Anisakis simplex: dangerous — dead and alive?

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The risk of infection with *Anisakis simplex* and related parasites of fish has been recognized for some time, but it is now emerging that ingestion of material from dead parasites in food is also potentially dangerous. The resulting allergic reactions range from rapid onset and potentially lethal anaphylactic reactions to chronic, debilitating conditions. This review discusses the problems and clinical implications associated with *A. simplex*, other related conditions, and the way in which disease manifestations vary from person to person.

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Helminth infections of humans are not usually accompanied by immune hypersensitivity reactions despite the association of helminths with high level immunoglobulin (IgE) responses [1]. Exceptions include infections with *Echinococcus granulosus* (traumatic or surgical breakage of hydatid cysts can lead to lethal anaphylaxis) and exposure to allergens from *Anisakis simplex*. Several species of helminth are also accidental parasites of humans through ingestion of raw, smoked or undercooked fish (Figs 1,2). The commonest ones are cestodes (genera *Diphyllobothrium* or *Diplogonoporus*), digeneans of the family Heterophyidae (*Heterophyes* spp. and *Metagonimus yokogawai*), Opisthorchiidae (*Clonorchis sinensis* and *Opistorchis* spp.) and the nematode genera *Anisakis* and *Pseudoterranova* from the family Anisakidae [2].

The disease anisakiasis was first recognized 40 years ago from an eosinophilic intestinal lesion in a patient suffering from severe abdominal pain [3]. The majority of such cases are from Japan, where consumption of raw fish is common [4], and ~2000 anisakiasis cases are diagnosed annually. The number of cases is, however, increasing worldwide, with ~50 cases reported annually in USA and ~500 cases have been recorded in Europe.

Anisakis simplex causes direct tissue damage following invasion of the gut wall, development of an eosinophilic granuloma, perforation of the gut [5,6] and strong allergic reactions. Japanese authors have described the onset of urticaria and anaphylactoid syndromes associated with gastrointestinal infection by *A. simplex* [7]. Subsequently, *A. simplex* was identified as an etiological agent of allergic reactions mediated by IgE [8]. In Spain, recent cases involved clinical symptoms ranging from isolated angioedema and urticaria to life-threatening anaphylactic shock have been described. Some authors described, as in Japan, syndromes combining allergy and infection simultaneously, with unexpected pathologies including eosinophilic gastroenteritis, occupational diseases, rheumatic manifestations and contact dermatitis. Moreover, cases are now being reported in which IgE against *A. simplex* appears sporadically without clear clinical signs of allergy (termed here as sensitized).

Urticaria and anaphylaxis in food allergy

Acute urticaria and angioedema affects 20% of the population at some time in their lives, particularly in young adults [9]. Usually self-limiting and not life threatening, the condition is nevertheless unpleasant as a result of the intense itching, inability to sleep and even disfigurement when angioedema is present. Angioedema is associated with urticaria in 30% of cases, and is potentially life threatening because of the risk of oedema of the glottis. Anaphylaxis is a rapid onset and dangerous syndrome characterized by urticaria and angioedema, collapse, shock, bronchoconstriction and severe gastrointestinal symptoms.

The medical history of a patient is crucial in establishing the diagnosis of urticaria, angioedema or anaphylaxis because this usually reveals antigenic exposure during the previous few hours (food and drugs are common causes). Food allergy was responsible for up to 55% of cases of children with urticaria [10]. In a study involving 13 children and teenagers with fatal and near-fatal anaphylaxis, ingested allergens (mainly nuts and egg) were the responsible agents [11]. These results were similar to a French study in which food was the probable cause of 10% of anaphylaxis cases [12]. The foods most commonly involved in triggering anaphylaxis include egg, fish, nuts, shellfish, milk and fruits.

Clinical manifestations of allergy to *A. simplex*

Since 1995, >150 cases of allergy to the parasite have been reported in Spain, and a role for IgE has been indicated in all such cases. *Anisakis simplex* is now included in the standard sets of allergens for the investigation of food allergy, anaphylaxis and even drug allergies. The first patient studied had repeated episodes of anaphylaxis, each following the consumption of hake (*Merluccius merluccius*) [8]. The elevated levels of total IgE on several such occasions and the detection of IgE against *Ascaris lumbricoides* in the absence of *Ascaris* infection suggested that fish parasites could provoke episodes of relapsing

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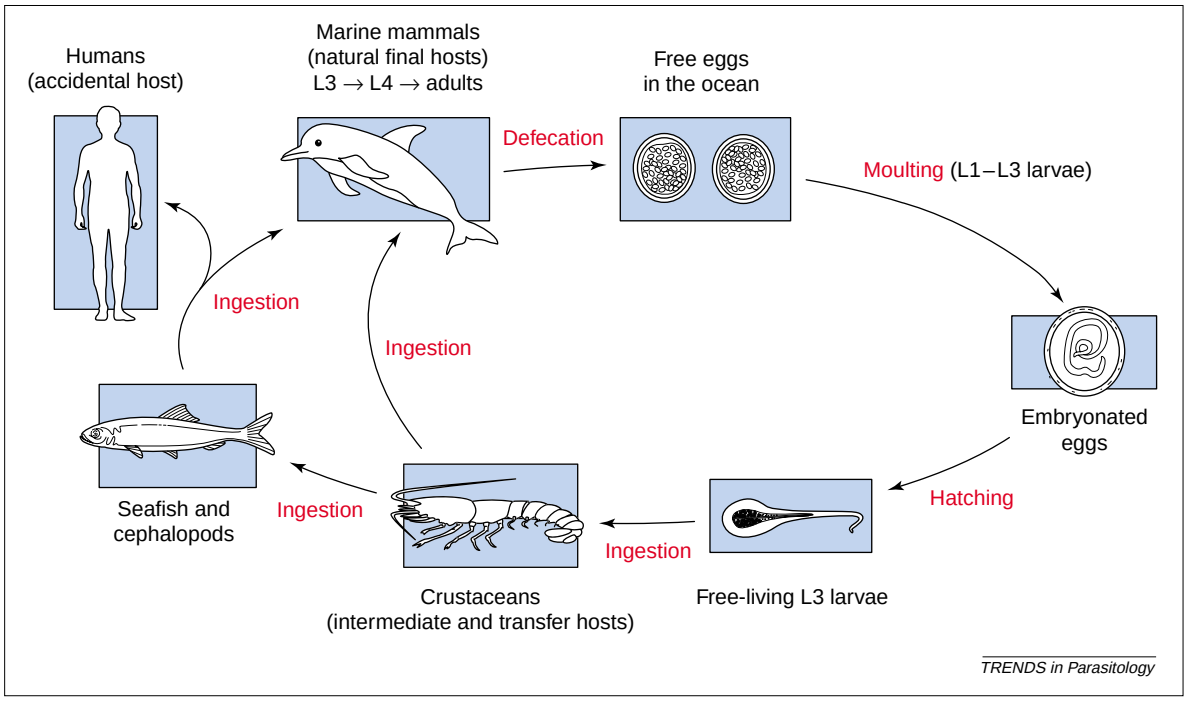

Fig. 1. Life cycle of *Anisakis simplex* including the intermediate host and food species which can be sources of human infection with the parasite. Fish can also become infected following ingestion of other infected fish. The precise details of the life cycles of *A. simplex* and other species of marine anisakid nematode have been the subject of some debate [63]. In the complete life cycle, there are three moults with each successive larval stage (L) being designated as larval L1, L2, L3, and L4,

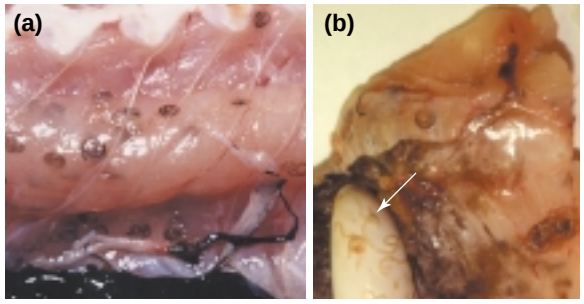

Fig. 2. (a) Fish muscle of hake infected with anisakid larvae. (b) *Anisakis simplex* larvae (indicated by an arrow) removed from infected hake with a gloved finger to illustrate scale. Larvae of *Anisakis* darken with age, but such dark coloured larvae could also be those of *Pseuterranova*, which is more common in northern seas.

anaphylaxis because *Ascaris* is antigenically cross-reactive with *Anisakis* [13]. The diagnosis of allergy to *Anisakis* was based on: (1) skin-prick testing with a somatic extract of *Anisakis* larvae (Fig. 3), (2) confirmation by detecting *Anisakis*-specific IgE (CAP™ assay, Pharmacia, Uppsala, Sweden) and (3) subsequent histamine release and immunoblot assays. IgE against the ascaridid nematodes *A. lumbricoides* and *Toxocara canis* has been detected in *Anisakis*-allergic patients [14], but the mean IgE levels against *A. lumbricoides* and *T. canis* were substantially lower than those against *A. simplex*. Worryingly, the *A. simplex* allergens are highly resistant to heat and freezing; therefore, cooking, which should kill the parasites, might not diminish the potency of their allergens [14,15]. These results suggested that: (1) episodes of anaphylaxis associated with the consumption of *A. simplex* can occur without previously recognized infections and (2) reactions to other parasite antigens from the order Ascaridoidae were cross-reacting with *Anisakis*.

Immunoblotting subsequently demonstrated the presence of *A. simplex*-specific antibody using sera from allergic patients [16] and also detected individual patterns of antigen recognition [17]. It was then asked whether there was a different pattern of IgE recognition in patients with allergic symptoms compared with sensitized patients without allergic manifestations. Sixty-one patients with positive IgE responses against *A. simplex* and a history of urticaria, angioedema or anaphylaxis were classified into three groups according to their history of previous consumption of fish and excluding other common causes of allergy: (1) allergic, (2) non-allergic controls and (3) patients with unexplained episodes (positive for IgE to *A. simplex* but no history of fish consumption in the 6–12 hours period before the allergic episode). Four discrete patterns were observed using IgE immunoblotting and a somatic extract of *A. simplex*. A predominant pattern was observed in *A. simplex* allergic patients (78%) – an antibody was detected against low and medium molecular weight allergens, whereas no reaction was observed in non-allergic controls. In the group with unexplained reactions, 40% showed evidence of reactions similar to those in allergic patients [18].

The Spanish cases

In the Basque region of Spain, ~90 g of fish is consumed per person per day, similar to that in Portugal (92 g), higher compared with the rest of Spain (85 g) but second only to Japan (239 g). These numbers could explain the recent description of >100 cases of allergy to *A. simplex* [19–21] in the north of Spain, but reports from central region of Spain are also becoming common [17,22,23]. Clinical manifestations vary from urticaria or angioedema (the

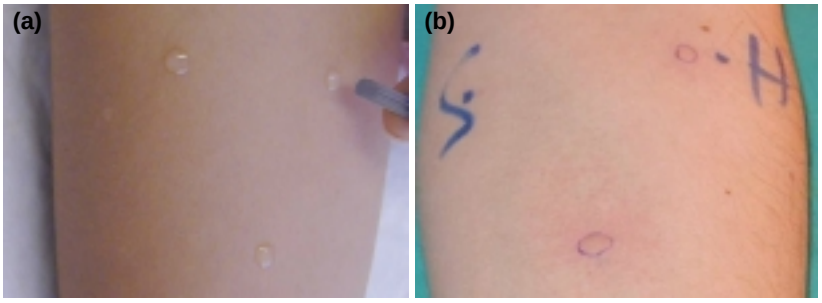

Fig. 3. Positive skin-prick test against *Anisakis simplex* allergen. (a) Initiation of test with saline [negative control indicated by S in (b)], 10 mg ml^{-1} histamine [positive control indicated by H in (b)], and 1 mg ml^{-1} *A. simplex* extract (unlabelled). The fluids are deposited onto the skin and introduced into the skin by pricking with a standard allergy lancet [8]. (b) A positive reaction can appear as a red wheal 15 m later. Resulting pauples (circled with blue pen) are measured, and a diameter of >3 mm represent a positive result indicated by the tested allergen (unlabelled) and positive control (H). Wheals are absent from the negative control (S).

majority of cases) to an almost fatal case of respiratory arrest. Alarmingly, anaphylaxis was reported in 27% of the patients. The first signs of an allergic reaction usually appear within 60–120 mins after ingestion of infected fish but can take up to six hours (classical anaphylaxis usually takes place within minutes to one hour of exposure, but the slower onset could be a result of the time taken for passage of allergens through the stomach and into intestinal tissue).

By contrast to common food allergies, some unexpected features were discovered in patients allergic to *A. simplex*, namely, a lack of previous atopic disease and an elevated mean age of the patient (aged 40–50 years) [17,19,23,24]. Other novel characteristics from their clinical histories include: (1) the patients and their general practitioners linked the symptoms with medications, although such causes were later rejected [17,19] and (2) the episodes occurred at night. In the north of Spain, *A. simplex* is now considered to be the main factor associated with urticaria and angioedema in adults following fish and shellfish consumption and is responsible for 8% of acute urticaria and angioedema cases [25] (M.D. Del Pozo, PhD thesis, University of Zaragoza, 1998). This constitutes a similar or even a higher prevalence compared with other sources of ingested allergens (fruits, nuts, shellfish and fish muscle).

It is noteworthy that >50% of *A. simplex*-allergic patients required emergency treatment, and five out of 64 were hospitalized [19] as a result of respiratory arrest (one case), severe shock (two cases) and persistent angioedema (three cases). The fish species most frequently involved in these cases were, in decreasing order, hake (*M. merluccius*), anchovies (*Engraulis encrasicholus*) and cod (*Gadus morhua*), although other studies have reported different rankings [23]. Half of the patients who presented symptoms had ingested raw fish; at least three patients were affected (presumably) by parasite material in canned fish and the remainder had eaten cooked fish.

Digestive manifestations

It is striking that the second most commonly reported symptoms in *A. simplex* allergy are confined to the digestive tract (~70%), whereas combined digestive and respiratory symptoms appear in only ~20% of allergies to fish muscle [26]. In some regions of Spain, cases designated 'gastroallergic anisakiasis' have been described because the allergic reaction (urticaria, angioedema and even anaphylaxis) and the infection were concomitant [23]. This raises the possibility that anisakiasis could be undiagnosed in such cases that were described as allergic only even if accompanied by digestive symptoms.

If we consider all the cases described so far, the need for a priming infection with a living parasite to induce sensitization cannot be excluded because a preceding episode of anisakiasis might have been undetected or wrongly attributed, and successive exposure to *Anisakis* allergens is highly likely in many populations as a result of the high prevalence of infection in fish [27]. Some authors consider that an infection is a prerequisite for *A. simplex* allergy [23], which is entirely feasible. There is experimental evidence indicating that some antigens from helminth parasites can generate IgE antibody responses without infection [28], although only a few cases of such autoallergenicity have been reported. Whether a prior infection is necessary or not, purified allergens from *A. simplex* are potent enough to cause anaphylaxis even as a result of a skin-prick test with an extract of the parasite [29].

Sensitization to *A. simplex* could also explain some cases of eosinophilic gastroenteritis in which the etiological agent had not been identified. In ten such cases reviewed by pathological studies, one of them possessed vestiges of larval nematodes [30].

Rheumatologic disorders and occupational association

Rheumatological symptoms have been described in association with *A. simplex* infection in a patient whose symptoms began with skin manifestations and arthritis [31]. A second allergic episode that involved skin symptoms and IgE positivity, the presence of parasites in the fish ingested on the day of the allergic reaction was confirmed. Cases of concomitant type III with type I hypersensitivity in humans have been described and were associated with exposure to parasite antigens, suggesting that *A. simplex* contributes towards rheumatic pathology [32].

Occupational cases of *A. simplex* allergy have been described in fishmongers [33], or were related to exposure (contact or inhalation) to fish meals in chicken feed [34]. Similarly, a case of protein-contact-dermatitis as a result of *A. simplex* has been described in a housewife in which both type I (IgE-mediated and immediate) and type IV (delayed) hypersensitivity were demonstrated [35].

Diagnosis of allergy to *A. simplex*

Over the past few years, *A. simplex* has progressed from being relatively unknown to a frequently recognized etiological agent of food allergy in the adult population [25]. The diagnosis of allergy to *A. simplex* is based on the following criteria: (1) a compatible history, such as urticaria, angioedema or anaphylaxis following fish consumption, (2) positive skin-prick test, (3) specific-IgE against *A. simplex* (radioimmunoassay) with values >0.7 kU l^{-1} considered as positive and (4) a lack of reaction to proteins from the host fish. Other foods and allergens should also be screened in such cases [36].

IgE immunoblotting with sera from allergic patients appears to be specific because cross-reactivity was not observed in sera from African patients suffering from other parasitoses [16]. However, when control populations (with no seafood consumption and with multiple parasitic diseases) were tested, the IgE immunoblot was susceptible to cross-reactions [37]. Interestingly, the *A. simplex* antigens recognized by the monoclonal antibody (mAb) UA3 were also detected in all patients with unambiguous *A. simplex* allergy [38]. It is therefore envisaged that the antigen recognized by this mAb could provide allergists with a tool for specific diagnosis of *Anisakis* allergy [37]. Currently, the best confirmation of food allergy is a double-blind challenge-test against a placebo [36,39]. This test has been evaluated in paediatric populations and correlates well with skin tests, radioimmunoassays and histamine release assays. In the study of *A. simplex* allergy, ethical considerations preclude exposure testing under the current regulations because parasites cannot be classified as food, despite being a common contaminant. Thus, the most appropriate diagnostic criteria for diagnosing *A. simplex* are: (1) compatible history of *A. simplex* allergy and skin-prick test, (2) specific IgE with or without an immunoblot and (3) antibody to *O*-deglycosylated UA3 epitope.

Sensitization without allergy symptoms

It is not rare to find positive IgE values against *A. simplex* in subjects who do not react allergically to this parasite. In these cases, the identification of specific IgE against the parasite cannot be considered as a reliable indicator of allergy, but can appear as a confusing factor because this antibody has been detected in 25% of otherwise healthy controls [25] (M.D. Del Pozo, op. cit.). Possible explanations for the existence of IgE against *A. simplex* without clinical manifestations could be: (1) cross-reactivity with other nematodes [13,14,40,41] (R. Iglesias, PhD thesis, University of Santiago de Compostela, 1998); (2) presence of a panallergen, such as tropomyosin, which occurs in crustaceans, insects and mites [42] (some authors also suggest that IgE can be stimulated by invertebrate tropomyosin in particular [43]); (3) cross-reactivity with carbohydrates or phosphorylcholine [44] and (4) cross-reactivity with glycans present in glycoproteins of other nematodes, or the presence of biotinyl-enzymes that can stimulate the production of IgE in some patients [37,45].

Serology

Serodiagnostic tests available for *Anisakis* reactivity include latex-based agglutination procedures, Ouchterlony tests and immunoelectrophoresis, immunofluorescence, indirect haemagglutination, complement fixation, immunoblotting and ELISA [46–53]. All of these methods use unfractionated or partially purified antigens, which can demonstrate poor specificity as a result of cross-reactivity with other parasite antigens [13,40]. This is particularly relevant to immunodominant carbohydrates that might be present in parasite glycoproteins [54]. There is still a need for a more specific serological assay, perhaps based on a cocktail of recombinant proteins.

Histology and endoscopy

The use of endoscopy, soon after the onset of gastric symptoms, has led to the observation of live larvae penetrating the mucosa [55]. When the larvae have already penetrated the wall (particularly in the intestines), a histopathological study of the resected area might prove useful in determining their presence. In the most advanced chronic cases, only a granulomatous lesion with larval cuticular debris remains [30,56]. In cases of acute abdomen with intestinal resections, pathologists should consider parasitism and search for parasite debris, and possibly also in eosinophilic gastroenteritis.

Epidemiology – populations most at risk

Out of the total ~14 000 cases of anisakiasis reported worldwide to date, ~95% are from Japan, where ~2000 cases are reported each year [4]. In Europe, the disease is (apparently) much less frequent, where only ~500 cases have been reported to date, of which >95% are from The Netherlands, Germany, France and Spain. In Spain, the number of reported cases has increased rapidly over the past few years as a result of increased awareness by physicians. In Japan, gastric anisakidosis is far more common than intestinal anisakidosis (95% of cases), whereas in Europe intestinal anisakidosis is more common. These differences might have resulted from a reporting bias, differences in diagnostic methods or epidemiological factors [57]. In Japan, there is more widespread use of endoscopic techniques and greater awareness of the disease [58].

The transmission of these food-borne pathogens is clearly related to traditions of consumption of raw or lightly cooked fish. Several fish dishes are considered to be high-risk, including Japanese *sushi* and *sashimi*, Dutch salted or smoked herring, Nordic *gravlax* (dry, cured salmon), Hawaiian *lomi-lomi* (raw salmon), South American *cebiche* and Spanish *boquerones en vinagre* (pickled anchovies).

Epidemiological studies in Japan indicated that anisakiasis was more frequent in coastal populations (particularly where people are involved in the fish industry) and in males aged 20–50 years [47]. The main sources of *A. simplex* were the spotted chub mackerel (*Scomber japonicus*) and Japanese flying squid (*Todarodes pacificus*) [59]. In western Europe, herring (*Clupea harengus*) is the main species involved [60], although cases with other species that were insufficiently cooked (microwaved, grilled or shallow-fried) have also been reported. In Spain, most cases were related to the consumption of pickled anchovies (*Engraulis encrasicholus*) and raw sardines (*Sardina pilchardus*) [23]. In the allergic cases from northern Spain, cooked hake predominates (*M. merluccius*) closely followed by

anchovies [19]. It is becoming clear that *A. simplex* is the most important food allergen in adult population of the Basque Country in northern Spain [25].

Japanese authors have also demonstrated that IgE responses to excretory or secretory antigens from *A. simplex* were produced in 87.5% of endoscopically diagnosed gastric-anisakiasis patients, in 75% of patients with urticaria induced by spotted chub mackerel, but only in 8.3% patients with urticaria of unknown origin, and in 19% of normal controls [61]. Results from a prevalence study of sensitization and allergy in a group of 150 cases of urticaria, angioedema or anaphylaxis with a control group (healthy blood donors) [25] indicated that the prevalence of sensitization to *A. simplex* was 26% in the group of acute urticaria patients and 12.6% in the blood donors group. Sensitization was considered clinically relevant only in 31% of the sensitization cases. Twelve patients diagnosed with an allergy to *A. simplex* would have been labelled as idiopathic if this new agent had not been investigated. Finally, IgE against *A. simplex* have also been detected in atopic dermatitis patients in Japan [61], hence there is a need to investigate the clinical impact of exposure to the *A. simplex* and its allergens.

Control measures

Current European Community regulations on food fish and products (Directive 91/493/EC and Decision 93/140/EC) require the visual examination of the fish, extraction of the visible parasites and the removal of those that are heavily parasitized from the market. Moreover, species intended for marinating or salting at temperatures <60°C must be stored at –20°C for 24 hours. The US Food and Drugs Administration (FDA) agency demands that all fish products not intended for cooking or processing at temperatures >60°C should be deep frozen at –35°C for >15 hours, or at –23°C for a minimum period of seven days [62]. This should reduce infection rates and, if *A. simplex* infection predisposes an individual to *Anisakis*-related allergic responses, there should also be a reduction in the prevalence of allergic reactions to ingested worm material in fish.

A simple but effective control measure is to inform the general public about *A. simplex* infection and the risks of eating raw or undercooked fish. Nevertheless, such measures might not be adequate considering the thermostability of the allergens involved in such allergic reactions. For many patients, it is difficult to avoid eating fish, but the simple precaution of avoiding whole small fish (e.g. anchovies) and the abdominal region of fish could be effective [24].

It still remains questionable whether the development of *Anisakis* allergy requires prior exposure to living parasites, and if desensitization is possible. The reason why some people vary in propensity to *Anisakis* allergy and the identification of the allergens involved still remains to be clarified.

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Resistance and susceptibility in human onchocerciasis – beyond Th1 vs Th2

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As research progress has led to programs for the elimination of onchocerciasis as a public health problem, research must now be intensified to protect elimination efforts. A profound understanding of the immunology in the human–parasite relationship is required for predicting the impacts of an altered immune response in a population post-microfilaricide treatment, and for the development of a vaccine against onchocerciasis, a highly desirable tool to guarantee sustained elimination success. This article summarizes the recent advancements in understanding the human immune mechanisms against onchocerciasis, and focuses on the new concept of T-cell suppressor responses as a major counterbalance mechanism for effector responses driven by T helper 1 and T helper 2 cells against the filarial worms.

Onchocerciasis, caused by the filarial nematode *Onchocerca volvulus*, is endemic in 37 countries and affects >17.7 million people. The infection is transmitted by blackfly (*Simulium*) species that acquire microfilariae (larval stage 1, L1) from infected humans during blood feeding. The L1 larvae mature into infective larvae stage 3 (L3) within 10–12 days in the vector. Within several months, L3 develop into female or male adult worms, which reside in the subcutaneous (s.c.) nodules (onchocercomas) for 10–15 years and produce millions of microfilaria (Mf). It is this Mf stage that is the cause of substantial morbidity. Blindness – the most devastating disease manifestation – is caused by inflammation in the eye as a result of Mf dying in the cornea. With visual impairment in 500 000 and blindness in 270 000 humans, onchocerciasis has remained the second most frequent cause of preventable blindness in Africa. Second in its impact on life, but a much more frequent disease manifestation is dermatitis, ranging from acute episodes of papular inflammation to chronic atrophy, lichenification and leopard skin.

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