Cell and cytokine profile in nasal secretions in cystic fibrosis

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Abstract

\textbf{Background:} Nasal polyposis (NP) frequently complicates the course of cystic fibrosis (CF). The aim of this study was to determine the pattern of inflammatory cells and mediators in nasal secretions from patients with or without NP compared to patients with idiopathic NP and healthy controls.

\textbf{Methods:} Eighteen CF patients with NP (NP+ group: 6 untreated, 12 treated with nasal steroids), and 15 without NP (NP− group) were included in this prospective study and compared to 9 patients with idiopathic NP and 12 healthy controls. Differential cell count eosinophil cationic protein (ECP), interleukin-5 (IL-5) and IL-8 were determined in nasal lavage fluids.

\textbf{Results:} The total cell count, the number and the percentage of neutrophils and eosinophils, the levels of IL-8, IL-5 and ECP were significantly higher in nasal secretions from both NP+ and NP− as compared with controls. No difference was found between untreated and treated CF patients with NP. No difference was found between NP+ and NP− groups. Compared to idiopathic NP group, both NP+ and NP− groups had higher percentage of neutrophils and lower percentage of eosinophils. There were no differences according to the use of topical steroids, systemic antibiotherapy, or the type of mutation. CF patients with positive nasal culture had a higher percentage of neutrophils than those with negative culture. CF patients with atopy had a higher percentage of eosinophils than non-atopic patients.

\textbf{Conclusion:} Our results demonstrate that nasal inflammation is a prominent feature in patients with CF and does not differ according to the presence of NP. IL-8 and IL-5 may play crucial roles in recruitment and activation of neutrophils and eosinophils in upper airways of CF patients.

\textbf{Keywords:} Cystic fibrosis; Nasal polyposis; Interleukin-5; Interleukin-8; Inflammation; Neutrophil; Eosinophil; Nasal lavage

1. Introduction

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disease in the Caucasian population [1]. Nasal polyposis (NP) complicates the course of CF up to 50% of the patients [2–4]. Clinical manifestations of NP are characterised by obstruction, anosmia, sneezing, rhinorrhea and itching, which significantly impair quality of life [5]. Treatment of NP is difficult in patients with CF; they are frequently not improved by the medical treatment, and thus require multiple surgical procedures [6,7]. Furthermore, NP may play a role in pulmonary function impairment during CF, as previously suggested in patients with NP and asthma [8,9].

Pathogenesis of NP occurring during CF as well as pathogenesis of idiopathic NP polyposis are unclear. The numerous hypotheses include chronic infection, inhalant or food allergies, T-cell disturbances, and aerodynamic factors [10,11]. Polyps from patients with CF are classically thought to be ‘neutrophilic’ as opposed to ‘eosinophilic’ polyps, occurring in patients without CF. Recent data have confirmed the presence of neutrophils as the major inflammatory cells present in polyps from patients with CF, but eosinophils were also found [12–14]. Different investigators have suggested a possible role of inflammatory cells and cytokines in the
genesis of idiopathic NP, especially a key role of eosinophils and interleukin-5 (IL-5) [11–15].

We hypothesised that beside neutrophils, an increased recruitment of eosinophils might differentiate CF patients who exhibited NP from CF patients without NP. To establish this, we examined nasal fluids, which provide a simple and non-invasive tool for studying cells and mediators involved in nasal inflammatory processes. Therefore, the aim of this study was to characterise the pattern of inflammatory cells, cell mediators and chemokines in nasal secretions from patients with CF associated or not with NP, and to compare this pattern to that observed in healthy controls and in idiopathic NP. Because of the presence of both inflammatory processes and infectious processes in CF, we evaluated nasal fluid IL-5, eosinophil cationic protein (ECP), IL-8. IL-5 is considered to have a pivotal role in the recruitment and activation of eosinophils; ECP is an eosinophil activation marker and IL-8 was selected as a major neutrophil recruitant [16–18].

2. Patients and methods

2.1. Patients

Thirty-three consecutive patients with cystic fibrosis were included in this prospective study. All patients were followed in the adult and paediatric CF units of the University Hospital of Lille. The diagnosis of CF was established on the basis of clinical features, positive sweat test and genotypic mutation. Nine untreated patients with idiopathic NP without nasal infection and 12 non-atopic age-matched healthy controls (mean age 18 years, 6 males, 6 females) without history of pulmonary, nasal or digestive diseases were included as control groups. Informed consent was obtained from all subjects and study protocol was approved by the institutional review board (CP 98/102).

NP was identified in 18 out of 33 CF patients by anterior rhinoscopy. Five nasal symptoms (obstruction, anosmia, sneezing, rhinorrhea, itching) were collected and scored from 0 to 3: 0 for no symptom, 1 for mild symptom (just noticeable), 2 for moderate symptom (annoying), and 3 for severe symptom (distress), so that the maximal nasal score was 15.

Atopic status was studied in all patients. Atopy was defined as the presence of at least one positive skin prick tests, i.e. wheal size superior to 3 mm to the negative control) against common environmental Aeroallergen from a standard battery of extracts (Laboratoire Stallergènes, Paris, France). The skin test battery included the following allergens. Dermatophagoides pteronyssinus and farinae, cat and dog extracts, German cockroach, Alternaria, Aspergillus, Cladosporium, Bermuda grass, birch tree, hazel, ash, privet, oak, poplar, nettle tree, mixed weeds ragweed and plantain. In case of positive skin tests, serum specific IgE antibodies against the relevant allergens were performed, using the Phadebas radioallergosorbent test (Pharmacia Diagnostics, Uppsala Sweden). Aspergillus sensitisation was present in 10 patients and characterised by increased total serum IgE levels (median: 228 kU/l) and the presence of specific IgE antibodies against Aspergillus antigens (median class 3). Only one of the patients with Aspergillus sensitisation developed allergic bronchopulmonary aspergillosis with clinical symptoms, blood eosinophilia, and Aspergillus fumigatus specific IgG antibodies. The use of topical nasal steroids or systemic treatment by antibiotics was recorded. Patients with previous history of nasal surgical procedure (such as polypectomy or ethmoidectomy) were excluded from the study.

2.2. Nasal lavage

Collection of nasal secretions was performed as previously described [19]. After clearing excess mucous by forceful exsuflation, the subjects extended their necks approximately 30° from the horizontal while in a sitting position. Six millilitres of normal saline (0.9%) was instilled into each nostril while the subjects did not breathe or swallow. After 10 s, the subjects flexed their necks, expelling the mixture of mucus and saline into a vessel, which was stored on ice until the end of the experiment. In addition, a bacteriological culture of nasal secretions was performed in all cases. Lavages were stored at 4 °C for a maximum of 2 h until processed. Nasal lavages were centrifuged at 1000 × g for 10 min. The cell pellet was resuspended in 500 μl RPMI (Gibco-BRL, Cergy-Pontoise, France) containing 0.4% N-acetyl cysteine (Sigma, St Louis, MI, USA). Total cell number was measured with a Thomas haemocytometer. Differential cell count was performed on cytocentrifuged smears stained by the May-Grünwald-Giemsa method. After centrifugation, supernatants were portioned and stored at −20 °C. Cytokine concentrations were measured directly on nasal lavages by using commercially available kits according to the manufacturer’s instructions (IL-5: Immunotech, Marseille, France; IL-8: Duoset, R&D Systems, England; ECP: Pharmacia Uppsala Sweden). Specific sensitivity of the ELISA kits was as follows: IL-5: 1 pg/ml; IL-8: 5 pg/ml, whereas specific sensitivity of the RIA for ECP was 2 μg/l.

2.3. Statistical evaluation

The statistical analysis was performed with a Macintosh computer (Apple Company, Cupertino, CA, USA) using the Statview IV.5 Software (Statview, Inc.). Results were expressed as median and interquartile range (IQR). The Kruskall–Wallis analysis of variance was applied first to the quantitative data of the groups. When this was significant, each pairing was examined by
Table 1
General characteristics of patients with cystic fibrosis (CF) with or without nasal polyposis (NP)

<table>
<thead>
<tr>
<th></th>
<th>CF with NP (n=18)</th>
<th>CF without NP (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>16.7±1.8</td>
<td>16.0±4.6</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>(10/8)</td>
<td>(8/7)</td>
</tr>
<tr>
<td>Nasal score</td>
<td>3.5±0.7</td>
<td>1.6±0.5†</td>
</tr>
<tr>
<td>Anosmia</td>
<td>0.5±0.2</td>
<td>0†</td>
</tr>
<tr>
<td>Obstruction</td>
<td>1.5±0.3</td>
<td>0.5±0.2*</td>
</tr>
<tr>
<td>Atopy</td>
<td>5/18</td>
<td>3/15</td>
</tr>
<tr>
<td>Nasal steroids</td>
<td>12/18</td>
<td>0/15</td>
</tr>
<tr>
<td>ΔF508/ΔF508</td>
<td>8/18</td>
<td>8/15</td>
</tr>
<tr>
<td>ΔF508 heterozygote</td>
<td>9/18</td>
<td>5/15</td>
</tr>
<tr>
<td>Other mutation</td>
<td>1/18</td>
<td>2/15</td>
</tr>
<tr>
<td>Rh-Dnase treatment</td>
<td>15/18</td>
<td>13/15</td>
</tr>
<tr>
<td>Systemic antibiotherapy</td>
<td>4/18</td>
<td>8/15</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>18/18</td>
<td>15/15</td>
</tr>
<tr>
<td>Total IgE (IU/l)</td>
<td>179±66</td>
<td>167±16</td>
</tr>
<tr>
<td>Positive Aspergillus RAST</td>
<td>4/18</td>
<td>6/18</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.E.M. †P=0.02; ‡P=0.03; *P=0.01.

The number and percentage of neutrophils and eosinophils in nasal lavage were significantly higher in CF patients than in controls, but there was no difference between CF subgroups. Compared to idiopathic NP group, CF groups had a higher percentage of neutrophils and a lower percentage of eosinophils (Table 2). IL-8, IL-5, and ECP concentrations were significantly higher in CF groups than in healthy controls, but there was no difference between CF groups.

3. Results

3.1. General characteristics

General characteristics of the patients with CF are shown in Table 1. CF patients with NP (NP+ group) and without NP (NP− group) did not differ in terms of age, sex ratio and type of mutation. Six CF NP+ patients were untreated whereas 12 CF NP+ patients were treated with nasal steroids. The number of patients with atopy or receiving systemic antibiotherapy, or with Aspergillus sensitisation was similar in the two groups (NP+ and NP−). Nasal obstruction score and anosmia scores were significantly higher in the NP+ group than in the NP− group.

3.2. Bacteriological findings in patients with cystic fibrosis

We found bacterial pathogens in the nasal lavages of 22 out the 33 patients with CF (66%). The number of pathogens per patient ranged from 1 to 4. There were P. aeruginosa (n=15), S. aureus (n=10), E. coli (n=4), Haemophilus influenzae (n=3), S. pneumoniae (n=1) and K. pneumoniae (n=1). The number of patients with positive bacterial culture, the type and the number of bacteria identified in nasal lavages were not different between CF with NP+ and NP− groups. Nasal cultures were negative in both control groups.

3.3. Cells and cytokines

The total cell count, the number and percentage of neutrophils and eosinophils in nasal lavage were significantly higher in CF patients than in controls, but there was no difference between CF subgroups. Compared to idiopathic NP group, CF groups had a higher percentage of neutrophils and a lower percentage of eosinophils (Table 2). IL-8, IL-5, and ECP concentrations were significantly higher in CF groups than in healthy controls, but there was no difference between CF groups.

We found a significant positive correlation between neutrophils and IL-8 (r=0.66, P<0.0001), neutrophils and IL-5 (r=0.27, P<0.05) and neutrophils and ECP.

Table 2
Cells in nasal lavage fluids from patients with cystic fibrosis with nasal polyposis (n=18) and without nasal polyposis (n=15), with idiopathic nasal polyposis (n=9) and healthy controls (n=12)

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Idiopathic NP</th>
<th>CF with NP on steroids</th>
<th>CF with NP without steroids</th>
<th>CF without NP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volume (ml)</td>
<td>6.00 (2.50)</td>
<td>4.5 (2)†</td>
<td>5.75 (3.75)</td>
<td>6.25 (1.50)</td>
<td>7 (4.15)§</td>
</tr>
<tr>
<td>Total cell number (per ml)</td>
<td>4410 (2362)</td>
<td>6879 9978</td>
<td>12277 (108435)†</td>
<td>11983 (37371)</td>
<td>8333 (84197)</td>
</tr>
<tr>
<td>Mononuclear cell (%)</td>
<td>6 (8.7)</td>
<td>7.5 (15)</td>
<td>3.7 (3.4)</td>
<td>3.8 (6.8)</td>
<td>4.7 (5.0)</td>
</tr>
<tr>
<td>Mononuclear cells/ml</td>
<td>253 (225)</td>
<td>637 (1260)</td>
<td>1181 (4747)</td>
<td>673 (1014)</td>
<td>317 (5587)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>6 (8)</td>
<td>25.5 (25.5)†</td>
<td>65 (65)§</td>
<td>70 (37)§</td>
<td>56 (63)§</td>
</tr>
<tr>
<td>Neutrophils/ml</td>
<td>196 (251)</td>
<td>2025 (3427)†</td>
<td>6468 (100128)†</td>
<td>10460 (33837)§</td>
<td>3890 (44699)‡</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0 (0)</td>
<td>4.3 (12)‡</td>
<td>0.7 (1.2)‡</td>
<td>0.5 (2)§</td>
<td>1 (1.8)§</td>
</tr>
<tr>
<td>Eosinophils/ml</td>
<td>0 (0)</td>
<td>232 (326)‡</td>
<td>140 (800)§</td>
<td>34 (408)‡</td>
<td>84 (902)†</td>
</tr>
<tr>
<td>Epithelial cells (%)</td>
<td>46 (35)</td>
<td>20 (22)†</td>
<td>18.5 (22)†</td>
<td>18 (25)</td>
<td>14 (20)†</td>
</tr>
<tr>
<td>Epithelial cells/ml</td>
<td>1867 (1487)</td>
<td>782 (2219)</td>
<td>2181 (6170)</td>
<td>972 (1052)</td>
<td>2900 (9629)</td>
</tr>
<tr>
<td>Squamous cells (%)</td>
<td>32 (32)</td>
<td>26 (33)</td>
<td>8 (33)†</td>
<td>4 (3.5)§</td>
<td>12 (31)§</td>
</tr>
<tr>
<td>Squamous cells/ml</td>
<td>1350 (1630)</td>
<td>962 (3727)</td>
<td>2057 (3805)</td>
<td>327 (393)</td>
<td>1150 (4144)</td>
</tr>
</tbody>
</table>

Definition of abbreviations: CF=cystic fibrosis; NP=nasal polyposis. Results are expressed as median and interquartile range (IQR). *P value of Kruskall Wallis test; †P<0.05 from controls; ‡P<0.005 from controls; §P<0.05 from idiopathic NP.
Fig. 1. Concentrations of IL-5, IL-8 and ECP in nasal lavages from controls (n=12), untreated patients with idiopathic nasal polyposis (n=9), CF patients with untreated nasal polyposis (n=6), nasal steroid treated CF patients with nasal polyposis (n=12) and CF patients without nasal polyposis (n=15). †P < 0.05 vs. controls; ‡P < 0.001 vs. controls; $P < 0.05 vs. idiopathic NP. ECP (μg/l); IL-5 (pg/ml); IL-8 (pg/ml). Data presented as box plots. Horizontal lines represent the median, squares the 25th and 75th percentiles, and bars the 10th and 90th percentiles.

When excluding the control group and idiopathic NP group, correlation tests remained similar, except for IL-5 and eosinophils (r = 0.54, P = 0.11) and IL-5 and neutrophils (r = -0.29, P = 0.13).

Total cell number differential cell count and concentration of cytokines were not different according to the use of systemic antibiotherapy, or the type of mutation. Interestingly, patients with positive nasal culture had a higher percentage of neutrophils than those with negative culture (P < 0.03). Patients with atopy had a higher percentage of eosinophils than non-atopic patients (P < 0.01) (Fig. 2).

4. Discussion

This study was developed to gain more insight into the knowledge of inflammatory processes present in the upper respiratory tract of CF patients with or without NP. Three main points are worth discussion: (1) we found a marked inflammation in nasal secretions from all patients with CF; (2) bacterial colonisation of nasal mucosa and atopy were associated with an increase in neutrophils or eosinophils, respectively; (3) the pattern of inflammatory cells and cytokines did not differ among patients with or without NP.

There are only few reports about nasal inflammation during CF. Pathological data suggest a prominent neutrophil inflammation, whereas the presence of eosinophils has also been reported [10,13,14,20–23]. Noah et al. did not find significant amounts of inflammatory cells or cytokines (IL-6, IL-8, IL-10) in nasal secretions from infants (median years: 2.4) with CF [20]. Other investigators showed high levels of various inflammatory mediators like ECP or leukotriene C4 in nasal secretions from adult patients with CF [21,22]. In our study, we found a marked inflammation in nasal secretions from CF patients characterised by the presence of an increased number of inflammatory cells, especially neutrophils and to a lesser extent eosinophils. This

Fig. 2. Left. Neutrophil percentage in nasal lavages according to positive or negative bacterial culture in cystic fibrosis. Right. Eosinophil percentage in nasal lavages according to atopic status in CF. *P = 0.03; **P = 0.009. Data presented as box plots. Horizontal lines represent the median, squares the 25th and 75th percentiles, and bars the 10th and 90th percentiles.
inflammatory pattern is clearly different from the one observed in idiopathic NP, which is characterised by more eosinophils and less neutrophils than in CF. In agreement with this finding, the increase of neutrophils in CF was associated with an increase of IL-8 levels, which was significantly higher than in idiopathic NP. Furthermore, a strong positive correlation was demonstrated between neutrophils and IL-8 concentration. These data are in keeping with previous reports showing that IL-8 is a major neutrophil chemoattractant, and also an activator of neutrophil degranulation [16]. The overproduction of IL-8 in CF has been widely reported in the lower airways of patients with CF [24–32] and has been reported even in the absence of infectious exacerbation, suggesting that bacterial infection is not the sole factor responsible for neutrophil inflammation [20,27,32]. A recent paper also reported increased levels of IL-8 in nasal fluids from CF patients without differences between CF patients with or without NP [33]. Thus, a neutrophil-dominated inflammation of the upper airways is present in CF even in patients without nasal bacterial pathogens, although nasal neutrophilia was largely increased in CF patients with bacterial colonisation.

Atopic status was also shown to significantly influence nasal eosinophils. This relation between atopy and eosinophils in mucosal secretions is well documented in diseases like allergic rhinitis or allergic asthma [34]. Wang and co-workers did not report evidence of eosinophilia in nasal secretions of CF patients but demonstrated significant amounts of ECP [21]. Their findings might be due to the fact that eosinophils were activated and released ECP but being degranulated they were not easily identified. In keeping with these results, Koller demonstrated increased ECP levels in serum without significant increase of blood eosinophils [35]. We also found increased levels of ECP in nasal secretions, which might reflect eosinophil degranulation but one cannot exclude that neutrophils also release significant amounts of ECP [36]. Several studies have suggested that IL-5 and eosinophils may play a key role in the pathogenesis of idiopathic NP [11,15,37]. Accumulation of activated eosinophils in the lamina propria leads to a release of wide arrays of cytokines as well as specific cell mediator like ECP, producing inflammation and oedema. In CF, the magnitude of eosinophilic inflammation in nasal secretions was similar both in patients with or without NP. Along this line, it is noteworthy to consider that nasal eosinophilia and increased IL-5 level have also been reported in nasal secretions of patients with active allergic rhinitis without nasal polyps [34].

Lastly, we found no significant difference in the pattern of inflammatory cells and cytokines in CF patients according to the presence of nasal polyps. Nasal neutrophils and IL-8, nasal eosinophil and IL-5 were present in CF as well as in patients with NP as in patients without NP. It is unlikely that this lack of difference is due to the small number of patients since the same observation was reported recently in a large series [33]. Taken together, all these results support the hypothesis that neither eosinophils nor neutrophils are the main factors responsible for the occurrence of NP in CF and suggest that other biological mechanisms are involved and/or responsible for the development of nasal polyps. In conclusion, nasal inflammation is prominent in patients with CF. In the present study, an increase of neutrophils and IL-8, and to a lesser extent an increase of eosinophils, IL-5 and ECP was clearly evidenced in nasal secretions. This inflammatory process is present not only in patients with NP, but also in patients without NP. The recruitment and the activation of neutrophils and/or eosinophils in nasal secretions may occur via chemokines such as IL-8 and IL-5.

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References


