Endothelial and Epithelial Expression of Eotaxin-2 (CCL24) in Nasal Polyps

D. Schaefer\textsuperscript{a} J.E. Meyer\textsuperscript{b} R. Pods\textsuperscript{b} W. Pethe\textsuperscript{b} J. Hedderich\textsuperscript{c} C. Schmidt\textsuperscript{d} S. Maune\textsuperscript{b}

\textsuperscript{a}Department of Allergology Medical Clinic III, Friedrich Alexander University of Erlangen-Nuremberg, Erlangen-Nuremberg, and \textsuperscript{b}Department of Otorhinolaryngology, Head and Neck Surgery, \textsuperscript{c}Department of Statistical Analyses and \textsuperscript{d}Department of General and Thoracical Surgery, Universitary Hospital Kiel, Christian Albrechts University Kiel, Kiel, Germany

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Abstract
Background: Nasal polyposis is mostly associated with eosinophilia of mucosal tissue. This points to the implication of CC chemokines in nasal eosinophilia. Recently the CC chemokine eotaxin-2 (CCL24) was identified. This study was initiated to localize the cellular source, analyze expression of mRNA, and quantify protein synthesis of CCL24. Methods: Specimens of nasal inferior turbinates from controls and polypous tissue from patients suffering from chronic polyposus sinusitis were collected. Furthermore, fibroblasts and epithelial cells were cultured. CCL24 protein was analyzed by immunohistochemistry and ELISA, expression of mRNA by SQ-RT-PCR. Results: CCL24 was observed in endothelial and epithelial cells. Specimens from patients expressed significantly (>2fold) more CCL24 mRNA than controls. Fibroblasts and unstimulated cells did not express CCL24 mRNA. Upon stimulation with TNF-\(\alpha\), INF-\(\gamma\), IL-4, or costimulation with TNF-\(\alpha\) and INF-\(\gamma\) CCL24 mRNA was significantly enhanced (3.2–19.6%). In controls, fibroblast, and un-

stimulated cells CCL24 protein was below detection limit. Most polyps comprised significant amounts of CCL24 (mean 0.24 ng/mg). TNF-\(\alpha\), INF-\(\gamma\) or IL-4 induced CCL24 protein (0.1–0.3 ng/ml) in epithelial cells. Costimulation with TNF-\(\alpha\) and IL-4 (0.1–30 and 1–30 ng/ml, respectively) synergistically induced synthesis of CCL24 protein (0.18–0.31 ng/ml). Conclusion: In nasal polyps endothelial and epithelial cells are obviously the main source of CCL24, which was shown for transcription (mRNA) and production (protein) levels and was associated with diseases. Results gave evidence of CCL24-directed migration of cells from inside (the bloodstream) to the epithelial side (mucosa) in eosinophilic inflammatory diseases, e.g. nasal polyposis.

Introduction
Nasal polyposis is a chronic inflammatory disease of the paranasal sinuses with a prevalence of 1–4% in the general population increasing with age. Polyposus sinusitis mostly causes nasal obstruction, secretion, headache, loss of smell, and reduced well-being [1]. Eosinophilic nasal polyposis is accompanied with aspirin intolerance...
(up to 25%) and intrinsic asthma (up to 40%). The triad of nasal polyps, asthma, and aspirin intolerance is known as ‘Widal syndrome’ [2]. Nasal polyps are characterized histologically by edema, fibrosis, decreased vascularization, and diminished glands [1–4].

The pathogenic explanation of eosinophilia in nasal polyposis tissue focused on an increased transendothelial migration and inhibition of apoptosis in eosinophilic granulocytes [5, 6]. Nevertheless, the pathogenesis of chronic polyposis in sinusitis is still unknown. Intolerance to nonsteroidal anti-inflammatory drugs (NSAIDs), chronic infection, epithelial disruption, epithelial cell defects, and inhalation/food-associated allergies are known to be associated with nasal polyps [6, 7]. Tos [8] proposed increased edema of submucosal tissue with rupture of the epithelial layer, prolapse into the sinus which is followed by reepithelization as a model of polyp development. Histomorphologically eosinophils are present in early stage manifestations of nasal polyps, forming a subepithelial cap over a pseudocyst area that was filled with albumin [9]. Eosinophils, in turn, are attracted and activated by chemokines. Activity of eosinophils is positively correlated to the concentration of chemokines and the number of activated eosinophils correlates directly with nasal polyp formation [10]. An increased expression and production of a variety of proinflammatory cytokines in nasal polyps were reported and are assumed to contribute to the chronic eosinophilic inflammation-regulating migration, survival, and activation of eosinophilic granulocytes.

Chemokines are known to have leukocyte subtype-selective properties in vitro. Among the four classes of chemokines CXC, CC, C, and CX3C – classified by the conserved cysteine residues of immature proteins – the CC chemokines RANTES, eotaxin (CCL11), and eotaxin-2 (CCL24) attract eosinophils in vitro. Therefore, these chemokines are major candidates of eosinophilic infiltration [11, 12]. Recently detected CCL24 induces chemotaxis of eosinophilic as well as basophilic granulocytes [13, 14]. Despite minor structural homologies of CCL11 and CCL24, both chemokines exclusively use the CCR-3 receptor and show the same efficiency in selective chemotraction of eosinophils, but a higher potency for CCL11 [13]. The expression of CCL11 mRNA is increased in nasal polyps of atopic and nonatopic patients [15]. Similar results were obtained in bronchial biopsies from atopic and nonatopic asthmatics [16]. As shown by our group and others cultured human nasal fibroblasts and epithelial cells express CCL11 mRNA, whereas mRNA of CCL24 was not expressed [15–17]. Infection by rhinovirus upregulated neither the expression of mRNA of CCL11 nor of CCL24 in bronchial epithelial cells [18, 19].

The aim of this study was to localize the cellular source, to analyze the basal as well as modulated mRNA expression. The production of CCL24 was quantified in unaffected human nasal mucosa as well as nasal polyps. These results obtained from native human nasal tissue in vivo were compared to those revealed from cultured human fibroblasts and epithelial cells to clarify the role of CCL24 in eosinophilic polyposis.

**Patients and Methods**

**Patients**

Specimens from 40 patients, 12 females (aged 18–70, mean 48) and 28 males (aged 10–74, mean 53) were investigated. Nine individuals served as controls (4 females, aged 20–73, mean 43; 5 males, aged 23–64, mean 49). Controls received treatment for noninflammatory diseases, such as deviation of the septum. Individuals of the control group had no signs of allergic symptoms, chronic or recurrent polyposis, asthma bronchiale, or other chronic diseases, inconspicuous CT scan and endoscopic investigation.

Thirty-one patients (16 females, aged 18–68, mean 45; 15 males, 26–74, mean 52) served as polyp group suffering from sinusitis accompanied with chronic nasal polyposis, which was diagnosed by patient history, endoscopic investigation, and CT scan. Nineteen patients suffered from allergic rhinitis (10 females, aged 12–65, mean 43; 9 males, aged 16–68, mean 51), diagnosed by patient history, and additionally by prick testing. Nine patients (4 females, aged 10–60, mean 48; 5 males, aged 23–74, mean 51) suffered from intrinsic asthma. Intolerance to NSAIDs was diagnosed in 3 patients (2 females, aged 36 and 42; 1 male, aged 45). All asthmatic patients used inhalational β-sympathomimetis as regular medication, 3 of them used inhalational corticoids (budesonide 0.2 mg/day b.i.d.) as an additional drug.

Patients of both control and nasal polyp group received neither systemic corticosteroids nor other immune-modulating drugs.

**Sampling of Tissue Specimens**

Tissue specimens were taken from the anterior part of the inferior turbinates (control group) or the middle meatus (polyp group). Specimens were obtained during endoscopic surgery of the sinus, immediately frozen in liquid nitrogen, and stored at ~80°C until further processing.

The patients included had visible polyps at the endoscopic endonasal investigation and a Lund-Mackay score (CT scans) of more than 14. Eosinophils in the tissue were shown histopathobiologically by hematoxylin-eosin staining using high power field investigation counting more than 100 eosinophils per high power field in each polyp. Five high power fields for each specimen were investigated, which was independently verified at the Department of Pathology, University of Kiel, and the arithmetic mean was documented.
Expression of Eotaxin-2 in Nasal Polyps

Cytokines, Antibodies, and Other Reagents
Recombinant human CCL24, TNF-α, INF-γ, and IL-4 were purchased from R&D Systems (Wiesbaden, Germany), peroxidase blocking reagent and 3-amino-9-ethylcarbazole (ACE) as chromogenic substrate from DAKO (DAKO Diagnostics, Hamburg, Germany), and o-phenylenediamine dihydrochloride (OPD) from Sigma (Deisenhofen, Germany). The antibodies used for this investigation were the following: polyclonal goat anti-human CCL24 (Natutec, Frankfurt, Germany), as well as a biotinylated rabbit anti-goat polyclonal antibody (Amersham, Braunschweig, Germany) for immunohistochemistry. Mouse IgG (Sigma) was used as negative control; polyclonal rat anti-CCL24 antibody (R&D Systems), monoclonal mouse anti-human CCL24 clone 2DS/G6 and 1D2/A12 (Department of Dermatology, University of Kiel, Germany), and peroxidase-conjugated rabbit anti-mouse (Jackson Immunoresearch Lab., West Grove, Pa., USA) were used for enzyme immunoassay. Primer of GAPDH and CCL24 were synthesized and purchase from MWG Biotech (Munich, Germany). The following primers were used: GAPDH forward primer 5′-CCA GCC CAT CGC-3′, GAPDH reverse primer 5′-ATG AGC CCA GCC TTC ACC AT-3′, CCL24 forward primer 5′-CTA CCG GCT CTG TGG TC-3′, CCL24 reverse primer 5′-GGT TTG GTT GCC AGG ATA-3′. Culture medium MEM and DMEM was obtained from PromoCell (Heidelberg, Germany). Chemicals for buffers were purchased from Merck (Darmstadt, Germany).

CCL24 Immunohistochemistry
Routine semithin sections (5 μm) were prepared from inferior turbinate and polypous tissue specimens stored in liquid nitrogen. Immunohistochemistry was performed as follows: samples were thawed (overnight, room temperature (RT)), air dried, and washed 3 times using PBS (pH 7.5, RT). Peroxidase blocking reagent was added (20 min, RT) followed by a washing step and blocking of unspecific binding sites by addition of diluted normal serum (1:100 in PBS, 30 min, RT). Subsequently goat anti-CCL24 antibody was added (0.25 μg/ml, overnight, 4°C). Following additional washing, sections were incubated with a secondary biotinylated goat antibody (1:100 in PBS, 45 min, RT). Then ACE substrate solution was added (1:100 diluted in substrate buffer, 15 min, RT; according to the instructions of the manufacturer). Followed by a final washing step, specimens were counterstained with hematoxylin-eosin (10 s, RT), rinsed in H2O (1 min, RT) and analyzed microscopically.

Cell Culture
For cell culture experiments the human bronchial tumor epithelial cell line A-549 was selected as a well-established representative human respiratory epithelial cell line. Furthermore, the hypothesis of the importance of chemokines in respiratory cells is well established in either bronchial or nasal inflammatory diseases, correlating with the eosinophilic infiltration in the respiratory mucosa. Therefore, A-549 cells were tested in former experiments by our group showing no differences to primary nasal cells concerning the chemokine expression. In addition, A-549 cells were selected as a relevant model cell line revealing reproducibility of in vitro experiments. A-549 was purchased form CLS (Heidelberg, Germany) and cultured under standard conditions in DMEM. Cellular viability were controlled by morphological criteria. Experiments were performed on subcultures between the third and seventh passage.

Fibroblast culture was performed as negative control experiments based on former results [17]. Nasal fibroblasts of inferior turbinates were obtained by standard enzymatic digestion as published previously [20] with minor modifications. Briefly, biopsy specimens were collected in MEM-containing gentamycin (50 mg/ml). Specimens were digested overnight at 4°C using trypsin solution (0.17%). Digestion was stopped by adding MEM containing 10% FCS. Cells were pelleted (7 min, 400 g, RT), resuspended in 10% FCS-conditioned MEM, and seeded in polystyrene culture dishes at a density of 5 × 10^5 cells. Cells were cultured under standard conditions (38°C, 5% CO2, humidified air). Medium was changed every second day.

Basal expression of CCL24 mRNA and CCL24 production in A-549 cells and nasal fibroblasts were analyzed from cells cultured in medium without any additives. The effects of various proinflammatory cytokines were analyzed upon 24 h of exposure to TNF-α (1–50 ng/ml), IFN-γ (1–300 U/ml), IL-4 (0.1–50 ng/ml), or a combination of TNF-α (0.1–30 ng/ml), IFN-γ (3–30 U/ml), and IL-4 (1–30 ng/ml) as indicated. Exposure to cytokines was done by exchange of culture media with or without the relevant concentration of cytokines.

Semiquantitative Reverse Transcriptase Polymerase Chain Reaction
Semiquantitative reverse transcriptase polymerase chain reaction (SQRT-PCR) was performed as described previously with minor modifications [21]. In brief, total RNA of nasal polypous specimens was isolated according to the protocol of Chomczynski and Sacchi [22]. RNA was quantified photometrically at 260 and 280 nm. The quality of isolated RNA was assured by both quantifying absorption ratio at 260/280 nm and verifying RNA in an 1% agarose gel. A photometric ratio of 1.7 to 2.0 in combination with two clear bands on the gel of the tested were used as criteria for selecting RNA for further processing. cDNA was amplified from 1 μg of total RNA by reverses transcriptase, followed by polymerase chain reaction using specific primers for CCL24 and GAPDH. Reaction mixture was preheated at 95°C for 1 min, 67°C for 1 min and 72°C for 2 min, followed by amplification of the targets using 31 cycles with denaturation at 95°C for 45 s and annealing at 60°C for 1 min. mRNA expression of GAPDH served as internal standard. The reaction product was separated in a 2% agarose gel and visualized by ethidium bromide under UV light. Gels were digitally scanned for computer-assisted data processing. The intensity of bands was densitometrically quantified using the software package Biometra BioDoc II. CCL24-specific bands were normalized to GAPDH. Results were given as percent relative (rel.) density.

Primer specificity was verified by extraction of CCL24 from the gel and by specifying the nucleotide sequence using the 310 Gentics Analyzer of Perkin Elmer (Weiterstadt, Germany). Data were compared to data from the gene bank of PubMed.

CCL24 ELISA
Concentration of CCL24 protein production was determined in duplicates by a sandwich enzyme-linked immunosorbent assay (ELISA). Therefore, samples were prepared from 200 mg of homogenized (1 min on ice by ultrasound) nasal polypous tissue specimens or of the nasal inferior turbinates frozen by liquid nitrogen and thereafter dissolved in citrate buffer (2 ml, pH 2.5). Followed by centrifugation and lyophilization, phosphate buffer (pH 7.0) was added, and solution was stored at −70°C until further processing.
As published recently [21], 96-well microtiter plates (Sarstedt, Nümbercht, Germany) were coated with polyclonal anti-CCL24 IgG (1 μg/ml, overnight, 4°C) dissolved in coating buffer (0.1 M Na₂CO₃, 0.2 M NaHCO₃, pH 10.5). Unspecific binding sites were blocked by addition of PBS-BSA (1% w/v, 1 h, RT). Subsequently, samples or standards of recombinant CCL24 and secondary mouse anti-CCL24 antibody (0.5 μg/ml) were added (100 μl/well, each) and incubated (90 min, RT), followed by incubation with peroxidase-labeled anti-mouse Ig (20 min, RT). Finally OPD substrate solution was added (100 μl/well, 25 min, RT) and reaction was stopped by adding of 3 M H₂SO₄ (100 μl/well). Each incubation step was followed 3 times by a washing step using PBS-Tween (5% v/v). Standard was analyzed in duplicates at seven concentration levels (9–5,000 pg/well). Optical density was measured at 492 nm using the MR-5000 microplate reader (Dynatech Laboratories, Denkendorf, Germany). Concentrations of samples were calculated by a computerized calibration cubic spline curve. Results obtained were normalized to the protein content of the relevant sample. They were expressed as arithmetic mean of duplicate measurements in ng/mg polypous tissue. The interassay variance was 14.3%, the intra-assay variance was 6.8%, calculated from 12 experiments performed in preliminary experiments before applying the assay for quantification of the samples.

Statistical Analyses
Densitometry analysis was performed using the software package Biometra BioDoc II. All data are expressed as mean ± SEM. Significance was determined using Student’s t test for comparison of two means. Means were considered significant (p < 0.05) or highly different (p < 0.01). Results of CCL24 mRNA expression and CCL24 synthesis were calculated as arithmetic mean of duplicate measurements. For respective conditions 3 up to 7 replicates from controls or patients were analyzed. Normal distribution of the control measurements could be assumed by the KS test.

Due to the small number of samples and the partially higher variation of the values of CCL24 mRNA or protein synthesis statistical tests could not be applied for all samples. Wherever applicable data were expressed as mean ± SEM. Results of respective measurements were presented as dots, and the arithmetic means were represented as horizontal lines, which was evaluated by the average value from the measurements plus 2 standard deviations (Institute of Statistical Analyses, University of Kiel, Germany).

Results
CCL24 Localization in Tissue Samples
The inferior nasal turbinate obtained from 9 patients of the control group were negatively immunohistochemically stained for CCL24 (fig. 1a) concerning epithelial, endothelial, connective, or glandular cells. In contrast, 96% of nasal polypous tissue specimens (30 out of 31 patients suffering from nasal polyposis accompanied by eosinophilia) showed immunohistochemically significantly (p = 0.05, compared to controls) positive CCL24 staining of epithelial and endothelial cells (up to 87 and 79%, respectively), without achieving a statistically significant
difference comparing these cell types. Only the specimen of 1 patient, suffering from chronic polyposis with intrinsic asthma, was negative for immunoreactive staining of CCL24. Connective tissue cells and glandular cells were immunohistochemically negative for in the polyp group (fig. 1b). The semiquantitative evaluation of eosinophil count in polypous specimens revealed negative results in the control group (i.e. 0–2% eosinophils per high power field), whereas the polyp group was characterized by intermediate to severe eosinophil counts (i.e. ~50–74% per high power field); in more detail there were severe eosinophils (53–74%) in allergic rhinitis, intermediate eosinophils (42–61%) in asthmatics, and low eosinophils (28–43%) in patients intolerant to NSAIDs. There was a flimsy correlation (R = 0.73) of CCL24 protein expression and eosinophil counts in polypous tissue specimens.

Expression of CCL24 mRNA in Nasal Tissue
All 40 specimens analyzed expressed CCL24 mRNA. Representative results of two controls and eight polypous tissue samples are presented in figure 2. The upper band (318-bp product) represents GAPDH expression and shows equal loading. The lower band (290-bp product) represents CCL24 mRNA. In polypous tissue CCL24 mRNA expression varied between 0.7 and 24.3% (mean 4.2%, median 3.6%), which was significantly higher (p < 0.05) than in controls [range <0.3 to 1.7% (mean 0.69%, median 0.78%)]. Two representative results of controls (0.9 and 1.4% rel. density; lanes 3 and 12, respectively) and 7 patients suffering from chronic polyposis sinusitis (2.6–24.3% rel. density; fig. 2, lanes 3–11, respectively) are shown in figure 2. The densitometric results of all experiments are summarized in figure 3.

Expression of CCL24 mRNA of polypous tissue did not differ statistically significantly between patient groups suffering from bronchial asthma (mean 4.1, median 3.9, range 0.9–22.1), aspirin intolerance (mean 3.8, median 3.2, range 0.7–24.3), and nasal polyposis (mean 4.3, median 4.0, range 0.8–23.7), due to the small number of samples investigated.

Synthesis of CCL24 in Nasal Tissue
The production of CCL24 protein was quantified in 7 out of 9 (77%) controls and in 23 out of 31 (74%) nasal polyps. The amount of CCL24 in controls was below the validated detection limit of the assay (<0.01 ng/ml). Nasal polyps produced significantly more CCL24 (median: 0.24 ng/mg; range: 0.05 up to 0.43 ng/mg; p < 0.01) than the tissue of controls (fig. 4).

Expression of CCL24 mRNA in Cultured Cells
The basal expression of CCL24 mRNA in A-549 cell line was marginal near the detection limit. A representative result is shown in figure 5. Proinflammatory cytokine TNF-α (10 ng/ml; lane 3), IFN-γ (10 U/ml; lane 4), or IL-4 (10 ng/ml, lane 5) significantly (p < 0.05) enhanced the expression of CCL24 (6.6, 4.3 and 2.3%, respectively). Costimulation with TNF-α and IFN-γ (0.1 ng/ml and 10 U/ml or 10 ng/ml and 100 U/ml; lane 6 or 7, respectively) or TNF-α and IL-4 (0.1 and 1 ng/ml or 10 and 20 ng/ml; lane 9 or 10, respectively) revealed slightly synergistic effects enhancing the expression of CCL24.
mRNA (7.8 and 7.6% or 2.7 and 4.6%, respectively) without reaching statistical significance ($p < 0.06$, $p < 0.07$, $p < 0.06$, respectively) when compared to separate exposure to TNF-α (6.6%), IFN-γ (4.3%) or IL-4 (2.3%).

Detailed dose-response effects of these proinflammatory cytokines on the expression of CCL24 mRNA are presented in figure 6a–e. TNF-α (0.1–50 ng/ml)-induced expression of CCL24 among 1–35.4% (mean 2–16.5%)
which was highest upon stimulation with 10–50 ng/ml TNF-α (mean 6–16.6% rel. density; fig. 6a). INF-γ (1–300 U/ml) caused indifferent effects on CCL24 expression ranging from 0.9 to 13.4% (mean 3.2–5.1% rel. density; fig. 6b). IL-4 (1–50 ng/ml) caused minute enhancement of CCL24 mRNA expression ranging from 0.8 to 14.2% (mean 1.1–8.1% rel. density; fig. 6c).

Costimulation with increasing doses of TNF-α (0.1–30 ng/ml) and INF-γ (3–300 U/ml) indifferently enhanced CCL24 mRNA expression from 0.9 to 16.8% (mean 2.3–11.7% rel. density). Increasing doses of co-stimulation with TNF-α (0.1–30 ng/ml) and IL-4 (1–30 ng/ml) tended towards a direct correlation of CCL24 mRNA expression (3.6–19.6%) without reaching statisti-
The most prominent effects were observed for costimulation with high concentrations of TNF-α and INF-γ (mean: 18.4–19.6% rel. density, p < 0.05, compared to single exposure to TNFα or IL-4; fig. 6e). Neither nasal fibroblasts of the inferior turbinates of controls nor fibroblasts of polypous tissue expressed detectable amounts of CCL24 mRNA (as expected from former experiments [15], data not shown).

Synthesis of CCL24 in Cultured Cells

A-549 cells were cultured with proinflammatory cytokines TNF-α, IFN-γ, or IL-4 (fig. 7). There was no well-defined dose response upon stimulation with IFN-γ by itself (fig. 7b). It has to be mentioned that there was a high variation in cellular CCL24 production (fig. 7a–e).

TNF-α (0.1–50 ng/ml) induced moderate synthesis of CCL24 (0.01–0.37 ng/ml). Exposure to 3 ng/ml TNF-α
caused a significant ($p < 0.05$) peak of CCL24 synthesis (0.21–0.35, mean 0.25 ng/mg; fig. 7a). High concentration TNF-α (10–30 ng/ml) caused weakly significant ($p < 0.051$) increased production of CCL24 (~0.25 to 0.23 ng/ml; fig. 7a). IFN-γ (1–300 U/ml) enhanced synthesis of CCL24 indifferently (<0.1–0.41, mean 0.20–0.23 ng/ml; fig. 7b). IL-4 (0.1–50 ng/ml) caused minute amounts of CCL24 synthesis (<0.01–0.41, mean 0.13–0.23 ng/ml; fig. 7c), and high concentration of IL-4 (50 ng/ml) caused significantly ($p < 0.05$) increased production of CCL24 (0.24 ng/ml; fig. 7c).

No synergistic or additive effect on CCL24 production was measured upon costimulation with TNF-α, IFN-γ, or IL-4 (fig. 7d, e), with the exception of costimulation using TNF-α (30 ng/ml) and IL-4 (30 ng/ml). This costimulation was significantly ($p < 0.05$) higher (0.31 ng/ml CCL24) than single stimulation using these cytokines (fig. 7e). Costimulation with TNF-α (0.1–30 ng/ml) and IFN-γ (3–300 U/ml) led to indifferent effects on CCL24 synthesis (<0.13–0.36, mean 0.16–0.24 ng/ml; fig. 7d). A synergistic effect was observed for costimulation with TNF-α (0.1–30 ng/ml) and IL-4 (1–50 ng/ml) with the most prominent effect following costimulation with 3–30 g/ml TNF-α and 10–50 ng/ml IL-4. Synthesis of CCL24 was induced at 0.15–0.45 ng/ml (mean 0.22–0.31 ng/ml; fig. 7e). The synthesis of CCL24 protein of nasal fibroblasts was not detected (data not shown).

**Discussion**

Immunoreactive CCL24 was observed in epithelial and endothelial cells of polypous tissue, whereas unaffected tissue of the inferior turbinate was negative. About 74% of polypous specimens expressed remarkable amounts of CCL24 mRNA (0.7–24.3% density of GAPDH) and CCL24 protein (mean 0.24 ng/mg). Cultured nasal fibroblasts did not express or synthesize CCL24, neither unstimulated nor upon stimulation with variable cytokines. In contrast, unstimulated epithelial cells expressed minute amounts of CCL24, which was (not well-defined) dose-dependently upregulated by the cytokines TNF-α, INF-γ, and IL-4 (1.8–19.6%). The synthesis of CCL24 protein was induced by these proinflammatory cytokines (0.1–0.3 ng/ml) with TNF-α and IL-4 (0.2–0.3 ng/ml) showing synergistic effects.

CC chemokines attract and activate eosinophilic leukocytes to the site of inflammation [11, 23]. In previous studies we extracted an eosinophilic chemotactic activity in nasal polyps [24]. Furthermore, the immunohistochemical results of the present study are in accordance with others demonstrating epithelial cells as the main source of CCL24 expression in nasal polyps [25]. In addition, CCL11 was immunohistochemically located in certain leukocytes as well as in human respiratory epithelium and in nasal polypous tissue, accompanied by an infiltration of eosinophils at sites of CCL11 upregulation [26]. Polypous nasal tissue synthesized significantly higher amounts of CCL11 and CCL24 than the middle turbinate [25]. CCL11 and CCL24 are most likely associated with Th2 immune response [27]. These findings are in accordance with our immunohistochemical results. In previous experiments, we demonstrated a dose- and time-dependent upregulation of CCL11 by proinflammatory cytokines [18], which could not be demonstrated for the expression and production of CCL24 for cultured cells in the recent investigation.

Chronic polyposis in patients suffering from sinusitis is mostly accompanied by mucosal eosinophilia of mucosal airways. The number of activated eosinophils is significantly higher in nasal polyps than in unaffected nasal tissue [10]. This points toward an impact of eosinophils and the importance of CC chemokines like CCL24, as shown in this study, although a further underlying chemotactic mechanism causing selective infiltration of eosinophils remains unknown.

Based on our recent and previous findings we conclude that the elevated mRNA expression and production of CCL24 protein, in association with additional/synergistic inflammatory mediators (e.g. TNF-α, INF-γ, and/or IL-4), points towards a prominent role of CCL24 as a relevant factor of persistent chronic inflammation in the pathogenesis of chronic nasal polyps accompanied by eosinophilia.

This statement has to be evaluated carefully due to the relatively small number of samples investigated for each group. However, the role of CCL24 points towards CCL24 as being a pivotal factor underlying persistent chronic inflammation like e.g. chronic polyposis. Further experiments were initiated and will have to prove the role of CCL24 and its natural receptor CCR-3 as a prominent factor in the pathogenesis of eosinophilia-associated inflammatory nasal diseases, which might initiate the development of new therapeutics.