Untangling the role of eosinophils in trichinellosis: a never ending story

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{848 words}

The role of eosinophils in inflammatory responses has long remained unknown. In the 1970s, *in vitro* models demonstrated their potential effector functions against different migrant stages of helminth parasites (including *Schistosoma mansoni* schistosomula, *Trichinella spiralis* newborn larvae (NBL) and *Onchocerca volvulus* microfilariae) [1].

Peripheral blood and tissue eosinophilia, as well as increased total IgE levels, are typical of human trichinellosis and of other helminth infections. Both processes are the consequence of T helper cell type 2 (Th2) activation [2].

After ingestion by a susceptible host, the larvae (L1) of the parasitic nematode *Trichinella spiralis* reach the intestine, where they mature into adult worms. After 4–6 days, these adult worms release (NBL) that pass through the intestinal mucosa and enter the lymph, and then the blood, from which they extravasate to invade the skeletal muscle fibres. After invasion, these fibres acquire a new phenotype known as the 'nurse cell' [3].

There is no doubt that, in both animal and human models, eosinophils can adhere to and kill *Trichinella spiralis* NBL *in vitro*, via antibody-dependent cellular cytotoxicity ADCC [4,5]. Several eosinophil-derived molecules (such as major basic protein, eosinophil cationic protein, eosinophil peroxidase, eosinophil-derived neurotoxin) are responsible for the cytotoxic effect. However, the role of these cells in host defense against *T. spiralis, in vivo,* has been a matter of debate [2].

A recent review on eosinophils and *Trichinella* [2], stated that 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'.

For example, in interleukin (IL)-5-deficient mice, IL-5 transgenic mice, or in mice depleted of eosinophils after treatment with specific monoclonal antibodies (mAbs) the worm burden during a primary infection was found to be moderately higher, or unchanged, compared to control animals [6–8]. More recently, mice deficient in CCR3 (one of the receptors for several eosinophil chemokines, constitutively present on the surface of eosinophils, together with CCR1), failing to recruit eosinophils to the nurse cell–parasite complex, after infection with *T. spiralis*, showed an

increase in the number of cysts and a reduction in necrotic nurse cells in histological sections of tongue, compared to wild-type animals [9], supporting a conclusion that eosinophils function in host defense against muscle-stage *T. spiralis*. The recruitment of eosinophils in response to chemokines such as eotaxin-1 (CCL11), for example, seems to play a relevant role also at mucosal level [10].

In a recent paper, Fabre *et al*. [11] studied *T. spiralis* infection in two models of eosinophil ablation: (i) mice bearing a deletion of the high-affinity double GATA site in the GATA-1 promoter (Δdbl-GATA$^{-/-}$), where the eosinophil differentiation is blocked; and (ii) eosinophil peroxidase diphtheria toxin transgenic mice (PHIL). PHIL mice were engineered to incorporate a coding sequence for the diphtheria toxin A chain in the eosinophil peroxidase locus.

Infiltrates surrounding infected muscle cells of wild-type mice were particularly rich in eosinophils, and eosinophils were (as expected) completely absent in the blood and skeletal muscles of genetically modified mice; surprisingly, however, *T. spiralis* muscle larvae died in large numbers, with significant reduction of larvae recovered from both animal models (67–77% reduction in PHIL mice, and 48% in Δdbl-GATA$^{-/-}$ mice**).** Reduction in the parasite burden correlated with enhanced Th1 response (as shown by increased levels of IFN-γ in lymph node cell cultures) and downregulated Th2 response (decreased IL-4 levels). By blocking inducible nitric oxide (NO) synthase (iNOS) with specific inhibitors, Fabre *et al*. observed improved larval survival, implicating NO production in parasite destruction. In double deficient IL-10$^{-/-}$/PHIL mice, there was a dramatic reduction in the larval burden (93%), compared with mice deficient only in IL-10. Furthermore, when these animals were treated with the iNOS inhibitor, in both IL-10$^{-/-}$ or IL-10$^{-/-}$/PHIL mice, the production of NO resulted lower in lymph node cell cultures, and larval improved survival. These results show that the destruction of muscle larvae is driven by Th1 response, which is down regulated by eosinophils. According to the results of the Fabre's paper eosinophils have a role of regulatory cells in immune response to parasites, as already described in allergic disease [1]. The results suggest that the parasite induces eosinophilia to protect itself.

This is just a new episode of the never-ending story regarding the understanding of the *in vivo* role of eosinophils versus helminth parasites, in particular *Trichinella*.

Two lessons can be learnt, however, from this recent paper: (i) experimental infection with *Trichinella* is more complex than those with other helminths, due to the important interaction of host cells with the parasite, at muscular level and (ii) infection in genetically modified animals has drawn completely opposite conclusions (eosinophils resulted host protective using CCR3 deficient mice where eosinophils, although present, are unable to extravasate to inflammation site and parasite protective when infection was carried out in Δdbl-GATA$^{-/-}$ mice which completely lack eosinophils).

Which is the truth, it is difficult to say, it is to note that in trichinellosis patients, however, a sudden fall in eosinophil levels is considered a sign of a bad prognosis, this would suggest an *in vivo* host-protective role of these effector cells [2].

References

1 Hogan, S.P. *et al*. (2008) Eosinophils: biological properties and role in health and disease. *Clin. Exp. Allergy* 38, 709–750

2 Bruschi, F. *et al*. (2008) Eosinophils and *Trichinella* infection: toxic for the parasite and the host? *Trends Parasitol.* 24, 462-467

3 Despommier, D.D. (1998) How does *Trichinella spiralis* make itself at home? *Parasitol. Today* 14, 318–323

4 Kazura, J.W. and Aikawa M. (1980) Host defense mechanisms against *Trichinella spiralis* infection in the mouse: eosinophil-mediated destruction of newborn larvae *in vitro*. *J. Immunol.* 124, 355–361

5 Gansmüller, A. *et al*. (1987) Antibody-dependent in vitro cytotoxicity of newborn *Trichinella spiralis* larvae. Nature of the cells involved. *Parasite Immunol.* 9, 281–292

6 Herndon, F.J. and Kayes, S.G. (1992) Depletion of eosinophils by anti-IL-5 monoclonal antibody treatment of mice infected with *Trichinella spiralis* does not alter parasite burden or immunologic resistance to reinfection. *J. Immunol.* 149, 3642–3647

7 Hokibara, S. *et al*. (1997) Marked eosinophilia in interleukin-5 transgenic mice fails to prevent *Trichinella spiralis* infection. *J. Parasitol.* 83, 1186–1189

8 Vallance, B.A. *et al.* (2000) Interleukin-5 deficient mice exhibit impaired host defence against challenge *Trichinella spiralis* infections. *Parasite Immunol.* 22, 487–492

9 Gurish, M.F. *et al.* (2002) CCR3 is required for tissue eosinophilia and larval cytotoxicity after infection with *Trichinella spiralis*. *J. Immunol.* 168, 5730–5736

10 Dixon, H *et al.* (2006) The role of Th2 cytokines, chemokines and parasite products in eosinophil recruitment to the gastrointestinal mucosa during helminth infection. *Eur. J. Immunol.* 36, 1753– 1763

11 Fabre, V. *et al.* (2009) Eosinophil Deficiency Compromises Parasite Survival in Chronic Nematode Infection. *J. Immunol*. 182, 1577–1583