into adult worms. Ingestion of infectious *Toxocara* eggs by paratenic hosts such as mice, results in somatic migration of the larvae remaining thereafter in the tissues. After predation of paratenic hosts by dogs and cats the larvae are released and develop into adult worms in the intestinal tract of these animals [12-14]. Humans are accidental host for *Toxocara* spp, meaning that although infection can be established, these parasites do not reach the adult stage in the human host. Infection is initiated, as in the dogs or cats, by the ingestion of infectious eggs. Larvae hatch in the small intestine, migrate to the liver and lungs but they do not reach the intestine and therefore do not mature to the adult stage. Instead the larvae migrate throughout the body, invading different organs such as the liver, lungs, eyes and brain. Although after infection most of the larvae eventually die, some of them can survive for several months, up to years. In experimentally infected rhesus monkeys *Toxocara* larvae have been reported to remain viable in tissues for at least 9 years [15].

Transmission

Transmission to humans occurs by ingestion of *Toxocara* infectious eggs present in soil, either directly by geophagia or indirectly by consumption of unwashed contaminated

*Address correspondence to this author at the Diagnostic Laboratory for Infectious Diseases and Perinatal Screening, Centre for Infectious Disease Control Netherlands, National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands; Tel: +31302744277; Fax: +312742971; E-mail: elena.pinelli.ortiz@rivm.nl

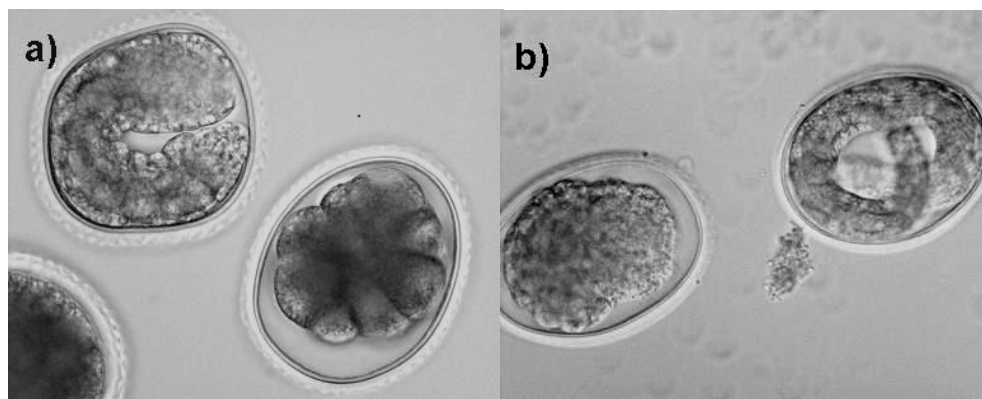


Fig. (1). *Toxocara canis* embryonated eggs (a) or containing infectious larva (b) (x 400).

fresh vegetables. Ingestion of *Toxocara* eggs present in the fur of dogs particularly the puppies of stray dogs, have been suggested as a potential transmission route for this parasite [16, 17]. The number of infectious eggs present in the fur of the studied animals were however, either low or absent [18].

Human infections with tissue larvae have also been described and can take place by consumption of raw or undercooked meat from potential paratenic hosts such as chicken, lambs, rabbits and cattle [15, 19-21].

Epidemiology and Risk Factors for Infection

Toxocara worms have a worldwide distribution and according to seroprevalence studies human toxocariasis is one of the most common zoonotic infections. The recorded seroprevalence however, varies among countries or even within countries. Seroprevalence can vary between 2,4% in Denmark to 92,8% in la Réunion, a French island located in the Indian Ocean [22, 23]. The exposure to *Toxocara spp.* in the Netherlands based on serological surveys have been reported to be 19 % on average, with 4 % to 15% in people younger than 30 years and 30 % for the age-group older than 45 years, (reviewed in [13]). In another study carried out with Dutch schoolchildren aged 4-6 years the *Toxocara* seroprevalence was found to be 6 % in the city of Rotterdam and 11 % in the city of The Hague [24]. The seroprevalence in children in the state of Connecticut, USA, varied from 6.1% in New Haven to 27.9 % in Bridgeport, indicating a high rate of exposure to *Toxocara spp.* in children living in urban areas. In this study, the only risk factors for *Toxocara* infection found were race and income [9]. Children are most at risk to be infected, especially when there is a history of pica (deliberate ingestion of non-food material, such as soil). Other risk factors include education, gender, socio-economic status, playing in sandpits and having dogs as pets (reviewed in [1]).

Toxocara infections in the definitive hosts are not only prevalent in dogs and cats but also in wild carnivores like foxes and wolves. The prevalence of *Toxocara spp.* in Poland has been reported to be 39% in cats, 32% in dogs and 16% in foxes [25] and in Spain 6,4% has been reported in wolves [26]. In the Netherlands prevalence ranges in household animals from 4,7% in cats and 2,9% in dogs to 21% in stray cats [13]. In Ciudad Bolívar (Bolívar State,

Venezuela) a city with a large dog population, 16.7 % of the stool samples and 55% of the soil samples taken from public squares and parks of the city were positive for *Toxocara* eggs [27]. Children's sandpits can be contaminated by *T. canis* and *T. cati*, depending on their maintenance [28]. Contamination of soil with *Toxocara* eggs vary from 17,4% and 60,3% in Brazil, 14,4% to 20,6% in de United States, 13,0% tot 87,1% in Europe, 30,3% to 54,5% in Africa and 6,6% tot 63,3% in Asia (reviewed in [1]).

Clinical Disease and Diagnosis

Toxocara infections are usually asymptomatic however high parasite loads can result in clinical disease. Three clinical syndromes of human toxocariasis have been described: visceral larva migrans (VLM); ocular larva migrans (OLM) and covert toxocariasis (CT) (reviewed in [29]). VLM a systemic disease caused by migration of the larvae through different organs is associated with non-specific clinical symptoms such as fever, malaise, weight loss, skin rash, respiratory complaints and hepatomegaly. Laboratory findings include eosinophilia, leucocytosis and hyperglobulinemia. Complications include myocarditis, nephritis and involvement of the central nervous system [30]. OLM occurs when the larvae migrate to the eye and it manifests mainly in older children. OLM usually appears as an unilateral vision disorder often accompanied by strabismus [31]. Invasion of the retina leading to granuloma formation is the most serious consequence of the infection and occurs peripherally or in the posterior pole [32]. CT is a less severe syndrome found in patients with clinical symptoms that are non-specific and do not match the VLM or OLM. Symptoms include cough, sleep disturbances, abdominal pain, headache and behavioural changes [33].

Diagnosis of toxocariasis is based mainly on serology since biopsies are rarely positive. The most common serological assays used for detection of antibodies against these parasites are enzyme-linked immunoabsorbent assay (ELISA) and Western blot (WB) (reviewed in [29]). The use of ELISA based on excretory-secretory products released by the second stage larvae (TES) for detection of IgG antibodies has been shown to have sufficient specificity (ranging from 91% - 93 %) and sensitivity (ranging from 78%- 91%) [34, 35]. The use of WB and the TES antigen overcomes issues with nematode cross-reactions because the low-molecular-

weight bands (24-32 kDa) are specific for *Toxocara* infection [36]. Serological analysis of paired serum samples is also recommended for the best serodiagnostic results [29]. OLM is diagnosed on clinical criteria during an ophthalmologic examination. Serological tests for antibodies are not as reliable for OLM as they are for VLM. A positive titre may be a diagnostic aid, but a negative titre may not exclude the diagnosis. IgG-ELISA and WB with specific anti-*Toxocara* IgE detection have been reported to be an accurate procedure for the immunodiagnosis of OLM, when performed simultaneously on serum and ocular fluid [36, 37]. Combining information obtained from the clinical, laboratory and serological evaluation is fundamental in order to make a complete diagnosis.

ALLERGY AND IMMUNOLOGICAL MECHANISMS

Allergy is a hypersensitive reaction initiated by immunological mechanisms in response to exposure to innocuous antigens (allergens). In the majority of cases, IgE is the antibody responsible for allergy, however not all IgE-mediated allergic reactions occur in atopic subjects. Atopy is a hereditary disorder characterized by the tendency to produce high levels of IgE antibodies and to develop localized immediate hypersensitivity reactions to allergens such as pollen, food *etc.* [38]. In non-IgE-mediated allergy the antibody can belong to the IgG isotype, as occurs in anaphylaxis due to immune complexes containing dextran. Allergic diseases include allergic asthma, allergic rhinitis, allergic conjunctivitis, dermatitis (eczema and contact dermatitis), allergic urticaria, food allergy, drug allergy and anaphylaxis [38].

It is estimated that over 20% of the world population suffers from IgE-mediated allergic diseases [39]. Among these diseases, asthma affects approximately 300 million people of all ages and ethnic backgrounds [40]. For many years it has been reported that allergic diseases including asthma was increasing in Western countries [41, 42]. However, international surveys completed by the International Study of Asthma and Allergy in Childhood (ISAAC) carried out in 2007 report on a decrease of asthma symptoms in Western countries. In contrast, the prevalence of asthma symptoms has increased in regions such as Africa, Latin America and parts of Asia where prevalence was previously low [43]. Although asthma symptom prevalence is no longer increasing in most Western countries, its global burden continues to rise.

Allergic reactions that are IgE-mediated are initiated when allergen is taken up by antigen presenting cells, such as dendritic cells (DC) that process fragments of the allergen and present it in the context of MHC class II to T cells, inducing a Th2 type of immune response. Allergen-specific B cells that take up allergen through the cell-surface immunoglobulin receptor may also initiate these allergic reactions. Activation of the allergen-specific Th2 cells leads to secretion of interleukin (IL)-4, IL-13 and subsequent switching to IgE synthesis by B cells. In addition, basophils secrete high levels of IL-4, IL-13 after activation and are suggested to play a role in polyclonal amplification of IgE production and in the differentiation of Th2 cells [44]. The binding of secreted IgE to mast cells *via* high-affinity Fc

receptors and subsequent cross linking of receptor-bound IgE by allergen triggers the release of pro-inflammatory mediators [45].

In allergic asthmatic patients, exposure to allergen leads to an early-phase reaction that involves IgE-mediated degranulation of mast cells and subsequent constriction of the airway smooth muscle. This is followed 4–18 hours later by the late-phase reaction, which is characterized by recruitment of eosinophils and T cells [46]. Th2 cells mediate IgE synthesis *via* IL-4, eosinophilic inflammation *via* IL-5 and the recruitment and growth of mast cells *via* IL-9, which, together with IL-13, contribute to airway hyper-responsiveness (AHR) and other clinical features of allergic disease [47]. Although lung DCs are sufficient to initiate and maintain the adaptive Th2 cell responses to inhaled allergens [48], it is now known that the epithelium cells and basophils play a central role. Studies have shown that the house dust mite (HDM) Der p 1 allergen activates airway epithelial cells through protease-activated receptor 2, C-type lectin receptors and Toll-like receptors leading to the production of thymic stromal lymphopoietin (TSLP), granulocyte-macrophage colony stimulating factor, and IL-33 [49]. TSLP induces immediate innate immune functions in DCs leading to chemokine-driven recruitment of Th2 cells and eosinophils to the airways. Epithelial cells produce CCL20 and IL-25 to further attract innate immune cells and Th2 cells to the lungs. TSLP and IL-33 induce DC migration to the mediastinal lymph nodes and stimulate the functions of mast cells and basophils. Induction of DC maturation by TSLP in the absence of IL-12, induces expression of OX40L, the ligand for the cell survival factor OX40, on DCs, and OX40-OX40L interactions are critical for the ability of the DCs to drive Th2 cell polarization. In addition to its effects on DCs, TSLP can also activate mast cells and basophils to produce IL-4 for Th2 cell development [49, 50]. In conclusion, effector Th2 cells control the features of asthma in combination with mediators released by eosinophils, mast cells and basophils.

HELMINTH INFECTIONS, ALLERGY AND THE FACTORS INVOLVED

The original *hygiene hypothesis* proposed that the lack of childhood infections results in a weaker Th1 cell responsiveness, allowing the expansion of the Th2 cell responses towards environmental allergens. However, studies with helminths which are characterized by the induction of Th2 cells, showed that infection with these pathogens can also protect against allergic diseases [51]. It has now become clear that the interaction between helminth infection and allergy often involves T regulatory (Treg) cells [52]. These cells have giving a new concept to the *hygiene hypothesis* based on their role in damping both Th1 and Th2 effectors responses. Multiple subsets of Treg cells have been identified in the last years. These cells are categorized according to their origin, function, and expression of cell surface markers: natural Treg cells (CD4+CD25+FOXP3+) and inducible Treg cells that include the IL-10-producing Tr1 cells and the Foxp3+ T cells induced in the periphery [53].

The inverse association between helminth infections and allergy has been extensively reported. Van den Biggelaar *et al.* have shown that chronic infection with *Schistosoma haematobium* in an endemic area in Gabon was negatively associated with skin-test reactivity to HDM. In addition, schistosome-specific IL-10 production was significantly higher in infected children and negatively associated with the outcome of skin-test reactivity to mite, suggesting an important role for this cytokine in suppressing atopy [54]. An important role for IL-10 was also found in another study where anti-helminthic treatment of *Schistosoma mansoni*-infected patients with asthma resulted in down-modulation of the Der p 1 specific IL-10 production *in vitro* [55]. In Brazil, Medeiros *et al.* have reported that the frequency of positive skin reactivity to HDM antigens in subjects with history of wheezing was significantly lower in a *S. mansoni* endemic area than in a non-endemic area [56]. Similar studies in Ethiopia found that hookworm infection protects against wheeze in atopic individuals and to a lesser extent, *Ascaris lumbricoides* infection [57]. A recent study has shown that in an area endemic for *Brugia malayi* infected individuals had a significantly reduced risk for atopic reactivity to cockroach [58].

The association between helminths and allergy is however not always consistent. In fact, infections with geohelminths have actually been found in some studies to be a risk factor for allergy. Cooper *et al.* suggest that high infection prevalence with geohelminths may confer protection against allergic disease whereas low prevalence infections is associated with increased risk for allergy [59]. Fig. (3) summarizes findings on the effect of different helminths on allergy. For instance, the prevalence of skin test reactivity was significantly lower among children that had heavy *Trichuris trichiura* infections compared to children with light or no infection [60]. In contrast, in a cross-sectional study of 2,164 children in China, the association between *A. lumbricoides* and asthma was investigated. Infection with this nematode was associated with increased risk of asthma, increased skin test reactivity, and increased airways responsiveness. Here, it was found that the intensity of *Ascaris* infection was light to moderate in the majority of the children studied [61]. Similar findings were obtained in a study in Brazil [62] however, in a study with Cuban children that had low prevalence and intensities of infection, no association between *A. lumbricoides* infection and asthma or positive skin prick test was found [63]. In a nested case-control study drawn from a survey of 7,155 children (1 to 4 years old) from urban and rural areas of Jimma, Ethiopia it was found that wheezing was significantly more prevalent in urban than rural children, and was less prevalent in those infected with *Ascaris*, particularly in those with high intensity of infection [64]. A meta-analysis study analysed the effects of parasite infection intensity of *A. lumbricoides*, *T. trichuria*, and hookworm on asthma and wheeze. Results from this study disclosed no effect of *T. trichuria*, non-significant reductions in risk at higher levels of infection with *A. lumbricoides*, and significant dose-related reductions in risk of both asthma and wheeze with hookworm infection [65].

The ability to induce specific host immune regulatory mechanisms may be partly determined by host genetics and environmental factors. A study suggests that genetic variants

of STAT6 can confer enhanced resistance to *Ascaris spp.* in environments where this helminths are prevalent [66]. Interestingly, Africans in rural Africa seem to suffer less from allergies while people of African ancestry living in affluent countries have higher prevalence and severity of allergic symptoms than natives of these host countries, raising important issues on genetic control of allergic diseases and the influence of environmental factors [67]. Different helminths may have different effects on allergy, such as *Toxocara spp.* which has been suggested to be a risk factor for allergic manifestations, as discussed below.

Animal models offer a great opportunity to analyze the interaction between helminths and allergic diseases. Using a well-defined model for allergic airway inflammation (using ovalbumin-OVA as the allergen) it has been shown that chronic, but not acute, schistosome infections can suppress allergic airway inflammation in a dose-dependent manner. Here, IL-10 was shown to play a central role in suppressing allergic airway inflammation after the adoptive transfer of splenocytes from chronically infected mice [68]. Another study showed that suppression of allergen-induced airway eosinophilia and reduction of eotaxin production were not observed in IL-10 deficient mice infected with *Nippostrongylus brasiliensis* in comparison to control mice. These results suggested that infection with this parasite suppresses the development of allergen induced airway eosinophilia and that this effect may be mediated by IL-10 [69].

Another animal model used to study the interaction between helminths and airway allergy is *Heligmosomoides polygyrus*, a natural intestinal parasite of mice. In this study the effect of Th2 cells induced by this gastrointestinal nematode, on experimentally airway allergy induced by OVA and HDM Derp1 was investigated. Infiltration of inflammatory cells in the lungs induced by both allergens was suppressed in infected mice compared to uninfected controls. Suppression was reversed in mice treated with antibodies to CD25. Most notably, suppression was transferable with mesenteric lymph node cells (MLNC) from infected animals to uninfected sensitized mice. MLNC from infected animals were found to have elevated numbers of CD4+CD25+FOXP3+ T cells producing TGF- β and IL-10. Thus, these data support the argument that helminth infections elicit a Treg cell population able to down-regulate allergen induced lung pathology *in vivo* [52].

There are also experimental studies that show that infection with other helminths have a positive association with allergy. A study using a murine model has shown that *T. canis* infection results in exacerbation of experimental airway inflammation [5], which supports finding from the epidemiological studies, as discussed below. Other studies using non-human primates have found that infection with *Ascaris suum* results in AHR and eosinophilia [70, 71]. The effect of this worm infection on an ongoing experimental allergic asthma remains to be investigated.

Taking all these studies together, it is clear that there are several factors that may influence the association between helminth infections and allergic manifestations [5, 72, 73]. These include 1. The helminth species involved: studies with different helminths suggest that depending on the pathogen, infection can either protect or exacerbate allergies.

2. Definitive vs accidental host: in an accidental host the parasite does not develop to the adult stage. It is likely that there are differences between parasites of humans that have evolved with their host and have developed strategies to survive without causing much damage compared to parasites such as *Toxocara spp.* in the accidental host. 3. Host genetics: gene polymorphisms have been found to be associated with susceptibility to different helminth infections. 4. Sporadic vs. chronic infection: chronic infections appear to result in immunosuppression not only against the parasite but also against other inflammatory diseases such as allergies. Whereas sporadic or transient infections may enhance allergic manifestations. 5. Intensity of infection: high parasite burden may induce a suppressive type of immune response compared to light infections. 6. Timing of infection in relation to allergen exposure: for certain geohelminths infection in the first years of life is crucial in order to induce the type of immune response required for protection against allergic diseases. Using murine models for toxocarasis however, we show that the timing of infection in relation to allergen exposure made no difference.

EPIDEMIOLOGICAL STUDIES ON THE INTERACTION BETWEEN TOXOCARA SEROPREVALENCE AND ALLERGY

Evidence from epidemiological studies appears to be conflicting. While a large number of studies suggest that infection with *Toxocara* worms contributes to the development of atopic diseases, few others suggest no association. Table 1 summarizes different epidemiological studies on the association between *Toxocara* seroprevalence and allergic manifestations. The majority of these studies use the ELISA based on TES antigen, to measure antibodies against this parasite. A positive association between *Toxocara* infections and allergic asthma has been reported already in 1981 by Desowitz *et al.* [4]. These authors analyzed the prevalence of antibodies to *T. canis* and *Dirofilaria immitis*, in asthmatic and non-asthmatic children born and raised in Hawaii. A total of 176 children from 1 to 18 years of age were included in this study. The children attended a children's hospital where asthma was diagnosed. Children of matched ages that were admitted to the same hospital with a diagnose other than asthma were included. The authors found a significant higher prevalence of parasite-specific IgE in the asthmatic compared to the non-asthmatic population. Since *Toxocara* serodiagnosis has been reported to cross-react with other ascarids, stool examination was performed. Results indicated that all the *Toxocara*-IgE positive asthmatic children were negative for ova of parasites including those of *A. lumbricoides*. The authors discuss that if indeed zoonotic helminth infections enhances allergic asthma this should be taken into account in the treatment of asthmatics who are serologically positive for *Toxocara spp.* [4]. Other studies reporting on a positive association between *Toxocara* seropositivity and allergic manifestations are the ones carried out in Malaysia [3, 74]. These are small studies in which blood samples were taken from children below the age of 10 years that were admitted to a hospital. A group of children were diagnosed with bronchial asthma and the age matched control group were children presenting other medical conditions. The prevalence of *Toxocara*-IgG antibodies for

children with bronchial asthma was found to be significantly higher compared to the non-asthmatic controls. In the Netherlands, Buijs and coworkers carried out cross-sectional studies among elementary school children aged 4-6 years [24]. These authors found that occurrences of asthma recurrent bronchitis and hospitalization due to asthma/recurrent bronchitis were significantly associated with *Toxocara* seropositivity. A marginally significant relation with eczema was also found. In this study IgE specific for inhaled allergens occurred significantly more often in the *Toxocara*-seropositive group. In another study, Buijs *et al.* investigated differences in *Toxocara* seroprevalence, allergic manifestations and the associations between these two, in children from urban and rural environments. In this study blood samples from 1,379 Dutch urban and rural elementary schoolchildren were taken and *Toxocara* antibodies, eosinophil numbers, total IgE concentrations, and the occurrence of inhaled allergen-specific IgE were measured. Questionnaires investigating respiratory health and putative risk factors for infection were used. Results from this study indicated that total serum IgE levels and blood eosinophils were significantly higher in the *Toxocara*-seropositive than in the seronegative group. Other results from this study were that inhaled allergen-specific IgE and asthma/recurrent bronchitis occurred significantly less often in rural than in urban areas, and significantly less often among girls than among boys [2]. Recently, Walsh carried out a study in the United States using data from the Third National Health and Nutrition Examination Survey, undertaken by the United States Department of Health and Human Services, during 1988-1994 [75]. The study aimed at determining the association between *Toxocara* infections and lung function. Results from this study suggest diminished lung function in the presence of *Toxocara* infection. This positive association was found after controlling for age, sex, education level, BMI, ethnicity, smoking status, whether the person was born in the USA or immigrated there, rural residence and dog ownership. The author stresses the urgent need for longitudinal studies to more clearly define the immunological mechanisms underlining *Toxocara* infection and its potential influence on lung function.

Studies that indicate no association between *Toxocara* infections and allergic manifestations include that of Shargi *et al.* [9]. The authors conducted a clinic based case-control study in which blood samples were collected from 95 children aged 2-15 years that had physician diagnosed asthma and from 229 children that did not have asthma. *Toxocara* IgG antibodies were measured using ELISA. Risk factors for asthma and *Toxocara* infection were assessed by a questionnaire. Significant associations were found between asthma and risk factors and between *Toxocara* infection and risk factors but not between *Toxocara* infection and asthma. In Spain a cross-sectional survey of 463 subjects from an adult population to study the association between *Toxocara* exposure and atopic features was conducted. Skin prick test to different aeroallergens, total IgE, allergen-specific IgE, blood eosinophil counts and serum *Toxocara*-IgG were determined. Information concerning respiratory symptoms was collected using a questionnaire. The main outcome from this study is that the *Toxocara* seropositive individuals showed higher total serum IgE levels and higher prevalence

Table 1. Epidemiological studies on the association between *Toxocara* seroprevalence and allergic manifestations.

Authors, Year	Country (City/State)	Sample Size	Population Age (Years)	Antibody Detected-Serological Assay*	Clinical Manifestations	Statistical Analysis	Association between <i>Toxocara</i> seroprevalence and Allergic Manifestations	Ref.
Desowitz <i>et al.</i> , 1981	USA (Hawaii)	176	Children (1-18)	IgG-CEP and IgE-RAST	Asthma	χ^2 test	Positive. for <i>Toxocara</i> -IgE	[4]
Hakim <i>et al.</i> , 1997	Malaysia (Kuala Lumpur)	45	Children (1-10)	IgG-ELISA	Bronchial asthma	χ^2 test and Mann-Whitney U test	Positive	[74]
Chan <i>et al.</i> , 2001	Malaysia	124	Children (mean age 8.1 $\pm$ 3.1)	Commercial IgG-ELISA	Bronchial asthma	Student's <i>t</i> -test, χ^2 test or Fishers exact test	Positive	[3]
Buijs <i>et al.</i> , 1994	The Netherlands (The Hague and Rotterdam)	712	Children (4-6)	IgG-ELISA	Recurrent bronchitis/ Asthma	Logistic Regression	Positive	[24]
Buijs <i>et al.</i> , 1997	The Netherlands (Utrecht and Brabant)	1.379	Children (4-12)	IgG-ELISA	Recurrent bronchitis/ Asthma	Logistic Regression, ANCOVA	Positive	[2]
Walsh, 2010	USA (National study)	11.606	Adults (18-65)	IgG-ELISA	Lung function as an indicator of asthma	Multiple linear regression, using different models	Positive	[75]
Shargi <i>et al.</i> , 2001	USA (New Haven and Bridgeport)	324	Children (2-15)	IgG-ELISA	Asthma	Multivariate model by stepwise logistic regression	No association	[9]
Gonzalez-Quintela <i>et al.</i> , 2006	Spain	463	Adults ($\geq$ 18)	Commercial IgG-ELISA	Respiratory Symptoms	Pearson χ^2 test, Student's <i>t</i> -test	No association with respiratory symptoms but a positive association with allergic sensitization	[8]
Fernando <i>et al.</i> , 2009	Sri Lanka	196	Children (5-12)	Commercial IgG-ELISA	Bronchial asthma	χ^2 test or Fishers exact test	Positive association in a univariate model but no association in a multivariate model.	[7]

*Serological assays include: Enzyme-linked immunosorbent assay (ELISA), Counter-electrophoresis (CEP) and Solid phase radio-allergosorbent test (RAST). For these serological tests, the excretory-secretory products from *Toxocara canis* second-stage larvae were used as antigen.

of aeroallergen sensitization compared to the *Toxocara* seronegative subjects. No association was found between *Toxocara* seropositivity and respiratory symptoms. Since the *Toxocara* exposure and allergic sensitization was restricted to mites the authors suggest that common antigens present in *Toxocara* and mites may favour mite sensitization [8]. Whether indeed there is cross-reactivity between *Toxocara* and mite antigens, remains to be investigated. Recently, Fernando *et al.* carried out a study with Sri Lankan children on the association between *Toxocara* seropositivity and asthma [7]. The studied population consisted of 196 children aged 5 – 12 years that were taken to the hospital. One group of children were confirmed to be suffering from bronchial asthma and the control group consisted of children attending

the same hospital for different reasons but who had never had bronchial asthma and were not suffering from upper respiratory tract infection at the time of the study. Findings from this study indicate that *Toxocara* seropositivity was identified as a significant risk factor in the development of asthma. However, this was only true when a univariate model was used to analyse the data. When a multivariate model was used there was no significant difference in the *Toxocara* seroprevalence for the group of children with asthma compared to the age, sex and ethnic group matched controls. The authors suggest an association between toxocariasis and other risk factors of asthma, rather than a direct association between toxocariasis and asthma.

The different conclusions drawn from all these studies could be due to differences in the study design, which include the size of the studied population, the different serological assays used and the statistical analysis employed (Table 1). Studies should therefore be carried out: a) in different countries; b) with larger sample size; c) for both children and adults; d) using a standardize serological assay to determine both IgG and IgE antibodies against this parasite; e) including other laboratory findings such as eosinophilia as well as information on clinical examination for both toxocariasis and asthma and f) using multivariate analysis of the data, corrected for well known risk factors for asthma such as smoking, lower respiratory tract infections and parents with asthma. Understanding any possible contribution of *Toxocara* infections to the pathogenesis of asthma is important since it would provide a potential strategy for prevention of this disease.

IMMUNE RESPONSES TO TOXOCARA INFECTIONS AND ITS ASSOCIATION WITH EXPERIMENTAL AIRWAY INFLAMMATION

Toxocara infection results in the induction of Th2 cells [76] that make cytokines such as IL-4, IL-5, and IL-13, which induce responses to the parasite such as increased IgE levels and eosinophilia [77]. Several studies using murine models for toxocariasis have shown that infection with *T. canis* lead to persistent pulmonary inflammation, eosinophilia, IgE production, airway hyper-reactivity and production of Th-2 type cytokines [78-80]. Pulmonary inflammation develops as soon as 48 hours after infection and it can persist up to 2 or 3 months [80, 81]. Granulomas develop within a week and could be found throughout the anterior musculature, in the liver, kidneys, heart and sometimes in the eye [82]. Analysis of cell composition in bronchoalveolar lavage (BAL) indicate that at two weeks post infection (p.i.) eosinophils account for more than 75% of the recovered cells compared to 25% in peripheral blood [83]. The parasite-specific antibody response peaks around 14 days after infection, but it depends on the load of administered eggs and the mice strain used [84]. Studies from our group indicate that infection of BALB/c mice with

1,000, 100 and 10 *T. canis* embryonated eggs resulted in a dose dependent response characterized by pulmonary inflammation (Fig. 2), increased levels of total IgE, and *Toxocara*-specific IgG1 that persisted up to 60 days p.i. Relative quantification of cytokine expression in lungs of mice infected with different doses showed proportional increased expression of the IL-4, IL-5, and IL-10 transcripts, whereas the expression of the IFN-gamma transcript was not different from that of uninfected controls. Results from this study indicate that infection of mice with *T. canis* results in chronic pulmonary inflammation and a dominant Th2 type of immune response, independent of the inoculum size [85].

We have also shown that infection of BALB/c mice with 1,000 embryonated eggs resulted in hyper-reactivity of the airways that persisted up to 30 days p.i. At 60 days p.i. the reactivity of the airways decreased to background levels however, pulmonary inflammation as well as increased levels of IgE and eosinophils in BAL persisted up to 60 days p.i. Evaluation of parasite burden revealed that few *T. canis* larvae were still present in the lungs of infected mice at 60 days p.i. which could explain the persistent immune response observed in these mice [80].

T cells characterized by the expression of CD4 and CD25 on the cell surface and the presence of Foxp3 has been shown to play an important role in regulating immunopathology including those caused by parasites [86, 87]. Recently, Othman *et al.* have described the kinetics of Foxp3-expressing cells during the course of experimental infection with *T. canis*. Findings indicate progressive increase in Foxp3-expressing cell counts in the liver starting from 5 weeks p.i. These cells were detected within and around *Toxocara*-induced granulomas as well as in isolated inflammatory foci in the portal tracts or within the hepatic parenchyma. The authors suggest a potential role for Foxp3-expressing regulatory cells in the *T. canis* induced immunopathology [88].

Common features in allergic asthma and toxocariasis are the induction of a Th2-cell mediated immune response including the production of high levels of IgE, inflammation of the airways, and the accumulation of eosinophils [89]. In

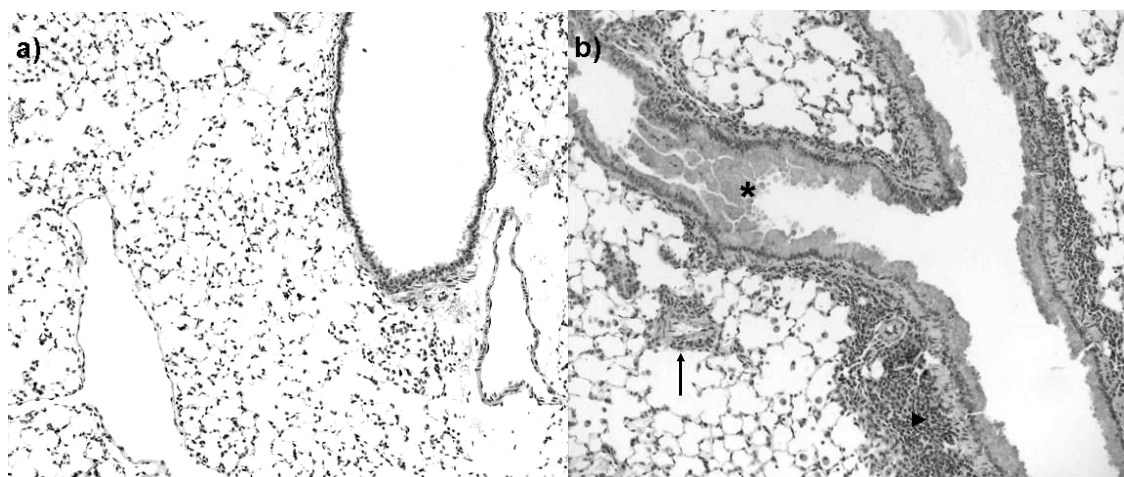


Fig. (2). Pulmonary inflammation induced by *Toxocara canis* infection. (a) Lung from an un-infected mouse showing bronchiole without any sign of inflammation. (b) Lung from a mouse 7 days after infection with 1,000 *T. canis* embryonated eggs. Perivascularitis (arrow), peribronchiolitis (arrow head) and bronchiolar lumen filled with mucus (*) are shown. Stained with haematoxylin-eosin (HE), $\times 160$.

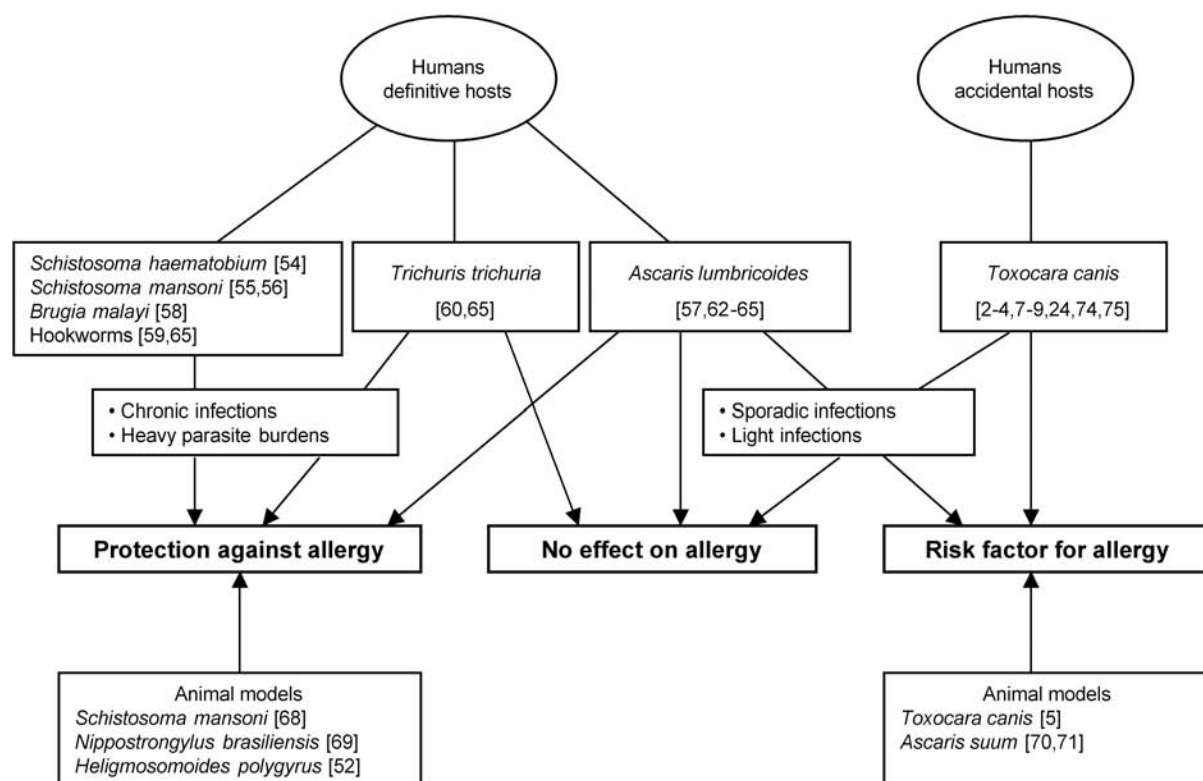


Fig. (3). Association between helminth infections and allergic manifestations. Several factors may influence the association between helminth infections and allergies. Infections with certain helminths have been reported to protect against allergies while others have the opposite effect or no effect at all. Being a definitive host could be beneficial while being an accidental host could be a risk factor for allergy. Chronic and heavy parasite burdens may be associated with protection against allergies compared to periodic and light infections.

addition, infections with this helminth share common clinical features with allergic asthma such as wheezing, coughs, mucus hyper-secretion and bronchial hyper-reactivity. In order to study the effect of *Toxocara* infection on allergic manifestations we combined two murine models namely, the murine model for toxocariasis and the well characterized experimental model for allergic airway inflammation [5]. For this study we infected BALB/c mice with 500 embryonated *T. canis* eggs and exposed them to OVA treatment. Results indicate that infection with *T. canis* in combination with OVA treatment led to exacerbation of pulmonary inflammation, eosinophilia, airway hyper-responsiveness, OVA specific and total IgE. Cytokines were also measured in this model by relative quantification indicating increased expression of IL-4 compared with mice that were only *T. canis* infected or OVA treated. The observed exacerbation of experimental allergic airway inflammation was independent of the timing of infection in relation to allergen exposure. In conclusion, a previous infection with *T. canis* leads to exacerbation of experimental allergic airway inflammation [5]. These findings confirm the epidemiological studies on the positive association between *Toxocara* seropositivity and allergic manifestations and have extended our knowledge on the immunological mechanisms underlying this association. Studies using murine models for toxocariasis are relevant since mice are natural (paratenic) hosts of *Toxocara canis*.

The precise underlying mechanism in the association between *Toxocara* infection and experimental allergic airway inflammation is still not clear. Fig. (4) proposes a

mechanism where *Toxocara* infections leads to a dominant type of Th2 response which is characterized by increased IL5 and IL4/IL13 production that results in eosinophilia and increased levels of IgE respectively [90]. The lungs are one of the organs where the larvae migrate to. Also in the lung a dominant type of immune response is observed and infiltration of inflammatory cells such as eosinophils, macrophages and mast cells takes place [80, 81]. After allergen exposure, activation of cells bearing allergen-specific IgE such as eosinophils, mast cells and macrophages will take place.

During asthma in humans it has been shown that in the early-phase reaction activated mast cells and macrophages rapidly release pro-inflammatory mediators such as histamine, eicosanoids, and reactive oxygen species. These mediators induce contraction of airways smooth muscle, mucous secretion, and vasodilation, contributing therefore to airflow obstruction. In the late-phase reaction which occurs between 4 to 18 h after allergen exposure, recruitment and activation of eosinophils, CD4⁺ T cells, basophils, neutrophils, and macrophages takes place (reviewed in [91]).

In mice we observe that once the *Toxocara*-infected animals are exposed to the allergen, exacerbation of allergic airway inflammation takes place [5]. Whether functional Tregs are present in the lungs of these animals and if so, what role do they play in disease, still remain to be investigated.

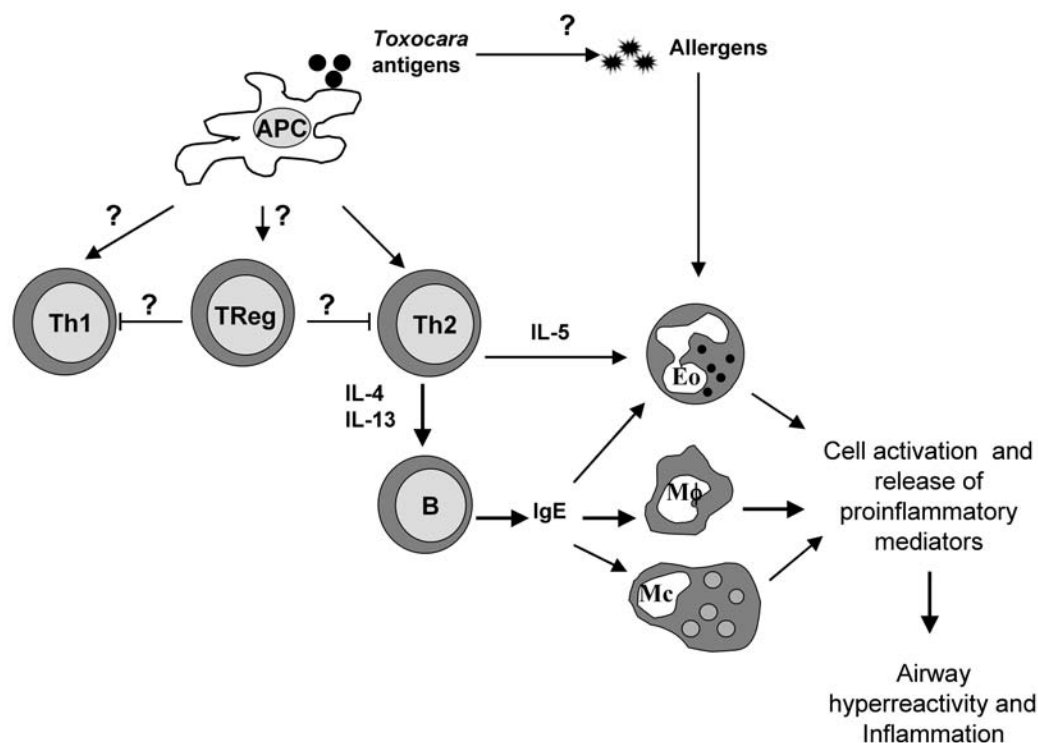


Fig. (4). Immune response in murine toxocariasis and possible association with allergic asthma. Infection with *Toxocara* spp. results in the induction of a dominant T-helper 2 (Th2) type of immune response characterized by the production of cytokines such as Interleukin-4 (IL-4), IL-13 and IL-5. *Toxocara* larvae migrate to the lungs and due to the Th2 type of immune response induced, infiltration of eosinophils (Eo), macrophages (Mφ) and mast cells (Mc), in addition to increased levels of IgE takes place. After allergen challenge, IgE interacts with specific allergen and bind to high and low affinity receptors on mast cells, eosinophils and macrophages that secrete several mediators involved in the induction of airway inflammation. FOXP3⁺ cells have been recently described to increase during pathogenesis of toxocariasis, however it is not known whether these are functional T-regulatory cells or their role in pathology. The role of Th1 in toxocariasis is still not clear: are parasite-specific Th1 cells induced during infection and if so, what role do they play in the induction, maintenance or suppression of allergic asthma? And finally, the question as whether *Toxocara* antigens could trigger allergic asthma in *Toxocara* infected individuals, still remain to be investigated. APC: antigen presenting cell; B: B cells. TReg: T-regulatory.

FINAL REMARKS

Helminths modulate the host immune response in order to survive in their host. A regulatory type of immune response has been suggested to be induced during chronic infections, which benefits parasite survival and at the same time benefits the host by suppressing other inflammatory diseases. Although this might be true for certain helminths, many factors may influence the association between helminth infections and allergy as discussed above. *Toxocara* spp. is a parasite of dogs and cats that can also infect humans where the larvae migrate but do not reach the adult stage. Migration of the larvae through different organs including the lungs may results in tissue damage and inflammation. Findings from human epidemiological studies are conflicting with some suggesting that *Toxocara* infections are a risk factor for allergy, whereas others find no association at all. More studies including larger population size, standardized serological assays and statistical analyses are required.

To establish and understand the relation between *Toxocara* infection and allergic asthma, the immunological and molecular mechanisms that can explain the observed association clearly need to be further investigated. Murine models have proven to be very valuable to investigate the

possible factors that contribute to the observed association between these two disorders. Future studies should focus on determining whether Treg cells are induced in toxocariasis and if so, what role they play in regulating pathology. Other questions that remain to be investigated include: 1. What is the effect of *T. canis* infection during allergen challenge? 2. Which parasite antigens exacerbate allergic airway inflammation? 3. Do *Toxocara* antigens have common structures with known allergens? 4. Can *Toxocara* antigens trigger allergic airway inflammation in *Toxocara* infected mice? Answer to these questions are essential in order to understand the relation between allergic airway inflammation and *Toxocara* infections and will contribute to development of alternative means to combat or prevent these inflammatory diseases.

REFERENCES

- [1] Rubinsky-Elefant, G.; Hirata, C.E.; Yamamoto, J.H. and Ferreira, M.U. (2010) Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. *Ann. Trop. Med. Parasitol.*, **104**, 3-23.
- [2] Buijs, J.; Borsboom, G.; Renting, M.; Hilgersom, W.J.; Van Wieringen, J.C.; Jansen, G. and Neijens, J. (1997) Relationship between allergic manifestations and *Toxocara* seropositivity: a cross-sectional study among elementary school children. *Eur. Respir. J.*, **10**, 1467-1475.

- [3] Chan, P.W.; Anuar, A.K.; Fong, M.Y.; Debruyne, J.A. and Ibrahim, J. (2001) *Toxocara* seroprevalence and childhood asthma among Malaysian children. *Pediatr. Int.*, **43**, 350-353.
- [4] Desowitz, R.S.; Rudoy, R. and Barnwell, J.W. (1981) Antibodies to canine helminth parasites in asthmatic and nonasthmatic children. *Int. Arch. Allergy Appl. Immunol.*, **65**, 361-366.
- [5] Pinelli, E.; Brandes, S.; Dormans, J.; Gremmer, E. and Van Loveren, H. (2008) Infection with the roundworm *Toxocara canis* leads to exacerbation of experimental allergic airway inflammation. *Clin. Exp. Allergy*, **38**, 649-658.
- [6] Asher, M.I. (2010) Recent perspectives on global epidemiology of asthma in childhood. *Allergol. Immunopathol. (Madr.)*, **38**, 83-87.
- [7] Fernando, D.; Wickramasinghe, P.; Kapilana, G.; Dewasurendra, R.L.; Amarasekiriya, M. and Dayaratne, A. (2009) *Toxocara* seropositivity in Sri Lankan children with asthma. *Pediatr. Int.*, **51**, 241-245.
- [8] Gonzalez-Quintela, A.; Gude, F.; Campos, J.; Garea, M.T.; Romero, P.A.; Rey, J.; Meijide, L.M.; Fernandez-Merino, M.C. and Vidal, C. (2006) *Toxocara* infection seroprevalence and its relationship with atopic features in a general adult population. *Int. Arch. Allergy Immunol.*, **139**, 317-324.
- [9] Sharghi, N.; Schantz, P.M.; Caramico, L.; Ballas, K.; Teague, B.A. and Hotez, P.J. (2001) Environmental exposure to *Toxocara* as a possible risk factor for asthma: a clinic-based case-control study. *Clin. Infect. Dis.*, **32**, E111-E116.
- [10] Smits, H.H.; Everts, B.; Hartgers, F.C. and Yazdanbakhsh, M. (2010) Chronic helminth infections protect against allergic diseases by active regulatory processes. *Curr. Allergy Asthma Rep.*, **10**, 3-12.
- [11] Strachan, D.P. (2000) Family size, infection and atopy: the first decade of the "hygiene hypothesis". *Thorax*, **55** Suppl 1, S2-10.
- [12] Despommier, D. (2003) Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. *Clin. Microbiol. Rev.*, **16**, 265-272.
- [13] Overgaauw, P.A.M. (1997) Aspects of *Toxocara* epidemiology: human toxocarosis - toxocarosis in dogs and cats. *Crit. Rev. Microbiol.*, **23**, 233-251.
- [14] Pinelli, E.; Kortbeek, L.M. and Van der Giessen, J. (2005) *Toxocara*. In *Parasitology* (Wakelin, D.; Cox, F.; Despommier, D. and Gilliespie, S. Eds), pp. 750. Hodder Arnold, London, UK.
- [15] Schantz, P.M. (1989) *Toxocara* larva migrans now. *Am. J. Trop. Med. Hyg.*, **41**, 21-34.
- [16] Aydeniz-Ozkayhan, M.; Yagci, B.B. and Erat, S. (2008) The investigation of *Toxocara canis* eggs in coats of different dog breeds as a potential transmission route in human toxocariasis. *Vet. Parasitol.*, **152**, 94-100.
- [17] Roddie, G.; Stafford, P.; Holland, C. and Wolfe, A. (2008) Contamination of dog hair with eggs of *Toxocara canis*. *Vet. Parasitol.*, **152**, 85-93.
- [18] Overgaauw, P.A.; Van Zutphen, L.; Hoek, D.; Yaya, F.O.; Roelfsema, J.; Pinelli, E.; Van Knapen, F. and Kortbeek, L.M. (2009) Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. *Vet. Parasitol.*, **163**, 115-122.
- [19] Morimatsu, Y.; Akao, N.; Akiyoshi, H.; Kawazu, T.; Okabe, Y. and Aizawa, H. (2006) A familial case of visceral larva migrans after ingestion of raw chicken livers: appearance of specific antibody in bronchoalveolar lavage fluid of the patients. *Am. J. Trop. Med. Hyg.*, **75**, 303-306.
- [20] Nagakura, K.; Tachibana, H.; Kaneda, Y. and Kato, Y. (1989) Toxocariasis possibly caused by ingesting raw chicken. *J. Infect. Dis.*, **160**, 735-736.
- [21] Salem, G. and Schantz, P. (1992) Toxocaral visceral larva migrans after ingestion of raw lamb liver. *Clin. Infect. Dis.*, **15**, 743-744.
- [22] Magnaval, J.F.; Michault, A.; Calon, N. and Charlet, J.P. (1994) Epidemiology of human toxocariasis in La Reunion. *Trans. R. Soc. Trop. Med. Hyg.*, **88**, 531-533.
- [23] Stensvold, C.R.; Skov, J.; Moller, L.N.; Jensen, P.M.; Kapel, C.M.; Petersen, E. and Nielsen, H.V. (2009) Seroprevalence of human toxocariasis in Denmark. *Clin. Vaccine Immunol.*, **16**, 1372-1373.
- [24] Buijs, J.; Borsboom, G.; Van Gemund, J.J.; Hazebroek, A.; Van Dongen, P.A.; Van Knapen, F. and Neijens, H.J. (1994) *Toxocara* seroprevalence in 5-year-old elementary schoolchildren: relation with allergic asthma. *Am. J. Epidemiol.*, **140**, 839-847.
- [25] Luty, T. (2001) Prevalence of species of *Toxocara* in dogs, cats and red foxes from the Poznan region, Poland. *J. Helminthol.*, **75**, 153-156.
- [26] Segovia, J.M.; Torres, J.; Miquel, J.; Llana, L. and Feliu, C. (2001) Helminths in the wolf, *Canis lupus*, from north-western Spain. *J. Helminthol.*, **75**, 183-192.
- [27] Devera, R.; Blanco, Y.; Hernandez, H. and Simoes, D. (2008) *Toxocara* spp. and other helminths in squares and parks of Ciudad Bolivar, Bolivar State (Venezuela). *Enferm. Infecc. Microbiol. Clin.*, **26**, 23-26.
- [28] Jansen, J.; Van Knapen, F.; Schreurs, M. and Van Wijngaarden, T. (1993) *Toxocara* ova in parks and sand-boxes in the city of Utrecht. *Tijdschr. Diergeneesk.*, **118**, 611-614.
- [29] Smith, H.; Holland, C.; Taylor, M.; Magnaval, J.F.; Schantz, P. and Maizels, R. (2009) How common is human toxocariasis? Towards standardizing our knowledge. *Trends Parasitol.*, **25**, 182-188.
- [30] Gutierrez, Y. (2000) Visceral larva migrans. In *Diagnostic Pathology of Parasitic Infections with Clinical Correlations* pp. 402-414. OUP USA, New York.
- [31] Gillespie, S.H.; Dinning, W.J.; Voller, A. and Crowcroft, N.S. (1993) The spectrum of ocular toxocariasis. *Eye (Lond)*, **7**, 415-418.
- [32] Taylor, M.R. (2001) The epidemiology of ocular toxocariasis. *J. Helminthol.*, **75**, 109-118.
- [33] Taylor, M.R.; Keane, C.T.; O'Connor, P.; Girdwood, R.W. and Smith, H. (1987) Clinical features of covert toxocariasis. *Scand. J. Infect. Dis.*, **19**, 693-696.
- [34] Glickman, L.; Schantz, P.; Dombroske, R. and Cypess, R. (1978) Evaluation of serodiagnostic tests for visceral larva migrans. *Am. J. Trop. Med. Hyg.*, **27**, 492-498.
- [35] Taylor, M.H.R.; O'Connor, P.; Keane, C.T.; Mulvihill, E. and Holland, C. (1988) The expanded spectrum of Toxocaral disease. *The Lancet*, **26**, 692-695.
- [36] Magnaval, J.F.; Malard, L.; Morassin, B. and Fabre, R. (2002) Immunodiagnosis of ocular toxocariasis using Western-blot for the detection of specific anti-*Toxocara* IgG and CAP for the measurement of specific anti-*Toxocara* IgE. *J. Helminthol.*, **76**, 335-339.
- [37] de Visser, L.; Rothova, A.; de Boer, J.H.; Van Loon, A.M.; Kerkhoff, F.T.; Canning-Van Dijk, M.R.; Weersink, A.Y. and Groot-Mijnes, J.D. (2008) Diagnosis of ocular toxocariasis by establishing intraocular antibody production. *Am. J. Ophthalmol.*, **145**, 369-374.
- [38] Johansson, S.G.; Bieber, T.; Dahl, R.; Friedmann, P.S.; Lanier, B.Q.; Lockey, R.F.; Motala, C.; Ortega, M.J.A.; Platts-Mills, T.A.; Ring, J.; Thien, F.; Van Cauwenberge, P. and Williams, H.C. (2004) Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J. Allergy Clin. Immunol.*, **113**, 832-836.
- [39] World Health Organization. Prevention of Allergy and Allergic Asthma. World Health Organization. 2003. http://www.worldallergy.org/professional/who_paa2003.pdf.
- [40] World Health Organization (2007) *Global Surveillance, Prevention and Control of Chronic Respiratory Diseases*. WHO Press, Switzerland.
- [41] Asher, M.I.; Keil, U.; Anderson, H.R.; Beasley, R.; Crane, J.; Martinez, F.; Mitchell, E.A.; Pearce, N.; Sibbald, B.; Stewart, A.W.; et al. (1995) International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur. Respir. J.*, **8**, 483-491.
- [42] Weiland, S.K.; Bjorksten, B.; Brunekreef, B.; Cookson, W.O.; von Mutius, E. and Strachan, D.P. (2004) Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. *Eur. Respir. J.*, **24**, 406-412.
- [43] Pearce, N.; Ait-Khaled, N.; Beasley, R.; Mallol, J.; Keil, U.; Mitchell, E. and Robertson, C. (2007) Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax*, **62**, 758-766.
- [44] Stone, K.D.; Prussin, C. and Metcalfe, D.D. (2010) IgE, mast cells, basophils, and eosinophils. *J. Allergy Clin. Immunol.*, **125**, S73-S80.
- [45] Shakib, F.; Ghaemmaghami, A.M. and Sewell, H.F. (2008) The molecular basis of allergenicity. *Trends Immunol.*, **29**, 633-642.
- [46] Lloyd, C.M. and Hessel, E.M. (2010) Functions of T cells in asthma: more than just T(H)2 cells. *Nat. Rev. Immunol.*, **10**, 838-848.

- [47] Kim, H.Y.; DeKruyff, R.H. and Umetsu, D.T. (2010) The many paths to asthma: phenotype shaped by innate and adaptive immunity. *Nat. Immunol.*, **11**, 577-584.
- [48] Van Rijt, L.S.; Jung, S.; Kleinjan, A.; Vos, N.; Willart, M.; Duez, C.; Hoogsteden, H.C. and Lambrecht, B.N. (2005) *In vivo* depletion of lung CD11c+ dendritic cells during allergen challenge abrogates the characteristic features of asthma. *J. Exp. Med.*, **201**, 981-991.
- [49] Lambrecht, B.N. and Hammad, H. (2009) Biology of lung dendritic cells at the origin of asthma. *Immunity*, **31**, 412-424.
- [50] Ziegler, S.F. and Artis, D. (2010) Sensing the outside world: TSLP regulates barrier immunity. *Nat. Immunol.*, **11**, 289-293.
- [51] Yazdanbakhsh, M.; Kremsner, P.G. and Van Ree, R. (2002) Allergy, parasites, and the hygiene hypothesis. *Science*, **296**, 490-494.
- [52] Wilson, M.S.; Taylor, M.D.; Balic, A.; Finney, C.A.; Lamb, J.R. and Maizels, R.M. (2005) Suppression of allergic airway inflammation by helminth-induced regulatory T cells. *J. Exp. Med.*, **202**, 1199-1212.
- [53] Belkaid, Y. and Chen, W. (2010) Regulatory ripples. *Nat. Immunol.*, **11**, 1077-1078.
- [54] Van den Biggelaar, A.H.; Van Ree, R.; Rodrigues, L.C.; Lell, B.; Deelder, A.M.; Kremsner, P.G. and Yazdanbakhsh, M. (2000) Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet*, **356**, 1723-1727.
- [55] Araujo, M.I.; Hoppe, B.; Medeiros, M.Jr.; Alcantara, L.; Almeida, M.C.; Schrieffer, A.; Oliveira, R.R.; Kruschewsky, R.; Figueiredo, J.P.; Cruz, A.A. and Carvalho, E.M. (2004) Impaired T helper 2 response to aeroallergen in helminth-infected patients with asthma. *J. Infect. Dis.*, **190**, 1797-1803.
- [56] Medeiros, M.Jr.; Figueiredo, J.P.; Almeida, M.C.; Matos, M.A.; Araujo, M.I.; Cruz, A.A.; Atta, A.M.; Rego, M.A.; de Jesus, A.R.; Taketomi, E.A. and Carvalho, E.M. (2003) *Schistosoma mansoni* infection is associated with a reduced course of asthma. *J. Allergy Clin. Immunol.*, **111**, 947-951.
- [57] Scrivener, S.; Yemaneberhan, H.; Zebenigus, M.; Tilahun, D.; Girma, S.; Ali, S.; McElroy, P.; Custovic, A.; Woodcock, A.; Pritchard, D.; Venn, A. and Britton, J. (2001) Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. *Lancet*, **358**, 1493-1499.
- [58] Supali, T.; Djuardi, Y.; Wibowo, H.; Van Ree, R.; Yazdanbakhsh, M. and Sartono, E. (2010) Relationship between different species of helminths and atopy: a study in a population living in helminth-endemic area in Sulawesi, Indonesia. *Int. Arch. Allergy Immunol.*, **153**, 388-394.
- [59] Cooper, P.J.; Barreto, M.L. and Rodrigues, L.C. (2006) Human allergy and geohelminth infections: a review of the literature and a proposed conceptual model to guide the investigation of possible causal associations. *Br. Med. Bull.*, **79-80**, 203-218.
- [60] Rodrigues, L.C.; Newcombe, P.J.; Cunha, S.S.; Alcantara-Neves, N.M.; Genser, B.; Cruz, A.A.; Simoes, S.M.; Fiaccone, R.; Amorim, L.; Cooper, P.J. and Barreto, M.L. (2008) Early infection with *Trichuris trichiura* and allergen skin test reactivity in later childhood. *Clin. Exp. Allergy*, **38**, 1769-1777.
- [61] Palmer, L.J.; Celedon, J.C.; Weiss, S.T.; Wang, B.; Fang, Z. and Xu, X. (2002) *Ascaris lumbricoides* infection is associated with increased risk of childhood asthma and atopy in rural China. *Am. J. Respir. Crit. Care Med.*, **165**, 1489-1493.
- [62] da Silva, E.R.; Sly, P.D.; de Pereira, M.U.; Pinto, L.A.; Jones, M.H.; Piteze, P.M. and Stein, R.T. (2008) Intestinal helminth infestation is associated with increased bronchial responsiveness in children. *Pediatr. Pulmonol.*, **43**, 662-665.
- [63] Wordemann, M.; Diaz, R.J.; Heredia, L.M.; Collado Madurga, A.M.; Ruiz, E.A.; Prado, R.C.; Millan, I.A.; Escobedo, A.; Rojas, R.L.; Gryseels, B.; Gorbea, M.B. and Polman, K. (2008) Association of atopy, asthma, allergic rhinoconjunctivitis, atopic dermatitis and intestinal helminth infections in Cuban children. *Trop. Med. Int. Health*, **13**, 180-186.
- [64] Dagoye, D.; Bekele, Z.; Woldemichael, K.; Nida, H.; Yimam, M.; Hall, A.; Venn, A.J.; Britton, J.R.; Hubbard, R. and Lewis, S.A. (2003) Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. *Am. J. Respir. Crit. Care Med.*, **167**, 1369-1373.
- [65] Leonardi-Bee, J.; Pritchard, D. and Britton, J. (2006) Asthma and current intestinal parasite infection: systematic review and meta-analysis. *Am. J. Respir. Crit. Care Med.*, **174**, 514-523.
- [66] Moller, M.; Gravenor, M.B.; Roberts, S.E.; Sun, D.; Gao, P. and Hopkin, J.M. (2007) Genetic haplotypes of Th-2 immune signalling link allergy to enhanced protection to parasitic worms. *Hum. Mol. Genet.*, **16**, 1828-1836.
- [67] Obeng, B.B.; Hartgers, F.; Boakye, D. and Yazdanbakhsh, M. (2008) Out of Africa: what can be learned from the studies of allergic disorders in Africa and Africans? *Curr. Opin. Allergy Clin. Immunol.*, **8**, 391-397.
- [68] Smits, H.H.; Hammad, H.; Van Nimwegen, M.; Soullie, T.; Willart, M.A.; Lievers, E.; Kadouch, J.; Kool, M.; Kos-Van Oosterhoud, J.; Deelder, A.M.; Lambrecht, B.N. and Yazdanbakhsh, M. (2007) Protective effect of *Schistosoma mansoni* infection on allergic airway inflammation depends on the intensity and chronicity of infection. *J. Allergy Clin. Immunol.*, **120**, 932-940.
- [69] Wohlleben, G.; Trujillo, C.; Muller, J.; Ritz, Y.; Grunewald, S.; Tatsch, U. and Erb, K.J. (2004) Helminth infection modulates the development of allergen-induced airway inflammation. *Int. Immunol.*, **16**, 585-596.
- [70] Pritchard, D.I.; Eady, R.P.; Harper, S.T.; Jackson, D.M.; Orr, T.S.; Richards, I.M.; Trigg, S. and Wells, E. (1983) Laboratory infection of primates with *Ascaris suum* to provide a model of allergic bronchoconstriction. *Clin. Exp. Immunol.*, **54**, 469-476.
- [71] Patterson, R.; Harris, K.E. and Pruzansky, J.J. (1983) Induction of IgE-mediated cutaneous, cellular, and airway reactivity in rhesus monkeys by *Ascaris suum* infection. *J. Lab. Clin. Med.*, **101**, 864-872.
- [72] Bourke, C.D.; Maizels, R.M. and Mutapi, F. (2011) Acquired immune heterogeneity and its sources in human helminth infection. *Parasitol.*, **138**, 139-159.
- [73] Cooper, P.J. (2009) Interactions between helminth parasites and allergy. *Curr. Opin. Allergy Clin. Immunol.*, **9**, 29-37.
- [74] Hakim, L. (1997) Prevalence of *Toxocara canis* antibody among children with bronchial asthma in Klang hospital, Malaysia (vol 91, pg 528, 1997). *Trans. Roy. Soc. Trop. Med. Hyg.*, **91**, 728.
- [75] Walsh, M.G. (2010) *Toxocara* infection and diminished lung function in a nationally representative sample from the United States population. *Int. J. Parasitol.*, **41**, 243-247.
- [76] Del Prete, G.F.; De Carli, M.; Mastromauro, C.; Biagiotta, R.; Macchia, D.; Falagiani, P.; Ricci, M. and Romagnani, S. (1991) Purified protein derivative of *Mycobacterium tuberculosis* and excretory/secretory antigen(s) of *Toxocara canis* expand *in vitro* human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. *J. Clin. Invest.*, **88**, 346-350.
- [77] Coffman, R.L. and Mosmann, T.R. (1991) CD4+ T-cell subsets: regulation of differentiation and function. *Res. Immunol.*, **142**, 7-9.
- [78] Buijs, J.; Egbers, M.W.; Lokhorst, W.H.; Savelkoul, H.F. and Nijkamp, F.P. (1995) *Toxocara*-induced eosinophilic inflammation. Airway function and effect of anti-IL-5. *Am. J. Respir. Crit. Care Med.*, **151**, 873-878.
- [79] Kayes, S.G. (1986) Nonspecific allergic granulomatosis in the lungs of mice infected with large but not small inocula of the canine ascarid, *Toxocara canis*. *Clin. Immunol. Immunopathol.*, **41**, 55-65.
- [80] Pinelli, E.; Withagen, C.; Fonville, M.; Verlaan, A.; Dormans, J.; Van Loveren, H.; Nicoll, G.; Maizels, R.M. and Van der Giessen, J. (2005) Persistent airway hyper-responsiveness and inflammation in *Toxocara canis*-infected BALB/c mice. *Clin. Exp. Allergy*, **35**, 826-832.
- [81] Buijs, J.; Lokhorst, W.H.; Robinson, J. and Nijkamp, F.P. (1994) *Toxocara canis*-induced murine pulmonary inflammation: analysis of cells and proteins in lung tissue and bronchoalveolar lavage fluid. *Parasite Immunol.*, **16**, 1-9.
- [82] Kayes, S.G. (1997) Human toxocariasis and the visceral larva migrans syndrome: correlative immunopathology. *Chem. Immunol.*, **66**, 99-124.
- [83] Kayes, S.G.; Jones, R.E. and Omholt, P.E. (1987) Use of bronchoalveolar lavage to compare local pulmonary immunity with the systemic immune response of *Toxocara canis*-infected mice. *Infect. Immun.*, **55**, 2132-2136.
- [84] Kayes, S.G.; Omholt, P.E. and Grieve, R.B. (1985) Immune responses of CBA/J mice to graded infections with *Toxocara canis*. *Infect. Immun.*, **48**, 697-703.

- [85] Pinelli, E.; Brandes, S.; Dormans, J.; Fonville, M.; Hamilton, C.M. and Van der Giessen, J. (2007) *Toxocara canis*: effect of inoculum size on pulmonary pathology and cytokine expression in BALB/c mice. *Exp. Parasitol.*, **115**, 76-82.
- [86] Sakaguchi, S. (2005) Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat. Immunol.*, **6**, 345-352.
- [87] Belkaid, Y.; Sun, C.M. and Bouladoux, N. (2006) Parasites and immunoregulatory T cells. *Curr. Opin. Immunol.*, **18**, 406-412.
- [88] Othman, A.A.; El Shourbagy, S.H. and Soliman, R.H. (2010) Kinetics of Foxp3-expressing regulatory cells in experimental *Toxocara canis* infection. *Exp. Parasitol.*, **127**(2), 454-459.
- [89] Pinelli, E.; Dormans, J. and Van Die, I. (2006) *Toxocara* and Asthma. In *Toxocara: The enigmatic parasite*. (Celia Holland and Huw Smith., ed), pp. 42-57. CABI Publishers, Oxfordshire, UK.
- [90] Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.*, **7**, 145-173.
- [91] Bousquet, J.; Jeffery, P.K.; Busse, W.W.; Johnson, M. and Vignola, A.M. (2000) Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am. J. Respir. Crit. Care Med.*, **161**, 1720-1745.

Received: 02 February, 2011

Accepted: 22 March, 2011

Copyright of Endocrine, Metabolic & Immune Disorders - Drug Targets is the property of Bentham Science Publishers Ltd. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.