Eosinophilia in Transgenic Mice Expressing Interleukin 5

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Summary

Experiments in vitro suggest that although interleukin 5 (IL-5) stimulates the late stages of eosinophil differentiation, other cytokines are required for the generation of eosinophil progenitor cells. In this study transgenic mice constitutively expressing the IL-5 gene were established using a genomic fragment of the IL-5 gene coupled to the dominant control region from the gene encoding human CD2. Four independent eosinophilic transgenic lines have thus far been established, two of which with 8 and 49 transgene copies, are described in detail. These mice appeared macroscopically normal apart from splenomegaly. Eosinophils were at least 65- and 265-fold higher in blood from transgenics, relative to normal littermates, and approximately two- or sevenfold more numerous relative to blood from mice infected with the helminth *Mesocestoides corti*. Much more modest increases in blood neutrophil, lymphocyte, and monocyte numbers were noted in transgenics, relative to normal littermates (less than threefold). Thus IL-5 in vivo is relatively specific for the eosinophil lineage. Large numbers of eosinophils were present in spleen, bone marrow, and peritoneal exudate, and were highest in the line with the greatest transgene copy number. Eosinophilia was also noted in histological sections of transgenic lungs, Peyer's patches, mesenteric lymph nodes, and gut lamina propria but not in other tissues examined. IL-5 was detected in the sera of transgenics at levels comparable to those seen in sera from parasite-infected animals. IL-3 and granulocyte/macrophage colony-stimulating factor (GM-CSF) were not found. IL-5 mRNA was detected in transgenic thymus, Peyer's patches, and superficial lymph nodes, but not in heart, liver, brain, or skeletal muscle or in any tissues from nontransgenics. Bone marrow from transgenic mice was rich in IL-5-dependent eosinophil precursors. These data indicate that induction of the IL-5 gene is sufficient for production of eosinophilia, and that IL-5 can induce the full pathway of eosinophil differentiation. IL-5 may therefore not be restricted in action to the later stages of eosinophil differentiation, as suggested by earlier in vitro studies.

The generation of blood leucocytes occurs from a population of multipotential stem cells, which undergo a series of differentiation steps in which their multilineage potential is progressively restricted. This gives rise to populations of progenitor cells committed to the different leucocyte lineages. More is known about the later stages of these pathways since the discovery of the CSFs, but little is known about the mechanisms controlling the production of the progenitor cells.

Eosinophilia is characteristic of allergy and infection by helminths. One of the intriguing aspects of eosinophilia is its biological specificity, in which an increase in eosinophils can occur in the absence of increases in other leucocytes (1, 2). This suggests a lineage specific control mechanism. However, studies in vitro suggest that the sequential action of IL1 and IL3 or granulocyte colony-stimulating factor (G-CSF)[1] are necessary for progenitor cell production (3–5), and that IL-5 is responsible only for the differentiation of these progenitor cells into mature eosinophils (6). As G-CSF stimulates the production of neutrophils and IL-3 is active on many other lineages, a role for these factors in eosinophilia is paradoxical.

To define the activity of IL-5 in vivo, transgenic mice have been produced in which transcription of the IL-5 gene is under the influence of the dominant control region (DCR) of the gene encoding human CD2 (8) (a T cell surface antigen). Detailed studies have shown that this region allows the expression of reporter genes in transgenic mice, which is constitutive, thymocyte and T cell specific, dependent on copy number, and independent of orientation or insertion site (8, 9).

In this study we compare two transgenic lines of mice, each expressing different levels of IL-5, with mice infected with the cestode *Mesocestoides corti*. We show that constitutive expression of the IL-5 gene is sufficient to induce lifelong high-level eosinophilia. The eosinophilia in both transgenic lines is at least as high as the peak eosinophilia observed in

[1] *Abbreviations used in this paper:* DCR, dominant control region; G-CSF, granulocyte colony-stimulating factor.

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M. corti infection. Despite the wide tissue distribution of eosinophils, the transgenic animals remain physically normal.

Materials and Methods

Production and Analysis of Transgenic Mice. The human genomic CD2 DCR (8, 9) was obtained as a 5-kb BamHI-XbaI fragment in the vector polyIII-I. After excision of a 3-kb HindIII fragment, the 10-kb HindIII IL-5 genomic fragment (10) was ligated into the opened vector (Fig. 1). The 12-kb hybrid gene was excised as a BamHI fragment and injected into CBA/Ca oocytes, which were then transferred to the oviducts of pseudopregnant (CBA/Ca × C57B6)F_1 mice using standard techniques (11). Mice were maintained by the Institute animal facility. Southern blots were performed with DNA prepared from the tails of 9–30-d-old mice, and probed with a 650-bp fragment of IL-5 cDNA (12). Densitometric analysis of Southern blots was performed using a Chromoscan 3 (Joyce Loebl, Deusseldorf, FRG).

Total RNA was prepared by acid guanidinium thiocyanate-phenol-chloroform extraction (13), and 5–20 μg/sample were electrophoresed through formaldehyde/agarose gels. Northern blot analysis was performed as described (14) using the IL-5 cDNA probe described above and a riboprobe prepared from actin cDNA (15). Restriction endonucleases and other enzymes were obtained from Boehringer Mannheim (Lewes, UK) or Northumbria Biologicals Ltd. (Cramlington, UK). All other reagents were from standard commercial sources. All techniques not specifically referenced were performed using standard methods (16).

Cytokine and Eosinophil Progenitor Assays. IL-5 was obtained from COS cells transfected with recombinant mouse (rm) IL-5 cDNA (10). IL-5 was assayed by the eosinophil differentiation assay (EDA) (17). Briefly, sample dilutions were incubated in microplates with bone marrow from *M. corti* infected mice for 5 d. Eosinophil numbers were determined indirectly by assaying for eosinophil peroxidase. Absorbance (A_{492}) was read in a plate reader, using cultures incubated in the absence of exogenous IL-5 as blanks.

Eosinophil progenitors were determined indirectly by titrating bone marrow cells in the presence of a predetermined optimum concentration of rmIL-5 and by bone marrow responsiveness to IL-5.

IL-3/GM-CSF levels were determined by using the factor-dependent cell line FDCP-2, which detects both of these cytokines (18).

Cytology and Histology. Slides for differential cell counts were prepared from single cell suspensions using a cytocentrifuge, or as standard blood films, fixed with methanol and stained with Giemsa. 2–5 × 10^2 cells were counted per slide. Tissues were fixed in neutral buffered formalin, embedded in paraffin and 4–6-μm sections were stained with Congo Red (19) and counterstained with haematoxylin.

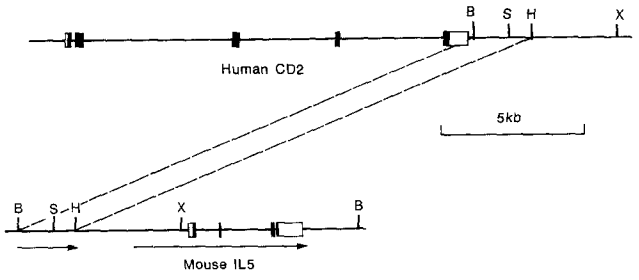

Figure 1. Map of human CD2 (5) and mouse CD2 IL-5 gene construct used to generate transgenic mice. Restriction enzyme sites: B, BamHI; S, SacI; H, HindIII; X, XbaI. Exons are shown as boxes.

Results

Transgenic Mouse Lines. Four transgenic offspring were detected, initially by high eosinophil counts in a smear of tail blood (counts in excess of 45% at 9 d of age, compared with a mean of 3% in normal littermates), and later by Southern analysis of tail DNA. Of 34 livebirths only these 4 animals had increased blood eosinophil counts, and only these 4 were transgenic by Southern analysis. Independent transgenic lines have been established from all four of these founder animals and are designated Tg(0IL5)C1, Tg(0IL5)C2, Tg(0IL5)C3, and Tg(0IL5)C4, abbreviated Tg5C1, Tg5C2, Tg5C3, and Tg5C4, respectively. Blood eosinophilia in transgenic offspring of each line was comparable to that seen in founders. Data for Tg5C1 and Tg5C2 are presented here. Southern analysis of SacI digests indicated they carry ~8 and 49 copies of the transgene, respectively (mean densitometry curve integrals of 4,626 and 28,272 U for Tg5C1 and Tg5C2, respectively, relative to a mean of 591 U for nontransgenic littermate DNA).

IL-5 Transgene Expression Is Tissue Restricted. Northern blot analysis identified IL-5 mRNA in the thymus, Peyer's patches, and subcutaneous (pooled inguinal and axillary) lymph nodes (Fig. 2 *a*). No IL-5 mRNA was detected in liver, heart, brain, or skeletal muscle tissues from transgenics (not shown), nor in any tissues from normal littermates. The presence of in-

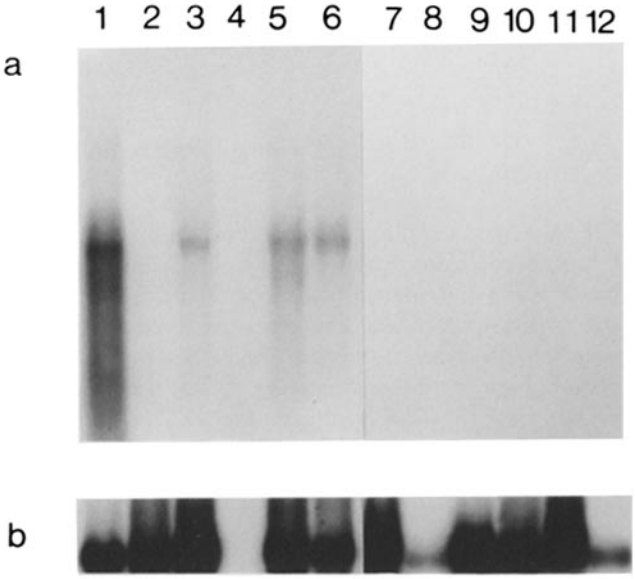

Figure 2. Northern analysis of IL-5 mRNA in tissues from transgenic mice (lanes *1–6*) and normal littermates (lanes *7–12*). (Lanes *1,7*) Thymus; (lanes *2,8*) bone marrow; (lanes *3,9*) Peyer's patches; (lanes *4,10*) spleen; (lanes *5,11*) mesenteric lymph nodes; (lanes *6,12*) pooled axillary and inguinal lymph nodes. (*a*) Using an IL-5 cDNA probe. (*b*) Using an actin RNA probe. mRNA from heart, kidney, brain, muscle, and liver were negative.

Table 1. *Comparative White Blood Cell Counts*

Mice (gene copy no.)	Spleen weight	Serum IL-5	Blood cells/ml ($\times 10^{-5}$) Total WBC	Differential counts (%) N	Eo	L	M
	mg	*U*					
Tg5C1 (8)	205 ± 3	39 ± 11	305 ± 14	35 (12)	130 (44)	130 (44)	5 (2)
Tg5C2 (49)	675 ± 166	73 ± 27	786 ± 16	60 (8)	530 (68)	210 (27)	9 (2)
M. corti	450 ± 35	55 ± 13	250 ± 35	85 (34)	70 (28)	33 (33)	13 (5)
Normal	58 ± 2	<1	119 ± 8	18 (16)	2 (2)	98 (82)	2 (2)

WBC, White blood cells; N, neutrophils; Eo, eosinophils; L, lymphocytes; M, monocytes. Values are means ± 1 SD, excepting differential counts (mean cells/ml and percent ± SD were also calculated, but added little to the analysis and so have been omitted). Spleen weight and serum IL-5 levels (n = 3). Blood cell counts for 9–12-wk-old transgenic mice (n = 5), *M. corti*–infected mice (n = 2), normal littermates (n = 5). Normal mice were counted for each category, but were not significantly different from the normal group presented.

tact mRNA was confirmed using an actin RNA probe (Fig. 2 *b*). Using three different preparative procedures intact mRNA was not obtained from transgenic spleen. This is thought to be due to RNase activity associated with the high levels of eosinophils in this tissue.

IL-5 Levels and Eosinophil Counts in the Blood. IL-5 levels in sera from transgenic mice were comparable to peak levels in sera from mice infected with *M. corti* (Table 1). No IL-3 or GM-CSF activity was detected in the same sera.

Tg5C1 and Tg5C2 lines had 65-fold and 265-fold, respectively, the blood eosinophils seen in normal littermates, while *M. corti*–infected mice were 35-fold over normal controls (Table 1). Transgenic mice also showed small but significant increases (up to threefold) in neutrophil, lymphocyte, and monocyte numbers relative to normal littermates.

Thus, comparing the two transgenic lines, eosinophilia and IL-5 levels were significantly higher in the line with the highest transgene copy number. Transgenic mice have been observed for up to 6 mo and eosinophilia was maintained at relatively stable levels during this period.

IL-5 Production in Transgenic Cell Cultures. Supernatants from unstimulated transgenic spleen cell cultures contained IL-5, indicating constitutive production, and this was increased markedly in the presence of ConA. None was detectable in supernatants from unstimulated normal spleen cells or spleen cells from mice infected with *M. corti* (Table 2). Surprisingly, although high levels of mRNA were detected in transgenic thymus, no IL-5 was constitutively produced in culture. However, when cultured with Con A, very high levels of IL-5 were detected (Table 2).

In contrast, the levels of IL-3/GM-CSF detected in transgenic spleen and thymus cell cultures were similar to normal spleen cultures, and negligible relative to supernatants from the IL-3-producing cell line WEHI-3D.

Tissue Distribution of Eosinophils. Total and differential counts on single cell suspensions prepared from various tissues revealed major eosinophil localisation in the spleen, bone marrow, and peritoneal exudate (Fig. 3). Both the bone marrow and the spleen contained many morphologically immature forms, indicating active eosinophil haemopoiesis in both organs. Transgenics had enlarged spleens (Table 1), with a high proportion of eosinophils relative to nontransgenic littermates. Both transgenic lines had a high percentage of eosinophils in the peritoneal exudate, although the total number was lower than from mice infected with *M. corti*. For example, the approximate total numbers of peritoneal eosinophils for Tg5C1, Tg5C2, and parasitized mice were 2 $\times 10^6$, 5 $\times 10^6$, and 20 $\times 10^6$, respectively. Eosinophils represented 1% of thymocytes and 3% of mesenteric lymph node cells recovered from transgenics. Although relatively low compared with the spleen these are significantly higher than normal littermates, where eosinophils were virtually undetectable.

Table 2. *Cytokine Production in Spleen and Thymocyte Cultures*

Cultures	IL-3/GM-CSF Control	IL-3/GM-CSF Con A	IL-5 Control	IL-5 Con A
Spleen				
Tg5C1	12 ± 1	26 ± 1	37 ± 6	339 ± 53
M. corti	3 ± 2	10 ± 1	<1	8 ± 7
Normal	14 ± 4	5 ± 3	<1	16 ± 15
Thymus				
Tg5C1	<1	6 ± 1	<1	241 ± 92
Normal	<1	2 ± 2	<1	<1

Values are means ± 1 SD of 50% endpoints of replicate culture supernatants in each assay. WEHI-3D–conditioned medium gave 5,744 U in the IL-3/GM-CSF assay.

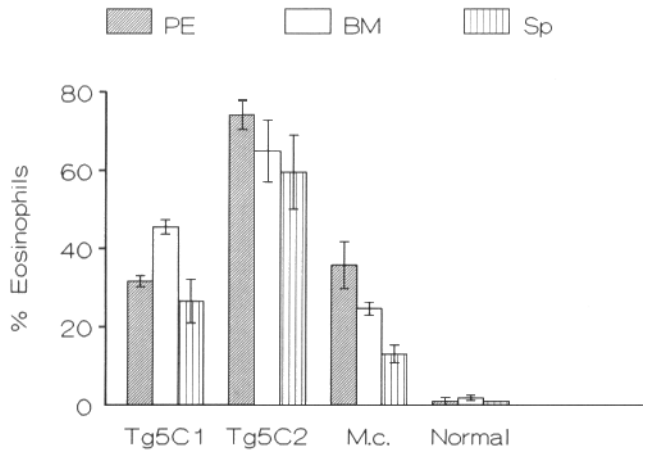

Figure 3. Tissue distribution of eosinophils in transgenic lines Tg5C1 and Tg5C2, *M. corti*–infected BALB/c mice (*M.c.*) and nontransgenic littermates (*Normal*). Representative data from one experiment with animals killed at 8 wk of age. PE, peritoneal exudate; BM, bone marrow; Sp, spleen.

Histological sections showed that most of the eosinophils in the spleen were located in the red pulp with very few in the white pulp area (Fig. 4, *a* and *b*; transgenic and normal, respectively). It was also noted that the lungs of transgenic but not of normal animals were heavily infiltrated with eosinophils (Fig. 4, *c* and *d*, respectively). Small but significant numbers of eosinophils were also observed in sections of mesenteric lymph nodes, Peyer's patches, and lamina propria of the small intestine from transgenics. None of the other tissue sections examined (heart, liver, skeletal muscle, or brain) showed increased numbers of eosinophils.

IL-5-responsive Eosinophil Precursors in Bone Marrow. Limiting dilution culture of bone marrow in the presence of IL-5 indicated that cells from transgenics had >10-fold higher capacity to produce eosinophils than normal littermates (Fig. 5). The capacity of transgenic marrow was also higher than that of bone marrow from mice infected with *M. corti*.

Another indirect measure of eosinophil progenitors is the sensitivity of the bone marrow to detect IL-5. This is illustrated in Fig. 6 where the bone marrow can be ranked in order of increasing sensitivity: normal, *M. corti*, Tg5C1 and Tg5C2. Although not obvious in these data the bone marrow from *M. corti*–infected mice is several orders of magnitude more sensitive than normal bone marrow (1).

Discussion

We report here the generation of four founder mice carrying transgenes for the cytokine IL-5. All four mice were eosinophilic, and independent lines have been established from each. Two of these lines carrying 8 or 49 copies of the transgene are reported here. The transgene was constructed by ligating a 2-kb fragment of human genomic DNA containing the DCR of the human CD2 gene (8) to a 10-kb DNA fragment containing the mouse IL-5 gene (10). The DCR was placed 5′ of the IL-5 gene. The murine fragment included flanking sequences of ~4 kb upstream from the IL-5 cap site, and 1.3 kb downstream from the polyadenylation attachment site of the IL-5 gene. These flanking regions are likely to contain many of the elements controlling the expression of the IL-5 gene, including the IL-5 promoter. Thus, while transcription is occurring from the IL-5 promoter, the CD2 DCR is acting to override other normal transcriptional controls on the IL-5 gene, allowing constitutive secretion of IL-5. The level of constitutive transgene expression has not been established, but may be lower than the induced expression of the endogenous gene. For example, the levels of IL-5 detected in transgenic sera are not as high as might be anticipated from the number of transgenes, when compared with the levels produced following expression by the single gene in normal mice infected with *M. corti*. It is possible that the transgenes retain the capacity to show normal inducible expression in response to activation. Some evidence for this is seen in the high levels of IL-5 produced by transgenic lymphoid organs after the addition of Con A.

Although the thymus of Tg5C1 contained high levels of IL-5 mRNA no IL-5 was secreted except after addition of Con A. This failure to secrete IL-5 constitutively in culture is puzzling, and it is not clear whether it represents a real inability to secrete, or simply a failure to secrete in vitro. Spleen cells secreted IL-5 constitutively in vitro and this was further increased after addition of ConA. In contrast little or no IL-3 or GMCSF was detected from either cell type.

The level of expression of other CD2 DCR transgene constructs is dependent upon transgene copy number and is T cell-specific (8, 9). Similarly, serum IL-5 levels and tissue eosinophilia were higher in the IL-5 transgenic line with the greatest copy number (Tg5C2). Further, IL-5 mRNA transcription patterns are consistent with transgene expression restricted to lymphoid tissues.

Both transgenic lines had profound, continuous eosinophilia in the blood, lymphoid, and hematopoietic tissues. Extensive eosinophilic infiltration of the lungs and intestinal lamina propria was also noted. Bone marrow from transgenics was rich in eosinophil progenitors as indicated by the number of immature cells observed, and by increased responsiveness to IL-5. Transgenics were compared with mice infected with the helminth *M. corti*, a pathogen known to induce high levels of eosinophilia and elevated serum IL-5 (1). In most tissues the numbers of eosinophils were higher in the transgenic lines compared with parasite-infected animals, although it must be remembered that eosinophilia in the parasitized mice is only transient and the animals included in this study may not have been at peak eosinophilia (1).

These transgenic mice raise some interesting points about the control of eosinophilia. Since its first characterization, studies in vitro have suggested that IL-5 is a late-acting factor in eosinophil differentiation (3–7). Eosinophils were produced only transiently in long-term bone marrow cultures stimulated with exogenous IL-5, suggesting that at least in these systems the progenitor cells were not being produced from stem cells. In addition, bone marrow from eosinophilic parasite-infected mice gives rise to many more eosinophils than bone marrow from normal mice, indicating that the

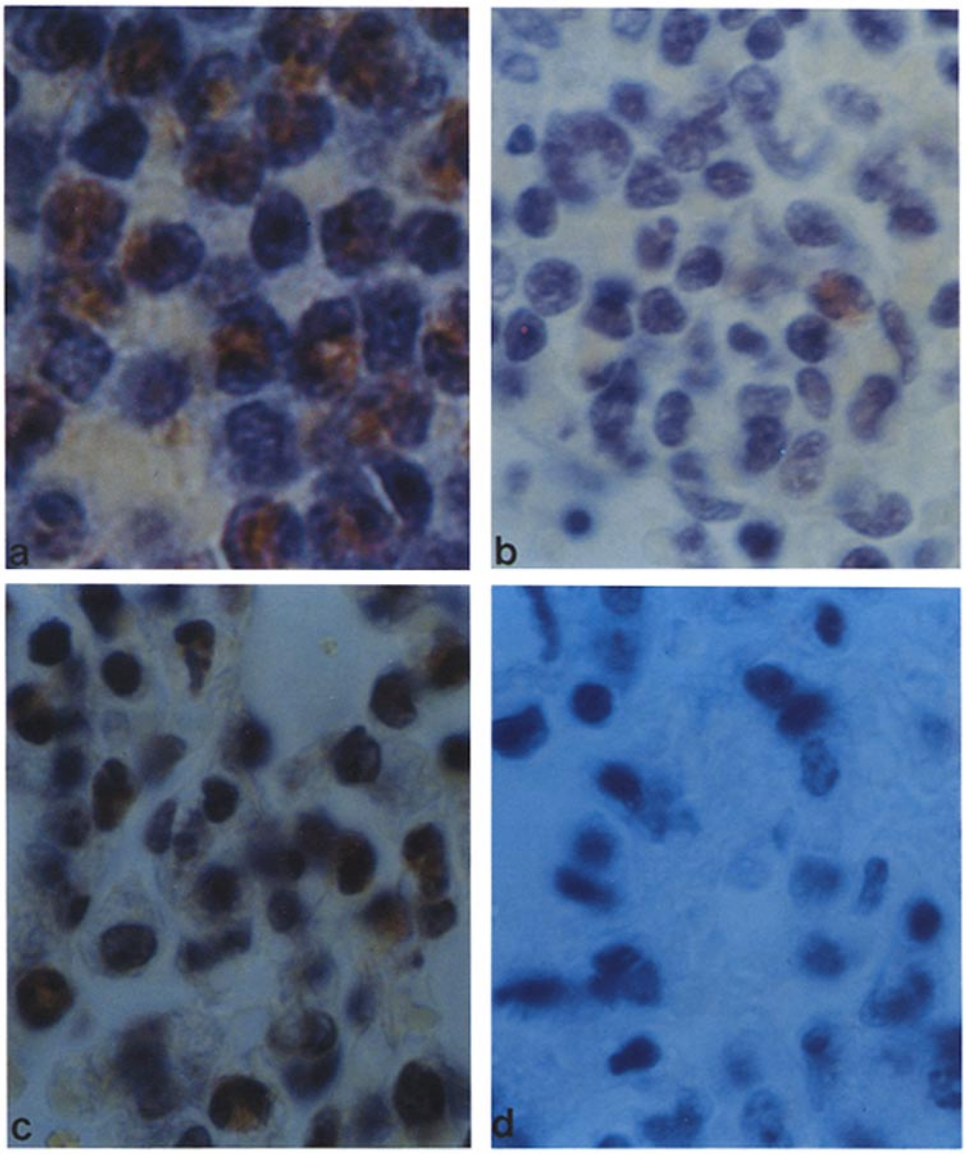

Figure 4. Histological sections of spleens and lungs from the Tg5C1 line (*a*, *c*, respectively) and a nontransgenic littermate (*b*, *d*, respectively), showing extensive eosinophilia in the transgenic tissue relative to nontransgenics. Eosinophil granules stain red-orange.

parasitic infection stimulates the production of progenitors. This suggests that other cytokines may be inducing the progenitors, and indeed it has been shown that IL-1, IL-3, and possibly G-CSF and GM-CSF are involved in this process (4, 6). Similarly, in studies with human bone marrow, both IL-3 and GM-CSF but not IL-5 induced eosinophil precursors, assayed as IL-5-responsive colony-forming cells (5, 7). However, the transgenics show that IL-5 expression is sufficient to induce eosinophilia, which suggests that IL-5 in vivo is able to control the production of eosinophil progenitors. Although a role for other cytokines in this process cannot be ruled out, they must be either constitutively expressed or their expression is induced by IL-5. It should be noted that no significant production of IL-3 or GM-CSF was detected, and so there is no apparent induction of these cytokines in the transgenic mice.

It has recently been shown that parasite-induced eosinophilia can be blocked by the in vivo administration of anti-IL-5 mAbs (20, 21). Thus, while both IL-3 and GM-CSF induce eosinophil production in vitro, these experiments in vivo suggest that IL-3 and GM-CSF do not operate in the development of eosinophilia in parasitic infections. Together with these results, the present study of IL-5 transgenic mice points to a major role for IL-5 in the control of eosinophilia. This provides an explanation for the biological specificity of eosinophilia (1, 2), as it appears that cytokines such as IL-3 and GM-CSF are not primarily involved in its induction. In both transgenic lines there was a small but significant increase in blood neutrophils and monocytes. It is not clear whether this represents a direct effect of IL-5 on these lineages, or whether it is secondary to the large turnover of eosinophils. It should be noted that studies in vitro showed a decrease in neutrophil numbers in bone marrow cultures containing IL-5 (3). IL-5 also has a well-characterized activity on B cells, with more recent reports of activities on T cells (reviewed in reference 22). Although there were also increases in lymphocyte

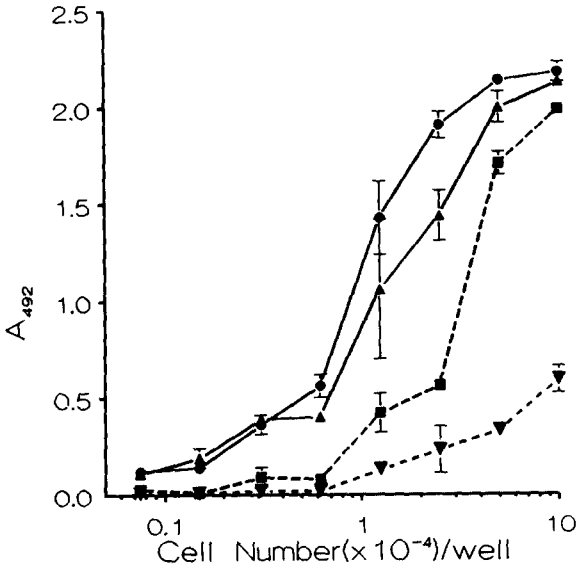

Figure 5. Eosinophils in bone marrow cultures after 5 d, detected by assay for eosinophil peroxidase (A_{492}). Serial titration of bone marrow cells in the presence of a constant amount of rmIL-5. Data indicate relative production of eosinophils in bone marrow from different sources. Normal mice (▼), *M. corti*–infected mice (■), Tg5C1 mice (▲), and Tg5C2 mice (●). Data are means ± 1 SD of duplicate cultures.

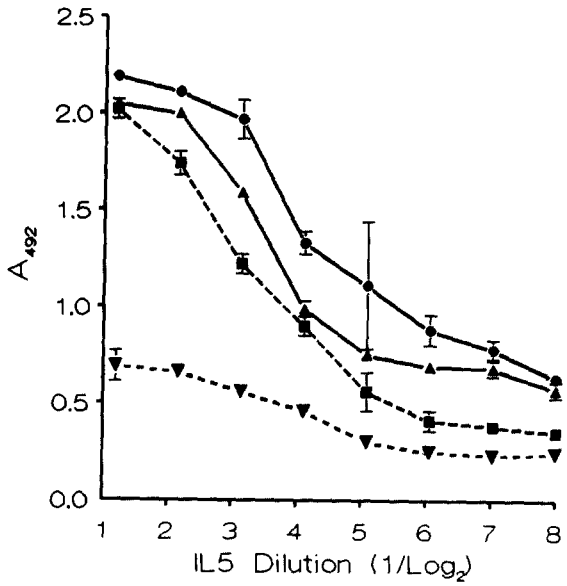

Figure 6. Eosinophils in bone marrow cultures after 5 d, detected by assay for eosinophil peroxidase (A_{492}). Serial titration of rmIL-5 (starting dilution 1 × 10^{-4}) added to 10^5 cells/well showing the relative sensitivity to IL-5 of bone marrow from different sources. Symbols and data presentation as for Fig. 5.

numbers in transgenics relative to nontransgenic controls, the changes were marginal compared with the magnitude of the eosinophil response. Preliminary flow cytometric analysis has not revealed any major selective increases in T or B cell subsets. Further experiments are being carried out to clarify these issues.

In the design of these transgenic animals we have been aware of the T cell dependence of eosinophilia, and the fact that IL-5 is normally a T cell product (23). The use of the CD2 DCR should ensure constitutive expression by T cells, and the data are consistent with this tissue-restricted expression. It seems remarkable that animals with such high levels of a potentially damaging leukocyte remain normal. This suggests that eosinophils require other factors, for example antigen-antibody complexes, for degranulation and subsequent tissue damage. As eosinophils play a role in allergic diseases in man (24), these animals will provide a model for testing modifiers of eosinophil production.

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