IL-4-Dependent Regulation of TGF-α and TGF-β1 Expression in Human Eosinophils[1]

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TGFs play important roles in wound healing and carcinogenesis. We have previously demonstrated that eosinophils infiltrating into different pathologic processes elaborate TGF-α and TGF-β1. Eosinophils infiltrating hamster cutaneous wounds were found to express TGFs sequentially. In this study, we examined the biologic mediators that may regulate the expression of TGF-α and -β1 by eosinophils. Eosinophils were isolated from the peripheral blood of healthy donors and cultured in the absence or presence of IL-3, IL-4, and IL-5. Cells were analyzed by in situ hybridization and immunohistochemistry. Supernatants from these cultures were assayed for secreted TGF-α and TGF-β1 using TGF-specific ELISAs. IL-3, IL-4, and IL-5 independently up-regulated TGF-β1 mRNA and product expression by eosinophils in all donors. Interestingly, TGF-α production by eosinophils was up-regulated by IL-3 and IL-5 but was down-regulated by IL-4. Consistent with the ability of IL-4 to regulate eosinophil responses, IL-4 signaling molecules are present in human eosinophils. The observation that IL-4 can differentially regulate the expression of TGF-α and TGF-β1 suggests that IL-4 may serve as a physiologic molecular switch of TGF expression by the infiltrating eosinophils in wound healing and carcinogenesis. ***The Journal of Immunology,*** **1998, 160: 6121–6127.**

Eosinophils are bone marrow-derived granulocytes and are predominantly tissue-dwelling cells. They are activated by a range of mostly T cell-derived factors that, among other functions, can stimulate eosinophils to secrete their stored granule contents and increase their longevity in tissues. Although it has recently been demonstrated that eosinophils can express and elaborate many different cytokines, their roles in normal physiology as well as in eosinophil-associated disease processes remain uncertain.

We have demonstrated that eosinophils in oral carcinomas can synthesize TGF-α (1, 2). We also showed that eosinophils from the peripheral blood of patients with idiopathic hypereosinophilic syndrome produce TGF-α as well as TGF-β1, whereas eosinophils from the peripheral blood of healthy donors contain little or no detectable TGFs (2, 3). We have further shown that eosinophils infiltrating hamster cutaneous wounds express TGFs sequentially; eosinophils express TGF-α in the early stages of wound healing, while eosinophil-derived TGF-β1 is more pronounced during the postacute phase of this process (4). Thus, it is conceivable that specific mediators released within the microenvironment of normal and diseased tissues can regulate the production of TGFs by eosinophils.

TGFs are multifunctional cytokines with various biologic and pathologic actions, such as stimulatory and inhibitory effects on epithelial cell growth, induction of angiogenesis, extracellular matrix remodeling, and regulation of inflammatory processes (5–8). TGF production by eosinophils, therefore, may be important in carcinogenesis as well as inflammatory disorders and wound healing. The ability to control TGF production by eosinophils may therefore have therapeutic benefits. However, little is known about the mechanisms that activate and regulate the production of TGFs by eosinophils.

The present study demonstrates that the basal level of TGF expression by peripheral blood eosinophils can be enhanced by IL-3 and IL-5. Even more interesting is the finding that IL-4 can inhibit the synthesis of TGF-α and concurrently up-regulate the synthesis of TGF-β1 by peripheral blood eosinophils.

Materials and Methods

Subjects

Blood (200 ml) was obtained from healthy volunteers (28–34 years old). These subjects had no evidence by history and physical examination of asthma or other allergic diseases.

Human eosinophil isolation

Eosinophils were isolated from the peripheral blood of six healthy donors. Whole blood was subjected to dextran sedimentation (Macrodex, Pharmacia, Piscataway, NJ), centrifugation through Ficoll-Paque (Pharmacia), and hypotonic lysis of erythrocytes as described previously (2). Eosinophils were enriched from granulocytes and passage via the MACS system by sequential incubation at 4°C with anti-CD16 mAb magnetic beads (Miltenyi Biotec, Auburn, CA) to deplete $CD16^+$ neutrophils as described by Lim and Weller (9).

Cytokine treatments

Eosinophils with purities of 95 to 99% were cultured for 18 h at 37°C in 24-well plates at 2×10^6 cells/ml in complete medium (RPMI 1640 with 5% dialyzed FBS) in the absence and presence of the following cytokines: IL-3 at 10^{-10} M (Genzyme, Cambridge, MA); IL-4 at 10^{-9} M (Genzyme); and IL-5 at 10^{-9} M (Amgen Biologicals, Thousand Oaks, CA). Following incubation, eosinophils were collected and mixed into 1% agar in 1× PBS and then fixed in 4% freshly prepared paraformaldehyde, processed, and embedded in paraffin as previously described (2). Five-micrometer sections

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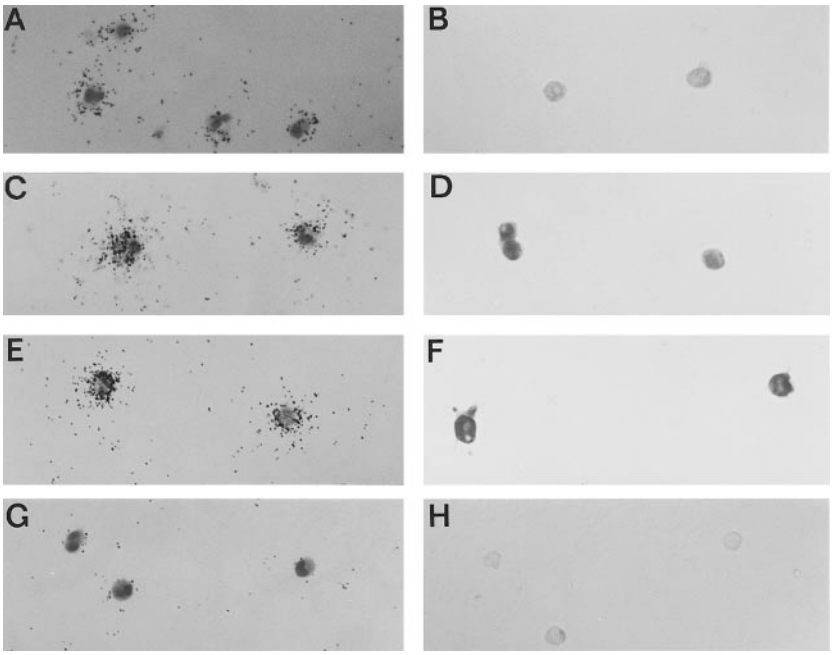


FIGURE 1. The effect of IL-3, IL-5, and IL-4 on the level of TGF-α expression of by human peripheral blood eosinophils demonstrated by in situ hybridization and immunohistochemistry. *A, C, E,* and *G* are light microscopy photomicrographs of representative fields of peripheral blood eosinophils (99% pure) from donor 1 incubated in the absence and presence of IL-3, IL-5 and IL-4. The sections were subjected to in situ hybridization with a ^{35}S-labeled TGF-α antisense riboprobe and counterstained with Giemsa. The eosinophils exhibit the characteristic pink cytoplasm and bilobed nucleus. TGF-α mRNA, demonstrated by the autoradiographic grains, is shown to be expressed by the eosinophils incubated in the absence of cytokines (*A*). The intensity of labeling is increased after incubation with IL-3 and IL-5 (*C* and *E*) and decreased significantly after incubation with IL-4 (*G*). *B, D, F,* and *H*, Light microscopy photomicrographs of sections adjacent to those shown in *A, C, E,* and *G* immunostained with the TGF-α mAb followed by detection using an alkaline phosphatase method and counterstaining with aniline blue. The staining demonstrating intracellular TGF-α immunoreactivity is noted in eosinophils that were incubated in the absence of cytokines (*B*). As demonstrated by the in situ hybridization results, the intensity of staining appears to increase after incubation with IL-3 and IL-5 (*D* and *F*) and to decrease significantly after incubation with IL-4. Magnification, $\times$250.

were mounted on gelatin-coated slides for in situ hybridization and immunohistochemistry. Supernatants from incubations were collected and stored at -80°C for ELISA analysis.

In situ hybridization

Our in situ hybridization procedures using the antisense and sense human TGF-α and TGF-β1 ^{35}S-labeled riboprobes have been described in detail (1, 2).

Immunohistochemistry

To detect human TGF-α, we used a mAb directed against the C terminus of the human TGF-α mature peptide (amino acids 34–50) (TGF-α:Ab-2; GF-10; Oncogene Science, Cambridge, MA). A mAb to the bacterial protein *trp*-E was used at the same concentration as the negative control. To detect TGF-β1, we used a rabbit anti-human TGF-β1 polyclonal Ab at 1:100 dilution (AB-20-PB; R&D Systems, Minneapolis, MN). An IgG fraction from a nonimmune normal rabbit (I-5006; Sigma Chemical, St. Louis, MO) was used at the same concentration as a negative control. Immunohistochemistry was performed using an alkaline phosphatase detection system. All sections were counterstained with 0.4% aniline blue (CI 42755) for 10 min to permit identification of eosinophils by UV fluorescence microscopy (2, 3).

Quantitative analysis of in situ hybridization data

In situ hybridization results were quantified using a computer imaging analysis system (MetaMorph, Universal Imaging, West Chester, PA). Slides with sections of eosinophils from the different treatment conditions were subjected to in situ hybridization. Following autoradiographic exposure for 3 days and counterstaining with Giemsa, slides were blindly coded and numbered in random order. Slides were imaged on a Nikon Microphot-FXA microscope with a 40 $\times$ 0.95 objective using Koehler illumination. Images of 50 cells from each condition were acquired from a Sony CCD/RGB color video camera DXC 151. Images were stored and analyzed using an Universal Imaging 46 PC computer running the MetaMorph Imaging System. At least 50 cells were quantified on each slide.

Statistical analysis

The differences between the experimental and control groups was analyzed by Student's *t* test.

Assessment of TGF protein release

TGF-α and TGF-β1 proteins released into culture supernatants were assessed by ELISA kits from Oncogene Science and R&D Systems, respectively. Each sample was tested in triplicate.

Flow cytometry

For flow cytometry, 2×10^5 purified eosinophils were incubated with 100 μl of mouse anti-human IL-4R α-chain mAb (Immunotech, Westbrook, ME) at a final concentration of 30 μg/ml (diluted in FACS buffer (HBSS without Ca/Mg + 0.5% BSA + 0.1% azide)) for 30 min on ice. Isotype control, myeloma protein (MOPC 21), was used at the same concentration. Cells were then washed twice in ice-cold FACS buffer and resuspended in 100 μl of 1/32 dilution FITC-conjugated goat anti-mouse (Sigma Chemical). After 30 min on ice, cells were washed twice in cold FACS buffer and fixed in 1% paraformaldehyde. FACS analysis (Becton Dickinson, Mountain View, CA) was performed on at least 10,000 cells.

Western blotting

Western blotting was performed according to the protocol of Harlow and Lane (10). Cells (1×10^8) were lysed with 1 ml of RIPA buffer in the presence of PMSF (100 μg/ml) and aprotinin (1 μg/ml) for 30 min on ice. The protein content of each lysate was determined by a Bio-Rad (Richmond, CA) DC protein assay. The rabbit polyclonal Ab to STAT6 was obtained from Transduction Laboratories (Lexington, KY), while the 4PS/IRS2 Ab was a kind gift from Dr. Morris White, Harvard Medical School, Boston MA. Signal detection was performed with the Kodak

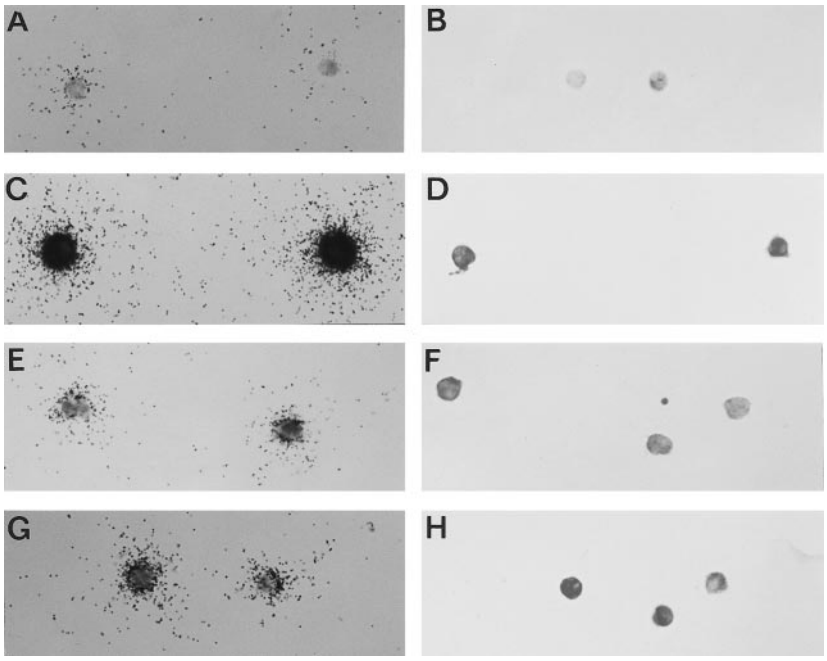


FIGURE 2. The effect of IL-3, IL-5 and IL-4 on the level of expression of TGF-β1 by human peripheral blood eosinophils demonstrated by in situ hybridization and immunohistochemistry. *A, C, E,* and *G,* Light microscopy photomicrographs of representative fields of peripheral blood eosinophils from the same donor as in Figure 1 (adjacent sections) incubated in the absence and presence of IL-3, IL-5, and IL-4. The sections were subjected to in situ hybridization with a ^{35}S-labeled TGF-β1 antisense riboprobe and counterstained with Giemsa. TGF-β1 mRNA, demonstrated by the autoradiographic grains, is detectable in one of the two cells representing the control group of eosinophils incubated in the absence of cytokines (*A*). The intensity of labeling is increased after incubation with IL-3 to the extent that the cells are completely masked by the autoradiographic grains (*C*) and is also increased after incubation with IL-5 as well as IL-4 (*E* and *G*). *B, D, F,* and *H,* Light microscopy photomicrographs of sections adjacent to those shown in *A, C, E,* and *G* immunostained with the TGF-β1 polyclonal Ab followed by detection by an alkaline phosphatase method and counterstained with aniline blue. The staining demonstrating intracellular TGF-β1 immunoreactivity is noted in eosinophils incubated in the absence of cytokines (*B*). As demonstrated by the in situ hybridization results, the intensity of staining appears to significantly increase after incubation with IL-3 and IL-5, as well as IL-4 (*D, F,* and *H*). Magnification, $\times$250.

Chemiluminescent Detection System and BioMax MR film (both from Eastman Kodak, Rochester, NY).

Results

Constitutive expression of TGF mRNA by peripheral blood eosinophils

Since our initial report, which demonstrated that eosinophils from the peripheral blood of healthy donors showed no detectable levels of TGF-α or TGF-β1 mRNA and protein, we have modified our procedure for eosinophil isolation by adding an incubation step with anti-CD16 magnetic beads to achieve an enriched population of 97 to 99% eosinophils (9). In the present study, in situ hybridization results using ^{35}S-labeled TGF-α and TGF-β1 antisense riboprobes showed that eosinophils harvested in this manner expressed a basal level of TGF-α and TGF-β1 mRNA (Figs. 1*A* and 2*A*).

TGF mRNA expression by eosinophils treated with IL-3, IL-4, and IL-5

Marked changes in the TGF mRNA expression were observed in peripheral blood eosinophils incubated in the presence of IL-3, -4, and -5 (Figs. 1 and 2). To quantify these differences, a computer-assisted analysis was used that allowed us to quantify the relative changes in TGF mRNA levels. IL-3-, IL-4-, and IL-5-treated eosinophils were obtained from six normal donors, subjected to in situ hybridization, and analyzed blindly as described in *Materials and Methods*. The results are summarized in Tables I and II. All of the cytokines used significantly increased TGF-β1 mRNA expression in all of the donors (Table II). IL-3 and IL-5, respectively, up-regulated the expression of TGF-α mRNA in five of the six donors. IL-4, however, significantly down-regulated the expression of TGF-α in all six donors.

Table I. *TGF-α mRNA expression by human eosinophils*

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
Control	1,825	3,435	1,950	2,720	1,882	2,142
IL-3	18,305***	4,975*	1,330	9,428**	7,904**	8,140**
IL-5	21,266***	2,765	2,220	12,240**	7,520**	2,540
IL-4	1,025***	1,375**	380**	1,435**	757**	664**

*$p < 0.05$; **$p < 0.005$; ***$p < 0.0005$. These values represent the areas of the total grain counts of 50 cells from each group.

Table II. *TGF-β1 mRNA expression by human eosinophils*

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
Control	2,325	3,150	1,615	2,247	3,678	1,960
IL-3	77,327**	15,850**	17,805**	65,498**	17,556**	18,970**
IL-5	29,567**	12,250**	5,790**	25,432**	15,667**	7,865**
IL-4	33,078**	4,455*	5,790**	42,008**	45,679**	38,972**

*$p < 0.005$; **$p < 0.0005$. These values represent the areas of the total grain counts of 50 cells from each group.

TGF production by eosinophils treated with IL-3, IL-4, and IL-5

Immunohistochemistry was used on adjacent sections to evaluate intracellular immunoreactivity for TGF-α and TGF-β1. Similar to the in situ hybridization results, eosinophils incubated in the absence of any of the exogenous cytokines demonstrated a basal level staining for TGF-α and TGF-β1 (Figs. 1*B* and 2*B*). Differences were observed in the intensity and numbers of eosinophils staining for both TGFs, depending on the cytokine used (Figs. 1 and 2). Since the intensity of immunoreactivity is difficult to quantify, the effect of each exogenous cytokine on the cellular levels of TGF-α and TGF-β1 proteins were quantified by comparing the percentage of cells stained for TGF-α and TGF-β1, examining 100 cells in each slide. The results are summarized in Tables III and IV. Consistent with our in situ hybridization results, eosinophils incubated with IL-3 and IL-5 demonstrated an increase in the percentages of cells that stained for TGF-β1 in all donors. IL-4-treated eosinophils demonstrated an increase in the percentage of cells stained for TGF-β1 in five of the six donors. Eosinophils incubated with IL-3 and IL-5 demonstrated an increase in the number of cells that stained for TGF-α; however, eosinophils incubated with IL-4 consistently showed a decrease in the intensities of staining as well as a decrease in the number of cells that stained for TGF-α in all six donors.

These results demonstrate that IL-4 increased the level of TGF-β1 protein in eosinophils from five of the six donors, while it consistently decreased the expression of TGF-α protein in eosinophils from all donors examined.

Effect of IL-3, IL-4, and IL-5 on TGF protein release by eosinophils

Human peripheral blood eosinophils from three donors were incubated in the absence and presence of IL-3, IL-4, and IL-5 for 18 h. Cell-free supernatants from these cultures were assayed for TGF-α and TGF-β1 protein release by specific ELISAs. The results demonstrated that IL-3 significantly enhanced TGF-α release by eosinophils in all three donors by 3.7- to 5.5-fold above the nonstimulated controls. IL-5 also induced an increase in TGF-α release in all three donors by 2.3- to 3-fold above control levels (Fig. 3*A*). IL-4 treatment led to a slight decrease of TGF-α release compared to nonstimulated control. The effects of IL-4 and IL-5 on TGF-α release by the treated eosinophils were not significant. IL-3, IL-4, and IL-5 treatment of human eosinophils led to a significant increase in TGF-β1 release in all donors examined (Fig. 3*B*).

Eosinophils express IL-4R and IL-4 signaling proteins 4PS and STAT6

Our observations demonstrated that eosinophils are responsive to IL-4 and therefore should express cell surface IL-4R. To determine whether human eosinophils express receptors to IL-4, peripheral blood human eosinophils were phenotyped for IL-4R by RT-PCR and flow cytometry. Figure 4*A* shows that when using human IL-4R PCR primers (Clontech, Palo Alto, CA) to amplify total RNA isolated from two normal donors, the expected 526-bp IL-4R PCR product was detected in both donors. For flow cytometry, 2×10^5 purified eosinophils were incubated with mouse anti-human IL-4R α-chain mAb (Immunotech). Compared with the control (black line), cells stained with anti-IL-4R α-chain mAb showed a unimodal increase in fluorescence, indicating that human eosinophils constitutively express IL-4R α-chain (Fig. 4*B*).

Since IL-4 is known to act in various cells via either the 4PS or STAT6 signaling pathways, Western blotting was used to evaluate these signal transduction proteins in lysates from peripheral blood human eosinophils. Figure 5 demonstrates the detection of STAT6 and 4PS signaling molecules in normal human eosinophils. The sizes of the human STAT6 and 4PS proteins are 100 and 170 kDa, respectively.

Discussion

We and others have previously shown that human eosinophils in either infiltrating tumors or inflammatory disorders express TGF-α and TGF-β1, whereas eosinophils from the peripheral blood of normal donors do not (2, 3, 11, 12). Furthermore, a sequential expression of TGF-α followed by TGF-β1 by eosinophils was demonstrated in cutaneous wound healing in the hamster (4). These observations led us to hypothesize that biologic mediators in the tissue can regulate the expression of eosinophil-derived TGFs. The results shown in this paper indicate that IL-3 and IL-5 can stimulate the expression of both TGF-α and TGF-β1 by normal peripheral blood eosinophils, whereas IL-4 up-regulates eosinophil synthesis of TGF-β1 but suppresses TGF-α expression at both the mRNA and protein level.

Table III. *TGF-α production by human eosinophils (%)*[a]

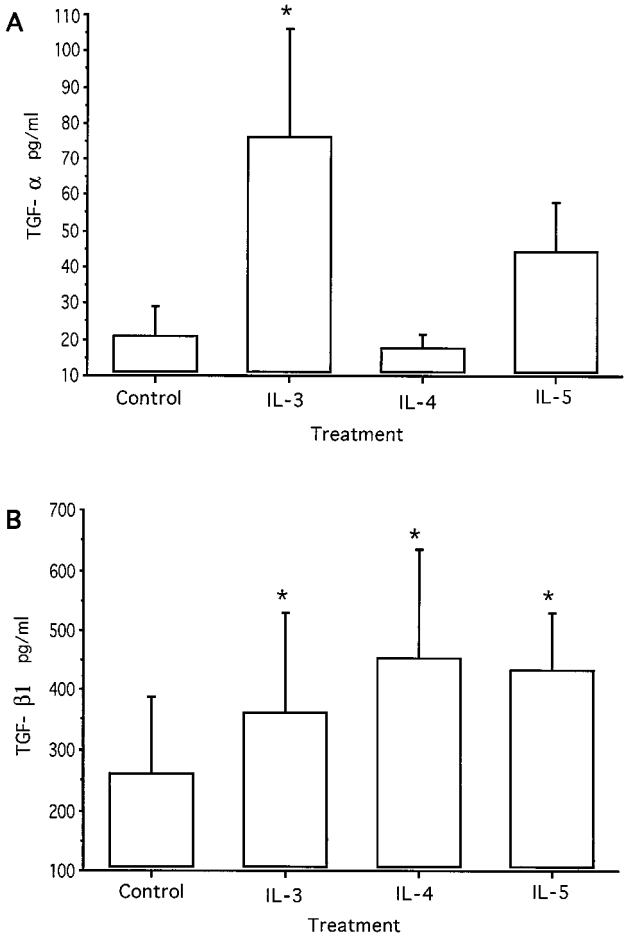
	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
Control	67	86	46	55	32	43
IL-3	89	97	70	82	67	87
IL-5	78	90	77	89	76	87
IL-4	18	84	2	22	9	14

[a] Percentages of eosinophils that stained with TGF-α Ab determined by counting 100 cells in each group.

Table IV. *TGF-β1 production by human eosinophils (%)*[a]

	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6
Control	67	78	0	43	36	57
IL-3	83	100	45	82	76	65
IL-5	89	100	82	75	82	88
IL-4	43	100	35	67	83	78

[a] Percentages of eosinophils that stained with TGF-β1 Ab determined by counting 100 cells in each group.



* P< 0.05

FIGURE 3. Effect of IL-3, IL-5, and IL-4 on the release of TGF-α (*A*) and TGF-β1 (*B*) proteins by peripheral blood eosinophils. Cells (5×10^6) were incubated in the absence (control) and presence of IL-3 (10^{-10} M), IL-4 (10^{-9} M), and IL-5 (10^{-9} M) for 18 h. Incubation media were analyzed for TGF-α and TGF-β1 proteins as described in *Materials and Methods*; results are given as means ± SEM of 3 donors (* indicates statistical significance $p < 0.05$).

Since the number of eosinophils is relatively small in the circulating blood, it is often a challenge to obtain sufficient numbers of purified cells to conduct studies both on the mRNA and protein levels. To overcome this difficulty, in situ techniques were employed enabling us to work with a small number of cells and facilitating specific identification of positive cells. The inability to accurately quantify the immunohistochemical staining intensities precluded a direct comparison with the more quantitative mRNA labeling results generated by computer-assisted analysis. Nevertheless, we noted differences in the percentage of cells that stained for both TGFs, which correlated with the in situ hybridization results.

The inhibitory effect of IL-4 on the expression of TGF-α was initially shown by in situ hybridization and immunohistochemistry, later confirmed by TGF-α-specific ELISA. Although the amount of TGF-α released by the IL-4-treated eosinophils did not show a significant reduction by ELISA, a slight decrease was observed. While the mechanism responsible for this apparent discrepancy is not clear, potential reasons for the observed finding include the sensitivity of the assays and increased stability of secreted TGF-α protein when treated with IL-4. In the cytokine-treated human eosinophils, IL-4 consistently up-regulated expression of TGF-β1, as shown by in situ hybridization, immunohistochemistry, and ELISA.

The activation of eosinophils and their accumulation in tissue sites is believed to be mediated by mechanisms that involve T cell-derived cytokines (13). In particular, a subset of helper T cells, Th2, secretes IL-5, which like IL-3 and GM-CSF is released by both Th1 and Th2 cells, influences eosinophil growth, maturation, and differentiation, and appears to be critical in prolonging the survival of eosinophils in tissues and allowing their movement into the tissues (14). Although these lymphokines have been shown to activate eosinophils and prime them for several other effector functions (15, 16), only a few studies have evaluated the effects of cytokines such as IL-3 or IL-5 on expression levels of eosinophil-derived cytokines. In agreement with our study, Brach et al. have shown that treatment with both IL-3 and IL-5 resulted in a severalfold increase of TGF-α mRNA expression and in protein release by eosinophils (17).

IL-4, originally defined as a B cell growth factor (18), has been demonstrated to be a pleiotropic cytokine with both stimulatory as well as inhibitory effects on monocytes. The regulation of mononuclear cells by IL-4 has been widely studied. IL-4 was shown to inhibit the production of cytokines such as IL-1, TNF-α, and IL-6, but to enhance the production of the IL-1 receptor antagonist, IL-1RA (19). Until now, however, the effect of IL-4 on the regulation of eosinophil-derived cytokines has not been examined. IL-4 may indirectly effect eosinophils accumulation in tissue by promoting the adherence of eosinophils but not neutrophils to endothelium

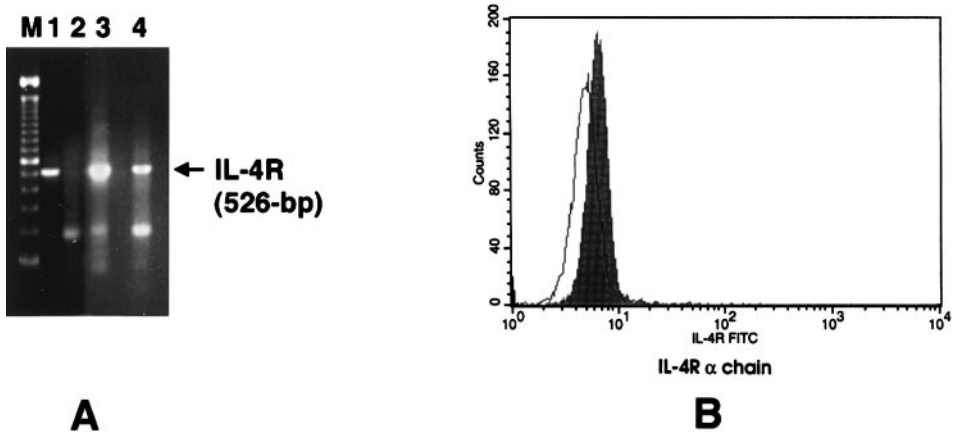


FIGURE 4. Eosinophils express IL-4 receptors. *A*, detection of IL-4R mRNA in two normal donors using RT-PCR. M = 100-bp m.w. marker; *lane 1*, PBMCs (positive control); *lane 2*, same as *lane 1* omitting reverse transcriptase (negative control); *lane 3*, normal donor 1; *lane 4*, normal donor 2. The size of the expected IL-4R PCR product is 526 bp. *B*, Flow cytometry of purified eosinophils incubated with mouse anti-human IL-4R α-chain mAb.

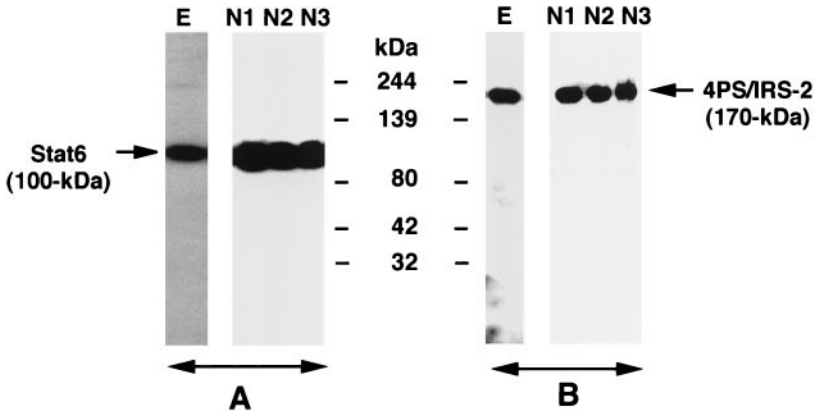


FIGURE 5. Detection of STAT6 and 4PS signaling proteins in normal human eosinophils by western blotting. *A*, STAT6 (100 kDa) in eosinophils from three normal donors. *B*, 4PS (170 kDa) in eosinophils from three normal donors. E = human eosinophil cell line EoL-1 used as a positive control.

(20). In this study, we have demonstrated that IL-4 can affect eosinophils directly and that it can differentially regulate the synthesis of TGF by eosinophil.

Consistent with the theory that IL-4 acts to regulate eosinophil function, we have shown by RT-PCR and flow cytometry that normal human eosinophils express IL-4R. Since IL-4 is known to mediate its pleiotropic effects on cellular responses via 4PS or STAT6 signal pathways, we have further demonstrated the presence of these signaling proteins in lysates obtained from peripheral blood eosinophils (Fig. 5). This is consistent with the recent results of Baskar et al. (26) and Dubois et al. (27), which demonstrated that mature eosinophils do respond to IL-4. In future studies, we plan to use these findings to further elucidate the molecular mechanisms involved in the regulation of TGF genes expression.

The findings described above may have important implications in the context of our in vivo wound-healing studies. We previously demonstrated a wound-healing model in the hamster in which eosinophils infiltrate into cutaneous healing wounds and elaborate both TGF-α and TGF-β1, with the expression of TGF-α preceding that of TGF-β1 (4). Our results in this study suggest that IL-4 may act as an in situ regulator of TGF expression by eosinophils. In the postacute phase of healing, IL-4 may shut off TGF-α production and concurrently enhance TGF-β1 production, which may promote extracellular remodeling and inhibit epithelial proliferation. Future investigations will be aimed at determining whether local expression of IL-4 in the healing wound correlates with the switching of TGF expression by the infiltrating eosinophils. In support of IL-4 as a facilitator of wound healing, Kucukcelebi et al. reported that exogenous application of IL-4 accelerated healing and improved the breaking strength of a wound (21).

In other studies, we have demonstrated that eosinophils infiltrating hamster and human squamous carcinomas represent a source of TGF-α (1, 2). In addition to its mitogenic effect on epithelial cells and its implication in malignant transformation, TGF-α has also been shown to be a potent angiogenic factor (8). Since eosinophils can express the inhibitory cytokine TGF-β1 as well, it is possible that in situ mediators such as IL-4 can induce eosinophils to produce TGF-β1 and not TGF-α, leading to inhibition of tumor cell growth. This hypothesis is interesting in light of recent reports by Tepper et al. (22, 23) describing an eosinophil-dependent mechanism for the anti-tumor effect of IL-4 (24).

In recent years, eosinophils have been shown to synthesize a wide array of cytokines, suggesting a functional versatility of these granulocytes. Little is known, however, about the control and regulation of eosinophil-derived cytokine production. In this study, we demonstrate that IL-4 can simultaneously up-regulate the synthesis of TGF-β1 and inhibit the synthesis of eosinophil-derived TGF-α, implying that the expression of cytokines by eosinophils can be differentially regulated. The expression of TGFs by eosinophils may be modulated in a selective manner by particular cytokines present in the microenvironment of various pathologic and physiologic processes, which may significantly affect eosinophil function in vivo (25). A better understanding of TGF regulation by these cells may have therapeutic implications for processes involving eosinophils such as wound healing, carcinogenesis, and allergic inflammation.

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