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Toxocara canis: Molecular basis of immune recognition and evasion

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ABSTRACT

Toxocara canis has extraordinary abilities to survive for many years in the tissues of diverse vertebrate species, as well as to develop to maturity in the intestinal tract of its definitive canid host. Human disease is caused by larval stages invading musculature, brain and the eye, and immune mechanisms appear to be ineffective at eliminating the infection. Survival of *T. canis* larvae can be attributed to two molecular strategies evolved by the parasite. Firstly, it releases quantities of 'excretory–secretory' products which include lectins, mucins and enzymes that interact with and modulate host immunity. For example, one lectin (CTL-1) is very similar to mammalian lectins, required for tissue inflammation, suggesting that *T. canis* may interfere with leucocyte extravasation into infected sites. The second strategy is the elaboration of a specialised mucin-rich surface coat; this is loosely attached to the parasite epicuticle in a fashion that permits rapid escape when host antibodies and cells adhere, resulting in an inflammatory reaction around a newly vacated focus. The mucins have been characterised as bearing multiple glycan side-chains, consisting of a blood-group-like trisaccharide with one or two O-methylation modifications. Both the lectins and these trisaccharides are targeted by host antibodies, with anti-lectin antibodies showing particular diagnostic promise. Antibodies to the mono-methylated trisaccharide appear to be *T. canis*-specific, as this epitope is not found in the closely related *Toxocara cati*, but all other antigenic determinants are very similar between the two species. This distinction may be important in designing new and more accurate diagnostic tests. Further tools to control toxocariasis could also arise from understanding the molecular cues and steps involved in larval development. *In vitro*-cultivated larvae express high levels of four mRNAs that are translationally silenced, as the proteins they encode are not detectable in cultured larvae. However, these appear to be produced once the parasite has entered the mammalian host, as they are recognised by specific antibodies in infected patients. Elucidating the function of these genes, or analysing if micro-RNA translational silencing suppresses production of the proteins, may point towards new drug targets for tissue-phase parasites in humans.

1. Introduction

Toxocara canis is the most prevalent intestinal roundworm of dogs, foxes and other canid species, with zoonotic potential for human beings. In many temperate countries, toxocariasis is the most common helminth infection and causes a significant morbidity (Despommier, 2003; Hotez and Wilkins, 2009; Rubinsky-Elefant et al., 2010; Smith et al., 2009).

The nematode has many salient biological features, which contribute to its continuing presence in animal and human populations. Firstly, it is able to invade an extraordinarily wide range of hosts, from invertebrates and poultry (Galvin, 1964) through to mice and man (Strube et al., 2013); for all but the definitive (canid) species, these represent intermediate hosts which can, through

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predation, allow the parasite to reach its final definitive host species. Secondly, the larval stage is able to enter a long-term developmental arrest, which allows it to suspend life cycle progression whilst in a paratenic host, and to resume maturation to the adult stage only once reaching a canid species. Thirdly, even in female canids, developmental arrest occurs so that larvae can dwell in tissues until pregnancy occurs, and then migrate transplacentally or *via* colostrum to infect the foetal or newborn pup (Schnieder et al., 2011).

T. canis is highly prevalent in all canid populations that are not treated regularly with anthelmintics, and its infectivity to wild species renders elimination almost impossible. Furthermore, the arrested state has remarkable longevity: in experimentally infected monkeys for example, larvae remained viable in the tissues for 9 years and were able to infect mice on transfer (Beaver, 1962).

Within the paratenic host, *T. canis* larvae can migrate widely including the liver, musculature and the central nervous system, causing the well-characterised syndrome of visceral larva migrans (VLM) (Beaver et al., 1952; Carvalho and Rocha, 2011; Schantz, 1989). The propensity to invade the brain and the eye has given rise to particular concern in the human population, with ocular toxocariasis (OT) a recognised syndrome (Good et al., 2004), and neurotoxocariasis (NT) inferred from cognitive deficits, higher prevalence among epilepsy cases (Quattrocchi et al., 2012), and finding larvae in post-mortem brain samples (Hill et al., 1985). Current options for treatment of humans infected with tissue-dwelling larvae are of uncertain efficacy because of the covert nature of the infection and the incomplete resolution of symptoms (Othman, 2012; Wiśniewska-Ligier et al., 2012).

2. Immune recognition

Pathogens are recognised initially by the innate immune system reacting to intrinsically foreign molecular signatures associated with xenogeneic species, such as bacterial lipopolysaccharide, unmethylated DNA and fungal carbohydrates. Such signals are essential for innate immune sentinel cells, such as dendritic cells, to react to the presence of infectious organisms and to initiate pathogen-specific responses from the adaptive immune system. Currently, few such "pathogen-associated molecular patterns" (PAMPs) have been defined for any helminth parasite, but their existence in *T. canis* can be surmised by the strong adaptive immune response that occurs in this infection. The major feature of this adaptive immune response is production of *T. canis*-specific antibodies, associated with CD4+ T-helper type 2 cell (Th2) activity (Del Prete et al., 1991).

Such a Th2 response is characterised by release of a specific subset of mediators, in particular the type 2 cytokines IL-4, -5, -10 and -13, during infection. Subsequently, IL-4 promotes B cell differentiation and antibody class-switching, while IL-5 drives the differentiation of eosinophils, a marked feature of human *Toxocara* spp. infection (Beaver et al., 1952). T cell responses to *T. canis* in human beings are clearly of the Th2 type, and are stimulated by the "excretory–secretory" (ES) antigens of the parasite (Del Prete et al., 1991). However, no individual T cell specific antigen or epitope has yet been defined from *T. canis*.

Recently, the distinction between innate and adaptive arms of immunity has become much more blurred with identification of innate lymphoid cells, which can rapidly produce Type-2 cytokines (Neill et al., 2010) without requiring activation by dendritic cells (Smith et al., 2012). Indeed, it has been known for some time that some innate populations of cells can participate in the "Th2 response" to *T. canis*, for example non-B-non-T cells produce IL-5, even in T cell-deficient nude mice infected with the parasite (Takamoto et al., 1995). Moreover, eosinophilia in infected mice shows a biphasic response with early (day 10) and late (day 21) peaks; while the later peak is absent in CD4+ T cell-deficient mice, the earlier one is intact, indicating that it is generated by the innate rather than the adaptive Type-2 response (Takamoto et al., 1998).

Generation of specific antibodies provides the most definitive evidence for infection and is the basis for all diagnostic tests for toxocariasis; with ELISA and Western blot reactivity to *T. canis* ES (TES) antigens generally employed. However, the diagnostic field is still evolving with respect to the optimal target antigens that should be used, and more critically to the interpretation of antibody reactivity to an infection which is frequently covert and in which symptoms do not necessarily correlate with infection intensity or antibody titre (Smith et al., 2009).

There are now extensive data on the seroprevalence of anti-TES antibodies in human populations around the world, using the established ELISA. In older studies, seropositivity in Europe ranged from 3 to 7% in adults and 7 to 23% in children (Gillespie et al., 1993), while in the USA 13.9% of those over 6 years of age were antibody-positive (Won et al., 2008). Remarkably, seroprevalences exceeded 50% in tropical settings such as the Caribbean (Bundy et al., 1987; Magnaval et al., 1994). More recent studies similarly report seroprevalences of 14.5% in Polish teenagers (Jarosz et al., 2010), 13% in Turkish children (Doğan et al., 2007) and 9% in Iranian under-10s (Fallah et al., 2007), rising to 27% in Brazilian Amazonia (Rubinsky-Elefant et al., 2008) and 45–82% in different rural districts of Sulawesi, Indonesia (Hayashi et al., 2005). Hence widespread toxocariasis remains of great concern in most parts of the world.

The question of which serum antibody isotypes are most relevant still remains to be explored. Most human infections generate antibodies of the IgG1 subclass, with significant levels of both IgM and IgE (Smith, 1993). The IgG4 isotype, which can be predominant in other, more intense, tissue helminth infections such as filariasis and schistosomiasis (Maizels et al., 1995) is less prominent, although reportedly more evident in active cases of visceral larva migrans (Obwaller et al., 1998). An unresolved issue is whether the expression of IgE correlates with active infection and/or invasion of certain tissue sites in the host, or possibly mediates only bystander allergic-type reactions such as infection-related rashes (Magnaval et al., 2006). Related to this question is whether particular isotypes are functionally important in tissue immunity, for example by trapping larvae or activating Fc-dependent protection mechanisms.