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Gene expression of interleukin-2 in purified human peripheral blood eosinophils

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SUMMARY

To verify the hypothesis that eosinophils produce interleukin-2 (IL-2), a cytokine essential for lymphocyte activation, the expression of IL-2 was examined in peripheral blood eosinophils obtained from normal, atopic, asthmatic and hypereosinophilic subjects. Purified blood cell preparations were >95% eosinophils, the remaining cells being neutrophils. Based on morphological observations and on CD3 expression, no lymphocytes were detected in these eosinophil preparations. The expression of IL-2 mRNA was detected by reverse transcriptase polymerase chain reaction (RT-PCR) in total RNA extracted from purified eosinophils stimulated with granulocyte–macrophage colony-stimulating factor (GM-CSF), with or without calcium ionophore (A23187). In-cell RT-PCR combined with *in situ* hybridization further confirmed that it was the eosinophils that expressed IL-2 mRNA. Moreover, in this experiment IL-2 mRNA expression increased upon costimulation with A23187 and GM-CSF suggesting that a steady-state level of IL-2 mRNA was inducible. Finally, IL-2 was detected in purified eosinophils by immunochemistry. These data, obtained by different techniques, demonstrate that eosinophils can express IL-2. An IL-2-mediated eosinophil–lymphocyte interaction could contribute to the chronic state of cell activation in inflamed tissues where these cells are implicated.

INTRODUCTION

Asthma is an inflammatory disease characterized by airway inflammation and infiltration with activated lymphocytes and eosinophils. T lymphocytes from bronchoalveolar lavage and bronchial biopsies of asthmatics have a helper T cell type 2 (Th2)-like pattern and express interleukin (IL)-3, IL-4, IL-5 and granulocyte–macrophage colony-stimulating factor (GM-CSF) mRNA.[1–5] These cytokines can upregulate a number of eosinophil functions, such as superoxide anion generation, leukotriene C_4 production, degranulation, antibody-dependent cytotoxicity, hyperadhesion and *in vitro* survival rate.[6–8] Eosinophils release a large array of mediators, including leukotriene C_4, platelet-activating factor, substance P and cationic proteins. These mediators can induce many physiopathological features of asthma: bronchoconstriction, increased vascular permeability and mucus production, epithelial damage and cell recruitment and activation.[9] Consequently, eosinophils are considered as potent effector cells the functions of which are modulated by lymphocytes. Moreover, eosinophils express mRNA or both mRNA and protein of many cytokines.[10–18] These cytokines may amplify the inflammatory process, activate eosinophils in an autocrine manner and promote functions of several other cell types such as fibroblasts, lymphocytes and mast cells. Therefore, eosinophils also have the potential for acting as effective modulatory cells at the site of inflammation.

IL-2, a potent lymphocyte activator produced by activated lymphocytes, acts by an autocrine mechanism.[19] This cytokine is a chemoattractant for eosinophils but, contrary to IL-5, IL-3 and GM-CSF, has no direct effect on eosinophil functions.[20] Whereas eosinophils were found to produce the Th2 lymphokines IL-5 and IL-6, no previous studies have shown that they can produce IL-2. Because of the close association between lymphocytes and eosinophils in the bronchial inflammation, and since eosinophils have the capacity to produce cytokines, we hypothesized that eosinophils could also produce IL-2. In this study, we demonstrated that purified peripheral blood eosinophils, stimulated with GM-CSF and/or calcium ionophore, express IL-2 mRNA as detected by reverse transcriptase-polymerase chain reaction (RT-PCR) and in-cell RT-PCR. The IL-2 protein was detected by immunochemistry in purified

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Abbreviations: dNTP, set of dATP, dGTP, dCTP, dTTP; DTT, dithiotreitol; EGF, epidermal growth factor; GM-CSF, granulocyte–macrophage colony-stimulating factor; M-MLV, Moloney murine leukaemia virus; PDGF, platelet-derived growth factor; PHA, phytohemagglutinin; RNAsin, RNase inhibitor; ssDNA, single strand deoxyribonucleic acid.

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eosinophils stimulated with or without phytohaemagglutinin (PHA).

MATERIALS AND METHODS

Eosinophil purification

Peripheral blood eosinophils were isolated from normal individuals, subjects with asthma of varied severity, atopic subjects and one person with drug allergy-induced hypereosinophilia. Peripheral blood cells were subjected to Dextran sedimentation and granulocytes were separated from mononuclear cells on a Ficoll–Paque gradient (Pharmacia, Sweden). Remaining red blood cells were lysed with water. The granulocytes were then suspended in Ca^{2+}/Mg^{2+}-free Hank's buffer sodium salt (HBSS) (Gibco BRL, Canada) and depleted from neutrophils in a magnetic cell sorter (Miltenyi Biotec GmbH, Germany) using immunomagnetic beads conjugated with anti-CD16.[21] Eosinophils were also purified with a Percoll gradient technique as modified by Laviolette *et al.*[22] The resulting cell suspensions were constituted of >95% eosinophils as assessed by Diff-Quik staining. The contaminating cells were neutrophils; no lymphocytes were identified on morphology or by immunochemistry with CD3 antibody.

Stimulation conditions

The eosinophil suspensions were incubated (10^6 cells/ml) in RPMI medium, fetal bovine serum (FBS) 10%, 1% penicillin-streptomycin (Gibco, USA), with or without calcium ionophore A23187 0·2 μM (Sigma, USA), and GM-CSF 100 U/ml (Genzyme, USA). Cells treated for 6 hr were harvested and used for RT-PCR or in-cell RT-PCR. In other experiments, purified eosinophils were incubated with or without PHA 10 μg/ml (Sigma, USA). Cells treated for 16 hr with GM-CSF/A23187 or PHA were used for IL-2 detection.

RT-PCR

Total cellular RNA was extracted from 1 to 5×10^7 cells by the guanidium thiocyanate method described by Chomczynski & Sacchi.[23] Reverse transcription (RT) was performed on 1 μg of total RNA using 2.5 μM of random hexamers (Cetus, Canada), 15 mM KCl, 1 mM set of dATP, dGTP, dCTP and dTTP (dNTP), 6 mM $MgCl_2$, 1 mM dithiotreitol (DTT), and 1 unit/μl of Moloney murine leukemia virus (M-MLV) reverse transcriptase (Gibco BRL, Canada), at 42° for 1 hr. After reverse transcription, the IL-2 cDNA was amplified using the forward primer (5′-TTTAAGTTTTACATGGCCAC3′) and the reverse primer (5′GATCCCTTTAGTTCCAGAAC3′). IL-2 primers were designed for specific IL-2 cDNA amplification using a forward primer spanning junction of exons 1 and 2 and the reverse primer spanning junction of exons 3 and 4 of the IL-2 gene. The β-actin cDNA was also amplified as a control of the PCR using forward ('5AGCCATGCCAATCTCATCTTGT3') and reverse ('5ACGGCTGCTTCCAGCTCCTC3') primers. PCR was performed in a reaction mixture comprising 50 pmol of each primer, 50 mM Tris–HCl (pH 8·3), 15 mM KCl, 1 mM dNTP, 1·5 mM $MgCl_2$ and 0·25 unit/μl ampli*Taq* DNA polymerase (Cetus, Canada). Thirty-five cycles of amplification were carried out on a DNA Thermal Cycler 480 (Cetus, Canada) using a standard step cycle: 95°, 30 s; 55°, 30 s; 72°, 1 min. Specific PCR products for IL-2 and β-actin were confirmed by Southern blot analysis.

In-cell RT-PCR

Following a 6 hr incubation with or without stimulation, purified eosinophils were washed twice in phosphate-buffered saline (PBS) (pH 7·4) and immediately fixed for 30 min, at room temperature, in a 4% (w/v) deionized paraformaldehyde/PBS solution.[24] After fixation, cells were permeabilized with 0·1 M glycine/0·05% (w/v) Nonidet P-40/PBS (pH 7·4) for 30 min on ice.[25,26] They were washed and equilibrated for 5 min in 50 mM Tris–Cl (pH 8·3), 15 mM KCl and 1·5 mM $MgCl_2$ containing 1 unit/μl RNAsin™ (Promega, Canada) prior reverse transcription. Reverse transcription was performed (10^6 cells/reaction) as described above for total mRNA, using 200 units of M-MVL reverse transcriptase. PCR conditions were as described above for total RNA, but using 40 cycles. After PCR, cells were fixed in 4% (w/v) paraformaldehyde/PBS and embedded in glycol methacrylate resin (GMA) using the JB4 embedding kit (Polysciences, USA). Sections of 2 μm were mounted on polarized slides (Fisher, Canada), and were kept at −20° with dessicant until used for *in situ* hybridization.

In situ *hybridization*

The RT-PCR-amplified IL-2 cDNA was detected by *in situ* hybridization (ISH) using the IL-2 plasmid cDNA pLW81 (American Type Culture Collection, USA). A 220 (bp) base-pair-specific PCR probe amplified from pLW81 cDNA was purified on a 5% acrylamide gel and labelled with [^{35}S]dCTP using the T7 Quick Prime™ kit (Pharmacia, Canada). Unincorporated nucleotides were removed by chromatography using a Bio-Spin 6 column (Bio-Rad, Canada). Slides were incubated in 2× SSC for 10 min, 2× SSC, 0·1% Triton-X-100 for 20 min and soaked in 2× SSC. Slides were then prehybridized in formamide 50% (v/v); 5× SSPE; sodium dodecyl sulphate (w/v) 0·1%, 5× Denhardt's solution; yeast-tRNA (Sigma, USA), 200 μg/ml; ssDNA, 200 μg/ml; poly A oligonucleotide, 2 μg/ml and Dextran sulphate (w/v) 4% for 2 hr, at room temperature. The cDNA amplified *in situ* was denatured by incubating slides at 95° for 5 min. Slides were hybridized in the same solution with the labelled IL-2 probe, using 2×10^6 c.p.m./slides/200 μl of hybridization solution for 16 hr at 37°. After incubation, the slides were soaked sequentially in 2× SSC, 1× SSC, and 0·5× SSC for 90 min each step. The slides were then incubated for 1 hr at 37° in 0·5× SSC, and soaked again in 0·5× SSC for 1 hr at room temperature, then dehydrated for 2 min in ethanol 70%. X-ray film was exposed to the slides for 3 days. After 7–21 days according to the parallel X-ray film signal, the slides dipped in photographic emulsion were then developed, and stained with haematoxylin and eosin. The number of grains per positive cells were counted and corrected according to cell surface area by image analysis (SAMBA, Canada).

Immunochemical detection

Eosinophils incubated for 16 hr with or without PHA (10 μg/ml) were fixed overnight in acetone with 1 mM of phenyl methyl sulphonyl fluoride at −20°, transferred into acetone and methylbenzol, immersed in GMA monomer, and polymerized overnight at 4° (Polyscience Inc., USA). Two micrometre sequential sections were cut from GMA blocks with an ultramicrotome (Reichart Ultracut S, Austria), and mounted on poly-L-lysine-coated slides. To block endogenous peroxidase, the slides were places in 0·1% sodium azide and 0·3%

hydrogen peroxide for 30 min, and rinsed in 0·05 M Tris-buffered saline (TBS). To prevent non-specific protein binding, specimens were incubated with normal horse serum for 30 min, prior to addition of primary antibodies. Cell sections were then incubated overnight at 4° with monoclonal antibodies: anti-IL-2 (Genzyme, USA), EG2 (Pharmacia, Canada) or anti-CD3 (Becton Dickinson, Canada). Cells were counted by image analysis using MOCHA software (Canada), the results of each preparation expressed as mean of three counts of 1000 cells.

Enzyme immunoassays

Supernatants of cells incubated for 16 hr with or without A231187 (0·2 μM) and GM-CSF (100 U/ml), or PHA (10 μg/ml) alone were tested for IL-2 secretion by enzyme-linked immunosorbent assay (ELISA) using a primary polyclonal hIL-2 antibody (range of detection: 100–4000 pg/ml) (Genzyme, USA) or a primary monoclonal hIL-2 antibody (range of detection: 10–1000 pg/ml) (Cayman, USA). For internal control, in some experiments, IL-2 was added (250 pg/ml) to culture medium to evaluate protein degradation due to eosinophil degranulation and activation.

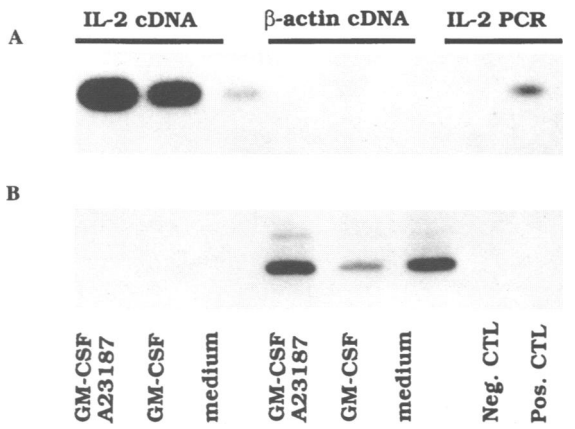

Figure 1. Southern blot analysis of amplified cDNA from purified blood eosinophil total RNA. Panel a represents IL-2 cDNA detection; panel b, β-actin cDNA detection. Cells were incubated with GM-CSF (100 units/ml) and A23187 (0·2 μM), GM-CSF (100 units/ml) or medium only. Lane 7 represents a negative control (CTL) where RNA was omitted and lane 8 a positive control. IL-2 cDNA amplification using the IL-2 cDNA clone pLW81.

RESULTS

RT-PCR for IL-2 mRNA detection

The IL-2 mRNA was detected by RT-PCR in total mRNA of purified blood eosinophils. The amplified IL-2 cDNA was confirmed by Southern blot and detected either spontaneously or after stimulation with GM-CSF and calcium ionophore or GM-CSF alone. Figure 1 shows the results obtained with a subject presenting a drug allergy-induced hypereosinophilia. The detected amount of IL-2 was higher following stimulation:

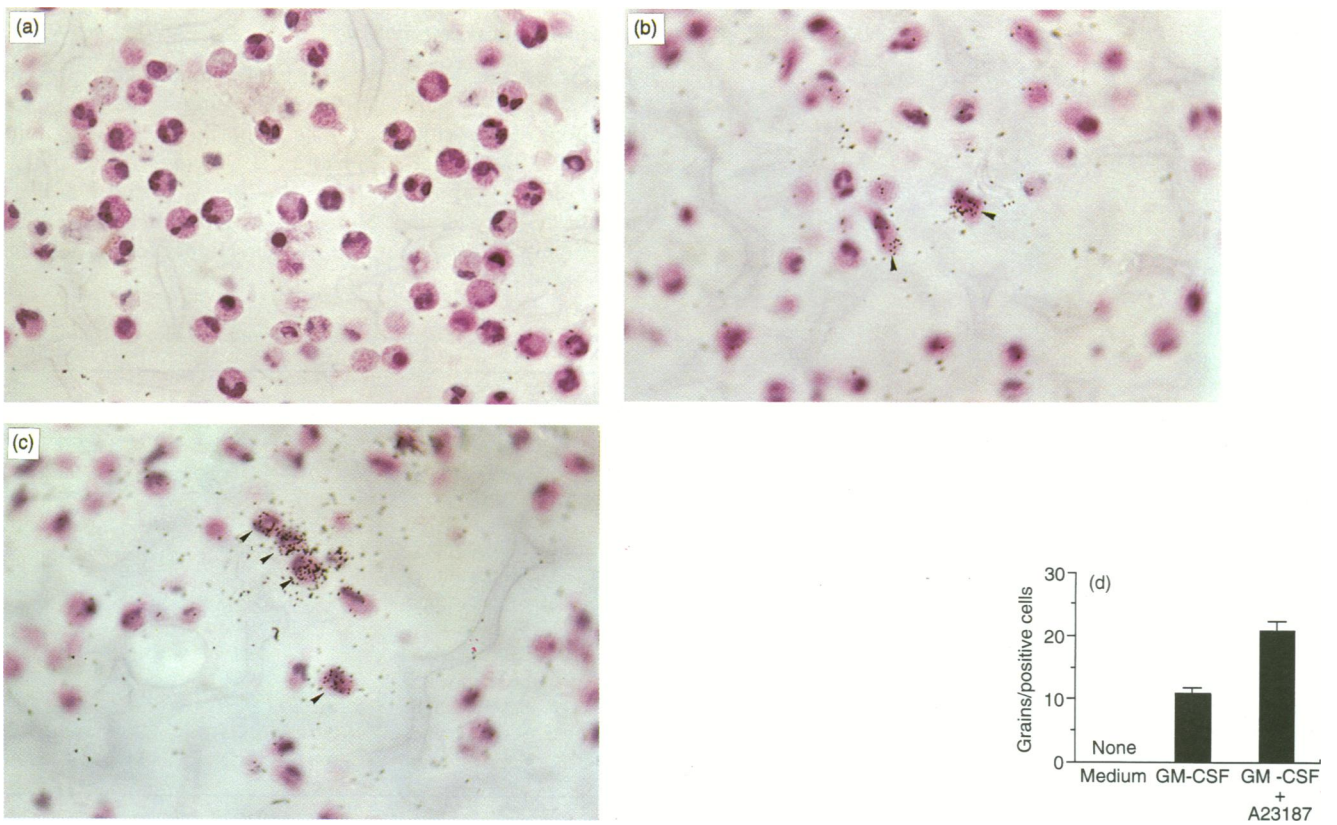

Figure 2. *In situ* hybridization for IL-2 cDNA detection on 2 μM sections of GMA-embedded purified blood eosinophils. Cells were incubated for 6 hr in RPMI (a), GM-CSF (100 units/ml) (b), or GM-CSF (100 units/ml) and A23187 (0·2 mM) (c) (original magnification ×500). Arrows indicate positive cells. Panel d represents the number of grains counted on positive eosinophils for each set of conditions, results are mean ± SEM of grain numbers per positive cells (n = 74).

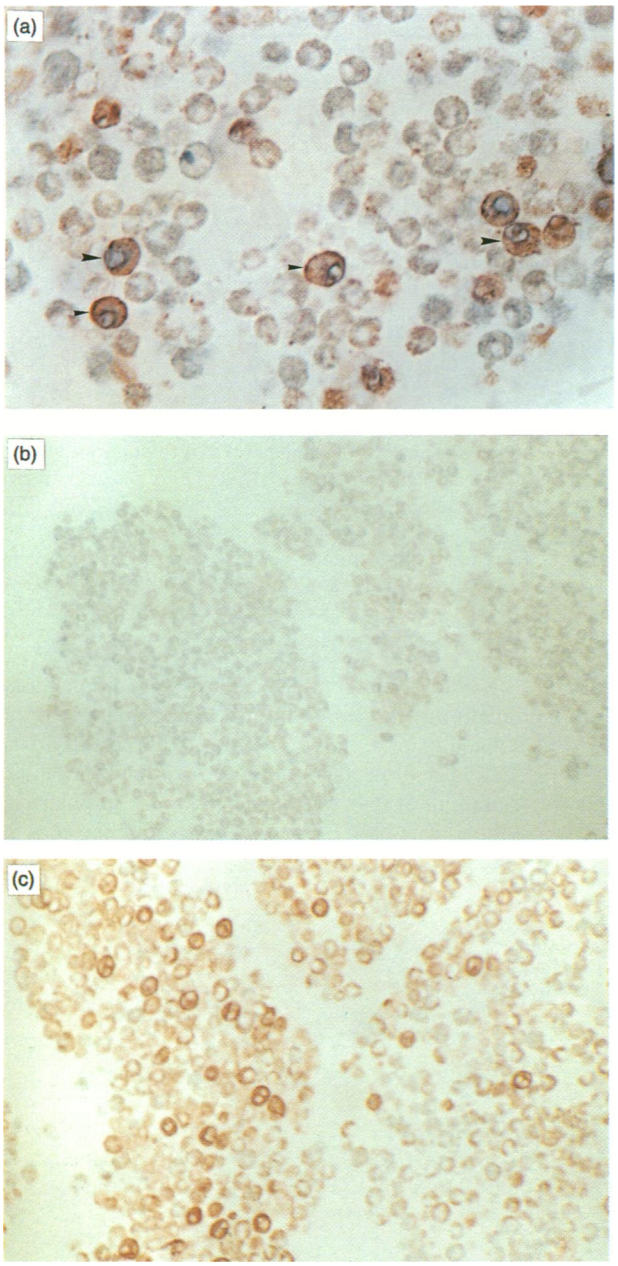

Figure 3. Immunochemical detection of IL-2 on 2 μM serial sections of GMA-embedded purified blood eosinophils from a severe asthmatic. Panel a, anti-IL-2 (original magnification × 500); panel b, anti-CD-3 (×125); and panel C, EG2 (×200). Large arrows indicate positive cells with cytoplasmic staining and thin arrows indicate positive cells with membrane-like staining.

GM-CSF alone or with ionophore A23187 compared to medium alone. Similar results were obtained with two asthmatic subjects (data not shown).

In-cell RT-PCR and *in situ* hybridization

An in-cell RT-PCR was performed on purified blood eosinophils using IL-2 cDNA-specific primers. The IL-2 cDNA PCR product present in the cells was detected by *in situ* hybridization. Positive cells were found only in eosinophils stimulated by GM-CSF and calcium ionophore or GM-CSF

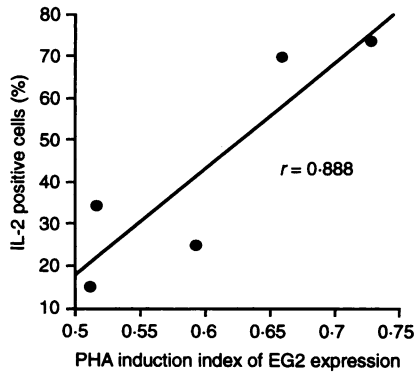

Figure 4. Correlation between the percentage of IL-2-positive eosinophils after PHA stimulation and the PHA induction index of eosinophil EG2 expression in severe asthmatics. PHA induction index of EG2 expression formula: (% of EG2-positive cells with PHA) − (% of EG2-positive cells without PHA)/(% of EG2 positive cells without PHA).

alone (Fig. 2a–c). No contaminating neutrophils were positive to IL-2 mRNA expression. No non-specific labelling were found as evaluated with purified eosinophils cultured in RPMI (Fig. 2a). According to the number of grains counted in positive eosinophils, the amount of hybridized IL-2 cDNA probes detected by *in situ* hybridization increased by twofold following GM-CSF/A23187 stimulation when compared with GM-CSF stimulation alone (Fig. 2d).

Immunochemical detection of IL-2

The expression of IL-2 was detected by immunochemistry on serial sections of purified eosinophils using a specific monoclonal antibody (Fig. 3a). The majority of positive cells (mean ± SEM), (17 ± 6%) had an IL-2 cytoplasmic staining. Some strongly positive cells also show a focal plasma membrane-like staining. No cells were positive to CD3 (Fig. 3b). Figure 3b also shows no residual endogenous eosinophil

Table 1. Immunochemistry analysis of purified blood eosinophils

		EG2		IL-2	
Subject	Clinical status	PHA$^-$	PHA$^+$	PHA$^-$	PHA$^+$
1	Atopic	56·7*	97·8	3·8	16·3
2	Mild asthma	87·5	92·8	0·9	27·4
3	Mild asthma	81·0	98·5	5·9	77·5
4	Severe asthma	53·8	81·3	7·5	15·2
5	Severe asthma	61·2	94·3	18·2	34·5
6	Severe asthma	57·1	90·9	11·0	25·0
7	Severe asthma	57·3	95·0	13·2	69·8
8	Severe asthma	53·8	92·9	73·5	19·0
9	Severe asthma	96·2	ND	20·8	ND
10	Severe asthma	78·0	ND	43·0	ND

*Results represent percentage of positive eosinophils: EG2, cells positive to ECP and EDN using the EG2 monoclonal antibody; IL-2, cells positive to IL-2 using an anti-IL-2 monoclonal antibody. Cells were incubated for 16 hr in RPMI with or without (+/−) 10 μg/ml PHA; ND, not determined.

peroxidase activity. The percentage of positive cells to IL-2 was increased after PHA stimulation (36 ± 8%, $P = 0{\cdot}005$) (Table 1). In all preparations, over 50% of the cells were positive to EG2 which identifies eosinophil cationic protein and eosinophil-derived neurotoxin in activated eosinophils.[27] The number of EG2-positive cells further increased after the PHA stimulation (93 ± 2%, $P = 0{\cdot}002$). In purified eosinophils of severe asthmatics, the percentage of positive cells to IL-2, after PHA stimulation, correlates with the induction of EG2 expression ($r = 0{\cdot}89$) (analysis of variance $P = 0{\cdot}04$) (Fig. 4).

IL-2 detection in the supernatant

Variable amount of IL-2 was detected by ELISA in some experiments, irrespective of the stimulus used, the incubation time and the donor status. The mean (± SEM) IL-2 detected by ELISA (Genzyme, USA) on eosinophil supernatants stimulated with GM-CSF and A23187 was 210 ± 100 pg/ml ($n = 7$). Since ELISA results remained variable, supernatants of eosinophils stimulated with GM-CSF, A23187, or PHA were assessed by ELISA using a monoclonal antibody for IL-2 detection ($n = 17$). In these assays, no IL-2 was detected, and no degradation of internal IL-2 standard was found.

DISCUSSION

Lymphocytes and eosinophils are intimately associated in allergic and asthmatic inflammatory processes.[2] Lymphocytes release cytokines which modulate eosinophil functions.[6–8] Eosinophils have been shown to produce many cytokines, including the Th2 cytokines IL-5 and IL-6.[10–18] This is the first demonstration of the capacity of the human eosinophils to produce IL-2, a Th1-type cytokine. This potent cytokine was hitherto believed to be produced exclusively by T lymphocytes.[19] The demonstration of IL-2 production by eosinophils suggests that these cells may activate lymphocytes in asthma and allergic diseases. This mode of amplifying lymphocytes via IL-2 originating from eosinophils could be a crucial factor sustaining the inflammatory process in tissues where these cells are seen.

The RT-PCR technique allowed a clear amplification of IL-2 cDNA from total RNA of blood eosinophils. We used highly purified eosinophil preparations where neutrophils were the only identified contaminating cells. However, since PCR is a powerful technique which can detect low levels of mRNA, detection of some IL-2 originating from very few unidentified contaminating cells remains theoretically possible. Thus the relative amount of IL-2 cDNA detected in these experiments should be interpreted cautiously. The in-cell/RT-PCR combined with *in situ* hybridization confirmed that eosinophils were the major source of IL-2 mRNA in these preparations. The GM-CSF stimulation alone was sufficient to detect IL-2 cDNA in those eosinophils. The increase in the level of IL-2 cDNA detected by *in situ* hybridization suggests that the steady-state level of IL-2 mRNA in eosinophils was up-regulated after costimulation with A23187 and GM-CSF.

The signal transduction of GM-CSF leading to IL-2 gene transcription is not fully understood. Recombinant hGM-CSF induces c-*fos* and c-*jun* gene transcription.[29] Accordingly, the higher level of IL-2 mRNA on GM-CSF-treated blood eosinophils may be due to an increase in AP-1 activity, resulting in an increase in IL-2 gene transcription; other nuclear factors may also be implicated. Alternatively, GM-CSF may additionally increase the amount of IL-2 mRNA by affecting their stability.

Immunostaining clearly demonstrated the presence of IL-2 in the cytoplasm of the purified eosinophils incubated with or without PHA stimulation. The correlation between the PHA induction of EG2 and IL-2 expressions after PHA strongly suggests that the eosinophils are activated by PHA and that this activation leads to an increased IL-2 expression. The presence of IL-2 in eosinophil cytoplasm is confirmed by works of Levi-Schaffer *et al.*[30] where IL-2 was detected by specific ELISA in unstimulated, highly purified disrupted eosinophils obtained from mild atopic asthmatic subjects. Furthermore, fractionation of the eosinophil population as well as immunogold labelling revealed that IL-2 was associated with the crystalline core of eosinophil granules.

The release of cytokine in supernatants of eosinophil suspensions was not steadily detected under the *in vitro* conditions used in this study. The degradation of secreted IL-2 by oxygen radicals, lytic enzymes, or proteases is very unlikely since no degradation of exogenous IL-2 was observed. They are possible explanations for the poor IL-2 secretion observed. First, the eosinophil is mostly a tissue-dwelling cell where it interacts with resident and/or inflammatory cells and extracellular matrix components. Therefore, eosinophils may release IL-2 only under specific physiological conditions found in tissue and not addressed by our experiments. Second, it is generally accepted that cytokines expressed by lymphocytes are secreted and act mainly by paracrine, autocrine and/or endocrine stimulation. However, the modes of intercellular signalling vary according to the target cell type. In juxtacrine intercellular signalling, the cytokine remains associated to the signalling cells providing a strict spatial control of the activation on the targeted cells.[31,32] The expression of tumour necrosis factor-α on human immunodeficiency virus-infected T cells and IL-8 on endothelial cells to activate B cells and adherent neutrophils respectively are suggested examples of this signalling pathway.[33,34] The focal membrane-like staining observed in highly purified eosinophils suggest that these cells could present IL-2 on their surface to activate lymphocyte by juxtacrine signalling in a localized cell to cell induction manner. Such interactions between eosinophils, lymphocytes and other tissue cell types and components need to be further documented.

In conclusion, using a combination of RT-PCR, *in situ* hybridization on in-cell RT-PCR and immunochemistry analysis on purified CD3-negative eosinophil preparations, this study shows that eosinophils produce IL-2, a potent lymphocyte activator. The ability of eosinophils to express IL-2 may play an important role in chronic diseases like asthma, where lymphocytes and eosinophils closely interact, enhancing and sustaining the inflammatory process possibly in a juxtacrine fashion.

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REFERENCES

1. Azzawi M., Bradley B., Jeffrey P.K. *et al.* (1990) Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* **142,** 1407.
2. Frigas E. & Gleich G.J. (1986) The eosinophil and the pathophysiology of asthma. *J Allergy Clin Immunol* **77,** 527.
3. Robinson D.S., Hamid Q., Ying S. *et al.* (1992) Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* **326,** 298.
4. Sousa A.R., Poston N.R., Lane S.J., Nakhosteen J.A. & Lee T.H. (1993) Detection of GM-CSF in asthmatic bronchial epithelium and decrease by corticosteroids. *Am Rev Respir Dis* **147,** 1557.
5. Hamid Q., Azzawi M., Ying S. *et al.* (1991) Expression of mRNA for interleukin-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* **87,** 1541.
6. Lopez A.F., Sanderson C.J., Gamble J.R., Campbell H.D., Young I.G. & Vadas M.A. (1988) Recombinant human interleukin-5 is a selective activator of human eosinophil function. *J Exp Med* **167,** 219.
7. Silberstein D.S., Owen W.F., Gasson J.C. *et al.* (1986) Enhancement of human eosinophil cytotoxicity and leukotriene synthesis by biosynthetic (recombinant) granulocyte/macrophage colony stimulating factor. *J Immunol* **137,** 3290.
8. Owen W.F., Rothenberg M.E., Silberstein D.S. *et al.* (1987) Regulation of human eosinophil viability, density, and function by granulocyte/macrophage colony stimulating factor in the presence of 3T3 fibroblasts. *J Exp Med* **166,** 129.
9. Makino S. & Fukuda T. (1993) *Eosinophils: Biological and Clinical Aspects.* 453 p., CRC Press Inc., Boca Raton, FL.
10. Weller P.F., Rand T.H., Barret T., Elovic A., Wong D.T. & Finberg R.W. (1993) Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 alpha expression. *J Immunol* **150,** 2554.
11. Moqbel R., Hamid Q., Ying S. *et al.* (1991) Expression of mRNA and immunoreactivity for the granulocyte/macrophage colony-stimulating factor in activated human eosinophils. *J Exp Med* **174,** 749.
12. Desreumaux P., Janin A., Colombel J.F. *et al.* (1992) Interleukin-5 messenger RNA expression by eosinophils in the intestinal mucosa of patients with coeliac disease. *J Exp Med* **175,** 293.
13. Broide D.H., Paine M.M. & Firestein G.S. (1992) Eosinophils express interleukin-5 and granulocyte/macrophage-colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics. *J Clin Invest* **90,** 1414.
14. Hamid Q., Barkans J., Meng Q. *et al.* (1992) Human eosinophils synthesize and secrete interleukin-6, *in vitro*. *Blood* **80,** 1496.
15. Ohno I., Lea G.R., Finotto S. *et al.* (1991) Granulocyte/macrophage colony-stimulating factor (GM-CSF) gene expression by eosinophils in nasal polyposis. *Am J Respir Cell Mol Biol* **5,** 505.
16. Costa J.J., Matossian K., Resnick M.B. *et al.* (1993) Human eosinophils can express the cytokine tumor necrosis factor-alpha and macrophage inflammatory protein-1 alpha. *J Clin Invest* **91,** 2673.
17. Wong D.T.W., Weller P.F., Galli S.J. *et al.* (1990) Human eosinophils express transforming growth factor α. *J Exp Med* **172,** 673.
18. Ohno I., Lea G.R., Flanders K.C. *et al.* (1992) Eosinophils in chronically inflamed human upper airway tissues express transforming growth factor β1 gene (TGFβ1). *J Clin Invest* **89,** 1662.
19. Sabath D.E. & Prystowsky M.B. (1990) The molecular basis of interleukin-2 action. In: *Lymphokines and the Immune Response.* (ed S. Cohen), p. 181. CRC Press Inc., Boca Raton, FL.
20. Rand T.H., Silberstein D.S., Kornfeld H. & Weller P.F. (1991) Human eosinophils express functional interleukin-2 receptors. *J Clin Invest* **88,** 825.
21. Hansel T.T., de Vries I.J.M., Iff T. *et al.* (1991) An improved immunomagnetic procedure for the isolation of highly purified human blood eosinophils. *J Immunol Meth* **145,** 105.
22. Laviolette M., Bossé M., Rocheleau H., Lavigne S. & Ferland C. (1993) Comparison of two modified techniques for purifying blood eosinophils. *J Immunol Meth* **165,** 253.
23. Chomczynski P. & Sacchi N. (1987) Single-step method of RNA isolation by guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162,** 156.
24. Haase A.T., Retzel E.F. & Stastus K.A. (1990) Amplification and detection of lentiviral DNA inside cells. *Proc Natl Acad Sci* **87,** 4971.
25. Nuovo G.J., Gallery F., MacConell P., Becker J. & Bloch W. (1991) An improved technique for the *in situ* detection of DNA after polymerase chain reaction amplification. *Am J Pathol* **139,** 1239.
26. Embleton M.J., Gorochov G., Jones P.T. & Winter G. (1992) In-cell PCR from mRNA: amplifying and linking the rearranged immunoglobulin heavy and light chain V-genes within single cells. *Nucl Acids Res* **20,** 3831.
27. Tai P.C., Spry C.J.F., Peterson C., Venge P. & Olsson I. (1984) Monoclonal antibodies distinguish between storage and secreted forms of eosinophil cationic protein. *Nature* **309,** 182.
28. Lucey D.R., Dorskyf D.I., Nicholson-Weller A. & Weller P.F. (1989) Human eosinophils express CD4 protein and bind HIV-1 GP120. *J Exp Med* **169,** 327.
29. Sato N., Sakamaki K., Terada N., Arai K.I. & Miyajima A. (1994) Signal transduction by the high-affinity GM-CSF receptor: two distinct cytoplasmic regions of the common β subunit responsible for different signaling. *EMBO J* **12,** 4181.
30. Levi-Schaffer F., Barkans J., Newman T.M. *et al.* (1995) Identification of interleukin-2 (IL-2) in human peripheral blood eosinophils. *J Allergy Clin Immunol* **95,** 342.
31. Bosenberg M.W. & Massague J. (1993) Juxtacrine cell signaling molecules. *Curr Opin Cell Biol* **5,** 832.
32. Zimmerman G.A., Lorant D.E., McIntyre T.M. & Prescott S.M. (1993) Juxtacrine intercellular signaling: another way to do it. *Am J Respir Cell Mol Biol* **9,** 573.
33. Macchia D., Almerigogna F., Parronchi P., Ravina A., Maggi E. & Romagnani S. (1993) Membrane tumour necrosis factor-α is involved in the polyclonal B-cell activation induced by HIV-infected human T cells. *Nature* **363,** 464.
34. Rot A. (1992) Endothelial cell binding of NAP-1/IL-8: role in neutrophil emigration. *Immunol Today* **13,** 291.