CHAPTER THREE

Cross-species immunomodulation by zoonotic helminths: The roles of excretory/secretory products and extracellular vesicles

Marilyn Zumbado-Salas[a], Alicia Rojas[a,b], Rick M. Maizels[c], and Javier Mora[a,b,*]

[a]Centro de Investigación en Enfermedades Tropicales, University of Costa Rica, San José, Costa Rica
[b]Laboratory of Helminthology, Faculty of Parasitology, University of Costa Rica, San José, Costa Rica
[c]School of Infection and Immunity, Centre for Parasitology, University of Glasgow, Glasgow, United Kingdom
*Corresponding author. e-mail address: javierfrancisco.mora@ucr.ac.cr

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Abstract

Parasitic helminths display remarkable plasticity in their interactions with the host immune system. Zoonotic species can elicit markedly different immune profiles depending on the host, ranging from balanced responses that allow long-term parasite persistence with minimal pathology to concomitant responses leading to rapid clearance accompanied by varying degrees of inflammation and/or fibrosis. Central to this host-specific immunomodulation are helminth-derived excretory/secretory products (ESPs) and extracellular vesicles (EVs), which carry a diverse repertoire of bioactive molecules capable of modulating key immune pathways. These mediators influence both innate and adaptive immunity, promoting regulatory, type 2, or mixed inflammatory responses according to the host context. This review synthesizes current evidence on how zoonotic helminths employ ESPs and EVs to

Current Topics in Membranes, Volume 96
ISSN 1063-5823, https://doi.org/10.1016/bs.ctm.2025.08.001

fine-tune immune outcomes across natural, accidental, and experimental hosts. Elucidating these host-dependent dynamics offers valuable insights into parasite adaptation, the clinical manifestations of zoonotic infections, and the potential use of helminth-derived molecules as innovative immunotherapeutics.

1. Introduction

Helminths are a diverse group of metazoa classically divided into two phyla: Nematoda and Platyhelminthes, with the latter comprising trematodes and cestodes (Cháves-González et al., 2022). These three major groups of organisms have been demonstrated to release excretion-secretion products (ESPs) and extracellular vesicles (EVs). ESPs correspond to a wide variety of helminth-derived proteins, carbohydrates and lipids released within mammalian hosts or into *ex vivo* culture media (Doyle & Wang, 2019) and can be actively produced by helminths to interact with their surroundings or passively distributed as part of their normal metabolism (Harnett, 2014). ESPs frequently modulate the host immune response which may result in the development of concomitant immunity or the tolerance and survival of parasites in their hosts for prolonged periods of time (Maizels et al., 2018; Stear et al., 2025). However, parasite-derived ESPs can act as targets for the death or expulsion of the helminth, and thus have provided valuable sources of antigens for helminth immunodiagnosis and vaccines against worm infections (Hewitson et al., 2015). On the other hand, EVs are lipid membranous nanoparticles secreted by different cell types to the extracellular space (Doyle & Wang, 2019) which are generally originated through the endosomal system or after shedding of the plasma membrane (Niel et al., 2018). EVs cargo include proteins, lipids, and nucleic acids of various types (Doyle & Wang, 2019; Liu & Wang, 2023), such as dsDNA, ssDNA, miRNA, tRNA or lcRNA, which have been demonstrated to be involved in the host-parasite interaction (Buck et al., 2014; Hoffmann et al., 2020). These structures can be categorized as exosomes, microvesicles, or apoptotic bodies (Doyle & Wang, 2019; Liu & Wang, 2023) based on their biogenesis, release pathways, size, content, and function (Doyle & Wang, 2019).

2. How do helminth release ESPs and EVs?

ESPs have now been analyzed in a number of zoonotic helminth species such as *Toxocara canis* (Wu, Liotta et al., 2024), *Ancylostoma caninum*

(Eichenberger et al., 2018), *Trichinella spiralis* (Yang et al., 2020), *Fasciola hepatica* (Sánchez-lópez et al., 2020) and *Echinococcus* spp. (Nicolao et al., 2019) (Fig. 1). ESPs are secreted from specialized excretory or secretory organs, as well as from cuticular and tegumental surfaces. For instance, esophageal glands have been involved in the ESP secretion in some nematodes of the genera *Chabertia, Necator, Nippostrongylus, Oesophagostomum, Ostertagia, Stephanurus* and *Trichostrongylus* (Ogilvie et al., 1972; Rhoads, 1981; Rothwell & Merritt, 1974). In addition, ESPs may also be released by stichocyte cells that surround the esophagus, as it happens in the adenophoreans *T. spiralis* and *Trichuris muris* (Jenkins & Wakelin, 1977). Moreover, it has been found that *T. canis* larvae release ESPs also from the anal secretory glands and excretory pores (Hogarth-Scott, 1966), with monoclonal antibodies to different ESPs localizing to the esophageal gland

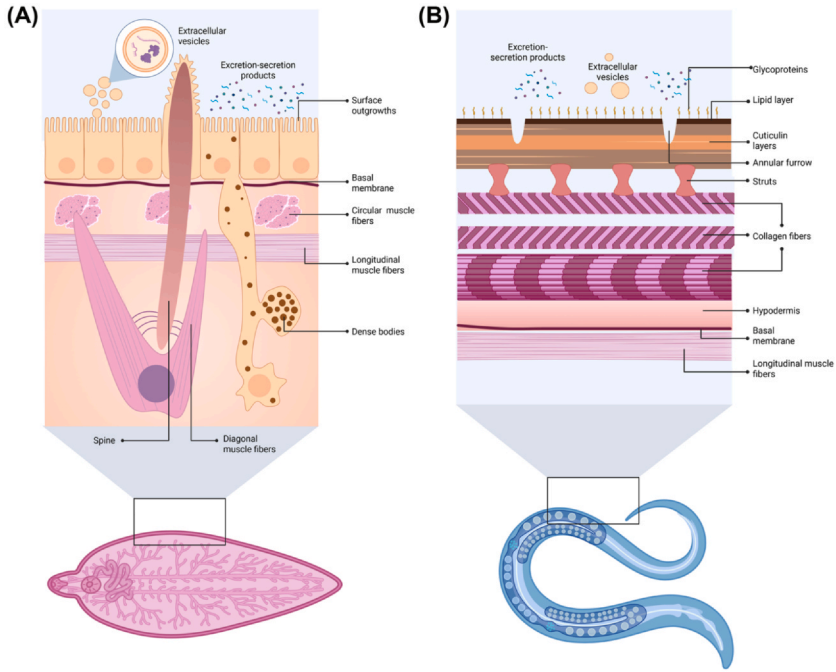

Fig. 1 Schematic representation of the outer surface of trematodes and nematodes. (A) The tegument is a metabolically active surface with different cells responding to stimuli with the release of excretion or secretion products or extracellular vesicles (EVs). Instead, the nematode´s cuticle (B) is formed by rich layers of cuticulin and collagen, thus is more impermeable and releases less number of EVs, when compared to platyhelminthes. *Adapted from Poddubnaya et al. (2019) and Blaxter and Robertson (1998). This figure was created with Biorender.*

and the midbody secretory column that leads into the excretory pore; secretory and amphidial pores were also bound by lectins recognizing secreted glcyoproteins (Page, Hamilton et al., 1992; Page, Rudin et al., 1992). In *Schistosoma mansoni* miracidia, ESPs can be shed from the apical and lateral penetration glands and across the eggshell by shell pores (Hang et al., 1974; Kloetzel, 1967) as well as the preacetabular glands of schistosomal cercariae (Doyle & Wang, 2019; Minard et al., 1977; Pino-Heiss & McKerrow, 1986; Stirewalt, 1934). Furthermore, there is also evidence of ESPs being released from other glandular tissues of *S. mansoni* (Minard et al., 1977; Pino-Heiss & McKerrow, 1986; Torre-escudero et al., 2019).

The biogenesis of EVs has not been well characterized particularly regarding their cellular source and secretion pathways, as well as the identification of the precise site of secretion (Shimomura et al., 2021). Proteins associated to EVs biogenesis have been analyzed by using mass spectrometry with the subsequent identification of the endosomal sorting complexes required for transport (ESCRT) components, lipid mediators, Rab proteins involved in the multivesicular body docking to the plasma membrane and other molecules described in EV biosynthesis and delivery in mammal systems (Jeppesen et al., 2023). Alternatively, some components of the ESCRT pathways have been found in the EV content or in genomes of certain organisms, suggesting that this pathway may be involved in EVs production in helminths (Rojas & Regev-Rudzki, 2024). In the case of *Heligmosomoides polygyrus*, mass spectrometric analysis of ESP EVs revealed multiple proteins associated with the worm intestinal epithelium, suggesting that tissue as a source of secreted EVs (Buck et al., 2014). In order to identify the anatomical locations where EVs are released from helminth surfaces, techniques such as transmission electron microscopy (TEM) (Drurey & Maizels, 2021; Harnett, 2014; Rooney et al., 2022) and fluorescence confocal microscopy using membrane-binding dyes (Shimomura et al., 2021) have also been used. Through the application of these strategies, EVs have been demonstrated to be released from different anatomical sites in metazoan parasites throughout their life cycle, depending on their metabolic requirements and the site of interaction with their hosts (Drurey et al., 2020). EVs may be released from the gut of helminths, or their outer surface, such as the cuticle in nematodes or the tegument of trematodes or cestodes.

The tegument and cuticle are structurally and metabolically different surfaces (Bennett et al., 2020) and these differences have a direct impact on the number and physicochemical characteristics of the vesicles produced as well as the lipid composition of the vesicle (Drurey & Maizels, 2021;

Sánchez-López et al., 2021) (Fig. 1). The tegument of platyhelminths is metabolically active due to the presence of a syncytial epithelium involved in absorption and secretion of different molecules, nutrients, and metabolites (Poddubnaya et al., 2020) (Fig. 1A). On the other hand, the nematode cuticle is not metabolically active since is composed of an extracellular matrix of cuticulin and collagen that cross-link and protect the worm´s body (Bennett et al., 2020) (Fig. 1B). This molecular difference is likely to account for the ability of platyhelminths to produce greater quantities of EVs when compared to nematodes (Sánchez-López et al., 2021). TEM images have shown EVs being released from the tegument of the cestode *Echinococcus multilocularis,* the lancet liver fluke *Dicrocoelium dendriticum,* and *S. mansoni* cercariae (Liu et al., 2023; Sánchez-López et al., 2021). In the case of nematodes, vesicles budding from the intestinal epithelium have been identified in the model nematode *H. polygyrus* (Buck et al., 2014). During the adult intestinal stages of this parasite, In addition to the tegument, EVs may also be secreted from inner organs like the gut in nematodes (Drurey & Maizels, 2021), as well as the gastrodermus (Drurey et al., 2020) or protonephridial cells (Sánchez-López et al., 2021) in trematodes prior to their release with other fluids in the anterior or posterior openings of these parasites (Drurey et al., 2020; Sánchez-López et al., 2021).

Studies using TEM in *F. hepatica* have confirmed the release of two subpopulations of nanoparticules: one recovered after 15,000 *x* g centrifugation (15k) and another one obtained after 120,000 *x* g ultracentrifugation (120k) (Cwiklinski et al., 2015). It is proposed that EVs obtained after 120k may have an endosomal origin and could be released from the protonephridial system whilst larger 15k EVs are released from the gastrodermal epithelial cells lining in the gut (Torre-escudero et al., 2019). These data suggest that EV release in helminths is highly complex, possibly due to the simultaneous activation of both ESCRT-dependent and -independent pathways (Abou-El-Naga & Mogahed, 2023), as well as the compartmentalization of worms into specialized tissues and systems from which EVs are secreted (Dagenais et al., 2021). Furthermore, EVs and ESPs from the same species may differ in size or biochemical composition, not only for the reasons mentioned above, but also due to the adaptation to different host species, internal host niches, external environmental conditions and the variety of interacting cell types. Considering these peculiarities, helminth-derived secretomes (including ESPs and EVs) are highly diverse, complex, dynamic and, to some extent, unpredictable (White et al., 2023).

3. Internalization of EVs by host cells

There is growing evidence that EVs released by helminths, such as *F. hepatica,* can be internalized by host cells through a series of intracellular signaling events (Torre-escudero et al., 2019), where endocytosis may be the main mechanism for the uptake of parasite-derived EVs by recipient cells (Boysen et al., 2020). Another proposed mechanism, is the fusion of EVs with the host's plasma membrane, which has been described for the protozoan *Trypanosoma brucei* (Torre-escudero et al., 2019). This mechanism has been suggested in helminths based on the description of certain proteins with fusogenic potential in the surface of *F. hepatica* EVs, such as myoferlin, EHD1 and GAPDH (Torre-escudero et al., 2019).

During the process of EV uptake, ligand-receptor interactions between EVs and the host cells are essential to enable vesicle coupling and the subsequent internalization. However, in helminths the molecular basis of these interactions, as well as potential active or passive mechanisms involved in EV uptake by host cells remain poorly understood (Sánchez-López et al., 2021). It has been proposed that internalization of *F. hepatica* EVs by macrophages may be influenced by surface-exposed proteins in EVs and the interaction of host lectins with carbohydrates exposed in these nanoparticles (Torre-escudero et al., 2019). Additionally, EVs released by *S. mansoni* contain glycoconjugates, which may be mediators of their internalization by monocyte-derived dendritic cells via DC-SIGN receptors, constituting some of the first findings related to EVs molecules in helminths that can act as ligands for receptors on host immune cells (Kuipers et al., 2020).

4. Immune effects of helminth-derived ESP and EV in different hosts

Most studies analyzing the interactions of helminth-derived ESPs and EVs with their hosts have been focused on their immunoregulatory potential using experimental in vitro and in vivo models (Drurey & Maizels, 2021). These studies have significantly advanced our understanding on the effects of these particles in their hosts, leading to their investigation as potential infection biomarkers or tools for immune regulation in specific diseases (Rooney et al., 2023). Immune responses induced by helminths, regulated mainly by ESPs and EVs, are complex and

depend on several factors such as degree of adaptation to the host, helminth localization within the host, stage of the infection, and parasite burden, among others.

The immune response against helminths is complex and highly regulated. Traditionally, it has been characterized as a type 2 response, primarily driven by the activation of Th2 cells and the secretion of cytokines such as IL-4, IL-5, IL-9, and IL-13 (Grencis, 2015). However, this response extends beyond T helper cell polarization and encompasses a broader type 2 immune program including the activation of innate immune cells such as eosinophils, basophils, mast cells, group 2 innate lymphoid cells (ILC2s), alternatively activated (M2) macrophages, and the production of IgE antibodies (Maizels & Gause, 2023). Type 2 immunity comprehends a spectrum of responses which may act closely in crosstalk with other immune mechanisms (Gause et al., 2020). This spectrum includes, but is not limited to, (a) Regulatory type 2 responses, often referred as anti-inflammatory Th2, combining type 2 mechanisms with production of cytokines such as IL-10 and TGF-β, and Treg activation; (b) Effector type 2 responses, which can be predominantly dominated by type 2 mediators and have a crosstalk with IL-17 driven, or type 1 immune mechanisms; (c) Tissue repairing type 2 responses, which are associated with mechanisms associated with wound healing and fibrosis; and (d) pathogenic or harmful type 2 responses, associated with type 2 unregulated immune responses and allergic reactions (Harris & Loke, 2017).

The complexity and heterogeneity of helminths are reflected in the intricate interactions with their hosts and the immune response induced by different species. In intestinal parasites a strong type 2 response is associated with host resistance and worm expulsion. However, even in helminths inhabiting the gut there are some differences in the elicited immune response. A canonical type 2 effector response is effective in eliminating parasites confined to the intestinal lumen. In contrast, resistance to helminths that penetrate the intestinal mucosa requires a coordinated interplay between IL-17–mediated mechanisms and a subsequent type 2 tissue repair response (Maizels & Gause, 2023; Zaiss et al., 2024).

On the other hand, in tissue-dwelling parasites, such as zoonotic helminths, the immune response is more complex and highly dependent on the specific niche within the host and the stage of the infection. In some cases, regulatory type 2 responses are associated with parasite survival, whereas a combination of type 2 effector responses with type 1 and tissue repair mechanisms are required for resistance. This heterogenicity increases

in zoonotic helminths that may have a different degree of adaptation to humans. The following sections will explore the effects of ESPs and EVs on the immune response in the natural host of zoonotic helminths, as well as in humans and experimental models.

4.1 Toxocara canis

Toxocara canis has an indirect life cycle with domestic and wild canids acting as definitive hosts. Therefore, full sexual development of adult worms usually occurs in young dogs where female *T. canis* adults in the dog's gut shed eggs which are released in the animal's feces. Approximately one week after deposition, eggs embryonate in soil and L2 larvae develop inside them, becoming fully infective to paratenic and canid definitive hosts (Despommier, 2003). In puppies, eggs hatch in the small intestine where L2 larvae perforate the intestinal wall, reach blood vessels, pass through the liver, lungs and heart and later disseminate through systemic circulation by a complex pathway known as the Loss cycle (Wu & Bowman, 2022). After they reach the lungs, they ascend the trachea as L3 larvae, where they are coughed and/or swallowed, until they get to the small intestine, and develop into L4 and adults. Importantly, the life cycle of *T. canis* larvae is halted by the immune response elicited in adult dogs, or in accidental or paratenic hosts such as humans or mice, respectively. In these cases, L3 larvae do not reach the intestine, and therefore, adults are not developed (Strube et al., 2013). Consequently, L3 can remain in an arrested phase or persist in tissues for long periods, causing significant tissue damage and inflammation (Mulvenna et al., 2009; Razaul & Khan, 2023).

In humans, *T. canis* larvae have been found in the liver, lungs, heart, eyes and brain, causing visceral, ocular or cerebral *larva migrans* (Ma et al., 2018; Yoshida et al., 2022). Additionally, larvae are usually encapsulated within granulomas where parasitic stages are destroyed or remain viable for years (Mulvenna et al., 2009; Razaul & Khan, 2023; Sun et al., 2019). Due to these fundamental biological differences in the life cycles in different hosts, it is suggested that findings in murine or canid models may not be extrapolated entirely to what may occur in humans.

T. canis ESPs, known as TES, are conjectured to be pathogen-associated molecular patterns (PAMPs) due to the strong immune response that occurs after this infection (Maizels, 2013). TES contain a large number of glycosylated molecules and proteins such as TES-26 (TcPEB-1), TES-32 (Tc-CTL-1), TES-70 (Tc-CTL-4) and TES-120 (MUC-1 to 5) (Maizels, 2013). These molecules are responsible of modulating immune responses

during infection and actively participating in different processes such as antigen presentation, apoptosis of astrocytes, immune cell adhesion, and T cell polarization (Chou & Fan, 2020; Hewitson et al., 2009; Jeppesen et al., 2023; Loukas et al., 2000).

Studies regarding the immune response induced by *T. canis* in canids are limited, most likely because of the difficulty of maintaining dogs in the laboratory. However, understanding in detail the molecular interactions between *T. canis* and its canid hosts would provide valuable information regarding ascarids interactions with their natural hosts, like *Ascaris lumbricoides* and humans. Helminths are specialized immune regulators in their natural hosts which is associated with a close co-evolution process (Moreau & Chauvin, 2010). Peripheral blood mononuclear cells from pregnant dogs and puppies infected with *T. canis*, showed an initial predominance of IL-10 and repression of IFN-γ after stimulation with *T. canis* L2 ESP antigens. This was observed especially during gestation, favoring the reactivation of larvae in pregnant dogs and the persistence of the disease in puppies (Torina et al., 2005). Moreover, transcriptional analysis of PBMCs from infected dogs during the lung infection period revealed that *T. canis* regulates the host immune response by affecting the Notch signaling pathway, Toll-like receptor signaling pathway, and ECM-receptor interaction pathway (Cai et al., 2022), suggesting an important immune regulation induced by *T. canis* in its natural hosts.

In mouse models, some similarities with the immune response observed in canids have been reported. TES have been associated with immunomodulatory activity since a reduced proinflammatory activity of macrophages was observed in murine models, accompanied by a low production of IL-12 and Th1 polarization (Kuroda et al., 2001), with a progressive increase in Foxp3-expressing cells that was observed within and around *Toxocara*-induced granulomas and inflammatory foci in infected Swiss albino mice (Othman et al., 2011). However, it is important to consider that the immune response observed depends on the mouse strain used as a model for Toxocariasis. In general, rather than a strong immunoregulatory response as described for canids above, the immune response against *T. canis* in murine models is characterized by a mixed response, with a predominance of type 2 immune activation. Thus, TES from migratory larvae are involved in triggering innate immune responses and recruiting neutrophils, eosinophils, macrophages, basophils and lymphocytes (Pinelli et al., 2005). Furthermore, a predominantly adaptive immunity is observed in murine models, characterized by the cellular differentiation of CD4 + T

helper cells to type 2 subsets, and the release of type 2 cytokines that mediate the differentiation of B cells (Ruiz-Manzano et al., 2019). However, a polarization towards Th2 and Th17, accompanied by the secretion of cytokines such as IL-4, IL-5, IL-6, IL-13, IL-17, and IL-33 produced by both innate and adaptive immune cells has also been described (Długosz et al., 2015). This cytokine cocktail promotes IgE production, strong eosinophilia, and M2 macrophage polarization, which are characteristic features of the immune response against helminths (Resende et al., 2015; Rieu et al., 1994).

Humans act as dead-end hosts for *T. canis* and their immune response halts the migration and development of L3 larvae, thereby mirroring what may occur in mice. In addition, humans usually show a strong adaptive response stimulated by TES. This response is associated with the activity of Th2-type CD4+ helper T cells (Del Prete et al., 1991), characterized by the release of IL-5, which promotes eosinophil differentiation (Maizels, 2013). Initially, there is an acute inflammation characterized by larvae partially surrounded by a collagen capsule, with eosinophils, neutrophils and, to a less extent, macrophages. In a chronic stage, larvae are usually encapsulated by mature granulomas, the central portion of which is composed of mononuclear cells. Later, the death of *T. canis* larvae triggers early and late hypersensitivity responses. Therefore, granuloma formation is considered a manifestation of delayed hypersensitivity mediated by Th1 cells (Kayes, 1997), but also favors, in some cases, the encapsulation of larva in host tissues promoting larvae permanence and prolonged infectivity.

Even though several responses against TES have been elucidated mainly by using murine models, many aspects of the effect of TES or TEVs are uncertain in the pathology observed in humans. In particular, very little is known about *T. canis* EVs effects on the pathogenesis and immune regulation induced by this parasite. Recently, the composition of *T. canis* EVs was described comparing the protein content of isolated EVs and EVs-depleted medium collected from cultured larvae (Wu, Fu et al., 2024). Interestingly, the molecules exclusively present in EVs include neprilysin-1, malate dehydrogenase, cathepsin D, and peroxiredoxin, which could have an important role in immune regulation at different levels. Mammalian neprilysin (NEP) has been reported to be associated with an increased inflammatory response (Atta et al., 2024). NEP is a membrane-bound protein with enzymatic activity, and considering that nematode neprilysin-1 has been reported to be the member of this family most similar to mammalian NEP (Spanier et al., 2005), and that proteins present in the membrane of EVs can

be expressed in the surface of host cells after EVs fusion or uptake (Papareddy et al., 2024), this protein may have an important role in the inflammatory response induced by *T. canis*. In the case of malate dehydrogenase, this enzyme catalyzes the interconversion between malate and oxaloacetate as a part of the tricarboxylic acid (TCA) and is also a critical component of the malate-aspartate shuttle that promotes oxidative phosphorylation through electron transport into the mitochondria during glycolysis (Wolyniak et al., 2024). T cells require a metabolic switch to glycolysis in order to become activated effector cells (Pearce, 2016), which may be supported by this enzyme since it has been reported that cytosolic malate dehydrogenase activity potentiates a glycolytic metabolism in cancer cells (Hanse et al., 2017). Moreover, the malate shuttle is known to maintain the balance of NAD^+/NADH between the cytosol and mitochondria. However, additionally it provides longevity to chronic-infection-induced exhausted T cells protecting them against ammonia-induced cell death (Kumar & Delgoffe, 2023). Therefore, this enzyme may play an important role modulating T cell activation in *T.canis* infections. Another protein exclusively found in *T. canis* EVs is cathepsin D, which plays a key role in antigen presentation and inflammation (Hausmann et al., 2004). Additionally, Cathepsin D may represent an immune evasion mechanism delivered in *T. canis* EVs inducing apoptosis of immune cells (Conus et al., 2008). Finally, EVs containing peroxiredoxin may be important in the inflammatory response induced by *T. canis*, since this enzyme can act as a cytoprotective antioxidant during inflammation, as well as a modulator of redox signaling, and as a DAMP or PAMP recognized by innate immune cells (Knoops et al., 2016). Even though functional studies are required to determine the importance of *T. canis* EVs cargo in the pathogenesis and immune response induced by this parasite, these initial data suggest that *T. canis* EVs contain important molecules capable of modulating intracellular and metabolic pathways in host immune cells with great potential to be targeted therapeutically in this zoonosis.

4.2 *Ancylostoma caninum, Ancylostoma braziliense* and *Ancylostoma ceylanicum*

Hookworms are a wide group of parasites from the family Ancylostomatidae, which have high host specificity and direct life cycles (Loukas & Prociv, 2001). Hookworm species naturally infecting humans include *Ancylostoma duodenale* and *Necator americanus*, whereas *A. caninum* or *A. braziliense* normally infect canid hosts (Loukas & Prociv, 2001). Moreover, *A. ceylanicum*

has been described in the past decade as a zoonotic intestinal pathogen found in dogs, cats and humans, producing gastrointestinal infections (Colella et al., 2021). Hookworm adults reproduce in the small intestine of their natural definitive hosts where eggs are shed in feces (Loukas & Prociv, 2001). Approximately 2 days later, larvae hatch and further develop in soil where they become infective L3 larvae. L3 larvae penetrate the skin of definitive hosts, and are transported through the blood vessels following the Loss cycle (Alex Loukas et al., 2016) until reaching the small intestine where they mature into adult worms. These adults are responsible for blood loss and anemia through their persistent attachment to the intestinal wall and intestinal arteria. Additionally, like *T. canis, Ancylostoma* spp. show transmammary and transplacental infection of L3 larvae from the mother to her offspring (Shepherd et al., 2018), thereby explaining infection in puppies or newborns.

Zoonosis occurs when *Ancylostoma* spp. associated with dogs infect humans using a percutaneous route. The main etiologic agents of cutaneous *larva migrans* are *A. caninum* and *A. braziliense* (Caumes, 2006). In these cases worms do not fully develop in humans since these represent accidental hosts for the canid-associated parasites (Loukas et al., 2016). Therefore, development of infective L3 is halted in skin, where strong eosinophilic inflammation is usually observed 0.5 mm below the epidermis (Beaver, 1956). Conversely, intestinal infections with decreased villi height and increased depth of Lieberkuhn crypts have been observed in hamsters during *A. ceylanicum* experimental infections, which according to some authors share a similar reaction to humans (Alkazmi & Behnke, 2010).

The main proteins isolated from *A. caninum* larvae belong to the *Ancylostoma* secreted protein (ASP) family, a cysteine-rich protein group that is part of the wider CAP class of proteins. ASPs are believed to act as neutrophil inhibitory factors due to the percentage of similarity with its homologue in humans. In addition, it is proposed that ASPs can inhibit the phagocytic ability of neutrophils (Rieu et al., 1994). ASPs are fast release proteins, which may suggest important functions in the parasite's early developmental stages. As modulators of host immune responses, other ASPs also show homology with the peptide toxin helothermine, suggesting a potentially toxic effect on the host's immune effector cells (Hawdon et al., 1996).

Mice treated with *A. caninum* ESPs and dextran sulfate sodium (DSS) as a colitis inducer, lead to the overexpression of MUC13 and MUC2 (Sotillo et al., 2017). Overexpression of MUC13 has been evident in the lamina propria, however, the role of this mucin is uncertain since it has been related

to inflammatory, anti-inflammatory and antiapoptotic effects (Sotillo et al., 2017). Additionally, MUC13 plays an important role in gastrointestinal homeostasis, and could be playing a key role in the regeneration of the intestinal barrier (Sotillo et al., 2017). MUC2 is the primary component of intestinal mucus and maintains the integrity of the epithelial barrier. It also contributes to intestinal homeostasis by producing tolerogenic signals that are crucial for protection against colitis. In addition, overexpression of MUC2 by goblet cells has been demonstrated after ASPs exposure in mice (Sotillo et al., 2017). These data reinforce the hypothesis that *A. caninum* ESPs can restore intestinal homeostasis and integrity during colitis (Sotillo et al., 2017), and induce a tolerogenic response in mice (Ferreira et al., 2013). This pathology suppression has been associated with the induction of type 2 cytokines, characterized by the co-expression of IL-4 and IL-10 by CD4 + T cells, decreased proinflammatory cytokine expression in draining lymph nodes and the colon, and the recruitment of alternatively activated macrophages and eosinophils (Ferreira et al., 2013). Correspondingly, similar results were obtained evaluating the role of *A. ceylanicum* ESPs in DSS-induced colitis in BALB/c mice showing reduced colonic inflammation and a downregulation of Th1 and Th17 cytokines (Cançado et al., 2011). Regarding canine models, an immunomodulatory role is also observed. In vitro studies of peripheral blood mononuclear cells exposed to L3 ESPs of *A. caninum* showed that these ESPs produce Foxp3$^{(high)}$ T cell modulation, induction of IL-10 secretion mainly by CD8 T lymphocytes, inhibition of polyclonal T cell proliferation and prevention of dendritic cell maturation, leading to a tolerant immune response, promoting an anti-inflammatory environment (Junginger et al., 2017).

ESPs associated with *A. ceylanicum* also include proteins enriched in CAP domains, which belong to sperm coat protein-like proteins. The function of these proteins is unclear, but in other hookworm species they have been implicated in larval skin penetration, the transition from free to infectious phase, and the modulation of the host immune response (Uzoechi et al., 2023). In particular, AceES-2 plays an important role in host-parasite interaction in a hamster model, and it has been proposed that targeting this molecule through immunization may be a useful strategy to mitigate hookworm-associated pathology (Bungiro et al., 2004). Moreover, *A. ceylanicum* fatty acid and retinol binding protein-1 (AceFAR-1) has been found abundantly in eggs, as well as in adult males and females. It was detectable in the hypodermis, ovaries, uterus, and testes. This suggests that Ace-FAR-1 may contribute to fatty acid uptake for cuticle integrity, egg

production, and embryonic development (Abuzeid et al., 2020). Retinol-binding proteins-1 (RBP-1) are considered both immunogenic and immunomodulatory. They are immunogenic because they have been recognized by antibodies in infected individuals and can induce a significant immune response (Zhan et al., 2018). At the same time, they possess immunomodulatory properties characterized by their ability to bind to host lipid signaling molecules, interfering with specific immune pathways (Ryan et al., 2022). This interference leads to suppression or redirection of inflammation, which favors parasite survival (Wong et al., 2025).

To date, there are no studies available characterizing the content of EVs of zoonotic Ancylostomas or their functional effect on immune regulation. However, analysis of EVs from the rodent parasite *Nippostrongylus brasiliensis*, which has been used as a model for human hookworm infection revealed the presence of several proteins with immunomodulatory potential in these vesicles. Additionally, *N. brasiliensis* EVs intraperitoneal injection reduced the inflammatory response in a DSS-induced colitis model suppressing proinflammatory cytokines such as IL-6, IL-1β, IFNγ, and IL-17a, and upregulated the expression of the anti-inflammatory cytokine IL-10 (Eichenberger et al., 2018). This suggests that EVs of *A. caninum* and *A. ceylanicum* may have immunomodulatory potential, being at least partially responsible of the findings explained above protecting against DSS-induced colitis in murine models using ESPs of these parasites, since the ESPs extracts used in these experiments may contain an important fraction of EVs.

Although the studies mentioned above have explored the therapeutic potential of ASPs against colitis, no mechanistic investigations have been conducted regarding infection tolerance or resistance in dead-end hosts such as humans, although a similar immune profile as in *T. canis* may be expected at least for *A. caninum*. Still, it remains unclear whether zoonotic hookworm ESPs or EVs play a role in the skin pathology observed during cutaneous *larva migrans*. Studying ESPs and EVs associated with *A. ceylanicum* may help identify key molecules that explain why some hookworms, such as *A. caninum* or *A. braziliense*, do not reach the bloodstream, while others, like *A. ceylanicum*, which is also associated with dogs, are able to breach this barrier and complete their life cycle in humans.

4.3 Trichinella spiralis

The life cycle of *T. spiralis,* the causative agent of trichinosis, starts with the ingestion of meat carrying encysted L1 larvae by humans or pigs. Once in

the stomach, gastric juices dissolve the capsule-shaped cyst and release the larvae, which passes to the small intestine (Mitreva & Jasmer, 2006). Then, larvae mature until they become adults. Female worms produce L1 larvae which migrate through the lymphatic vessels and enter the general circulation to the small capillaries, where they finally reach the muscle fibers (Mitreva & Jasmer, 2006). There, L1 encyst becoming infective in 15 days, but can remain dormant for years (Mitreva & Jasmer, 2006). Pigs, rodents and humans can act as both definitive and intermediate hosts. Pathogenicity of *T. spiralis* is higher in these species than in other carnivores, such as wild boars, dogs and domestic cats (Pozio et al., 2009) due to the higher number of newborn larvae produced by females (Pozio et al., 1992) and the more intense immune reaction induced in humans (Bruschi et al., 1999; Gomez Morales et al., 2002).

ESPs from different stages of *T. spiralis* reduce the ability of mouse macrophages to express proinflammatory cytokines in response to lipopolysaccharide stimulation by inhibiting nuclear factor-κB translocation to the nucleus and the phosphorylation of both extracellular signal-regulated protein kinase 1/2 and p38 mitogen-activated protein kinase (Bai et al., 2012). The inhibition of proinflammatory cytokine production and the polarization of macrophages towards an alternatively-activated phenotype may favor worm survival (Bai et al., 2012). In addition, it has been demonstrated that ESPs released by *T. spiralis* L1 larvae modulate inflammatory cytokine production in the colon of mice, suggesting that they inhibit Th1 immune responses and enhance Th2 and Treg responses in TNBS-induced colitis in mice (Jin et al., 2019). In addition, strong Treg cell responses have been observed in mice and have been characterized by increased CD4+CD25+Foxp3+ and CD4+CD25-Foxp3+ cells accompanied by high levels of IL-10 and TGF-β (Sun et al., 2019). Furthermore, under the influence of L1-associated ESPs, human dendritic cells acquire a tolerogenic phenotype, characterized by the low expression of HLA-DR, CD83 and CD86, as well as moderate expression of CD40 and elevated production of IL-10 and TGF-β (Ilic et al., 2018). The interaction between L1-derived ESPs and dendritic cells also modulates the expression of Toll-like receptors TLR-2 and −4, inducing the observed phenotypic and functional changes in the dendritic cells, as well as enhancing their capacity to polarize T cells towards Th2 profile, while reducing their allostimulatory capacity to induce Th1 polarization (Ilic et al., 2018). Altogether, these properties may create a regulatory environment that benefits the survival of the parasite in the host.

Regarding the effect of *T. spiralis* EVs, vesicles derived from *T. spiralis* L1 muscle larvae were shown to increase IL-10 and IL-6 production in human PBMCs (Kosanović et al., 2019). In addition, *T. spiralis* EVs, also improved TNBS-induced colitis in mice, alleviated intestinal epithelial barrier damage, reduced proinflammatory cytokine secretion and neutrophil infiltration, and increased immunoregulatory cytokine expression in colonic tissue. Furthermore, EVs modulated the adaptive immune response inducing a significant reduction of Th1 and Th17 cell populations and an increase in CD4+IL-4+ Th2 and CD4+IL-13 + Th2 cell populations. Furthermore, EVs showed a significant and reproducible increase in the percentage of CD4+CD25+Foxp3+ Treg cells, which play a critical role in maintaining immune tolerance and reducing the intensity of inflammation (Yang et al., 2020).

In the published mouse and human models used to investigate the response to *T. spiralis* ESPs and EVs, an immunomodulatory effect is evident, facilitating the parasite´s development within the host. Interestingly, as mentioned above, the pathogenicity of *T. spiralis* is higher in these species, suggesting that other factors may be involved in the pathogenic response induced in these hosts rather than a strong inflammatory response induce by ESPs or EVs. Further research is needed on the immune response elicited by ESPs and EVs in pigs, key hosts of *T. spiralis* life cycle, and to compare their effects to what has been observed in mice and humans.

4.4 Fasciola hepatica

Adult flukes of *F. hepatica* develop in bile ducts of their definitive hosts, including cattle and humans. Accordingly, humans and cattle become infected by the ingestion of metacercariae encysted in aquatic vegetation. These metacercariae excyst in the duodenum, migrate to the bile ducts and develop into adult stages. From here, adult worms shed eggs that after being carried by bile reach the small intestine and are expelled by feces until reaching an aquatic environment (Moazeni & Ahmadi, 2016). If conditions are suitable, eggs hatch and release ciliated miracidium which swim towards its first intermediate host, snails of the genus *Lymnaea*. In snails, miracidia asexually reproduce into sporocysts, rediae and free-swimming cercariae. Cercariae typically encyst on aquatic plants, becoming metacercariae, which are infectious to definitive hosts (Moazeni & Ahmadi, 2016). In humans, flukes may remain viable between 9 and 13.5 years (Cutress & Cutress, 2019) whereas in cattle adults may persist up to 2 years (Forbes, 2017) suggesting

different adaptations of the parasite to their hosts. In this sense, adaptative Th2 responses have been associated with increased protection against *F. hepatica* infection in murine models (Fernández, 2023).

Juvenile stages and adults of *F. hepatica* release a variety of ESPs with immunomodulatory potential when infecting their definitive host tissues (Wildblood et al., 2005). Many different compounds have been identified within the secretome of *F. hepatica* including antioxidants, proteases, mucin-like peptides, as well as helminth defense molecules (HDMs). HDMs are a conserved group of proteins that interact with cell membranes without cytotoxic effects or antimicrobial activity, suggesting a specific immunomodulatory role of these molecules (Ryan et al., 2020).

In murine models, the immune profile induced by *F. hepatica* infection is strain- and dose- dependent. A Th2 response characterized by high levels of IL-4 and IL-5 was observed in spleen cells from BALB/c and 129 Sv/Ev mice infected with a low-dose of metacercariae, whereas a mixed Th1/Th2 response was observed in spleen cells from C57BL/6 mice. Interestingly, using a higher dose of infective metacercariae, BALB/c, 129 Sv/Ev, C57BL/6 exhibited a clear Th2 response, suggesting that this parasite may secrete molecules that downregulate Th1 responses (O'Neill et al., 2000).

Regarding initial innate immune responses induced by *F. hepatica* ESPs, heat-shock proteins and fatty-acid binding proteins are considered damage-associated molecular pattern molecule (DAMP) homologues (Medzhitov, 2007; Robinson et al., 2010) which are actively secreted in trematodes similarly to what has been described for *Leishmania spp.* (Silverman et al., 2010). For instance, the DAMP thioredoxin peroxidase (TPx) has been identified in *F. hepatica* ESPs, and has been shown to modulate dendritic cell activation and cytokine production (Donnelly et al., 2005) which in turn has been considered as a pathogen-associated molecular pattern molecule (PAMP).

Metacercariae ESPs and recombinant TPx in mice induce the alternative activation of macrophages characterized by the production of high levels of IL-10 and prostaglandin E2 and low levels of IL-12, suggesting a key role of TPx in the induction of the type 2 responses observed in mice infected by *F. hepatica* (Donnelly et al., 2005). Additionally, alternative activation of macrophages by TPx (Giuliani et al., 2010; Marcilla et al., 2012; Robinson et al., 2010) promotes Th2 responses and suppresses Th1-driven inflammation, inducing an enhanced secretion of IL-4, IL-5, and IL-13 from naive CD4 T cells (Donnelly et al., 2008).

Furthermore, metacercariae ESPs in mice have been implicated in the maturation of eosinophils acting in a similar fashion to IL-5 on the precursors of these cells (Milbourne & Howell, 1993). Additionally, eosinophil peroxidase (EPO) activity is increased in the presence of *F. hepatica* ESPs in mouse models. EPO normally acts as a marker of eosinophil functional maturity and has been suggested to be a key factor for the maintenance of the proeosinophil populations over time (Milbourne & Howell, 1993). The immune response towards *F. hepatica* ESPs in mice varies depending on the mice strain. However, a clear type-2 response is observed, characterized by high levels of IL-4 and IL-5 (Donnelly et al., 2005). Treatment of bone marrow-derived dendritic cells of mice with metacercaria ESPs, leads to the differentiation to tolerogenic dendritic cells capable of driving naïve T cells to Th2 regulatory phenotypes (Milbourne & Howell, 1993). *In vitro* studies of adult-derived ESPs in mice demonstrated that these molecules lead to apoptosis of peritoneal macrophages and eosinophils, which appears to be a parasitic strategy to evade host immune responses (Guasconi et al., 2012).

Considering the importance of this infection in cattle, some studies have used sheep or bovines as an experimental model, where a similar potent regulatory effect of *F. hepatica* has been observed. It has been shown that PBMCs from *F. hepatica*-infected bovines stimulated with ESPs, elicit a strong IL-4 and IL-10 production. Additionally, ESPs favor the polarization towards alternative activated macrophages and reduced IFN-γ and nitric oxide production in macrophages after LPS stimulation (Flynn & Mulcahy, 2008) In sheep, *F. hepatica* infection induced an increase in systemic eosinophilia, and a strong IL-10 production by ESPs-stimulated PBMCs (Zhang et al., 2005). Furthermore, ESPs of *F. hepatica* have been shown to inhibit concanavalin A-induced proliferation of sheep PBMC's generating anergia of specific T-cell subsets (Moreau et al., 2002).

In humans, the available information regarding the immune profile induced by *Fasciola hepatica* remains limited. Nevertheless, existing evidence suggests that the parasite's immunoregulatory capabilities also extend to the human host. For instance, although an initial inflammatory response may occur during the early phase of infection, up to 50 % of infected individuals remain asymptomatic (Dalton et al., 2013) pointing to the presence of effective immune evasion mechanisms. Additionally, high levels of IgG4 antibodies specific to *F. hepatica* antigens observed in humans are indicative of a Th2-skewed immune response (O'Neill et al., 1999). Furthermore, one of the *F. hepatica* ESPs, the fatty acid binding protein, has been shown

to promote macrophage polarization toward an M2 phenotype, leading to IL-10 overexpression and suppression of LPS-induced proinflammatory cytokines such as TNF-α, IL-12, and IL-1β (Figueroa-Santiago & Espino, 2014). Therefore, *F. hepatica* ESPs exhibit a wide range of modulatory and tolerogenic effects observed in mice, bovids, and humans which are likely critical for parasite's survival and long-term persistence.

Adult *F. hepatica* EVs exhibit a wide range of morphological variations (i.e., tubular, round-shaped, and as lamellar bodies), and this divergence can be related to their lipid composition, which can contribute to variations that benefit their uptake rate by host cells (Sánchez-lópez et al., 2024). The proteome of EVs derived from adult *F. hepatica* contains a high number of proteases, such as cathepsins and leucine aminopeptidase (LAP) as well as detoxifying enzymes i.e. heat-shock proteins, and fatty-acid binding proteins (Marcilla et al., 2012). Also, proteins like thioredoxin, HSP70, annexin, and hexokinase, have been found to be among the most abundant proteins identified in *F. hepatica* EVs. Universal stress proteins (USPs) have been previously described as critical for enabling parasites to withstand unfavorable environmental conditions such as oxidative stress, temperature fluctuations, low pH, or hypoxia during its complex developmental life cycle (Murphy et al., 2020). Proteins involved in locomotion, membrane trafficking and cellular physiology were also identified from newly-excysted juvenile *F. hepatica* EVs, including actin-2, tubulins, tropomyosin, dynein beta chain, the small GTP-binding protein Rab1, ADP-ribosylation factor, and metabolic enzymes such as GAPDH, enolase, pyruvate kinase, triosephosphate isomerase, and phosphodiesterase-nucleotide pyrophosphatase, as well HSP70 and HSP90 chaperones. These EV-associated molecules may influence the rapid metabolic adaptation to the newly encountered environment and evasion of host innate immune responses (Sa et al., 2022) which leads to the successful infection of newly excysted *F. hepatica*.

The proteins identified in adult *F. hepatica* EVs constitute 52 % of the total secretome (Marcilla et al., 2012). A large overlap in the protein content of *F. hepatica* secretome and EVs was observed suggesting that these vesicles are the primary mechanism of protein release of these worms (Marcilla et al., 2012). In contrast with previous analysis of *F. hepatica* immunomodulatory capabilities, murine bone marrow derived dendritic cells treated with EVs from adult *F. hepatica* showed increased expression of TNF-α, and induction of a semi-mature dendritic cell phenotype, with increased expression of CD80, CD86, CD40, OX40L, and SIGNR1.

Furtherore, they do not induce the production of IL-13 or IL-10 from co-cultured CD4 + cells unlike other major components of the secretome (Murphy et al., 2020). This suggests that isolated EVs do not promote Th2 or Treg polarization, however further characterization of their specific effect in immunomodulation is required.

On the other hand, adult *F. hepatica* EVs have a cytostatic effect on hepatic stellate cells and induce the secretion of extracellular matrix and anti-inflammatory responses in hepatocytes of the LX-2 human HSC line, the HepG2 human hepatoma cell line and the Caco-2 human colon epithelial cell line (Sánchez-lópez et al., 2024). Additionally, it was shown that *F. hepatica* EVs induced anti-inflammatory effect in LPS-activated human macrophages (Sánchez-López et al., 2023). Therefore, these interactions can have a great influence on the progression of the disease, generating conditions that favor the establishment of helminths, which additionally provide initial valuable information to understand the prolonged half-life of adult flukes and the immunoregulatory role of their EVs in human hosts.

The *F. hepatica* secretome, including ESPs and EVs, exhibits an important immunomodulatory effect in the different definitive hosts, such as humans or bovids. Proteomic similarities in ESPs and EVs suggest that both play a key role in the regulation of the immune response resulting in parasite survival and long-term infections. Different molecules contained in *F. hepatica* secretome have been proposed as potential candidates for vaccine development or as targets for immunotherapy against liver flukes (Dalton et al., 2013), thus harnessing their immunoregulatory potential, which has been characterized over the years, to enhance both the treatment and prevention strategies against this parasitic disease.

4.5 Taenia solium

Taenia solium infects humans as definitive or intermediate hosts and pigs as intermediate hosts to complete its life cycle. Adult *T. solium* attaches to the human intestinal wall where they produce eggs which are later shed with feces. Pigs and humans become infected by the ingestion of food or water contaminated with the parasite´s eggs. These latter stages develop as cysticerci in different tissues of their intermediate hosts, with a predilection for the central nervous system, causing a severe disease known as neurocysticercosis (Cervi et al., 2009; Flynn & Mulcahy, 2008). In humans, cysticerci silently invade different tissues by mechanisms poorly understood, that may be favored by immunomodulatory components derived from cysts (Othman et al., 2011; Prodjinotho et al., 2020). During infection of intermediate hosts, most

invading oncospheres are destroyed while passing through the liver or removed at arrival into non-immunologically privileged sites like muscle and subcutaneous tissues. Nevertheless, some oncospheres survive, preferentially in protected places like the central nervous system or the eye (Garcia et al., 2014).

The immune response induced by *T. solium* is very complex and it is dependent on different factors such as localization in the host, parasite burden, stage of the infection, and parasite viability. Most of the findings regarding this have been obtained in the context of neurocysticercosis. In general, *T. solium* induces a predominantly type 2 immune response in infected hosts, especially during asymptomatic stages, with the activation of regulatory mechanisms that help to prevent excessive inflammation. This response is characterized by the secretion of cytokines with immunomodulatory properties such as IL-4, IL-10, TGF-β, and IL-13, which influence innate immune cells like dendritic cells, macrophages, and monocytes (Chavarría et al., 2003; Grewal et al., 2000). This immune profile is similar to the one reported in patients with viable cysts (Grewal et al., 2000), but it can change during infection and in the appearance of symptoms. In cases of symptomatic neurocysticercosis, particularly when cysts begin to degenerate either spontaneously or following anthelmintic treatment, a more inflammatory profile, with increased production of IL-1β, TNF-α, and IFN-γ is observed (Grewal et al., 2000). Afterwards, the host´s immune response progresses to a mixed Th1/Th2 cytokine environment associated with fibrosis and granuloma formation around the cysts with expression of cytokines such as IFNγ, IL-18, IL-4, IL-10, IL-13, and TGF-ß (Restrepo et al., 2001; Robinson et al., 2002).

ESPs derived from *T. solium* metacestodes are key inducers of parasite-driven immune modulation. In human macrophages, ESPs induce a complex cytokine response with both Th1 and Th2 features but overall ESPs promote alternative activation of macrophages and Th2 polarization (Arora et al., 2023; Rodríguez-Sosa et al., 2002). This is accompanied by suppression of TLR4 expression and downregulation of AKT-mediated ROS production, providing a permissive environment for parasite survival (Arora et al., 2023). ESPs also influence T cell dynamics by activating TGF-β and IL-2/STAT5 pathways in porcine CD4 T cells, thereby suppressing Th17 differentiation and promoting immune evasion, (He et al., 2023), suggesting a similar role of ESPs in humans and pigs.

Accordingly, EVs released by *T. solium* larvae play a key role in immunoregulation. In a murine model, EVs ameliorate DSS-induced colitis in C57BL/6 mice by preserving colonic architecture, demonstrating

their potent immunosuppressive capacity. In vitro, human THP-1 macrophages treated with these EVs exhibit increased expression of anti-inflammatory Th2-associated molecules such as IL-4, IL-10, and Arginase-1, without significant changes in IL-1β or TNF-α levels. These EVs are enriched in metabolites that inhibit the PI3K/AKT signaling pathway. Upon internalization by macrophages, they promote lysosomal degradation of AKT and mTOR through enhanced ubiquitination. This process reduces ROS production and impairs bacterial killing capacity, ultimately compromising macrophage function (Rawat et al., 2024), These findings align with the above-described immunomodulatory effects of ESPs, suggesting that both EVs and ESPs may share similar immunoregulatory components.

Collectively, the ESPs and EVs released by *T. solium* exhibit potent immunomodulatory properties, primarily promoting regulatory type 2 responses and disrupting pro-inflammatory signaling pathways. These effects appear to be largely host-independent, yet they display substantial complexity and variability depending on the stage of infection and the viability of the parasite.

4.6 Echinococcus spp

The genus *Echinococcus* includes several species of human importance such as *E. granulosus, E. multilocularis, E. oligarthra, E. vogeli, E. shiquicus, E. equinus,* and *E. canadensis* (Peón et al., 2016). The definitive hosts of *Echinococcus* spp. are mainly carnivores of the families Canidae and Felidae, although a large range of wild carnivores have been reported with adult parasites (Carmena & Cardona, 2014). Moreover, the intermediate hosts of *Echinococcus* spp. are mainly mammals, and vary according to the *specific species*. (Lymbery & Thompson, 1996). For instance, adult *E. granulosus* lives in the small intestine of its canid definitive host where eggs are shed in feces and later ingested by an intermediate or accidental host, like humans. In the latter, eggs hatch in the small intestine, oncospheres penetrate the intestinal wall and are transported by the circulatory system to various organs, such as lung, heart, liver, large intestine and bone, and develop into hydatid cysts (Thompson, 1979). Similarly, *E. multilocularis* migrates to different tissues within human hosts, but develops into a different metacestode, i.e. alveolar cysts (Thompson, 1979).

The zoonotic importance of *Echinococcus* species resides in the capability of these parasites to infect humans as intermediate hosts. Therefore, most efforts have been focused on understanding the immunoregulation induced

by *Echinococcus spp* using murine models or analyzing samples form patients infected with these parasites. Experimental infections with *E. granulosus* in BALB/c mice elicit a strong Th2 regulatory immune response, characterized by the production of antibodies targeting carbohydrate epitopes and the secretion of IL-4, IL-5, IL-13, and IL-10. Specifically, a carbohydrate-rich fraction derived from the parasite promoted IL-10 production and suppressed mitogen-induced lymphocyte proliferation, suggesting a role in early immune escape and the establishment of parasite tolerance (Dematteis et al., 2001). In *E. multilocularis*, the infection course involves a transition from initial Th1 responses, characterized by IL-2 and IFN-γ secretion and limited parasite growth, to a mixed Th1/Th2 phase, and finally a third phase of strong immunosuppression. This terminal stage is marked by impaired lymphocyte proliferation, decreased expression of co-stimulatory molecules (e.g., CD40) in antigen-presenting cells, and increased T-cell apoptosis mediated in part by parasite-derived antigens like Em492, resulting in reduced clonal expansion of effector T cells and diminished control over metacestode cell proliferation (Emery et al., 1996; Mejri & Gottstein, 2006; Walker et al., 2004).

In humans, a similar immune profile is observed, although increased complexity arises from inter-individual variability. Patients with active alveolar echinococcosis (AE) frequently exhibit elevated levels of regulatory cytokines such as IL-10 and TGF-β, both in serum and in PBMC cultures. IL-10 is considered a hallmark of progressive disease, as its spontaneous secretion by CD4 , CD8 , and non-T/non-B cells is consistently associated with advanced lesions. In contrast, lower IL-10 levels have been reported in patients with abortive or contained lesions, suggesting its critical role in modulating immune tolerance and parasite survival (Vuitton & Gottstein, 2010; Zhang et al., 2008).

Despite the dominance of Th2 and regulatory profiles, the human immune response to *E. multilocularis* is not exclusively anti-inflammatory. In most studies, a mixed cytokine profile has been observed with significant levels of Th1 cytokines (IL-12, IL-2, IFN-γ), indicating ongoing immune activation even in chronic stages. This mixed response has been associated with the absence of protoscoleces in humans. Interestingly, enhancing Th1-related immune responses have resulted in increased resistance to *E. multilocularis* infection in murine models. Therefore, this complex cytokine milieu likely reflects attempts to balance protective inflammation and parasite-induced immune regulation (Aumüller et al., 2004).

Echinococcus spp ESPs and EVs are central in the regulation of the immune response against these parasites. For instance, the Em492 antigen from *E. multilocularis* suppresses splenocyte proliferation and induces anti-CD3 dependent apoptosis of T cells, contributing to local immunoregulation at the host-parasite interface (Vuitton & Gottstein, 2010; Walker et al., 2004). Likewise, ESPs derived from *E. granulosus* protoscoleces have been shown to induce regulatory IL-10 producing B cells in a TLR-2-dependent manner (Pan et al., 2018). These products also influence the differentiation of IL-17A-producing B cells and Th17 cells, with effects dependent on the structural integrity of ESPs, particularly their carbohydrate components (Pan et al., 2017).

The immunomodulatory capacity of ESPs is complemented by the functions of EVs, which are released both in vitro and in vivo by various stages of the parasite. Proteomic analyses of EVs isolated from cyst fluid and protoscoleces culture supernatants revealed a diverse cargo with 1026 and 38 proteins that were exclusively identified in the EVs, respectively. These proteins include antigens, signaling molecules, and immunomodulatory proteins. The potent immunoregulatory role of Echinococcus derived EVs was demonstrated during a co-culture of protoscolex-derived EVs with murine PBMCs inhibiting the proliferation of T cells and the production of IFN-γ, IL-6, IL-17A, and TNF-α (Zhou et al., 2019). Furthermore, the immunoregulatory role of *E. granulosis* derived EVs was also confirmed in a murine model of allergic airway inflammation. EVs treatment reduced airway resistance, as well as the levels of eosinophils and Th2/Th17-related cytokines in bronchoalveolar fluid. Additionally, pretreatment with EVs decreased the number of IL-4+ CD4 + T cells and increased the number of Treg cells in the lymph nodes and spleen (Jeong et al., 2021), highlighting the importance of differentiating between anti-helmintic Th2 responses and harmful type 2 inflammatory responses.

Interestingly, a recent approach suggests that the immunoregulatory role of EVs can be modified to be used therapeutically in this zoonosis. The cargo from EVs derived from protoscoleces treated with metformin- and albendazole sulfoxide showed differences both in the proportion of specific proteins and in the appearance of unique proteins after treatment. Additionally, drug treated-EVs can shift the immune response towards a pro-inflammatory Th1 profile, with increased expression of IL-12, TNF-α, and IL-6 in dendritic cells, while suppressing TGF-β and IL-23, highlighting their potential for immune-based therapeutic strategies (Nicolao et al., 2023).

Altogether, these findings underscore the sophisticated immune manipulation strategies employed by Echinococcus spp. in their intermediate hosts. By shaping host responses through ESPs and EVs, the parasite not only ensures its survival and chronicity but also provides promising targets for immunotherapy. The ability of parasite-derived EVs to modulate key immune pathways suggests novel avenues for treating echinococcosis, either by enhancing protective immunity or by harnessing their immunosuppressive properties for unrelated inflammatory diseases.

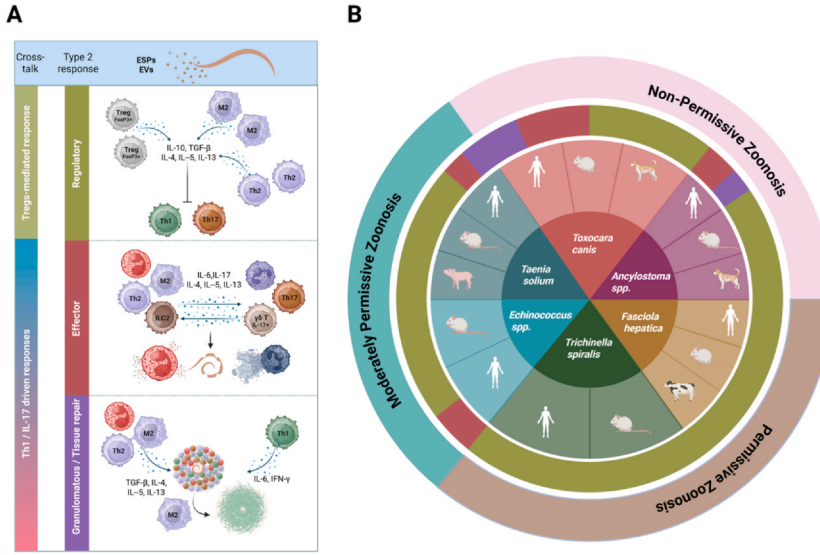

Fig. 2 Type 2 immune response according to zoonotic helminth species and the vertebrate hosts in which experiments have been conducted. (A) The spectrum of type-2 responses, including regulatory response characterized by type-2 cytokines with a strong cross-talk with T regulatory associated mechanisms mediated mainly by IL-10; concomitant or effector type-2 response with a cross-talk with IL-17 mediated mechanisms; and granulomatous or tissue-repair type-2 response which act in combination with Th1 profile features. (B) The outer circle describes the division of zoonotic parasites into: (i) those producing a non-permissive zoonosis in their hosts with high pathology, such as *Ancylostoma* spp. or *Toxocara canis* in humans; (ii) those capable of fully developing in humans, although humans are not their natural hosts or reservoirs, such as *Taenia solium* and *Echinococcus* spp., in which moderate pathology is observed; and (iii) those that fully develop in humans with mild pathology, producing a permissive zoonosis, such as *Trichinella spiralis* and *Fasciola hepatica*. The middle circle indicates the type 2 response associated with each model: regulatory (green), effector (red), and granulomatous or tissue-repair associated (purple).

5. Conclusions

Excretion-secretion products in helminths have been studied for their crucial role in host-pathogen interactions, as well as their potential use in diagnostics and vaccine development. Their biogenesis and effects are much better understood than those of EVs, largely due to the inherent challenges of working with helminths in vitro, obtaining and maintaining their different developmental stages, the availability of suitable animal models and the complexity of EV isolation methods (White et al., 2023). Nevertheless, the study of helminth-derived EVs holds similar promise to ESPs in terms of their potential as disease biomarkers and their role in modulating host immune processes. For instance, helminth EVs have been proposed as key mediators in the interaction with the host gut microbiome, potentially altering its composition or function (Rooney et al., 2022). Therefore, future research should aim to unravel these complex trans-microbe interactions (Rojas, 2025).

In zoonotic helminths the immunomodulatory role of ESPs and EVs is generally associated with the induction of type 2 regulatory immune responses that favor parasite persistence and survival. This immunological pattern is particularly evident in natural definitive hosts and in permissive experimental models, where ESPs and EVs promote a tolerogenic environment that limits inflammation and tissue damage, ultimately allowing the completion of the parasite's life cycle (Fig. 2).

However, this immunoregulatory signature is not universal when cross-species immune responses are analyzed for certain parasites. In non-permissive or accidental hosts, such as humans in the case of some nematodes like *T. canis* and *Ancylostoma* spp., the immune response often shows mixed profiles, including type 1 and IL-17 driven responses and a granulomatous reaction, which limits parasite development and survival. These differences highlight how host-specific factors influence the outcome of helminth infection and the effectiveness of immunomodulatory potential of ESPs and EVs (Fig. 2B).

Interestingly, in helminths such as *T. spiralis*, and *Fasciola* hepatica, regulatory type 2 responses are largely conserved across species, suggesting a robust and adaptable set of ESP- and EV-mediated mechanisms. Nonetheless, the pathology observed in humans during zoonotic infections can originate from the effect of the presence and metabolic activity of these parasites in specific tissues such as muscle or the biliary tract, rather than an insufficient immunomodulatory capacity of ESPs and EVs (Fig. 2B).

Moreover, in infections caused by *T. solium* and *Echinococcus* spp., the interplay between regulatory and type 1 responses appears to be critical. While ESPs and EVs mediated immunoregulation supports chronic infection, evidence from human cases suggests that a stronger Th1 response may correlate with parasite control and developmental arrest in the case of *Echinococcus* spp, and that, pro-inflammatory responses, tissue remodeling, and mixed Th1/Th2 profiles may emerge after cysts of *T.solium* are no longer viable resulting in different pathologic presentations that are tissue-dependent (Fig. 2B).

Investigating the immune regulatory effect of ESPs or EVs from zoonotic parasites in natural hosts, humans and experimental models not only provides valuable insights on the underlying mechanisms that determine completion or disruption of the parasite's life cycle, and the associated pathogenicity, but also reveals the degree of adaptation of these helminths to the human host and allows the identification of promising candidates for diagnostic testing, vaccine development or therapeutic applications.

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