Brief Definitive Report

Interleukin 5 Messenger RNA Expression by Eosinophils in the Intestinal Mucosa of Patients with Coeliac Disease

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Summary

Interleukin 5 (IL-5), the major factor involved in eosinophil differentiation, is produced by T cells or mast cells. In the present study, we found that eosinophils infiltrating the mucosa of four patients with active coeliac disease also express the IL-5 mRNA. No positive signal was obtained in normal duodenum tissues and in the cell infiltrate from patients submitted to gluten restriction. The identification of labeled mucosal cells as eosinophils relied on their typical morphology. Moreover, highly purified blood eosinophils from three out of four patients with eosinophilia were also strongly labeled with the IL-5 antisense but not with the corresponding sense probe. Together, these results suggest that eosinophils have the capacity to synthesize IL-5, which could contribute to paracrine interactions with T and B cells and, in autocrine fashion, locally participate, through binding to the IL-5 receptor, to eosinophil differentiation and activation. These data might have implications not only in the pathology of coeliac disease but also in other diseases associated with eosinophil infiltration.

IL-5 supports the proliferation and terminal differentiation of eosinophilic precursors (1) as well as the prolonged survival of eosinophils in vitro (2). It is also a potent activator of eosinophil functions such as cytotoxicity or mediator release (3). The effects of IL-5 on B cells and Ig synthesis have been studied in more detail in rodents. Both human and mouse IL-5 and IL-6 enhance in vitro IgA secretion from isolated murine cells of mucosal origin (4). More recently, it has been reported that both IL-5 and IL-6 augment local IgA production in vivo, in the tears of rats (5). However, the effect of IL-5 on human B cells remains controversial (6).

Coeliac disease (CD) is defined as a gluten-sensitive enteropathy that results in intestinal damage with varying degrees of villous atrophy. Morphometric studies have shown that the eosinophil population is markedly expanded in the CD mucosa and diminished under gluten restriction (7). Another feature of CD is a dense infiltration of the jejunum by IgA-secreting plasma cells and an enhanced production of secretory IgA antibodies (8).

By in situ hybridization, IL-5 messenger RNA (mRNA) was detected in skin and mucosal bronchial biopsies of patients with eosinophil infiltration, but without identification of the labeled cells (9, 10). In the present study, we analyze by a similar approach the expression of IL-5 mRNA in duodenojejunal mucosa from patients with CD. Our results show the presence of IL-5 mRNA in eosinophils and suggest that IL-5, classically produced by T cells, can arise from eosinophils and participate to eosinophil activation in CD, as well as in other diseases.

Materials and Methods

Tissue Processing. Biopsy specimens were collected by endoscopy from the terminal duodenum of four patients with clinically active CD. The biopsies showed crypt hyperplasia and total villous atrophy. In two of these patients, biopsy specimens were also obtained endoscopically at an inactive phase of the disease after treatment by gluten restriction. Negative controls were samples of normal duodenum obtained during investigation of three patients with functional abdominal pain. Intestinal fragments were properly oriented, fixed immediately in fresh 4% paraformaldehyde/PBS, and processed for paraffin embedding by standard techniques.

Cell Preparations. Eosinophils were purified from the blood of four patients with eosinophilia of different etiologies: Hodgkin's disease, idiopathic hypereosinophilic syndrome, systemic mastocytosis, and episodic angioedema. A technique of centrifugation on discontinuous metrizamide gradients was used as previously de-

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scribed (11). Activated hypodense eosinophils, which sediment in the low density layers, were collected. The degree of eosinophil purity was evaluated by May Grünwald Giemsa differential counting on cytocentrifuged preparations and reached between 75% and 95% eosinophils. These cells were resuspended at a concentration of 0.8 × 10^6 cells/ml of HBSS and loaded on gelatin-coated slides (0.8 × 10^5 cells/slide) by cytocentrifugation. The cytopreparations were immediately fixed for 20 min in 4% paraformaldehyde and stored at −20°C before being analyzed by in situ hybridization.

Preparation of Labeled Probes. The cDNA for human IL-5 and IL-6 were subcloned into the Blue Script vector by standard techniques. Linearized plasmids were used as templates for the synthesis in vitro of ^{35}S-labeled RNA probes complementary to the cellular IL mRNA (antisense probes). RNA was also transcribed from the opposite direction and used as a negative control (sense probes).

In Situ Hybridization. For a given RNA, antisense or sense probes (4,150 cpm/mm^2) were used and hybridization was performed with duodenojejunal biopsies and controls as previously described (12). To inhibit the nonspecific binding of ^{35}S, tissues and cells were acetylated in triethanolamine (0.1 M) for 5 min and then in acetic anhydride, 0.25% triethanolamine for 10 min. Then, to avoid nonspecific binding to eosinophils, prehybridization was carried out with a solution containing a nonradiolabeled S-UTP irrelevant probe for at least 2 h at 42°C, DTT was added to the hybridization buffer, and RNAse A was used for posthybridization washings (13). After development of the emulsion, tissue sections or cytospin preparations were then stained with May Grünwald Giemsa or Hoechst for examination in bright or dark field illumination, respectively.

Results and Discussion

In situ hybridization, using a ^{35}S-labeled antisense IL-5 RNA as the probe, revealed that numerous cells infiltrating the mucosa of patients with active CD highly express IL-5 mRNA. The results of one patient are shown in Fig. 1 *A*; however, a similar label intensity was obtained in the mucosa of the three other patients. Dark field illumination (Fig. 1 *B*) confirmed the strong level of hybridization and indicated that the positive hybridization signal was mainly located around the crypts. Examination of the tissues at a higher magnification revealed that the majority of eosinophils infiltrating active CD mucosae were significantly labeled with the IL-5 antisense probe (Fig. 1 *C*). The presence of eosinophils in the tissue samples was detected by immunohistochemical analysis with the mAb EG2, which is directed against eosinophil cationic protein (14), on serial sections of each biopsy (data not shown). Moreover, a reduced time of incubation before development was used in order to more precisely identify the labeled cells, eosinophils being characterized by their bilobed nuclei (Fig. 1 *D*). Very interestingly, no positive signal was detected with the IL-5 antisense probe in the mucosa during remission stage of the disease, although infiltrating eosinophils were present (Fig. 1 *E*). Similar negative results were obtained in normal duodenum samples. The absence of hybridization signal, in active CD patients, with the ^{35}S-labeled IL-5 sense probe (Fig. 1 *F*) as well as with IL-6 sense or antisense probes (not shown) indicated that binding of the IL-5 antisense probe was specific. Since the identification of eosinophils relied on morphologic criteria alone, we wanted to gain further evidence that eosinophils can express IL-5 mRNA. Therefore, highly purified blood eosinophils (up to 95% purity), were also studied by in situ hybridization. The majority of eosinophils from three out of four patients spontaneously expressed IL-5 mRNA (Fig. 1 *G*) with the highest level of expression in eosinophils from a patient with the hypereosinophilic syndrome, whereas no hybridization was detected on eosinophils from a patient with episodic angioedema. No positive signal was detected with the control IL-5 sense probe on purified blood eosinophils from any of these patients (Fig. 1 *H*).

Previous reports have established that IL-5 mRNA expression, regarded as being characteristic of activated T cells, can be detected in cell populations like mast cells (15) or Reed-Sternberg cells (16). The present data indicate that human eosinophils, which have been reported to produce TGF-α (13), can also synthesize IL-5. It is interesting to note that in all cases, eosinophils from tissues or peripheral blood that expressed IL-5 mRNA were activated. This state of activation was suggested by the altered morphological appearance of eosinophils detected in the tissues during CD by electron microscopy (17). In the case of the blood eosinophils, the selected hypodense populations correspond to the activated phenotype, previously characterized by immunological and biochemical properties (11).

These new findings raise the possibility that expression by eosinophils of IL-5 mRNA might result from an activation process due to cell interaction or release of mediators in situ. It has been recently reported that expression of TGF-α by eosinophils might be under microenvironmental regulation (13). It will clearly be of interest to search for the factors that influence the expression of IL-5 mRNA by eosinophils. Among these, cytokines or growth factors, as well as antibodies, might be involved. It is known that different regulatory mechanisms exist in mast cells for the transcription of differents groups of cytokines; in this model, increased levels of IL-5 mRNA are obtained by IgE–anti-IgE stimulation (15).

The identification of IL-5 mRNA, which provides evidence of gene expression by eosinophils, suggests but does not prove active synthesis of IL-5. Preliminary results using an IL-5-dependent cell line indicate that 99% pure eosinophils can secrete a significant amount of IL-5 (up to 3 U/2 × 10^6 cells). Taken together, these results provide evidence for the cellular localization of IL-5 mRNA in activated eosinophils, suggesting another role for eosinophils in the immune response. IL-5 can contribute to paracrine interactions with T and B cells. In CD, the local secretion of IL-5 can explain, at least in part, the enhanced synthesis of IgA. Autocrine effects of IL-5 can also be discussed, since IL-5 membrane receptors have been described, specially expressed on hypodense blood and tissue eosinophils (18). An enhanced secretion of IL-5 might influence the local stimulation of eosinophil proliferation, differentiation, and activation. In addition, the eosinophil infiltrate observed in CD might be due to the effects of IL-5 on eosinophil adhesion molecules recently described (19). Two recent reports have suggested the production of granulocyte/macrophage colony-stimulating factor and IL-3 by peripheral blood eosinophils, but only after in vitro stim-

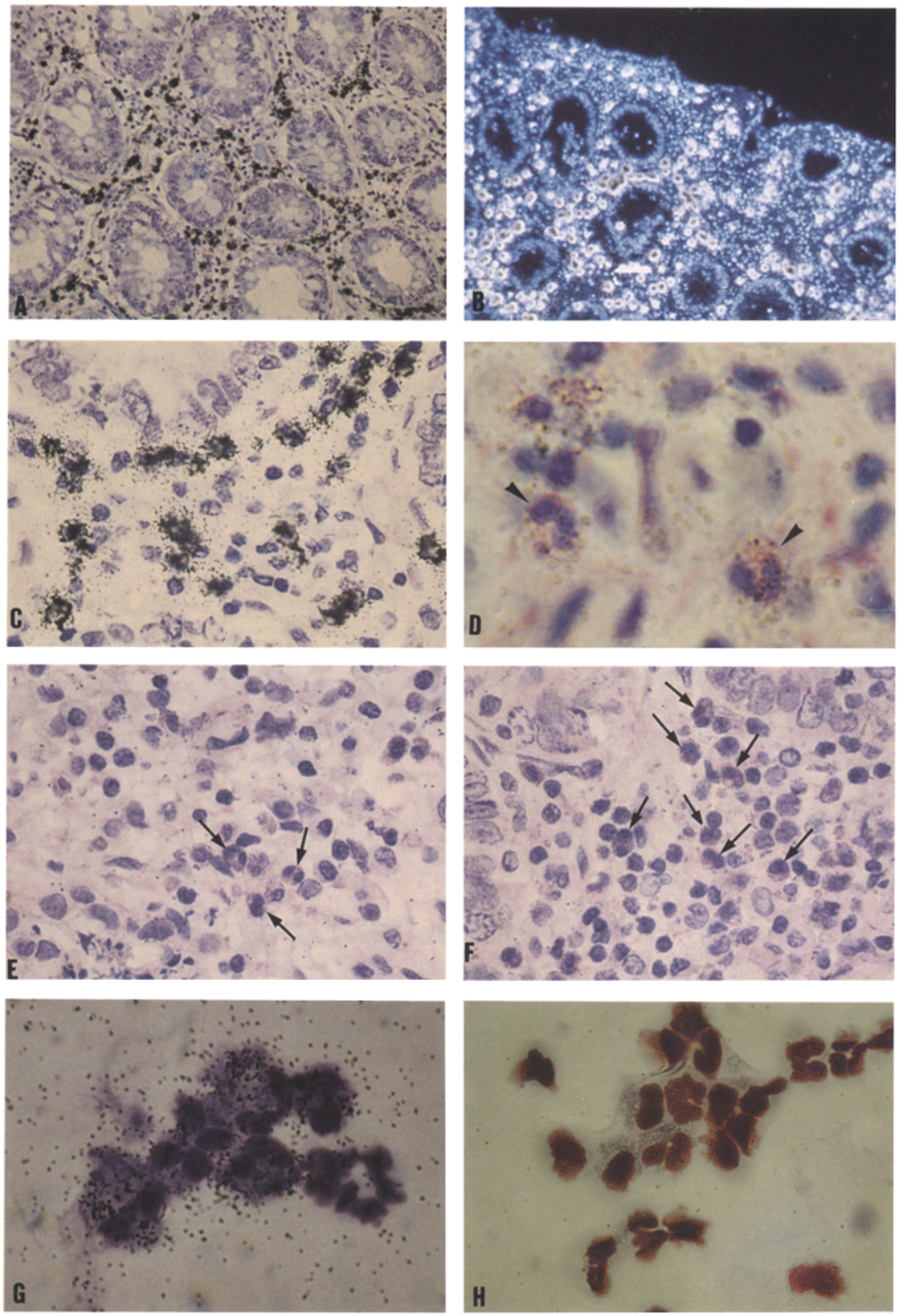

Figure 1. Detection of IL-5 mRNA in human eosinophils by in situ hybridization. (*A–F*) Sections of the duodenojejunal mucosa of a patient with coeliac disease. (*A–D*) Positive in situ hybridization with a ^{35}S-labeled antisense IL-5 RNA probe. Exposure time was 11 d, except for *D* (7 d). (*B*) Dark-field illumination. (*C*) At a higher magnification, note the presence of positively labeled cells with bilobed nuclei. (*E*) Negative hybridization signal with the IL-5 antisense probe from the same patient after treatment. (*F*) Negative control with the sense IL-5 probe. Arrows indicate eosinophils. (*G* and *H*) Cytopreparations of purified blood eosinophils from a patient with hypereosinophilic syndrome. (*G*) Strong hybridization signal with the IL-5 antisense probe on the six eosinophils. (*H*) Similar preparation treated with the IL-5 sense probe.

ulation with calcium ionophore or ionomycine (20, 21). Our data indicate that tissue eosinophils from patients with CD spontaneously express IL-5 mRNA in a defined pathological situation. In addition to their inflammatory and effector functions, eosinophils may thus serve as a source of growth and regulatory factors having a broad range of biologic effects.

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References

1. Yamaguchi, Y., T. Suda, J. Suda, M. Eguchi, Y. Miura, N. Harada, A. Tominaga, and K. Takatsu. 1988. Purified interleukin 5 supports the terminal differentiation and proliferation of murine eosinophilic precursors. *J. Exp. Med.* 167:43.
2. Yamaguchi, Y., Y. Hayashi, Y. Miura, T. Kasahara, S. Kitamura, M. Torisu, S. Mita, A. Tominaga, K. Takatsu, and T. Suda. 1988. Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs in vitro survival. *J. Exp. Med.* 167:1737.
3. Lopez, A.F., C.J. Sanderson, J.R. Gamble, H.D. Campbell, I.G. Young, and M.A. Vadas. 1988. Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J. Exp. Med.* 167:219.
4. Beagley, K.W., J.H. Eldridge, F. Lee, H. Kiyono, M.P. Everson, W.J. Koopman, T. Hirano, T. Kishimoto, and J.R. McGhee. 1989. Human and murine interleukin 6 induce high rate IgA secretion in IgA-committed B cells. *J. Exp. Med.* 169:2133.
5. Pockley, A.G., and P.C. Montgomery. 1991. In vivo adjuvant effect of interleukins 5 and 6 on rat tear IgA antibody responses. *Immunology.* 73:19.
6. Clutterbuck, E., J.G. Shields, J. Gordon, S.H. Smith, A. Boyd, R.E. Callard, H.D. Campbell, I.G. Young, and C.J. Sanderson. 1987. Recombinant human interleukin 5 is an eosinophil differentiating factor but has no activity in standard human B cell growth factor assays. *Eur. J. Immunol.* 17:1743.
7. Marsh, M.N., and J. Hinde. 1985. Inflammatory component of celiac sprue mucosa. 1. Mast cells, basophils, and eosinophils. *Gastroenterology.* 89:92.
8. Colombel, J.F., F. Mascart-Lemone, J. Nemeth, J.P. Vaerman, C. Dive, and J.C. Rambaud. 1990. Jejunal immunoglobulin and antigliadin antibody secretion in adult coeliac disease. *Gut.* 31:1345.
9. Kay, A.B., S. Ying, V. Varney, M. Gaga, S.R. Durham, R. Moqbel, A.J. Wardlaw, and Q. Hamid. 1991. Messenger RNA expression of the cytokine gene cluster, interleukin 3 (IL-3), IL-4, IL-5, and granulocyte/macrophage colony-stimulating factor, in allergen-induced late-phase cutaneous reactions in atopic subjects. *J. Exp. Med.* 173:775.
10. Hamid, Q., M. Azzawi, S. Ying, R. Moqbel, A.J. Wardlaw, C.J. Corrigan, B. Bradley, S.R. Durham, J.V. Collins, P.K. Jeffery, D.J. Quint, and A.B. Kay. 1991. Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J. Clin. Invest.* 87:1541.
11. Prin, L., M. Capron, A.B. Tonnel, O. Bletry, and A. Capron. 1983. Heterogeneity of human peripheral blood eosinophils: variability in cell density and cytotoxic ability in relation to the level and the origin of hypereosinophilia. *Int. Arch. Allergy Appl. Immunol.* 72:336.
12. Peuchmaur, M., D. Emilie, M.C. Crevon, P. Solal-Celigny, M.C. Maillot, G. Lemalgre, and P. Galanaud. 1990. IL-2 mRNA expression in Tac-positive malignant lymphomas. *Am. J. Pathol.* 136:383.
13. Wong, D.T.W., P.F. Weller, S.J. Galli, A. Elovic, T.H. Rand, G.T. Gallagher, T. Chiang, M.Y. Chou, K. Matossian, J. McBride, and R. Todd. 1990. Human eosinophils express transforming growth factor α. *J. Exp. Med.* 172:673.
14. Tai, P.C., C.J.F. Spry, C. Peterson, P. Venge, and I. Olsson. 1984. Monoclonal antibodies distinguish between storage and secreted forms of eosinophil cationic protein. *Nature (Lond.).* 308:182.
15. Plaut, M., J.H. Pierce, C.J. Watson, J. Hanley-Hyde, R.P. Nordan, and W.E. Paul. 1989. Mast cell lines produce lymphokines in response to cross-linkage of Fc ER1 or to calcium ionophores. *Nature (Lond.).* 339:64.
16. Samoszuck, M., and L. Nansen. 1990. Detection of interleukin-5 messenger RNA in Reed-Sternberg cells of Hodgkin's disease with eosinophils. *Blood.* 1:13.
17. Colombel, J.F., A. Janin, and G. Torpier. 1990. Activated eosinophils in coeliac disease. *Gut.* 31:583.
18. Chihara, J., J. Plumas, V. Gruart, J. Tavernier, L. Prin, A. Capron, and M. Capron. 1990. Characterization of a receptor for interleukin 5 on human eosinophils: variable expression and induction by granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 172:1347.
19. Walsh, G.M., A. Hartnell, A.J. Wardlaw, K. Kurihara, C.J. Sanderson, and A.B. Kay. 1990. IL-5 enhances the in vitro adhesion of human eosinophils, but not neutrophils, in a leucocyte integrin (CD11/18)-dependent manner. *Immunology.* 71:258.
20. Moqbel, R., Q. Hamid, S. Ying, J. Barkans, A. Hartnell, A. Tsicopoulos, A.J. Wardlaw, and A.B. Kay. 1991. Expression of mRNA and immunoreactivity for the Granulocyte/macrophage colony-stimulating factor in activated human eosinophils. *J. Exp. Med.* 174:749.
21. Kita, H., T. Ohnishi, Y. Okubo, D. Weiler, J.S. Abrams, and G.J. Gleich. 1991. Granulocyte/macrophage colony-stimulating factor and interleukin 3 release from human peripheral blood eosinophils and neutrophils. *J. Exp. Med.* 174:745.