

# The Role of Eosinophils in Parasitic Helminth Infections: Insights from Genetically Modified Mice

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*Eosinophilia – an increase in the number of eosinophils in the blood or tissues – has historically been recognized as a distinctive feature of helminth infections in mammals. Yet the precise functions of these cells are still poorly understood. Many scientists consider that their primary function is protection against parasites, although there is little unequivocal in vivo evidence to prove this. Eosinophils are also responsible for considerable pathology in mammals because they are inevitably present in large numbers in inflammatory lesions associated with helminth infections or allergic conditions. In this review, Carolyn Behm and Karen Ovington outline some of the cellular and biological properties of eosinophils and evaluate the evidence for their role(s) in parasitic infections.*

Eosinophils or 'eosinophilic granulocytes' normally comprise only a small fraction (<1–5%) of circulating leukocytes. They were so-named by Paul Ehrlich in 1879, when he observed the affinity of their cytoplasmic granules – small 'bombs' containing cytotoxic proteins – for the red acid dye eosin, which stains their cytosol a distinctive granular pink. Eosinophils develop in the bone marrow and are released constitutively at a low rate into the circulation. They are terminally differentiated cells that do not appear to multiply after leaving the bone marrow<sup>1</sup>; their half-life in blood is about 18 h<sup>2</sup>. Most of the eosinophil population is found in the tissues, predominantly those at the surfaces of the body that interact with the external environment, ie. the skin and mucosal surfaces of the gut, respiratory and reproductive systems<sup>3,4</sup>. The normal life span of eosinophils in healthy tissue is not known but they are believed to survive for several days<sup>5</sup>, possibly weeks<sup>3</sup>. During helminth infections or in allergic conditions, eosinophils are released more rapidly from the bone marrow (within 1 h of stimulation<sup>6</sup>), their survival in tissues is enhanced<sup>7,8</sup> and the rate of bone marrow eosinophilopoiesis increases dramatically. The rate of entry of eosinophils into infected and inflamed tissues, and perhaps mucosal sites in general, is considerably upregulated; this results in tissue eosinophilia.

Eosinophils arise in the bone marrow from haematopoietic CD34<sup>+</sup> precursor cells<sup>4</sup>. The early stages of their differentiation are controlled by the cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin 3 (IL-3), which also control the development of other granulocytes such as neutrophils, basophils and mast cells. The later stages of differentiation and maturation of most of the eosinophil population are controlled by the cytokine IL-5, which is produced by activated T cells and mast cells. Recently,

it has been shown that IL-5, found at high levels in helminth-infected hosts during the T-helper type 2 (Th2) cytokine-biased immune response, appears to be important in mucosal immune responses and is responsible for helminth-induced eosinophilia. IL-5 presents quite a puzzle for immunologists. It has been highly conserved during mammalian evolution – mouse IL-5, for example, has 71% amino acid identity with human IL-5 – which suggests it has important function(s) that have been selected during evolution. However, functions exclusive to IL-5 are not numerous, and none appears to be essential for survival, at least for mice living in laboratory conditions. In mice, IL-5 controls or influences the development of two major cell types: the elevated rate of development, maturation and survival of eosinophils during a Th2 cytokine response and the maturation of peritoneal and intestinal B-1 lymphocytes. Even for these cells, IL-5 is not absolutely essential. Maturation of B-1 cells was delayed only slightly in (uninfected) IL-5-knockout mice<sup>9</sup> and more extensively in IL-5 receptor  $\alpha$ -knockout mice<sup>10</sup>. Furthermore, there is a minor population of IL-5-independent eosinophils that develops and functions in the absence of functional receptors for IL-5, GM-CSF and IL-3 (Ref. 11). Therefore, current evidence leads us to hypothesize that the most important, and apparently exclusive, function of IL-5 is the control of eosinophilia, with the question of any essential role in the development of B-1 cells still open.

### Properties and functions of eosinophils

What is special about eosinophils and what is the evolutionary importance of eosinophilia? Do eosinophils have essential roles at mucosal sites? Why is the terminal development of eosinophils controlled independently of the other granulocytes? These and many other questions remain to be answered. Eosinophils are clearly multifunctional cells. They possess in their granules and lipid bodies a battery of potent cytotoxic and proinflammatory agents, and they express receptors for and also secrete a large variety of immunologically important molecules (Box 1). Under the influence of the Th2-cell environment, they respond to chemoattractants and other signals by leaving the blood vessels and homing in to inflammatory or helminth-infected sites, where they become activated and secrete cytokines, proinflammatory lipid and other mediators, degranulate to release cytotoxic products, and phagocytose particulate material. Although eosinophils phagocytose and kill bacteria, they are unable to clear a bacterial infection in the absence of neutrophils<sup>3</sup>. Their primary function is considered to be defence against organisms that are too large to be phagocytosed, particularly parasitic helminths. They might also be involved in wound healing and repair, in fibrosis, and are thought to act as antigen-presenting cells<sup>12</sup>. As well as host-derived immunoglobulins and

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components of complement on the surface of their targets, eosinophils might bind and respond to carbohydrate ligands expressed on the parasite surface, such as the Lewis<sup>x</sup>-related molecules, and cell-adhesion molecules similar to selectins that have, for example, been demonstrated on schistosomula<sup>13</sup>. After becoming activated during the homing process, they degranulate on to or around their targets; they then die by apoptosis and are phagocytosed by other cells such as macrophages. The turnover of eosinophils is quite rapid at inflammatory sites, where they may survive for only 4–5 days.

### Eosinophils in parasite infections

The hypothesis that the primary function of eosinophils is to defend hosts against infection by relatively large organisms such as parasitic helminths is based on the accumulation of observations that: (1) eosinophils degranulate on to and can kill helminths *in vitro* in the presence of antibody and/or complement; (2) they move from the blood and aggregate in the locality of helminths *in vivo*; (3) large numbers of eosinophils are often seen in close association with both intact and damaged helminths *in vivo*; and (4) they clearly degranulate in the vicinity of, or on to the surfaces of, helminths *in vivo*<sup>14</sup>. Further evidence is provided by epidemiological studies showing correlations between eosinophilia and protection against schistosome infections in Africa<sup>15,16</sup>. However, direct evidence of a role for eosinophils in host protection against helminths *in vivo* is lacking, and the debate continues (see below and Ref. 17).

A variety of studies has been carried out in which monoclonal antibodies (mAbs) that neutralize IL-5 have been administered to mice. This treatment greatly reduced the development of eosinophilia upon infection with parasitic helminths, but had little effect on the survival or reproduction of a number of nematodes and trematodes – primary infections of *Nippostrongylus brasiliensis*<sup>18</sup>, *Schistosoma mansoni*<sup>19,20</sup>, *S. japonicum*<sup>21</sup>, *Trichinella spiralis*<sup>22</sup>, *Toxocara canis*<sup>23</sup>, *Trichuris muris*<sup>24</sup>, migrating larvae of *Strongyloides stercoralis*<sup>25</sup> and secondary infections of *Heligmosomoides polygyrus*<sup>26</sup>. However, in a minority of studies with other parasite species, anti-IL-5 antibody treatment exacerbated the infection. The survival and distribution of parasites in certain tissues was increased in primary infections of mice with the rat parasites *Strongyloides venezuelensis* or *Angiostrongylus cantonensis* after treatment with anti-IL-5 mAbs<sup>27,28</sup> which, as expected, ablated eosinophils. Anti-IL-5 mAb treatment compromised both the killing, by eosinophils, of *Onchocerca volvulus* infective larvae implanted in diffusion chambers of vaccinated mice<sup>29</sup> and the clearance of microfilariae of *O. lienalis* from immunized mice<sup>30,31</sup>. Similarly, killing of third-stage larvae of *S. stercoralis*, the parasite that infects humans, within diffusion chambers in immunized mice was ablated by IL-5 mAb treatment<sup>25</sup>. It might be significant that most of these parasites do not naturally infect mice. The conclusion to be drawn from this evidence is that IL-5-dependent immune responses, including eosinophilia, might limit infection with a relatively small number of species of nematodes generally in hosts other than their natural hosts. For species for which no discernible effect of ablation of IL-5 could be detected, IL-5 might be either functionally unimportant, or redundant.

### Studies with genetically modified mice

The recent availability of genetically modified mice has somewhat altered our views. Transgenic mouse strains constitutively overexpressing the gene encoding IL-5 (*IL5*) have been developed using different transgene constructs by two research groups (Table 1). One group<sup>32</sup> coupled the *IL5* gene to the promoter of the human *CD2* gene, which encodes a dominant T-cell surface antigen (in CBA/Ca mice). The other group<sup>33</sup> used the metallothionein promoter to drive *IL5* expression inducibly in various organs (eg. liver, kidney, intestine, heart and spleen) in C3H/HeN mice. Thus, the regulation of overexpression of *IL5* is different in the two sets of transgenic mice. Both sets display constitutive high blood and tissue eosinophilia (up to 80–90% of total leukocytes in peripheral blood), but in the CBA/Ca strains, normal serum levels of all subclasses of IgG, IgM, IgA and IgE were reported in uninfected mice<sup>34</sup>, whereas uninfected mice of the C3H/HeN transgenic strain had elevated serum IgM and IgA but not IgG1 or IgG2a<sup>33</sup>. The C3H/HeN transgenic mice also exhibited preferential growth of a distinctive and inducible splenic (but not peritoneal) population of B cells that expressed the IL-5 receptor (IL-5R) and IgM along with weak expression of B220 and Ly-1 surface antigens. The eosinophils of the transgenic mice appeared to be fully functional but did not cause overt disease in uninfected mice<sup>34,35</sup>.

These mice have now been infected experimentally with a variety of parasites, with quite variable outcomes (Table 2). For four parasite species – *T. canis*<sup>36–38</sup>, *T. spiralis* (in the C3H/HeN background)<sup>39</sup>, *S. mansoni* (in the C3H/HeN background)<sup>40</sup> and *Mesocestoides corti*<sup>32,41</sup> there were no differences in the worm burdens in IL-5 transgenic mice. However, in primary infections with *N. brasiliensis* and *Angiostrongylus cantonensis*, parasite burdens were dramatically decreased<sup>10,37,42,43</sup>, indicating an IL-5-dependent host-protective effect. Furthermore, many of the *N. brasiliensis* worms that did establish in the intestine of the transgenic mice failed to thrive and produce eggs<sup>37</sup>, and there was evidence<sup>37,44</sup> that many of the nematodes were damaged in the skin before passage through the lungs, as well as in the gut. Worm burdens in secondary *N. brasiliensis* infections of normal and IL-5 transgenic mice were similar, however, indicating no essential role for IL-5 in immunological memory in this infection. In the *A. cantonensis* infections, fewer intracranial worms established; worms were killed more rapidly and female worms were smaller than in normal C3H/HeN mice<sup>10,43</sup>. The effects were correlated with greatly intensified eosinophil infiltration into the cerebrospinal fluid of transgenic mice, clear evidence of their degranulation on to the worms, and increased parasite antigen-specific serum IgG1 and IgA. Although the increase in IgG1 occurred probably too late in the infection to have a significant antiparasitic effect, the elevation of IgA occurred within 5–7 days post-infection (p.i.). IgA is reported to be the most effective stimulator of degranulation of human eosinophils<sup>45</sup>.

One hypothesis to account for these experimental observations<sup>36</sup> is that helminths with rapid transits through the tissues and intestines do not normally encounter large numbers of activated eosinophils as it takes the host seven days or more p.i. to mount an eosinophilopoietic response. Therefore, these parasites

## Box 1. A Survey of Immunologically Important Molecules Expressed or Secreted by Eosinophils<sup>a</sup>

### Molecules expressed on cell surface<sup>b</sup>

#### Receptors for:

##### *Immunoglobulins*

	Ligands	Refs
Fc <sub>ε</sub> RI	IgE (high affinity) (not detected in mice)	4,54
Fc <sub>ε</sub> RII	IgE (low affinity) (not detected in mice)	4,54
Fc <sub>γ</sub> RI, Fc <sub>γ</sub> RII, Fc <sub>γ</sub> RIII	IgG	4,63
Fc <sub>α</sub> R	IgA (highest affinity is for secretory IgA)	4,64
Mac-2	IgE (not detected in mice)	54,65

##### *Complement fragments*

	C1q, C3b/C4b, iC3b, C5a	3,4
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##### *Cytokines*

	IL-2, IL-3, IL-4, IL-5, IL-13, IL-16, GM-CSF, IFN- $\alpha$ , IFN- $\gamma$ , TNF- $\alpha$	4,58,66 67
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##### *Chemoattractants, immunomodulators and chemokines*

Eicosanoid receptors	Leukotrienes, lipoxins	3,4
Formyl peptide receptor	FMLP	68
PAF receptor	PAF	3,4
CCR1	RANTES, MIP-1 $\alpha$ , MCP-2, MCP-3	69,70
CCR3	Eotaxin, eotaxin-2, MCP-2, MCP-3, MCP-4, RANTES	71-75
CXCR2	IL-8	73,75

### Cell-surface adhesion molecules:

L-selectin	MAdCAM-1, GlyCAM-1, CD34	76
ICAM-1	Mac-1, LFA-1	77

##### *Integrins*

$\alpha_4\beta_1$ (VLA-4)	VCAM-1, fibronectin CS-1	4
$\alpha_4\beta_7$	VCAM-1, fibronectin, MAdCAM-1	4
$\alpha_5\beta_1$ (VLA-5)	Fibronectin	78
$\alpha_6\beta_1$ (VLA-6)	Laminin	60
$\alpha_M\beta_2$ (Mac-1)	ICAM-1, iC3b, fibrinogen, ICAM-3	79
$\alpha_L\beta_2$ (LFA-1)	ICAM-1, ICAM-2, ICAM-3, ICAM-4, ICAM-5	3
$\alpha_d\beta_2$	ICAM-3, VCAM-1	60
$\alpha_x\beta_2$ (p150,95)	Fibrinogen, iC3b, lipopolysaccharide	3

##### *Carbohydrates*

sLe <sup>x</sup> and others	E- and P-selectins	4
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##### *Surface glycoproteins*

CD4	MHC Class II, IL-16	3,79
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##### *MHC Class II molecules*

HLA-DR	T-cell receptor	3,4
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### Intracellular receptors:

Oestrogen receptors	Oestrogens	3,4
Steroid receptors	Glucocorticoids	3,4

### Molecules released by eosinophils

#### Released from granules:

	Putative functions	
MBP	In crystalline core; no known enzymatic activity, toxic to helminths, tumour and host cells; activates platelets, neutrophils, mast cells, basophils	3,79
ECP	Non-core matrix; bactericidal and toxic to helminths and host cells	3,79
EDN	Non-core matrix; ribonuclease catalytic activity	3,79
EPO	Non-core matrix; peroxidase activity catalysing synthesis of hypohalous acids; toxic to helminths, protozoa, bacteria, tumour and host cells	3,79
Lysosomal hydrolases	Digestive functions	3,79
Lysophospholipase	Present in primary granules and cell membrane; hydrophobic protein that forms the Charcot-Leyden crystals; constitutes about 5% of total eosinophil proteins; membrane digestive function	3,79
Bactericidal/permeability increasing protein	Bactericidal (Gram-negative bacteria)	62

#### Secreted:

##### *Lipid mediators*

LTC <sub>4</sub> , LTD <sub>4</sub> , PGE <sub>2</sub>	Stimulate vasoactivity, smooth muscle contraction, secretion of mucus	4,57
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PAF	Stimulates vasoactivity, microvascular leakage, smooth muscle contraction; eosinophil chemoattractant; stimulates effector functions of eosinophils, neutrophils, macrophages, platelets	80
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Lipoxins	Anti-inflammatory immunomodulators	4
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**Box 1. A Survey of Immunologically Important Molecules Expressed or Secreted by Eosinophils<sup>a</sup> (cont'd)**

<i>Peptide mediators</i>		
Substance P	Proinflammatory, increases vascular permeability, eosinophil chemoattractant and activator	3,4
<i>Cytokines and chemokines —may be stored as preformed pools within specific granules</i>		
IL-2, IL-4, IL-10, IL-12, IL-16, IFN- $\gamma$	Potential regulators of immune response	4,5,9,81
GM-CSF, IL-3, IL-5, LCF (IL-16), RANTES, MIP-1 $\alpha$ , eotaxin	Growth factors and chemokines	4,5,9,61
TGF- $\alpha$ , TGF- $\beta$ 1, VEGF/VPF, TNF- $\alpha$ , IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-8	Involved in inflammation, fibrosis, wound healing and tissue repair	4,5,9,82
<i>Proteases</i>		
Matrix metalloprotease-9	Degrades intercellular matrix	83
<i>Reactive oxygen metabolites and nitric oxide</i>		
	Microbicidal, damage membranes and macromolecules	57,84

<sup>a</sup> Compiled from the sources listed. The list is not exhaustive and differences occur between species, animal strains and individual humans.

<sup>b</sup> Abbreviations: ECP, eosinophil cationic protein; EDN, eosinophil-derived neurotoxin; EPO, eosinophil peroxidase; FMLP, formyl-methionyl-leucyl-phenylalanine; GlyCAM, glycosylation-dependent cell adhesion molecule; GM-CSF, granulocyte-macrophage colony stimulating factor; ICAM, intercellular cell adhesion molecule; IFN, interferon; Ig, immunoglobulin; IL, interleukin; LCF, lymphocyte chemoattractant factor (IL-16); LFA, lymphocyte function-associated antigen; LT, leukotriene; MAdCAM, mucosal addressin cell adhesion molecule; MBP, major basic protein; MCP, macrophage chemoattractant protein; MHC, major histocompatibility complex; MIP, macrophage inflammatory protein; PAF, platelet activating factor; PG, prostaglandin; RANTES, regulated upon activation normal T cell expressed and secreted; sLe<sup>x</sup>, sialyl Lewis<sup>x</sup> tetrasaccharide; TGF, transforming growth factor; TNF, tumour necrosis factor; VCAM, vascular cell adhesion molecule; VEGF/VPF, vascular endothelial cell growth factor/vascular permeability factor; VLA, very late antigen.

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would not have been under evolutionary pressure to express protective mechanisms against eosinophilic attack during rapid migration through the tissues. Thus, when larvae of rapid-transit parasites such as *N. brasiliensis* encounter large numbers of eosinophils within hours of inoculation into the IL-5 transgenic mice, they have inadequate protective mechanisms and are damaged or killed. Such a phenomenon has been observed in challenge *Strongyloides ratti* infections of normal Wistar rats, a biologically more natural host–parasite system: infective larvae were killed in

the skin, surrounded by large aggregations of eosinophils in close contact with the larval cuticle, within several hours of a subcutaneous sixth challenge infection<sup>46</sup>. If the ‘rapid-transit’ hypothesis is true, one prediction would be that helminths that reside in the host tissues for longer periods, such as *T. canis*, *M. corti*, *A. cantonensis* or *S. mansoni*, would be the ones selected during evolution to express protective mechanisms that allow them to survive eosinophilic attack, and thus would not be adversely affected in hypereosinophilic mice. We should note,

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Table 1. IL-5 transgenic and gene knockout mice: phenotypic properties in uninfected mice<sup>a</sup>

Mice	Genetic background	Construct	Promoter	Phenotypic effects	Refs
IL-5-knockout	C57BL/6	Neomycin resistance gene inserted into exon 3 of gene encoding IL-5 ( <i>IL5</i> )	Mouse IL-5	Reduced constitutive eosinophil population; absence of eosinophilopoietic response to helminth infection; delayed development of CD5 <sup>+</sup> B-1a lymphocytes; depleted intestinal IgA <sup>+</sup> B-1 cells; normal serum antibody profiles	9,55
IL-5R $\alpha$ -knockout	(129/Ola $\times$ C57BL/6) F2 hybrid	Neomycin resistance gene and translational stop codon inserted into exons 6 and 5, respectively, of gene encoding IL-5R $\alpha$	Mouse IL-5R $\alpha$	Reduced constitutive eosinophil population; delayed development of peritoneal CD5 <sup>+</sup> B-1a cells; fewer mucosal B-1 cells; fewer, and impaired development of, mucosal IgA <sup>+</sup> B-1 cells; low serum IgM and IgG3; low mucosal secretory IgA; normal serum IgA; delayed development of thymocyte populations; impaired eosinophilopoietic response to nematode infection or exogenous IL-5; impaired response to T-cell-independent antigens	10,56
IL-5 transgenic	CBA/Ca	Additional copies of murine <i>IL5</i> gene; several strains with different gene copy numbers available	Dominant control region of human <i>CD2</i> gene (a T-cell surface antigen)	Constitutive blood and tissue eosinophilia; splenomegaly; B-cell population and serum antibody profiles normal; homozygotes fail to breed	32,34, 41
IL-5 transgenic	C3H/HeN	Murine <i>IL5</i> cDNA inserted into exon of rabbit gene encoding $\beta$ -globin; about 40 copies integrated	Mouse metallothionein	Inducible blood and tissue eosinophilia; elevated serum IgM, IgA; inducible elevated splenic Ly-1 (CD5) <sup>+</sup> B-cell population	33

<sup>a</sup> Abbreviations: IL-5, interleukin 5; IL-5R $\alpha$ , IL-5 receptor  $\alpha$  subunit; Ig, immunoglobulin.

however, that natural exposure to helminths is very different from that generally used experimentally. Under natural conditions, hosts are repeatedly exposed to small numbers of infective larvae. The first exposure would induce eosinophilia, which could still be present, or more rapidly induced, when the host was next exposed to infective larvae.

Although the results from infecting transgenic mice with *T. canis*, *M. corti* and *A. cantonensis* have supported this hypothesis, the surprises came with the results from infection of mice in the CBA/Ca background with *S. mansoni*<sup>34</sup> and *T. spiralis*<sup>36</sup>, both parasites that reside in the mammalian host for long periods. Whereas the general interpretation of the earlier IL-5 antibody studies<sup>19,20,22</sup> had suggested that ablation of IL-5 had little impact on the outcome of these two infections, this strain of IL-5 transgenic mice showed a tendency to increased worm burdens in these infections, which does not support the 'rapid-transit' hypothesis. In the *S. mansoni* infections, IL-5 transgenic mice also responded less effectively to vaccination with irradiated cercariae. This implies that IL-5, and hence perhaps eosinophilia, is in some way parasite-protective in these infections. This is very interesting in the light of earlier studies showing a requirement for host tumour necrosis factor (TNF) in egg-laying in *S. mansoni* infections of severe combined immunodeficient (SCID) mice<sup>47,48</sup>. Schistosomes are apparently very well adapted to take advantage of the immune responses of the murine host. These results appear to contradict the human epidemiological evidence that shows a correlation between eosinophilia and protection against schistosomiasis: clearly, we need

to probe this relationship further to determine whether these observations represent fundamental differences in the antischistosomal immune responses or in the functional capabilities of eosinophils between mice and humans.

More recently, mice have become available that are genetically deficient in IL-5 (Ref. 9) or in the  $\alpha$ -subunit of the IL-5 receptor (IL-5R $\alpha$ )<sup>10</sup> (Table 1). The IL-5R $\alpha$  is exclusive to the IL-5 receptor, and is expressed in cells responsive to IL-5, particularly eosinophils and CD5<sup>+</sup> B-1 cells. IL-5- and IL-5R $\alpha$ -deficient mice harbour very small populations of apparently normal eosinophils, termed IL-5-independent eosinophils, and fail to develop an eosinophilopoietic response when infected with any of the helminths tested to date<sup>49</sup>. They also exhibit delayed development of the peritoneal B-1 cell population, and IL-5R $\alpha$ -deficient mice have reduced levels of serum IgM and IgG3 and mucosal secretory IgA. The outcome of infection of these mice with a large variety of parasites has now been determined (Table 3). To date, the patterns of worm burdens in primary and secondary infections are, as might be expected, the opposite of those reported for IL-5 transgenic mice. Thus, worm burdens and distributions in primary infections of IL-5-deficient mice with *M. corti* and *T. canis* were similar to wild-type mice, although we did observe reduced pathology in *T. canis* infections<sup>9,50</sup>. Reduced jejunal smooth muscle hypercontractility and a slight delay in expulsion of intestinal adults were also observed in infections of IL-5-deficient mice with *T. spiralis*, although worm burdens were similar<sup>51</sup>. In IL-5-deficient mice, no difference was found in the outcome of *Fasciola hepatica* infection, which has not yet

Table 2. Outcome of helminth infections in IL-5 transgenic mice compared with normal littermates

Parasite	Infection	Mouse genetic background	Parasitological outcome	Refs
<b>Cestodes</b>				
<i>Mesocestoides corti</i>	Primary	CBA/Ca	Similar number of tissue larvae	32,41
<b>Trematodes</b>				
<i>Schistosoma mansoni</i>	Primary	CBA/Ca	Increased liver-stage larvae	34
<i>S. mansoni</i>	Immunized	CBA/Ca	Impaired parasite clearance	34
<i>S. mansoni</i>	Primary	C3H/HeN	Similar recovery of adult worms	40
<i>S. mansoni</i>	Immunized	C3H/HeN	Similar recovery of adult worms	40
<b>Nematodes</b>				
<i>Angiostrongylus cantonensis</i>	Primary	C3H/HeN	Smaller and fewer intracranial worms	10,43
<i>Nippostrongylus brasiliensis</i>	Primary	CBA/Ca	Smaller and fewer intestinal worms; reduced egg output	36,37
<i>N. brasiliensis</i>	Secondary	CBA/Ca	Similar number of intestinal worms	36
<i>N. brasiliensis</i>	Primary	C3H/HeN	Reduced number of larvae in lungs and adults in intestine	42
<i>N. brasiliensis</i>	Primary	C57BL/6	Reduced number of larvae in lungs and adults in intestine	42
<i>Toxocara canis</i>	Primary	C3H/HeN	Similar number of tissue larvae	38
<i>T. canis</i>	Immunized with larval antigens	C3H/HeN	Similar number of tissue larvae	38
<i>T. canis</i>	Primary	CBA/Ca	Similar number of tissue larvae	36,37
<i>Trichinella spiralis</i>	Primary	CBA/Ca	Increased number of tissue larvae	36
<i>T. spiralis</i>	Primary	C3H/HeN	Similar number of intestinal adult worms; similar female fecundity; similar number of tissue larvae	39
<i>T. spiralis</i>	Immunized with larval antigens	C3H/HeN	Similar number of intestinal adult worms; similar female fecundity; similar number of tissue larvae	39

been tested in IL-5 transgenic mice. As would be predicted from the IL-5 transgenic experiments, infection of IL-5R $\alpha$ -deficient mice with *A. cantonensis* yielded a greater number of larger intracranial worms than was seen in normal mice<sup>10,43</sup>. However, in both *S. ratti*<sup>52</sup> and *H. polygyrus* (D. Morgan, unpublished) infections, we found significant differences in parasite burdens between IL-5-deficient and normal mice. *Strongyloides ratti* causes an acute infection in mice, the worms being expelled by Day 10 p.i., whereas *H. polygyrus* infections are chronic, lasting months, during which the intestinal population gradually declines. Worm establishment and fecundity were increased in *S. ratti* infections

of IL-5-deficient mice, as was host pathology. Unchanged, however, was the duration of the infection, from which we conclude that IL-5 has no essential role in the rapid expulsion of *S. ratti* adults from mice. In *H. polygyrus* infections, however, the situation is different. IL-5-deficient mice had more worms that were more fecund and persisted for longer.

The *S. ratti* experiments<sup>52</sup> provide compelling *in vivo* evidence for a protective role of eosinophilia against an intestinal nematode. We have drawn this conclusion on the basis of: (1) considerably reduced numbers of eosinophils in IL-5-deficient mice; (2) the presence of large accumulations of eosinophils – the first leukocytes

Table 3. Outcome of helminth infections in IL-5 and IL-5R $\alpha$  knockout mice compared with normal mice<sup>a</sup>

Parasite	Infection	Comparative outcome	Refs
<b>IL-5 knockout mice</b>			
<b>Cestodes</b>			
<i>Mesocestoides corti</i>	Primary	Similar number of worms and host pathology	9
<i>Hymenolepis diminuta</i>	Primary	Similar; worms failed to develop and persist	49
<b>Trematodes</b>			
<i>Fasciola hepatica</i>	Primary	Similar establishment and host pathology	49
<b>Nematodes</b>			
<i>Strongyloides ratti</i>	Primary	Increased worm burden; more fecund parasites; increased host pathology	52
<i>S. ratti</i>	Secondary	Similar host protection	52
<i>Trichinella spiralis</i>	Primary	Slightly delayed expulsion of intestinal adult worms; similar number of intestinal adults and muscle larvae; slightly reduced enteric smooth muscle hypercontractility	51
<i>Toxocara canis</i>	Primary	Similar number of tissue larvae; reduced lung pathology	50
<i>Heligmosomoides polygyrus</i>	Primary	Increased worm burden; more fecund worms; delayed expulsion	b
<i>H. polygyrus</i>	Secondary	Similar host protection	b
<b>IL-5 receptor <math>\alpha</math> knockout mice</b>			
<b>Nematodes</b>			
<i>Angiostrongylus cantonensis</i>	Primary	Elevated intracranial worm burdens; larger worms	10,43

<sup>a</sup> Abbreviations: IL-5, interleukin-5; IL-5R $\alpha$ , IL-5 receptor  $\alpha$ .

<sup>b</sup> D. Morgan, unpublished.



to appear – in the vicinity of gut nematodes in wild-type mice from Day 4 p.i., compared with the accumulation of very low numbers of eosinophils in IL-5-deficient mice; and (3) the absence of any other persistent deficiency identified to date in IL-5-deficient mice. This is still not indisputable evidence of a protective role for eosinophils, however, as the evidence remains circumstantial. Until all the *in vivo* functions of IL-5 have been identified and detailed in infected and uninfected mice, some uncertainty remains. The role of the IL-5-dependent intestinal IgA<sup>+</sup> B-1 cells, for example, is yet to be evaluated, so it is important to test eosinophil function in hosts with normal B-1 cell populations. Further work is needed to identify and characterize molecules essential and exclusive to the development or function of all eosinophils – including the IL-5-independent population – followed up by genetic or antibody-based inactivation of these molecules and dissection of the phenotypic consequences in parasite infections. Perhaps the toxic granule proteins, major basic protein (MBP) and eosinophil cationic protein, would be good candidates for this type of study.

The 'rapid-transit' hypothesis<sup>34</sup> that eosinophilia is host-protective only after about Day 7 p.i., when eosinophilopoiesis in the bone marrow has been upregulated, is not supported by our results in *S. ratti* infections in IL-5-deficient mice<sup>52</sup>. We saw clear differences in parasite establishment in the gut from Day 4 p.i. At Day 6 p.i., there were many more eosinophils in the gut in wild-type infected mice. This suggests that eosinophils from the constitutive population were recruited to the site of infection early in the infection before mature eosinophils were available from upregulated bone-marrow eosinophilopoiesis. Thus, we hypothesize that in mice the constitutive bone-marrow eosinophil population is released early in infection by helminths, and that it is sufficient in *S. ratti* infections (but not many others) to affect the establishment and fecundity of the worms. This leads us to hypothesize that this constitutive bone marrow eosinophil population, in concert with the eosinophil population normally resident in mucosal tissues, could be important in determining whether incoming helminth larvae can become established in mammalian hosts; i.e. that eosinophils contribute to an important 'first line of defence' and thus to the determination of the host range of parasites. Such a function is likely to be selected during evolution. Factors important in determining the host range of parasites would, like the constitutive mucosal and bone marrow eosinophil populations, be constitutive, act early in the infection and generally operate independently of immunological memory.

## Conclusions

What do these new studies in mice tell us about the role of eosinophils in helminth infections? Clearly, IL-5 and eosinophils have different impacts on different helminth infections and general conclusions are difficult to propose from the diversity of species combinations under study. Murine studies are particularly problematical because mice are not the natural hosts of many of the parasites used experimentally. Nonetheless, three major conclusions can be drawn: (1) in some infections, such as those with *M. corti* and *T. canis*, IL-5 and IL-5-dependent eosinophils do not substantially affect the parasitological outcome of the

infection, although IL-5 might cause increased pathology in *T. canis* infections; (2) there is a level of IL-5-dependent, and hence possibly eosinophil-dependent, host protection in infections with the nematodes *S. ratti*, *H. polygyrus* and *N. brasiliensis*; and (3) in murine *S. mansoni* infections, under some circumstances IL-5, and hence perhaps IL-5-dependent eosinophils, are parasite-protective by yet-to-be-determined mechanisms.

These studies in mice give insight into *in vivo* functions of IL-5 and eosinophils, but how useful are they in providing compelling evidence for a hypothesized essential role of IL-5 during the evolution of mammals? Are mice the best model to use? Murine eosinophils are less effective than rat eosinophils in killing schistosomes in the presence of IgG<sup>34</sup>. They are unusual in not binding IgE or expressing receptors for IgE (FcεRI, FcεRII or Mac-2)<sup>53,54</sup>, yet IgE is important in effector functions of human eosinophils. Are mice sufficiently representative of other mammals to use for testing eosinophil function? Furthermore, laboratory mono-infection experiments using inbred strains of well-fed and sheltered mice do not closely imitate the real world, where exposure of outbred, free-ranging and possibly undernourished hosts to many different parasites is a continuous process. More realistic field studies, possibly using anti-IL-5 antibody treatment of hosts, under circumstances where natural host-parasite interactions will occur, might answer this question. In the field situation, the IL-5-dependent effects on helminth fecundity might be shown to be particularly important at the population level, perhaps more important than protective effects on individual hosts.

## Acknowledgements

We apologize to the authors whose work has not been specifically referenced in this article owing to the restricted number of references permitted. We thank Elizabeth Milbourne, Klaus Matthaei and Ian Young for many discussions and collaboration, and the Australian Research Council, the National Health and Medical Research Council and the Clive and Vera Ramaciotti Foundation for financial support of our work.

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