

Expression of the neutrophil-activating CXC chemokine ENA-78/CXCL5 by human eosinophils

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Summary

Background Eosinophils are seen at sites of inflammation in diseases such as helminthic infestation, asthma, ulcerative colitis and some neoplastic diseases. They are also associated with connective tissue remodelling, for example in longstanding asthma. In the present study, we investigated whether eosinophils express the CXC chemokine epithelial cell-derived neutrophil activating peptide (ENA-78/CXCL5), a chemokine that can activate neutrophils and in addition possesses angiogenic properties.

Immunocytochemistry detected CXCL5 in eosinophils and the peptide was localized in the specific granules by immunoelectron microscopy.

Methods and Results In eosinophil lysates, 12 ± 2 pg (mean $\pm$ SEM) of CXCL5 was detected per 10^6 cells by enzyme-linked immunosorbent assay (ELISA). Weak constitutive expression of CXCL5, as well as the related CXC chemokine IL-8/CXCL8, could be detected in freshly isolated eosinophils by RT-PCR. However, during prolonged incubation of eosinophils, a strong increase in both CXCL5 and IL-8/CXCL8 expression was seen, as detected by RT-PCR, and increasing amounts of CXCL5 peptide with time were detected in the incubation medium by ELISA. Addition of TNF- α neutralizing antibodies during prolonged incubation significantly inhibited CXCL5 production, demonstrating involvement of auto- and paracrine effects from TNF- α produced by eosinophils themselves. Addition of IFN- γ showed a strong inhibitory effect on CXCL5 synthesis.

Conclusion These findings suggest that, through expression of CXCL5, eosinophils can recruit and activate CXC receptor 2 (CXCR2)-bearing cells such as neutrophils at sites of inflammation. Eosinophils may also promote connective tissue remodelling through release of this peptide.

Keywords allergy, chemokine, eosinophils

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Introduction

Eosinophils are a typical component of the inflammatory response seen in diseases such as allergic asthma, ulcerative colitis, helminthic infestation and some neoplastic diseases [1]. In many conditions where eosinophils accumulate, there are also neutrophils, mast cells and macrophages [2]. As eosinophils may have a long lifespan in tissues and can elaborate several cytokines and chemokines, they are likely to have regulatory functions and interact with other immune cells at sites of inflammation [3].

Chemokines are a superfamily of proinflammatory peptides that attract and activate leucocytes by binding to G protein-coupled receptors on the cell surface. They can be divided into four subfamilies – C, CC, CXC and C(X)₃C – based on the arrangement of conserved cysteine residues [4]. The CXC subfamily can be divided further into ELR⁺ and ELR[−] based on the presence or absence of the sequence motif of glutamic

acid–leucine–arginine (ELR) N-terminal to the first cysteine [4]. All ELR⁺ CXC chemokines attract and activate human neutrophils *in vitro*. Epithelial cell-derived neutrophil-activating peptide (ENA-78/CXCL5) is a CXC chemokine that is structurally and functionally related to another CXC chemokine, IL-8/CXCL8 [5]. Like IL-8/CXCL8, it is a potent neutrophil-activating peptide, inducing chemotaxis, cytoskeletal reorganization and modulation of neutrophil adhesion molecule expression [6]. However, while IL-8/CXCL8 binds to both CXC receptor 1 (CXCR1) and CXCR2, CXCL5 binds only to CXCR2, which may result in different responses of the target cells [7]. In addition to their neutrophil-activating properties, ELR⁺ CXC chemokines are potent angiogenic factors that can regulate endothelial cell proliferation [8,9]. This may be important in connective tissue remodelling and tumour growth.

In this study, we show that eosinophils produce CXCL5, and they may therefore contribute to recruitment and activation of neutrophils at sites of inflammation. This finding can also be one of the explanations for effects from eosinophils seen in connective tissue remodelling and promotion of tumour growth.

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Materials and methods

Special reagents

Recombinant human eotaxin, IL-5, IFN- γ , IL-1 β , TNF- α , monoclonal antibodies against CXCL5, IL-1 α , TNF- α isotype-matched control antibodies and enzyme-linked immunosorbent assay (ELISA) kits for the measurement of CXCL5 and TNF- α were purchased from R & D Systems Europe (Abingdon, UK). Actinomycin D and cycloheximide were from Sigma (St Louis, MO, USA).

Isolation of cells

Blood was obtained from volunteers, either healthy or allergic, after informed consent and approval of the protocol by the ethical committee at Lund University (no. LU 724-01). Some donors were characterized with respect to allergy, including history of allergic symptoms, measurement of total IgE, eosinophil blood counts and specific IgE antibodies against grass and tree pollen allergen, to confirm absence or presence of allergy. Eosinophils were isolated essentially as previously described [10]. Mononuclear cells were removed by centrifugation over Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden). After lysis of the erythrocytes, the granulocytes were washed in Hank's balanced salt solution (HBSS) without calcium and magnesium. Immunomagnetic beads coated with antibodies to CD16 (Miltenyi, Gladbach, Germany) were used to retrieve the neutrophils in a magnetic column, allowing the isolation of highly purified eosinophils (viability was >99% as judged by trypan blue exclusion, and purity >98%; contaminating cells were lymphocytes and neutrophils, as judged by May-Grünwald-Giemsa staining).

Immunocytochemistry

Purified eosinophils were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, dehydrated and embedded in paraffin. Immunohistochemistry was performed essentially as described [11]. In short, sections 3 μ m in thickness were cut and after deparaffinization and rehydration, these were incubated with an antibody against CXCL5 or an isotype-matched irrelevant monoclonal antibody. This was followed by incubation with a secondary ALP-conjugated goat anti-mouse antibody (Dakopatts, Glostrup, Denmark). To visualize bound antibodies, the sections were developed by exposure to a mixture containing naphthol phosphate, fast red and levamisole (all from Sigma). The sections were counterstained with Mayer's haematoxylin.

Immunoelectron microscopy

Purified eosinophils were fixed for 24 h in 4% paraformaldehyde in PHEM buffer (120 mM PIPES, 50 mM HEPES, 8 mM MgCl₂, 40 mM EGTA, pH 6.9) and then processed for ultrathin cryosectioning as previously described [12]. Forty-five-nanometer cryosections were cut at -125 °C using diamond knives (Drukker, Cuijk, the Netherlands) in an ultracryomicrotome (Leica, Vienna, Austria) and transferred with a mixture of sucrose and methylcellulose onto formvar-coated copper grids [13]. The grids were placed on 35-mm Petri dishes containing 2% gelatine. Cryosections were incubated with a specific monoclonal antibody against CXCL5, followed by incubation with rabbit anti-mouse IgG and then incubated with 10-nm protein

A-conjugated colloidal gold (EM Laboratory, Utrecht University, the Netherlands). After immunolabelling, the cryosections were embedded in a mixture of methylcellulose and uranyl acetate and examined with a Philips CM 10 electron microscope (Philips, Eindhoven, the Netherlands). For the control, an isotype-matched monoclonal antibody replaced the specific primary antibody.

Measurement of CXCL5 in cell lysates and in supernatants

The CXCL5 and TNF- α content of medium and cell lysates were measured by ELISA. The measurements were performed in duplicate. According to the manufacturer, the CXCL5 ELISA does not recognize related peptides such as IL-8/CXCL8, GRO- α , GRO- β , GRO- γ , MCP-1, MIP-1 α , MIP-1 β , or RANTES. The range of the CXCL5 ELISA was 15–2000 pg/mL and the range of the TNF- α ELISA was 4–1000 pg/mL.

Prolonged incubation

Eosinophils were incubated in 24- or 96-well plates (Nunc, Roskilde, Denmark) at 2×10^6 cells/mL, in RPMI-1640 (Gibco, Life Technologies, Täby, Sweden) supplemented with 10% fetal calf serum (FCS) and gentamycin at 10 μ g/mL. After incubation for 16, 24 or 48 h at 37 °C in a 5% CO₂ atmosphere, the cells were pelleted by centrifugation. The cell viability was >98% after 16 h, >93% after 24 h and >88% after 48 h of incubation, as judged by trypan blue exclusion. In selected experiments, eosinophils were incubated in the presence or absence of the following cytokines: TNF- α (10 ng/mL), IFN- γ (500 U/mL), eotaxin (6 nM), or IL-5 (1 nM) or the following monoclonal antibodies: TNF- α or IL-1 α neutralizing antibody (10 μ g/mL) or irrelevant isotype-matched control antibody (10 μ g/mL). The cell-free supernatants were collected from each well after centrifugation at 500 g for 7 min, and thereafter 1% (v/v) Triton X-100 and 10 mM benzamidinium in PBS was added to each well to lyse the remaining cells. The duration of cell lysis was 20 min on ice. The cell lysates were cleared from insoluble matter by centrifugation at 21000 g for 10 min. The supernatants and cell lysates were stored at -80 °C until assayed by ELISA. All experiments were performed in duplicate.

Detection of CXCL5 and IL-8/CXCL8 expression by RT-PCR

Total cellular RNA was isolated using a kit based on a modified single-step procedure by acid guanidinium thiocyanate-phenol-chloroform extraction (Total RNA Isolation kitTM; BD Biosciences, Stockholm, Sweden). RT-PCR to detect CXCL5 expression was performed essentially as described previously [14]. The primer sequences for detection of CXCL5 expression by RT-PCR are intron-spanning. The primer sequences for CXCL5 were 5'-CTGTGTTGAGAGAGCTGC-GTTGC-3' (sense) and 5'-GTTTTCCTTGTTTCCACCGTCC 3' (antisense), generating a product of 216 bp, and the intron-spanning primer sequences for IL-8/CXCL8 were 5'-ATGACTTCCAAGCTGGCCGTG-3' and 5'-GGAG-TATGTCTTTATGCACTGACATCTA-3', generating a product of 122 bp. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control. The intron-spanning GAPDH primers were 5'-ACCACCATG-GAGAAGGCTGG-3' (sense) and 5'-CACAGTGTAGCC-CAGGAT-GC-3' (antisense), generating a product of 530 bp.

The RT-PCR reactions were performed using pre-mixed, pre-dispensed reactions tubes to which 100 ng of RNA and primer-pairs were added (Ready-To-Go RT-PCR Beads™; Pharmacia, Uppsala, Sweden). Ten-microlitre samples of the PCR-reaction mixtures were loaded on a 2% agarose gel and stained with ethidium bromide. The PCR products were detected by UV light in a computer-based gel-documentation system (Gel Doc 2000; Bio-Rad, Hercules, CA, USA).

In situ hybridization

Detection of CXCL5 gene expression on a single cell level was performed on cytospin preparations of eosinophils that had been incubated for 16 h by use of a digoxigenin-labelled 36-mer oligonucleotide with the sequence 5'-GCCAGGGCC-CATCGCCAGCGCT- GGTCCTGCCGCTGC-3'. This oligonucleotide was searched to exclude significant similarities with other human nucleotide sequences using the software BLAST version 2.0.10 [15]. A sense oligonucleotide served as a negative control. *In situ* hybridization with digoxigenin-labelled oligoprobes was performed as described previously [16]. In short, slides were hybridized with probe (200 ng/mL)

overnight at +30 °C and thereafter washed at highly stringent conditions. To detect hybridization signals, the sections were incubated with alkaline phosphatase-conjugated Fab fragments from antidigoxigenin IgG (Roche). After thorough washing, sections were developed by exposure to nitroblue tetrazolium chloride and levamisole. For control purposes, adjacent sections were hybridized with digoxigenin-labelled oligo-dT to ensure successful fixation and hybridization.

Statistics

Student's *t*-test for paired data was used for statistical calculations. Statistical significance was set at $P=0.05$, $P \leq 0.01$ and $P \leq 0.005$.

Results

Detection of CXCL5 in freshly isolated eosinophils

Sections of freshly isolated eosinophils showed immunologically detectable CXCL5 peptide by immunohistochemistry (Fig. 1a). Replacement of the specific primary antibody with

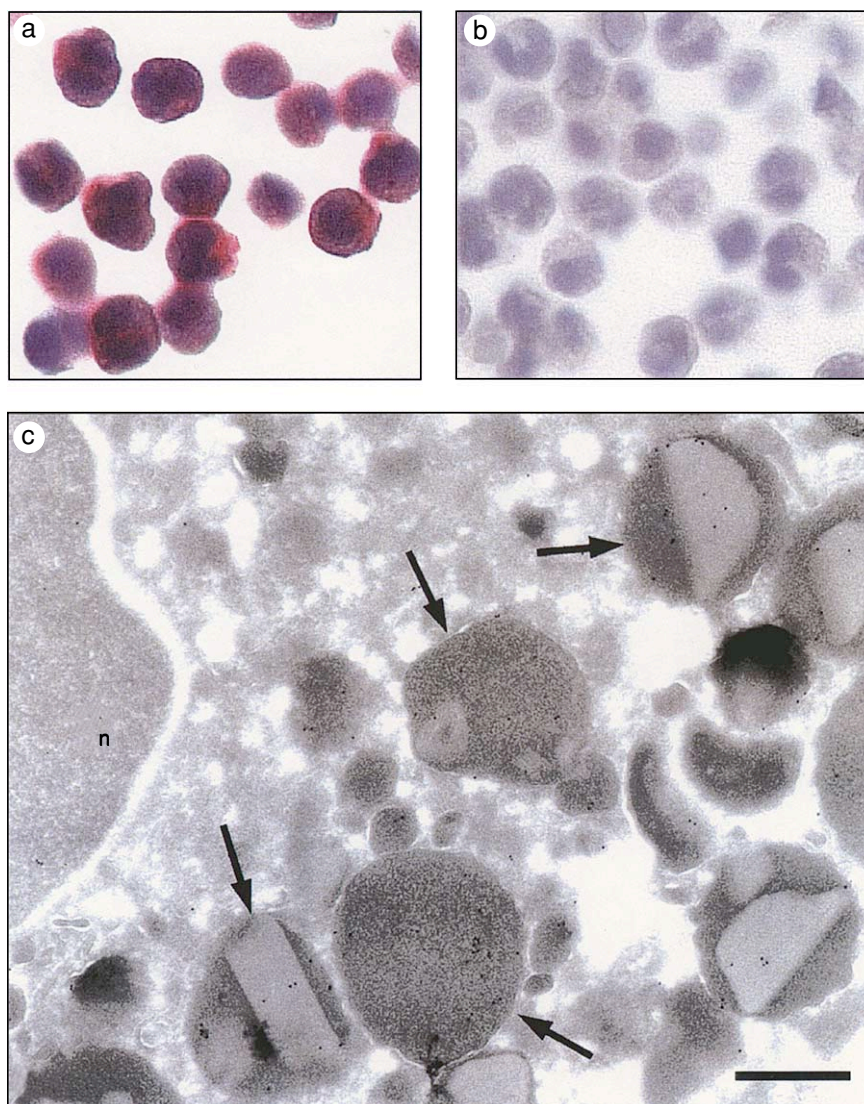


Fig. 1. Detection of CXCL5 in eosinophils by immunocytochemistry and immunoelectron microscopy. (a) Immunocytochemical detection of CXCL5 in eosinophils. Sections of purified eosinophils were incubated with a monoclonal antibody against CXCL5 and bound antibodies were visualized by a secondary alkaline phosphatase reaction resulting in red staining of the cells. (b) Identical incubation of sections as in (a) but the primary antibody has been replaced by an irrelevant isotype-matched monoclonal antibody, resulting in loss of staining. (c) Electron micrograph showing intracellular distribution of CXCL5 in freshly isolated eosinophils. Ultrathin cryosections of eosinophils were incubated with monoclonal antibody against CXCL5 and 10 nm protein A-gold. Labelling is seen within the specific granules of the cytoplasm. Bar = 400 nm.

an irrelevant isotype-matched antibody resulted in loss of staining (Fig. 1b). Subcellular localization of CXCL5 in eosinophils was performed by use of immunogold electron microscopy on eosinophils fixed immediately after isolation. CXCL5 was detected in the specific granules of the cytoplasm (Fig. 1c). Replacement of the specific primary antibody with an irrelevant isotype-matched antibody resulted in loss of labelling (not shown).

In homogenates of freshly isolated eosinophils, 12 ± 2 pg (mean $\pm$ SEM, $n = 3$) of preformed CXCL5 was detected per 10^6 cells by ELISA.

Increased expression and release of CXCL5 during prolonged incubation

During prolonged incubation, increasing amounts of CXCL5 were detected in the medium by ELISA (Fig. 2a). There was also a slight increase in the intracellular content as measured in cell homogenates. However, the intracellular content accounted only for a small fraction of the total CXCL5 content during prolonged incubation and the main portion of the newly synthesized CXCL5 was released into the medium. In addition, incubation of eosinophils in the presence of actinomycin D and cycloheximide, inhibitors of gene transcription and translation, respectively, resulted in non-detectable levels of CXCL5 in the medium (data not shown). This further demonstrates that the released CXCL5 is mainly synthesized *de novo*.

Weak constitutive CXCL5 gene expression was detected by RT-PCR using RNA isolated from freshly isolated blood eosinophils (Fig. 2b). In addition, a higher constitutive expression of the related CXC chemokine IL-8/CXCL8 was detected. After 16 h of incubation, a strong increase in gene expression of both CXCL5 and IL-8/CXCL8 was seen (Fig. 2b). The degree of constitutive expression or the size of increase with respect to gene expression during prolonged incubation was not related to the presence or absence of allergy (not shown). CXCL5 gene expression increased by 4, 8 and 16 h, as detected by RT-PCR (Fig. 2c). Cytospin preparations of eosinophils were subjected to *in situ* hybridization to detect expression of CXCL5 after 16 h of incubation. There was no marked difference between cells, thus showing that the whole population of eosinophils, and not only a subpopulation, increased their CXCL5 expression (Fig. 2d). As a control, incubation of eosinophils with a complementary oligonucleotide showed a weak signal representing background (Fig. 2e).

Auto- and paracrine effects on CXCL5 expression from TNF- α

TNF- α and IL-1 can induce expression of CXCL5, at least in some cells [17]. Because eosinophils can elaborate both TNF- α and IL-1 α and possess the corresponding receptors, we investigated whether TNF- α or IL-1 α produced by eosinophils themselves could influence CXCL5 production in an auto- and

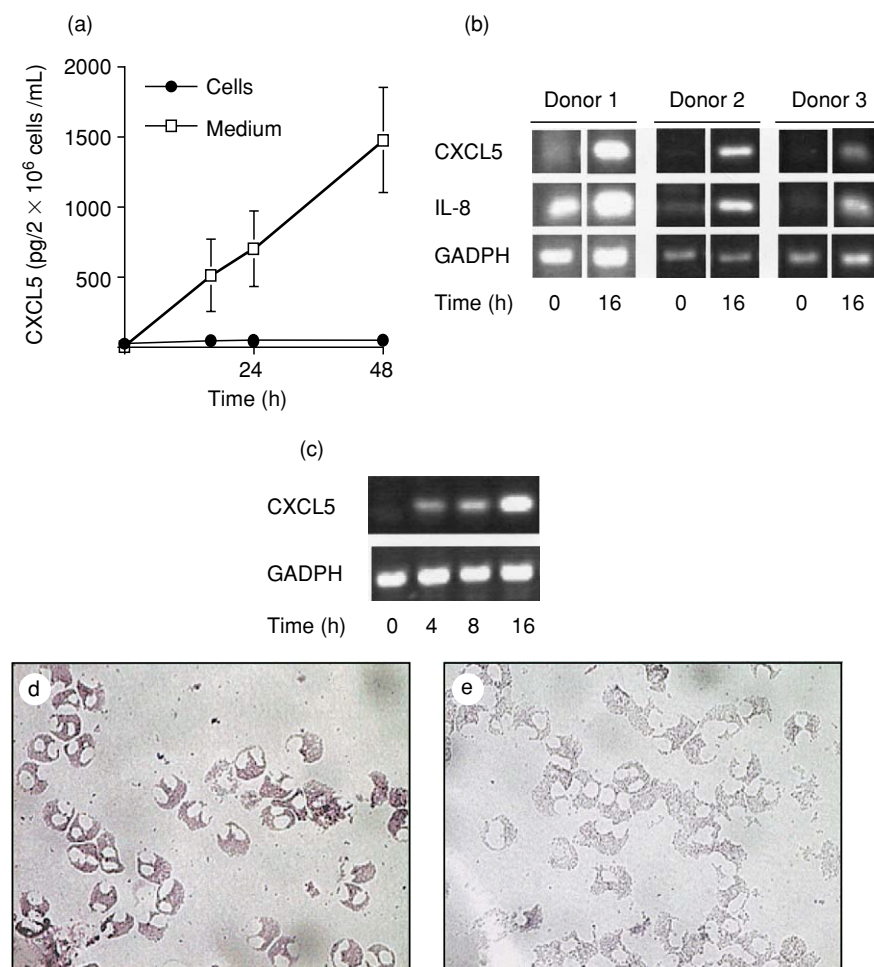


Fig. 2. Up-regulation of CXCL5 gene expression and synthesis during prolonged incubation *in vitro*. (a) Eosinophils (2×10^5 cells/mL) were incubated in medium. Supernatants and cell lysates were collected at the indicated time-points and the CXCL5 content was measured by ELISA. (b) RNA was isolated from freshly isolated eosinophils and after 16 h of incubation, RT-PCR was performed to detect transcripts for CXCL5, IL-8/CXCL8, and the housekeeping gene GADPH. The results obtained from three representative donors are shown. A strong increase in both CXCL5 and IL-8/CXCL8 expression was seen after 16 h of incubation *in vitro*. (c) Kinetics of the up-regulation of CXCL5 gene expression during prolonged incubation. RT-PCR was performed on RNA isolated at the indicated time-points. A gradual increase in CXCL5 gene expression was seen during the period observed. The CXCL5 expression in eosinophils from one representative out of three donors is shown. (d) Cytospin preparations of eosinophils after 16 h of incubation were subjected to *in situ* hybridization using a digoxigenin-labelled antisense oligonucleotide specific for the CXCL5 transcript. Hybridization signals are detected by an alkaline-phosphatase reaction product, resulting in a purple staining of the cytoplasm of the cells. (e) Cytospin preparation of eosinophils incubated with a control oligonucleotide, but otherwise treated as in (d), resulting in low hybridization signals, representing background.

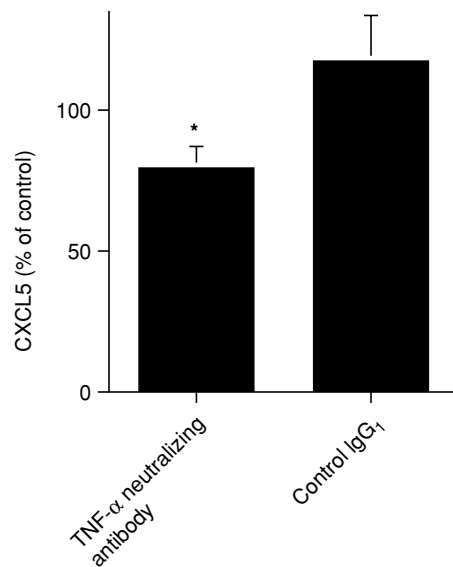


Fig. 3. Auto- and paracrine effects from TNF- α on CXCL5 expression. Eosinophils were incubated in medium alone, in the presence of a TNF- α -neutralizing monoclonal antibody (10 μ g/mL), and in the presence of an isotype-matched irrelevant monoclonal antibody (10 μ g/mL). The values obtained were normalized against the CXCL5 content of supernatants where cells were incubated in medium alone, which was set to 100%. The TNF- α -neutralizing antibody significantly inhibited CXCL5 production (* P < 0.05), while the control antibody had no significant effect. The data shown represent the mean $\pm$ SEM of six separate experiments.

paracrine manner during prolonged incubation [18–20]. The CXCL5 production by eosinophils in medium alone was compared with the production by cells in the presence of the TNF- α or IL-1 α neutralizing antibodies, or an isotype-matched control antibody, after 24 h of incubation. Incubation in the presence of the TNF- α -neutralizing antibody significantly reduced CXCL5 production, while the IL-1 α (not shown) or control antibodies did not have any significant effects (Fig. 3). In addition, significant amounts of TNF- α were detectable in culture supernatants after 24 h of incubation (data not shown).

Effects on eosinophil CXCL5 synthesis from cytokines

Eosinophils were incubated in medium alone or in the presence of the prototypic Th1 cytokines IFN- γ or TNF- α , and the eosinophil-activating Th2 cytokines eotaxin or IL-5 (Fig. 4) [21]. IFN- γ showed a strong inhibitory effect on CXCL5 expression, as detected by RT-PCR and measurement of the peptide in medium by ELISA (Figs 4a and b). IFN- γ did not negatively affect cell viability as judged by trypan blue exclusion test compared with the other cytokines tested or with cells in medium alone. TNF- α showed a non-significant trend towards a stimulatory effect on CXCL5 production as detected by ELISA (Fig. 4b). No certain effects on the level of CXCL5 gene expression could be seen after incubation of eosinophils in the presence of eotaxin or IL-5, as detected by RT-PCR (Fig. 4a).

Discussion

In the present study, we show that human eosinophils have a weak constitutive expression of the CXC chemokine CXCL5 and that the expression increases during prolonged incubation

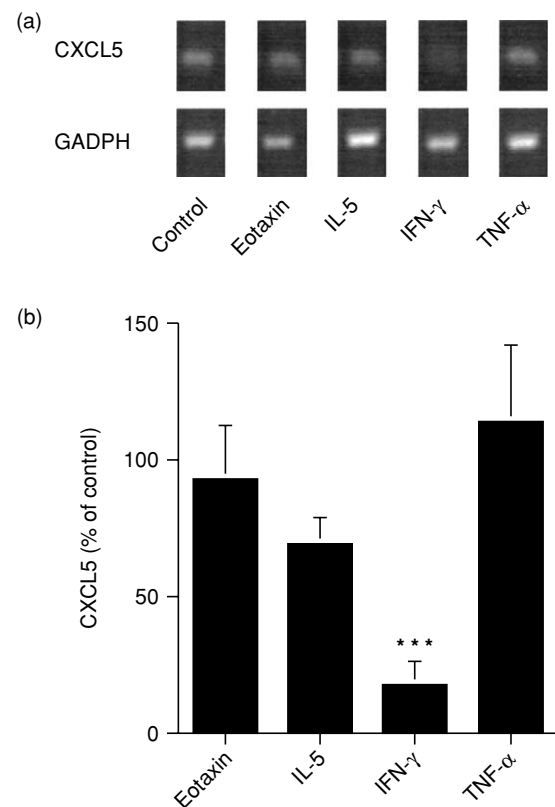


Fig. 4. Effects from eotaxin, IL-5, IFN- γ and TNF- α on CXCL5 expression. Eosinophils were incubated in medium alone or in the presence of eotaxin (1 nM), IL-5 (1 nM), IFN- γ (500 U/mL), or TNF- α (10 ng/mL). (a) RNA was isolated from eosinophils after 16 h of incubation and was subjected to RT-PCR. Cells incubated in the presence of IFN- γ showed a reduced expression of CXCL5 compared to the control, while no apparent effect was seen after incubation in the presence of the other cytokines investigated. The result shown is from one representative out of three experiments. (b) Supernatants were collected after 24 h of incubation and the CXCL5 content was determined by ELISA. The CXCL5 content in medium obtained during the various conditions used was normalized against the CXCL5 content obtained from cells incubated in medium alone (which was set to 100%). IFN- γ showed a strong inhibitory effect on CXCL5 production compared to cells incubated in medium alone (*** P < 0.001), while eotaxin, IL-5, or TNF- α had no significant effect. The data shown are the mean $\pm$ SEM of four separate experiments.

of eosinophils *in vitro*. Auto- and paracrine effects from TNF- α produced by eosinophils themselves can, at least in part, explain the increased expression. Furthermore, CXCL5 synthesis was down-regulated by IFN- γ , possibly via reduced production and release of TNF- α .

Instead of being only a target for cytokines produced by other cells, it has been shown in recent years that eosinophils can elaborate several cytokines and chemokines themselves, suggesting important immunoregulatory roles for these cells at sites of inflammation [3]. Eosinophils have been reported to express at least four different CC chemokines, viz. MCP-1, MIP-1 α , RANTES and eotaxin [22–24]. Among CXC chemokines, only expression of IL-8/CXCL8 has been reported hitherto in eosinophils [25, 26]. There are varying reports concerning the pre-formed content of IL-8/CXCL8 in eosinophils, from less than 3 pg/10⁶ cells to 70–140 pg/10⁶ cells, and the content of CXCL5 in this study (12 pg/10⁶) is in the range of other cytokines synthesized by these cells [3, 26, 27]. However, the *de novo* synthesis of IL-8/CXCL8 by mature eosinophils seems

to be relatively high compared to that reported for the CC chemokines [27,28]. Therefore, the relatively high expression of CXCL5 reported in the present study suggests that eosinophils may have a propensity for synthesis of CXC chemokines. This may be important at sites of inflammation and suggests important interactions with neutrophils, for example.

We found that the expression of CXCL5 was homogeneous among eosinophils both initially and after 16 h of incubation, and not only present in a subpopulation of cells, as detected by *in situ* hybridization. However, eosinophils isolated from peripheral blood are heterogeneous with respect to their state of activation, and a larger population of resting cells as well as a subpopulation with an activated phenotype can be identified [29,30]. In a previous report, IL-8/CXCL8 expression of freshly isolated eosinophils was detected only in cells from asthmatic patients, while we found a low constitutive expression of both CXCL5 and IL-8/CXCL8 regardless of the presence or absence of an atopic constitution of the donor [26]. The difference may be attributed to the sensitivity of the means of detection. The relatively low amounts of RNA and the high content of ribonucleases in mature eosinophils present difficulties when performing investigations based on techniques involving the isolation of mRNA such as RT-PCR, northern blot and ribonuclease protection assays [1].

Several factors may influence the up-regulation of CXCL5 gene expression *in vitro*. During prolonged incubation, a subpopulation of activated eosinophils may be important for the activation of neighbouring resting eosinophils. Eosinophils can express several cytokines for which they themselves possess the corresponding receptors, making auto- and paracrine stimulation possible. One example is the proinflammatory cytokine TNF- α , for which eosinophils have the corresponding receptors, TNF-receptor (R)I and TNF-RII [18,20].

Concomitant production of CXCL5 and IL-8/CXCL8 takes place in response to stimulation with TNF- α , and both genes are subject to regulation by the transcription factor NF- κ -B in transfected human embryonic 293 cells [17]. In the present study, we show that auto- and paracrine TNF- α production is of importance through the effect from added neutralizing antibodies against TNF- α . We were also able to detect significant amounts of TNF- α in the supernatants after incubation of cells (data not shown). Other factors such as homotypic aggregation or adhesion to surfaces may also influence gene expression in eosinophils [31,32]. The importance of eosinophil adhesion for autocrine production of granulocyte-macrophage colony-stimulating factor (GM-CSF) and thereby delayed apoptosis has been demonstrated *in vitro* [33].

At the moment, the only characterized receptor for CXCL5 is CXCR2, while IL-8/CXCL8 binds to both CXCR1 and CXCR2 [7]. The apparent redundancy among chemokines with respect to interaction with chemokine receptors may be present to assure important biological responses [34]. Eosinophils themselves do not possess CXCR1 and CXCR2 receptors and CXC chemokines seem to activate predominantly neutrophils, but CXC receptors are also expressed on other cells, e.g. monocytes/macrophages, T cells, NK cells, mast cells and endothelial cells [4,35]. On the other hand, CC chemokines, such as RANTES, MCP-4 and eotaxin, have eosinophils, basophils and T lymphocyte subsets as targets [4,36].

The cellular responses following stimulation of CXCR2 varies depending on the context in which it appears. For

example, CXCR2 is essential for neutrophil recruitment to the cornea in helminth-mediated keratitis (river blindness) using an animal model with gene-specific knockout mice [37]. In this model, eosinophils participate in the inflammatory response. In addition, using a synthetic and selective CXCR2 antagonist, neutrophil chemotaxis induced by IL-8/CXCL8 can be inhibited [38]. Activation of CXCR2 on mast cells results in a migratory response, while CXCR1 is not present on these cells [39]. The latter finding may have a bearing on the regulation of allergic inflammation, a context where presence of eosinophils is a hallmark.

In the present study, we found that IFN- γ down-regulated CXCL5 production. To investigate if the inhibitory effect from IFN- γ on CXCL5 production could be mediated by a decrease in TNF- α release, eosinophils were incubated in the absence or presence of IFN- γ . After 24 h of incubation, the TNF- α content of the supernatants was measured by ELISA. In the case of a higher TNF- α content in the supernatants of control cells, IFN- γ showed a strong inhibitory effect, whereas in the case of low TNF- α content, no firm conclusions could be made as to whether IFN- γ had any effect (data not shown). Therefore, further work is needed to elucidate how the inhibitory effect from IFN- γ is mediated.

Eosinophils are a characteristic component of the allergic inflammatory response, which is characterized by the presence of Th2-derived cytokines such as IL-5 and eotaxin [40]. IFN- γ is a typical component of the Th1 immune response and this cytokine can suppress allergic inflammation in favour of a Th1 response [21]. Similarly to what we found in eosinophils, IFN- γ down-regulates the production of CXCL5 in monocytes [41]. A possible role for eosinophils during allergic inflammation could be to recruit and activate neutrophils. Therefore, IFN- γ produced by macrophages or T cells may serve as a switch during the recruitment of inflammatory cells, down-regulating the release of neutrophil chemoattractants in favour of factors that attract monocytes and lymphocytes [41].

Taken together, the finding that eosinophils can express CXCL5 represents an additional biological effector function that can be exerted by these cells, connecting eosinophils to the recruitment and activation of neutrophils as well as connective tissue remodelling and interaction with tumours.

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References

- 1 Rosenberg HF. The eosinophil. In: Gallin, JI, Snyderman, R, eds. *Inflammation: Basic Principles and Clinical Correlates*, pp. 61–77. Philadelphia: Lippincott Williams & Wilkins, 1999.
- 2 Kay AB, Corrigan CJ. Asthma. Eosinophils and neutrophils. *Br Med Bull* 1992; 48:51–64.

- 3 Lacy P, Moqbel R. Eosinophil cytokines. *Chem Immunol* 2000; 76:134–55.
- 4 Murdoch C, Finn A. Chemokine receptors and their role in inflammation and infectious diseases. *Blood* 2000; 95:3032–43.
- 5 Walz A, Burgener R, Car B, Baggiolini M, Kunkel SL, Strieter RM. Structure and neutrophil-activating properties of a novel inflammatory peptide (ENA-78) with homology to interleukin 8. *J Exp Med* 1991; 174:1355–62.
- 6 Walz A, Schmutz P, Mueller C, Schnyder-Candrian S. Regulation and function of the CXC chemokine ENA-78 in monocytes and its role in disease. *J Leukoc Biol* 1997; 62:604–11.
- 7 Ahuja SK, Murphy PM. The CXC chemokine growth-regulated oncogene (GRO) α , GRO β , GRO γ , neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 are potent agonists for the type B, but not the type A, human interleukin-8 receptor. *J Biol Chem* 1996; 271:20545–50.
- 8 Strieter RM, Polverini PJ, Kunkel SL et al. The functional role of the ELR motif in CXC chemokine-mediated angiogenesis. *J Biol Chem* 1995; 270:27348–57.
- 9 Koch AE, Volin MV, Woods JM et al. Regulation of angiogenesis by the CXC chemokines interleukin-8 and epithelial neutrophil activating peptide 78 in the rheumatoid joint. *Arthritis Rheum* 2001; 44:31–40.
- 10 Hansel TT, De Vries IJM, Iff T et al. An improved immunomagnetic procedure for isolation of highly purified human blood eosinophils. *J Immunol Methods* 1991; 145:105–10.
- 11 Cordell JL, Falini B, Erber WN et al. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984; 32:219–29.
- 12 Egesten A, Calafat J, Knol EF, Janssen H, Walz TM. Subcellular localization of TGF- α in human eosinophil granulocytes. *Blood* 1996; 87:3910–8.
- 13 Liou W, Geuze HJ, Slot JW. Improving structural integrity of cryosections for immunogold labeling. *Histochem Cell Biol* 1996; 106:41–58.
- 14 Rieder G, Einsiedl W, Hatz RA, Stolte M, Enders GA, Walz A. Comparison of CXC chemokines ENA-78 and interleukin-8 expression in *Helicobacter pylori*-associated gastritis. *Infect Immun* 2001; 69:81–8.
- 15 Altschul SF, Madden TL, Sch  ffer AA et al. Gapped BLAST, PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997; 25:3389–402.
- 16 Bjartell A, Malm J, M  ller C, Gunnarsson M, Lundwall   , Lilja H. Distribution and tissue expression of Semenogelin I and II in man as demonstrated by *in situ* hybridization and immunocytochemistry. *J Androl* 1996; 17:17–26.
- 17 Chang M, McNinch J, Basu R, Simonet S. Cloning and characterization of the human neutrophil-activating peptide (ENA-78) gene. *J Biol Chem* 1994; 269:25277–82.
- 18 Costa JJ, Matossian K, Resnick MB et al. Human eosinophils can express the cytokines tumor necrosis factor- α and macrophage inflammatory protein-1 α . *J Clin Invest* 1993; 91:2673–84.
- 19 Weller PF, Rand TH, Barrett T, Elovic A, Wong DT, Finberg RW. Accessory cell function of human eosinophils. HLA-DR-dependent, MHC-restricted antigen-presentation and IL-1 α expression. *J Immunol* 1993; 150:2554–62.
- 20 Bracke M, van de Graaf E, Lammers JW, Coffey PJ, Koenderman L. *In vivo* priming of Fc ϵ R functioning on eosinophils of allergic asthmatics. *J Leukoc Biol* 2000; 68:655–61.
- 21 Lucey DR. Evolution of the type-1 (Th1)–type-2 (Th2) cytokine paradigm. *Infect Dis Clin North Am* 1999; 13:1–9.
- 22 Izumi S, Hirai K, Miyamasu M et al. Expression and regulation of monocyte chemoattractant protein-1 by human eosinophils. *Eur J Immunol* 1997; 27:816–24.
- 23 Ying S, Meng Q, Taborda-Barata L, Corrigan CJ et al. Human eosinophils express messenger RNA encoding RANTES and store and release biologically active RANTES protein. *Eur J Immunol* 1996; 26:70–6.
- 24 Nakajima T, Yamada H, Iikura M et al. Intracellular localization and release of eotaxin from normal eosinophils. *FEBS Lett* 1998; 434:226–30.
- 25 Braun RK, Franchini M, Erard F et al. Human peripheral blood eosinophils produce and release interleukin-8 on stimulation with calcium ionophore. *Eur J Immunol* 1993; 23:956–60.
- 26 Yousefi S, Hemmann S, Weber M et al. IL-8 is expressed by human peripheral blood eosinophils. Evidence for increased secretion in asthma. *J Immunol* 1995; 154:5481–90.
- 27 Nakajima H, Gleich GJ, Kita H. Constitutive production of IL-4 and IL-10 and stimulated production of IL-8 by normal peripheral blood eosinophils. *J Immunol* 1996; 156:4859–66.
- 28 Lim KG, Wan HC, Bozza PT et al. Human eosinophils elaborate the lymphocyte chemoattractants IL-16 (lymphocyte chemoattractant factor) and RANTES. *J Immunol* 1996; 156:2566–70.
- 29 Blom M, Tool AT, Mul FP, Knol EF, Roos D, Verhoeven AJ. Eosinophils isolated with two different methods show different characteristics of activation. *J Immunol Meth* 1995; 178:183–93.
- 30 Winqvist I, Olofsson T, Olsson I, Persson AM, Hallberg T. Altered density, metabolism and surface receptors of eosinophils in eosinophilia. *Immunology* 1982; 47:531–9.
- 31 Koenderman L, Kuijpers TW, Blom M, Tool AT, Roos D, Verhoeven AJ. Characteristics of CR3-mediated aggregation in human eosinophils: effect of priming by platelet-activating factor. *J Allergy Clin Immunol* 1991; 87:947–54.
- 32 Anwar AR, Walsh GM, Cromwell O, Kay AB, Wardlaw AJ. Adhesion to fibronectin primes eosinophils via α 4 β 1 (VLA-4). *Immunology* 1994; 82:222–8.
- 33 Anwar AR, Moqbel R, Walsh GM, Kay AB, Wardlaw AJ. Adhesion to fibronectin prolongs eosinophil survival. *J Exp Med* 1993; 177:839–43.
- 34 Mantovani A. The chemokine system: redundancy for robust outputs. *Immunol Today* 1999; 20:254–7.
- 35 Petering H, Gotze O, Kimmig D, Smolarski R, Kapp A, Elsner J. The biologic role of interleukin-8: functional analysis and expression of CXCR1 and CXCR2 on human eosinophils. *Blood* 1999; 93:694–702.
- 36 Hochstetter R, Dobos G, Kimmig D, Dulkys Y, Kapp A, Elsner J. The CC chemokine receptor 3 CCR3 is functionally expressed on eosinophils but not on neutrophils. *Eur J Immunol* 2000; 30:2759–64.
- 37 Hall LR, Diaconu E, Patel R, Pearlman E. CXC chemokine receptor 2 but not CC chemokine receptor 1 expression is essential for neutrophil recruitment to the cornea in helminth-mediated keratitis (river blindness). *J Immunol* 2001; 166:4035–41.
- 38 White JR, Lee JM, Young PR et al. Identification of a potent, selective non-peptide CXCR2 antagonist that inhibits interleukin-8-induced neutrophil migration. *J Biol Chem* 1998; 273:10095–8.
- 39 Nilsson G, Mikovits JA, Metcalfe DD, Taub DD. Mast cell migratory response to interleukin-8 is mediated through interaction with chemokine receptor CXCR2/interleukin-8RB. *Blood* 1999; 93:2791–7.
- 40 Rothenberg ME. Eosinophilia. *N Engl J Med* 1998; 338:1592–600.
- 41 Schnyder-Candrian S, Strieter RM, Kunkel SL, Walz A. Interferon- α and interferon- γ downregulate the production of interleukin-8 and ENA-78 in human monocytes. *J Leukoc Biol* 1995; 57:929–35.