Human Eosinophils Express Transforming Growth Factor α

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Summary

Transforming growth factor α (TGF-α) is a pleuripotential cytokine with diverse biological effects, including the ability to influence the proliferation of normal cells or neoplastic epithelial cells. Eosinophils are a subset of granulocytes that normally enter the peripheral tissues, particularly those beneath gastrointestinal, respiratory, and urogenital epithelium, where they reside in close proximity to the epithelial elements. In this study, we demonstrate that the great majority of eosinophils infiltrating the interstitial tissues adjacent to two colonic adenocarcinomas and two oral squamous cell carcinomas labeled specifically by in situ hybridization with a ^{35}S-riboprobe for human TGF-α (hTGF-α). No other identifiable leukocytes in these lesions contained detectable hTGF-α mRNA. We also examined leukocytes purified from a patient with the idiopathic hypereosinophilic syndrome. 80% of these eosinophils, but none of the patient's neutrophils or mononuclear cells, were positive for hTGF-α mRNA by in situ hybridization, and 55% of these eosinophils were positive by immunohistochemistry with a monoclonal antibody directed against the COOH terminus of the mature hTGF-α peptide. Finally, the identification of the purified eosinophil-associated transcript as hTGF-α was confirmed by polymerase chain reaction product restriction enzyme analysis followed by Southern blot hybridization. In contrast to eosinophils from the patient with hypereosinophilic syndrome, the peripheral blood eosinophils from only two of seven normal donors had detectable TGF-α mRNA and none of these eosinophils contained immunohistochemically detectable TGF-α product. Taken together, these findings establish that human eosinophils can express TGF-α, but suggest that the expression of TGF-α by eosinophils may be under microenvironmental regulation. Demonstration of TGF-α production by tissue-infiltrating eosinophils and the eosinophils in the hypereosinophilic syndrome identifies a novel mechanism by which eosinophils might contribute to physiological, immunological, and pathological responses.

Transforming growth factor α (TGF-α) is a 50 amino acid peptide that was initially discovered as an activity in retrovirus-transformed cells that can compete with epidermal growth factor (EGF)[1] for binding to the EGF-receptor (1). Most malignant epithelial tumors have been found to express TGF-α (2), which may contribute to the transformed phenotypes of these cells through autocrine mechanisms (3). More recently, TGF-α has been demonstrated in certain normal tissues and cell types as well, including normal human skin keratinocytes (4, 5), bovine anterior pituitary cells (6), rat material decidua (7), mouse blastocysts (8), and activated macrophages (9, 10). These findings suggest that TGF-α is not only involved in neoplasms but may also contribute to interactions among normal cells, possibly through autocrine and/or paracrine mechanisms (11).

The studies described herein were prompted by an unexpected observation made during an investigation of mRNA for the cytokine TGF-α in experimental oral carcinomas in Syrian hamsters (12, 13). When in situ hybridization was used to identify the cellular localization of TGF-α mRNA in these lesions, we found that a significant fraction of the

[1] *Abbreviations used in this paper:* DAPI, 4',6-diamidino-2-phenylindole; EGF, epidermal growth factor; h, human; mRNA, messenger RNA.

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eosinophils infiltrating the oral carcinomas were specifically labeled (Elovic, A., S. J. Galli, P. F. Weller, et al., unpublished data). Eosinophils are a distinct lineage of granulocytes that arise in the bone marrow, circulate in the blood, and emigrate into peripheral tissues (14). Although eosinophils normally represent only ~3% of circulating leukocytes in humans, large numbers of eosinophils ordinarily reside in certain tissues. In fact, the peripheral tissues rather than the blood and bone marrow contain the majority of mature eosinophils, which are especially abundant near the mucosal surfaces of gastrointestinal, respiratory, and genitourinary tracts (14). Moreover, greatly increased numbers of eosinophils appear in the blood and tissues in association with a variety of immune responses or disease processes (14, 15). In some of these responses, such as immune reactions to helminthic parasites, eosinophils function as effectors of host defense (16). In other settings, eosinophil-derived mediators probably contribute to the pathogenesis of disease (15). In yet other reactions, such as host responses to neoplasms (17–21), the specific role of the eosinophil remains obscure.

Because TGF-α is a multifunctional cytokine that has been implicated in the regulation of proliferation of both normal and neoplastic cells (3), as well as in other processes such as angiogenesis (22), we believed that it would be of interest to determine whether human eosinophils could elaborate this cytokine. We therefore used in situ hybridization to search for the expression of TGF-α mRNA in eosinophils infiltrating the interstitial tissues adjacent to human colonic or oral carcinomas. We also used in situ hybridization, Northern blotting, PCR product restriction enzyme analysis, RIA, and immunohistochemistry to demonstrate TGF-α mRNA and product in eosinophils from patients with the idiopathic hypereosinophilic syndrome.

Materials and Methods

Eosinophil-rich Human Tumors. Four freshly resected human colonic adenocarcinomas and 10 oral carcinomas were obtained from the Pathology Department at the Beth Israel Hospital, and the Department of Oral and Maxillofacial Surgery at the Massachusetts General Hospital, respectively. They were immediately fixed in freshly prepared 4% paraformaldehye and then processed for paraffin embedding. Representative sections from each of these tumors were examined for the presence of eosinophils in the stroma. Two of the colonic adenocarcinomas and two of the oral carcinomas contained many stromal eosinophils. These four eosinophil-rich human tumor specimens were used for in situ hybridization studies.

Human Eosinophil Isolation. Granulocytes were isolated from the sodium citrate anticoagulated blood of patients with idiopathic hypereosinophilic syndrome by dextran sedimentation, centrifugation through Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, NJ), and hypotonic lysis of erythrocytes (23). Unfractionated granulocytes were used for the in situ hybridization and immunohistochemistry studies. For PCR, Northern blot analyses, and TGF-α RIA, granulocytes were enriched for eosinophils by sequential incubation at 4°C with the anti-CD-16 mAb Leu11a (Becton Dickinson & Co., Mountain View, CA) and magnetic beads conjugated with goat anti-mouse IgG (Advanced Magnetics, Cambridge, MA) to deplete $CD16^+$ neutrophils. Leukocytes were also isolated by dextran sedimentation from the peripheral blood of seven healthy male donors, aged 27–44 yr. These preparations consisted of 1–5% eosinophils, 52–83% neutrophils, and 16–55% mononuclear cells.

Cell Culture. The TGF-α–producing human A431 epidermoid carcinoma cell line was maintained at 37°C, 5% CO_2 in DMEM medium supplemented with 10% FCS and antibiotics (penicillin 100 U/ml, streptomycin 100 μg/ml, amphotericin B 0.25 μg/ml) (Gibco Laboratories, Grand Island, NY).

RNA Isolation and Northern Blot Hybridization. Total RNA was isolated from human eosinophils and A431 cells using the guanidine isothiocyanate method described by Davis et al. (24). Details of Northern blot analysis using the Zetabind membrane were described previously (25). Random priming was used to label the cDNA inserts.

Molecular Probes. hTGF-α cDNA is a 0.9-kb EcoRI fragment obtained from G.I. Bell of Chiron Corp. (Emeryville, CA). The chicken β-actin cDNA is a 1.7-kb PstI fragment obtained from D.W. Cleveland, San Francisco, CA (26).

TGF-α mRNA Phenotyping. We used the mRNA phenotyping procedure of Rappolee et al. (10). A primer-extended hTGF-α–specific cDNA was constructed using a 21-mer hTGF-α–specific primer (3′-CGACGGTGAGTCTTTGTCACC-5′) from nucleotide position 492 to 512 according to the numbering system by Derynck et al. (27). The resultant cDNA was analyzed for the presence of hTGF-α sequences by the use of two hTGF-α–specific primers (5′ primer: 22-mer 5′-GGCCTTGGAGAACAGCACGTCC-3′ from nucleotide position 97 to 118; 3′ primer: 20-mer 3′ GCTCTTCGGGTCGCGGGAGG-5′ from nucleotide position 454 to 473), flanking the coding sequence of the mature TGF-α (nucleotide position 151 to 300). The 3′ primer is 19-bp upstream to the primer used for the cDNA library construction. The expected size of the hTGF-α PCR product is 377 bp.

In Situ Hybridization. Details of our in situ hybridization procedure using the antisense and sense hTGF-α riboprobes were described previously (13). Immediately following isolation, blood leukocytes or purified human eosinophils were embedded into 1% agar in 1× PBS and then fixed, processed, and embedded in paraffin as previously described (13).

Two reports indicate that under some conditions of hybridization, eosinophils can nonspecifically bind DNA (28) or RNA (29) probes used for in situ hybridization. We therefore performed preliminary experiments to define experimental conditions allowing the specific labeling of eosinophil mRNA by in situ hybridization. We found that hybridization at temperatures of <50°C and/or durations of <12 h resulted in nonspecific labeling of eosinophils. However, such nonspecific binding did not occur when sections of paraformaldehyde-fixed, paraffin-embedded tissues were incubated in a prehybridization solution containing S-UTP–labeled cold probe for at least 2 h at 42°C, and when RNase A and T1 were used in posthybridization washes. For the work reported here, sections were prehybridized for 2 h at 42°C in the presence of S-UTP–labeled pGEM3 single-stranded RNA. Hybridization was at 50°C for 12 h.

Immunohistochemistry. Blood leukocytes or purified eosinophils were prepared as described for in situ hybridization. The paraffin-embedded sections (6 μm) were subjected to staining for hTGF-α protein using a mAb directed against the COOH terminus (residues 34–50) of the mature 50 amino acid hTGF-α peptide (TGF-α: Ab-2; GF-10; Oncogene Science, Manhasset, NY). 12 μg/ml of the mAb was used to stain each section. A mAb to the bacterial protein β-galactosidase (Ab-1; OBO2; Oncogene Science) at the same concentration was used as a negative control. The Vectastain ABC-alkaline phosphatase (mouse IgG AK-5002) and alkaline phosphatase substrate I (SK-5100) (Vector Laboratories, Burlingame, CA)

in the presence of 1.25 mM of levamisole (SP-5000) were used for signal detection. All sections were stained with 0.2% aniline blue for 10 min to identify eosinophils (30).

TGF-α RIA. A TGF-α RIA kit from Biotope (Redmond, WA) was used to determine the presence of immunoreactive TGF-α in the supernatants from eosinophils of hypereosinophilic donors. The immunogen was a synthetic peptide conjugate of the COOH-terminal 17 residues (34–50) of rat TGF-α. Tracers and standards are made from bioactive synthetic rat TGF-α. The assay does not cross-react with rodent or human EGF. Appropriate controls indicated that the concentrated supernatants did not affect assay of 5 nM TGF-α standard or alter nonspecific binding (0.8%).

Results

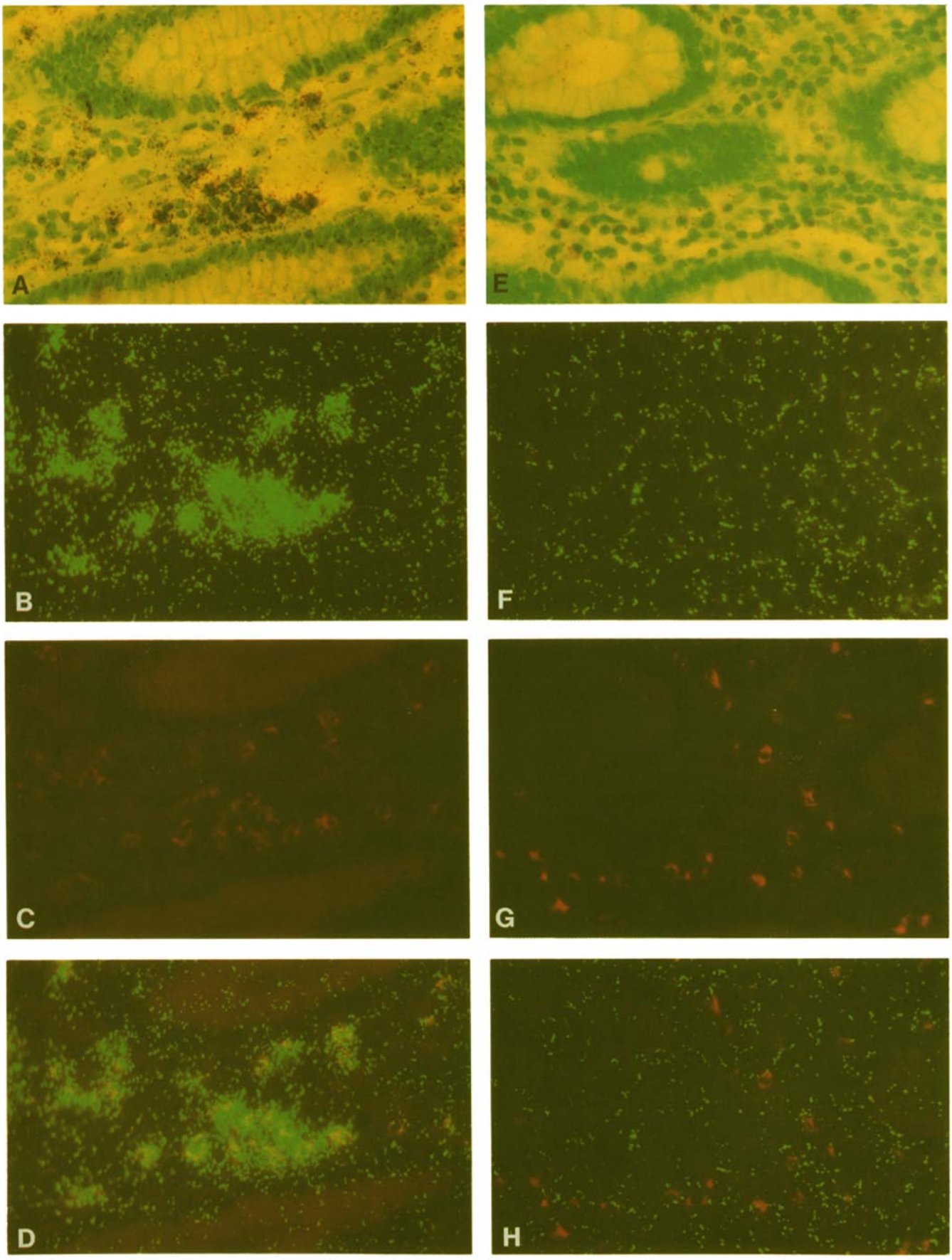
Eosinophils in Human Carcinomas Contain TGF-α mRNA. Our finding that eosinophils infiltrating into developing hamster oral carcinomas express TGF-α prompted us to determine if eosinophils infiltrating into human cancer could elaborate this cytokine. It is well known that human colonic adenocarcinomas and oral carcinomas are frequently associated with eosinophil-rich inflammatory infiltrates (18–21). Four freshly resected human colonic adenocarcinomas and 10 human oral squamous cell carcinomas were obtained. Two tumors from each of these anatomical sites contained many stromal eosinophils. These four specimens were used for in situ hybridization studies.

Eosinophils in the connective tissue adjacent to each of the four human tumors hybridized specifically with the ^{35}S-labeled antisense hTGF-α riboprobe, whereas hybridization of contiguous sections with the ^{35}S-labeled sense hTGF-α riboprobe demonstrated no specific labeling. Fig. 1, *A–H*, shows the in situ TGF-α mRNA labeling results from one of the two eosinophil-rich human colonic adenocarcinoma specimens. *A* to *D* are photomicrographs taken from a section of normal mucosa adjacent to the colonic adenocarcinoma hybridized with the ^{35}S-labeled antisense hTGF-α riboprobe. *A* is the bright-field view of this tumor demonstrating cells in the lamina propria labeling prominently with the ^{35}S-labeled antisense hTGF-α riboprobe. The identity of these labeled cells cannot be easily recognized from this photomicrograph. Fig. 1 *B* is the same field photographed with dark-field illumination and a green filter highlighting the autoradiographic grains. To define further the identity of the labeled cells, we exploited the fact that the eosinophilic cytoplasmic granules exhibit an autofluorescence that is much more intense than that of any other nucleated cell (31). We found that human eosinophils stained with a Fisher Giemsa stain and viewed with rhodamine filters at 552 nm exhibited an especially brilliant red fluorescence, as shown in Fig. 1 *C*. Fig. 1 *D* is a composite exposure demonstrating that most of the cell-hybridized TGF-α–specific autoradiographic grains were localized to the fluorescent cells. Analysis of such preparations indicated that ~90% of infiltrating eosinophils labeled with the ^{35}S-labeled antisense hTGF-α riboprobe. *E–H* show a contiguous section of the same specimen hybridized with the ^{35}S-labeled sense hTGF-α riboprobe. No specific autoradiographic signals are localized to any of the cells in this section.

TGF-α mRNA Phenotyping and Characterization in Purified Human Eosinophils from a Hypereosinophilic Patient. An hTGF-α–specific, primer-extended cDNA library was constructed by the mRNA phenotyping procedure (10), using total RNA isolated from a purified human eosinophil preparation (74% eosinophils and 26% neutrophils). Total RNA from the human epidermoid carcinoma A431 cell line, which is known to contain TGF-α mRNA, was used as a positive control (2). The resultant cDNA libraries were subjected to the PCR to search for TGF-α sequences. The two primers specific for human TGF-α used for the PCR reaction flank the coding sequence of the mature TGF-α and should produce a 377-bp PCR product if authentic human TGF-α cDNA is present. Lanes *1* and *7* in Fig. 2 demonstrate that a ~377-bp PCR product was detected in both the human eosinophil and A431 cells. The negative control lanes *4–6* contain all the reagents except for the starting human eosinophil or A431 cell RNA templates. To demonstrate the specificity of the PCR product, we used the fact that the human TGF-α sequence has a PvuII site 37 bp upstream from the 3′ end of the 377-bp PCR product (27). Upon subjecting the respective PCR products to a PvuII cut, we observed a slight but definite decrease in size of the parental band to ~340 bp (the other, 72-bp product is too small to be resolved discretely by the 2% agarose gel used). This finding supports the identification of the TGF-α PCR product as derived from human TGF-α. We next subjected the PCR products to a PstI digestion because there are two PstI sites within the coding sequence for mature TGF-α (27). PstI digestion of the 377-bp hTGF-α–PCR product therefore should give rise to three fragments with sizes of 52, 67, and 258 bp. Lanes *3* and *9* in Fig. 2 demonstrate the appearance of the predicted 258-bp PstI-digested fragments in both the HE and A431 samples (the 52- and 67-bp PstI digested fragments are too small to be resolved discretely by the 2% agarose gel used). Subsequent Southern blot hybridization with a ^{32}P-labeled human TGF-α cDNA revealed that the parental, PvuII-digested, and PstI-digested PCR products in both human eosinophils and A431 cells hybridized specifically at the expected locations (data not shown). These results demonstrate both the presence of human TGF-α mRNA in the preparation of purified human eosinophils and that the coding sequence of the mature cytokine is part of the expressed sequence.

Detection of TGF-α mRNA in Human Eosinophils by Northern Blotting. We then determined whether specific TGF-α mRNA sequences could be detected by Northern blot hybridization in total RNA derived from the same human eosinophil preparation used for PCR analysis. Using a ^{32}P-labeled human TGF-α cDNA probe, a TGF-α hybridizable RNA band was detected at ~4.5 kb (Fig. 3), identical in size to the TGF-α hybridizable RNA band in the A431 cells (2). Hybridization of the same blot with a ^{32}P-labeled chicken β-actin cDNA revealed that approximately the same amount of RNA had been loaded onto each lane and that the quality of the isolated RNA was satisfactory.

Localization of TGF-α mRNA to Human Eosinophils by In Situ Hybridization. Although human eosinophils can be isolated in purities of >75%, residual contamination by other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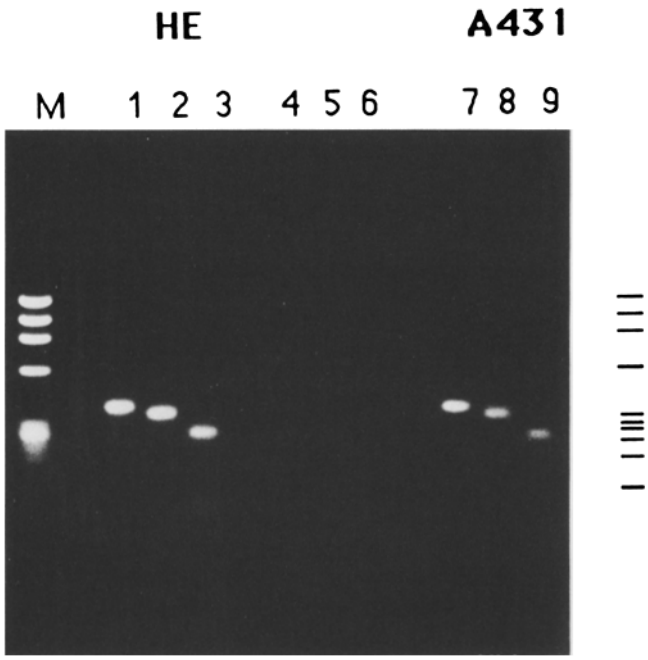

Figure 2. Human TGF-α mRNA phenotyping and characterization of total RNA isolated from a human eosinophil preparation (see text). (Lanes *1–3*) *HE*, human eosinophils; (lanes *4–6*) negative control (no RNA templates); (lanes *7–9*) A431, human epidermoid carcinoma cell line. (Lanes *1*, *4*, and *7*) PCR products of respective samples. (Lanes *2*, *5*, and *8*) PvuII digestion of respective PCR products. (Lanes *3*, *6*, and *9*) PstI digestion of respective PCR products. *M*, φχ-174 HaeIII-digested molecular weight DNA markers electrophoresed at the same time on this 2% agarose gel (from top to bottom: 1,353, 1,078, 872, 603, 310, 281, 271, 234, 194, 118, and 72 bp).

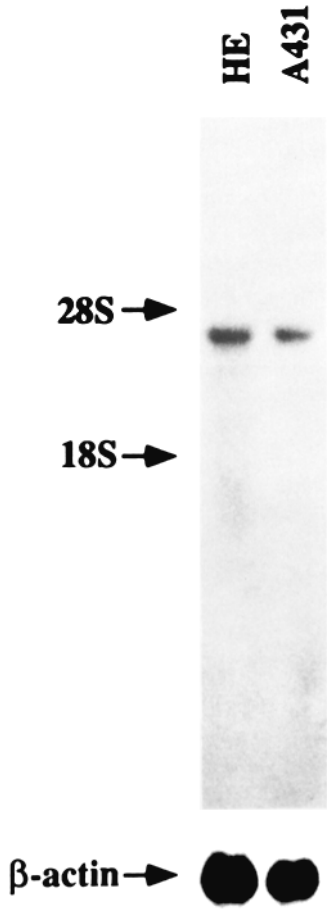

Figure 3. Detection of TGF-α transcripts in preparations of human eosinophils (*HE*) and human A431 epidermoid carcinoma cells. 12 μg of total RNA was loaded onto each lane. The full-length blot was hybridized to a ^{32}P-labeled hTGF-α cDNA. The size of the detected TGF-α hybridizable bands is ~4.5 kb. The arrows labeled 18S (~2 kb) and 28S (~5 kb) show the positions of ribosomal RNA markers that were electrophoresed at the same time. The same blot was rehybridized to a ^{32}P-labeled chicken β-actin shown in the lower blot. The size of the detected human β-actin transcript is ~2 kb.

granulocytes (almost exclusively neutrophils) is a common finding. The human eosinophil preparation used for PCR and Northern blot analyses contained 74% eosinophils, with neutrophils representing the only identifiable contaminating cells. To localize precisely the cellular source(s) of the TGF-α mRNA detected in the human eosinophil preparation, we performed in situ hybridization on unfractionated blood leukocytes from the same patient (Fig. 4). This preparation contained 56% eosinophils, 29% neutrophils, 15% mononuclear cells. 80% of the eosinophils hybridized with the ^{35}S-labeled antisense hTGF-α riboprobe. No neutrophils, monocytes, or lymphocytes were detectably labeled with this probe. No cells were seen labeled with the ^{35}S-labeled sense hTGF-α riboprobe on contiguous sections. *A–D* in Fig. 4 are from a Giemsa-stained section demonstrating typical hybridization with a ^{35}S-labeled antisense hTGF-α riboprobe. The bright-field photomicrograph in Fig. 4 *A* shows two cells with bi- or multi-lobulated nuclei. The one with eosinophilic cytoplasm (*upper right*) was prominently labeled with the riboprobe, which is especially apparent in the dark-field photomicrograph viewed with a green filter (Fig. 4 *B*). Giemsa fluorescence was used to confirm the identification of eosinophils (Fig. 4 *C*). Notice that only the cell in the upper right exhibits fluorescence. A double exposure simultaneously demonstrating fluorescence and in situ hybridization labeling confirms that the labeled cell is an eosinophil (Fig. 4 *D*). By morphology, the unlabeled cell is a neutrophil. *E–H* show a similar section hybridized to the control ^{35}S-labeled sense hTGF-α riboprobe. The two cells in the field have eosinophilic cytoplasm and exhibit fluorescence, but neither labeled with the [^{35}S]hTGF-α sense riboprobe. These findings demonstrate the specificity of our in situ hybridization results identifying TGF-α mRNA in human eosinophils.

Detection of TGF-α Protein in Human Eosinophils by Immunohistochemistry. To determine if TGF-α mRNAs detected in eosinophils were translated into TGF-α protein, immuno-

Figure 1. Detection of TGF-α mRNA in human eosinophils associated with colonic adenocarcinoma by in situ hybridization. (*A* and *D*) Section of normal colonic mucosa immediately adjacent to the adenocarcinoma hybridized to a ^{35}S-labeled antisense hTGF-α riboprobe. (*E–H*) hybridized to a ^{35}S-labeled sense hTGF-α riboprobe. Exposure time was for 48 h at 4°C. Original magnification ×12. (*A* and *E*) Bright-field visualization. (*B* and *F*) Dark-field visualization highlighting the autoradiography using a green filter. Notice a nonspecific faint green fluorescence from all of the cells present. (*C* and *G*) Fluorescence visualization using rhodamine filters demonstrating the fluorescence of human eosinophils. (*D* and *H*) Double exposure visualization, first with dark-field followed by rhodamine fluorescence.

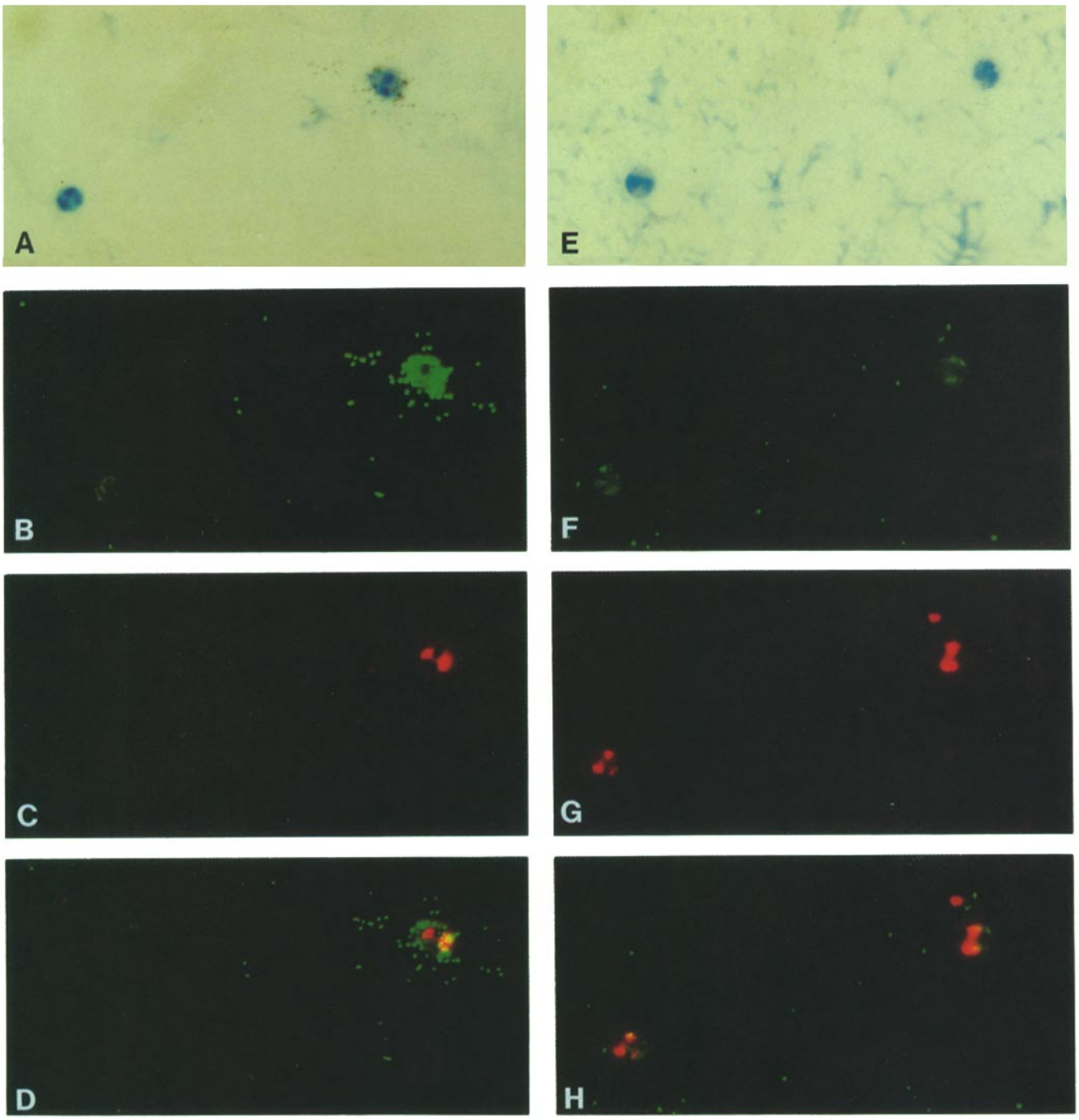

Figure 4. Localization of cellular sources of TGF-α transcripts in the human eosinophil preparation (see text). (*A* to *D*) Hybridized to a ^{35}S-labeled antisense hTGF-α riboprobe. (*E* to *H*) Hybridized to a ^{35}S-labeled sense hTGF-α riboprobe. Exposure time was for 48 h at 4°C. Original magnification, ×500. (*A* and *E*) bright-field visualization. (*B* and *F*) Dark-field visualization using a green filter to highlight the autoradiographic grains. Notice a nonspecific faint green fluorescence associated with all of the cells. (*C* and *G*) Fluorescence visualization using rhodamine filter demonstrating fluorescence in the eosinophils. (*D* and *H*) Double exposure visualization, first with dark-field followed by rhodamine fluorescence.

histochemistry was performed on paraffin-embedded sections of human eosinophil preparation using a mAb directed against the COOH terminus (residues 34–50) of the 50 amino acid mature sequence of hTGF-α. All sections were counterstained with a 0.2% aniline blue solution. Eosinophil cytoplasmic granules stained with this dye emit a blue fluorescence when viewed with a DAPI filter at 365 nm (30). Moreover, we find that the aniline blue fluorescence of eosinophil cytoplasmic

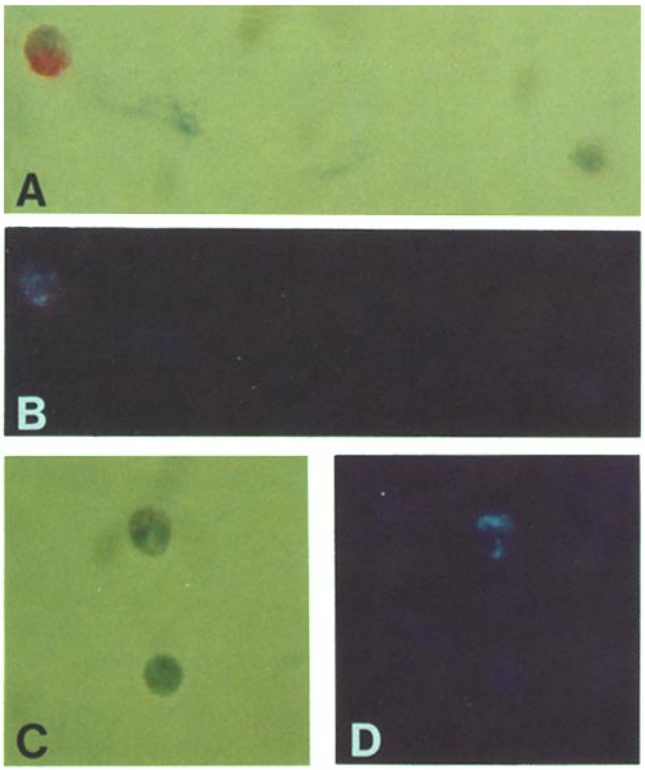

Figure 5. Immunohistochemical detection of hTGF-α protein in human eosinophils. (*A* and *B*) stained with a hTGF-α mAb at 12 μg/ml. (*C* and *D*) stained with a control (bacterial β-galactosidase) mAb at 12 μg/ml. Magnification ×500. (*A* and *C*) Bright-field visualization. (*B* and *D*) Visualization of aniline blue fluorescence of eosinophils by fluorescent microscopy at 365 nm with DAPI filter. Only the eosinophils demonstrate staining with the hTGF-α mAb. Eosinophils do not stain with the control mAb.

granules is not abolished by the red substrate staining of our immunohistochemical procedure. As a result, both procedures can be used on the same preparation to determine specifically whether eosinophils contain detectable TGF-α protein.

Approximately 55% of all aniline blue fluorescent eosinophils were stained by the hTGF-α mAb. No nonfluorescent cells (i.e., neutrophils and mononuclear cells) were detectably stained by the hTGF-α mAb. Staining of similar preparations with the control mAb (bacterial β-galactosidase) resulted in no detectable staining of eosinophils or any other cells. *A* and *B* in Fig. 5 show a section stained with the hTGF-α mAb detected with an alkaline phosphatase method. Fig. 5 *A* is a bright-field photomicrograph showing that one of the two cells in the field is positive for the red reaction product. Viewing the same field with DAPI filters at 365 nm (Fig. 5 *B*) clearly shows that only the hTGF-α mAb positive cell (on the left) exhibits blue fluorescence. This finding establishes the identity of the TGF-α positive cell as an eosinophil. *C* and *D* in Fig. 5 show a similar section containing two cells with bi- or multi-lobulated nuclear morphology stained with the control mAb (bacterial β-galactosidase). Fig. 5 *C* shows that no immunohistochemical positivity was observed in either of these cells. Fig. 5 *D* shows that one of the two cells exhibits aniline blue fluorescence and is thus an eosinophil.

Assay of TGF-α Activity in Supernatants of Human Eosinophils. We investigated whether eosinophils released detectable TGF-α peptide by using a commercial TGF-α RIA kit (Biotope, Redmond, WA). Eosinophils from two patients with hypereosinophilic syndrome (purified to 62 and 100%) were cultured at 5×10^5 cells/ml in RPMI 1640 for 18 h. Cell-free supernatants were concentrated against an Amicon YM-2 membrane and assayed for TGF-α by the TGF-α RIA kit. The amounts of TGF-α in the two supernatants were equivalent to 0.19 and 0.08 nM of rat TGF-α. These data indicate that eosinophils purified from patients with the hypereosinophilic syndrome release TGF-α when cultured in vitro.

TGF-α mRNA and Product in Eosinophils from Healthy Donors. For each of the seven normal donors, 400 peripheral blood leukocytes, containing 1–5% eosinophils, were examined for TGF-α mRNA and product, exactly as described above for the cells of the hypereosinophilic patient. None of the eosinophils, nor any other leukocyte in these specimens, exhibited detectable TGF-α product by immunohistochemistry (data not shown). When in situ hybridization results were examined in specimens exposed for 24 h, under conditions in which TGF-α mRNA was detected in 80% of the eosinophils from the patient with the hypereosinophilic syndrome, no eosinophils or other cells were positive for TGF-α mRNA. When the exposure was extended to 7 d, TGF-α mRNA positive eosinophils were detected in two of the seven preparations. In these two specimens, TGF-α mRNA-positive eosinophils accounted for 67 and 80% of the total eosinophils identified in the same preparation by Giemsa-enhanced fluorescence (data not shown). No cell type other than the eosinophil was positive for TGF-α mRNA after 7-d exposure of all seven preparations (data not shown).

Discussion

This report presents several lines of evidence demonstrating that human eosinophils can express TGF-α. TGF-α mRNA was detected by in situ hybridization in the eosinophils infiltrating interstitial tissues adjacent to each of the four different carcinomas tested (two adenocarcinomas of the colon, two squamous cell carcinomas of the oral cavity). To confirm the results obtained with tissue eosinophils, we examined eosinophils isolated from a patient with the hypereosinophilic syndrome. By Northern blotting, purified eosinophils exhibited a TGF-α hybridizable RNA band of the same size (~4.5 kb) as that detected in the human A431 carcinoma cell line, a population known to contain TGF-α mRNA (2). The identification of the eosinophil-associated transcript as TGF-α was confirmed by PCR product restriction enzyme analysis followed by Southern blot hybridization. Using supernatants of eosinophils purified from two patients with the hypereosinophilic syndrome, we detected TGF-α by RIA. Comparable amounts were detected by an EGF receptor radioassay (data not shown). Finally, the potential cellular sources

of TGF-α in the purified human eosinophil preparations were assessed by in situ hybridization and by immunohistochemistry with a mAb specific for the COOH terminus of the mature hTGF-α peptide. By either in situ hybridization or immunohistochemistry, only eosinophils were positive for TGF-α mRNA or product.

The detection of TGF-α cytokine expression in human eosinophils is a novel finding. Examination of human hematopoietic cell lines, including the HL-60 promyelocytic leukemia cell line, did not reveal detectable levels of TGF-α mRNA (2). However, Gottlieb et al. (5), using a mAb (mAb A1.5) against human TGF-α, detected TGF-α in cells in the dermal infiltrates associated with inflammatory, hyperproliferative (psoriasis), and neoplastic skin diseases. Although the identity of the TGF-α positive cells was not determined, Gottlieb et al. suggested that they may have been macrophages, Langerhan's cells, and/or activated T cells.

In our specimens, eosinophils represented the only identifiable infiltrating leukocyte positive for TGF-α mRNA by in situ hybridization. Moreover, PCR restriction enzyme analysis, immunohistochemistry, and RIA suggest that eosinophils express the mature, biologically active, form of the cytokine. Eosinophils thus may represent a major nonepithelial source of TGF-α. On the other hand, it remains to be established whether the form of TGF-α expressed by eosinophils is fully identical in structure and pattern of biological activity to TGF-α derived from other sources.

We found that TGF-α mRNA was detectable in ~90% of tumor-associated eosinophils and in the majority (80%) of the eosinophils isolated from our patient with the idiopathic hypereosinophilic syndrome. By immunohistochemistry, 55% of the eosinophils purified from the hypereosinophilic patient were positive for TGF-α product. By contrast, none of eosinophils purified from the peripheral blood of normal donors exhibited detectable TGF-α product and most of them lacked detectable TGF-α mRNA. Indeed, no eosinophils positive for TGF-α mRNA were detectable in five of the seven normal donors. On the other hand, in the two donors who had peripheral blood leukocytes positive for TGF-α mRNA, all of the positive cells were eosinophils.

Thus, the majority of the peripheral blood eosinophils of normal donors, as well as subpopulations of eosinophils from the hypereosinophilic patient or in the infiltrates associated with carcinomas, lacked detectable expression of TGF-α. These findings raise the possibility that the expression of TGF-α by eosinophils is subject to microenvironmental regulation. This would not be surprising, in view of what is known about the expression of TGF-α or other pleuripotential cytokines by other cell types. For example, TGF-α expression can be detected in human alveolar macrophages stimulated with LPS, but not in the unstimulated cells (9). Similarly, the expression by mouse mast cells of mRNA or product for a wide variety of cytokines is markedly augmented when the cells are stimulated via the IgE receptor (32–35). Finally, human eosinophils have been shown to synthesize specific proteins upon appropriate stimulation in vitro. When eosinophils purified from nine donors were examined for HLA-DR expression by flow cytometry, the cells of eight donors lacked HLA-DR expression and only 23% of the eosinophils of the ninth donor exhibited the antigen. But when the eosinophils were tested after incubation in vitro with recombinant human granulocyte/macrophage colony-stimulating factor (rhGM-CSF) and 3T3 fibroblasts, HLA-DR expression was induced on eosinophils from each donor (23).

It will clearly be of interest to search for factors that influence TGF-α expression by eosinophils. While such studies will be important for identifying the specific signals regulating TGF-α expression by eosinophils, we already have demonstrated that TGF-α mRNA is present in the majority of eosinophils infiltrating four spontaneous neoplasms occurring in two distinct anatomical sites in four different patients. This finding indicates that the expression of TGF-α by tissue eosinophils may not be an unusual occurrence, and permits speculation about novel mechanisms by which eosinophils might affect epithelial proliferation during physiological or pathological responses.

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