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Granulocyte–macrophage colony-stimulating factor and interleukin-3 induce surface expression of interleukin-2 receptor p55-chain and CD4 by human eosinophils

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SUMMARY

In this report it is shown by immunoflourescence analysis, biochemical analysis and mRNA hybridization that human eosinophils express surface CD4 and interleukin-2 receptor (IL-2R) (CD25) when exposed to eosinophil activators granulocyte–macrophage colony-stimulating factor (GM-CSF) and IL-3. Although the functional role of eosinophil CD4/CD25 expression has to be elucidated, it will be of interest in further studies to investigate whether *in vivo* induction of these molecules occurs in association with certain disease processes such as the hypereosinophilic syndrome or in immunological responses during allergic and helminthic parasitic diseases.

INTRODUCTION

The CD4 molecule, a 55,000–60,000 molecular weight (MW) glycoprotein, is expressed on the surface of a helper-inducer subset of human T cells that mediates the interaction of T cells with cells bearing major histocompatability complex (MHC) class II proteins during an immune response (Terhorst *et al.*, 1980). In addition to its expression on subsets of T cells, CD4 can also be detected on the surface of cells of the monocyte–macrophage lineage. Similarly, the p55 chain of the interleukin-2 receptor (IL-2R) can be induced on both T cells and monocytes (Herrmann *et al.*, 1985) and acts as a low-affinity binding site for IL-2. Eosinophils are notable participants in immunological responses during allergic reactions and helminthic infections (Silberstein & David, 1987). However, a co-operative mechanism for eosinophil stimulation by lymphocytes has so far not been defined. Recently it has been shown that mature human eosinophils have the capacity to express MHC class II protein (Lucey, Nicholson-Weller & Weller, 1989), providing the first clues of a possible T-cell–eosinophil interaction. In this study it is shown that normal donor-derived human eosinophils express binding sites for anti-CD4 and anti-CD25 (p55 IL-2 receptor) monoclonal antibodies (mAb) when exposed to T-cell lymphokines IL-3 and granulocyte-macrophage colony-stimulating factor (GM-CF), previously shown to promote eosinophil activation (Silberstein & David, 1987).

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MATERIALS AND METHODS

Purification of eosinophils

Peripheral blood eosinophils were obtained from three normal donors (1–4% blood eosinophils) and enriched to 70–92% purity by Percoll density gradient centrifugation, as described previously (Yazdanbaksh *et al.*, 1987). In brief, granulocytes were purified from leucocyte-rich buffy coats of 500 ml of blood anti-coagulated with 0·4% v/v trisodiumcitrate by centrifugation of buffy coats over Percoll (Pharmacia, Uppsala, Sweden) (specific density 1·077 g/cm^3). Thereafter, the cells were centrifuged after resuspension in Percoll with a specific density of 1·084 g/cm^3. This suspension was layered on top of Percoll with a specific density of 1·1 g/cm^3, overlaid with 0·2 ml phosphate-buffered saline (PBS) and centrifuged (20 min, 1000 ***g***, 27°). Eosinophils were collected from the lower interface of the gradient.

Culture of eosinophils

$0{\cdot}5 \times 10^6$/ml enriched eosinophils were cultured in RPMI-1640, 10% fetal calf serum FCS (standard culture medium) in the presence or absence of recombinant human (rh) colony-stimulating factors (CSF) (i.e. GM-CSF, 25 ng/ml; IL-3, 25 ng/ml; kindly provided by Behringwerke, Marburg) with an adherent monolayer of Swiss 3T3 fibroblasts (ATCC, Rockville, MD). By Day 3 of culture, >95% of all cells, except the 3T3 fibroblast, were eosinophils (Fig. 1) and >90% of eosinophils remained viable (trypan blue dye exclusion) when cells were cultured on 3T3 fibroblasts (both in the presence of rhGM-CSF or IL-3).

Flow cytometric analysis

Flow cytometry of 10^4 eosinophils (Day 6 of culture) was performed after 10^6 cells were stained with FITC-conjugated

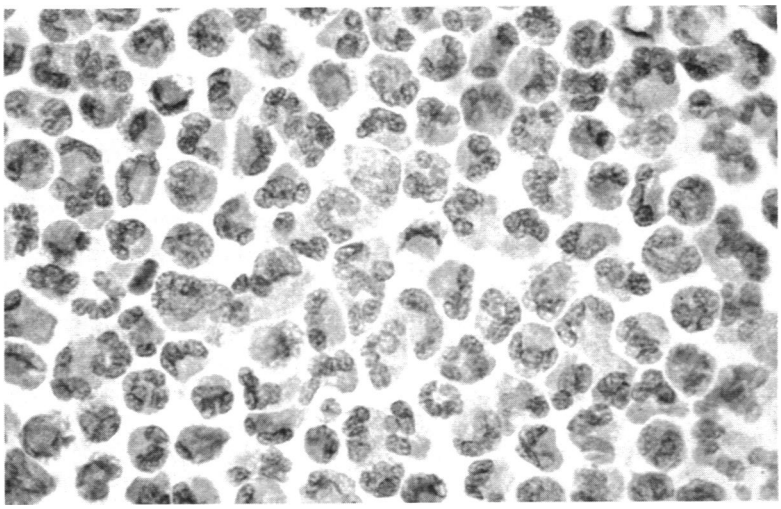

Figure 1. Morphological analysis of purified human eosinophils.

antibody to the p55 chain of the IL-2R (mAb anti-IL-2R1; Coulter Electronics, Hialeah, FL) or to CD4 (mAb anti-T4; Coulter Electronics). The absence of both T cells and monocytes in eosinophil cultures was confirmed by finding no more than background staining with FITC-conjugated antibodies to CD3 and CD14 (Herrmann *et al.*, 1985).

Immunoprecipitation of CD4 from eosinophil membranes

10^7 eosinophils were harvested on Day 4 of culture with adherent 3T3 cells in the presence or absence of rhGM-CSF and were subjected to radioiodination with the lactoperoxidase-catalysed method (Herrmann *et al.*, 1985). After disruption of eosinophils in RIPA buffer (radioimmunoprecipitation assay buffer; 0·1 M NaH_2PO_4, 1 mM PMSF, 10 mM EDTA, 10 mM EGTA, 10 mM NaF, 1% sodium DOC, 1% Triton X-100; all materials from Sigma, Munich), their soluble extract was immunoabsorbed with various non-binding mAb (CD1, CD2, CD3, CD19; Coulter Electronics) and immunoprecipitated with CD4/protein A–Sepharose (Pharmacia) complex. Immunoprecipitates were resolved by $NaDodSO_4$/PAGE and visualized by autoradiography of gels for 7 days.

mRNA analysis of p55 IL-2R in eosinophils

Total cellular RNA of eosinophils that were harvested at various intervals after treatment with appropriate compounds, was purified by the guadinium isothiocyanate cesium chloride method, analysed by gel-electophoresis, transferred to nitrocellulose paper and hybridized to the p55 IL-2R-specific cDNA (0·9 kb EcoRI fragment of Psp 65; kindly provided by Dr T. Waldmann, NIH, Bethesda, MD). The probe was labelled by primer extension with [alpha-^{32}p] dCTP (3.000 Ci/mmol) to yield a specific activity of 10^8 c.p.m./μg. Washed filters were exposed to Kodak XAR-5 films for 1–7 days. In selected experiments (not shown), eosinophil-derived RNA was control hybridized with a c-fms (provided by J. Sherr, St Jude Childrens Hospital, Memphis, TN) and T-cell receptor beta-chain cDNA (provided by H. D. Royer, German Cancer Research Center, Heidelberg) to exclude contamination with monocytes or T cells (Herrmann *et al.*, 1989).

RESULTS

Eosinophil surface expression of CD4 and CD25

Freshly separated eosinophils and eosinophils that survived in culture with adherent Swiss 3T3 fibroblasts expressed negligible amounts of CD4 and CD25 on their surface. However, when eosinophil-enriched cells were placed in culture with both rhGM-CSF or rhIL-3 on monolayers of 3T3 fibroblast, > 50% of eosinophils bound anti-CD4 mAb and > 20% expressed binding sites for anti-CD25 (p55 chain of the IL-2R) mAb (Table 1).

rhGM-CSF and rhIL-3 induce IL-2R mRNA accumulation in eosinophils

As shown in Fig. 2 freshly separated eosinophils lacked a detectable message of IL-2R by Northern blot analysis. When eosinophils were exposed to rhGM-CSF and also to rhIL-3, however, IL-2R-specific transcripts of 3·5 and 1·5 kb in size

Table 1. GM-CSF and IL-3 induce surface expression of CD25 and CD4 on normal donor-derived human eosinophils cultured with an adherent monolayer of Swiss 3T3 fibroblasts

Culture conditions*	CD25 (anti-p55 IL-2R) (%)	CD4 (anti-T4) (%)
3T3 fibroblasts	3 ± 2	5 ± 3
3T3 fibroblasts + GM-CSF	21 ± 4	59 ± 5
3T3 fibroblasts + IL-3	22 ± 4	50 ± 10

* Enriched eosinophils ($0·5 \times 10^6$/ml) were cultured for 6 days in standard culture medium in the presence or absence of rhGM-CSF (25 ng/ml) or rhIL-3 (25 ng/ml) with an adherent monolayer of Swiss 3T3 fibroblasts. On Days 2 and 4, half of the culture medium was exchanged with fresh medium ± GM-CSF/IL-3. Prior to cytometry (Day 6), cell viability (> 80%) was assessed with trypan blue and morphology by luxol fast blue staining.

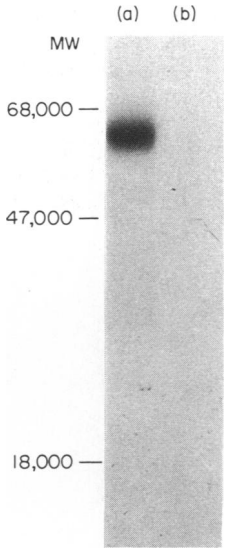

Figure 2. Northern blot analysis probing for p55 IL-2R mRNA in unactivated enriched eosinophils (c), eosinophils exposed to rhIL-3 for 24 hr (a) and to rhGM-CSF (b) for the same period. (d–f) Represent control blots obtained from T-cell RNA that were stimulated with phytohaemagglutinin/phorbol myristate acetate (1% v/v, 10^{-8} M), respectively, for 3 hr (f), 12 hr (e) or that were unstimulated (d).

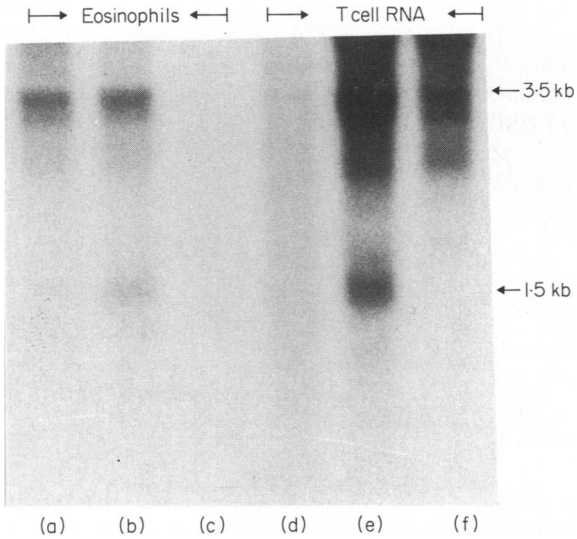

Figure 3. NaDodSO$_4$/PAGE of CD4 (55,000–60,000 MW) immunoprecipitated with anti-T4 mAb from enriched eosinophils cultured in the presence (a) or absence (b) or rhGM-CSF with adherent Swiss 3T3 fibroblasts for 6 days.

became detectable. Eosinophil-derived mRNA failed to hybridize to c-fms and the T-cell receptor β-chain gene probe, thus being devoid of monocyte or T-cell contamination (not shown).

Anti-CD4 mAb precipitates a ^{125}I-labelled 55,000–60,000 MW polypeptide from eosinophil membranes

As shown in Fig. 3 immunoprecipitation studies with anti-CD4 revealed that CD4 was precipitable from eosinophil membranes and migrated on SDS–PAGE with a MW of 55,000–60,000, identical to that previously determined for CD4 on T lymphocytes and monocytes. A prerequisite for the immunoprecipitation of CD4 from eosinophils was the exposure to rhGM-CSF. Eosinophils that were cultured on 3T3 fibroblast only failed to display precipitable CD4.

DISCUSSION

Following antigenic challenge, T lymphocytes produce different cytokines that modulate functions of a variety of cells. Among these molecules, GM-CSF is known to stimulate the functional repertoire of mature eosinophils (Silberstein & David, 1987), involving the enhancement of antibody-dependent cytotoxicity (Lopez *et al.*, 1986), synthesis of leukotriene C_4 in response to calcium ionophore, and induction of surface expression of HLA-DR molecules (Lucey *et al.*, 1989). Another lymphokine, IL-3, which is exclusively produced by activated T cells (Oster *et al.*, 1989), enhances eosinophil cytotoxicity, phagocytosis of yeast and production of superoxide in response to stimulation with formyl-methionyl-leucyl-phenylalanine (Silberstein & David, 1987).

In the present study, it was shown that rhGM-CSF and rhIL-3 have the capacity to induce surface expression of CD4 and CD25 molecules by eosinophils after 6 days of co-culture with 3T3 fibroblasts. These findings provide additional evidence to show that mature eosinophils retain the capacity of actively transcribing new messenger RNA and synthesizing new proteins. Since GM-CSF and IL-3 are products of activated T lymphocytes, and T cells co-operate in inflammatory or allergic lesions, an enhancement of CD4 and CD25 on the eosinophil surface may also occur *in vivo* as a result of an induction by soluble T-cell products. In the case of CD4, its expression could allow CD4$^+$ eosinophils to interact with class II MHC-bearing cells. CD4 could function as a signal transducing ligand on these cells, as it does on other CD4$^+$ cells (Sattentau & Weiss, 1988). Since the eosinophils can also be induced to express HLA-DR on their surface, it might be speculated that the interaction of HLA-DR-expressing eosinophils with CD4$^+$ T cells could trigger these cells to secrete cytokines such as IL-2, which in turn would interact with IL-2-receptive eosinophils. However, it has still to be established whether there is functional ligand binding of IL-2 to a high-affinity binding site on the eosinophil surface. It will also be of interest in further studies to investigate whether *in vivo* induction of these molecules may occur in the hypereosinophilic syndrome or in the immunological response during allergic or helminthic parasitic diseases.

ACKNOWLEDGMENTS

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