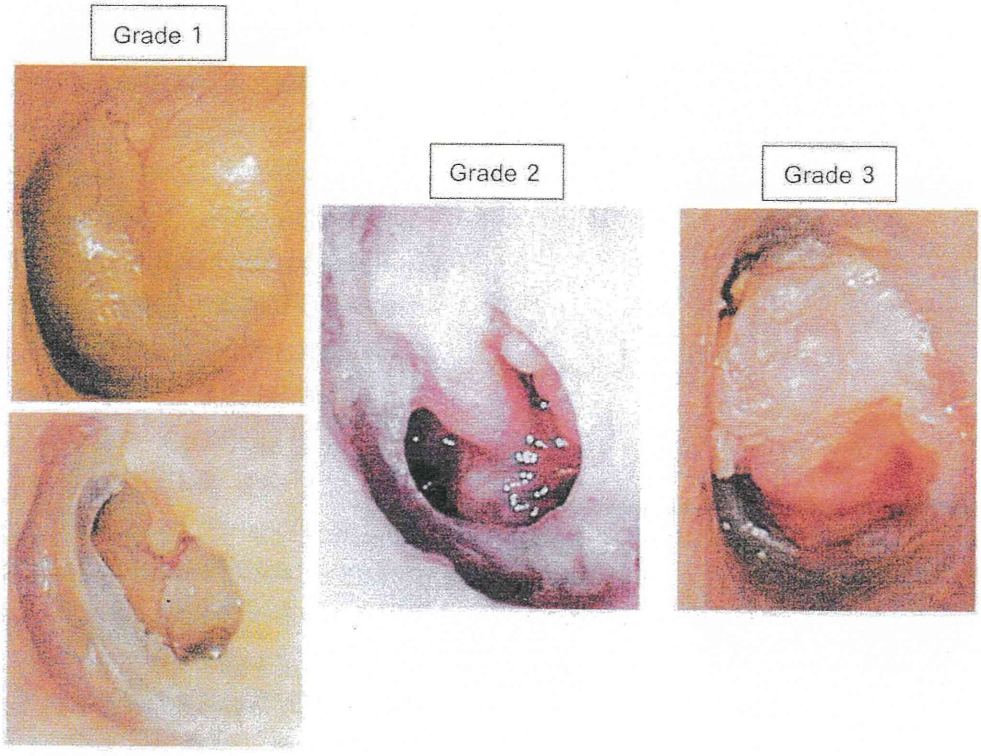

図1　中耳粘膜 Grade（G）分類別鼓膜所見
中耳粘膜 Grade 分類（表2）に沿った鼓膜の所見を示す．
（筆者提供）

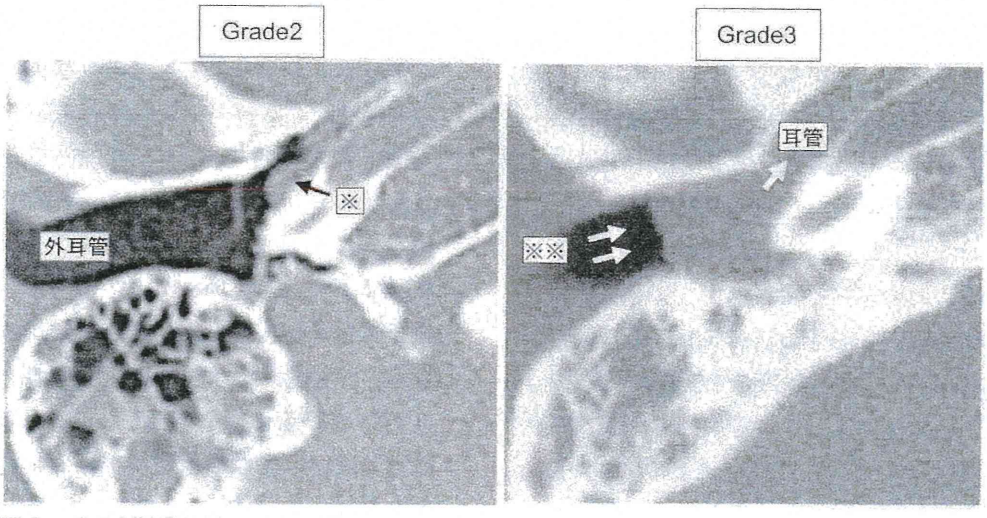

図2　CT（側頭骨軸位断，耳管レベル）
*：中耳粘膜は耳管鼓室口入口部付近から肥厚し，次第に耳管機能が低下する．
**：中耳粘膜肥厚は鼓膜を越え外耳道側へ張り出してくる．
（筆者提供）

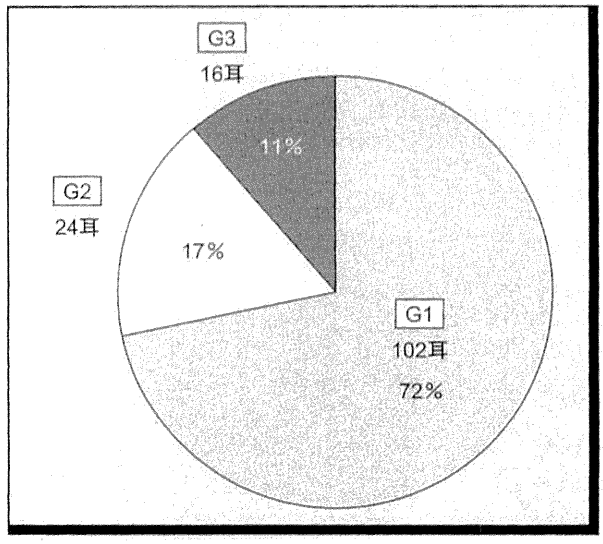

図3　当院外来通院患者における Grade（G）分類別内訳（全 71 人，141 耳）
平均観察期間4年6カ月（7カ月～8年3カ月）
多くは Grade 1 であった．
（筆者作成）

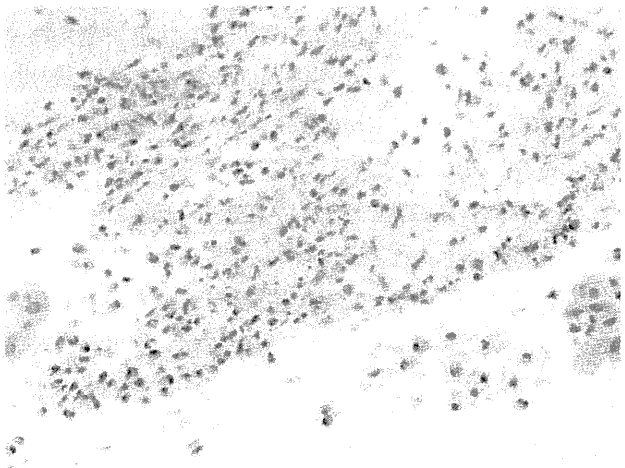

図4　好酸球性中耳炎における中耳貯留液（HE 染色像）
好酸球性中耳炎の診断には中耳貯留液の好酸球浸潤を確認する．
（筆者提供）

Ⅱ．病態

1．中耳腔における局所 IgE について

好酸球性中耳粘膜には，活性化された多数の好酸球浸潤がみられ，中耳貯留液中には IgE（immunoglobulin E）や IL（interleukin）-5，eotaxin，ECP（eosinophil cationic protein）等の好酸球性炎症と密接に関連する物質が検出される[13), 14)]。この病態は合併する好酸球性副鼻腔炎の病態と基本的には同様であることが推察されている[4), 5)]。実際これら IgE や好酸球の遊走因子は血清中では上昇しておらず，局所で好酸球性炎症が活発に生じていることを示している[14), 15)]。なお，中耳貯留液中からは黄色ブドウ球菌に対するスーパー抗原であるエンテロトキシンA，Bや真菌に対する特異的 IgE 抗体が検出されているが，後者については鼓室内貯留液の真菌培養から抗原そのものは検出されていない（**表3**）[15)]。局所で抗真菌抗体が作用する類似疾患として，アレルギー性気管支肺アスペルギルス症やアレルギー性真菌性副鼻腔炎がある[16), 17)]。しかし，好酸球性中耳炎と異なる点として，これらは検鏡や真菌培養にて真菌そのものが容易に同定され，自体も局所炎症に強く関与している。

2．耳管機能

好酸球性中耳炎症例では鼻をかんだ後に耳が聴こえなくなった，あるいは鼻をかむとすぐに耳に抜ける等，耳管開放症あるいは閉鎖不全症を疑う症状の訴えが多い傾向にある。好酸球性中耳炎症例の耳管機能を音響耳管法で調べると，対照例と比較して有意に耳管の開放時間が長い[18), 19)]。私どもはこの耳管開放状態が本症の発症病態と深く関与している可能性があると考えている。すなわ

IgE（immunoglobulin E）　IL（interleukin）
ECP（eosinophil cationic protein）

表3　中耳貯留液 / 血清中の特異的 IgE 抗体検出結果と中耳貯留液における細菌・真菌培養結果

特異的 IgE 抗体	血清	中耳貯留液	真菌 / 細菌培養（中耳貯留液中）
ダニ	11	13	−
アスペルギルス	0	9	0
アルテルナリア	0	9	0
カンジダ	0	11	1
ムコール	0	8	0
ペニシリウム	0	7	0
クラドスポリウム	0	8	0
黄色ブドウ球菌		7	4
エンテロトキシンA		5	3
エンテロトキシンB			

中耳貯留液中では真菌やエンテロトキシンA，Bが検出されたが，鼓室内貯留液の真菌 / 細菌培養では，真菌抗原そのものは検出されなかった．

（文献 15 より）

ち気管支喘息患者を代表するようなTh2優位な個体において，開放気味の耳管が存在すれば，容易に好酸球性炎症を惹起するような異物が中耳腔内に侵入可能となる[20]。一方好酸球はムチンの産生を増強し，さらに好酸球由来の細胞障害性蛋白は上皮細胞を障害する。よって好酸球等の浸潤細胞や上皮細胞の cell debris と過剰に産生されたムチンが混在し，さらに粘稠な貯留液を生じると考えられる。

一方，気管支喘息，好酸球性副鼻腔炎，好酸球性中耳炎の3つを合併する患者における各発症年齢の平均は，それぞれ（この順に）39.7 歳，38.1 歳，49.4 歳であり，好酸球性中耳炎は前者2つより約 10 年遅れて発症すると石戸谷は述べている[4]。自験例でも 31 人における検討において，同期間は約 16.8 年であった。耳管を介し中耳に粘膜病変が生じるまでに一定の期間を要することから，私どもは，気道，特に上気道における慢性好酸球性炎症の終末期像ではないかと考えている[18]。

3．鼻内視鏡手術後発症した場合

鼻内視鏡手術後発症する好酸球性中耳炎については，鼻副鼻腔粘膜が耳管を介して中耳粘膜に連続しているため，耳管粘膜も様々な影響を受け，通常より耳管が開放気味になっている可能性がある[18]。ここで圧をあげた鼻洗浄の使用や，強い擤鼻により，炎症自体が中耳腔内に波及し，中耳炎を発症することがある[19]。実際好酸球性副鼻腔炎に対する副鼻腔手術前に音響耳管法で耳管開放時間を確認すると，耳管が開放気味の症例が存在する。この場合は術後慎重に経過観察とし，場合によっては鼻洗浄の水圧を下げるように指示する。耳症状が出現した場合には洗浄自体の中止を指示し，頻回の鼻処置・ネブライザー吸入のみとする。このように対処しても再燃を繰り返す場合には，後述する適切な治療を早急に行うことで，進行を止めることができる。また日頃より習慣となっている鼻すすりや強い擤鼻を中止するように徹底した指導を行う。

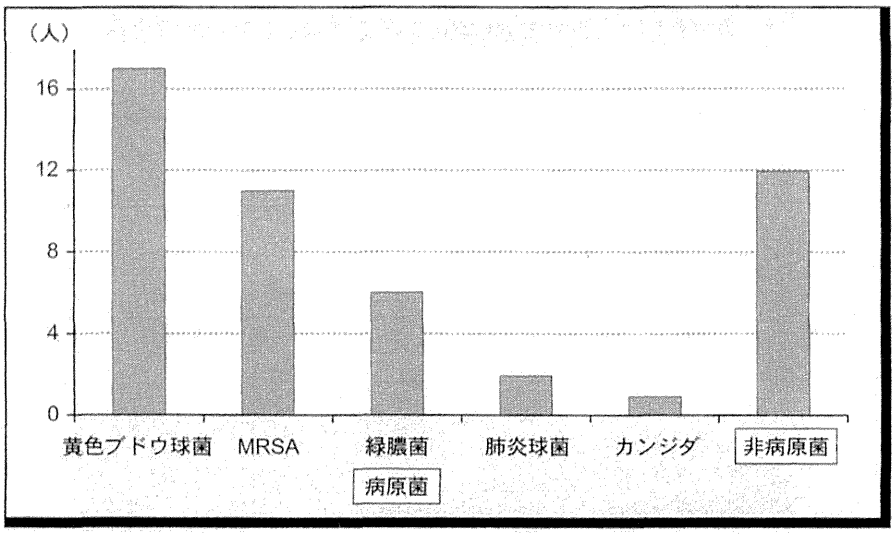

図5　当院外来通院患者（G2，3）の中耳貯留液（耳漏）における細菌・真菌培養結果
黄色ブドウ球菌やMRSA，緑膿菌などの病原菌が多く検出される．

（筆者作成）

Ⅲ．治療

好酸球性炎症の制御には全身あるいは局所での副腎皮質ステロイドの投与が有効である。なるべく副腎皮質ステロイドの局所投与を行うのが望ましい。不適切な副腎皮質ステロイドの全身投与が頻回に行われ，副腎機能不全に陥るケースもある。必要な場合は内科医との連携のもとに行うべきである。もちろん合併している喘息の治療がしっかりなされていることが大切である[21]。

1．鼓膜穿孔のない症例（滲出性中耳炎型）

中耳粘膜肥厚は軽度であり，大半がG1症例である。貯留液が粘稠で大量に存在する場合は，鼓膜切開を行いその貯留液を排除する。鼓膜チューブを留置してもすぐ閉塞するため，チューブ留置の適応ではない。この際には，デキサメタゾンあるいはベタメタゾンの点耳液を鼓室内に注入後，Pneumatic otoscopeを用い，加圧して薬液を耳管に逆通気する。鼓膜切開時にトリアムシノロンアセトニドを鼓室内注入した場合，鼓膜が菲薄化している例では鼓膜の永久穿孔を起こすことがあるので注意する。1～2週間後に鼓膜切開口が閉鎖すれば，以後トリアムシノロンアセトニドの鼓室内注入が可能となる[22]。1mLの注射器とカテラン針を用い，鼓膜の前上象限に鼓室穿刺し少量注入する。同様にPneumatic otoscopeを用い，加圧して薬液を耳管に逆通気する[12]。

その後は定期的に観察し，貯留液が少しみられてくればトリアムシノロンアセトニドの鼓室穿刺による注入を行う。1カ月に1回必要な場合や，徐々に間隔を空けることが可能なケースも多い。穿刺を繰り返すことによる小穿孔を防ぐために，穿孔部に少量のワセリンを綿棒で塗布することで，早期閉鎖を促すことができる場合もある。

2．鼓膜穿孔のある症例

穿孔が存在する場合は，感染し黄色ブドウ球菌，緑膿菌，MRSA（メチシリン耐性黄色ブドウ球菌）等が検出されることが多い。G2，3症例の自験例

表4　好酸球性中耳炎重症度スコア

1．中耳貯留液もしくは耳漏の量（左・右）
（鼓膜穿孔がない場合）
スコア0 なし
スコア1 中耳貯留液あるが，中鼓室内の一部に含気がみられる
スコア2 中耳貯留液により中鼓室内が充満している
（鼓膜穿孔を伴う場合）
スコア0 なし
スコア1 耳漏が中鼓室内に限局している
スコア2 耳漏が外耳道側へ排出されている
2．中耳粘膜の状態（中耳粘膜 Grade 分類）（左・右）
スコア0（G1）ほとんど正常もしくは浮腫が軽度にみられる
スコア1（G2）浮腫状もしくは肥厚している
スコア2（G3）強く肥厚し肉芽になり，外耳道側へ張り出している
3．副腎皮質ステロイドの鼓室内投与回数（左・右）
スコア0 過去3カ月間に0回
スコア1 過去3カ月間に1回
スコア2 過去3カ月間に2回もしくはそれ以上
4．副腎皮質ステロイドの全身投与回数
スコア0 過去3カ月間になし
スコア1 過去3カ月間に7日もしくはそれ以下
スコア2 過去3カ月間に7日以上
5．抗菌薬の全身投与回数
スコア0 過去3カ月間になし
スコア1 過去3カ月間に7日もしくはそれ以下
スコア2 過去3カ月間に7日以上

1～3については左右それぞれで計算する．合計最大16点となる．
（文献12より）

における耳漏培養結果を**図5**に示す。黄色ブドウ球菌，緑膿菌に感受性のあるニューキノロン薬が選択されることが多い。MRSAに対しては，耳洗浄やトリアムシノロンアセトニド鼓室内投与で膿性耳漏が改善せず骨導低下も出現する場合には，バンコマイシンなどの点滴を目的とした入院加療が推奨される。急性感音難聴を併発している場合には，耳洗浄に加えて，突発性難聴に準じた副腎皮質ステロイド全身投与も行う。感受性のある抗菌薬で感染がコントロールされてからトリアムシノロンの鼓室内注入に切り替える。感染があると中耳貯留液の粘性は落ち，感染がなくなると粘稠になる[20]。粘膜肥厚が外耳道側へ張り出し肉芽（G3）になっている場合は，上記のような消炎後，肉芽を鉗除し，トリアムシノロンアセトニドが鼓室内注入できるスペースを耳管鼓室口付近に作る。

なお，G1あるいはG2で粘膜肥厚が軽度の症例は，鼓膜形成術（接着法）で穿孔を閉鎖すること

MRSA（メチシリン耐性黄色ブドウ球菌）

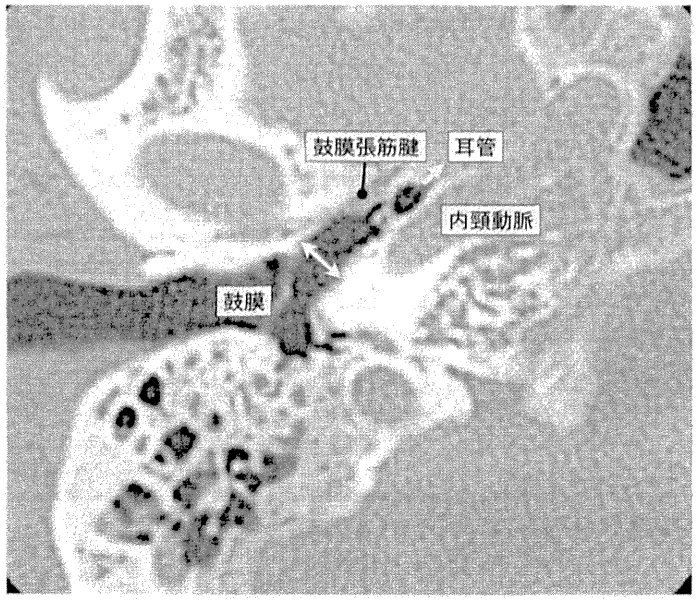

図6　耳管鼓室口骨部直径幅の計測(0.5 mmスライス側頭骨CT軸位断，耳管レベル)

好酸球性中耳炎の重症度算出に際して，独立変数の1つとして耳管鼓室入口部の骨部直径（前後径）も測定した．

（文献11より）

により，感染の機会が減り良好な状態を維持できる。しかし，中耳腔に感染の残った状態で施行すると，急性増悪し作成した鼓膜が脱落し再穿孔となる可能性が高く，適応については慎重に判断する。なお鼓膜形成術の際には，術前後に副腎皮質ステロイドの全身投与を行う。

Ⅳ．重症例の存在

G2，3症例の中で，中耳粘膜が耳小骨周囲～乳突洞へかけて肥厚した粘膜を正常化させることは困難である。特にG3症例に関しては上記のような現行加療を行い，一旦G2の状態まで改善しても再燃（感染増悪）するケースが多い。私どもは，中耳粘膜肥厚Grade分類に加えて，副腎皮質ステロイド（鼓室内，全身）の使用回数，抗菌薬の使用回数を用いて重症度スコアを算出した（**表4**）。そして，これに対し重症化に関係すると思われる因子（独立変数）を用いて重回帰分析を行い，その影響の大きさについて検討した[11]。この際の独立変数の1つとして，耳管鼓室口入口部の骨部直径（前後径）も測定した（**図6**）。この結果，影響の強い順に，BMI，喘息の罹病期間，アスピリン喘息の存在，Lund-Macky（**LM**）スコア（マイナスの相関）であることが判明した。BMIの関与については，日本人女性（成人）における難治性喘息のタイプが肥満と強く関係していることが近年判明している[23]。一方で中耳における肥満との関係についてはまだ不明である。LMスコアとのマイナ

LM (Lund-Macky)

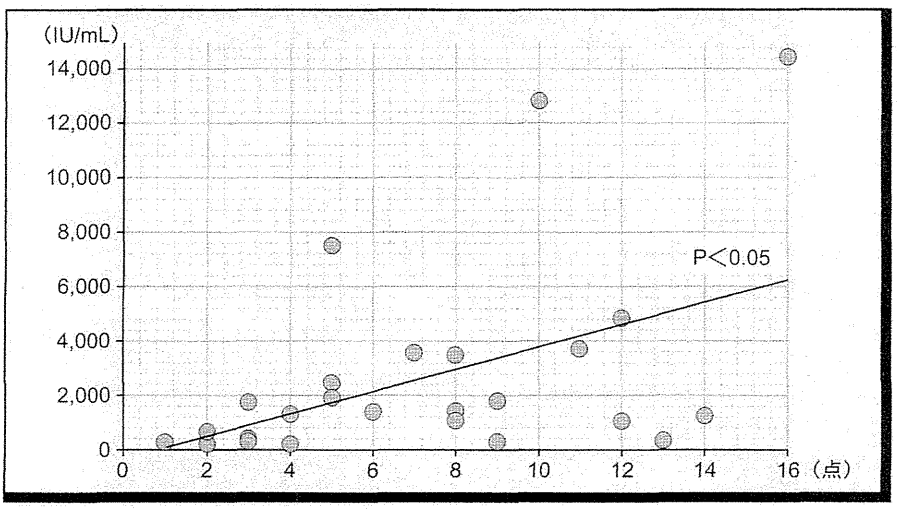

図7　好酸球性中耳炎重症度スコアと中耳貯留液中 IgE 値 ($p < 0.05$)
重症度スコアは中耳貯留液中の総 IgE と相関する.

(文献 15 より)

スの相関は，副腎皮質ステロイドの内服が今回の重症度指数の中に加えられており，この影響を考えている。副腎皮質ステロイドは好酸球性副鼻腔炎の再燃時にも効果があるといわれており[5]，今回の結果より，好酸球性副鼻腔炎は好酸球性中耳炎よりもより効果的に副腎皮質ステロイドが作用すると推察される[18]。一方この重症度スコアは中耳貯留液の総 IgE 値と相関し[15]（**図7**），治療内容も含めた評価として，今後も利用できると考えている。

その他治療法として，抗 IgE 抗体の報告がある[10]。効果がでるまで期間を要し，G2，3 症例は投薬を中止すると再燃する傾向がある。長期投与に対する報告はまだなく，使用方法なども含め今後検討が必要である。

Ⅴ．鑑別疾患

難知性中耳炎の 1 つとして，近年 ANCA（抗好中球細胞質抗体）関連血管炎症候群が重要視されている[24]。好酸球性中耳炎として治療途中から，急激な急性進行性感音難聴や顔面神経麻痺の出現，全身症状がみられ診断に至るケースがある。好酸球性中耳炎の確実な診断以外に，特に重症例については四肢のしびれや浮腫等の症状の確認を診察時に行うとともに，MPO-ANCA（ミエロペルオキシダーゼ抗体）や PR3-ANCA（プロテナーゼ 3-ANCA）や血中好酸球値を定期的にチェックする。

ま と め

好酸球性中耳炎は日本から発信した疾患であり，近年ようやく他国においても認知されるようになってきた[25],[26]。早期に診断し治療を開始することで，多くの症例は安定した状態を維持することができるが，発症からある程度時間が経過している重症例については現治療法だけでは不十分であり，新たな治療法が期待される。耳鼻咽喉科

ANCA（抗好中球細胞質抗体）
PR3-ANCA（プロテナーゼ 3-ANCA）
MPO-ANCA（ミエロペルオキシダーゼ抗体）

医のみならず，喘息にかかわる医療従事者が，この疾患の存在について認知することを望む。

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Differential expression of periostin in the nasal polyp may represent distinct histological features of chronic rhinosinusitis

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ABSTRACT

Objective: Chronic rhinosinusitis (CRS) is thought to be a multifactorial disease, and it is classified into a number of subtypes according to clinicohistological features. Periostin, a 90-kDa secreted protein, was reported to exist in nasal polyps (NPs) associated with CRS. We compared the expression of periostin with the degree of eosinophilic infiltration as well as tissue remodeling.

Materials and methods: Tissue samples were collected from 28 patients of CRS with NPs, and clinicohistological features were evaluated. The pattern of periostin expression was assessed immunohistochemically.

Result: Two patterns of periostin expression was observed in nasal polyps: "diffuse type", in which periostin was expressed throughout the lamina propria starting just below the basement membrane, and "superficial type", in which the protein was detected only in the subepithelial layers between the basement membrane and the nasal gland. The average infiltrated eosinophil count in the diffuse type was significantly higher than that in the superficial type (diffuse type 360.5 ± 393.0 vs. superficial type 8.46 ± 13.81, $p = 0.001$). Tissue remodeling was observed in 17 (85.0%) of the 20 diffuse-type nasal polyps, but only in one (12.5%) of the eight superficial-type nasal polyps ($p < 0.001$).

Conclusion: At least two distinct patterns of periostin expression were observed in the nasal polyps associated with CRS in accordance with the heterogeneous mechanisms underlying the pathogenesis of CRS with NPs.

1. Introduction

Chronic rhinosinusitis (CRS) is thought to be a multifactorial disease. According to the Guidelines of Rhinosinusitis in North America and Europe [1,2], CRS is classified into two subgroups, one is chronic rhinosinusitis with nasal polyps (CRSwNP), and the other is chronic rhinosinusitis without nasal polyps (CRSsNP). The histological features of the nasal polyps in cases of CRSwNP are massive infiltration of eosinophils, increased basement membrane thickness, epithelial damage, and goblet cell hyperplasia. These histological features of the nasal polyps are similar to those of the bronchial mucosa in asthmatic patients known as mucosal remodeling. The clinical features of CRSwNP include concomitant bronchial asthma and responsiveness to systemic steroid administration. In East Asia, including Japan, CRSwNP is further classified into two subgroups: one is characterized by eosinophilic inflammation (eosinophilic rhinosinusitis: ECRS) and the other is characterized by neutrophilic inflammation [3–8]. ECRS has features similar to CRSwNP as defined in North America and Europe [3,4]. On the other hand, the latter is characterized by infiltration of neutrophils and lymphocytes into nasal polyps, responsiveness to macrolide therapy, which was proven to be effective for diffuse pan-bronchitis in Japan [9,10], and a low rate of nasal polyp recurrence after ESS [11]. In East Asia, more than 50% of the CRS patients have been reported to have non-ECRS [5], suggesting that at least two different mechanisms are involved in CRSwNP pathogenesis.

Periostin, a 90-kDa secreted protein, was reported to play an important role in remodeling after tissue injury in various organs [12], including the lower airway [13,14]. This protein was reported

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to exist in intact nasal mucosa as well as in nasal polyps associated with CRSwNP [15]. There have been no reports on the differential expression of periostin between the intact nasal mucosa and the nasal polyps, and the role of periostin in the CRSwNP pathogenesis remains to be elucidated.

In this study, we examined the expression pattern of periostin in nasal polyps from CRS patients and sought to clarify the relationship between the expression pattern and a number of clinicohistological parameters.

2. Materials and methods

2.1. Tissue samples

Tissue samples of nasal polyp were collected from 28 patients with CRSwNP who had undergone ESS from January 2002 to December 2005 at the Department of Otorhinolaryngology, Yokohama City University Medical Center. Clinical parameters including patients' age, sex, blood eosinophil ratio, serum Immunoglobulin (Ig) E level, and the presence of seasonal or perennial allergic rhinitis were collected by a retrospective chart review. The presence of bronchial asthma was assessed by pulmonary physicians. The diagnosis of ECRS was obtained according to the previously reported clinical criteria [8]. Briefly, the criteria include three features: (1) blood eosinophilia (>6% of total WBC count), (2) the presence of soft tissue density in the olfactory cleft on computed tomography, and (3) the presence of soft tissue density in the posterior ethmoid sinus. This study was approved by the Internal Review Board of the Yokohama City University Medical Center.

2.2. Histological analysis

Tissue samples obtained during surgery were immediately fixed with 4% formaldehyde, and then embedded in paraffin, sliced into 5 μm sections, and stained with hematoxylin and eosin (H&E stain). The mean eosinophil count in five regions in high power field (400×) was calculated. To evaluate tissue remodeling, we examined the thickness of the basement membrane. Tissue remodeling was considered positive when the basement membrane was thicker than the size of nucleus of basal cells in the area where the epithelium was cut perpendicularly.

2.3. Immunohistochemical analysis

Immunohistochemical analysis was performed to evaluate the expression of periostin in the nasal polyp using the formaldehyde-fixed paraffin-embedded sections as mentioned above. After deparaffinization and rehydration, the sections were treated by proteinase K for 15 min at room temperature to activate antigen reactions. The sections were placed in 0.1% hydrogen peroxide to quench any endogenous peroxide activity and treated with a blocking reagent. The sections were then incubated with anti-periostin rabbit polyclonal antibody (kindly provided by laboratory of Izuhara, Saga Medical School, Japan [14]) at 1:1000 to 1:3000 dilution for an hour at room temperature. Negative controls for immunohistochemistry were performed by incubation with normal rabbit immunoglobulin G as a primary antibody. Periostin was detected by a standard process using the ENVISION+/HRP (DAB) kit system (Dako Cytomation, Glostrup, Denmark). The expression pattern of periostin was evaluated by comparison with the histological section in low power field (40×).

2.4. Statistical analysis

Statistical analysis was performed by using Welch's *t*-test and Spearman's rank correlation coefficient test. A *p*-value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Patient characteristics

Patient characteristics are summarized in Table 1. The blood eosinophil ratio varied from 0.2% to 28% (median 5.4%). The number of patients with allergic rhinitis, bronchial asthma and ECRS was 13 (48.1%), 6 (21.4%) and 6 (21.4%), respectively. No patient was diagnosed with aspirin intolerance. Systemic steroid were administered after ESS in 13 (46.3%) patients. Ten patients (35.7%) experienced nasal polyp recurrence. Histological analysis revealed that the mean infiltrated eosinophil count in the nasal polyps varied from 0 to 1239/high power field (mean 217.1 ± 292, median 70.0). Tissue remodeling, indicated by basement membrane thickening, was observed in 18 patients (64.3%).

3.2. Immunohistochemical analysis

The expression of periostin was detected in all of the 28 samples. We noticed that there were two patterns of periostin expression in the nasal polyps. In one pattern, the periostin was expressed throughout the lamina propria starting just below the basement membrane (Fig. 1a: immunohistochemistry, low power field, b: H&E stain, low power field, c: immunohistochemistry at

Table 1
Patient characteristics and the relationship between a periostin expression type and clinico-histological factors.

Characteristics or factors	Total (n=28)	Diffuse type (n=20)	Superficial type (n=8)	p-Value
Gender				
Male (n)	20 (71.4%)	15	5	N.S.
Female (n)	8 (28.6%)	5	3	
Mean age (year)	50.9	50.1	54.6	N.S.
Allergic rhinisis (n)	13 (48.1%)	11	2	N.S.
Seasonal (n)	11 (39.3%)	9	2	N.S.
Perennial (n)	7 (25.0%)	6	1	N.S.
Bronchial asthma (n)	6 (21.4%)	6	0	0.010
Aspirin intolerant (n)	0 (0%)			
ECRS (n)	6 (21.4%)	6	0	0.009
Polyp recurrence (n)	10 (35.7%)	7 (35.0%)	3 (37.5%)	N.S.
Steroids administration (n)	13 (46.4%)	10 (50.0%)	3 (37.5%)	N.S.
Serum IgE (average, IU)	228.2	281.6	90.7	0.033
Blood eosinophil ratio (average, %)	6.9	7.7	4.8	N.S.
Tissue remodeling (n)	18 (64.3%)	17 (85.0%)	1 (12.5%)	<0.001

IgE, immunogulobulin E; ECRS, eosinophilic chronic rhinosinusitis; N.S., not significant.

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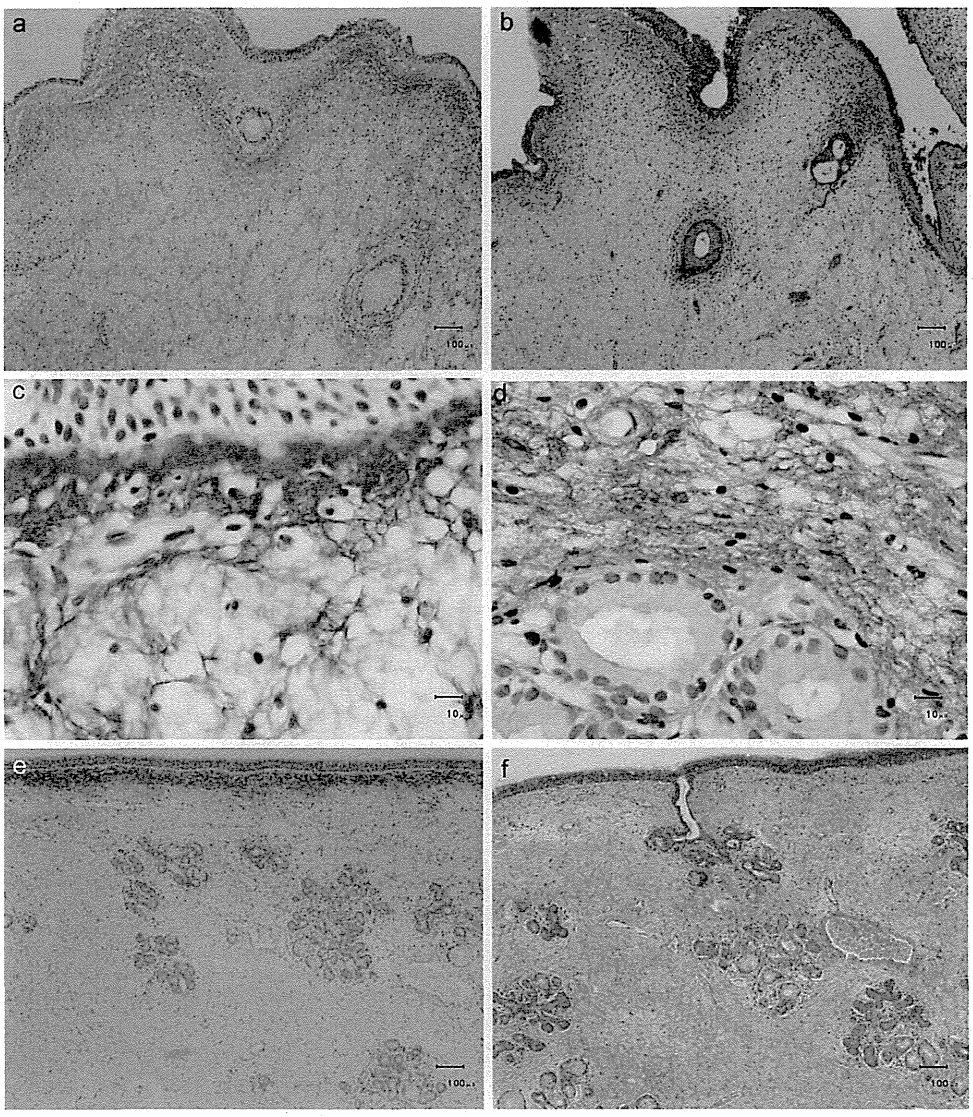

Fig. 1. (a–d) A diffuse type. (a) Periostin immunohistochemistry of low power field (40×), (b) H&E stain (40×), (c) Periostin immunohistochemistry at subepithelial portion of high power field (400×), and (d) at submucosa of high power field (400×). (e and f) A superficial type. (e) Periostin immunohistochemistry of low power field (40×) and (f) H&E stain of low power field (40×).

subepithelial portion, high power field, and d: immunohistochemistry at submucosa, high power field). In the second pattern, periostin was only detected in the subepithelial layers between the basement membrane and the nasal glands (Fig. 1e: immunohistochemistry, low power field and f: H&E stain, low power field). In this study, we designated the former pattern as "diffuse type", and the latter pattern as "superficial type". In the nasal polyps with a superficial type, very little periostin expression was observed in the lamina propria compared with that in the superficial area just below the basement membrane. A mixed pattern of diffuse and superficial periostin expression was observed in a single patient. This case was classified as a superficial type because the area with superficial expression was much greater than that with diffuse expression. The number of patients with diffuse and superficial types was 20 (71.4%) and 8 (28.6%), respectively. All the polyps with no more than three infiltrated eosinophils were classified as superficial type.

3.3. *The relationship between a periostin type and clinicohistological factors*

Next, we examined whether a periostin expression type was associated with specific clinical and histological features. The average infiltrated eosinophil count in the diffuse-type nasal polyps was significantly higher than that in the superficial type (diffuse type 360.5 ± 393.0 vs. superficial type 8.46 ± 13.81, $p = 0.001$, Fig. 2). The relationships between periostin expression type and other clinical and histological features are shown in Table 1. Tissue remodeling was observed in 17 (85.0%) of the 20 diffuse-type nasal polyps, whereas it was observed in only one (12.5%) of 8 superficial-type nasal polyps ($p < 0.001$). All of the patients with bronchial asthma showed diffuse-type periostin expression ($p = 0.010$). The same is true for those with ECRS ($p = 0.009$). Serum IgE level in the patients with diffuse-type expression was significantly higher than that in those with superficial type (diffuse type 281.6 ± 321.5 vs. superficial type 90.7 ± 95.5,

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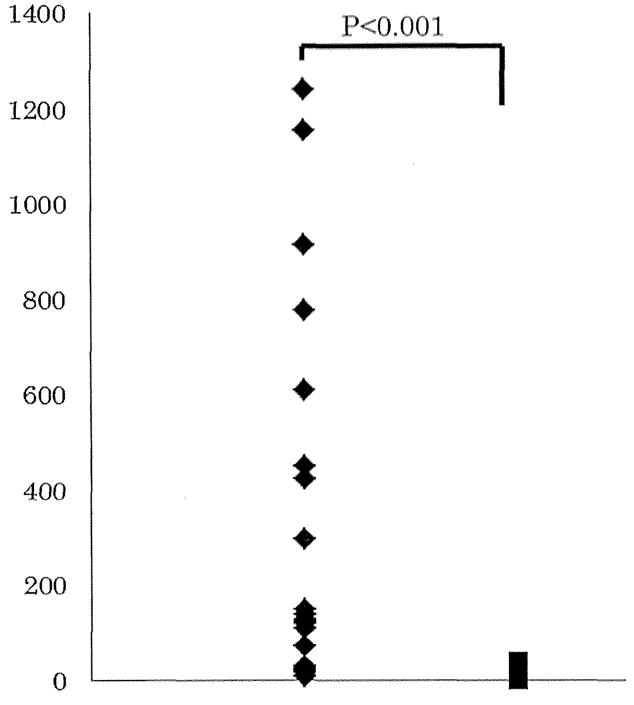

Fig. 2. Comparison of the number of infiltrating eosinophil in nasal polyps between in the diffuse (left side, ◆) and superficial (right side, ■) types.

$p = 0.033$). There were no significant differences in the other clinicohistological factors between the two types of expression.

4. Discussion

Stankovic and colleagues [15] first reported the existence of periostin in intact nasal mucosa and nasal polyps associated with CRSwNP. They reported that periostin expression in intact nasal mucosa was observed in a limited area between the basement membrane and nasal gland, whereas that in nasal polyps was observed diffusely in the lamina propria. In the present study we noticed that, even in nasal polyps, there were two types of periostin expression pattern: a diffuse type and a superficial type. This is the first study to report that a superficial pattern of periostin expression exists in nasal polyps. A diffuse type was considered to be similar to the periostin expression pattern in nasal polyps reported by Stankovic's group. Given the results that all polyps with no more than three infiltrated eosinophils were superficial type, it appears that when eosinophilic inflammation is absent, periostin is expressed at only very low levels in the lamina propria.

In East Asia including Japan, CRSwNP is classified into two subgroups: ECRS and non-ECRS [3–7]. More than 50% of the CRSwNP patients have been reported to have non-ECRS [5,7], suggesting one aspect of CRSwNP heterogeneity. As non-ECRS is rare in North America and Europe, it is speculated that nasal polyps with a superficial pattern of periostin expression may be found only in East Asia. The fact that medication and surgery are effective in the treatment of patients with non-ECRS may indicate milder inflammation in the sinonasal mucosa in those patients. This milder inflammation may be associated with a superficial pattern of periostin expression.

Periostin, a 90-kDa secreted protein recognized as a matricellular protein, was reported to have an important role in wound repair after tissue damage, known as remodeling, in various organs [12]. Yuyama and colleagues [13] reported that the periostin mRNA level was 8 times higher in asthmatic epithelial cells than in control epithelial cells after stimulation by interleukin (IL)-4 and IL-13. Takayama et al. [14] reported that the secretion of periostin from lung fibroblast related to subepithelial fibrosis in a mouse model of asthma, and that this subepithelial fibrosis was absent in periostin knockout mice. These reports support the idea that periostin has an essential role in mucosal remodeling in the lower airway. In the present study, the number of cases showing basement membrane thickening in the superficial type was statistically lower than that in diffuse type, suggesting that the degree of remodeling might be milder when periostin expression is observed only in the superficial layer of the nasal polyp.

On the other hand, in all cases having bronchial asthma and/or ECRS, periostin was expressed diffusely throughout the lamina propria. In the lower airway, eosinophils are the main source of TGF-beta and can induce tissue fibrosis through the TGF-beta signaling pathway [16]. Moreover, TGF-beta can stimulate epithelial cells and fibroblasts to secrete periostin [14,17]. Therefore, eosinophils are thought to increase the expression of periostin in the lower airway. There is a possibility that eosinophils can also induce increased periostin expression in the upper airway. In cases with bronchial asthma and/or ECRS, eosinophilic inflammation has been shown to occur simultaneously in the upper and lower airway and massive infiltration of eosinophils is observed in nasal polyps [2,3]. This may cause diffuse periostin expression in the lamina propria of the sinonasal mucosa, leading to the formation of nasal polyps.

Four of the eight cases in which the number of eosinophils ranged from 5 to 39 were classified as diffuse type. In the cases with mild eosinophilic inflammation, there may be other factors contributing to the periostin expression pattern. Besides eosinophil infiltration, possible factors for the promotion of periostin expression are mechanical stress [18], hypoxic stimulation [19,20], vascular injury [21], and histamine [22]. If such stimuli exist in the polyp mucosa, diffuse periostin expression may be observed in the lamina propria regardless of only mild eosinophil inflammation. Further study on these stimuli in the sinonasal mucosa may help explain the mechanisms underlying polyp formation in association with CRSwNP.

Ishida and colleagues [23] reported that periostin was up-regulated in the nasal mucosa of patients with allergic rhinitis and in the nasal polyps of patients with CRS or AIA, and concluded that periostin might be a therapeutic target for CRSwNP. On the other hand, based on a mouse experimental model, Kim et al. reported that periostin may play a protective role in the development of ECRS [24]. In this report, polyp-like lesions were observed more frequently in the periostin-null mice than in the wild type. This report suggested that periostin had an important role in the mechanisms for remodeling in the sinus mucosa, and periostin deficiency led to prolongation of healing from polyp-like lesions. Periostin was also reported to have a role in elastic collagen formation in asthma [17] and could, therefore, protect the inflammatory mucosa from developing polyp-like lesion through collagen fibrillogenesis.

In conclusion, the present study revealed that there were at least two different patterns of periostin expression in the nasal polyps associated with CRSwNP in accordance with the heterogeneous mechanisms underlying CRSwNP pathogenesis. However, further study is required to elucidate the exact role of periostin in the pathogenesis of CRSwNP.

Conflict of interest

No authors have a conflict of interest.

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Abstract

Nitric oxide is a possible reliable marker for evaluation of nasal allergy and chronic sinusitis.

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Abstract

Backgrounds

Nitric oxide (NO) has a variety of roles in human airways relevant to airway defense mechanisms, as well as being an inflammatory mediator. In asthmatic patients, it has been proposed to use fractional concentrations of exhaled NO (FeNO) as a marker to diagnose asthma, to monitor the response to antiinflammatory medications, and to predict upcoming disease exacerbations. The standardization of measurements by the American Thoracic Society/European Respiratory Society has opened the gate for the accumulation of comparable FeNO data in normal subjects and diseased patients. In the present study, we carried out a series of studies on the oral and nasal FeNO levels in inflammatory upper airway diseases of the Japanese population by using a handheld electrochemical NO analyzer, NObreath®.

The role of NO in human nasal cavity

Although the human paranasal sinuses are known to be a sizable source of intrinsic NO production, the origin of nasal NO measured from human nasal airway has been controversial, and there are some indications that favor the paranasal sinuses rather than the mucosa of the nasal cavity and vice versa. However, there are still several issues to be solved before FeNO measurement becomes a reliable and valid marker for the diagnosis of allergic rhinitis (AR) and chronic rhinosinusitis (CRS). They include 1) complicated anatomical structure of paranasal sinuses and gas exchange through the narrow sinus ostia, 2) the balance between maintaining optimal mucociliary clearing function by the ciliary epithelium and modulating inflammatory conditions by excess NO production, 3) inhibitory effects of gaseous NO diffusion into the air-filled sinus caused by excess secretions and thick aqueous epithelial lining in case of sinusitis. We consider that nasal FeNO represents a fraction of endogenous NO with contaminated air passing through the nose with a constant and relatively high flow rate. We have found that the reproducibility of this measurement technique with NObreath® is acceptable from a clinical point of view and the intraindividual variability within each measure is

usually minor in a cooperative and trained subject.

NO monitoring in nasal allergy and chronic sinusitis

AR patients have been considered to be associated with increased FeNO levels mainly by the increased expression of inducible nitric oxide synthase (iNOS) in the inferior turbinate. The perennial AR patients tended to show higher oral FeNO levels as compared with the normal subjects. The nasal FeNO levels in the AR patients were significantly higher than in the normal subjects and vasomotor rhinitis patients. There also existed positive correlations between the nasal symptom scores and FeNO levels.

Nasal NO levels generally decrease in most CRS patients. However, it is unclear to what extent nasal NO levels contribute to sinusitis pathology especially pertinent to different CRS types. We have recently shown that eosinophilic CRS (ECRS) patients show significantly higher oral FeNO levels compared to non-ECRS patients and normal subjects. In addition, nasal FeNO levels of the non-ECRS patients were significantly lower than those of the ECRS patients and normal subjects. Positive correlations existed between the blood eosinophil percentage and FeNO levels in ECRS patients. Immunohistological analysis revealed that higher FeNO levels in ECRS patients closely correlated with augmented iNOS expression and were accompanied by the excretion of NO metabolites into the paranasal sinus mucosa.

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ORIGINAL ARTICLE

Comparison of Nasal Nitric Oxide Levels between the Inferior Turbinate Surface and the Middle Meatus in Patients with Symptomatic Allergic Rhinitis

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ABSTRACT

Background: Because of the anatomical complexity and the high output of the human nose, it has been unclear whether nasal nitric oxide (NO) serves as a reliable marker of allergic rhinitis (AR). We examined whether nasal NO levels in the inferior turbinate (IT) surface and the middle meatus (MM) differ in symptomatic AR patients.

Methods: We measured fractional exhaled NO (FeNO) and nasal NO in normal subjects (n = 50) and AR patients with mild symptoms (n = 16) or moderate or severe symptoms (n = 27). Nasal NO measurements were obtained using an electrochemical analyzer connected to a catheter and an air-suction pump (flow rate 50 mL/sec).

Results: Compared to the normal subjects, the AR patients showed significantly higher nasal FeNO and nasal NO levels in the IT area. No significant difference in the MM area was observed among the three groups. The MM area showed higher NO levels than the IT area in all three groups. The ratio of nasal NO levels of the MM area to the IT area (MM/IT ratio) was significantly lower in the AR groups. The moderate/severe AR patients showed significantly higher nasal NO in the IT area (104.4 vs. 66.2 ppb) and lower MM/IT ratios than those in the mild AR patients. The analysis of nasal brushing cells revealed significantly higher eosinophil cationic protein and nitrotyrosine levels in the AR groups.

Conclusions: Nasal NO assessment in the IT area directly reflects persistent eosinophilic inflammation and may be a valid marker to estimate the severity of AR.

KEY WORDS

allergic rhinitis, exhaled nitric oxide, inferior turbinate, nasal nitric oxide, nitrotyrosine

ABBREVIATIONS

AR, allergic rhinitis; ECP, eosinophil cationic protein; FeNO, fractional exhaled nitric oxide; IT, inferior turbinate; MM, middle meatus; NO, nitric oxide; NT, nitrotyrosine; NOS, nitric oxide synthase; ppb, parts per billion.

INTRODUCTION

The standardization of measuring techniques by the American Thoracic Society/European Respiratory Society (ATS/ERS) has opened the way for the collection of comparable airway data on nitric oxide (NO) in normal subjects and those with disease states.[1,2] Allergic rhinitis (AR) has been considered to be asso-

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ciated with increased NO levels. However, it has not yet been determined whether nasal NO serves as a reliable index of disease severity, or to what extent NO concentrations inside the nose contribute to pathologies of AR.[3-5] Nasal NO is not routinely measured in daily clinical practice. One reason may be the heterogeneous results found in previous studies, mainly due to the complexity of the anatomical and physiological features of the human nose and the lack of consensus concerning the suitable measurement technique.[6-8]

In the present study, the fractional exhaled nitric oxide (FeNO) and nasal NO levels were examined in a population of normal subjects and AR patients. We used a newly designed method to measure nasal NO locally based on the anatomical features of the nasal cavity. For this purpose, the NO analyzer was connected to a suction catheter and a fixed-quantity suction pump in an out-patient clinic setting. The handheld device with an electrochemical sensor for nasal NO measurement has been shown to be reliable and simple to use at a lower cost.[9,10]

Here we examined whether local gradients of NO concentration in different areas inside the nasal cavity differ among normal subjects and AR patients classified by subjective symptom severity. We also obtained nasal brushing cells from the surface of the inferior turbinate mucosa and analyzed the concentrations of the extracted inflammatory mediators related to eosinophil activation and NO metabolism. We note that the nasal NO assessment described here is noninvasive, quickly performed, and may directly reflect the degree of allergic inflammatory conditions adjacent to the surface mucosa of the inferior turbinate.

METHODS

SUBJECTS

Ninety-three subjects were included in this cross-sectional, between-group and method comparison study. The subjects were 43 patients with perennial AR without bronchial asthma (28 males and 15 females; mean age 27.7 years) and 50 normal volunteers without nasal symptoms (30 males and 20 females; mean age 32.1 years). The AR patients were recruited on an outpatient setting and subdivided into two groups based on disease severity: the group of 16 patients with mild symptoms (mild group) and the group of 27 patients with moderate or severe symptoms (moderate/severe group).

The diagnosis of AR was based on clinical history, the presence of subjective nasal symptoms together with positive nasal eosinophil tests, and positive skin reactions or serum allergen-specific IgE antibody measurements against house dust mites. Nasal endoscopy was performed for all subjects before measuring nasal NO in order to assess the degree of nasal septum deviation and patency of middle meatus, and to exclude the presence of nasal polyposis. We excluded current-smokers and patients who had been treated with any allergen-specific immunotherapy. The patients did not receive any anti-allergic medication in the 30 days before the study. The patients' subjective symptoms were recorded at the time of the NO measurement. They include the average number of paroxysmal sneezing, episodes of nose blowing, and the degree of nasal blockage. The severity of the disease was then determined as mild, moderate or severe based on the classification proposed by the Japanese guideline for allergic rhinitis.[11]

The study protocol was approved by the Institutional Review Board at the Hiroshima University School of Medicine (Project approval #181-1). The purpose of the research and experimental protocols was explained to all participants, and written informed consent was obtained prior to the study.

NITRIC OXIDE MEASUREMENTS

The subjects' NO levels were measured using a handheld electrochemical analyzer (NObreath®, Bedfont Scientific, Rochester, UK) according to the ATS/ERS guidelines.[1] For the oral FeNO measurements, the subjects exhaled at a flow rate of 50 mL/s through a mouthpiece assisted by visual cues. For the nasal FeNO measurements, the subjects were instructed to exhale transnasally with their mouth closed into a nose adaptor at the same flow rate, as described.[12] The nasal FeNO measurements were carried out for the right and left nasal cavities separately, with the other nostril closing in turn. We also measured nasal NO in all of the subjects by directly aspirating air from the nasal cavity. For this purpose, the NO analyzer was connected to a suction catheter via a sterile syringe filter and a portable air-suction pump (MP-Σ300N, Sibata Science, Saitama, Japan), which could be set at constant flow levels (Fig. 1). The aspiration flow rate was fixed at a rate of 50 mL/sec, and the tip of the catheter was placed inside the nasal cavity under direct vision during the sampling period. Two different target areas were set based on the anatomical features of the nasal cavity, i.e., near the anterior surface of the inferior turbinate (IT area) and the front of the middle meatus (MM area). The subjects were advised to breathe through the mouth with their soft palate elevated to cease the choanal airflow. Nasal NO levels were measured separately for the left and right side, leaving the other nostril open, in alignment with ATS recommendations.[1] The measurements were performed in the same clinic under constant environmental conditions. The measurement was repeated three times and the mean value was used for analysis.

NASAL CELL SAMPLING

Nasal brushing cell specimens were obtained for an enzyme-linked immunosorbent assay (ELISA) from 27 of the 50 normal subjects and 31 of the 43 AR pa-

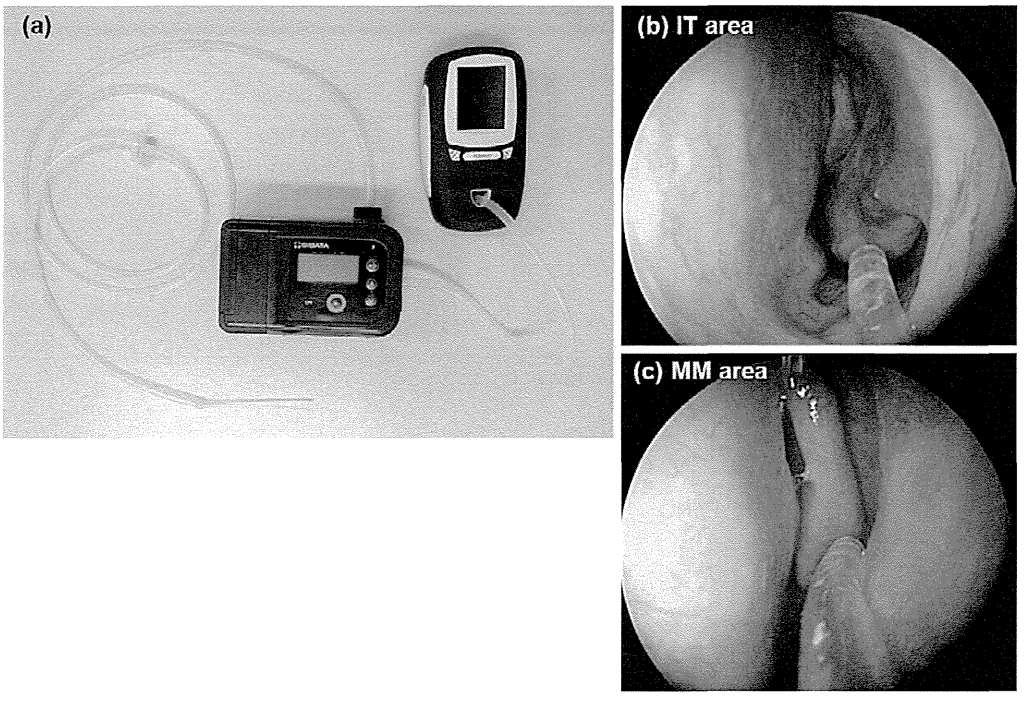

Fig. 1 (**a**) Configuration of the nose catheter, silicon tubes, and the air-suction pump used for the direct nasal NO measurement. The tip of the catheter was placed (**b**) in the anterior surface of the inferior turbinate (IT) or (**c**) in front of the middle meatus (MM) during the aspiration period.

tients (10 in the mild group and 21 in the moderate/severe group) who agreed to participate. There was no artificial bias for patient selection in cell sampling. A topical decongestant was applied to the nose just after the completion of the series of NO measurements, and secretions were carefully removed. Cells were then obtained by scraping of the medial wall of the IT under direct vision using a Cytobrush® (Medscand Medical, Malmo, Sweden). The cells were placed immediately in a 2-mL volume of chilled phosphate buffered saline (PBS) and then stored at -80℃ until further use. Protein extraction from the cell suspension was performed by a tissue homogenizer using bead-beating technology (Precellys® 24, Bertin Technologies, Montigny-le-Bretonneux, France) in 2-mL tubes with 1.4-mm prefilled glass beads (6000 rpm, two cycles for 20 s each).

The concentrations of eosinophil cationic protein (ECP) and nitrotyrosine (NT) from the supernatant were measured quantitatively by the ELISA method. Commercially distributed kits for ECP (ECP ELISA, Aviscera Bioscience, Santa Clara, CA, USA) and for NT (OxiSelect™ Nitrotyrosine ELISA Kit, Cell Biolabs, San Diego, CA, USA) were used according to the instructions supplied by the manufacturer.

DATA ANALYSIS

Group data are expressed as means ± standard deviations (SD). For multiple comparisons, a screening of data for differences was first carried out using the Kruskal-Wallis test. If the analysis gave a significant result, a further comparison was done by the Mann-Whitney U-test for the between-group analysis. The comparison of paired nasal NO levels between different areas was assessed with the Wilcoxon rank sum test. Spearman rank correlation was used in evaluating correlations. *P*-values < 0.05 were considered significant.

RESULTS

DIRECT MEASUREMENT OF NASAL NO LEVELS

The demographics and clinical background of the study population are summarized in Table 1. No significant difference between the normal and AR groups was found in the baseline data of gender or age distribution. The distribution of the oral and nasal FeNO values in each group is shown in Figure 2. Compared to the normal subjects, the patients in both AR groups showed significantly higher levels of nasal FeNO. There was no significant difference in nasal FeNO levels between the mild and the moderate/severe AR groups. The AR patients tended to show higher levels of oral FeNO, but the difference was not significant. The direct measurement of nasal NO levels from the IT area and the MM area inside the nasal cavity was successfully achieved using the current setting for all of the subjects. We had difficulty measuring the nasal NO levels in the MM area of the unilateral side in four normal subjects and three AR patients because of severe nasal septum deviation.

Table 1 Demographics and clinical background of the study population

	Normal volunteers	AR patients	
		Mild group	Moderate/severe group
number (male/female)	50 (30/20)	16 (11/5)	27 (17/10)
age	32.1 (12.5)	26.1 (5.8)	28.7 (8.4)
subjective nasal symptom score			
sneezing	-	0.31 (0.47)	1.48 (1.08)
nose blowing	-	0.81 (0.4)	2.26 (1.09)
nasal blockage	-	0.87 (0.34)	2.44 (1.05)
total score	-	2 (0.63)	6.19 (2.76)

Data are shown as mean with standard deviations in parenthesis.

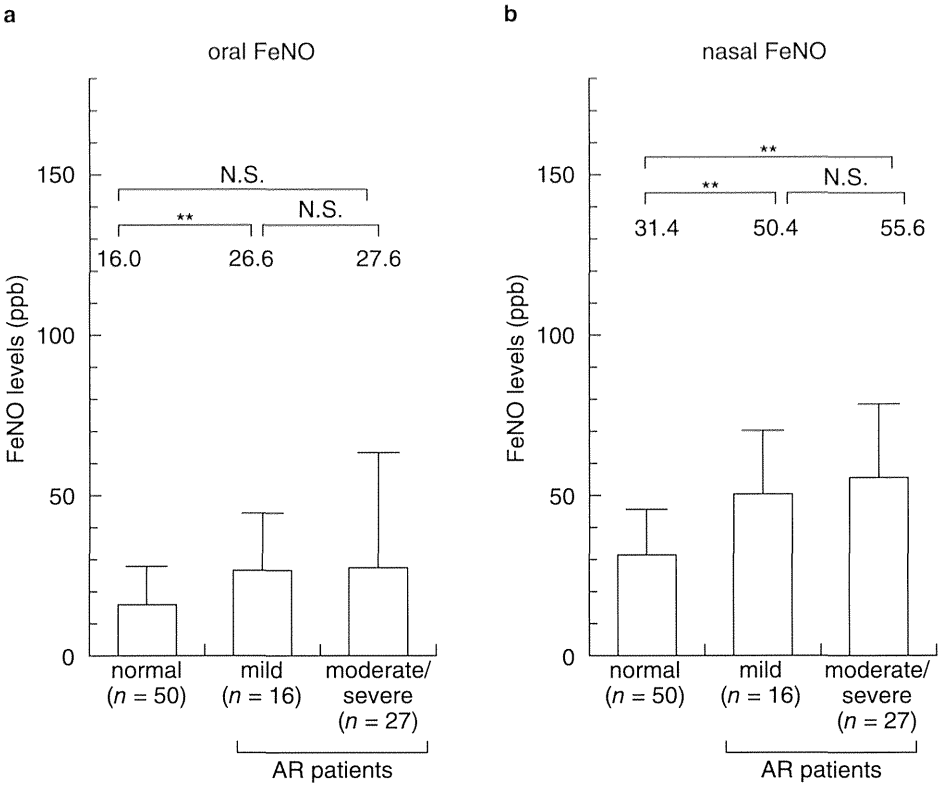

Fig. 2 (**a**) Oral and (**b**) nasal FeNO levels in the normal subjects (n = 50) and AR patients in the mild group (n = 16) and in the moderate/severe group (n = 27). The average of right and left nasal cavities was used for the nasal FeNO level of each individual. Error bars = mean values and SD. *p < 0.05; **p < 0.01; N.S., no significance; FeNO, fractional exhaled nitric oxide.

Therefore, 96 sides of the nasal cavity in the normal group and 83 sides in the AR group were processed for analysis. None of the subjects reported adverse effects after the procedure.

As shown in Figure 3, the nasal NO levels in the IT area in both groups of AR patients were significantly higher than those of the normal subjects. The AR patients in the moderate/severe group also showed significantly higher nasal NO in the IT area compared to the mild AR patients (104.4 ppb vs. 66.2 ppb). However, there was no significant difference in nasal NO levels in the MM area among the three groups. Consequently, the ratio of nasal NO levels of the MM area to the IT area (MM/IT ratio) was significantly lower in the two AR groups than in the normal group (Fig. 3c), with the moderate/severe AR group showing significantly lower MM/IT ratios compared to the mild AR group. When the same nasal cavity was compared for each subject, the MM area showed higher NO levels than the IT area, and the differences were

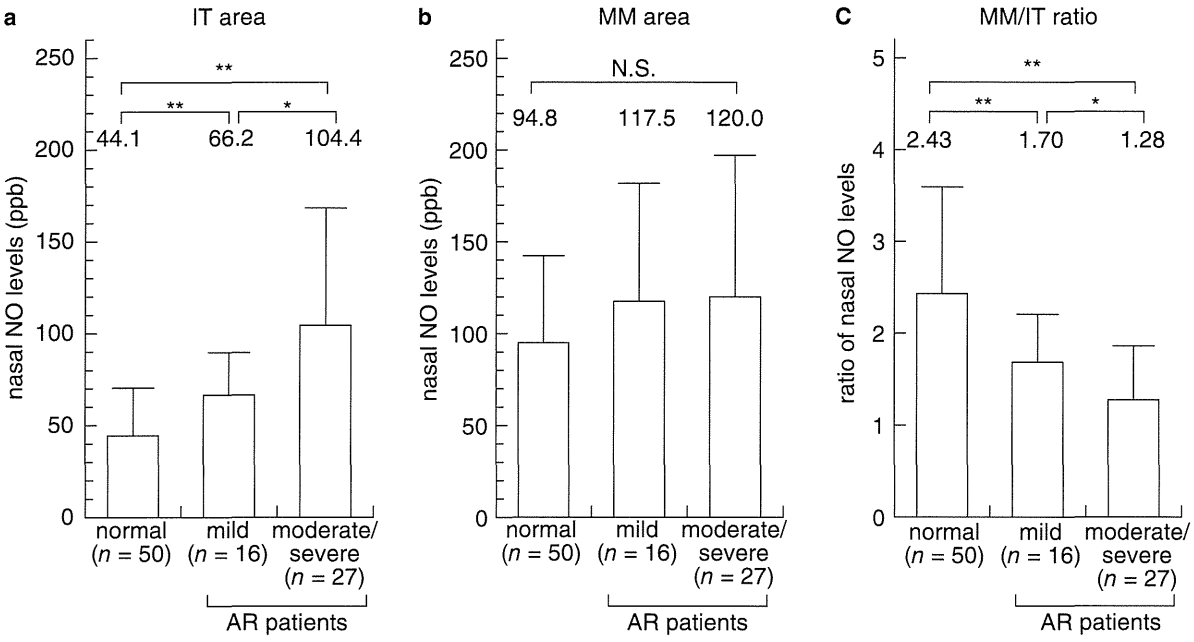

Fig. 3 Nasal NO levels (**a**) in the IT area and (**b**) in the MM area in the normal subjects and AR patients in the mild and moderate/severe groups. (**c**) The nasal NO ratio of the MM area to the IT area (MM/IT ratio) for each group. Measurement of the nasal NO levels and the calculation of the MM/IT ratio were carried out separately for the left and right sides of the nose, and the average of the two cavities was used for each individual. Error bars = mean values and SD. $*p < 0.05$; $**p < 0.01$; N.S., no significance.

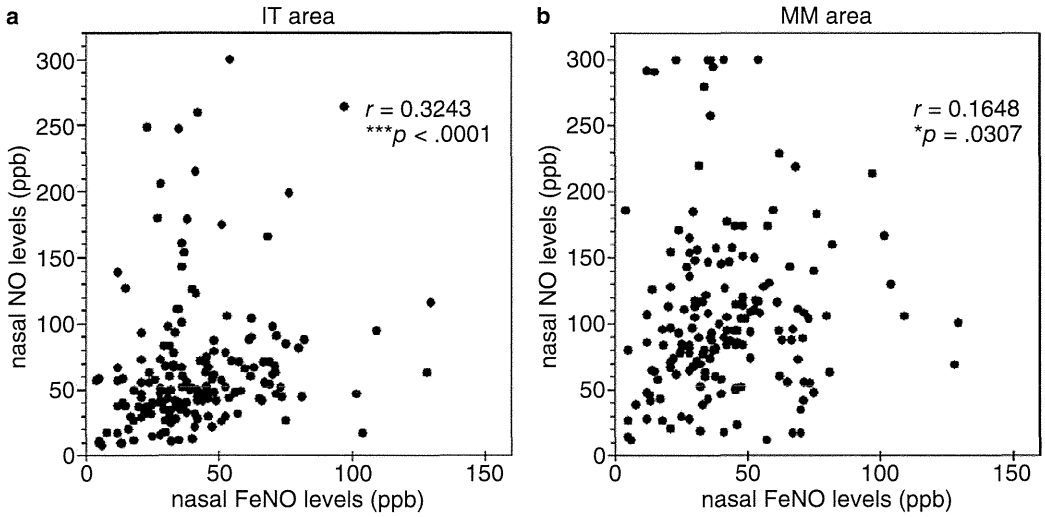

Fig. 4 Correlation between nasal FeNO levels and nasal NO levels (**a**) in the IT area and (**b**) in the MM area for each nasal cavity in all subjects ($n = 179$).

significant in all three groups. The mean differences in the nasal NO levels in the IT area between the right and left cavities were 16.1 ppb (SD 21.1) in the normal group, 18.9 ppb (SD 10.8) in the mild AR group, and 39.7 ppb (SD 35.7) in the moderate/severe AR group. The mean differences in the nasal NO levels between the cavities were more pronounced in the MM area: 30.5 ppb (SD 33.8) in the normal group, 38.5 ppb (SD 42.5) in the mild AR group, and 35.4 ppb (SD 35.1) in the moderate/severe AR group. The correlations between nasal FeNO levels and nasal NO levels in the IT and MM areas for each nasal cavity are shown in Figure 4. We found positive correlations between the paired two parameters, but the coefficient was markedly higher for nasal NO in the IT area than that in the MM area ($r = 0.3243$ vs. $r = 0.1648$).