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Interleukin-3 and c-Kit/stem cell factor are required for normal eosinophil responses in mice infected with *Strongyloides venezuelensis*

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To evaluate the potential roles of Interleukin-3 (IL-3) and c-Kit, the tyrosine kinase receptor for stem cell factor (SCF), in eosinophil responses *in vivo*, we examined eosinophil numbers in uninfected or nematode-infected wild-type mice, IL-3−/− mice, and IL-3−/− mice that also have a marked reduction in SCF/c-Kit signaling (ie, Kit^W/Kit^{W-v}, IL-3−/− mice). We found no significant differences in the numbers of eosinophils in the blood, bone marrow or various tissues of IL-3−/− *vs* IL-3+/+ mice, either at baseline or after the induction of bone marrow, blood or tissue eosinophilia in response to infection with *Strongyloides venezuelensis* (*S.v.*) or *Nippostrongylus brasiliensis* (*N.b.*). However, in mice with markedly impaired SCF/c-Kit signaling, IL-3 contributed significantly to the increased numbers of eosinophils that were observed in multiple tissues during *S.v.* infection, but not during infection with *N.b.*
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Interleukin-3 (IL-3) can promote the development of various hematopoietic cells *in vitro*, through effects on both multipotential and more mature, lineage-committed progenitor cells.[1] While many studies have shown that IL-3 can enhance the development, survival and functional activation of mouse or human eosinophils *in vitro*,[2–6] the importance of IL-3 in eosinophil development *in vivo* has been difficult to assess. In part, this reflects the fact that three cytokines, IL-3, IL-5 and GM-CSF, whose genes are closely linked and which all can be expressed by activated T cells or mast cells, express overlapping functions.[7–10] For example, all three cytokines can promote eosinophil development *in vitro*.[5,6] Moreover, all three of these cytokines bind to cell surface receptors which are heterodimers consisting of the same 'common' β chain (β_c);[3] this β_c forms high affinity receptors for IL-3, IL-5 or GM-CSF when paired with the appropriate specific ligand-binding α chain.[11,12] Mice, but not humans, also express an IL-3-specific β chain (β_{IL-3}).[13–15] The mouse β_{IL-3} is 91% identical to the mouse β_c at the amino-acid level, but it does not form functional high-affinity heterodimers with the mouse IL-5 or GM-CSF-specific α chains.[11,12]

Work with mice which lack either of the two β receptor genes[7–9] as well as studies in IL-5 −/− or IL-5R α chain −/− mice[16,17] indicate that IL-3 cannot overcome the marked defect in eosinophil production that is associated with a lack of signaling via the receptor for IL-5. Nevertheless, four considerations prompted us to reassess the potential role of IL-3 in mouse eosinophil responses *in vivo*. First, because

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β_c −/− mice and β_c −/− × IL-3 −/− mice lack IL-5 signaling, and therefore have markedly impaired eosinophil production, such animals do not represent an optimal setting in which to search for potentially important effects of IL-3, for example, enhancement of IL-5-dependent eosinophil development.[4,18] Second, we recently reported that eosinophil recruitment after i.p. allergen challenge in ragweed allergen-immunized mice was greater in IL-3 −/− mice than in IL-3 +/+ mice.[19] That IL-3 might, under certain circumstances, suppress eosinophil development and/or migration is supported by studies showing that mice deficient in both IL-3 and GM-CSF, but not single knockouts, exhibit increased numbers of circulating eosinophils.[20] Third, we reported that while IL-3 −/− mice were normal in many aspects of hematopoiesis *in vitro* or *in vivo*[21] as would have been predicted based on the phenotype of β_c −/− × IL-3 −/− mice,[10] IL-3 −/− mice showed marked impairment of two important features of their Th2-associated hematopoietic response to infection with the nematode *Strongyloides venezuelensis* (*S.v.*): bone marrow basophil production and tissue mast cell hyperplasia.[22] However, the eosinophil responses of these mice were not assessed. Fourth, stem cell factor (SCF), the ligand for the tyrosine kinase receptor c-Kit, has also been reported to enhance eosinophil development *in vitro*, but only when used in combination with other cytokines such as IL-3.[23,24] SCF also can stimulate VLA-4-mediated adhesion of human eosinophils to fibronectin and VCAM-1 *in vitro*.[25] SCF is required for physiological mast cell development in mice, as mast cells are essentially absent under baseline conditions in the tissues of Kit^W/Kit^{W-v} mice that have markedly reducing c-Kit signaling.[26,27] However, by using Kit^W/Kit^{W-v}, IL-3 −/− mice, we showed that the modest numbers of mast cells that can appear in the intestines or spleen of Kit^W/Kit^{W-v} mice infected with *S.v.* are dependent on IL-3.[22] This work indicated that Kit^W/Kit^{W-v}, IL-3 −/− mice can be useful for revealing biological responses that may depend on both IL-3 and c-Kit/SCF signaling for their full expression.

We therefore used IL-3 −/− and wild-type mice, as well as Kit^W/Kit^{W-v}, IL-3 −/− mice and the corresponding Kit^W/Kit^{W-v}, IL-3 +/+ mice, to assess the extent to which IL-3 might act alone or in concert with c-Kit/SCF to promote eosinophil production and tissue accumulation at baseline or during infection with nematodes.

Materials and methods

Mice

Unless otherwise specified, IL-3 −/− mice were fourth backcross generation on the C57BL/6 or BALB/c background.[21,22] Kit^W/Kit^{W-v}, IL-3 −/− mice were produced as previously described.[22] Briefly, WBB6F1-$Kit^{W/+}$ and $Kit^{W-v/+}$ mice were bred with

C57BL/6-IL-3 −/− mice and the appropriate F2 and F3 offspring were identified and bred selectively to generate double-mutant mice; WBB6F1-Kit +/+ mice (Jackson Laboratory) were used as controls. All mouse experiments were conducted according to guidelines of the AAALAC-accredited BIDMC IACUC and NIH.

Infection with Parasites

S. venezuelensis (*S.v.*) or *Nippostrongylus brasiliensis* (*N.b.*) were maintained by serial passage in male Wistar rats or female BALB/c mice, respectively. Third-stage infective larvae (L3) of *S.v.* were obtained by fecal culture on filter paper,[28] whereas L3 of *N.b.* were obtained by the charcoal-vermiculite fecal culture method.[29] Mice were infected by subcutaneous (s.c.) inoculation with 400, 2000 or 10 000 L3 of *S.v.*, or were infected by s.c. inoculation with 600 L3 of *N.b.*

Counting of Blood Leukocytes

Total leukocytes were counted in samples of retro-orbital blood using the UNOPETTE Test Kit (Becton Dickinson and Company, Franklin, NJ, USA). Smears were prepared of retro-orbital blood and were stained with May-Grünwald-Giemsa. A 300-cell leukocyte differential count was performed and the number of eosinophils was calculated with reference to the total leukocyte count for that animal. Bone marrow cells were obtained by washing the cells from a single femur from each mouse and cytospin preparations of these cells were stained with May-Grünwald-Giemsa. The percentage of eosinophils in 500 bone marrow cells was determined for each sample. Bone marrow basophils were identified and quantified by flow cytometry.[30]

Counting of Eosinophils and Mast Cells in Jejunum, Ileum, Spleen and Lung

Mice were killed by CO_2 inhalation before parasite infection ('baseline') or on various days after parasite infection and segments of jejunum and ileum were taken from an area 8–10 cm distal to the pylorus or from an area 4–6 cm proximal to the ileocecal junction, respectively. Segments of intestine, lung (taken near the main bronchus) and spleen were fixed in either Carnoy's fluid or in 10% neutral buffered formalin, pH 7.4. The fixed tissues were embedded in paraffin and 4 μm tissue sections were deparaffinized and dehydrated. To demonstrate mast cells, the Carnoy's fixed sections were stained with 1% alcian blue, pH 1.0 (spleen) or 0.5% alcian blue, pH 0.3 (jejunum and ileum) at 37°C for 1 h.[22] They were rinsed briefly in distilled water and counterstained with eosin-Y for 30 s and then dehydrated and mounted. To demonstrate

eosinophils, the formalin-fixed sections were stained with hematoxylin-2 (Richard-Allan Scientific, Kalamazoo, MI, USA) for 5 s then rinsed briefly in distilled water and immersed in alkaline sodium chloride solution (1.0% sodium hydroxide and saturated sodium chloride in 80% ethanol) for 20 min and stained in 0.5% Congo red solution (0.5% Congo red in 50% ethanol) for 20 min and dehydrated and mounted.[31] Mast cells and eosinophils were counted by light microscopy by a single observer who was unaware of the identity (mouse genotype) of the individual sections. Mast cells and eosinophils in the jejunum and ileum were counted in at least 10 consecutive villus crypt units and the results were expressed as the mean ($\pm$ s.e.m.) cells per villus crypt unit. The spleen and lung mast cells or eosinophils were counted in 4–6 randomly selected sections and the results were expressed as the mean ($\pm$ s.e.m.) cells per mm^2.

Statistics

The data were analyzed for statistical significance (defined as $P < 0.05$) using the two-tailed unpaired Student's *t*-test; unless otherwise specified, all data are expressed as mean $\pm$ s.e.m.

Results

IL-3 $-/-$ Mice Exhibit Normal Eosinophil Levels at Baseline and Unimpaired Eosinophil Responses to Infection with the Nematodes *S.v.* or *N.b.*

S.v. is a naturally occurring parasite of murine rodents that is rejected by a T-cell-dependent immune response which is associated with extensive mast cell hyperplasia and eosinophil infiltration in the affected intestinal mucosa.[32–34] We reported that IL-3 $-/-$ mice infected with 2000 *S.v.* L3, in comparison with the corresponding wild-type mice, exhibited significantly prolonged pro-

duction of the parasites' eggs, as well as two marked abnormalities in their hematopoietic effector cell response to the infection: a virtual lack of parasite-induced hyperplasia of bone marrow basophils and a significant reduction in parasite-induced mast cell hyperplasia in the intestines and spleen.[22]

When tissues derived from some of the mice used in the study of Lantz *et al*[22] were processed into Congo red-stained sections, we found that BALB/c IL-3 $-/-$ and IL-3 $+/+$ mice that were infected with 2000 *S.v.* L3 exhibited nearly identical levels of tissue eosinophilia in the jejunum, ($\sim$ 2-fold the corresponding levels in uninfected control mice), as well as similar levels of blood eosinophilia (Table 1). Similar results were obtained in a different experiment with C57BL/6 IL-3 $-/-$ and IL-3 $+/+$ mice (Table 1).

To address the possibility that eosinophil responses in IL-3 $-/-$ mice might differ from those of IL-3 $+/+$ mice when the animals were infected with numbers of parasites that are different from that (ie, 2000 L3) used in our standard experiments, we infected groups of C57BL/6 IL-3 $-/-$ or IL-3 $+/+$ mice with 400 or 10 000 *S.v.* L3. No parasite egg production was observed in any of the mice infected with 400 *S.v.* L3, indicating that this number of *S.v.* failed to establish a detectable infection. However, as shown in Figure 1a, substantial parasite egg production occurred in IL-3 $-/-$ or IL-3 $+/+$ mice that were infected with 10 000 *S.v.* L3. In accord with our findings in mice infected with 2000 *S.v.* L3,[22] the IL-3 $-/-$ mice exhibited a significant delay in their ability to clear the infection, with parasite egg counts dropping to undetectable levels on day 15 of infection in the IL-3 $-/-$ mice *vs* day 12 for the wild-type mice.

However, on days 7 and 8 of the infection with 10 000 *S.v.* L3, parasite egg production in the IL-3 $+/+$ mice was $\sim$ 3-fold that in the IL-3 $-/-$ mice. This finding, that was not observed in IL-3 $-/-$ and IL-3 $+/+$ mice which had been infected with 2000 *S.v.* L3,[22] indicates that the effects of IL-3

Table 1 Tissue and blood eosinophil numbers in wild-type (IL-3 +/+) *vs* IL-3 $-/-$ mice, with or without infection with *S.v.*

Mice	Eosinophils[a]			
	Jejunum (number/vcu)		Blood (number/mm³)	
	Control	*2000 S.v.*	*Control*	*2000 S.v.*
BALB/c				
IL-3 +/+	12$\pm$0.7 (n=3)	24$\pm$0.4[‡] (n=4)	63$\pm$7 (n=4)	120$\pm$6[†] (n=4)
IL-3 $-/-$	12$\pm$1.0 (n=3)	25$\pm$0.6[‡] (n=4)	57$\pm$3 (n=8)	103$\pm$7[‡] (n=4)
C57BL/6				
IL-3 +/+	8.5$\pm$0.5 (n=3)	16$\pm$0.5[‡] (n=8)	50$\pm$5 (n=8)	192$\pm$28[†] (n=8)
IL-3 $-/-$	9.2$\pm$0.8 (n=3)	17$\pm$0.4[†] (n=3)	52$\pm$11 (n=3)	124$\pm$25[*] (n=3)

[a]Blood and jejunal eosinophils in IL-3 $-/-$ *vs* IL-3 $+/+$ mice, either not infected with *S.v.* ('Control') or on day 11 or 13 of infection with *S.v.* All data are mean $\pm$ s.e.m.
[*]$P < 0.05$, [†]$P < 0.001$, [‡]$P < 0.0001$ *vs* the corresponding baseline values.
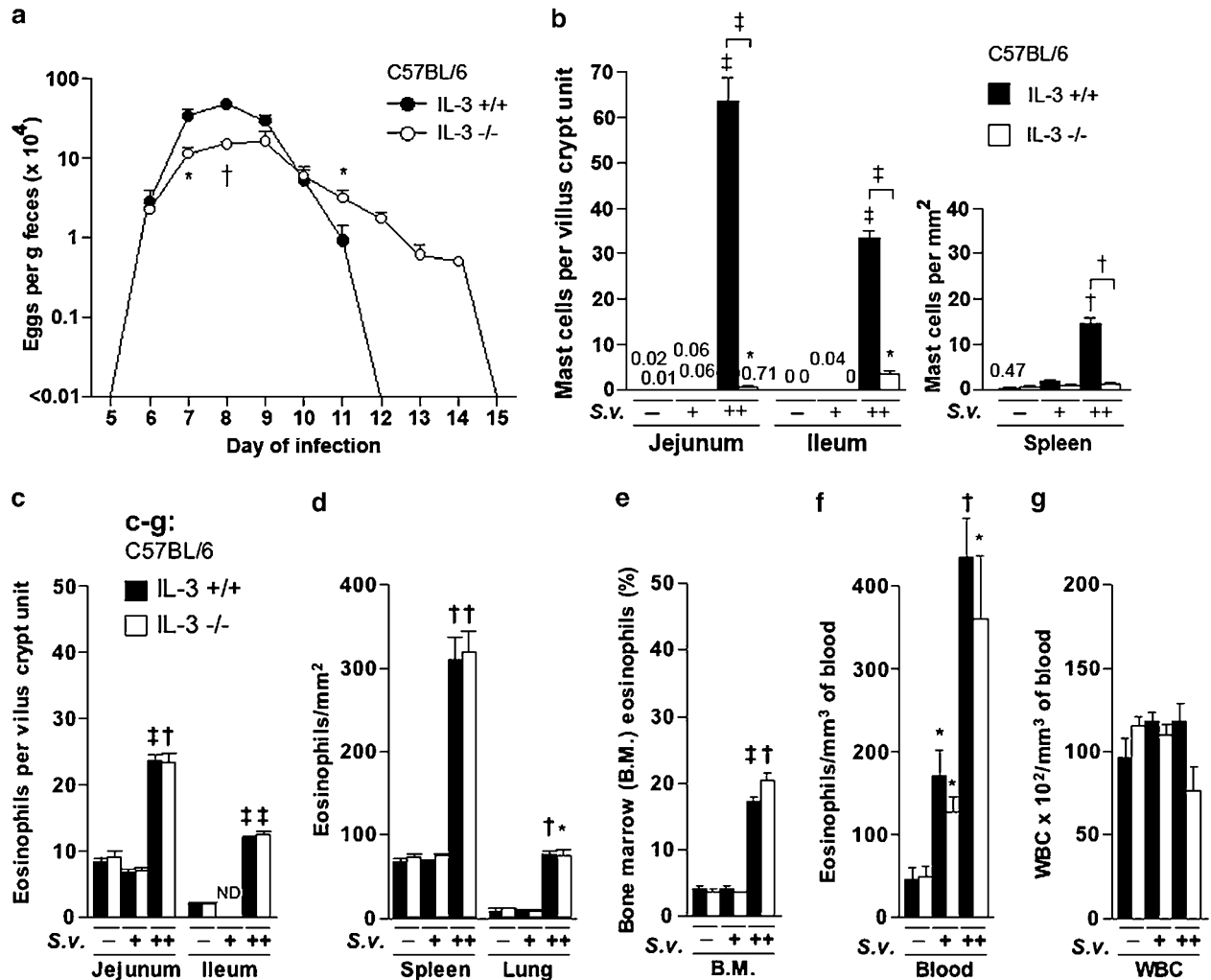
Figure 1 (**a**) Daily fecal egg production in female 8 to 10-week-old C57BL/6 IL-3 +/+ vs IL-3 −/− mice after infection with 10 000 L3 of *S.v.* All data are mean±s.e.m. ($n = 3–6$ per group). *$P < 0.05$, †$P < 0.001$ vs corresponding values for IL-3 +/+ mice. All mice were killed on the day of clearance of infection. (**b–g**) Numbers of mast cells (**b**), eosinophils per villus crypt unit in jejunum and ileum (**c**), eosinophils per mm² in sections of spleen and lung (**d**), % of bone marrow (BM) eosinophils (**e**), and numbers of eosinophils (**f**), and WBC (white blood cells) (**g**) per mm³ of blood, at baseline (−) or after infection with 400 (+) or 10 000 (++) L3 of *S.v.* in 8 to 10-week-old female C57BL/6 IL-3 +/+ vs IL-3 −/− mice. All data are mean±s.e.m. ($n = 3–6$ per group), except that only mean values are given for very low values. *$P < 0.05$, †$P < 0.001$, ‡$P < 0.0001$ vs corresponding baseline values for mice of the same genotype or (as indicated by brackets) vs corresponding values for mice of the other genotype. Data for mice infected with 10 000 L3 are from the same mice shown in (**a**). ND means not done.

during primary infection with *S.v.* may be quite complex: IL-3 can contribute to the rapid resolution of the infection, but, under certain circumstances, IL-3 may also enhance egg production at early stages of the infection.

In accord with results obtained in mice infected with 2000 *S.v.* L3,[22] IL-3 +/+ mice infected with 10 000 *S.v.* L3 exhibited a significant increase in numbers of bone marrow basophils by the time parasite egg expulsion was complete, whereas the identically challenged IL-3 −/− mice did not ($2.4 ± 0.2$ vs $0.86 ± 0.05\%$ basophils, $P < 0.001$). By contrast, infection with 400 *S.v.* L3 produced no significant changes in bone marrow basophil levels ($0.64 ± 0.06$ or $0.65 ± 0.02\%$ in infected IL-3 +/+ or IL-3 −/− mice, vs corresponding baseline values in

uninfected IL-3 +/+ or IL-3 −/− mice of $0.60 ± 0.04$ or $0.55 ± 0.08\%$). Infection with 10 000 *S.v.* L3 also induced a substantial mast cell hyperplasia in the jejunum and ileum of the IL-3 +/+ mice, and resulted in greatly increased numbers of mast cells in the spleen, whereas the changes in mast cell numbers at these sites in the IL-3 −/− mice were minimal (Figure 1b).

In contrast to the dramatic differences exhibited by IL-3 −/− and IL-3 +/+ mice in changes in bone marrow basophil or tissue mast cell levels during *S.v.* infection, the eosinophil responses to infection in mice of either genotype (and the baseline eosinophil levels in uninfected mice) were statistically indistinguishable (Figure 1c–f). Blood eosinophil levels were slightly lower after *S.v.* infection in

IL-3−/− mice than in IL-3+/+ mice, but this difference was not statistically significant (Figure 1f); eosinophil levels in all of the other tested sites were virtually identical in the IL-3−/− and IL-3+/+ mice (Figure 1c–e). Note that mice infected with only 400 *S.v.* L3 also developed a significant blood eosinophilia (albeit to levels substantially lower than those in mice infected with 10 000 L3), and the levels in the IL-3−/− mice were slightly, but not significantly lower than those in the IL-3+/+ mice (Figure 1f). Thus, of the three hematopoietic lineages examined (eosinophils, basophils, and mast cells), the eosinophil was the most sensitive indicator of the presence of parasites.

We also examined eosinophil responses in mice infected with the nematode *N.b.*, which, like *S.v.*, induces a Th2-type immune response that is associated with blood and tissue eosinophilia.[32,35] However, even though mice infected with *N.b.* develop an intestinal mast cell hyperplasia, several lines of evidence indicate that mast cells have little or no role in the resolution of *N.b.* infection.[34–36] We found that BALB/c IL-3−/− or IL-3+/+ mice infected with 600 *N.b.* L3 exhibited a virtually identical pattern of parasite egg production, with complete clearance of the infection in mice of either genotype by day 11 (data not shown). However, as in mice infected with *S.v.*, *N.b.*-infected IL-3+/+ mice developed increased numbers of bone marrow basophils (∼100% increase) and increased numbers of mast cells in the jejunum, ileum and spleen, whereas the *N.b.*-infected IL-3−/− mice failed to develop increased numbers of bone marrow basophils and exhibited greatly impaired expansion of intestinal and splenic mast cell populations (data not shown). By contrast, both IL-3−/− and IL-3+/+ mice developed substantial, and virtually identical, increases in levels of bone marrow, blood, jejunal, ileal and splenic eosinophils in response to *N.b.* infection (Figure 2).

These results strongly support the conclusion that, despite its well-documented ability to promote eosinophil development *in vitro*, IL-3 can be dispensable for eosinophil production and tissue accumulation in mice *in vivo*. In fact, we have shown using a mouse model of ragweed-induced allergic inflammation known to be associated with a selective tissue accumulation of eosinophils that, under some circumstances, IL-3 can actually have a suppressive effect on eosinophil development and/or migration.[19] While the potential role of IL-3 in regulating eosinophil production or tissue accumulation of eosinophils in the context of *S.v.* infection had not previously been investigated, Madden *et al*[32] examined the effects of the administration of polyclonal antibodies to mouse IL-3 on the blood eosinophilia, and enhanced levels of tissue mast cells, which developed in mice that were infected with *N.b.* They found that treatment with anti-IL-3 antibodies reduced *N.b.*-induced increases in tissue mast cells by ∼40%, but had no detectable effect on blood eosinophilia on days 11 or 13 of the infection.[32]

The results of this study, and those reported by Lantz *et al*,[22] clearly show that the requirements for IL-3 for expansion of basophil, intestinal mast cell and eosinophil populations during Th2-type responses to intestinal helminths in mice are distinct; IL-3 is required for the basophil response and importantly contributes to the mast cell response, but makes little or no detectable contribution to the eosinophil response. This conclusion is supported by our findings derived from mice which had been subjected to infection with a wide range of *S.v.* third
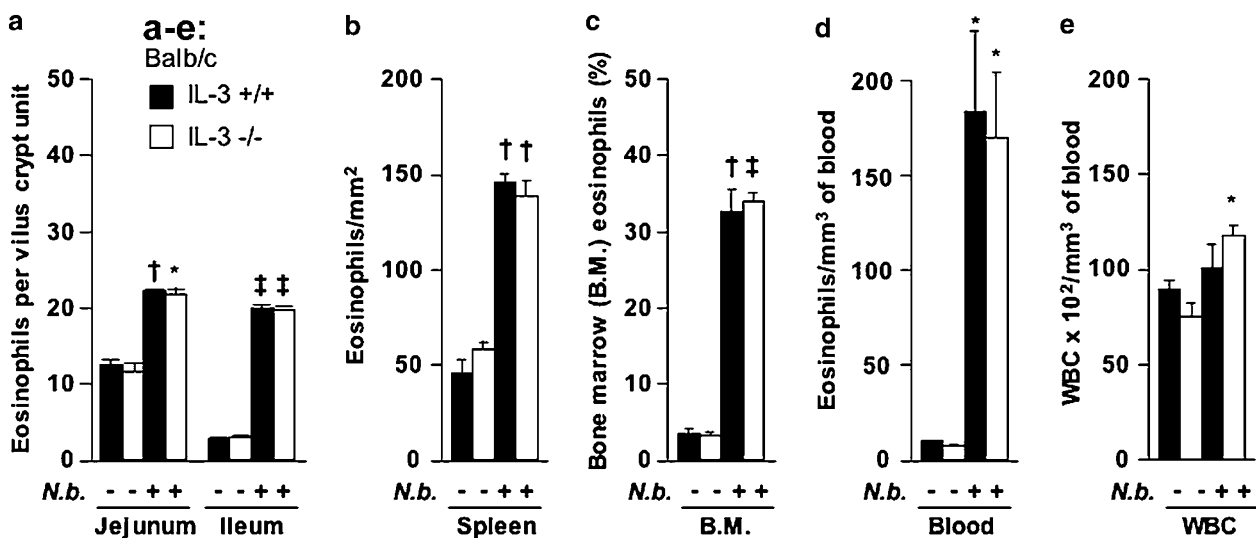
Figure 2 Numbers of eosinophils per villus crypt unit in jejunum and ileum (**a**), eosinophils per mm² in sections of spleen (**b**), % of bone marrow (BM) eosinophils (**c**), and numbers of eosinophils (**d**) and WBC (white blood cells) (**e**) per mm³ of blood, at baseline (−) or at day 11 after infection with 600 L3 of *N.b.* in 3 to 4-month-old male or female BALB/c IL-3 +/+ *vs* IL-3 −/− mice. All data are mean ± s.e.m. (*n* = 3–5 per group). *$P < 0.05$, †$P < 0.001$, ‡$P < 0.0001$ *vs* the corresponding baseline values for mice of the same genotype.

stage larvae: 400, 2000 or 10 000, as well as by our results and those of Madden *et al*[32] in *N.b.*-infected mice.

Infection with *S.v.*, but not *N.b.*, Induces Substantially Less Tissue Accumulation of Eosinophils in Kit^W/Kit^{W-v}, IL-3−/− Mice Than in Kit^W/Kit^{W-v}, IL-3+/+ Mice

Our findings in IL-3−/− and +/+ mice do not exclude the possibility that IL-3 might contribute to eosinophil responses in settings different than those we tested. For example, we reported that mast cell development and parasite immunity are impaired even more severely in *S.v.*-infected IL-3−/− mice that have markedly reducing c-Kit/SCF signaling (ie Kit^W/Kit^{W-v}, IL-3 −/− mice) than in similarly infected IL-3−/− or Kit^W/Kit^{W-v}, IL-3+/+ mice.[22] We found that baseline numbers of blood, bone marrow and tissue eosinophils were essentially identical in Kit^W/Kit^{W-v}, IL-3−/− and Kit^W/Kit^{W-v}, IL-3+/+ mice (Figure 3). By contrast, *S.v.* infection induced a significant increase in tissue eosinophil levels in Kit^W/Kit^{W-v}, IL-3+/+ mice but, with the exception of the bone marrow, not in the Kit^W/Kit^{W-v}, IL-3−/− mice (Figure 3). Although blood eosinophil levels were comparable in uninfected and *S.v.*-infected Kit^W/Kit^{W-v}, IL-3+/+ mice, blood eosinophil levels were significantly lower in *S.v.*-infected Kit^W/Kit^{W-v}, IL-3−/− mice as compared to uninfected Kit^W/Kit^{W-v}, IL-3−/− mice (Figure 3d). It is important to note that all mice were killed when Kit^W/Kit^{W-v}, IL-3+/+ mice cleared their infection (days 18 or 19), a point in the infection in which Kit^W/Kit^{W-v}, IL-3−/− mice still retained most of their parasites.[22] Thus, Kit^W/Kit^{W-v}, IL-3−/− mice exhibited a significantly impaired tissue eosinophil response despite the persistent stimulus of a heavy parasite burden.

We also examined eosinophil production in Kit^W/Kit^{W-v}, IL-3−/− mice infected with the nematode *N.b.* In contrast to our previous observations using *S.v.*,[22] Kit^W/Kit^{W-v}, IL-3−/− or Kit^W/Kit^{W-v}, IL-3+/+ mice infected with 600 *N.b.* L3 exhibited no significant differences in parasite egg production as compared with *Kit* +/+, IL-3+/+ mice, with clearance of the infection ranging from days 11 to 14 (data not shown). These results are in agreement with other reports indicating that the primary host response to *N.b.* infection is indistinguishable or only slightly delayed in Kit^W/Kit^{W-v} mice as compared with wild-type mice.[36] On the other hand, as in mice infected with *S.v.*,[22] we found that *N.b.* infection induced an enhancement of bone marrow basophil production that was primarily depended on IL-3 (Figure 4a) and striking increases in mast cells in the intestines and spleen that were primarily dependent on c-Kit (Figure 4b). However, the eosinophil responses induced by infection of Kit^W/Kit^{W-v} mice with *N.b.* or *S.v.* differed in their requirement for IL-3. Thus, in contrast to our results with *S.v.* (see Figure 3), the levels of bone marrow, jejunal, ileal, lung and splenic eosinophils that developed in *N.b.*-infected Kit^W/Kit^{W-v}, IL-3−/− and Kit^W/Kit^{W-v}, IL-3+/+ mice were virtually identical (Figure 4c–e).

Although our results indicate that IL-3 makes little or no contribution to eosinophil responses to *N.b.* infection in either wild-type (Figure 2c–f) or Kit^W/Kit^{W-v} (Figure 4c–f) mice, they do suggest that c-Kit/SCF significantly contributes to eosinophil responses in this setting. Thus, *N.b.* infection of Kit^W/Kit^{W-v}, IL-3+/+ or −/− mice resulted in significantly lower levels of eosinophils in the blood and in all tissues (except the bone marrow) than did *N.b.* infection of wild-type (*Kit* +/+, IL-3+/+) mice (Figure 4c-f). The extent to which c-Kit/SCF may contribute to eosinophil responses to other
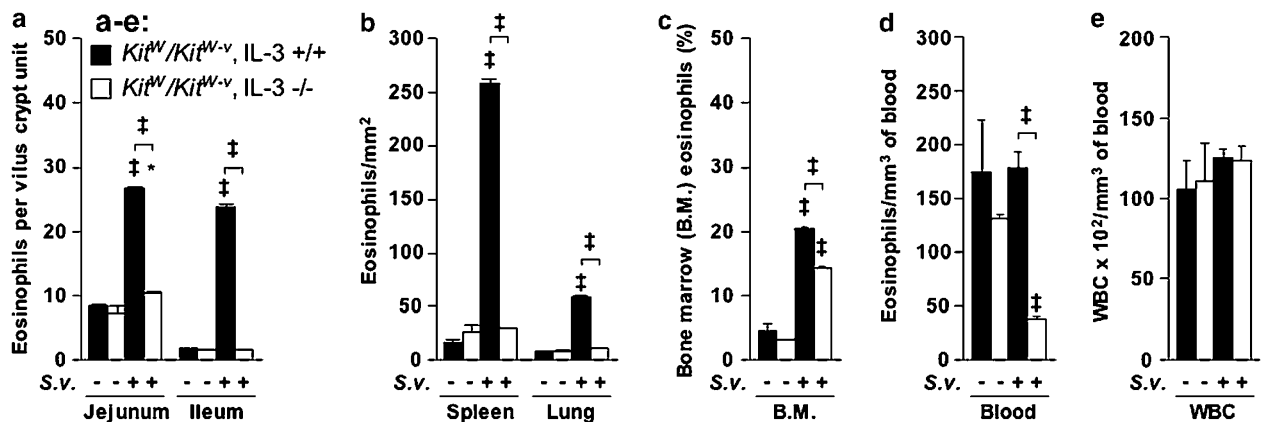
Figure 3 Numbers of eosinophils per villus crypt unit in jejunum and ileum (**a**), eosinophils per mm² in sections of spleen and lung (**b**), % of bone marrow (BM) eosinophils (**c**), and numbers of eosinophils (**d**) and WBC (white blood cells) (**e**) per mm³ of blood, at baseline (−) or at days 18 or 19 after infection with 2000 L3 of *S.v.* in 3 to 4-month-old male or female Kit^W/Kit^{W-v}, IL-3 +/+ *vs* Kit^W/Kit^{W-v}, IL-3 −/− mice. The data for *S.v.*-infected mice are from the same groups of mice depicted in Figure 3a in Lantz *et al*.[22] All data are mean±s.e.m. ($n=3$–9 per group). *$P<0.05$, ‡$P<0.0001$ *vs* the corresponding baseline values for mice of the same genotype or (as indicated by brackets) *vs* corresponding values for mice of the other genotype.
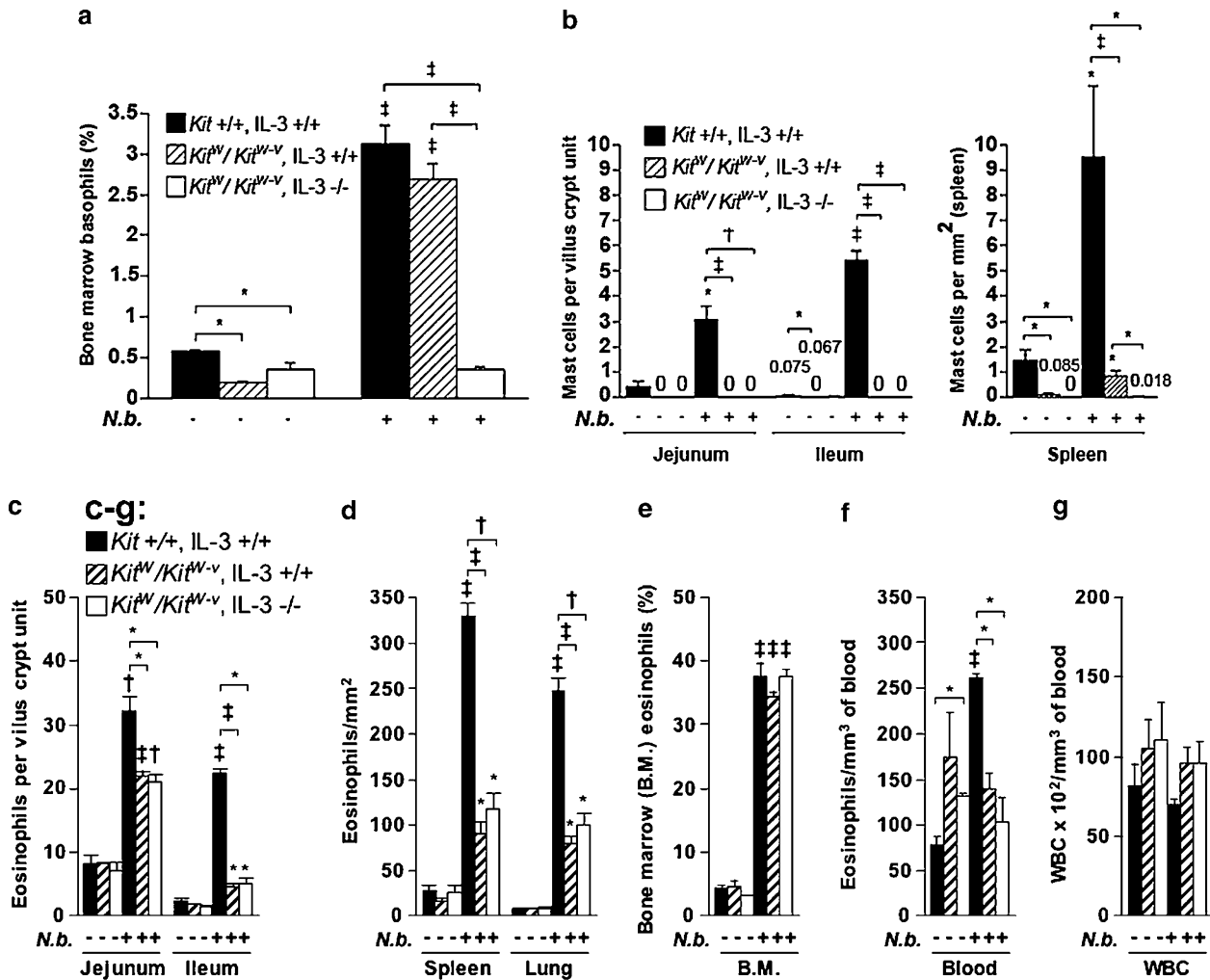
Figure 4 (**a–g**) Percentage of bone marrow basophils (**a**), numbers of mast cells (**b**), and numbers of eosinophils per villus crypt unit in jejunum and ileum (**c**), eosinophils per mm² in sections of spleen and lung (**d**), % of bone marrow (BM) eosinophils (**e**), and numbers of eosinophils (**f**) and WBC (white blood cells) (**g**) per mm³ of blood, at baseline (−) or at days 11–14 after infection with 600 L3 of *N.b.* in 3 to 4-month-old male or female *Kit* +/+, IL-3 +/+ *vs* Kit^W/Kit^{W-v}, IL-3 +/+ *vs* Kit^W/Kit^{W-v}, IL-3 −/− mice. All data are mean ± s.e.m. (*n* = 3–10 per group), except that only mean values are given for very low values (**b**). *$P < 0.05$, †$P < 0.001$, ‡$P < 0.0001$ *vs* the corresponding baseline values for mice of the same genotype or (as indicated by brackets) *vs* corresponding values for mice of another genotype. Baseline data for uninfected mice are from the same groups of mice depicted in Figure 3d in Lantz *et al*[22] (**a** and **b**) or from Figure 3(c–g).

nematodes remains to be determined. Wild-type mice were not infected with *S.v.* during the experiment shown in Figure 3, however, the eosinophil responses the *S.v.*-infected Kit^W/Kit^{W-v}, IL-3 +/+ mice depicted in Figure 3a–d were similar to those observed in the *S.v.*-infected wild-type (IL-3 +/+) mice shown in Table 1 and Figure 1c-f. Taken together, these results indicate that the eosinophil responses to *N.b.* infection in mice may be more dependent on c-Kit than are those observed during infection with *S.v.*

Table 2 summarizes some of our key findings regarding the roles of IL-3 and c-Kit signaling in influencing the numbers of blood and tissue eosinophils in mice subjected to the two different models of parasite infection analyzed in this study.

Discussion

IL-3 appeared to make no detectable contribution to eosinophil numbers in normal mice, in that we found no significant differences in the numbers of eosinophils in the blood, bone marrow or various tissues of IL-3−/− *vs* IL-3+/+ mice, either at baseline or after the induction of bone marrow, blood or tissue eosinophilia in response to infection with *S.v.* or *N.b.* However, in Kit^W/Kit^{W-v} mice, which have markedly impaired SCF/c-Kit signaling, our results indicate that IL-3 contributed significantly to the increased numbers of eosinophils that were observed in multiple tissues during *S.v.* infection, but not during infection with *N.b.*

A number of mechanisms may contribute to the inability of Kit^W/Kit^{W-v}, IL-3−/− mice to develop a

Table 2 Key findings regarding the influence of IL-3 and/or c-Kit signaling on levels of blood or tissue eosinophils in mice infected with *S.v.* or *N.b.*

Setting	Key findings
1. IL-3 deficiency in otherwise normal mice infected with *S.v.* or *N.b.*	At the times after infection analyzed, a lack of IL-3 had no significant effect on the substantially increased numbers of blood eosinophils, % of bone marrow eosinophils, or numbers of tissue eosinophils (in jejunum, ileum or spleen) in C57BL/6 mice infected with 10 000 L3 of *S.v.* (Figure 1) or in BALB/c mice infected with 600 L3 of *N.b.* (Figure 2); IL-3 also had no significant effect on the increased numbers of blood eosinophils or numbers of eosinophils in the jejunum in C57BL/6 or BALB/c mice infected with 2000 L3 of *S.v.* (Table 1).
	These findings indicate that, under the conditions examined, the presence or absence of IL-3 in otherwise normal C57BL/6 or BALB/c mice had little or no effect on the increased numbers of bone marrow, blood or tissue eosinophils which were observed as a result of infection with either of the two species of nematodes analyzed.
2. IL-3 deficiency in Kit^W/Kit^{W-v} mice infected with *S.v.*	At the times after infection analyzed, a lack of IL-3 markedly and significantly reduced the increases in the numbers of eosinophils in the jejunum, ileum, spleen and lung that were observed as a result of infection of Kit^W/Kit^{W-v} mice (that are markedly defective in c-Kit signaling) with 2000 L3 of *S.v.* (Figure 3); a lack of IL-3 in Kit^W/Kit^{W-v} mice also resulted in significantly fewer blood eosinophils and a significantly smaller increase in the % of bone marrow eosinophils that were observed during such infection (Figure 3).
	These findings indicate that, in mice with markedly diminished c-Kit signaling, IL-3 can contribute significantly to the increases in bone marrow and, particularly, tissue, eosinophils associated with infection with *S.v.*, at least under the conditions tested.
3. IL-3 deficiency in Kit^W/Kit^{W-v} mice infected with *N.b.*	At days 11–14 after infection with 600 L3 of *N.b.*, the presence or absence of IL-3 had little or no additional effect on the significant reductions in the elevated levels of tissue (jejunum, ileum, spleen or lung) or blood eosinophils which occurred in Kit^W/Kit^{W-v} mice (with markedly defective c-Kit signaling) as compared to the corresponding values in wild-type mice; however, the % of bone marrow eosinophils was elevated to the same extent in *N.b.*-infected wild-type, Kit^W/Kit^{W-v} or Kit^W/Kit^{W-v}, IL-3 −/− mice (Figure 4).
	These findings indicate that mice with markedly diminished c-Kit signaling exhibit significant reductions in the numbers of blood and tissue, but not bone marrow, eosinophils associated with infection with *N.b.*, but that the presence or absence of IL-3 has little or no effect on the values for these eosinophil populations in Kit^W/Kit^{W-v} mice, at least under the conditions tested.

robust eosinophil response to *S.v.* IL-3 can expand numbers of eosinophils generated from hematopoietic stem cells *in vitro*[2], an effect which can be enhanced by SCF.[23,24] Therefore, both IL-3 and SCF may be required for increased commitment of progenitor cells to the eosinophilic lineage during a primary infection with *S.v.* Alternatively, or in addition, IL-3 and SCF may be required to promote eosinophil accumulation during *S.v.* infection by influencing eosinophil migration. Indeed, Yuan *et al*[25] have provided evidence that SCF is an agonist for eosinophil adhesion to endothelial cells. Other studies suggest that eosinophil migration is in part regulated by the ability of resident mast cells to secrete chemoattractants for eosinophils, such as eotaxin and leukotrienes.[37–40] Modest numbers of mast cells do develop in the intestinal mucosal tissue or spleen of Kit^W/Kit^{W-v}, IL-3 +/+ mice during infection with *S.v.*,[22,33] however, mast cell are virtually absent from the corresponding tissues of *S.v.*-infected Kit^W/Kit^{W-v}, IL-3 −/− mice.[22] Thus, the presence of low levels of eosinophils in *S.v.*-infected Kit^W/Kit^{W-v}, IL-3 −/− mice may in part reflect the virtual absence of mast cells in these animals.

Our findings show that IL-3 can cooperate with c-Kit/SCF to increase numbers of eosinophils in response to *S.v.* infection. This is clearly shown in Figure 3, which demonstrates that the ability of *S.v.* infection to enhance eosinophil numbers

in the tissues is almost undetectable in Kit^W/Kit^{W-v}, IL-3−/− mice. Although the precise mechanism(s) by which IL-3 and c-Kit/SCF regulate the eosinophil responses during *S.v.* infection remain(s) to be determined, one important implication of our findings is that the impaired immunity to *S.v.* expressed by Kit^W/Kit^{W-v}, IL-3−/− mice[22] may in part reflect the combined contributions of mast cells, basophils and eosinophils.

We also noted that, on days 7 and 8 of the infection with 10 000 *S.v.* L3, parasite egg production in IL-3+/+ mice was ∼3-fold that in the IL-3−/− mice. Thus, in addition to contributing to the rapid resolution of the infection, under certain circumstances, IL-3 may also have effects that can enhance egg production at early stages of the infection. While the explanation for this observation remains to be determined, two studies have shown that egg production during nematode infections can be significantly higher in rats which have normal numbers of mast cells than in rats which are mast cell-deficient either because of loss-of-function mutations in c-*kit*[41] or because of mast cell depletion as a result of the administration of an anti-SCF antibody.[42] In the context of these findings in the rat, and in light of evidence that IL-3 can both promote increased numbers of mast cells and basophils[22] and also can enhance the function of these cells,[43,44] as well as have effects on eosinophils, we can speculate about at least one mechanism by which

IL-3 might promote increased nematode fecundity. Perhaps, in certain stages of such infections, the net effect of IL-3-dependent enhancement of mast cell, basophil and/or eosinophil numbers and/or function is to promote parasite fecundity. This might occur, for example, if IL-3 promoted the increased local release by mast cells, basophils and/or eosinophils of mediators that can enhance intestinal vascular or epithelial cell permeability, thereby favoring parasite nutrition, health and egg production.

Acknowledgements

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