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Effect of IL-4 and interferon-gamma (IFN-γ) on IL-3- and IL-5-induced eosinophil differentiation from human cord blood mononuclear cells

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SUMMARY

IL-4 and IFN-γ positively and negatively regulate allergic inflammation. To determine the regulatory mechanisms of eosinopoiesis by cytokines, we examined the effect of recombinant IL-4 and IFN-γ and of anti-IL-4 and anti-IFN-γ antibodies on IL-3- and IL-5-induced eosinophil differentiation from human umbilical cord blood mononuclear cells. rhIL-4 (10–300 U/ml) inhibited IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells on day 28 of culture by 62–81% in a concentration-dependent manner. rhIFN-γ (5–500 U/ml) also inhibited IL-3- and IL-5-induced eosinophil differentiation by 80–99% in a concentration-dependent manner. The inhibitory effect of rhIL-4 and rhIFN-γ was observed only when rhIL-4 or rhIFN-γ were present in the culture from day 0 to day 14, but not from day 15 to day 28. Addition of anti-IL-4 antibody to the culture enhanced IL-3- and IL-5-induced eosinophil differentiation on day 28 of culture by 30%, whereas anti-IL-2 MoAb and anti-IFN-γ MoAb had no significant effect. These results indicate that IL-4 and IFN-γ have inhibitory effects on IL-3- and IL-5-induced eosinophil differentiation from its progenitor cells.

Keywords eosinophil differentiation IL-4 IFN-γ cord blood mononuclear cells

INTRODUCTION

It has been shown that IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are eosinopoietic cytokines that induce the proliferation and differentiation of eosinophils from progenitor cells [1–8]. However, the regulatory mechanisms for eosinopoiesis have not been fully elucidated, although several cytokines such as transforming growth factor-beta (TGF-β) have been shown to be involved in regulating eosinopoiesis [9]. IL-4 has been shown to have an enhancing effect on granulocyte/macrophage colony formation induced by granulocyte colony-stimulating factor (G-CSF) [10,11], whereas IL-4 has an inhibitory effect on IL-3-dependent colony formation by granulocyte and macrophage progenitor cells and by multipotential progenitor cells [11]. IFN-γ has also been shown to inhibit granulocyte/macrophage colony formation of human bone marrow cells [12,13] and also inhibit IL-3-, IL-4- or GM-CSF-induced proliferation of murine bone marrow cells [14].

In this study, in order to elucidate the regulatory mechanisms of eosinopoiesis by cytokines, we examined the effect of recombinant IL-4 and IFN-γ and of anti-IL-4 and anti-IFN-γ antibodies on IL-3- and IL-5-induced eosinophil differentiation from human umbilical cord blood mononuclear cells. Our results indicate that IL-4 and IFN-γ have inhibitory effects on IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells.

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MATERIALS AND METHODS

Cytokines and antibodies

Recombinant human IL-3 (rhIL-3), rhIL-4, and rhIFN-γ were obtained from Genzyme Inc. (Boston, MA). rhIL-5 was kindly provided by Suntory Corp. (Tokyo, Japan). Murine anti-human IL-2 MoAb (IgG1), rabbit polyclonal anti-human IL-4 antibody, and murine anti-human IFN-γ MoAb (IgG2a) were purchased from Genzyme Inc. Control mouse IgG and rabbit IgG were obtained from Cappel Labs. (Malvern, PA).

IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells

Mononuclear cells were isolated from heparinized umbilical cord blood by Ficoll–Paque (1·077 g/ml) (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient centrifugation method [7]. Cells were then suspended at 2×10^6 cells/2 ml in RPMI 1640 medium supplemented with 10% fetal calf serum

(FCS) and were cultured in the presence of rhIL-3 ($3{\cdot}5 \times 10^3$ colony-forming units (CFU)/ml) and rhIL-5 (250 ng/ml) in a 24-well flat-bottomed tissue culture plate at 37°C for 7–35 days in a humidified 5% CO_2 and 95% air atmosphere. Prostaglandin E_1 (0·3 mM) (Sigma Chemical Co., St Louis, MO) was also added to the culture only on day 0. Half of the culture medium was replaced weekly with freshly prepared medium containing the same concentration of rhIL-3 and rhIL-5. At 7, 14, 21, 28, and 35 days after the culture, total cell number and differential count of cells were determined by examining 200 cells stained with Wright–Giemsa solution.

Effect of IL-4 and IFN-γ on IL-3- and IL-5-induced eosinophil differentiation

To determine the regulatory role of IL-4 and IFN-γ in the differentiation of eosinophils from progenitor cells, we examined the effect of rhIL-4 and rhIFN-γ on IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells. In these experiments, rhIL-4 (10, 30, 100 and 300 U/ml) or rhIFN-γ (5, 50, 150 and 500 U/ml) were added to the IL-3- and IL-5-stimulated mononuclear cell culture from day 0 to day 28.

To determine whether the suppressive effect of IL-4 and IFN-γ on IL-3- and IL-5-induced eosinophil differentiation is exerted in the early or late differentiation stage of the progenitor cells, we also examined the effect of early and late addition of rhIL-4 or rhIFN-γ to the culture on IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells. In these experiments, rhIL-4 (100 U/ml) or rhIFN-γ (50 U/ml) were added to the IL-3- and IL-5-stimulated mononuclear cell culture from day 0 to day 14 or from day 15 to day 28. In the experiment of the addition of rhIL-4 or rhIFN-γ to the culture from day 0 to day 14, cells were washed twice with RPMI 1640 medium containing 10% FCS and then resuspended in the culture medium containing rhIL-3 and rhIL-5 on day 15.

Effect of anti-IL-2, anti-IL-4, and anti-IFN-γ antibodies on IL-3- and IL-5-induced eosinophil differentiation.

To determine whether IL-2, IL-4, and IFN-γ that are produced from IL-3- and IL-5-stimulated mononuclear cells exert a regulatory role in eosinophil differentiation from progenitor cells, we examined the effect of antibodies to IL-2, IL-4 and IFN-γ on IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells. Anti-human IL-2 MoAb (10 μg/ml), anti-human IL-4 antibody (10 μg/ml), anti-human IFN-γ MoAb (10 μg/ml) or control rat or rabbit IgG (10 μg/ml) were added to the IL-3- and IL-5-stimulated mononuclear cell culture. Half of the culture medium was replaced weekly with freshly prepared medium containing rhIL-3, rhIL-5, and each antibody.

Flow cytometry

T cell subsets of cultured cells were determined by the presence of CD3, CD4, and CD8, respectively, using the corresponding murine MoAb conjugated to PE (Becton Dickinson, Mountain View, CA) by a fluorescence-activated cell sorter. Briefly, cultured cells ($0{\cdot}5–1 \times 10^6$) were incubated with the PE-conjugated MoAb or control murine IgG at 4°C for 30 min. After washing with PBS containing 1% FCS and 0·1% NaN_3, the intensity of the fluorescence of cells was calibrated by FACStar (Becton Dickinson).

Release of eosinophil cationic protein

Eosinophils derived from the IL-3- and IL-5-stimulated mononuclear cell culture on day 28 (1×10^6/ml, 1 ml) were incubated with 10^{-5} M platelet-activating factor or Hanks' balanced salt solution (HBSS) (control) in a plastic tube at 37°C for 30 min. The amount of eosinophil cationic protein (ECP) released into the culture supernatant was measured by a radioimmunoassay [15] using the Pharmacia ECP RIA kit (Pharmacia). The assay was performed in duplicate according to the manufacturer's instructions.

Statistical analysis

Data are summarized as mean ± s.d. The paired Student's *t*-test was used for statistical comparison of the data, and $P < 0{\cdot}05$ was considered significant.

RESULTS

IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells

The time course of IL-3- and IL-5-induced eosinophil differentiation from human umbilical cord blood mononuclear cells is shown in Fig. 1. Mononuclear cells freshly isolated from cord blood consisted of 75% lymphocytes, 16% monocytes, 8% neutrophils, and 1% erythroblasts. Eosinophils began to increase 2 weeks after culturing cord blood mononuclear cells with rhIL-3 ($3{\cdot}5 \times 10^3$ CFU/ml) and rhIL-5 (250 ng/ml) (Fig. 1), and the differential count of the cultured cells at 2 weeks after the culture was 31 ± 6% eosinophils, 32 ± 7% basophils, 3 ± 1% neutrophils, 12 ± 2% macrophages, 13 ± 2% lymphocytes, and 9 ± 2% blasts (mean ± s.d., $n = 4$ experiments). The number and purity of eosinophils became maximal at 4 weeks after the culture (purity 85 ± 8% of total cells) (Fig. 1), and the rest of cultured cells were basophils. The amount of ECP released from the eosinophils by stimulation with platelet-activating factor (10^{-5} M) was $4{\cdot}7 \pm 1{\cdot}2$ ng/10^6 cells ($n = 3$).

Effect of IL-4 on IL-3- and IL-5-induced eosinophil differentiation

rhIL-4 (10–300 U/ml) inhibited IL-3- and IL-5-induced eosi-

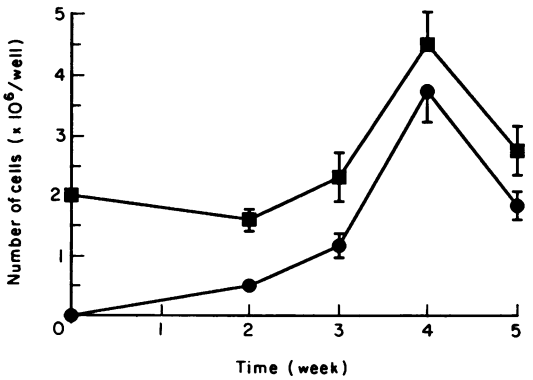

Fig. 1. Time course of IL-3- and IL-5-induced eosinophil differentiation from human umbilical cord blood mononuclear cells. Mononuclear cells from human umbilical cord blood (1×10^6 cells/ml) were cultured in the presence of rhIL-3 (3500 colony-forming units (CFU)/ml) and rhIL-5 (250 ng/ml) for 1 to 5 weeks. ■, Total cells, ●, eosinophils. Data are means ± s.d. for four experiments.

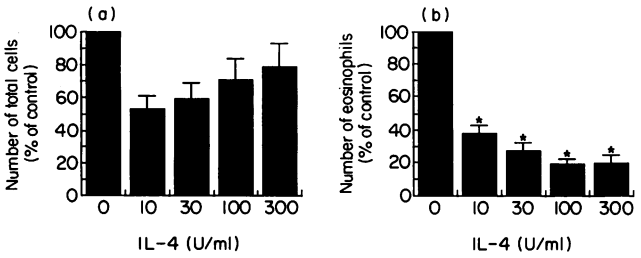

Fig. 2. Effect of IL-4 on IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells. rhIL-4 (10, 30, 100 and 300 U/ml) was added to IL-3- and IL-5-stimulated cord blood mononuclear cell culture from day 0 to day 28, and total cells (a) and eosinophils (b) were then counted. The axis is normalized to the control values. Data are means ± s.d. for five experiments. * Significantly different from the mean value of the control response, $*P < 0.001$.

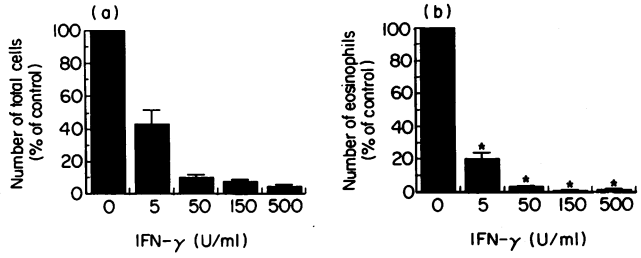

Fig. 4. Effect of IFN-γ on IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells. rhIFN-γ (5, 50, 150 and 500 U/ml) was added to IL-3- and IL-5-stimulated cord blood mononuclear cell culture from day 0 to day 28, and total cells (a) and eosinophils (b) were then counted. The axis is normalized to the control values. Data are means ± s.d. for five experiments. * Significantly different from the mean value of the control response, $*P < 0.001$.

nophil differentiation from cord blood mononuclear cells on day 28 of culture by 62·3–80·8% in a concentration-dependent manner ($n = 5$, $P < 0.001$) (Fig. 2). The inhibitory effect of rhIL-4 was observed when rhIL-4 was present in the culture from day 0 to day 14, whereas rhIL-4 had no significant effect when present in the culture from day 15 to day 28 (Fig. 3).

In contrast, rhIL-4 significantly increased T cells on day 28 of culture (71% $CD4^+CD8^-$, 12% $CD4^-CD8^+$, and 16% $CD4^+CD8^+$ T cells) (Fig. 2).

In addition, the addition of anti-IL-4 antibody restored the rhIL-4-induced inhibition of IL-3- and IL-5-induced eosinophil differentiation, whereas the addition of anti-IL-2 MoAb and anti-IFN-γ MoAb had no effect (data not shown).

Effect of IFN-γ on IL-3- and IL-5-induced eosinophil differentiation

rhIFN-γ (5–500 U/ml) also inhibited IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells on day 28 of culture by 80·2–99·1% in a concentration-dependent manner ($n = 5$, $P < 0.001$) (Fig. 4). The inhibitory effect of rhIFN-γ was observed only when rhIFN-γ was present in the culture from day 0 to day 14, but not from day 15 to day 28 (Fig. 5). The remaining cells were mainly macrophages (Fig. 4). Anti-IFN-γ MoAb but not anti-IL-2 MoAb or anti-IL-4 antibody restored the rhIFN-γ-induced inhibition of IL-3- and IL-5-induced eosinophil differentiation (data not shown).

Effect of anti-IL-2, anti-IL-4 and anti-IFN-γ antibodies on IL-3- and IL-5-induced eosinophil differentiation

Addition of anti-IL-4 antibody to the culture enhanced IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells on day 28 of culture by 29·9% ($n = 5$, $P < 0.05$), whereas anti-IL-2 MoAb and anti-IFN-γ MoAb had no significant effect (Fig. 6).

DISCUSSION

IL-4 and IFN-γ are regulatory cytokines that mutually control Th1 and Th2 cells and thereby positively and negatively

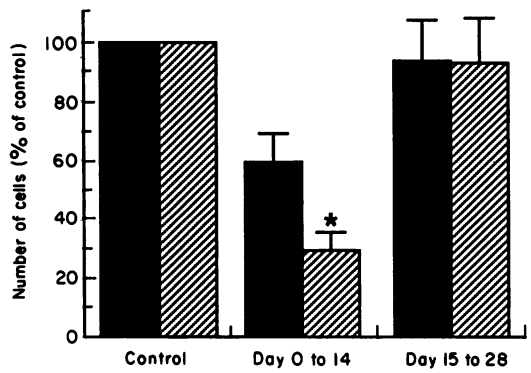

Fig. 3. Suppressive effect of early addition of IL-4 on IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells. rhIL-4 (100 U/ml) was added to IL-3- and IL-5-stimulated cord blood mononuclear cell culture from day 0 to day 14 (early addition) or from day 15 to day 28 (late addition). ■, Total cells; ▨, eosinophils. The axis is normalized to the control values. Data are means ± s.d. for five experiments. * Significantly different from the mean value of the control response, $*P < 0.001$.

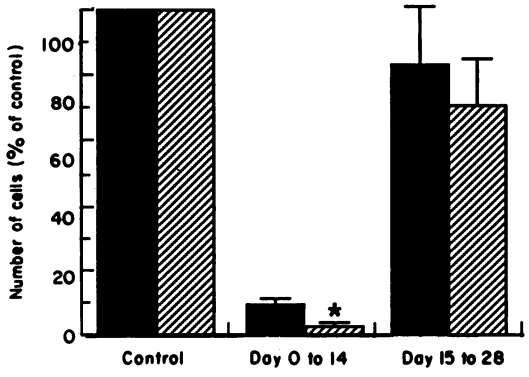

Fig. 5. Suppressive effect of early addition of IFN-γ on IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells. rhIFN-γ (50 U/ml) was added to IL-3-stimulated cord blood mononuclear cell culture from day 0 to day 14 (early addition) or from day 15 to day 28 (late addition). ■, Total cells; ▨, eosinophils. The axis is normalized to the control values. Data are means ± s.d. for five experiments. * Significantly different from the mean value of the control response, $*P < 0.001$.

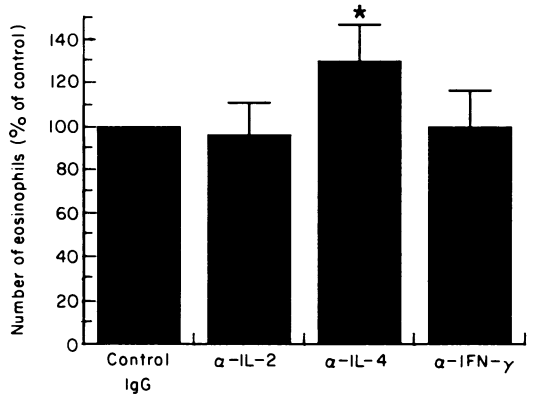

Fig. 6. Effect of anti-IL-2, anti-IL-4, and anti-IFN-γ antibodies on IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells. Anti-IL-2 MoAb (10 μg/ml), anti-IL-4 antibody (10 μg/ml), anti-IFN-γ MoAb (10 μg/ml) or control rat or rabbit IgG (10 μg/ml) were added to IL-3- and IL-5-stimulated cord blood mononuclear cell culture. The axis is normalized to the control values. Data are means ± s.d. for five experiments. * Significantly different from the mean value of the control response, $*P < 0.001$.

regulate allergic inflammation [16]. In this study, we show that IL-4 and IFN-γ both exert inhibitory effects on eosinophil differentiation from its progenitor cells. We found that rhIL-4 suppressed IL-3- and IL-5-induced eosinophil differentiation from human umbilical cord blood mononuclear cells (Figs 2 and 3) and that eosinophil differentiation was significantly increased by the addition of anti-IL-4 antibody (Fig. 6). We also found that rhIFN-γ inhibited IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells (Figs 4 and 5).

IL-4 has been shown to exhibit an enhancing effect on the proliferation of haematopoietic progenitor cells [10,11]. Peschel *et al.* [10] reported that murine IL-4 enhanced granulocyte/macrophage colony formation induced by G-CSF, and erythroid-burst formation and mixed colony formation induced by erythropoietin. Rennick *et al.* [11] also demonstrated that murine IL-4 enhanced erythroid, granulocyte, macrophage, and mast cell colony formations in the presence of erythropoietin, G-CSF, macrophage colony-stimulating factor, and IL-3, respectively. These observations suggest that IL-4 enhances colony formation by committed progenitor cells. In contrast, IL-4 has been shown to suppress IL-3-dependent colony formation by granulocyte and macrophage progenitor cells and by multipotential progenitor cells [11]. Therefore, our finding that IL-4 suppresses IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells suggests that the inhibitory effect of IL-4 might be exerted on IL-3-sensitive, relatively immature progenitor cells. Indeed, it has been shown that a murine IL-3-dependent haematopoietic cell line expresses IL-4 receptors [17]. In addition, it is unlikely that the suppressive effect of rhIL-4 on IL-3- and IL-5-induced eosinophil differentiation is due to the change in the kinetics of eosinophil differentiation by IL-4, because rhIL-4 also suppressed IL-3- and IL-5-induced eosinophil differentiation on day 14 and day 21 of culture (data not shown).

We also showed that rhIFN-γ inhibited IL-3- and IL-5-induced eosinophil differentiation from cord blood mononuclear cells (Fig. 4), and that this suppressive effect on eosinophil differentiation was exerted at the early differentiation stage of eosinophils from progenitor cells (Fig. 5). It has previously been shown that IFN-γ inhibits granulocyte/macrophage colony formation in cultures of human bone marrow cells [12,13], consistent with our present finding. Gajewski *et al.* [14] also suggested that using murine IL-3-dependent bone marrow-derived cell lines, there are subpopulations of haematopoietic progenitor cells which differ in terms of susceptibility or resistance to IFN-γ-mediated inhibition. Therefore, our finding that IFN-γ efficiently suppresses IL-3- and IL-5-induced eosinophil differentiation suggests that the progenitor cells for eosinophils are sensitive to IL-3 and IFN-γ. The failure of anti-IFN-γ MoAb to enhance IL-3- and IL-5-induced eosinophil differentiation might be due to deficiency of IFN-γ production in cord blood mononuclear cells [18,19]. In contrast to the suppressive effect of IFN-γ on IL-3- and IL-5-induced eosinophil differentiation from progenitor cells, it is interesting that IFN-γ is an activator for mature eosinophils [20,21].

In conclusion, we have shown that IL-3- and IL-5-induced eosinophil differentiation from progenitor cells is controlled by IL-4 and IFN-γ.

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