Eosinophils and *Trichinella* infection: toxic for the parasite and the host?

Fabrizio Bruschi[1], Masataka Korenaga[2] and Naohiro Watanabe[3]

[1]Department of Experimental Pathology, Medical Biotechnologies, Infectivology and Epidemiology, Università di Pisa, Medical School, Via Roma 55, 56126 Pisa, Italy
[2]Department of Parasitology, Kochi Medical School, Kochi University, Nankokushi, Kochi 783-8505, Japan
[3]Department of Tropical Medicine, Jikei University School of Medicine, 3-25-8 Nishi-shinbashi, Minatoku, Tokyo 105-8461, Japan
Corresponding author: Bruschi, F. (fbruschi@med.unipi.it).

Peripheral blood and tissue eosinophilia characterize trichinellosis in humans, and present in addition to the increased total IgE levels that occur in many helminth infections. Both processes are the consequence of T-helper 2 activation. Blood and tissue eosinophilia begins with eosinophilopoiesis in the bone marrow, which is followed by the migration of eosinophils through the circulatory system, the eosinophil infiltration of tissues at the inflammatory foci and, finally, degranulation and cell death. Recently, some aspects of eosinophilia caused by *Trichinella spiralis* infection have been elucidated; however, the protective role of this population of cells against *Trichinella* parasites remains controversial. Furthermore, when eosinophils are numerous, they can be toxic for host tissues. This review discusses these issues in both human and rodent infection models.

Eosinophilopoiesis

Trichinellosis is a worldwide zoonosis, and there are an estimated ten million people at risk for this parasitic infection [1]. The life cycle of *Trichinella* parasites consists of the enteral phase, which lasts 3–4 weeks in humans, and the parenteral phase, which starts after the arrival of migrant larvae in the striated skeletal muscle fibers. At the beginning of the parenteral phase, an increase in blood and tissue eosinophil levels is observed [2].

Trichinella antigens stimulate T cells to produce cytokines that induce eosinophilopoiesis in the infected host [3,4] (Figure 1). When spleen lymphocytes that were obtained from mice 4–6 weeks after infection with *Trichinella spiralis* were cultured with somatic antigens [5] or excretory and/or secretory products from muscle larvae (ML) [6], the conditioned medium induced eosinophil production from normal, nonadherent syngeneic bone-marrow cells. Eosinophilic responses in *Trichinella*-infected mice were dependent on the release of soluble products from lymphocytes that were sensitized to the parasite antigen, whereas the addition of the antigen to either non-sensitized lymphocytes or lymphocytes sensitized by the Calmette-Guérin bacillus did not induce colony-stimulating activity [5]. However, a direct injection of the antigen failed to induce eosinophilia in rats [7].

Interleukin-5 (IL-5) is an important cytokine that is involved in eosinophilopoiesis in mice that are infected with *T. spiralis* [8] (Table 1). Immunization with ML antigens combined with adjuvant induces a type-2-biased cytokine response and generates a high level of IL-5 [9–11]. Wang *et al.* [12] suggested that the interactions between antigen-presenting cells and $CD4^+$ T cells through co-stimulatory CD86 are crucial to IL-5 production in *T. spiralis* infections. The intraperitoneal injection of *Trichinella*-infected BALB/c mice with anti-CD80 and/or anti-CD86 monoclonal antibody (moAb) suppressed eosinophilia. The combination of both moAbs also effectively reduced the *in vitro* production of IL-5 in mesenteric lymph node cells that were stimulated with somatic antigen; however, when further studied, the *in vitro* production of IL-5 was only reduced in the presence of anti-CD86 where anti-CD80 alone was ineffective. When co-stimulatory factor interactions between intercellular adhesion molecule-1 and lymphocyte function antigen-1, or vascular cell adhesion molecule (VCAM) and very late antigen-4 were blocked *in vitro*, the production of IL-5 was not suppressed as effectively as it was in anti-CD86 moAb treatment.

Eosinophil migration and infiltration to tissues

Although homogenates of *T. spiralis* ML attract eosinophils [13], the migration of eosinophils into tissues is also the result of the action of chemokines, in particular the CCR3 ligands; adhesion molecules, especially those mediated by P-selectin and α4 integrins; and IL-5. CCR3 expressed on eosinophils in humans and mice mediates both chemotaxis and adhesion to endothelial cells *in vitro* and *in vivo*. Although CCR3 deficiency does not affect the capacity of mice to generate peripheral blood eosinophilia in response to *T. spiralis*, eosinophils are not recruited to the jejunal mucosa, nor are they present in the striated muscle adjacent to encysted larvae [14]. P-selectin mediates IL-13-induced eosinophil transmigration but not eotaxin production in mice [15]; however, the regulatory role of P-selectin in eosinophil transmigration during *T. spiralis* infection is unclear.

The trafficking of leukocytes to the gut-associated lymphoid tissue depends on the interaction of the α4β7 integrin with its ligand, the mucosal vascular addressin cell-adhesion molecule-1 (MAdCAM-1) that is present on

the endothelium [16]. Both eosinophils and mast cells express α4β7 [17,18]. During *T. spiralis* infection, intestinal eosinophilia was delayed and reduced in β7-integrin-deficient mice but not in mice that were treated with moAb to integrin β7, whereas the suppression of mastocytosis was observed in both experimental models [19,20]. These facts indicate that the contribution of β7 integrin to eosinophil tissue migration is inferior to that of the mast cells. Galectin-3 (Gal-3) has a crucial role in eosinophil recruitment and airway allergic inflammation of mice [21]. In humans, eosinophils from allergic donors express elevated levels of Gal-3, which co-localizes with α integrin and increase the rolling and firm adhesion of eosinophils on the VCAM-1 of endothelial cells [22].

Eotaxin is a fundamental regulator of physiological eosinophil trafficking during healthy states [23] and inflammation [24]. Ecalectin (galectin-9), which is produced by antigen-stimulated T cells, is a powerful eosinophil-specific chemoattractant *in vitro* and *in vivo* [25]. The role of these chemokines in eosinophilia that is caused by *T. spiralis* infection has been partially clarified. Eotaxin-1 (CCL11) is important in intestinal-tissue eosinophilia but not in peripheral eosinophilia during infection with *T. spiralis* [13]. In addition, eotaxin-2 (CCL24) is only induced in the infected intestine. When $CD4^+OX22^-$ T cells from rats infected with *T. spiralis* were transferred to uninfected rats and followed by a challenge infection, the cells that migrated into the intestinal epithelium induced intestinal eosinophilia [26]. There is a possibility that $CD4^+OX22^-$ T cells produce more chemokines that have chemotactic activity against eosinophils than $CD4^+OX22^+$ T cells.

In other helminthic diseases, such as that induced by *Brugia malayi*, alternatively activated macrophages (AAM) under the control of T-helper 2 (Th2)-dependent cytokines express unique products such as Arg 1 (arginase 1), FIZZ 1 (resistin-like α) and YM1 (chitinase-like molecule), the functions of which have not been fully elucidated. Furthermore, AAM and/or YM1 are involved in eosinophil chemotaxis and might stimulate the migration of cells to parasites [27,28]. Induced AAM and YM1 have been shown in both mice and guinea pigs infected with *T. spiralis* [29,30].

Degranulation of eosinophils

Eosinophils contain several types of granules and secretory organelles that are packed with major basic protein, eosinophil cationic protein, eosinophil-derived neurotoxin and eosinophil peroxidase. These proteins perform various biological activities [31]. It has been suggested that the immunoglobulins IgG and IgA (including secretory IgA) are able to trigger eosinophil degranulation [31]. However, the presence of IgE receptors on eosinophils remains controversial [31–33]. Recently, T cells have also been shown to have a role in eosinophil degranulation. $Rag\text{-}1^{-/-}$ mice that lacked T and B cells were infected with *Nippostrongylus brasiliensis*, and IL-4-expressing eosinophils were recruited to pulmonary tissues but failed to degranulate [34]. Reconstitution with $CD4^+$ T cells promoted the accumulation of degranulated IL-4-expressing eosinophils, but only if the T cells were stimulated with a cognate antigen. These facts indicate that T-helper cells confer antigen specificity on eosinophil cytotoxicity but not on the cytokine responses. Whether a similar mechanism is invoked during the infection with *T. spiralis* has not been studied.

Cytotoxic granules injure both the host tissue and the parasite. Eosinophils are thought to be involved in myocarditis in rats that are infected with *T. spiralis* [35]. Eosinophils are probably recruited by mediators, such as platelet-activating factor and eosinophil-chemotactic factor for anaphylaxis, that are released by degranulated mast cells that are activated by cognate antigen binding to IgE.

Apoptosis of eosinophils

Gon *et al.* [36] demonstrated that a considerable percentage of eosinophils in non-infected rats undergo apoptosis within a few hours, whereas the apoptosis rate of eosinophils from *Trichinella*-infected rats is notably lower. The inhibition of eosinophil apoptosis during the later stages of a *Trichinella* infection is related to the presence of IL-5. By contrast, the inhibition of eosinophil apoptosis during the earlier phases of the infection is thought to be unrelated to IL-5, possibly because of the presence of IL-3 and the granulocyte-macrophage colony-stimulating factor (GM-CSF). At 2–5 weeks post-infection, when the recovery phase of a *T. spiralis* infection occurs, excess eosinophils disappear slowly from the jejunum of mice, whereas apoptotic eosinophils remain. The draining mesenteric lymph nodes also contain large numbers of apoptotic eosinophils [37]. Therefore, it is believed that jejunal eosinophils undergo apoptosis locally once the parasite-specific T cells cease to be prominent in the intestine and after the adult worms have been expelled.

Protection by eosinophils in murine models

It is evident that host defenses to *T. spiralis* are mediated through the action of $CD4^+$ T-cell-derived cytokines [38–42]. Studies using IL-4 or IL-4-receptor-α deficient mice revealed that IL-4 has a crucial role in worm expulsion [43,44]. However, it is not clear whether IL-5 and/or eosinophils can be implicated in the protective response of the host against the parasite. Neither IL-5 nor eosinophils were essential to control the parasite in either primary or challenge infections of CF1 mice using anti-IL-5 moAb [8]. Furthermore, when non-immunized C3H/HeN and IL-5 transgenic (Tg) mice were compared to C3H/HeN and IL-5 Tg mice that were immunized with parasite somatic antigen, no differences were found in the recovery of ML or adult worms in the small intestine, the fecundity of female adult worms, or the infectivity of newborn larvae among the groups [45].

However, protective immune responses against the parasite should be analyzed carefully. Experimental models using CCR3-deficient BALB/c [14], CCL11- and IL-5-deficient BALB/c [13], and IL-5-deficient C57BL/6 mice [46] show that eosinophils are not essential for the expulsion of adult worms after a primary infection. By contrast, IL-5 and/or eosinophils are involved in the expulsion of adult worms after a secondary infection in IL-5-deficient C57BL/6 mice [47]. Worm burdens in the skeletal muscle were increased and the frequency of

encysted larvae that exhibited necrosis was reduced in CCR3-deficient mice, compared with wild-type mice, during a primary infection [14]. This observation supports the hypothesis that eosinophils are cytotoxic versus the larval stage in mice [48,49]. Mouse eosinophils adhere to and kill *T. spiralis* newborn larvae in an antibody-dependent cellular cytotoxicity system [49] (Figure 2 and Figure 3).

Untangling the role of eosinophils in experimental trichinellosis

The discrepancy of the role of eosinophils in protection might be due to the mouse strains, the stages of the parasites and/or the experimental models used. Recently, strain-dependent resistance in the asthma model in mice has been reported to correlate with the apoptosis rate of lung-derived eosinophils [50]. Another possibility might be the difference in the degree of eosinophil activation. Hypodense eosinophils are commonly found in infected tissue, and cytotoxic activity against parasites is increased in this population of eosinophils [51,52]. Eosinophils can release large amounts of powerful mediators to kill parasites, injure tissues and remodel the damaged tissue. Thus, it is possible that eosinophils might be important in host defense. To validate this hypothesis, however, the interactions between each stage of the parasite and the eosinophils will require further study. In addition, a comparison of different *Trichinella* spp. might shed some light on host defense mechanisms [53–55]. For example, both adult worms and newborn larvae of *Trichinella pseudospiralis* exhibit stronger neutrophil chemotactic activity than those of *T. spiralis* [56].

Eosinophilia in humans

Eosinophilia is present, with few exceptions, in most cases of human trichinellosis, inasmuch as it is the earliest and most important host response. Even in asymptomatic cases, increases in eosinophilia of up to 15% have been observed [2].

The mechanisms of eosinophil production in humans are similar to those described in experimental animals. Recently, a type-2 cytokine pattern of lymphocyte activation has been described during the muscular phase of infection in patients that are infected by either *T. spiralis* or *Trichinella britovi* [57]. Type-2 cytokines were elevated during the first nine months of infection. In particular, IL-5 mRNA expression and protein production were both overproduced in peripheral blood mononuclear cells that were stimulated with *T. spiralis* or *T. britovi* antigens. The increased mRNA expression lasted at least one year; however, IL-5 protein levels peaked at two months and declined sharply after, even though considerable levels remained after 14 months [58].

Eosinophilia appears at an early stage of infection, before the development of general clinical signs and symptoms, and it increases between the second and fifth weeks of infection [59]. Eosinophilia might be low (<1000 cells per μl), moderate (1000–3000 cells per μl) or high (>3000 cells per μl, and up to 19 000 cells per μl have been reported) [2]. Eosinophilia declines slowly and remains at low levels for several weeks, up to three months post-infection. The level of eosinophilia generally correlates with the degree of myalgia [60] and is significantly higher in people with neurological complications (i.e. neurotrichinosis). In fact, eosinophilia presenting with more than 4000 cells per μl was observed in 87.5% of patients with cerebral damage and in only 35.2% of those with no central neurological complications [61].

A significant correlation was observed between the presence of specific IgE in the patient sera during the first period of infection and blood eosinophilia levels. In fact, 73% of patients with blood eosinophilia after four months of infection were positive for specific IgE compared to only 28% of patients without eosinophilia [61]. During the acute stage of infection, a massive decrease of eosinophils in people with severe trichinellosis is a reasonable warning of a severe outcome, and a sudden decrease to 1% or less might even predict patient death [2]. The mechanism underlying this phenomenon is not known but is likely to be related to a massive peripheral migration.

Human eosinophils could have a protective role because they are cytotoxic against newborn larvae in an antibody-dependent cellular cytotoxicity system [62]. Whether eosinophils are more cytotoxic than neutrophils is controversial. It is difficult to say whether the cytotoxic ability of these cells can have a meaningful role in protection against the parasite. The observation that eosinophil number decreases massively in severe infections [59] is in favor of a protective role *in vivo* as well.

There is no doubt that the increase in activated eosinophil levels is responsible for damage to the vascular walls (probably because of the release of the major basic protein, which is elevated in patients with eosinophilia [63]), and this explains tissue damage in the central nervous system (CNS) [60] and other tissues. Figure 4 shows a rich eosinophil infiltrate in the myocardial tissue of one patient who died because of severe trichinellosis. There is a correlation between eosinophil levels and muscle enzyme (creatine phosphokinase and lactate dehydrogenase) levels in skeletal muscles, and between eosinophil levels and myalgic score in patients infected by *T. britovi*; this indicates a relationship between eosinophil levels, and tissue damage and pain [59].

Concluding remarks

Clinical observations indicate that allergic manifestations are typical of human trichinellosis. At the same time, eosinophils typically increase in number during infection. Is this phenomenon a side-effect of Th2 activation, or can these cells kill parasites *in vivo* and *in vitro*? The rapid decrease in eosinophil levels as a predictor of patient death is consistent with a protective role for eosinophils in humans.

However, a chronic exposure to activated eosinophils might cause tissue damage in the muscle, myocardium and CNS, as exemplified in the most severe form of human trichinellosis, the so-called 'neurotrichinosis'. Further studies are needed at a basic level to improve knowledge about mechanisms of eosinophil neurotoxicity.

Despite the lack of controlled studies, corticosteroids are used by infectious-disease specialists to prevent immediate-type reactions during trichinellosis.

Corticosteroids should always be used in combination with anthelmintics to prevent an increased worm burden caused by delayed worm expulsion. Such anti-inflammatory drugs are also used to treat vasculitis, owing to its effects in inhibiting eosinophil activation and degranulation [31].

Acknowledgements

We would like to thank Dante Zarlenga for his critical reading of the manuscript and Y. Hashiguchi, Kochi Medical School for his encouragement in this work. A part of this study was supported by the grant-in-aid for scientific research from the Japanese Ministry of Education, Science, Technology, Culture and Sport (no. 14570216) and by the Uehara Memorial Foundation. F.B. dedicates this article to Gino Malvaldi, to acknowledge the support and advice received during his career.

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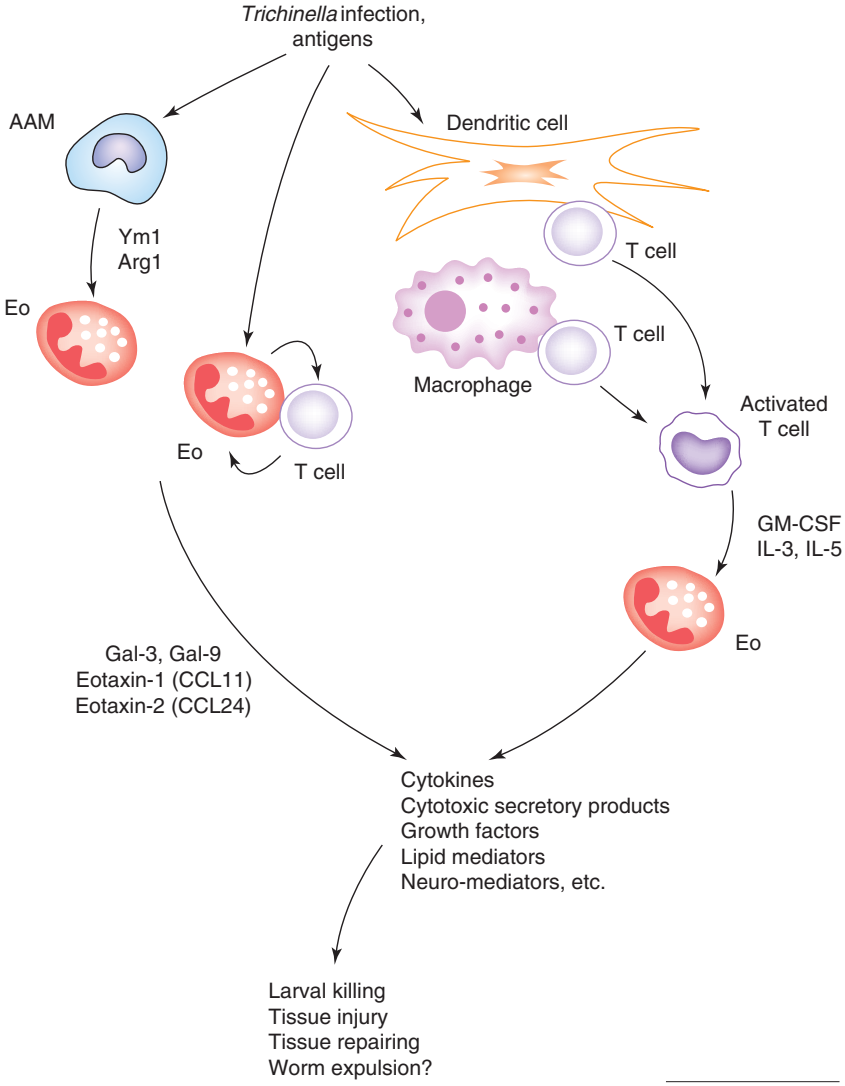

Figure 1. Eosinophil induction, recruitment and products during *Trichinella spiralis* infection. *Trichinella* antigens stimulate dendritic cells and classic macrophages to interact with T cells. Activated T cells produce cytokines including GM-CSF, IL-3 and IL-5. In the bone marrow, precursors proliferate and differentiate into eosinophils. Eosinophil migration from the bone marrow to the vessels is controlled mainly by IL-5. *Trichinella* antigens might attract and affect eosinophils directly to interact with T cells. AAMs also attract eosinophils through Ym1 and/or Arg1. Strong chemoattractants for eosinophils are Gal-9, eotaxin-1 and eotaxin-2. Gal-3 functions as a cell-surface adhesion molecule to support eosinophil rolling and adhesion. Eosinophils around *T. spiralis* in the tissues produce cytokines, cytotoxic secretory products, growth factors, lipid mediators and neuro-mediators. These molecules might be involved in larval killing, tissue injury and/or tissue repair, together with various cells. The involvement of eosinophils in worm expulsion from the gut is still obscure. Abbreviations: AAM, alternatively activated macrophage; Arg1, arginase 1; Eo, eosinophil; Gal-3, Galectin 3; Gal-9, Galectin 9; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL-3, interleukin 3; IL-5, interleukin 5; YM1, chitinase-like molecule.

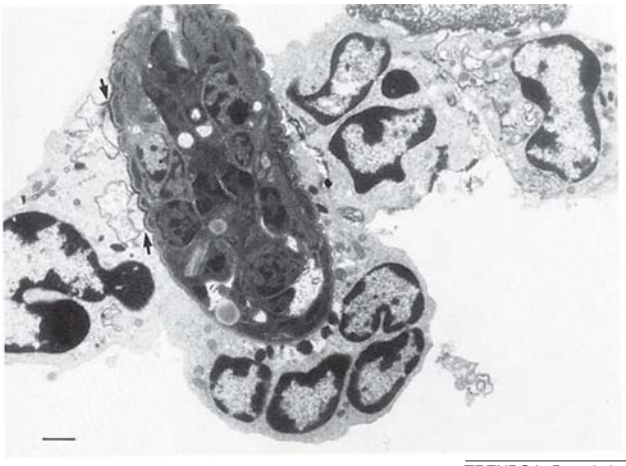

Figure 2. Electron micrograph of eosinophils adhering to *Trichinella spiralis*. Eosinophils from normal mice adhering to a *Trichinella spiralis* newborn larva incubated with immune sera. The arrows indicate intracellular vacuoles in contact with the cell basal membrane. Scale bar represents 1.3 μm. Reprinted, with permission, from Ref. [49].

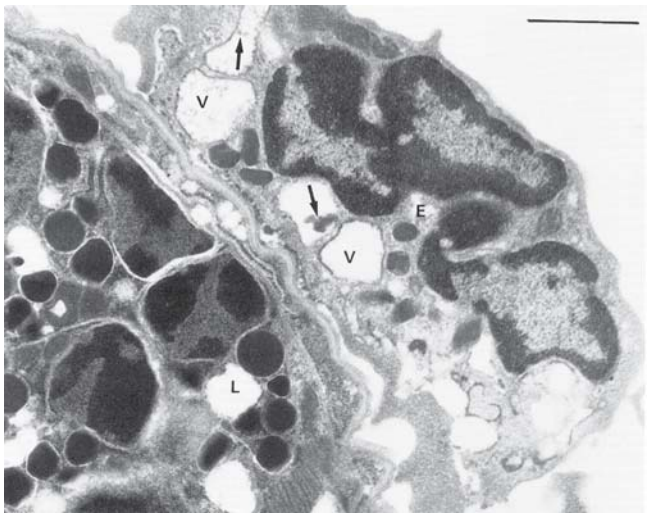

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Figure 3. Electron micrograph of a degranulated eosinophil. Eosinophil (E) from a normal mouse, degranulated after adhesion to a *Trichinella spiralis* newborn larva (L) incubated with immune sera. 'V' indicates intracellular vacuoles with remnants of eosinophil granule content (arrows). Scale bar represents 1.25 μm. Reprinted, with permission, from Ref. [49].

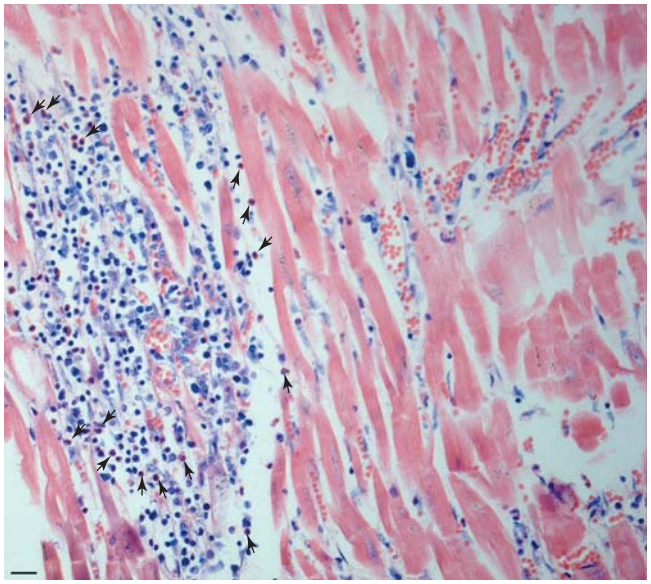

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Figure 4. Eosinophils infiltrating the myocardial tissue of a trichinellosis patient. Eosinophils are indicated with arrows. This patient died of complications from trichinellosis. Tissue sample provided by Wanda Kociecka. Sample stained with May-Grunwald–Giemsa. Scale bar represents 50 μm.

Table 1. Molecules involved in eosinophil differentiation, trafficking, survival and activation during *Trichinella* infection

Molecule	Physiological role	Role in *Trichinella* infection	Refs
IL-5	Differentiation and growth factor, eosinophil maturation and migration, the inhibition of eosinophil apoptosis	Protective[a] Protective in re-infection	[8,45] [46]
IL-3	Differentiation and growth factor, the inhibition of eosinophil apoptosis	—	[36]
GM-CSF	Differentiation and growth factor, the inhibition of eosinophil apoptosis	—	[36]
CD80	Induction of eosinophilia	—	[12]
CD86	Induction of eosinophilia	—	[12]
CCR3	Eosinophil migration	Protective to newborn larvae	[14]
β7 integrin	Eosinophil migration	—	[19,20]
Eotaxin-1 (CCL11)	Eosinophil trafficking	—[b]	[13]
Eotaxin-2 (CCL24)	Eosinophil trafficking	—	[13]
MBP	Eosinophil degranulation product	Protective to newborn larvae, and damaging to blood vessels and tissues	[59,60,64]
ECP	Eosinophil degranulation product	Protective to newborn larvae, and damaging to blood vessels and tissues	[59,60,64]
Ym1	AAM product, Th2 induction		[28]
Arginase	AAM product, Th2 induction		[29]

[a]Available data are not conclusive.

[b]Disruption of the CCL11 gene does not alter the expulsion.

Abbreviations: AAM, alternatively activated macrophage; CCL, CC chemokines ligand; CCR, CC chemokine receptor; CD, cluster differentiation; ECP, eosinophil cationic protein; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; MBP, major basic protein; Th, helper T cell; YM1, chitinase-like molecule.