

Endothelial L-selectin ligand expression in nasal polyps

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Keywords

antro-choanal polyps; chronic disease; cystic fibrosis; eosinophils; L-selectins; nasal polyps; rhinosinusitis; sialyl Lewis x.

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Abstract

Background: L-selectins on leukocytes and their counter-receptors on endothelial cells have been shown to be involved in leukocyte recruitment in chronic rhinosinusitis without nasal polyps (NP).

Objectives: The purpose of this study was to evaluate the expression level of functionally active endothelial L-selectin ligands in NP obtained from patients with NP of different etiology [simple NP, antro-choanal polyps (ACP) and cystic fibrosis (CF) NP] and inferior turbinate specimens of healthy controls and to compare these levels to the presence of various leukocyte subsets.

Methods: Nasal polyp specimens and healthy nasal mucosa specimens were obtained from patients undergoing surgery and were immunohistochemically stained with monoclonal antibodies detecting CD34, sialyl Lewis x (sLe^x) of sulfated extended core 1 lactosamines and various leukocyte subsets.

Results: All NP are characterized by a decrease in the number of CD34+ vessels. The number of eosinophils and the percentage of vessels expressing endothelial sulfated sLe^x epitopes is upregulated in all groups of simple NP. Tissue eosinophilia is increased in those patients with increased disease severity (acetyl salicylic acid intolerance), but the percentage of endothelial sulfated sLe^x epitopes is not. Results on CF NP are similar to those observed for simple NP. Antro-choanal polyps, on the contrary, are characterized by low numbers of tissue eosinophils and relatively few vessels expressing endothelial sulfated sLe^x epitopes.

Conclusions: Our results suggest that functionally active L-selectin ligands might play a role in guiding leukocyte traffic into NP in patients with simple NP and CF NP but not ACP.

Nasal polyposis is a multifactorial disease, associated with asthma, cystic fibrosis (CF), primary ciliary dyskinesia, acetyl salicylic acid (ASA) intolerance and possibly allergy, affecting

between 1% and 4% of the general population (1, 2), up to 56% of patients with CF (3) and up to 60% of patients with ASA intolerance (1, 2). Although in general occurring bilaterally and originating from the ethmoid cells, nasal polyps (NP) do exist unilaterally. Originating from the maxillary sinus mucosa and protruding into the choana, these polyps, frequently observed on only one side of the nasal cavity, are called antro-choanal polyps (ACP) and constitute a distinct disease entity (4).

Although many hypotheses have been suggested, the pathogenesis of all NP is still largely unknown. Macroscopically,

Abbreviations

ACP, antro-choanal polyps; AP, alkaline phosphatase; ASA, acetyl salicylic acid; CF, cystic fibrosis; CRS, chronic rhinosinusitis; HEV, high endothelial venules; mAb, monoclonal antibody; MBP, major basic protein; NM, control nasal mucosa (inferior turbinate); NP, nasal polyps; PBS, phosphate-buffered saline; RT, room temperature; sLe^x, sialyl Lewis x; SPT, skin prick test.

NP are characterized by the presence of edematous masses of inflamed mucosa, predominantly localized in the middle meatus and prolapsing into the nose, leading to nasal obstruction, secretion, loss of smell, headache/facial pain, and a reduced quality of life. Histologically, NP are characterized by a cover of respiratory epithelium, large quantities of extracellular edema and a dense inflammatory cell infiltrate consisting of mast cells, eosinophils, lymphocytes, neutrophils, and plasma cells, which release a variety of pro-inflammatory mediators, including cytokines, histamines, prostanoids, and leukotrienes. Although macroscopically and clinically remarkably similar, careful analysis of the literature reveals important histopathologic differences between subtypes of NP (4–7). In the majority of bilaterally occurring NP from patients without CF (in this study called simple NP), eosinophils are the most abundant inflammatory cell type. In contrast, CF NP, occurring bilaterally, and ACP, generally occurring unilaterally, are classically thought of as being neutrophilic (4, 5, 8–10).

Leukocyte recruitment in acute and chronic inflammation is characterized by sequential cell adhesion and activation events (11). Low-affinity adhesive interactions between selectins and their counter-receptors (glycosylated ligands) initially tether leukocytes to the vessel wall and cause tethered leukocytes to roll along endothelial cells lining the inside of the vessel, thereby facilitating activation of leukocytes by chemokines, leading to increased levels of expression of leukocyte integrins, resulting in firm adhesion of leukocytes to the vessel wall, arrest of rolling and, ultimately, the process of transmigration (11–15).

Selectins are a family of type I membrane-spanning glycoproteins that are essential for leukocyte recruitment. Each selectin recognizes related but distinct counter-receptors displayed by leukocytes, and/or endothelial cells. E-selectin is expressed by endothelial cells, P-selectin is expressed by endothelial cells and platelets and L-selectin is constitutively expressed by most types of circulating leukocytes. L-selectin differs from E- and P-selectins in that it, besides mediating low-affinity interactions leading to rolling, recognizes glycan-dependent endothelial-cell-derived counter-receptors and glycan-dependent counter-receptors expressed by leukocytes that have previously adhered to the endothelium. L-selectin counter-receptors include CD34, sulfated glycoprotein of 200 kDa (Sgp200), endomucin, glycosylation-dependent cell adhesion molecule 1 (GlyCAM-1), podocalyxin, endoglycan, mucosal addressin cell-adhesion molecule 1 (MAdCAM-1), and P-selectin glycoprotein ligand 1 (PSGL-1), expressed by endothelial cells of high endothelial venules (HEV) of lymph nodes and Peyer patches, activated endothelial cells and other leukocytes (16–19). All of these ligands are sialomucin in nature and contain a predominance of *O*-linked carbohydrate chains (*O*-glycans). The adhesive ‘activities’ of counter-receptors require specific glycan-based post-translational modifications, determined largely by glycosyl-transferases. The result is decoration with specific sialylated, sulfated, and fucosylated oligosaccharides, situated on C-6 positions of galactose, and *N*-acetylglucosamine residues on numerous *O*-glycans (20–27). Most L-selectin ligands such as CD34 present sulfation of the

sialyl Lewis x (sLe^x) tetrasaccharide (Sia α 2,3Gal β 1,4-[Fuc α 1,3]GlcNAc) on the C-6 position of GlcNAc (6-sulfo-sLe^x) as a recognition determinant within their heavily glycosylated mucin domains (28). Under normal conditions, properly glycosylated L-selectin ligands are expressed along HEV in lymphatic tissues only (29). However, in rodents and humans undergoing allograft rejection as well as in patients suffering from chronic inflammatory diseases such as asthma and chronic rhinosinusitis (CRS), induction of sLe^x and 6-sulfo-sLe^x decorated L-selectin ligands is seen in postcapillary endothelial cells in tissues other than lymphatic tissue (29–33).

Previously, we demonstrated that, in CRS patients without NP, the expression level of functionally active L-selectin ligands clearly correlates with the extent of tissue eosinophilia and severity of inflammation (33). Based on these observations, we postulate that it is the level of functionally active L-selectin ligands that determines the extent of tissue eosinophilia in simple NP as well. Elaborating on this, we suggest that increased levels of functionally active L-selectin ligands explain the increase in tissue eosinophilia observed in simple NP patients with asthma and/or ASA intolerance [classically thought of as a more severe phenotype of (simple) nasal polyposis] when compared with simple NP patients without asthma and/or ASA intolerance.

Material and methods

Subjects

Nasal polyps were obtained from patients undergoing endoscopic sinus surgery. Prior to surgery, a full ear, nose, and throat history was taken. Diagnosis of CF was established on the basis of clinical features, a positive sweat test and/or characteristic genotype abnormalities. Diagnosis of ACP was established by nasal endoscopy and computed tomography-imaging. Diagnosis of asthma was based on clinical features and when necessary on pulmonary function tests. Diagnosis of ASA intolerance was made on the basis of a clear-cut history of asthma attacks precipitated by nonsteroidal anti-inflammatory drugs. Diagnosis of atopy was based on skin prick test (SPT) positivity. Those patients with a positive SPT against one or more common aeroallergens were excluded. In addition, those patients treated with glucocorticoids (both oral and topical nasal) in the 4 weeks prior to inclusion were excluded. Control nasal mucosa (NM) samples (inferior turbinate) were obtained from patients undergoing corrective surgery for turbinate hypertrophy or septoplasty. None of the patients in this control group suffered from allergy, asthma, or ASA intolerance, and none of them was treated with glucocorticoids. Patient characteristics are summarized in Table 1.

Tissue handling

All specimens, obtained at the time of surgery, were immediately snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Immunohistochemistry

Alkaline phosphatase staining of CD68, CD117, MBP, elastase, functionally active L-selectins, and CD34

Serial sections, 5- μ m thick, of all snap-frozen specimens were cut on a Microm HM560 frigocut cryostat (Microm/Thermo Scientific, Walldorf, Germany) and transferred to amino-phosphate-ethylsilane coated slides (Starfrost, Braunschweig, Germany), dried, and stored at -70°C until analysis. Upon analysis, tissue sections were defrosted to room temperature (RT), dried, and fixed in acetone (cold as ice) for 10 min at RT. After fixation, tissue sections were rinsed with phosphate-buffered saline (PBS, pH 7.8), placed in a semi-automatic stainer (Sequenza; Shandon, Thermo Scientific, Walldorf, Germany) and incubated with 10% normal goat serum (CLB, Amsterdam, The Netherlands) in blocking reagent (Roche 10961760; Roche Diagnostics, Almere, the Netherlands) for 10 min. Following the blocking procedure, sections were incubated with primary antibody for 60 min at RT. Mouse anti-human monoclonal antibodies (mAbs) directed against CD68, CD117, major basic protein (MBP), and elastase, functionally active L-selectins (as detected with mAbs HECA-452 and MECA-79) and endothelial cells (CD34) were used (Table 2). All primary antibodies were

diluted in PBS containing 1% (w/v) blocking reagent (Roche 10961760) to block endogenous avidin and biotin activity. Following incubation with primary antibody, sections were rinsed with PBS for 5 min and incubated with biotinylated goat anti-mouse antiserum (1 : 50; Klinipath, Duiven, The Netherlands) for 30 min at RT. Next, sections were rinsed with PBS and incubated with streptavidin alkaline phosphatase (ss-AP, Biogenics 1 : 50; Klinipath) for 30 min at RT. Sections were then rinsed with PBS containing tris hydroxymethylaminoethane (TRIS) buffer (0.2 M, pH 8.5) and incubated with new fuchsin (Chroma, Kongen, Germany) substrate (containing levamisole to block endogenous AP enzyme activity) for 20 min at RT. Sections were counterstained with Gill's hematoxylin (CD68, CD117, MBP, elastase) or Mayer's Hämalaun (CD34, mAbsHECA-452, and MECA-79), rinsed with distilled water and mounted in Vecta Mount (Vector, Burlingame, CA, USA). Control staining was performed by substituting primary antibody with PBS in case of CD68, CD117, MBP, elastase, and CD34. mAbs 7C7 (mouse IgM) and TIB-146 (rat IgM) were used as negative controls for mAbs HECA-452 and MECA-79 respectively.

Tyramide signal amplification staining for Basogranulin

A sensitive protocol was used based on the AP method described above. Sections were cut and defrosted as described above. Sections were fixed in acetone (cold as ice) for 10 min, placed in the semi-automatic stainer, and incubated with normal goat serum as described above. Slides were incubated with mouse anti-human mAb directed against basogranulin, diluted in PBS containing 1% (w/v) blocking reagent (Table 2). After incubation with biotinylated goat anti-mouse antiserum, endogenous peroxidase was blocked using 0.2% (w/v) azide, 0.02% (v/v) hydrogen peroxide and 50% (v/v) methanol in PBS. Following the blocking procedure, slides were incubated with streptavidin conjugated peroxidase (NEN, Boston, MA, USA) for 30 min at RT, rinsed with PBS, and incubated with biotinyl tyramide in TRIS buffer for 10 min in order to amplify the signal. Next, slides were rinsed once again in PBS and incu-

Table 1 Patient characteristics

Group	No. patients	Male sex, n (%)	Age, median (IQ range)
ACP	8	3 (38)	51.0 (11.0–65.0)
CF NP	8	6 (75)	13.0 (9.5–16.5)
NM	22	17 (71)	29.0 (26.5–40.5)
Simple NP	9	4 (40)	46.0 (41.5–55.5)
Simple NP + asthma	2	1 (50)	68.5 (66.0–71.0)
Simple NP + asthma + ASA intolerance	8	6 (75)	47.0 (37.0–52.0)

ACP, antro-choanal polyps; CF NP, cystic fibrosis nasal polyps; NM, nasal mucosa; ASA, acetyl salicylic acid.

Table 2 Monoclonal antibodies

Antibody	Specificity	Cell type	Titer	Source
KI-M6	CD68	Macrophages	1 : 200	DAKO, Glostrup, Denmark
YB5.B8	CD117	Mast cells	1 : 65	Becton Dickinson, Alphen a/d Rijn, the Netherlands
BB1	Basogranulin	Basophils	1 : 150	A.F. Walls, South Hampton, UK
AHN-10	Elastase	Neutrophils	1 : 32000	Chemicon, Temecula, CA, USA
BMK-13	MBP	Eosinophils	1 : 100	Sanbio, Uden, The Netherlands
HECA-452	2,3-sialylation & 1, 3-fuco-sylation of lactosamine	Functionally active endothelial L-selectins	1 : 100	BD Biosciences Pharmingen, CA, USA
MECA-79	extended sulphate core 1 lactosamine	Functionally active endothelial L-selectins	1 : 100	BD Biosciences Pharmingen, CA, USA
CD34	CD34	Endothelial cells	1 : 100	DAKO, Glostrup, Denmark

bated with AP conjugated goat-anti-biotin antiserum (Sigma, Zwijndrecht, the Netherlands). Sections were then rinsed with PBS containing TRIS buffer (0.2 M, pH 8.5) and incubated with new fuchsin (Chroma, Kongen, Germany) substrate (containing levamisole to block endogenous AP enzyme activity) for 20 min at RT. Sections were counterstained with Gill's hematoxylin, rinsed with distilled water and mounted in Vecta Mount (Vector, Burlingame, CA, USA). Control staining was performed by substituting primary antibody with PBS.

Light microscopic evaluation

All sections were examined with an Olympus BX51 light microscope (Olympus Nederland B.V., Zoeterwoude, the Netherlands) by two independent observers blinded to the experimental conditions, as described previously. Positively stained leukocytes and vessels were counted in the lamina propria at a final magnification of 200 $\times$. Results are expressed as the mean number of positive leukocytes or vessels (CD34+) per mm². Mean numbers of mAb HECA-452 positive and mAb MECA-79 positive vessels were divided by the mean number of CD34+ vessels from the same specimen to yield the percentage of sLe^x- or sulfated lactosamine reactive vessels (31).

Data analysis

Statistical analysis was carried out by using SPSS 12.01 (SPSS Inc., Chicago, IL, USA). Data are expressed as median and interquartile range. Kruskal–Wallis nonparametric tests were performed to check for significant in-between-group variability. In case of significant in-between-group variability, Mann–Whitney *U*-test nonparametric tests were performed for between-group comparisons. Spearman's rank-order correlation was used to assess bivariate association. The correlation value (ρ) indicates the strength of correlation and varies between 0 and 1, in which 1 means a perfect correlation. Two-tailed *P*-values of <0.05 were considered statistically significant.

Results

Expression of leukocyte subsets and functionally active endothelial L-selectin ligands in control inferior turbinate (NM), simple NP, ACP, and CF NP

In the first part of this study, we compared the expression levels of functionally active endothelial L-selectin ligands and studied the extent of tissue eosinophilia, tissue neutrophilia, and the level of macrophages, basophils, and mast cells in control NM samples (20 patients), simple NP samples (nine patients), ACP samples (eight patients), and CF NP samples (eight patients). As asthma, allergy, and ASA intolerance are rarely present in patients with CF NP and ACP, those patients suffering from these comorbidities were not included in the simple NP group. Patients in the CF NP and NM group were younger than those in the simple NP group

($P = 0.01$ and $P = 0.01$ respectively). In addition, CF NP patients were younger than NM patients ($P = 0.05$). No differences in age were observed between patients suffering from ACP and all other patients. Gender distribution was similar in all groups ($P = 0.49$). Compared with control NM, all three groups of NP were characterized by decreased numbers of CD34+ vessels. For ACP, this difference reached significance (Fig. 1 and 4). Compared with control NM, simple NP as well as CF NP were characterized by an increased percentage of vessels expressing sulfated sLe^x epitopes (functionally active L-selectin ligands) as analysed with mAbs HECA-452 and MECA-79 (Fig. 2 and 4). In contrast, ACP were characterized by decreased levels of functionally active L-selectin ligands when compared with control NM, simple NP, and CF NP ($P < 0.05$).

Although the number of eosinophils was clearly increased in simple NP (Fig. 3 and 4), this increase was not significant when compared with control NM, CF NP, and ACP. When compared with control NM, CF NP are characterized by a significant increase in the number of neutrophils and macrophages (Table 3). Although numbers are not significant, similar results are observed for ACP (Table 3). Simple NP as well as ACP are characterized by a significant decrease in the number of mast cells (Table 2). No differences were observed in the number of basophils present.

When studying the correlation between the percentage of mAb MECA-79+ vessels and the presence of leukocytes in this group of tissue specimens, a clear correlation between mAb MECA-79 positivity and the presence of eosinophils was observed ($P = 0.02$, $r = 0.39$, data not shown), thereby suggesting a possible role for sulfated endothelial sLe^x oligosaccharides to preferentially guide eosinophils to diseased sinuses. No correlation was observed between the percentage of mAb MECA-79+ vessels and other leukocyte subsets and no correlation was observed for mAb HECA-452+ vessels (data not shown).

Expression of leukocyte subsets and functionally active endothelial L-selectin ligands in control NM and simple NP of patients with or without asthma and/or ASA intolerance

Patients in the NM group were younger than those patients in all other groups ($P = 0.05$ when compared with simple NP, $P = 0.03$ when compared with simple NP with asthma, and $P = 0.04$ when compared with simple NP with asthma and ASA intolerance). In addition, patients with simple NP with asthma were older than those simple NP patients without comorbidities and those simple NP patients with asthma and ASA intolerance ($P = 0.03$ and $P = 0.04$ respectively). Gender distribution was similar in all groups ($P = 0.49$). As has been described above, simple NP (nine patients) was characterized by decreased numbers of CD34+ vessels when compared with control NM (20 patients). The percentage of vessels expressing sulfo sLe^x epitopes as analysed with mAbs HECA-452 and MECA-79 was shown to be increased. Although a similar trend is observed for NP obtained from patients with asthma (two patients) and patients with

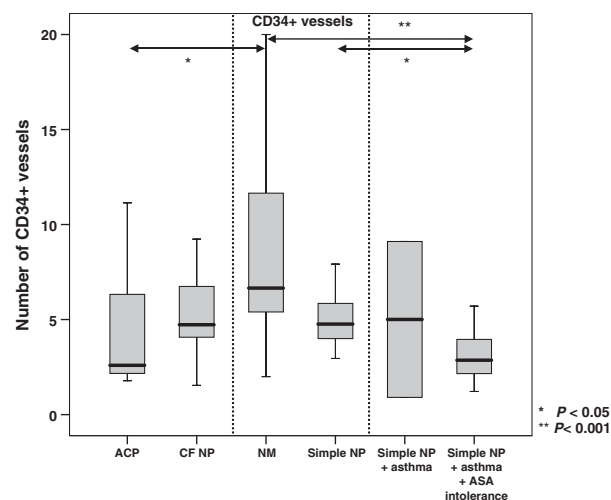


Figure 1 Number of CD34+ vessels in antro-choanal polyps (ACP), CF nasal polyps (CF NP), control nasal mucosa (NM), simple nasal polyps (simple NP), simple nasal polyps of patients with asthma (simple NP + asthma) and simple nasal polyps of patients with asthma and ASA intolerance (simple NP + asthma + ASA intolerance).

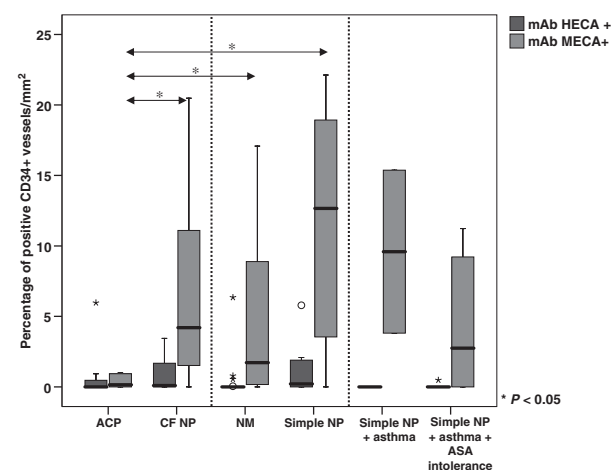


Figure 2 Percentage of mAb HECA-452+ and mAb MECA-79+ CD34+ vessels in control nasal mucosa (NM), simple nasal polyps (simple NP), simple nasal polyps of patients with asthma (simple NP + asthma) and simple nasal polyps of patients with asthma and ASA intolerance (simple NP + asthma + ASA intolerance).

asthma and ASA intolerance (eight patients), the number of CD34+ vessels and the percentage of mAb HECA-452+ and mAb MECA-79+ CD34+ vessels is lower when compared with simple NP specimens obtained from patients without asthma and ASA intolerance (Figs 1 and 2). Although the number of eosinophils is significantly increased in polyps from patients with NP with asthma and ASA intolerance (Fig. 3), no correlation between mAb MECA-79 positivity and the presence of eosinophils in NP tissue is

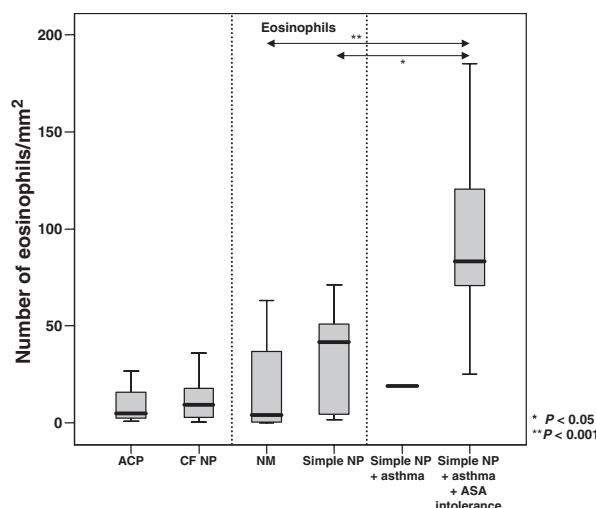


Figure 3 Absolute number of eosinophils per mm² in control nasal mucosa (NM), simple nasal polyps (simple NP), simple nasal polyps of patients with asthma (simple NP + asthma) and simple nasal polyps of patients with asthma and ASA intolerance (simple NP + asthma + ASA intolerance).

observed (data not shown). The percentage of mAb MECA-79+ vessels did not correlate with other subsets of leukocytes and no correlation was observed for mAb HECA-452+ vessels (data not shown).

Discussion

All NP are characterized by a decrease in the number of CD34+ vessels per mm². This lower density of CD34+ vessels clearly reflects the edema observed in all NP and is most obvious in those polyps obtained from simple NP patients with asthma and ASA intolerance as was shown previously by others (34).

In healthy NM, the majority of vessels does not express sulfated sLe^x glycans as detected by mAb HECA-452 (2,3-sialylation and 1,3-fucosylation of lactosamine). Although the percentage of vessels expressing sulfated lactosamines as detected with mAb MECA-79 (extended sulphate core 1 lactosamine) is higher, the overall percentage is low. In comparison to control maxillary sinus mucosa (33), we currently demonstrate that the percentage mAb MECA-79+ vessels is higher in healthy inferior turbinate specimens although no direct comparison was made. As inferior turbinate mucosa is in constant contact with the surrounding environment, filtering air from air-borne microbes and irritants, this slight upregulation of endothelial sulfated sLe^x oligosaccharides in control inferior turbinate mucosa may result from a low-level inflammation as a result of this exposure.

When compared with control NM, the expression of sulfated sLe^x oligosaccharides (as detected by both mAb HECA-452 and mAb MECA-79) and the number of eosinophils are upregulated in all groups of simple NP. Similarly,

Table 3 Presence of subsets of leukocytes per mm²

No. leukocytes/ mm ²	NM (median, min.–max.)	Simple NP (median, min.–max.)	ACP (median, min.–max.)	CF NP (median, min.–max.)
Eosinophils	4.0 (0.0–140.9)	41.6 (1.6–137.6)	4.8 (0.8–26.6)	9.4 (0.5–36.1)
Neutrophils	23.4 (4.3–297.4)	25.6 (4.0–251.8)	104.9 (40.8–157.8)	124.5 (63.0–267.6)
Macrophages	47.8 (17.3–129.1)	35.5 (16.1–138.9)	152.5 (65.7–239.4)	82.8 (51.3–158.9)
Mast cells	28.4 (7.1–63.2)	11.9 (2.3–41.0)	11.4 (9.6–18.3)	26.0 (17.9–83.1)
Basophils	1.1 (0.0–7.6)	0.4 (0.0–5.6)	0.6 (0.5–0.7)	1.5 (0.4–3.0)

NM, nasal mucosa; ACP, antro-choanal polyps; CF NP, cystic fibrosis nasal polyps.

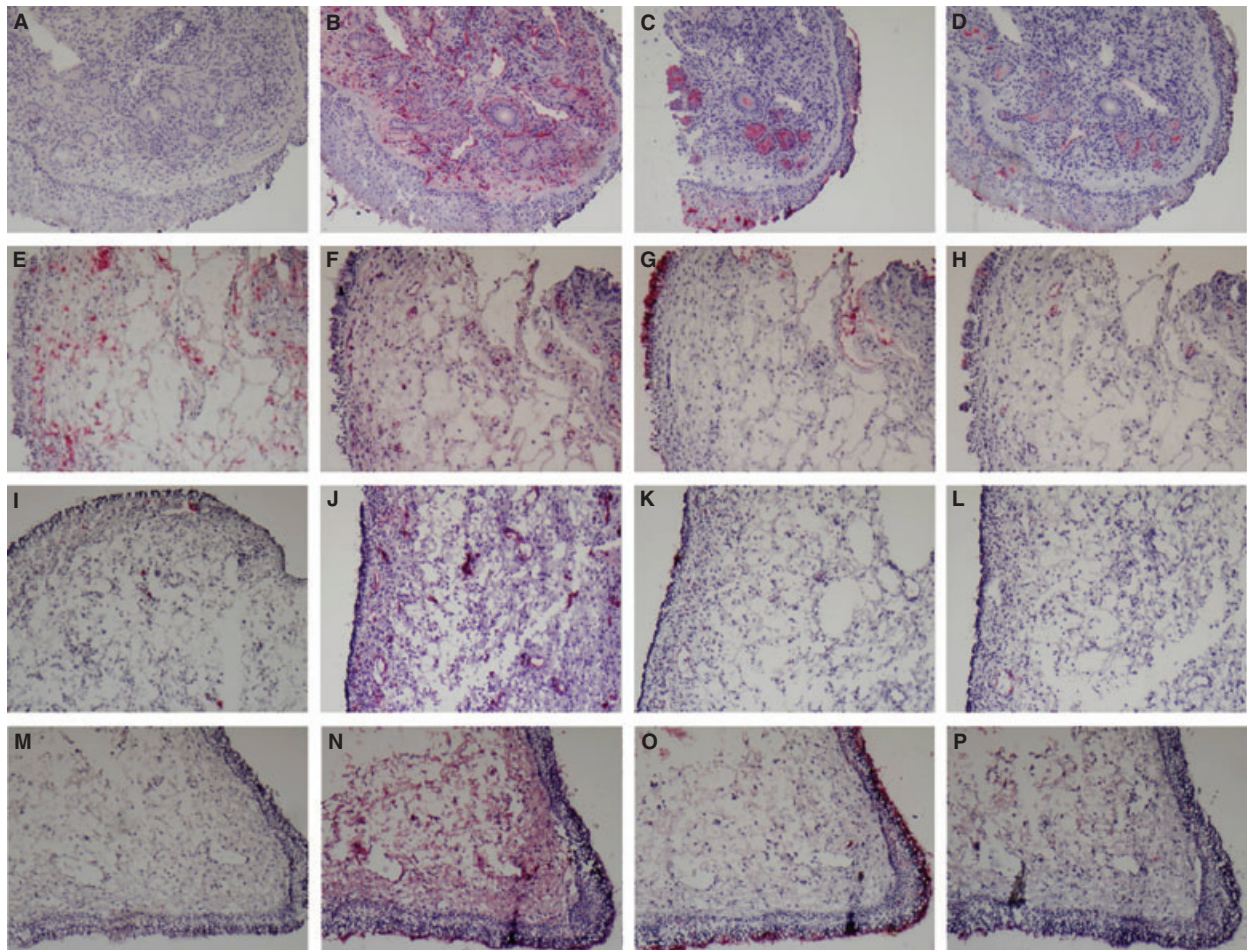


Figure 4 Presence of eosinophils, CD34+ vessels, mAb HECA+ cells and mAb MECA+ cells (magnification 10×). Control nasal mucosa (A–D), simple nasal polyp (E–H), CF nasal polyp (I–L) and antro-choanal nasal polyp (M–P) stained for eosinophils (MBP+: A, E, I, and M), endothelial cells (CD34+: B, F, J, and N) and functionally active L-selectins (mAb HECA+: C, G, K, and O; mAb MECA+: D, H, L, and P). Note high numbers of eosinophils in simple nasal polyps, abundant CD34+ endothelial cells in control nasal mucosa and relative absence of CD34+ endothelial cells in all nasal polyps and the relative abundance of mAb MECA+ CD34+ endothelial cells in simple nasal polyps.

although less extreme, the expression of sulfated sLe^x oligosaccharides and the number of eosinophils are upregulated in CF NP. All together, these data confirm previous data from our group, in which we showed *de novo* induction of sulfated sLe^x oligosaccharides in bronchial mucosa of asthmatic

patients and maxillary sinus mucosa of patients suffering from CRS (31, 33). From these observations one may conclude that simple NP and CF NP share (even though tissue eosinophilia is more profound in simple NP) a common pathophysiologic pathway.

In contrast to simple NP and CF NP, the expression of sulfated sLe^x oligosaccharides is extremely low in ACP. In addition, low numbers of eosinophils are observed. The number of neutrophils is however high. Based on these result, one may conclude that ACP are a distinct histopathologic entity separate from both simple NP and CF NP.

Previously, it was suggested that the amount of sulfated sLe^x oligosaccharides correlates with the presence of specific leukocyte subsets, especially eosinophils, in inflamed sinus mucosa. Although both eosinophils and sulfated sLe^x oligosaccharides are increased in simple NP, especially in those patients without asthma and ASA intolerance, thereby suggesting a correlation between functionally active L-selectin ligands and the presence of tissue eosinophilia, the percentage of functionally active L-selectin ligands decreases with disease severity in simple NP whereas the number of eosinophils clearly increases. In addition, although increased numbers of eosinophils are present in CF NP (as are sulfated sLe^x oligosaccharides), it is the tissue neutrophilia that is characteristic of CF NP. Taken together, these data suggest that functionally active L-selectin ligands are not a major determinant of tissue eosinophilia in NP. As no cor-

relation was observed between the amount of sulfated sLe^x oligosaccharides and all other leukocytes subsets studied, it is highly unlikely that functionally active L-selectin ligands are key players in guiding a single leukocyte subset to diseased sinus mucosa. Sulfated sLe^x oligosaccharides may however play some role in guiding leukocytes (including eosinophils) to diseased sinoNM in NP patients. Their presence is however not rate-limiting, as can be concluded from the decrease in sulfated sLe^x oligosaccharides in simple NP obtained from patients with asthma and ASA intolerance.

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