Immune Imbalance in Nasal Polyps of Caucasian Chronic Rhinosinusitis Patients

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1 Introduction

Chronic rhinosinusitis (CRS) affects about 5–15% of the population in Europe and the USA (Fokkens et al., 2012) thus represents a popular health problem with significant medical costs (Hastan et al., 2011). For many years, different medications did not offer a healing for every CRS patients and the effort of researchers to find a single treatment for CRS failed, because there is no general pathophysiology for CRS. CRS is just an umbrella term for many forms of chronic inflammation of the nose (Fokkens et al., 2012). In current national and international guidelines, CRS is subdivided in chronic rhinosinusitis with nasal polyps (CRSwNP) and chronic rhinosinusitis without nasal polyps (CRSsNP) (Fokkens et al., 2012; Stuck et al., 2007). CRSwNP is characterized as a chronic inflammatory condition of the nasal and paranasal sinuses with nasal polyp growth and a prevalence about 1–4% of the general population (Fokkens et al., 2012; Settipane et al., 2013). Nasal polyps are characterized by grape-like structures in the upper nasal cavity and typical histological features of nasal polyps, like a dense inflammatory infiltrates and loose fibrous connective tissue with substantial tissue edema (Fokkens et al., 2012; Stuck et al., 2007). However, nasal polyps exhibit four histological patterns, in which the edematous, eosinophilic polyp is the most common form of nasal polyps with a probability of 65–90% (Couto et al., 2008; Davidsson & Hellquist, 1993; Hellquist, 1996; Stuck
et al., 2007). Predominately eosinophilic cellular infiltration and eosinophilic Th2 inflammation are the major phenotypic markers of CRSwNP, while the inflammatory process is characterized by interleukin (IL)-4, IL-5, eosinophil cationic protein (ECP) and eotaxin-1/-2/-3 expression (Fokkens et al., 2012; Plager et al., 2010; Van Zele et al., 2006). During the last years, several T cell subsets were well characterized. Thus, for example CD4+ T cells are able to differentiate into T helper (Th)1, Th2, Th9, Th17, Th22 and T follicular helper (Tfh) effector cell subsets, but the balance between T helper subsets is essential (Annunziato & Romagnani, 2009; Zygmunt & Veldhoen, 2011). However, inflammation can destroy this balance and generate specific inflammatory pattern.

The innate immune system responds to inflammation with recruitment of eosinophils and other leukocytes from blood vessels into the site of inflammation. This process, called leukocyte adhesion cascade, is mediated by adhesion molecules and tightly regulated with multiple steps involving leukocyte adhesion, rolling along the surface of activated endothelial cells and transendothelial migration (Schmidt et al., 2013; Tam et al., 2011). The recruitment mainly appears in postcapillary blood vessels and starts with capturing of flowing leukocytes, followed by rolling along the blood vessel wall. Both, capture and rolling, are mediated by adhesions molecules called selectins, which interact with selectin ligands on leukocytes (Ley et al., 2007). The selectin family consists of three members, L(leukocyte)-selectin, P(latelet)-selectin and E(endothelial)-selectin. While L-selectin is expressed by most leukocytes, E-selectin and P-selectin are expressed by inflamed endothelial cells. P-selectin glycoprotein ligand 1 (PSGL1) has a dominant role as a ligand for all three selectins and is expressed on almost all leukocytes as well as endothelial cells (da Costa Martins et al., 2007). Leukocyte arrest during rolling is triggered by chemokines and other chemoattractants like CC-chemokine ligand 5 (CCL5, also known as RANTES), CXC-chemokine ligand 4 (CXCL4) and CXCL5 (Ley et al., 2007). This is mediated by binding of leukocyte integrins to immunoglobulin superfamily members, like intercellular adhesion molecule 1 (ICAM1) and vascular cell-adhesion molecule 1 (VCAM1), which were expressed by endothelial cells (Campbell et al., 1998; Campbell et al., 1996).

The pathogenesis of CRSwNP remains unclear, especially the ongoing chronic inflammation. The known role of the leukocyte adhesion cascade for the pathophysiology of inflammatory processes led us to investigate mRNA and protein expression profiles of leukocyte adhesion cascade related components in nasal polyps of chronic rhinosinusitis patients. Our results could represent a promising strategy in the future.

2 Results

Inflammation is an immune response of the organism to injury or tissue damage. During this process, native T-cells are activated and are able to differentiate into Th1-, Th2- or Th17-cells. Inflammation can affect the balance between these T-helper-cells and generate specific inflammatory pattern. Th1-cells primarily produce IL2, TNFα and INFγ, whereas Th2-cells predominantly produce IL4, IL5, IL10 and IL13. Th17- cells produce inter alia IL6, IL17 and TNFα. However it is possible that different cytokines
are produced by more than one T-helper subgroup, like IL2, IL6 and TNFα. Chronic rhinosinusitis with nasal polyps is usually a Th2 related inflammatory disease and microarray analysis showed significantly ($p \leq 0.05$) increased expression of specific genes, which were involved in immune-regulatory and inflammatory processes. Detailed analysis revealed a significant up-regulation of Th-2 related genes in nasal polyps when compared to associated inferior turbinates (Figure 1). Scatterplots point out the increased expression of Th-2 related genes, like IL4 (3.44-fold), IL5 (14.75- fold), IL10 (1.73- fold) and IL13 (21.15- fold) while Th1 and Th17 specific genes were unregulated between nasal polyps and inferior turbinates (Figure 2). Quantitative real-time PCR confirmed the increased expression of IL4 (3.93-fold, $p \leq 0.05$), IL5 (24.35-fold, $p \leq 0.01$), IL10 (2.77-fold, $p \leq 0.01$) and IL13 (31.01-fold, $p \leq 0.01$) in nasal polyps (Figure 3).

![Gene expression of specific inflammatory patterns displayed as heat map. Expression of particular genes where assigned to specific T-helper subgroups (Th1, Th2 and Th17). Gene expression alterations are color-coded as relative gene expression. Each line represents a gene and each column a tissue sample.](image)

**Figure 1:** Gene expression of specific inflammatory patterns displayed as heat map. Expression of particular genes where assigned to specific T-helper subgroups (Th1, Th2 and Th17). Gene expression alterations are color-coded as relative gene expression. Each line represents a gene and each column a tissue sample.

Further analysis of leukocyte adhesion cascade related components showed higher gene expression, P-selectin (1.62-fold, $n=7$), PSGL1 (1.74-fold, $n=7$) and VCAM1 (2.16-fold, $n=7$), while ICAM1 and CCL5 were unregulated. Surprisingly, E-selectin was strongly down-regulated in nasal polyps (0.32-fold, $n=7$) as well as CXCL4 (0.36-fold, $n=7$) and CXCL5 (0.12-fold, $n=7$) (Figure 4a). To confirm the data obtained by microarray analysis, we studied mRNA expression of adhesion molecules and chemokines.
Figure 2: Detailed expression of Th1 (IL2, IL12A, IL18, IFNγ, TNFα, T-bet), Th2 (IL2, IL4, IL5, IL6, IL10, IL13, GATA-3) and Th17 (IL6, IL17(A), IL23, TNFα, G-CSF, TGF-β1, RORγt) related cytokines in nasal polyps compared to associated inferior turbinates by microarray analysis. Each single dot shows the relative expression of the target molecule in nasal polyps compared to associated inferior turbinates of one patient. Median is indicated as horizontal bar.
Figure 3: Expression of Th1 (IL2, IFNγ, TNFα) and Th2 (IL4, IL5, IL10, IL13) related cytokines in nasal polyps compared to associated inferior turbinates validated by quantitative real-time PCR. Each single dot shows the relative expression of the target molecule in nasal polyps compared to associated inferior turbinates of one patient. Median is indicated as horizontal bar.

The expression of E-selectin was significantly decreased (0.22-fold, \(n=14, p \leq 0.01\)) as well as CXCL4 (0.15-fold, \(n=10, p \leq 0.05\)) and CXCL5 (0.017-fold, \(n=10, p \leq 0.05\)), while P-selectin expression was significantly increased (2.37-fold, \(n=15, p \leq 0.01\)) in nasal polyps compared to associated inferior turbinates (Figure 4b). Expression levels of PSGL1, CCL5, ICAM1 and VCAM1 did not significantly differ between nasal polyps and inferior turbinates.

E-selectin, P-selectin, PSGL1, ICAM1 and VCAM1 are expressed by inflamed endothelial cells, due to this we determined the expression of CD31, a specific endothelial cell marker, in nasal polyps and inferior turbinates of seven patients. CD31 was not altered expressed (0.93-fold) in nasal polyps compared to inferior turbinates (Figure 5a). Additionally, we normalized our data to CD31 (Figure 5b) and alterations were insignificantly compared to the β-actin normalized data (Figure 5c, Table I). Most chemokines are known to be deposit by platelets onto the inflamed endothelium (Ley et al., 2007). In this case, CCL5, CXCL4 and CXCL5 expression normalized to CD31 showed only insignificantly alterations when compared to the β-actin normalized data.

At the protein level, nasal polyps showed strongly decreased expression levels of E-selectin, while P-selectin was slightly up-regulated or equal expressed in nasal polyps when referenced to GAPDH and compared to inferior turbinates (Figure 6). After quantification and compared to inferior turbinates, protein expression of P-selectin was increased (145.8 % ± 30.3 %) and E-selectin was significantly decreased (62.6 % ± 6.96 %, \(p=0.018\)) in nasal polyps (Figure 7). Additional chemokine expression analysis revealed no significant altered availability of CCL5, CXCL4 and CXCL5 between nasal polyps and inferior turbinates (Figure 8).
Figure 4: Expression of leukocyte adhesion cascade related components in nasal polyps compared to associated inferior turbinates using microarray (a) and validated by quantitative real-time PCR (b). Each single dot shows the relative expression of the target molecule in nasal polyps compared to associated inferior turbinates of one patient. Median is indicated as horizontal bar.
Figure 5: Expression of CD31 normalized to β-actin (a) and leukocyte adhesion cascade related components normalized to CD31 (b) and β-actin (c) in nasal polyps compared to associated inferior turbinates using quantitative real-time PCR. Each single dot shows the relative expression of the target molecule in nasal polyps compared to associated inferior turbinates of one patient. Median is indicated as horizontal bar.

<table>
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<th>Gene</th>
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<th>Relative Expression Normalized to β-actin</th>
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<tr>
<td>E-selectin</td>
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<tr>
<td>P-selectin</td>
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<td>VCAM1</td>
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Table 1: Comparison of CD31 and β-actin normalized qPCR data.
Figure 6: Western blotting exhibited lower E-selectin expression in nasal polyps (P) than in the inferior turbinate (IT) of chronic rhinosinusitis patients (n=8). P-selectin was slightly up-regulated or equal expressed (n=8).

Figure 7: Quantification of E-selectin and P-selectin protein expression in nasal polyps referenced to endogenous control and compared to inferior turbinates (n=8).

Figure 8: Availability of chemokines CCL5, CXCL4 and CXCL5 in nasal polyps compared to associated inferior turbinates (n=3).
Expression of E-selectin, P-selectin and ICAM1 was detected on the endothelium of nasal polyps and inferior turbinates (Figures 9, 10, 11). E-selectin was expressed at high levels in the inferior turbinates, whereas in nasal polyps E-selectin was irregularly expressed and always at a low level (Figure 9). P-selectin was expressed at high levels in the endothelium of all observed vessels in both inferior turbinates and nasal polyps (Figure 10). Additional staining of ICAM1 revealed similar high expression in both, nasal polyps and inferior turbinates (Figure 11).

Further examinations of stained sections revealed that eosinophil counts were significantly ($p \leq 0.01$) higher in nasal polyp tissues, and to a lesser extent in inferior turbinate tissues. Neutrophil counts did not significantly differ between nasal polyps and inferior turbinates (Figure 12). Strikingly, eosinophil and neutrophils counts significantly ($p \leq 0.01$) differ in nasal polyp tissues, whereas inferior turbinate tissues exhibited balanced counts of eosinophils and neutrophils. The mean values of eosinophils and neutrophils in nasal polyps and inferior turbinates are summarized in Table 2.

**Figure 9:** Expression and localization of E-selectin ($n=10$) in inferior turbinate (A/B) and nasal polyp (C/D) of chronic rhinosinusitis patients.
Figure 10: Expression and localization of P-selectin ($n=10$) in inferior turbinate (A/B) and nasal polyp (C/D) of chronic rhinosinusitis patients.

Figure 11: Expression and localization of ICAM1 ($n=10$) in inferior turbinate (A/B) and nasal polyp (C/D) of chronic rhinosinusitis patients.
Figure 12: Eosinophils and neutrophils counts in nasal polyps and inferior turbinate (n=8). Each single dot represents the mean of 10 parts of high-power fields (HPF) at 400x magnification of one patient. Mean is indicated as horizontal bar.

<table>
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<th>Tissue</th>
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<th>Neutrophils [cells/HPF]</th>
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<td>Nasal polyps</td>
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<td>9.8±7.84</td>
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<tr>
<td>Inferior Turbinates</td>
<td>7.43±7.8</td>
<td>8.55±7.18</td>
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Table 2: Mean (±SD) values of eosinophils and neutrophils counts in nasal polyp and inferior turbinate tissues (n=8).

3 Discussion

Chronic rhinosinusitis is a multifunctional disease with several hypotheses that have been put forward regarding the pathogenesis of chronic rhinosinusitis with nasal polyps. However, there is a lack of detailed understanding of the basis of inflammation, especially the mechanisms responsible for recruitment and activation of leukocytes in nasal polyps. Inflammation in CRSsNP and CRSwNP are characterized by distinct inflammation patterns, showing a relatively Th1 biased inflammation pattern for CRSsNP, while CRSwNP portrays Th2-biased inflammation (Fokkens et al., 2012). But this does not apply worldwide. While the clinical nature of nasal polyps persists equally between Caucasians and Asians, the inflammatory patterns differ between geographical areas. 65–95% of European nasal polyps are eosinophilic (Couto et al., 2008; Stuck et al., 2007), while the most of Asian nasal polyps are predominantly noneosinophilic and shows different immunopathologic features with Th1 and Th17 cytokine profiles (Bachert et al., 2010; Cao et al., 2009; Kim et al., 2007; Zhang et al., 2008), but the reason for this geo-
graphical differences remains unclear. In the current study we focused our examinations on Caucasian nasal polyps of chronic rhinosinusitis patients with the typical eosinophilic and Th2-biased inflammation. The recruitment of leukocytes from the blood into the sites of inflammation involves integrins, selectins and chemokines (Ley et al., 2007). Several studies suggest a role of adhesion molecules for the pathogenesis of allergic, chronic and acute inflammatory diseases (Ciprandi et al., 1993; Kyan-Aung et al., 1991; Ley, 2003; Montefort et al., 1992) as well as tumor growth and metastasis (Biancone et al., 1996; Laubli & Borsig, 2010). E-selectin, ICAM1 and VCAM1 have been suggested to play a role in the pathophysiology of inflammatory airway diseases (Lukacs et al., 2002). But also chemokines are thought to initiate and perpetuate the chronic inflammatory response in CRSwNP (Hulse et al., 2014). For a better understanding of the mechanisms responsible for recruitment and activation of leukocytes in nasal polyps, we studied the expression profile of leukocyte adhesion cascade related components in nasal polyp and associated inferior turbinate tissue.

Selectins mediate the first adhesive contact of leukocytes to the endothelium under flow conditions (Schmidt et al., 2013). P-selectin preferentially promotes the recruitment of eosinophils, because P-selectin is known to activate α4β1 integrin on eosinophils, resulting in adherence to VCAM1 on activated endothelial cells (Johansson & Mosher, 2011). In line with our results, others have reported that P-selectin is well expressed in nasal polyp tissue (Hamilos et al., 1999; Symon et al., 1994). However, the selective recruitment of eosinophils in nasal polyps cannot be explained by a strong expression of P-selectin, because P-selectin is also able to bind neutrophils. E-selectin primarily promotes the recruitment of neutrophils and can be considered as the counterpart of P-selectin, which primarily promotes the recruitment of eosinophils (Chase et al., 2012), but E-selectin is also able to bind eosinophils. Accordingly, both, E-selectin and P-selectin, are able to support binding of both neutrophils and eosinophils, but E-selectin is most efficient at raising the affinity of CD18 integrins that support neutrophil deceleration and trafficking to sites of acute inflammation (Chase et al., 2012). Eosinophils bind significantly less efficiently to E-selectin than neutrophils, because neutrophils express more of the ligands for E-selectin (sialyl Lewis X or sialyl dimeric Lewis X antigen) on their cell surface than eosinophils (Beck et al., 1996; Bochner et al., 1994; Jahnsen et al., 1995). PSGL1 has a dominant role as a ligand for all three selectins in an inflammatory environment and is expressed on almost all leukocytes as well as endothelial cells (da Costa Martins et al., 2007; Ley et al., 2007). It is the best studied selectin ligand and important for neutrophil recruitment (Schmidt et al., 2013). Our results revealed an equal expression of PSGL1 in nasal polyps and inferior turbinates. However, the current literature does not involve PSGL1 in the pathogenesis of CRSwNP, so far the role of PSGL1 in CRSwNP remains unclear. Between first adhesive contact of leukocyte and leukocyte arrest, chemokines are crucial for further conformational changes of leukocytes, which mediates leukocyte arrest (Ley, 2014). Several chemokines have been linked to the selective recruitment of inflammatory cells into the tissue and to chronic rhinosinusitis with nasal polypos (Fokkens et al., 2012). Most of them are chemoattractants for eosinophils or are related to eosinophils. CCL5 was one of the first chemokines which was identified to be up-regulated in nasal polyps (Beck et al., 1996; Davidsson et al., 1996). CCL5 is a
strong chemoattractant for eosinophils and T lymphocytes, but not for neutrophils. Recently, Cavallari et al. showed that CCL5 gene expression was up-regulated in eosinophilic nasal polyps compared to healthy controls (Cavallari et al., 2012). We used the inferior turbinate of the same patient as internal control due to the fact that it is not affected by nasal polyp growth and shares the same chronic inflammatory conditions as nasal polyps. However, we did not find a significantly altered expression of CCL5. Additionally, CXCL4 and CXCL5 gene expression was significantly down-regulated, but not at the protein level. CXCL4, a member of the CXC chemokine family, is produced by cells of the megakaryocytic lineage, but is also constitutionally produced by human monocytes (Lasagni et al., 2007; Schaffner et al., 2005). CXCL4 has many signaling functions and is immunomodulating, but most of the effects are on monocytes (Deuel et al., 1981; Fleischer et al., 2002; Gewirtz et al., 1989; Han et al., 1997; Horton et al., 1980; Marti et al., 2002). Furthermore, CXCL4 is a chemoattractant for monocytes, but also neutrophils, and prevent monocytes from apoptosis (Deuel et al., 1981; Flad et al., 1998). Additionally, in conjunction with IL4, monocytes differentiate into macrophages and antigen-presenting cells, like dendritic cells (Fricke et al., 2004; Scheuerer et al., 2000; Xia & Kao, 2003). CXCL5 is an epithelial cell-derived neutrophil-activating peptide, which mainly attracts neutrophils and is produced by a wide variety of cells, including macrophages, dendritic and epithelial cells, at sites of inflammation (Charo & Ransohoff, 2006; Middleton et al., 2002; Schwerk et al., 2013; Suzuki et al., 2002). Both, CXCL4 and CXCL5, are able to attract neutrophils and gene expression was down-regulated in nasal polyps of CRS patients, but not at the protein level. The current literature does not imply CXCL4 and CXCL5 expression in the pathophysiology of nasal polyps and needs to be further investigated. In this context and due to the fact that most chemokines are deposited by platelets onto the inflamed endothelium (Ley et al., 2007), platelets may also play an important role in the pathogenesis of CRSwNP, as already demonstrated in allergic diseases like asthma, or allergic rhinitis. Platelets have an important role in the recruitment of leucocytes into the inflamed tissue and it was indicated that platelets contribute inflammation by modulating processes involved in airway remodelling (Page & Pitchford, 2014).

The immunoglobulin superfamily members ICAM1 and VCAM1 convey the adhesion of different leukocytes. ICAM1 is known for a selective recruitment of eosinophils and neutrophils, whereas VCAM1 plays a favored role for eosinophil extravasation in chronic inflammatory conditions (Beck et al., 1996; Fokkens et al., 2012; Jahnsen et al., 1995). We did not find a significant alteration in VCAM1 and ICAM1 gene expression, but others reported an increased expression of VCAM1 in nasal polyps (Hamilos et al., 1999; Jahnsen et al., 1995). ICAM1 plays a dominant role in allergic rhinitis and asthma. It initiates and modulates different intercellular signaling events and cellular functions and is highly related to the proinflammatory infiltrate (Gorska-Ciebiada et al., 2006; Stanciu & Djukanovic, 1998). Nonetheless, ICAM1 is not specific for the lymphocyte response mainly of the Th2 type and its role was discussed controversially. On the one hand increased ICAM1 expression was found in nasal polyp tissue (Doner et al., 2004; Kong et al., 1999; Rogala et al., 2000; Zhou et al., 2004), on the other hand ICAM1 expression did not differ between nasal polyps and healthy controls (Bujia & Rasp, 1997;
Cavallari et al., 2012; Corsi et al., 2008; Jahnsen et al., 1995). Several assumptions have been made to interpret this conflict, like different patient cohorts, differences in staining methods or preoperative corticosteroid medication. In our study we can exclude corticosteroid medication or associated diseases, which could affect the expression of several genes and proteins. Additionally, we used the inferior turbinate of the same patients as control, because it represents a good internal control due to the fact that it is not affected by nasal polyp growth and shares the same chronic inflammatory conditions as nasal polyps. However, we find a well expression of ICAM1, which did not significantly altered between nasal polyps and inferior turbinates. Further investigations are needed to clarify the role of ICAM1 in CRSwNP. When we put these results together, both P-selectin and VCAM1 predominantly recruit eosinophils and were up-regulated in nasal polyps. This could conceivably explain such selective recruitment of eosinophils, but not the high immune imbalance between eosinophils and neutrophils, because P-selectin as well as VCAM1 secondary recruit neutrophils.

However, a down-regulation of the neutrophil attracting chemokines CXCL4 and CXCL5 could contribute to this immune imbalance. Additionally, we found a down-regulation of E-selectin, which may be a crucial factor in the pathogenesis of nasal polyps, because E-selectin optimizes the mechanics and kinetics for the recognition of multiple ligands. The bond strength of E-selectin is more durable than that of P-selectin, because the membrane tethered with the substrate transmits force to the bonds with E-selectin ligands under shear forces of blood flow (Chase et al., 2012). Once inhibited, E-selectin was not able to recruit neutrophils in a primate model, the influx of eosinophils was unaffected (Beck et al., 1996; Symon et al., 1994). In line with the down-regulation of E-selectin, we demonstrated a selective recruitment of eosinophils in nasal polyps and a final balance of eosinophils and neutrophils in inferior turbinates. The role of E-selectin in immune surveillance may be to amplify the sensitivity to locally activate neutrophil arrest and migratory function (Chase et al., 2012). Neutrophils were suggested to play a significant role in the resolution of inflammation as well as for the pathology of the chronic inflammatory state (Mantovani et al., 2011). Additionally, as it has been reported for bronchial biopsies and serum of asthmatic patients, we have expected increased expression of E-selectin in nasal polyps, following the concept of united airway disease (Bentley et al., 1993; Gosset et al., 1995; Kobayashi et al., 1994; Yamashita et al., 1997). However, we found a down-regulation of E-selectin in the endothelium of nasal polyps, like human squamous cell carcinomas of the skin, which down-regulated vascular E-selectin to evade the immune response (Clark et al., 2008).

As mentioned above and consistently with our results, recent studies have demonstrated that CRSwNP is a T-helper-2 (Th2) disorder (Fokkens et al., 2012), but this does not apply worldwide. In European countries 65-95% of nasal polyps are eosinophilic (Couto et al., 2008; Stuck et al., 2007) but nasal polyps in Asia show different immunopathologic features with Th1 and Th17 cytokine profiles and neutrophilic inflammation (Bachert et al., 2010). E-selectin is responsible for the cellular influx of neutrophils and is down-regulated by the Th2 cytokine IL-4 (Raab et al., 2002). Another study from China demonstrated an up-regulation of E-selectin in Asian nasal polyps (Kang et al., 2006). So E-selectin may play an important role in the immune balance of eosinophils
and neutrophils and so on in the pathophysiology of nasal polyps.

In summary, an imbalance between Th1 and Th2 responses leads to a chronic inflammatory answer and to establish the final balance between Th1 and Th2 may be essential for the enhancement or protection of disease (Delcenserie et al., 2008; Nicolo et al., 2006). Caucasian nasal polyps exhibit a Th2 inflammation and the inflammatory infiltrate is mostly dominated by eosinophils. Different therapeutic enhancements could represent a promising strategy in the future to reestablish the final balance between Th1 and Th2, and thus a balance between eosinophils and neutrophils. Investigations in platelet activation pathways could provide novel anti-inflammatory therapies without affecting haemostasis. This could, for example result in a better understanding in deposition of chemokines, and up- or down-regulation of specific chemokines could resolve inflammation. Anti-inflammatory therapies, like anti-IL5 or anti-IL13, were already tested in asthmatic patients (Hua et al., 2015; Mukherjee et al., 2014) and could be a promising strategy for Caucasian nasal polyps. An anti-IL5 therapy was already tested in patients with nasal polyps with a successful reduction of the size of nasal polyps (Gevaert et al., 2006), but further examinations are necessary to evaluate the effectiveness of an anti-IL5 therapy. On the basis of our results, we suggest that an up-regulation of E-selectin and the associated influx of neutrophils may be essential to reestablish the immune balance in Caucasian nasal polyps, which are mostly dominated by eosinophils. On the other hand, a reduction of eosinophils is also a possible therapeutic therapy. P-selectin is able to activate α4β1 integrin on eosinophils, which result in greater adherence to VCAM1 and influx into the inflamed tissue. A down-regulation of P-selectin could reduce the influx of eosinophils and reestablish the immune balance between eosinophils and neutrophils in Caucasian nasal polyps, like we detected in inferior turbinates. However, further studies are necessary to validate the best therapy in the pathogenesis of nasal polyps in CRS, and if so, whether its therapeutic effectiveness could represent a promising strategy in the future.

4 Materials and Methods

All patients were treated surgically at the Department of Otorhinolaryngology, University Hospital Schleswig-Holstein, Campus Lübeck, and have given their written informed consent. The study was approved by the local ethics committee of the University of Lübeck and conducted in accordance with the ethical principles for medical research formulated in the WMA Declaration of Helsinki. Nasal polyp tissue and associated inferior turbinate tissue, as internal control, were harvested from 34 Caucasian patients (28 males and 6 females, mean age 49.32 ± 14.10) who underwent functional endoscopic sinus surgery (FESS) or septoplasty with resection of the inferior turbinates. Fresh tissue samples were flash frozen in liquid nitrogen immediately after resection, stored at -80 °C before RNA and protein extraction. All patients had a history of sinusitis of more than three months and did not respond to conservative therapy. Patients were skin tested for pollens, molds, dust mites, and pets using standardized extracts (Allergopharma Joachim Ganzer KG, Reinbek, Germany) within a time frame of four
weeks before surgery. Eosinophilic CRSwNP was determined by histopathologic examination and patients with mucoviscidosis or neutrophilic nasal polyps were not included in this study. All patients had been free of steroid medication for at least four weeks before surgery and had no history of atopy, bronchial asthma or salicylate intolerance/aspirin-exacerbated respiratory disease. Microarray, quantitative real-time PCR, western blot and immunohistochemistry were performed as previously published (Könnecke et al., 2014). Additional material and methods are shown in Table 3, Table 4 and Section 4.1.

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**Table 3:** Additional TaqMan® Gene Expression Assays used for qPCR.

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**Table 4:** Additional antibodies used for western blot and immunohistochemistry.

### 4.1 Chemokine Detection

Chemokines were detected using the Proteome Profiler™ Human Chemokine Array Kit (R&D Systems, Inc., NE, MN/USA, #ARY017) according to the manufacturer instructions. 200 µg of protein from nasal polyps and inferior turbinates were used for chemokine detection. For data analysis, pixel density of each spot was measured and average signals calculated. The background was subtracted and the corresponding signals were compared to determine the relative change in chemokine levels between nasal polyps and inferior turbinates.

### Acknowledgement

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References


Markers, 23(2), 115–120.


