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Review article

The role of cytokines in infectious sinusitis and nasal polyposis

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Sinusitis is one of the three most common disorders (1) and entails high socioeconomic costs and lost school or working days for both children and adults. The prevalence of nasal polyposis has been estimated to be about 4% in the general population, but was reported to be much higher in selected populations (2). These high prevalences have led to an increasing interest in the pathophysiology of different forms of sinusitis as the basis for better treatment modalities. Sinusitis has been understood as inflammation of the paranasal mucous membranes due to bacterial infection, which might be based on impaired ventilation and drainage of the sinuses due to either viral infection in acute sinusitis or obstruction of the ostiomeatal complex in chronic sinusitis (3–5). In contrast, nasal polyposis seems to be only partially related to infection, but is mainly related to eosinophilic inflammatory mechanisms as in aspirin sensitivity or asthma (2, 6, 7).

Inflammatory processes in the sinus cavities constitute a serious clinical problem, and research activities have mostly focused on mucociliary clearance, microbiology, and morphology of the sinuses. This knowledge has recently led to the development of new concepts of sinus physiology, followed by the introduction of sophisticated surgical techniques which take functional aspects into account (5). However, failure of treatment or recurrence of disease is still seen after restitution of ventilation and drainage, and this is obviously linked to underlying inflammatory processes. Therefore, understanding the pathomechanisms of inflammation, especially in chronic sinusitis and nasal polyposis, seems to be crucial for further success in disease treatment.

Recently, an increasing body of knowledge of the role of cytokines, chemokines, and adhesion receptors has emerged from studies on different models of nasal inflammation such as viral or allergic rhinitis. It is no longer than 5 years since the first studies on these biologic factors were performed in chronic sinusitis (8–11) and, because of the relationship to eosinophilic inflammation in asthma, also in nasal polyposis (12–17). This review aims to update these findings, but also to demonstrate the contradictory results and interpretations which have resulted from the small database available today. These discrepancies in findings may be due to insufficient characterization of patients, lack of a valid classification of sinus disease, and use of different techniques for investigation. Thus, this review aims to stimulate further research in this field, but also points to the importance of relating this research to clear-cut clinical findings.

Viral rhinitis and acute sinusitis

Viral rhinitis may not only precede an acute sinusitis episode, but also involve the sinuses regularly, as was recently shown by computed tomography (CT) evaluation (18). This involvement may be partially explained by the obstruction of the natural sinus ostia and the impairment of mucociliary drainage, but also may be caused by the effects of inflammatory cytokines on the nasal and paranasal mucosa and the cells contained therein. It is therefore interesting to look at cytokines and chemokines released due to a viral infection of the nose and to compare these observations with those of the sinus mucosa in acute inflammation.

In a pilot study comparing 20 patients with a naturally acquired common cold to five control subjects, we found significantly increased concentrations of the proinflammatory cytokines interleukin (IL)-1β, IL-6, and IL-8, but not of the allergy-related cytokine IL-4 (19). We then undertook a study including 130 normal subjects, in whom two baseline nasal lavages were carried out. Thirty-nine subjects who had developed symptoms of naturally acquired common cold within 6 months came back to the center on day 2 of symptoms, and were lavaged daily until day 5; 3–5 weeks after the episode, without any symptoms present, another lavage was performed. Various mediators, cytokines, and chemokines were measured in nasal lavages to monitor the inflammatory reaction (paper in preparation).

As was reported before (20), the concentrations of kinins were elevated from day 2 to 5 of symptoms, but failed to reach significance in our study. However, in accordance with our pilot study, the proinflammatory cytokines IL-1β, IL-6, and tumor-necrosis factor (TNF)-α were significantly elevated on days 2 and 3 of symptoms. MCP-1 and IL-8, both potent neutrophil chemoattractants, significantly increased on days 2–4 and were strongly correlated to MPO (myeloperoxidase), a specific neutrophil marker. All of these cytokines returned to the baseline values within 3 weeks after the common cold. In contrast, IL-1RA, a naturally occurring IL-1-receptor antagonist, which can be regularly found in nasal secretions in concentrations about 1000-fold higher than the agonist (21), did not show any significant alterations due to the infection.

These findings are in accordance with neutrophilic inflammatory reaction of the nasal mucosa due to viral infection (22) and illustrate the regulatory mechanisms behind this cell-migration phenomenon, as will be discussed later. Increased levels of IL-1 in nasal secretions have also been demonstrated due to experimentally induced rhinovirus infection and were shown to be unresponsive to prophylactic glucocorticoid treatment (23). The source of the cytokine release most probably is the respiratory epithelium, as was shown by Noah et al. (24). During viral upper respiratory tract infection, transcripts for IL-1β, IL-8, and IL-6 were shown to be increased in epithelial cells, and the protein concentrations of these cytokines in nasal secretion were markedly elevated compared to baseline.

Not only the proinflammatory cytokines, but also interferon (IFN)-γ and IL-10 were upregulated on days 2 and 3 and day 2 of disease, respectively. These findings are in accordance with a predominant IFN-γ and minor IL-10 production by activated T cells after bronchial infection with human respiratory syncytial virus in mice (25). Increased concentrations of IFN-γ in nasal secretions were also found after coronavirus-induced mucosal infection in symptomatic subjects (26). IFN-γ is known to possess strong antiviral activities and also to inhibit viral replication by various mechanisms (27), such as macrophage activation and class II antigen expression on accessory cells, but it also indicates the involvement of T cells of the TH1-type. IL-10, in contrast, is secreted in higher quantities from TH2 lymphocytes, and can suppress the synthesis of IFN-γ, but not of IL-4 (28). Apart from its anti-inflammatory effect by suppressing macrophage functions and indirect inhibition of TH1 and TH2 cells, IL-10 might under certain conditions also lead to a shift to TH2 cells by supporting the selection of IL-4-dependent lymphocytes. This may lead to the start of clinical symptoms of allergy in predisposed subjects.

Another marker of inflammation, the soluble intercellular adhesion molecule sICAM-1, was found to be upregulated on days 2 and 3 of viral infection. Cell-bound ICAM-1 has been identified as the major human rhinovirus receptor (29) and is expressed constitutively by basal respiratory epithelial cells. Whether this shedding of the molecule into nasal secretions represents only a secondary effect due to the stimulation of the epithelium by IL-1, IFN-γ, and TNF-α, or substantially contributes to the antiviral defense by binding rhinovirus particles, as was shown *in vitro* (30), remains to be elucidated.

In order to compare these findings with the cytokine pattern in acute sinusitis, sinus mucosa from the maxillary sinus of patients (n=10) was sampled during surgical procedures for complications of acute sinusitis. Turbinate mucosa from patients without infectious disease (e.g., during septal surgery) was used for control (n=7). The protein content of various cytokines was measured in tissue homogenates by ELISA.

In these samples, we found a significantly elevated protein concentration of IL-8 in acute sinusitis

mucosa (187.8±51.9 pg/ml, mean ± SEM) compared to controls (69.7 ± 13.7 pg/ml). Similar results were obtained for IL-1β and IL-6, both just missing statistical significance because of a large interindividual variance and the small number of samples. None of the other cytokines were upregulated, and granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-5 were not measurable in any of the samples. According to these findings, the proinflammatory cytokines IL-8, IL-1β, and IL-6 may play a pivotal role not only in viral rhinitis, but also in acute sinusitis (Table 1).

As was shown for allergic and viral rhinitis, proinflammatory cytokines such as IL-1β and TNF-α play a prominent role in ongoing inflammatory reactions (19, 31) by acting as activating factors for endothelial cells, T cells, and others. The activation of endothelial cells leads to the expression of cell adhesion molecules, such as selectins and members of the immunoglobulin supergene family, and initiates a complex procedure called the "adhesion cascade" (32). Proinflammatory cytokines also induce the release of various cytokines, such as IL-8, from endothelial cells, thus promoting the expression of corresponding adhesion receptors on peripheral blood granulocytes, which initiates the transendothelial migration of cells into the inflamed tissue. IL-8 is known to be a potent neutrophil chemotactic protein (33) and has been shown to be constantly synthesized in the nasal mucosa (21), probably by epithelial cells, in order to maintain neutrophils on the mucosal surface as an important part of the nasal defense mechanism. In acute sinusitis, the increased synthesis of large amounts of IL-8 may be related to the strong tissue neutrophilia seen in the mucosa. We suggest that the elevated IL-8 levels described here are at least partially the result of an early release of IL-1β and TNF-α. In all subjects with acute sinusitis, the samples were not obtained before day 5 of symptomatic infection, and we may therefore have missed the first events in the acute inflammation, such as the bulk release of proinflammatory cytokines, but still were able to record the secondary release of IL-8.

Table 1. Survey of key cytokine protein levels in sinusitis and nasal polyposis according to our data

Cytokine	Viral rhinitis	Acute sinusitis	Chronic sinusitis	Nasal polyposis
IL-1β	**Sign**	C (tendency)	C	C
IL-1RA	C	n.d.	n.d.	C
TNF-α	**Sign**	n.d.	C	C
IL-6	**Sign**	C (tendency)	C	C
IL-8	**Sign**	**Sign**	C	C
IL-3	C	C	**Sign**	C
GM-CSF	C	C	C	C
IL-5	C	C	C	**Sign**
IL-4	C	C	C	C
IL-13	n.d.	C	C	C
IL-10	**Sign**	n.d.	n.d.	C
IFN-γ	**Sign**	n.d.	n.d.	C
MCP-1	**Sign**	n.d.	n.d.	n.d.
RANTES	C	n.d.	n.d.	C

Sign: significantly upregulated compared to control; C: comparable to control; n.d.: not determined.

These data clearly characterize both viral rhinitis and acute sinusitis as strong, unspecific inflammatory reactions which naturally limit themselves within a few days. However, in the case of predisposing hereditary or anatomic factors, these diseases may act as a starting signal for the development of a chronic inflammatory reaction or as a trigger for immunologic changes within the mucosa, paving the way for allergic disease.

Chronic sinusitis

Chronic sinusitis in adults has been recently defined (34) as a disease of 8 weeks or more of persistent symptoms and signs, or four episodes per year of recurrent acute sinusitis, each lasting for at least 10 days, in association with persistent changes in CT. CT of the sinuses should be done 4 weeks after medical therapy without intervening acute infection. In children, the respective figures are 12 weeks of symptoms or more than six episodes of sinusitis per year. The symptoms and signs are nasal congestion, discharge, headache, facial pain or pressure, and olfactory disturbance, with fever and halitosis as minor symptoms, and cough and irritability as possible symptoms in children only. Furthermore, underlying diseases and factors such as abnormalities of mucociliary clearance, asthma, immune deficiency, atopy, or smoking may be defined.

Clinically, rhinologists use the terms "recurrent acute sinusitis", "chronic sinusitis", and "nasal polyposis" to distinguish among disease entities. For the purpose of this review, we will consider the first two groups together as "chronic sinusitis", as there are few data so far to distinguish between them in terms of cytokines, but we will discuss nasal polyposis in a separate section. Moreover, there are no data available today on cytokines in chronic sinusitis with respect to underlying diseases.

Acute sinusitis is considered a bacterial infection. *Streptococcus pneumoniae* and *Haemophilus influenzae* are the major bacterial causes of acute community-acquired sinusitis, accounting for up to 75% of cases (3). All other bacterial species are relatively infrequent causes of infection compared to these two species. Anaerobic bacteria and

Moraxella catarrhalis are next in frequency. In chronic maxillary sinusitis, however, anaerobic bacteria alone or mixed infections by anaerobic, facultative, and aerobic bacteria are predominant (4, 35). These observations, together with CT findings of anterior ethmoidal cells and regular obstruction of the "ostiomeatal complex" in chronic sinusitis (5), suggest that the development of chronic sinusitis is a two-step process involving infection and obstruction.

In the sinus fluid of patients with chronic sinusitis undergoing surgery, inflammatory cells are mainly neutrophils, as normally observed in acute sinusitis, but a low percentage of eosinophils, mast cells, and basophils may also be observed (36, 38). High concentrations of histamine, leukotrienes C_4, D_4, and E_4, and prostaglandin D_2 were found, suggesting mast-cell/basophil activation in chronically inflamed sinuses. However, in patients with allergy and/or asthma and chronic hyperplastic sinusitis, the paranasal tissue was extensively infiltrated by eosinophils, and extracellular deposition of major basic protein (MBP) was found to be associated with the damage to sinus respiratory epithelium (39). It might be suspected that some of these patients belonged to the group of nasal polyposis and asthma subjects, which will be discussed later. In chronic maxillary sinusitis, the neutrophilic infiltrate can be altered to a mononuclear infiltrate that appears together with goblet cell hyperplasia, sub-basement-membrane connective-tissue deposition, submucosal gland hyperplasia, and subepithelial edema (36). Infiltrating lymphocytes, about 40% of which are T cells, are an important component of the mononuclear infiltrate (37). CD8+ cells (suppressor/cytotoxic T cells) seem to be more frequent than CD4+ cells (helper T cells), especially in mucosa with a fibrotic histologic appearance.

Cytokine investigations have so far focused on the neutrophilic aspect of chronic sinus inflammation. IL-1β mRNA expression was found in some extravascular polymorphonuclear cells (PMNs) and in mononuclear leukocytes in chronic maxillary sinusitis mucosa, together with upregulation of ICAM-1 and of E-selectin on endothelial cells (8). The authors conclude that PMNs, participating in the release of IL-1β, may upregulate adhesion receptors to stimulate PMN infiltration in chronic sinusitis. However, proper controls were not carried out, making the interpretation of these results difficult. In another study, however, only a small proportion of tissues from patients with chronic sinusitis showed the presence of IL-1β, ICAM-1, and E-selectin by immunohistochemistry, but the cytokine and the adhesion receptors were found to be regularly expressed in the mucosa of frontoethmoidal mucoceles (9). By Northern blot analysis, IL-8 mRNA was found to be expressed in 5/11 maxillary mucosa samples of patients suffering from chronic sinusitis, but was not found in the inferior turbinates (10). This result seems questionable, as IL-8 protein is regularly found in nasal secretions and in homogenized inferior turbinate mucosa (19, 21, 31). IL-8 protein concentrations in nasal discharge from chronic sinusitis patients were significantly higher than in allergic rhinitis patients in a study also involving immunohistochemistry and *in situ* hybridization (11). Corresponding with the protein data, IL-8 immunoreactivity and mRNA could be demonstrated in PMNs, gland duct cells, and epithelial cells of chronic sinusitis patients, but only a little in the allergic subjects. The authors conclude that PMNs, once chemoattracted into the inflamed mucosa, elicit further neutrophil migration into the sinus by synthesizing IL-8, leading to a prolongation of disease. Thus, viral infections also could pave the way for subsequent chronic sinusitis, as IL-8 has been shown to be released in the course of the common cold (19). In fact, this relation is supported by clinical observation. Apart from IL-8, neutrophils have been shown to produce IL-1, IL-6, IFN-α, and TNF-α *in vitro* (40–42), further contributing to the chemotaxis and activation of PMNs.

We investigated the sinus and turbinate mucosa of chronic sinusitis patients (n=7), characterized according to the above mentioned criteria, for their cytokine protein content (IL-1β, IL-3, IL-4, IL-5, IL-6, IL-8, IL-13, GM-CSF, and IFN-γ) in comparison with normal turbinate tissue. From these cytokines, only IL-3 was found to be significantly increased in sinus mucosa, whereas in the inferior turbinate samples, IL-6 concentrations were higher than in controls. IL-8 protein was not found to be increased in chronic sinusitis samples, although the other study (11) suggested that it played a prominent role (Table 1). Different definition criteria and techniques of investigation may account for this discrepancy.

IL-3, the major source of which may be activated T cells, but also possibly mast cells and eosinophils, in the sinus mucosa, has multi-colony-stimulating factor (CSF) activities and stimulates the differentiation and activation of macrophages, neutrophils, mast cells, eosinophils, and other cells (43). Thus, IL-3 might be involved in the local defense and repair of the chronically inflamed sinus mucosa by supporting various cell populations and inducing the release of various mediators, but the cytokine may also indirectly contribute to fibrosis and constant thickening of the mucosa in the case of an ongoing repair, in the sense of "overrepair" (44). Today, there is little or no knowledge of the regulation of mucosal remodeling in the key area of sinus ventilation and drainage, the ostiomeatal

unit. A better understanding of this regulation seems to be crucial to clarify chronic sinusitis pathophysiology. Furthermore, another field of research related to cytokines and nasal airway remodeling urgently needs work: namely, the postoperative healing of nose and sinus mucosa wounds, which may be strongly influenced and even disturbed by underlying disease and inflammation (45).

Polyposis nasi

Nasal polyposis (NP), which is believed to be a multifactorial disease, is frequently associated with asthma and other respiratory diseases such as cystic fibrosis, primary ciliary dyskinesia, and aspirin sensitivity. The pathogenesis and pathomechanism of this disease are, so far, ill-defined (6, 12). The numerous hypotheses on the pathogenesis of eosinophilic nasal polyps include chronic infections, inhalant or food allergies, T-cell disturbances, and aerodynamic factors (7). Corticosteroid-dependent asthma, aspirin sensitivity, and NP form the so-called Samter triad. Clinically, NP is characterized by edematous masses in the nasal and paranasal cavities, originating from the ethmoidal cells and the middle meatus, and leading to nasal obstruction, secretion, loss of smell, headache, and reduced general well-being.

Histomorphologic characterization of polyp tissue reveals frequent epithelial damage, thickened basement membrane, and edematous to fibrotic tissue appearance with a reduced number of vessels and glands, but virtually no neural structures. In most nasal polyps, tissue eosinophilia is a striking finding, the pathomechanism of which is not understood. Histologic studies to elicit type and morphology of the infiltrating cells have shown that about 80–90% of the polyps are characterized by abundant eosinophils that are partially activated, as judged by their EG2 positivity (46). However, the claim that mAb EG2 provides reliable immunohistochemical discrimination between resting and activated eosinophils has recently been challenged (47). Other cells such T cells and mast cells are also regularly found in the polyp tissue and may contribute to the pathophysiology (6, 48).

The abundance of eosinophils in nasal polyps is the first key question to answer in order to understand this disease. It may be explained in two different ways: first, by an increased migration of eosinophils into the tissue; second, by a prolonged survival of these cells, or – most likely – by a combination of both. The second key question concerns the precise pathomechanism by which eosinophils may contribute to the tissue damage, inflammation, and polyp formation.

To answer these questions, various techniques, such as *in situ* hybridization, immunohistochemistry, measurement of protein content, and biofunctional models, have been used in biopsies taken from diseased tissues that may differ in terms of nomenclature or stage of disease. It is not surprising that these techniques and sources may lead to different conclusions on the role of a certain cytokine or chemokine. In this section, a survey of the literature is given, together with some of our own results.

Recently, *in situ* hybridization techniques have allowed the identification and localization of messenger RNA (mRNA) of cytokines, chemokines, and growth factors that may be involved in the formation of nasal polyps. These studies have shown that mRNA for GM-CSF, IL-3, TNF-α, MIP-1α, IL-1, and transforming growth factor (TGF)-β can be detected in nasal polyps. Liu et al. (49) and Hamaguchi et al. (50) have detected IL-1β and IL-1α in nasal polyps and proposed their significance in the pathogenesis. Tissue eosinophils from nasal polyps have also been shown to be strongly positive for TNF-α, another proinflammatory cytokine, and MIP-1α mRNA (13, 15). Eosinophils were also suggested as a major source of TGF-α and -β1 in nasal polyps, contributing to structural abnormalities such as stromal fibrosis and basement-membrane thickening (14, 16). Ohno et al. (51) have detected protein of GM-CSF in polyp fluids and have also shown that about 30% of the eosinophils in nasal polyp tissue expresses GM-CSF mRNA. Apart from eosinophils, fibroblasts from NP may add to the GM-CSF synthesis (52). It has further been suggested that polyp tissue expresses more GM-CSF mRNA than turbinate nasal mucosa (12), and that there is a correlation between the number of "activated" EG2+ eosinophils, IL-3 mRNA, and the amount of GM-CSF mRNA-positive cells. IL-5 mRNA was found only in low quantities in this study, whereas IL-5 protein was detected in high concentrations in nasal polyp samples when compared to other sinus diseases and normal controls in our studies (17, 53).

We investigated 23 subjects with eosinophilic polyps and 18 controls for their cytokine protein content (54). Nasal polyp samples were obtained during routine endonasal surgery. Nasal mucosal biopsies of the inferior turbinates from patients who underwent surgical procedures for septal deviations were used as controls. Subjects were tested for allergy, aspirin sensitivity, and asthma. The cytokine protein content of tissue homogenates was measured by the method of Biuret. As the mean total protein concentration (per 0.1 g of tissue) was 514.62±55.27 μg/ml in polyp tissue and 669.43±35.25 μg/ml in nasal mucosa, giving a significant difference between these groups (P=0.049),

we decided to normalize cytokine concentrations for this parameter.

Compared to turbinate nasal mucosa, the concentrations of IL-1β and IL-1RA, a naturally occurring receptor antagonist, were significantly lower in nasal polyps. As was shown before for nasal secretions from perennial allergic rhinitis patients, the concentration of IL-1RA exceeded that of IL-1β more than 1000-fold (21, 31). The relation of the cytokine to its natural antagonist, however, did not differ between mucosa and polyp tissue, indicating an identical net effect. TNF-α, IL-6, and IL-10 were detected in all specimens, without significant differences between groups. In addition, the concentrations of the chemokines IL-8, GRO-α, another CXC chemokine, and RANTES did not differ between groups.

Eosinophil migration mechanisms in nasal polyposis

Several studies have demonstrated the importance of cytokines and chemokines in mediating the migration of inflammatory cells *in vitro*. As an accumulation of eosinophils, but not of neutrophils, is regularly found in NP tissue, it is tempting to speculate on selective recruitment strategies. Based on the identification of specific eosinophil chemotactic factors or eosinophil adhesion to cytokine-activated endothelial cells, mechanisms of selective eosinophil recruitment have been postulated (33, 55–64). Among others, IL-1, IL-4, IL-5, IL-8, RANTES, and eotaxin have been shown to deliver signals that support or cause selective influx of eosinophils.

The proinflammatory cytokines IL-1β and TNF-α can lead to increased transendothelial migration of eosinophils, an effect that is nevertheless not specific for this cell type (33). IL-8 is known as a chemotactic factor for neutrophils, but may under certain circumstances also be chemotactic for eosinophils (56). RANTES is a member of the CC branch of the chemokine supergene family, and induces eosinophil chemotaxis, transendothelial migration, production of reactive oxygen species, and the release of eosinophil cationic protein (ECP) *in vitro* (57–60). RANTES is believed to be expressed on the luminal surface of endothelial cells (66), bound to proteoglycan components, to activate leukocyte integrins during the process of adhesion. RANTES immunoreactivity was reported in nasal polyp homogenates (66), and has been identified as eosinophil-chemotactic activity in human nasal polyps (67). However, in our study, RANTES protein concentrations did not differ between the NP and control tissue, bringing into question the importance of RANTES as a major recruitment factor for eosinophils in nasal polyps. Instead, RANTES may be involved in the localization of these cells within the area of the polyp, or act over a short distance in key areas only. It is also possible that a small amount of a chemokine may be sufficient to control a minor, but constant, transendothelial migration of primed cells (59).

Apart from RANTES, eotaxin has been shown to be a selective chemoattractant *in vitro*, and to induce eosinophil migration *in vivo* (68). In an *in vivo* model system, it also cooperates with IL-5 in inducing eosinophil infiltration (69). Eotaxin operates through activation of a serpentine G-coupled receptor, CC CKR3, which is preferentially expressed on eosinophils and is also used by other CC chemokines (70). However, it may not only play a role in attracting eosinophils to the site of inflammation, but also contribute to tissue damage by its capacity to induce the release of reactive oxygen species (71). Immunohistochemistry on human nasal polyps with antieotaxin mAB recently indicated its presence on leukocytes and epithelial cells (64). Further studies are clearly needed to evaluate the role of this chemokine in comparison to others.

Jahnsen et al. (72), using an elegant three-color immunofluorescence-staining technique to investigate selective recruitment mechanisms for eosinophils, found a significantly increased expression of VCAM-1 (vascular cell adhesion molecule) on microvascular endothelium in nasal polyps compared to turbinate mucosa of the same patient, whereas the expression of ICAM-1 and E-selectin was not increased. Furthermore, they found a high correlation of the relative number of extravasated eosinophils with the proportional expression of VCAM-1 on polyp vessels. From these findings, they speculated that IL-4 may be involved in the induction of VCAM-1, the ligand of which, very-late antigen (VLA)-4, is found on eosinophils, but not on neutrophils (32). A possible source of IL-4 may be not only the T cell, but also the mast cell or the eosinophil. It has recently been shown that about 80% of IL-4-positive cells within nasal polyps may represent eosinophils (73). However, in our studies (54, 74), IL-4 protein was not detectable in NP tissue. In a later experiment using a highly sensitive ELISA for IL-4, some polyp samples expressed this protein above detection level, but control mucosa did also. Since IL-4 was not significantly produced in human nasal polyp tissue, an IL-4-dependent specific recruitment mechanism for eosinophils seems unlikely. VCAM-1 upregulation in nasal polyp tissue was recently confirmed by immunohistochemistry, but correlated strongly with the density of TNF-α mRNA+ cells (75). The tissue density of TNF-α mRNA+ cells was found to be significantly higher in nonallergic than allergic nasal polyp tissue.

In nonallergic asthma, another disease with eosinophilia, but without increased IL-4 expression, an IL-4-independent eosinophil recruitment has also been suggested (76). Furthermore, Nakajima et al. (77) showed that pretreatment of asthmatic mice with mAb to IL-4 decreased the antigen-induced VCAM-1 expression by only 27%, suggesting a more complex upregulation mechanism for this adhesion molecule. The role of the vascular cell adhesion molecule-1/very late activation antigen-4 interaction for eosinophil recruitment within the human airway mucosa is not completely understood. Whereas Bachert et al. (31) found a significant upregulation of E-selectin and ICAM-1, but not VCAM-1, in seasonal allergic rhinitis samples, together with an increase in eosinophil numbers, Montefort et al. (78) showed an upregulation of ICAM-1 and VCAM-1, but not E-selectin, in perennial allergy, although a parallel increase of eosinophils was not observed. In a primate model, Wegner et al. (61) reported neutralizing mAb to ICAM-1 to attenuate airway eosinophilia and hyperresponsiveness after multiple allergen challenge. Symon et al. (79) found only weak or absent expression of VCAM-1 in their polyp samples, but could almost completely inhibit eosinophil adherence to NP endothelium by blocking vascular P-selectin. However, P-selectin may not be considered an eosinophil-selective adhesion receptor.

Taken together, there is evidence of several eosinophil-related recruitment mechanisms, the precise role and cooperation of which remain to be established. In considering the second hypothesis of eosinophil accumulation in human nasal polyps, prolonged survival of the cells, the reader may also be confronted with distinct pathomechanisms.

Eosinophil survival and the role of IL-5

Hamilos et al. (80) recently proposed distinct mechanisms of eosinophilia in NP patients with or without allergy. From *in situ* hybridization experiments and the calculation of cell densities, they concluded that the production of TH2-type cytokines, including GM-CSF, IL-3, IL-4, and IL-5, by infiltrating T cells would contribute to the "allergic mechanism" of eosinophilia, whereas the nonallergic mechanism would involve GM-CSF, IL-3, and IFN-γ. According to their findings, polyp eosinophilia in aspirin-sensitive subjects would be independent of IL-4 and IL-5, but would involve IFN-γ. This model follows the hypothesis of the predominance of TH2-type cytokines in allergic rhinitis, but is in clear contrast to recent investigations using *in situ* hybridization, protein measurements, immunohistochemistry, and neutralizing antibody experiments (54, 74).

In the above mentioned study (54), IL-5 was detected in 18/23 nasal polyps, but in only 1/18 control turbinate samples. The median IL-5 protein concentration in the polyp group was 11.45 pg/mg. As determined by the quantitative polymerase chain reaction technique (qPCR), mRNA expression for IL-5 correlated well with the protein content in the tissue samples (unpublished). In a later investigation, we compared nasal polyp tissue with inferior turbinate mucosa of the same patient and again found IL-5 only, but constantly, in the polyp samples (unpublished). Within the group of polyp samples, we tested for differences of IL-5 protein concentrations in patients with different underlying diseases. In contrast to the study of Hamilos et al. (80), who were looking for mRNA, we found no significant difference between polyps from allergic (mean 11.96 pg/mg) and nonallergic patients (10.73 pg/mg, P=0.54) in terms of protein concentrations. Although not reaching statistical significance, mean IL-5 concentrations were higher in aspirin-sensitive (19.24 pg/mg) than in nonsensitive patients (7.57 pg/mg, P=0.10). However, IL-5 concentrations in polyps from asthmatic (19.23 pg/mg) were significantly higher than those from nonasthmatic subjects (5.26 pg/mg, P=0.037). The three highest IL-5 concentrations were all found in polyp patients with asthma and aspirin sensitivity. IL-3 protein was not detectable, and GM-CSF could be found in only one control and three polyp samples in concentrations close to the limit of detection. IFN-γ was detectable in small amounts in two of the polyp samples and one mucosal control. As mentioned, IL-4, too, was not found to be increased in polyp samples compared to controls (Table 1). This fact supports the epidemiologic finding that NP is nonallergic rather than allergic.

The antrochoanal polyp (ACP) is defined as a polyp that originates in the maxillary sinus and passes through an accessory sinus ostium, and it may be distinguished from nasal polyposis by its localization. Histologic features of the ACP reveal inflammatory cell infiltration, especially of neutrophils, significant reduction of submucosal glands, and stromal edema (81). In contrast to nasal polyposis, ACPs lack eosinophils. In correspondence to this observation, we detected not elevated IL-5 protein concentrations in ACPs, but increased levels of IL-1β, IL-6, and IL-8 compared to control mucosa (although not reaching statistical significance). In conclusion, ACPs can be distinguished from nasal polyposis not only by site and histology, but also by cytokine pattern (Table 1).

Cytokines such as IL-3, IL-5, GM-CSF, and IFN-γ dramatically increase the life span of eosinophils by inhibition of the programmed cell death *in vitro*. It is tempting to speculate that this is also true *in*

vivo (82–85). Simon et al. (74) used an elegant culture model of nasal polyp tissue to mimic the *in vivo* microenvironment, with the exception of eosinophil recruitment. Apoptosis was detected by *in situ* labeling of nuclear DNA fragmentation. Evidence of apoptosis was obtained by days 8–12 of tissue culture in nasal polyp samples, by days 2–3 in control mucosa, and within 24 h in purified blood eosinophils. From these findings, the authors concluded that eosinophil apoptosis was indeed delayed in nasal polyps compared to nasal mucosa or blood eosinophils. Tissue treatment with neutralizing concentrations of antibodies to IL-5, but not to GM-CSF or control mAb, induced eosinophil apoptosis and decreased tissue eosinophilia, again pointing to IL-5 as the major support for human nasal polyp eosinophilia. Tissue eosinophils not exposed to survival factors undergo rapid apoptosis, whereas stimulated eosinophils have an increased life span. As a consequence, tissue eosinophilia may develop.

To determine the cellular source of IL-5, we used immunohistochemistry to investigate selected samples with abundant numbers of eosinophils (54). Polyp specimens were stained for MBP, cleaved ECP (EG2 mAb), and IL-5 (the mAb was a gift from Glaxo Wellcome, UK) in sequential slides. More than 50% of the eosinophils were IL-5-positive, representing about 70% of IL-5-positive cells. Eosinophils of the human respiratory tract have been shown to be capable of synthesizing this cytokine (86). As was shown before for eosinophilic heart disease by Desreumaux et al. (87), immunohistochemistry in our study suggested that eosinophils may represent a major source of IL-5. These findings do not exclude the possibility that there is also a substantial synthesis of IL-5 by other cells such as T cells, as these cells do not store the protein and therefore are not expected to be positive in immunohistochemical investigations. Simon et al. (74) also observed small amounts of IL-5 protein expression in mast cells and lymphocytes in polyp tissue.

As shown by immunohistochemistry (87) and mRNA analysis (86), eosinophils may be a major source of IL-5 in man, and this is also evident in human nasal polyps. Thus, eosinophils may be able to create an autocrine loop for their activation and survival within the tissue (Fig. 1). It may be speculated that at the beginning of the disease, T cells are the major source of IL-5, but with the aging of NP, the eosinophils may more and more overtake IL-5 synthesis. As all the studies have been performed with mature polyps so far, the pathomechanisms valid at the start of the disease are completely unknown. This opens an interesting field for research in the near future.

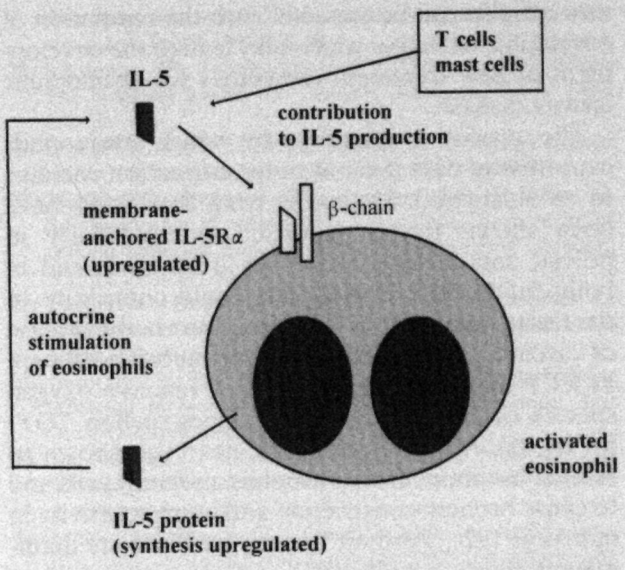

Fig. 1. Eosinophils may be able to create autocrine loop for their activation and survival within nasal polyp tissue.

Furthermore, these studies convincingly argue that IL-5 is the main target for future selective therapy of human nasal polyps, and also represents the key to further understanding of the pathophysiology of this disease (88). Treatment strategies may involve mAb to IL-5 (89), protein synthesis inhibitors (90), or the use of receptor subunits with antagonistic properties.

By binding to cell-bound ligands, the specific receptors, eosinophil-related cytokines report potent activating or suppressing signals to the target cell. The IL-5 receptor is a heterodimer (or oligomer), of which both subunits belong to the cytokine receptor superfamily. Devos et al. (91) and Tavernier et al. (92) previously reported that the α-subunit is ligand-specific, but that the β-chain is shared with the GM-CSF and IL-3 receptor complexes. It is well established that both receptor subunits are essential for high-affinity binding of IL-5 and for signal transduction. In contrast to the ubiquitous expression of the β-subunit within the hematopoietic system, the α-subunit is uniquely expressed on eosinophils and on basophils in man. Unexpectedly, in mature human eosinophils and basophils, the major transcript for the IL-5Rα-subunit encodes a secreted variant, which has antagonistic properties *in vitro*. It inhibits the proliferation of IL-5-dependent cell lines and also blocks the IL-5-induced differentiation of eosinophils from cord-blood cultures (93). Regulated alternative splicing dictates which receptor isoform is expressed.

By the comparison of IL-5 synthesis and IL-5-receptor expression on eosinophils from nasal polyps, from controls (normal blood eosinophils) and from temporary eosinophilic disease (perennial rhinitis),

new insights can be expected into the regulation of eosinophil activation which may lead to the development of new treatment modalities for eosinophilic airway disease.

The precise mechanism by which eosinophils cause tissue damage and polyp formation remains to be elucidated. Mediators from these cells have been shown to be deposited extracellularly in polyps, and raised levels have been measured in body fluids (94). Eosinophils could contribute to the tissue damage and inflammation via the release of vasoactive, neuro- and cytotoxic substances, such as ECP and MBP, the release of reactive oxygen species, or the synthesis of cytokines such as TGF-β (95, 96). MBP, for example, has been shown to release histamine from basophils and mast cells and to cause bronchoconstriction and hyperreactivity in monkeys (97). Purified human ECP affects mammalian ciliary activity (98) and may be linked to the depletion of innervation, as shown for primary esophageal achalasia (99). TGF-α and -β1 have been found in nasal polyps, possibly contributing to structural changes such as stromal fibrosis (14, 16). On the other hand, chemokines and cytokines, such as eotaxin, RANTES, and IL-5 – most probably in concert – contribute to the activation and release of mediators from eosinophils. Studies on the effects of eosinophils and their products on human nasal epithelial cells, vasculature, nerval structures, and fibroblasts may clarify the pathogenesis of polyp formation.

Concluding remarks

Acute sinusitis is clinically linked to viral rhinitis, and proinflammatory cytokines are upregulated in both cases to orchestrate mucosal defense and to limit infection. As a consequence, these diseases are characterized by an acute inflammatory cell reaction that ends without major sequelae for the sinus mucosa. In chronic sinusitis, IL-8, as neutrophil chemoattractant, and also IL-3 with multi-CSF activities have been described as prominent cytokines. These cytokines are probably involved in the regulation of local defense and repair, but may also contribute to the obstruction of the sinuses by fibrosis and thickening of the mucosa in the ostiomeatal complex, if the inflammatory reaction is ongoing. So far, no data are available on the regulation of cytokines in wound healing after sinus surgery, a field which is clearly of high interest to the clinician.

Tissue eosinophilia, a prominent feature in most nasal polyps, may be explained by an increased migration of eosinophils into the tissue, by a prolonged survival of these cells, or by a combination of both. Our data point to IL-5 as a key protein in the pathomechanism of tissue eosinophilia, enhancing the activation and survival of eosinophils. It is now widely accepted that eosinophils play an important role in the pathogenesis of bronchial asthma, a disease that is frequently associated with nasal polyps. Interestingly, IL-5 protein concentrations in nasal polyps were significantly higher in asthmatic subjects than nonasthmatic subjects, and as a tendency, were higher in aspirin-sensitive than nonsensitive subjects. These associations represent another link between asthma, nasal polyps, and aspirin sensitivity. Furthermore, eosinophils may be the major source of IL-5 in late-stage disease, thus creating a possible autocrine loop for activation and survival. Therefore, IL-5 represents a main target for therapy of polyps, and nasal polyposis may serve as a good model for other eosinophil-related diseases, such as asthma, and for investigating their response to therapy, i.e., in steroid resistance, with respect to the regulation of IL-5 synthesis and IL-5-receptor expression.

The investigation of cytokine patterns may help not only to link diseases of the upper airways to those of the lower respiratory tract, but also to distinguish between sinusitis subgroups, e.g., nasal polyposis and ACP. Clinically, chronic sinusitis is heterogeneous, and this notion has now been supported by cytokine data. Thus, cytokine investigations may help in classifying sinus diseases. We expect that research in the field of cytokines, chemokines, and adhesion receptors and their effects, not only on inflammatory cells, but also on the mucosal tissue itself, will tremendously change and enlarge our knowledge of the pathophysiology of acute and chronic sinusitis in the next decade.

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