

Review article

Nasal polyposis: a cellular-based approach to answering questions

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Nasal polyposis (NP) is a common chronic inflammatory disease of the nasal mucosa that has a major impact on patients' lives. NP is characterized by benign polypous tissue swellings in the nose that originate from the paranasal sinuses, most often from the anterior ethmoid complex (Figs 1 and 2) (1). From there the polyps can descend between the middle turbinate and the lateral nasal wall into the nasal cavity causing symptoms such as nasal congestion, rhinorrhea, hyposmia and facial pressure (2).

Treatment with corticosteroids alleviates symptoms, but no curative treatment exists. Often patients require recurrent operations and this, in combination with the symptoms, has a significant effect on the patients' quality of life (3, 4). When tested by means of a disease-independent questionnaire (SF-36), the quality of life in these patients is worse than in patients suffering from hypertension, migraine, angina pectoris and head and neck cancer. NP patients have comparable quality of life scores as patients with chronic obstructive pulmonary disease (5). Unfortunately, the aetiology of NP is largely unknown. Although some hypotheses have focused on the possible involvement of micro-organisms in the aetiology of NP, this has not yet developed into a successful treatment alternative.

This review aims at discussing some of the difficulties and pitfalls in NP research, and to identify the important cellular players and interactions in the pathophysiology of NP. We would also like to suggest potential relevant

future directions for research. Understanding the pathogenesis of NP may lead to new treatment options for this incapacitating disease.

Difficulties in NP research

Fundamental research into the pathogenesis of NP is hampered by two problems. First, it is unclear how the many different clinical phenotypes of NP influence the pathogenesis. Secondly, it is not clear whether NP should be considered a local disease or a local manifestation of a systemic disease.

Many co-morbidities have been described in NP that affect the prevalence of NP. In the general population the prevalence is 0.5–4.3%, making it one of the most common chronic diseases of the upper respiratory tract. The prevalence of NP is increased in patients with asthma (7–15%), cystic fibrosis (39–56%) or aspirin intolerance (36–96%). Interestingly, although the prevalence is increased in asthma, this does not seem to hold true for patients with allergic rhinitis, where the prevalence of NP is unchanged (0.5–4.5%) (2, 6). Chronic rhinosinusitis (CRS) almost always coexists with NP, but the converse is not true, only about 20% of the patients with CRS develop nasal polyps (7). Evidence accumulates that CRS with NP and CRS without NP actually are two different disease entities (8, 9).

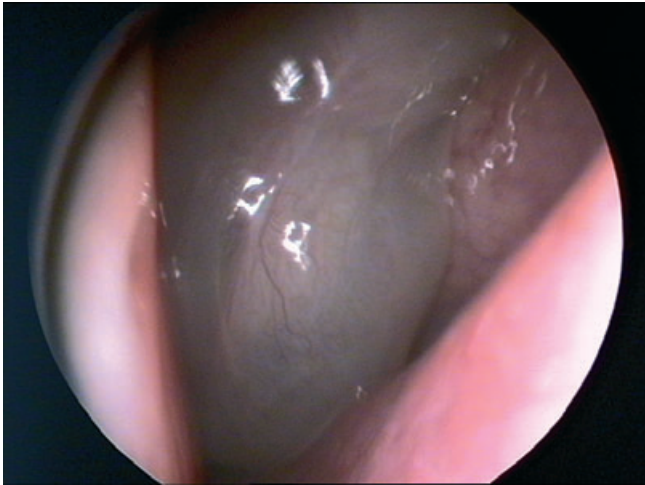


Figure 1. Nasal endoscopic view of the left nasal cavity. Nasal polyps are transparent, pale grey oedematous projections originating from the nasal ethmoid mucosa, descending between the middle turbinate and the lateral nasal wall into the nasal cavity. This polyp just touches the superior border of the inferior turbinate.

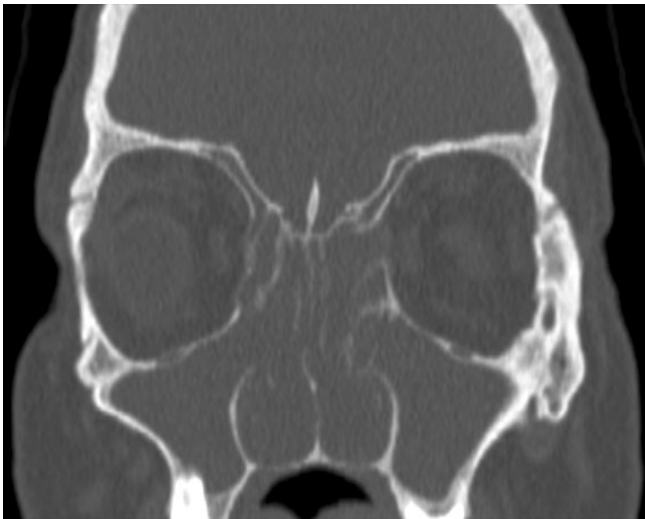


Figure 2. Coronal reconstruction of computed tomography (CT) scanning image of nasal polyposis in a 48-year-old Turkish man. There is opacification of all sinuses and complete obstruction of both nasal cavities.

As these different clinical NP phenotypes have a clear influence on the prevalence of NP, they cannot be discarded. We do not know whether features seen in NP are a local manifestation of these co-morbidities. For instance, similar typical findings can be found in microscopic examinations of nasal polyps, when compared with the bronchial mucosa of patients with asthma. In both tissues there is epithelial damage, goblet cell hyperplasia, thickening of the basement membrane, accumulation of extracellular matrix, fibrosis and eosinophil-dominated

inflammation. The link between these two diseases is further made plausible by the observation that the nasal polyp eosinophilic inflammation is significantly higher in NP patients with concomitant asthma when compared with nonasthmatic NP patients (10–14).

Another issue in these NP phenotypes is that different possible mechanisms can lead to the same polyp formation. For instance, as will be seen in the section Eosinophilic inflammation: a ‘toxic’ source of mediators, there appears to be a difference in eosinophil recruitment between allergic (IL-5) and nonallergic nasal polyps [granulocyte macrophage-colony stimulating factor (GM-CSF)] (15). Yet the amount of tissue eosinophilia seems to be the same in both groups.

These examples indicate that NP is a heterogeneous disease. In order to get to the bottom of the NP mystery we must study the differences as well as the similarities between the different co-morbidities. We have to be very cautious when interpreting data from experiments, where NP phenotypes are combined in one group and compared with healthy nasal mucosa. It is very difficult, if not impossible, to determine which factors can be attributed to the heterogeneity of the underlying diseases and which factors are essential in the development of NP. Designing future experiments to seek actively for differences as well as similarities between the clinical phenotypes of NP will shed more light on the pathogenesis of NP.

A similar discussion is possible on the topic of local versus systemic disease. As will also be seen in the section Eosinophilic inflammation: a ‘toxic’ source of mediators, the inflammatory process (eosinophils, lymphocytes, etc.) varies significantly in polyps, the middle turbinate and the inferior turbinate in the same patient. This may suggest that NP is a local disease, or that the inflammatory process in the middle turbinate is (i) different from that in the inferior turbinate and (ii) probably closer to the process in NP. This is an important finding, as much NP research has focused solely on inferior turbinate samples from healthy patients as control tissue for NP. We have to make sure that the ‘healthy’ control tissue used is from the exact same source as the diseased nasal polyp. As nasal polyps mostly originate in the ethmoid sinuses near the middle turbinate, this would either be healthy sinus or middle turbinate mucosa. Future experiments should be designed to identify the best control tissue.

Cellular basis of the pathological mechanism in nasal polyposis

As the previous section highlighted, there are two interesting similarities between NP and asthma. First of all there is eosinophil-dominated inflammation. Secondly, there are structural modulations of the nasal mucosa, with the myofibroblast being thought to play an important role. This section addresses these two features. We will also explain the central coordinating role of the

epithelium in these two processes. A better understanding of these essential interactions can help us to understand the pathogenesis of NP better.

Eosinophilic inflammation: a 'toxic' source of mediators

The polyp tissue is infiltrated predominately by eosinophils, lymphocytes, plasma cells and mast cells (16–19).

The activated infiltrating eosinophils produce a large amount of toxic proteins, such as eosinophilic cationic protein (ECP) and major basic protein (MBP). In addition to these toxic mediators, eosinophils are also capable of producing a variety of cytokines, chemokines and growth factors. For instance, they produce interleukin-5 (IL-5), GM-CSF, RANTES and GRO- α (15, 17, 20–23). So they seem to extend their own lifespan and increase tissue infiltration in an autocrine fashion.

It is, however, not known what initiates the influx of activated eosinophils into the nasal polyp. It is widely accepted that eosinophils are also a hallmark of allergy. In patients with NP and co-existing allergic rhinitis the eosinophils seem to be attracted mainly by the release of IL-5 (15). Preliminary data in nonallergic, nonasthmatic and aspirin-tolerant NP patients however do not show increased levels of IL-5-producing cells (unpublished results). By contrast, in the absence of allergy, eosinophils appear to be recruited mainly by the release of GM-CSF (15). Nevertheless, the resulting eosinophilic influx appears to be the same for both atopic and nonatopic NP (24–27). This does not hold true for asthmatic and aspirin-intolerant patients with NP. Bachert et al. found significantly more eosinophilic infiltration in NP samples containing high total IgE tissue concentrations (10). These high total IgE levels were more frequently found in asthmatic and aspirin-intolerant NP patients. Other researchers have confirmed that the eosinophilic influx is higher in asthmatic patients when compared with nonasthmatic patients (10–14). This difference in eosinophilic influx is even more marked in aspirin-intolerant asthmatic patients (26, 28–30). This suggests a more aggressive inflammatory response in those patients who apparently have more extensive polyposis and many recurrences after surgery. This correlation between the extent of eosinophilia and the disease severity is also seen in asthma itself. Patients with a higher degree of eosinophilia have significantly more severe symptom scores and residual airway obstruction after bronchodilatory therapy (31–34).

Given that nasal polyps are formed in a defined region in the nose it is important to note that there seems to be a specific distribution of eosinophils in the nasal cavity. As can perhaps be expected, there are significantly more activated eosinophils in polyp samples compared with middle and inferior turbinates from healthy controls (16, 35, 36). However, several authors have specifically investigated the distribution of eosinophils throughout the nose in NP patients. They report that polyp samples

contain a significantly higher amount of eosinophils than the middle and inferior turbinate samples in the same NP patients, with a significantly higher number of eosinophils in the middle turbinate than in the inferior turbinate (16, 36, 37).

In conclusion, we can state that it is not known what initiates the primary recruitment of eosinophils to the site of nasal polyps. In allergic rhinitis there is a role for Th2-lymphocytes producing IL-5 and this may apply to NP. To address this question the cellular sources of IL-5 need to be identified.

Structural modulations of the nasal mucosa: the role of the myofibroblast

Myofibroblasts are atypical stromal cells that play a crucial role in the pathological tissue changes seen in both NP and asthma. (Electron) microscopic examinations of nasal polyps reveal a number of typical findings. First, the surface area of the polyp can vary considerably. The polyp surface is partially covered by normal (ciliated) respiratory epithelium, interrupted by areas of erosion/damage and squamous metaplasia. Typically, there is goblet cell hyperplasia. Secondly, underneath this partially damaged epithelium, there is thickening of the basal membrane (lamina reticularis). And thirdly, the stroma of the polyps is characterized by massive oedema, pseudocysts, and (subepithelial) fibrosis with an accumulation of extracellular matrix (38–43).

Interestingly, these observations are very similar to the observations in the sinus mucosa of CRS patients without NP (13, 27), and to observations in the lower airways of asthmatic patients (44–49). These typical findings are more pronounced in the nasal mucosa of CRS/NP patients with concomitant asthma (13).

Both in asthma and NP, pathological cells have been observed underneath the thickened basal membrane that are not present in 'healthy' bronchial or nasal mucosa. (12, 50–53). They are myofibroblasts, which can be seen as an activated phenotype of fibroblasts. Upon stimulation with transforming growth factor- β (TGF- β), resident fibroblasts in skin, cardiac, lung and nasal tissue differentiate into active myofibroblasts where, in non-pathological circumstances they are involved in wound repair and tissue differentiation (54–58).

Myofibroblasts produce large amounts of extracellular matrix molecules, such as collagens (type I, III, IV and VIII) and fibronectin. This extracellular matrix secretory function is essential in tissue repair and wound healing. The cytoplasm of the myofibroblast contains a fibronexus that connects the stress fibres of α -smooth muscle actin, through a trans-membrane $\alpha\beta$ integrin, to the fibronectin in the extracellular matrix. Contraction of the myofibroblasts pulls the extracellular matrix together and reduces the physical size of a damaged area. The tissue repair process is completed by the apoptosis of the myofibroblast.

Myofibroblasts play an important role in several diseases. One of those is asthma, in which an interaction between the epithelium and myofibroblasts is at the basis of the underlying disease mechanism. We hypothesize that similar interactions are of importance in the pathogenesis of NP. The epithelium is a crucial factor in these interactions, as will be clarified in the following section.

Epithelium: active participant in the inflammatory and structural response

Traditionally, the nasal epithelium is seen as a passive barrier lining the nasal cavity, protecting the tissue against all sorts of pathogens and allergens. However, there is a growing awareness that it should rather be seen as an active participant in the immunological response. In NP, the epithelium is both an active player and a 'passive' target in the pathology. It plays a central role in the interactions with eosinophils and myofibroblasts, as will be explained below.

The epithelium is 'passively' under attack by the infiltrating eosinophils. The activated eosinophils produce reactive oxygen radicals, as well as toxic proteins (ECP and MBP) that have been shown to damage respiratory epithelium (59, 60). Ciliary beat frequency, as well as epithelial cell membrane integrity, is significantly decreased by activated eosinophils (61–67). Furthermore, cell proliferation is significantly higher in the polyp epithelium compared with inferior turbinate epithelium from the same patients (68). Epithelial damage caused by inflammatory mediators induces this proliferation via epithelial repair processes.

It seems that the epithelium itself is partly responsible for the toxic eosinophilic invasion. In a model where epithelial damage is mimicked by nonenzymatic induction of loss of cell–cell contacts, a pleiotropic induction of mediators is seen. These mediators are known to be involved in repair and fibrosis (IL-1 β , TGF- β , epidermal growth factor [EGF], vascular endothelial growth factor [VEGF], insulin-like growth factor-binding protein 3 [IGF-BP3], TIMP-1 and TIMP-2), but they are also involved in the recruitment and activation of cells of the immune system (IL-6, IL-8, G-CSF, IL-1 β , γ -interferon, tumour necrosis factor- α , IL-4, IP-10, leukemia inhibitory factor (LIF) and GRO- α) (A.B. Vrolijk, personal communication.). Several researchers have shown that polyp epithelial cells additionally produce a number of eosinophil chemo-attractant mediators. These include GM-CSF, RANTES and eotaxin (15, 69–73).

Not only can epithelial cells attract eosinophils to the polyps, they can also increase their lifespan. When incubating peripheral blood eosinophils from healthy subjects with human nasal polyp epithelium cell conditioned medium (HECM), eosinophil survival increased significantly as a result of the inhibition of apoptosis. It was possible to block this effect almost completely through the addition of anti-GM-CSF to the HECM

(69, 70, 72). This indicates that GM-CSF is the most important eosinophil survival enhancer produced by the nasal polyp epithelial cells.

The epithelium also plays a very active role in the interaction with myofibroblasts. Under normal circumstances this interaction is essential to organogenesis. For instance, epithelial–myofibroblast interactions are critical in embryologic lung development. They secrete soluble mediators, growth factors and interstitial matrix and/or basement membrane molecules. In this process, platelet-derived growth factor (PDGF) is essential for the myofibroblast proliferation. Bostrom et al. demonstrated that postnatal surviving PDGF-A-deficient mice develop pulmonary emphysema secondary to the failure of alveolar septation. This is caused by the loss of alveolar myofibroblasts and associated elastin fibre deposits (74). Given that TGF- β is important for myofibroblast differentiation it should be noted that it also induces PDGF receptors on the myofibroblast. Other mediators considered to be important in myofibroblast activation and proliferation are TGF- α , EGF, GM-CSF, FGF and IGF (75).

Myofibroblasts are frequently present in asthma, where a specific interaction between epithelium and these fibroblasts is thought to be part of the underlying disease mechanism. As mentioned before, asthma (that often co-exists with NP) shares many similar histopathological features with CRS and/or NP. There is epithelial damage, thickening of the lamina reticularis, hyperplasia of goblet cells and smooth muscle cells, accumulation of extracellular matrix, and fibrosis. This structural reorganization in the bronchial mucosa of asthmatic patients is known as 'airway remodelling' and is responsible for the irreversibility of the disease. An essential component of this pathological remodelling process is the interaction between epithelial cells and myofibroblasts. This may take place through a reactivation of the 'epithelium mesenchymal trophic unit' that plays an important role in normal lung development (46, 49). As in the process of deranged wound repair and tissue fibrosis, it is not clear what triggers this revival of a system that is essential to normal organ development. The higher prevalence of NP in asthmatic patients is, however, a striking finding that forces us to consider the possibility that similar pathology might underlie both diseases.

Myofibroblasts are found in pathological conditions that may have some bearing on the presence of these cells in NP. First, under conditions of unchecked, deranged or repeated tissue repair, the myofibroblasts do not go into apoptosis. This could lead to fibrosis (75). In NP, the highest density of myofibroblasts is found in the pedicle area of the polyp. This area, where the polyps can be thought to 'grow', also contains the highest density of TGF- β -positive cells (51). High levels of TGF- β are secreted by the infiltrating eosinophils, and very likely by the epithelium also (76, 77). The polyp epithelium might be blocked in a 'repair phenotype', with the continuous release of proliferative and profibrotic mediators. Zhang

demonstrated this *in vitro*. He chemically damaged cultured bronchial epithelial cells with poly-L-arginine, which is a surrogate for ECP. In a co-culture system these damaged epithelial cells significantly increased myofibroblast differentiation through the release of high amounts of TGF- β , bFGF, IGF-1 and PDGF (78). When mechanically damaging cultured guinea-pig bronchial epithelial cells, Morishima et al. saw a similar increase in myofibroblast proliferation, accompanied by the up-regulation of collagen type I and III synthesis by co-cultured (myo-) fibroblasts (79). In this way, fibrotic disease can be seen as a major pathological end point of activated and proliferating myofibroblasts. Indeed, myofibroblasts have been identified as major players in fibrotic diseases such as: pancreatic fibrosis, liver fibrosis and cirrhosis, sclerosing glomerulonephritis, fibrosis in Crohn's disease, pulmonary fibrosis and many others. In NP however, fibrotic changes do not dominate. Fibrosis is only evident in the peduncle area, where most myofibroblasts are found. Most of the polyp stroma, however, is predominantly oedematous. Nevertheless, the presence of myofibroblasts in NP points to a pathological damage-repair response in which the created molecular environment allows them, together with the activated eosinophils, to escape normal apoptosis. But what derails this 'normal' repair process? What exactly triggers the epithelium to persist in this 'activated' phenotype and to inhibit myofibroblast and eosinophil apoptosis?

Unfortunately, we do not know the answers to the above formulated questions. As will be discussed in the section Concluding remarks and future directions, we intend to design several experiments that may enhance our understanding of the complex interaction of epithelium and myofibroblasts in NP. We expect to gain more insight into the pathophysiology of NP, with the aim of identifying potential new targets for treatment.

Therapeutic options

The reason for identifying new treatment targets is abundantly clear; there still is no curative treatment for NP. This section will expound on the present treatment options in NP. We will discuss the golden standard, the 'steroid-based treatment', as well as on 'micro-organism based treatment'. Finally we will discuss a recently investigated example of a 'mediator-based treatment' in order to explain how fundamental research can lead to new potentially therapeutic targets.

Steroid-based treatment

In an extensive review of the literature on treatment modalities in NP, the European Position Paper on Rhinosinusitis and Nasal Polyps proposes an evidence-based treatment strategy for NP (2). Steroids are the cornerstone for treating NP. This can range from topical

steroid sprays or drops in mild to moderate polyposis, to a short course of systemic steroids in severely affected patients. There is a good amount of evidence that topical as well as systemic steroids are effective in reducing the size and symptoms of nasal polyps (80–84). Nevertheless, a substantial number of patients appear to be refractory to steroid treatment, or seem to develop a decreasing steroid sensitivity along the way. So why do some patients respond to treatment while others do not? One important factor is whether the local application of steroid reaches the polyp. In a direct comparison of steroid sprays and droplets, 50% of the patients who did not respond to nasal spray did respond to the same medication when applied as a droplet (85). Most likely, the 'lying head back position' allows the steroid drop to reach the nasal polyps better than a common spray. However, not all steroid unresponsiveness originates from poor application as some patients are also unresponsive to a systemic course of steroids. van Camp and Clement reported an overall success rate of 72% for systemic steroid treatment (86). This means that approximately one of three patients with NP will not benefit from this treatment and need operative removal of the polyps. Some asthma studies have hinted that the ratio between two distinct glucocorticoid receptors (GR α and GR β) is responsible for the failure of steroid treatment in some patients. Glucocorticoid-insensitive asthma is associated with a significantly higher number of GR- β ⁺ inflammatory cells (87, 88). Although this avenue has not been fully explored, the same appears to be true for glucocorticoid-insensitive nasal polyps (89). It is not known if NP patients who have concomitant 'glucocorticoid-insensitive asthma', also have 'glucocorticoid insensitive polyps'.

Micro-organism-based treatment

Several micro-organisms have been investigated to determine their causal role in CRS and NP. Ponikau et al. showed that the presence of fungi could be found in 96% of 210 consecutive patients with CRS (90). Braun et al. found similar high incidences of fungal colonization in CRS patients (91%) but, together with Ragab et al., demonstrated that fungal colonization is also frequently encountered in healthy control subjects (91–100%) (91, 92). Although two uncontrolled studies (93, 94) initially indicated promising effects and a placebo-controlled study from the same group could demonstrate a small reduction in tissue swelling on CT (95), later multicentre studies failed to corroborate these findings. Two double-blind placebo-controlled randomized trials were performed to test the effectiveness of topical antifungal treatment in CRS with NP (96, 97). Both an antifungal spray (96) and antifungal nasal lavages (97), when administered over a period of 2–3 months, were ineffective in reducing patients' symptom and nasal endoscopy scores. Furthermore, a 6-week course of terbinafine orally in patients with CRS without NP also failed to bring

about any improvements in patients' symptom scores or CT scores (98).

Other frequently encountered pathogens in the mucus of NP patients are bacteria, especially *Staphylococcus aureus* (99–101). Van Zele et al. reported that *S. aureus* colonization of the middle nasal meatus is higher in patients with NP (64%) compared to patients with CRS (27%) and healthy controls (33%) (99). These *S. aureus* colonization rates paralleled the presence of specific IgE antibodies to *S. aureus*-derived enterotoxins (SAEs). Tissue concentrations of specific IgE against SAEs were highest in NP patients with asthma and aspirin intolerance. The significantly highest levels of nasal polyp eosinophilic infiltration and IgE production were also found in these groups. This IgE production appears to be multiclonal, indicating that SAEs can act as superantigens that can activate large subpopulations of T lymphocytes (10). *Staphylococcus aureus* is also one of the predominant bacteria in patients with atopic dermatitis (102). In patients with atopic dermatitis, the eradication of *S. aureus* has been shown to improve symptomatology significantly, although temporarily (103). This potential therapeutic effect of *S. aureus* eradication has not yet been studied in NP, but large-scale double-blind placebo-controlled studies are currently being conducted.

Old studies have suggested the possible involvement of viruses in the pathogenesis of NP. After initial interest, these ideas have been abandoned, mostly because viruses were found in both healthy individuals and NP patients. Consequently, no anti-viral treatment has been considered or is currently being explored.

Mediator-based treatment (anti IL-5)

In NP, eosinophils are the main infiltrating inflammatory cells. Attempting to stop this toxic invasion is therefore a logical therapeutic goal. In asthma, this has been tried by means of the intravenously administration of anti-IL-5 monoclonal antibody. This resulted in a 100% decrease in circulating blood eosinophils and in a reduction of approximately 50% of airway eosinophils (104, 105). There was also a significant reduction in the number of TGF- β^+ airway eosinophils, and in the TGF- β concentration in bronchial alveolar lavage fluid (106). Nevertheless, anti-IL-5 treatment did not result in a reduction in asthma-related symptoms. It was concluded that anti-IL-5 could reduce but not deplete airway eosinophils. This suggests that IL-5 is either not the most prominent, or not the only, factor responsible for eosinophil infiltration and survival in the asthmatic bronchial mucosa.

Recently, Gevaert et al. tested the intravenous administration of anti-IL-5 monoclonal antibody in the treatment of NP (107). In a double-blind placebo-controlled randomized trial, nasal polyp score was measured by means of nasal endoscopy and compared before and after treatment, together with symptom scores and nasal peak inspiratory flow values. There were no significant changes

in the patient symptom scores or in the nasal peak inspiratory flow values between the treatment groups compared with the placebo group. Just like in the asthma model, these researchers observed a complete reduction in blood eosinophilia, but only a partial reduction in tissue eosinophilia.

Some interesting observations came from subgroup analysis. Depending on the endoscopic improvement in the nasal polyp score within 4 weeks after dosing, the patients who received the anti-IL-5 treatment were sorted into responders and nonresponders. At baseline, the responders were shown to have significantly higher IL-5 concentrations in the nasal lavage than nonresponders. Hamilos et al. demonstrated that IL-5 is not the only molecule responsible for eosinophil recruitment. In the absence of allergy, eosinophils in nasal polyps seem to be recruited mainly by the release of GM-CSF rather than IL-5 (15). Taken together, these observations suggest that the treatment effect of anti-IL-5 could perhaps be restricted to those patients whose atopic status means they have increased levels of IL-5. Treatment would then be equivalent to the reduction in 'allergic' eosinophilic infiltration. Unfortunately, the atopic status of the patients participating in the anti-IL-5 trial has not been described.

Concluding remarks and future directions

Despite a substantial amount of experimental research, the elementary processes responsible for the formation of polyps still need to be unravelled, and many (partly) unanswered questions remain: (i) As NP is so frequently seen with CRS, is NP part of the CRS-spectrum, or are these two distinct diseases? (ii) As polyps are found to originate in such a defined region, should we see NP as a local disease, or are the polyps a local expression of a systemic disease? (iii) In what way do the co-morbidities affect the occurrence of nasal polyps?

This review describes important cellular players in the pathogenesis of NP and, on that basis, attempts to identify potential new targets for treatment. To summarize our discussion briefly, it would seem clear that there is no single entity responsible for NP. To put it more strongly, even our limited overview shows that there is a complex interplay involving tissue-resident cells (epithelium), inflammatory cells (e.g. eosinophils) and pathogenic cells (myofibroblast). The interaction between these players is not one way. Toxic products from eosinophils can damage epithelium, yet factors from the epithelium attract and maintain eosinophils. Similar interactions can be seen between epithelium and myofibroblasts. These interactions seem to define a cascade, a feedback loop, with a sole outcome: worsening of the disease. A better understanding of these complex interactions may help us to stop this negative spiral and provide new treatment options.

Major cellular players, treatment options and caveats

Based on our understanding of the pathogenic role of eosinophils in (allergic) disease and the control of their differentiation and activation, the treatment with anti-IL-5 was seen as a valid option. Unfortunately, this approach has only been partly successful, as not all patients have benefited from treatment. Subgroup analysis suggests that only patients with high levels of IL-5 have responded. NP is a heterogeneous and very complex disease, in which IL-5 is clearly not the only molecule relevant for eosinophils. As this review showed, IL-5 may even be unimportant in nonallergic NP. Often, research has focused on a limited number of molecules as few techniques allow for a comprehensive analysis of multi-players. The development of multi-plex ELISAs, however, enable us to screen for up to 30 protein mediators simultaneously, so that even in circumstances where limited amounts of material are available, a more comprehensive picture can be obtained. A similar benefit can be obtained by the application of micro-array analysis, where expression profiles of 55 000 genes can be acquired simultaneously.

Could it be that the success of the anti-IL-5 treatment is dependent on IL-5-driven eosinophilia, as seen in allergy or asthma? This aspect is unclear, but it may well be that the anti-IL-5 treatment interferes with a co-morbidity aspect of NP. In patients with asthma, the prevalence of polyposis is not only higher, but these patients also have more severe symptoms. Irrespective of the mechanism, it is clear that in the analysis of NP one should carefully consider co-morbidities, not only because they may confuse interpretations, but also because they may shed light on the pathogenesis of the disease. The available data suggest that this same reasoning may hold true for the type of control tissue used. Inflammatory processes may well be different depending on the position in the nose where they are investigated. The cellular influx is different in the inferior and middle turbinates, and may be different again from the influx seen in nasal polyps. Without a better understanding of regional differences in the nose it is not clear whether inferior turbinate from healthy individuals are the most appropriate controls.

The outcome of the anti-IL5 treatment should not dishearten us. Without the efforts of many researchers, IL-5 would never have been considered as a treatment target. However, it also shows that a more comprehensive understanding of NP is required.

Future directions

Given the many parallels between remodelling seen in asthma and polyposis, greater attention is warranted for the interaction between the polyp epithelium and (myo)fibroblasts. This interaction may explain epithelial damage and the proliferation, recruitment and survival of inflammatory cells, differentiation of myofibroblasts, deposition

of extracellular matrix, etc. A proteomic and genomic approach seems best suited to identify the different players involved in this vicious circle that could underlie the pathogenesis of NP.

Until now, micro-array has not been used frequently in NP research. Fritz et al. investigated the difference in gene expression between nasal mucosa from patients with allergic rhinitis with and without NP (108). They found 34 genes that were expressed significantly different between the two groups. Several of these genes coded for inflammatory mediators, but the genes with the greatest differential expression were described in association with various forms of neoplasia, such as mammary-globin. Similar results were found by Liu et al., who compared the gene profiles of NP samples with normal sphenoid sinus mucosa (109). They also found differential expression of several inflammation-associated genes, as well as genes possibly involved in apoptosis inhibition. Unfortunately, the NP patients were made up of a heterogeneous population of patients, with some having concomitant asthma, allergy or aspirin intolerance. Furthermore, the samples used for analysis included multiple cell types, each with their typical expression profile. Differences in cellular influx between conditions make it difficult to draw firm conclusions from the data.

Because we want to establish a better picture of the relevant interactions referred to above, we plan to take a close look at the NP epithelium, which seems to be such a central player. Not only as a potential target for toxic or environmental factors that may trigger disease, but also because of its potential role in the maintenance of disease through its interaction with myofibroblasts and its anti-apoptotic effects. Focusing on a single cell type in combination with strict patient selection can make the micro-array a very powerful and reliable tool that may help us to answer multiple questions.

None of the patients in this micro-array experiment suffer from co-morbidities such as allergy, asthma, aspirin intolerance or cystic fibrosis. We have defined three groups. The first group contains healthy subjects (CRS-, NP-) from whom biopsies will be taken from the middle turbinate as well as the sphenoid sinus. The second group consists of patients with CRS, without NP (CRS+, NP-). Only middle turbinate biopsies from these patients will be studied. The third group consists of patients with CRS and NP (CRS+, NP+). In addition to the NP samples, we will also harvest biopsies from the middle turbinates. The epithelial cells will be isolated and cultured until confluence.

Epithelial cell gene expression is compared between the different tissue samples and patient groups (Fig. 3). This enables us to answer several relevant questions. First, what factors produced by the epithelium may contribute to the pathogenesis of NP and CRS? Answers will be found in comparing polyps with healthy sinus (Fig. 3A), NP middle turbinate with healthy middle turbinate (Fig. 3B) and CRS middle turbinate with healthy middle turbinate (Fig. 3C).

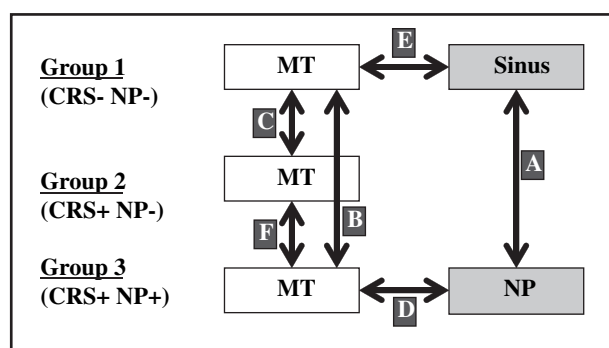


Figure 3. Micro-array experimental set-up. Epithelial cell gene expression is compared between middle turbinate (MT), nasal polyp (NP) and sinus mucosa (sinus) in the three different patient groups (comparisons A–F).

Secondly, is NP a local disease or a systemic disease? Comparing the polyp samples with the middle turbinate sample from NP patients (Fig. 3D) will shed more light on this question. Thirdly, can we use the middle turbinate as representative control tissue for a sinonasal disease?

In other words, is there a significant difference in gene expression between the sinus and middle turbinate mucosa in a healthy person (Fig. 3E)? Finally, is the mucosal disease in a patient with CRS and polyposis different from CRS patients without polyposis? Comparing the middle turbinate from NP patients with the middle turbinate from CRS patients will answer this interesting question (Fig. 3F). In a parallel experiment, we will try to answer the question of whether NP is a single disease modified by the existing co-morbidities or whether nasal polyps are a common structure found in different disease entities. Determining the differences between the subgroups of NP (allergy, asthma, aspirin intolerance or cystic fibrosis) will allow us to answer this question. Furthermore, by comparing the similarities between these subgroups we may further refine the observations of experiment 1 and pinpoint common factors.

Finally and most importantly, potential new leads will be investigated in more detail in an *in vitro* cell culture system we have developed to study their contribution to the interaction between epithelium, fibroblasts and inflammatory cells.

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