Enterocyte Expression of the Eotaxin and Interleukin-5 Transgenes Induces Compartmentalized Dysregulation of Eosinophil Trafficking**

Anil Mishra‡§, Simon P. Hogan‡§, Eric B. Brandt‡, Norbert Wagner‡, Michael W. Crossman‡, Paul S. Foster‡, and Marc E. Rothenberg‡**

From the ‡Department of Pediatrics, Children’s Hospital Medical Center, Cincinnati, Ohio 45229, the §Cologne Institute for Genetics, University of Cologne, Cologne 50931, Germany, and the ¶Division of Biochemistry and Molecular Biology, John Curtin School of Medical Research, Australian National University, Canberra, Australian Capital Territory 0200, Australia

Eosinophils accumulate in the gastrointestinal tract in a number of medical disorders, but the mechanisms involved are largely unknown. To understand the significance of cytokine expression by enterocytes, enterocyte transgenic mice that overexpressed the eosinophil-selective cytokines eotaxin and interleukin (IL)-5 were generated. Transgenic mice, generated by utilizing the rat intestinal fatty acid-binding protein promoter (Fabpi), overexpressed the mRNA for these cytokines in the small intestine. Overexpression of IL-5 resulted in marked increases of eosinophils in the bone marrow and blood, whereas eotaxin overexpression resulted in similar levels compared with nontransgenic control mice. In contrast, both IL-5 and eotaxin transgenic mice had significant accumulation of eosinophils in the gastrointestinal mucosa compared with control mice. Eotaxin-induced gastrointestinal eosinophilia was substantially higher than that induced by IL-5 and was especially prominent within the lamina propria of the villi. Interestingly, genetic rescue of eotaxin deficiency (by transgenic overexpression of eotaxin in eotaxin gene-targeted mice) resulted in significant restoration of gastrointestinal eosinophil levels. Finally, the intestinal eosinophilia induced by the eotaxin transgene was β7 integrin-dependent. Taken together, these results demonstrate that expression of eotaxin and IL-5 in intestinal epithelium induces compartmentalized dysregulation of eosinophil trafficking and the important role of the β7 integrin in gastrointestinal allergic responses.

** To whom correspondence should be addressed: Div. of Allergy and Immunology, Dept. of Pediatrics, Children’s Hospital Medical Center, Cincinnati, OH 45229. Tel.: 513-636-7210; Fax: 513-636-3310; E-mail: rothenberg@chmcc.org.

† The abbreviations used are: IL, interleukin; VLA, very late antigen; Fabpi, rat intestinal fatty acid-binding protein promoter; Fabpi, rat intestinal fatty acid-binding protein; hGH, human growth hormone; PBS, phosphate-buffered saline.

Received for publication, October 30, 2001
Published, JBC Papers in Press, November 30, 2001, DOI 10.1074/jbc.M110424200

This paper is available on line at http://www.jbc.org
Eotaxin and IL-5 Intestine Transgenic Mice

**MATERIALS AND METHODS**

**Generation of Fabpi Eotaxin and IL-5 Transgenic Mice—Oligonucleotides containing BamHI sites were ligated to both ends of a 330-bp fragment containing the entire coding region of the murine eotaxin cDNA. A 415-bp fragment of the entire coding region of murine IL-5 cDNA was amplified by PCR incorporating an improved Kozak consensus sequence and BamHI restriction sites on both ends. Both cDNAs were ligated into the BamHI site of the PBSIF1178-hGHpgkNeo plasmid (28, 33), which contained a 3.5-kb EcoRI fragment containing nucleotides −1178 to +28 of rat Fabpi promoter linked to nucleotides +3 to +2150 of human growth hormone (hGH) gene (except for its 5′ regulatory sequences) as represented by Fig. 1. The transgene plasmid was propagated in Escherichia coli DH5α and in Escherichia coli DH11001 (using the QIAEX DNA extraction kit, Qiagen Inc., Chatsworth, CA).**

**Bone and Bone Marrow Eosinophil Analysis**—Bone marrow analysis was performed by irrigating each femur with 5 ml of PBS, pH 7.4, using a 23-gauge needle to isolate femoral marrow leukocytes.**
Eotaxin and IL-5 Intestine Transgenic Mice

A. iET Transgene

B. IL-5 Transgene

Fig. 1. The Fabpi transgenic constructs. The cDNA fragment encoding the open reading frame of murine eotaxin (mEot) or murine IL-5 (mIL-5) was cloned into the BamHI site present in a 3.5-kb EcoRI fragment containing nucleotides −1178 to +28 of rat Fabpi promoter linked to nucleotides +3 to +2150 of the hGH gene (except for its 5′ regulatory region). The position of the PCR primers used for the detection of the Fabpi-eotaxin (iET) (P1 and P2) and the Fabpi-IL-5 (iIL-5) transgenes (P2 and P3) are indicated.

Statistical Analysis—Data are expressed as mean ± standard deviation (S.D.). Statistical significance comparing different sets of mice was determined by Student’s t test.

RESULTS

Generation of Fabpi Eotaxin and IL-5 Transgenic Mice—Enterocytes have been demonstrated to be a chief source of select chemokines (e.g. IL-8, MCP-1, ENA-78) in inflammatory lesions in patients with diverse gastrointestinal inflammatory disorders (32, 38, 39). We were interested in examining the consequences of expressing eotaxin-active cytokines by intestinal enterocytes. The 1.2-kb 5′-flanking region of the rat Fabpi gene contains all of the necessary elements to promote specific expression of transgenes into enterocytes in the small intestine (28, 40). We initially generated transgenic mice that utilized this promoter to direct expression of eotaxin or IL-5 in the intestine (see transgenic construct (Fig. 1)). Three different eotaxin transgenic founder lines were established (designated iET-1–3) that had −1, 4, and 10 copies of the transgene insert (for iET-2, iET-1, and iET-3, respectively) as assessed by Southern analysis. Northern blot analysis with an eotaxin-specific cDNA probe revealed increased expression of eotaxin in the intestinal mRNA of transgenic mice (Fig. 2A). The wild-type mRNA species was detected as a predominant single band of ∼1 kb, whereas the transgenic mice had eotaxin mRNA migrating as multiple mRNA species. The predicted mature mRNA was detectable at ∼500 base pairs. The higher molecular weight mRNA species may represent the presence of chimeric eotaxin-hGH mRNA in the transgenic construct (28, 40).

In addition, five different founder lines for the Fabpi-IL-5 transgenic mice (designated iIL-5-1–5) were also derived. These mice were found to have transgene copy numbers of −6, 15, 7, 5, and 9 for each iIL-5 line, respectively. Northern blot analysis (Fig. 2B) revealed markedly increased levels of IL-5 mRNA in the transgenic mice compared with wild-type mice. In wild-type mice, no IL-5 mRNA was detectable, whereas the transgenic lines had readily detectable IL-5 mRNA species present, migrating as multiple mRNA species.

The Effect of Intestinal Transgene Expression on Eosinophil Levels in the Hematopoietic Tissues—We were next interested in testing the hypothesis that overexpression of IL-5 or eotaxin in intestinal enterocytes would have consequences on eosinophil levels in hematopoietic organs. To initially analyze this, eosinophil levels were determined in the bone marrow of wild-type and transgenic mice. In the six lines of iIL-5 transgenic mice, marked increases in bone marrow eosinophil levels were seen compared with wild-type mice. For example, eosinophils accounted for 2.4 ± 0.85% and 22 ± 10% (mean ± S.D.; n = 4; p < 0.01) of the bone marrow cells in wild-type and iIL-5 transgenic mice, respectively. In contrast, overexpression of eotaxin did not affect eosinophil levels in the bone marrow (3.4 ± 2.1 and 2.8 ± 1.5% eosinophils for wild-type and iET mice, respectively; p > 0.1). We were next interested in analyzing the level of circulating eosinophils in the transgenic mice. Consistent with the expansion of eosinophils in the bone marrow, eosinophil levels in the peripheral blood of iIL-5 transgenic mice were increased 17 ± 12-fold (mean ± S.D., n = 11) compared with wild-type mice when combining data from all transgenic lines. In contrast, eotaxin transgenic mice had no significant alteration in the level of circulating eosinophils. Circulating eosinophil levels from representative transgenic and wild-type mice are shown in Fig. 3. Taken together, these results demonstrate that ectopic expression of IL-5 in intestinal enterocytes, in contrast to overexpression of eotaxin, dramatically increases eosinophils in the bone marrow and peripheral circulation.

The Effect of Transgene Expression on Gastrointestinal Eosinophil Levels—It was relevant to determine whether overexpression of eotaxin or IL-5 in the intestine would induce the accumulation of eosinophils specifically in the intestine. We focused our attention on eosinophils in the jejunum because this is the intestinal region with the highest level of Fabpi transgene expression (Refs. 28 and 40 and data not shown). We first examined eosinophil levels in the gastrointestinal tract of IL-5 transgenic mice. Mice overexpressing IL-5 had a 3.0 ± 1.1-fold (mean ± S.D., n = 4) increase in gastrointestinal eosinophil levels compared with wild-type mice. Eotaxin transgenic mice had an even larger increase in the levels of gastrointestinal eosinophils (p < 0.05 for iET versus iIL-5). Mice overexpressing eotaxin had 8.5 ± 4.4-fold (mean ± S.D., n = 10) increase in eosinophils compared with wild-type mice. Eosinophil accumulation occurred within the lamina propria in the submucosa and villus (Fig. 4). Anti-MBP immunohistochemistry revealed that eosinophils were not extensively degranulating, as demonstrated by the predominant cell-associated anti-MBP staining (Fig. 5 and data not shown). The tissue
eosinophilia induced by overexpression of eotaxin with the Fabpi promoter was restricted to the small intestinal mucosa because eosinophil levels in the stomach (37 ± 16 versus 30 ± 10 (mean ± S.D., n = 4 mice) eosinophils/mm²), colon (57 ± 7 versus 55 ± 15 (mean ± S.D., n = 5) eosinophils/mm²), and the bronchoalveolar lavage fluid (560 ± 590 versus 320 ± 280 (mean ± S.D., n = 4) eosinophils/ml for wild-type and transgenic mice, respectively) were comparable between transgenic and control mice.

Eotaxin transgenic mice developed a change in the normal distribution of gastrointestinal eosinophils. In wild-type control mice, jejunum eosinophils predominantly resided in the lamina propria associated with the submucosa and the crypts of Lieberkuhn consistent with previous studies (4, 5). However, in the iIL-5 and iET mice, eosinophils were noted to be frequently present within the villus. Morphometric analysis allowed quantification of eosinophils within the lamina propria of the total area of the gastrointestinal tissue and within the lamina propria of the villi only. Comparison of eosinophil levels within the lamina propria of the total gastrointestinal tissue to levels within the villi alone revealed a relative accumulation within the villi (Fig. 4). Eosinophil levels in the villi increased by 23 ± 7-fold (mean ± S.D., n = 4) and 74 ± 10-fold (mean ± S.D., n = 3) compared with wild-type mice in iIL-5 and iET mice, respectively (p < 0.001).

Eotaxin-IL-5 Bitransgenic Mice—We were next interested in determining the consequences of overexpressing both IL-5 and eotaxin in the intestine. We hypothesized that the effect of the eotaxin transgene would be enhanced in the presence of the IL-5 transgene. To test this hypothesis, we crossed the iET and iIL-5 mice and identified offspring that contained both transgenic constructs. However, bitransgenic mice had eosinophil levels that were comparable with mice engineered with the eotaxin transgene alone. For example, wild-type, iIL-5, iET, and iET/iIL-5 bitransgenic mice had 32 ± 12, 91 ± 14, 258 ± 37, and 185 ± 139 (mean ± S.D., n = 4–11) eosinophils/mm².

---

**Eotaxin and IL-5 Intestine Transgenic Mice**

**Fig. 3. Peripheral blood eosinophil levels in wild-type and Fabpi transgenic mice.** Eosinophil levels in the peripheral blood of wild-type, intestine-eotaxin (iET), and intestine-IL-5 transgenic (iIL-5) mice are indicated. The results are representative of three separate experiments and are presented as the mean ± S.D. (n = 4 mice).

**Fig. 4. Gastrointestinal eosinophil levels.** Eosinophil levels in wild-type (WT), eotaxin transgenic (iET), and IL-5 transgenic (iIL-5) mice were determined by morphometric analysis. Eosinophil levels were analyzed in the total area of the lamina propria and in the lamina propria area only within the villi. Eosinophil levels are expressed as the mean eosinophils/mm² ± S.D. (n = 9–14 mice) and are representative of two separate experiments.

**Fig. 5. Immunohistochemical staining of jejunum sections from wild-type and transgenic mice.** The jejunum from wild-type (WT; A), Fabpi-IL-5 transgenic (iIL-5; B), or Fabpi-eotaxin transgenic (iET; C) mice was stained with anti-MBP. Representative tissue sections are shown, and representative eosinophils, recognized by black staining, are indicated by the arrows. Original magnification, ×125.
Taken together, these data indicate that overexpression of eotaxin in the intestine has a dominant role in regulating the local accumulation of eosinophils and that the level of eosinophils is not further enhanced by local overexpression of IL-5.

Transgenic Rescue of Eotaxin Gene Deletion—Eotaxin gene-targeted mice have a specific deficiency of eosinophils in the gastrointestinal tract (2). We were interested in testing the hypothesis that transgenic expression of eotaxin in intestinal enterocytes would restore the eosinophil deficiency seen in the eotaxin gene-targeted mice. The generation of iET mice allowed us to test this hypothesis by a genetic rescue approach. Mice genetically deficient in eotaxin were crossed with the iET mice to allow the generation of mice that were deficient in the wild-type eotaxin gene but contained the eotaxin transgene under the control of the Fabpi promoter. To determine the effect of the genetic rescue, the level of intestinal eosinophils was compared in the rescued, wild-type, and eotaxin-deficient mice (Fig. 6). Compared with eotaxin-deficient mice (which have a reduction in the level of eosinophils compared with wild-type mice) (4), genetically rescued mice had eosinophil levels that were restored (p < 0.05) to values comparable with wild-type mice. For example, eosinophil levels in the total lamina propria area were 75 ± 23, 53 ± 5.3, and 43 ± 41 eosinophils/mm² (mean ± S.D., n = 11–17) for wild-type, eotaxin-deficient, and iET × eotaxin (−/−) mice, respectively. When eosinophils were quantified only in the lamina propria of the villi, eosinophil levels were increased 23- and 8-fold compared with eotaxin deficient and wild-type mice, respectively (Fig. 6).

Tissue distribution of eosinophils within the intestinal tract. Under base-line conditions, most eosinophils reside within the lamina propria of the villi, consistent with the expression of the Fabpi transgene in well differentiated villus-associated enterocytes (28, 40). Increased expression of eotaxin in enterocytes likely induces the selective migration of lamina propria eosinophils from the base to within the villus. Third, our results establish that the eosinophil deficiency seen in eotaxin-deficient animals can be phenotypically corrected by genetic rescue of eotaxin with the iET transgene.

Critical Role of the β7 Integrin in Eotaxin-induced Intestinal Eosinophilia—Eosinophils express several classes of adhesion molecules including β1, β2, and β3 integrins (41). The β7 integrin family has been demonstrated to be critical for lymphocyte trafficking to the gastrointestinal tract (21). Because eosinophils reside in similar locations to gastrointestinal lymphocytes (e.g. lamina propria) (2), we hypothesized that the β7 integrin would also be important in eosinophil trafficking to the gastrointestinal tract. To test this hypothesis, we analyzed the level of intestinal eosinophils in wild-type, β7 integrin−/− gene-targeted mice (35), and in eotaxin transgenic mice engineered to be genetically deficient in the β7 integrin (Fig. 7). Initial analysis of β7−/− deficient animals revealed that their level of intestinal eosinophils was not significantly different from wild-type mice, indicating that this integrin was not essential for the constitutive homing of eosinophils into the gastrointestinal tract. In contrast, the gastrointestinal eosinophilia induced by the eotaxin transgene was found to be completely dependent upon the β7 integrin (Fig. 7).

DISCUSSION

Although enterocytes have been demonstrated to express diverse chemokines, the specific involvement of enterocyte-derived chemokines in the gastrointestinal immune system and in the pathogenesis of disease is largely unknown. Gastrointestinal inflammatory events have been examined in chemokine receptor-deficient mice, but the consequences of overexpressing chemokines in the gastrointestinal tract have not been directly addressed (39, 42). In the current study, we dissect the consequences of overexpressing eotaxin in enterocytes using a well established enterocyte-specific promoter (Fabpi) (28, 40). For comparison, Fabpi transgenic mice were also generated, which overexpress IL-5, a cytokine that is increased in lesions of patients with eosinophil-associated inflammatory gastrointestinal disorders (11, 43). Experimental analysis of eotaxin and IL-5 intestine transgenic mice has established several fundamental principles. First, overexpression of each cytokine is demonstrated to have a significant impact on regulating eosinophil accumulation in the small intestine. The effect of eotaxin is especially potent compared with IL-5 and is elicited by a unique mechanism because eotaxin transgenic mice have no alterations in eosinophil levels in the hematopoietic organs, whereas IL-5 transgenic mice have marked expansion of eosinophils in the bone marrow and peripheral blood. These later results are consistent with previous studies demonstrating that ectopic expression of IL-5 in pulmonary epithelial cells induces both lung and circulating eosinophilia (44). Second, our study demonstrates that expression of eotaxin in enterocytes induces a change in the regional distribution of eosinophils within the intestinal tract. Under base-line conditions, most eosinophils reside within the lamina propria at the base of the crypts (4). In contrast, eotaxin transgenic mice have a marked accumulation of lamina propria eosinophils within the villi, consistent with the expression of the Fabpi transgene in well differentiated villus-associated enterocytes (28, 40). Increased expression of eotaxin in enterocytes likely induces the selective migration of lamina propria eosinophils from the base to within the villus. Third, our results establish that the eosinophil deficiency seen in eotaxin-deficient animals can be phenotypically corrected by genetic rescue of eotaxin with the iET transgene.
Eotaxin and IL-5 Intestine Transgenic Mice

rescue with the Fabp1-eotaxin transgene. This indicates that expression of eotaxin in enterocytes is able to compensate for the normal eotaxin-dependent recruitment of eosinophils to the gastrointestinal tract. Finally, our results establish a critical role for the β2 integrin in promoting the eotaxin transgene-mediated gastrointestinal eosinophilia.

We have previously reported an obligatory role for eotaxin in regulating eosinophil homing to segments of the gastrointestinal tract wherein they normally reside (e.g. stomach and intestine) (2, 4, 5). However, the expression of eotaxin is not sufficient for eosinophil trafficking, as exemplified by readily detectable eotaxin in the esophagus despite the absence of eosinophils from this gastrointestinal segment (5). In addition, although eotaxin is an allergen-induced gene product in the lungs and contributes to antigen-induced pulmonary eosinophil recruitment (26, 45), we have found that transgenic overexpression of eotaxin in the lung does not promote eosinophil accumulation (6). It was thus critical to generate Fabp1-eotaxin transgenic mice to determine the consequences of eotaxin overexpression in the mucosal tissue where our previous data had suggested a dominant role for this chemokine (45). The results revealed marked tissue-specific eosinophil recruitment by eotaxin overexpression in the intestine. IL-5 also promoted gastrointestinal eosinophilia, but the effect of IL-5 was not tissue-specific. Our previous finding that systemic delivery of IL-5 induces intestinal eosinophilia (46) suggests that the effect of the Fabp1-IL-5 transgene is not solely because of gastrointestinal-specific mechanisms. Eotaxin and IL-5 have been demonstrated to cooperate in the induction of pulmonary eosinophilia because IL-5 promotes an increased pool of eotaxin-responsive eosinophils and primes eosinophils to respond to CCR3 ligands (7, 8). To address the cooperation of these two pathways in the gastrointestinal tract, mice transgenic for both eotaxin and IL-5 were generated. However, analysis of these bitransgenic mice revealed that eosinophil recruitment could not be further enhanced by the presence of both transgenes compared with the eotaxin transgene alone. These results suggest that there may be a limit to the steady state homing of eosinophils into the gastrointestinal tract. This limitation may be imposed by the level or activation of specific adhesion molecules and/or homing receptors. Eosinophils express members of the β1, β2, and β3 integrin family (41). Extensive studies have focused on analysis of β1 and β2 integrins and have demonstrated the critical role for the CD18-associated β2 integrin molecules and the α4-associated β1 integrin (VLA-4) (41). The α4β2 integrin is expressed on both murine and human eosinophils, but the specific role of this adhesion molecule on eosinophils has not been reported (13, 41). We hypothesized that the β2 integrin was critical for eosinophil homing into the gastrointestinal tract because this molecule is essential in the homing of intestinal lymphocytes, cells that reside in similar locations as gastrointestinal eosinophils (2). Importantly, MacCAM-1, the receptor for α4β2, is expressed by the endothelial cells residing in the intestinal lamina propria and Peyer’s patches (23, 24). To address the role of this integrin, we generated mice that were eotaxin transgenic and genetically deficient in the β2 integrin. Analysis of gastrointestinal eosinophil levels in these mice revealed that eotaxin-transgene-induced eosinophilia was completely dependent upon the β2 integrin. Previous reports have established that β2-deficient animals have impaired intestinal hypersensitivity responses (including eosinophilia) in response to helminthic infection (27). However, the specific role of the β2 integrin in regulating eosinophil recruitment was not addressed because these mice also had impaired T cell recruitment.

Eosinophils accumulate in the gastrointestinal tract in diverse diseases including eosinophilic gastroenteritis, eosinophil-associated protein-sensitive enteropathy, and inflammatory bowel disease (2, 47, 48). In some of these disorders, eosinophils are thought to be principle effector cells (47, 49). In experimental oral antigen-induced eosinophilic gastrointestinal inflammation, a critical role for eotaxin and eosinophils in the pathogenesis of specific manifestations of the disease has been recently reported (14). However, there is also clinical literature indicating that increased levels of intestinal eosinophils can be found in apparently healthy individuals and in atopic individuals with no evidence of gastrointestinal disorders (50–52). Our results are consistent with the hypothesis that overproduction of eotaxin specifically in the intestinal tract is causally related to eosinophil recruitment, but may not be the only event that occurs in patients that have co-existing systemic eosinophilia. In the latter case, IL-5 overexpression is likely to also occur (rather than just eotaxin overexpression). Interestingly, only one-third of patients with eosinophil-associated gastrointestinal disorders have co-existing peripheral blood eosinophilia (2, 53). Importantly, eosinil and IL-5 intestine transgenic mice had no obvious clinical manifestations. Taken together, these data suggest that IL-5 and eotaxin are likely to cooperate with other factors involved in the pathogenesis of eosinophil-associated pathology. For example, in experimental oral antigen-induced eosinophil-associated gastrointestinal allergy, antigen-specific IgG, IgE, and IgA are produced, which are likely to provide an additional mechanism for activation of the effector arm of the immune response (14).

In summary, we have developed a transgenic model of cytokine-induced eosinophil-associated gastrointestinal accumulation. Although no murine model adequately mimics human disease, our transgenic system offers an experimental framework to analyze the events associated with eosinophilic intestinal inflammation. Our results establish that eotaxin or IL-5 overexpression in intestinal enterocytes induces compartmentalized dysregulation of eosinophil trafficking and provides a model to explain the dissociation between peripheral blood and gastrointestinal eosinophilia, involving dysregulated overproduction of these cytokines. Our results also establish a critical role for the β2 integrin in regulating eosinophil gastrointestinal trafficking. These results, which provide insight into the molecular pathogenesis of gastrointestinal allergy, suggest a critical role for enterocytes, eotaxin, IL-5, and the β2 integrin in eosinophil-associated gastrointestinal inflammation and have significant therapeutic implications.

Acknowledgments—We thank Andrea Lippelman for editorial assistance and Drs. Mitchell Cohen, Jeffrey Whitsett, Susan Wurtz, and Nives Zimmermann for helpful discussions and review of the manuscript. We thank Michael Royalty and Jessica Kavanaugh for technical assistance. We thank Drs. James and Nancy Lee (Mayo Clinic, Scottsdale, AZ) for the generous supply of anti-MBP and Dr. Jeffrey Gordon for the original fatty acid-binding protein transgenic constructs.

REFERENCES

10. Desreumaux, P., Bliege, P., Seguy, D., Capron, M., Cortet, A., Colombel, J. P.,
Eotaxin and IL-5 Intestine Transgenic Mice

37. Dicosmo, J. (1940) Lancet i, 185