（松浪硝子、大阪）に伸展、接着しヘマトキシリン・エオジン染色、免疫染色に用いた。一部はRNA later®(Life Technologies, Carlsbad, CA, US)で処理し、PCR解析用検体とし、一部は生標本のまま-80℃で凍結させ、ELISA解析用検体とした。

3．ELISA

採取された鼻粘膜は凍結解凍後、CelLytic ™ MT Cell Lysis Reagent (Sigma-Aldrich Japan)、protease inhibitor cocktail (P8340 Sigma-Aldrich Japan)、benzonase endonuclease (E1014 Sigma-Aldrich Japan)の混合液でホモジェナイズした。ホモジェナイズされたサンプルは4℃、15,000回転で10分間遠心し、上澄をELISA測定に用いた。吸光度は450nmマイクロプレートリーダーを用いて測定した。IL-33、ST2についてタンパク定量を行い、それぞれabcam社（東京）のキットを使用した。

4．免疫染色と組織解析

組織切片に下記の抗体による単染色を施行した。

・抗IgE抗体 (rabbit polyclonal, against ε-Heavy Chain, from Nichirei, Tokyo, Japan)

・抗eosinophil major basic protein (MBP)抗体 (mouse monoclonal, clone BMK-13, Millipore, CA, USA)

・抗Fcε receptor1抗体 (mouse monoclonal, clone 9E1, from abcam, Tokyo, Japan)

・抗Fcε receptor2（CD23）抗体 (mouse monoclonal, clone 1B12, from Nichirei, Tokyo, Japan)

・抗mast cell tryptase抗体 (mouse monoclonal, clone AA1, Thermo Fisher Scientific, CA, USA)

・抗ヒト形質細胞（plasma cell）抗体 (mouse monoclonal, clone VS38c, Dako Cytomation Japan, Kyoto, Japan)

・抗CD3（T細胞）抗体 (rabbit monoclonal, clone SP7, Nichirei, Tokyo, Japan)

・抗CD20（B細胞）抗体 (rabbit monoclonal, clone L26, Nichirei, Tokyo, Japan)

・抗IL-33抗体 (mouse monoclonal, clone Nessy-1, from Alexis Biochemicals, CA, USA)

・抗ST2抗体 (mouse monoclonal, clone HB12, from Medical & Biological Laboratories, Nagoya, Japan)

またST2陽性細胞の細胞種を同定するため、MBPとST-2、mast cell tryptaseとST2、CD3とST2の2重染色をそれぞれ施行した。

陽性細胞数の計測は染色標本を光学顕微鏡にて高倍率（400倍）で観察し、無作為に5視野で陽性細胞を数え、平均した。

5．定量PCR

RNA later®で処理された組織をISOGEN (Nippon Gene, Tokyo, Japan)で溶解し、総RNAを抽出した。mRNA発現はApplied Biosystems 7500 Real Time PCR System (PE Applied Biosystems, Foster City, CA)で測定した。プライマー及びプローブはhuman β-actin、IL-4、5、13、33、ST2、FcεR1α、CD23についてPE Applied Biosystemsで市販されているものを購入しmRNA発現はTaqMan detection systemで測定した。Nuclease-free waterを陰性コントロールとして使用した。

相対的定量のためΔΔCt法を用いた。ハウス

キーピング遺伝子(housekeeping gene, HKG) をリファレンスとして mRNA 量の補正を行った。HKG としては β-actin (ACTB)をを用いた。サイトカインと HKG の threshold cycle (ΔCt sample, ΔCt control) を測定し、ΔCt value (ΔΔCt, ΔCt sample – ΔCt control) を計算した。その値より relative quantitation (RQ) value ($RQ = 2^{-\Delta\Delta Ct}$) を計算した。

6．統計解析

統計処理は SPSS 統計ソフト (SPSS, Chicago, IL, USA)を用いた。 全てのデータは、各群の平均±標準誤差で表わした。グループ間の比較は Kruskal-Wallis test を、2 群間の比較は Mann-Whitney U test を用いた。IL-33、ST2 の ELISA については各群間の比較に t 検定を用いた。有意差は $p < .05$ の場合とした。

（倫理面への配慮）

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C．研究結果

1．鼻粘膜中炎症細胞の組織学的解析

好酸球数 (H-E 染色)、マスト細胞数 (mast cell tryptase 陽性細胞)、形質細胞数 (VS38c 陽性細胞)、T 細胞数 (CD3 陽性細胞)、B 細胞数 (CD20 陽性細胞)、IL-33 陽性細胞、ST2 陽性細胞数、IgE 陽性細胞数、 FcεR1 陽性細胞数、 Fc ε R2 陽性細胞数 (CD23 陽性細胞) を好酸球性副鼻腔炎群ポリープ、非好酸球性副鼻腔炎群ポリープ、非副鼻腔炎コントロール群正常鼻粘膜で計測した。

好酸球数は好酸球性副鼻腔炎群ポリープ(n=18, 127.6; 53.2-385.2) でコントロール(n=8, 0.0; 0-3.8; $p < .001$)、非好酸球性副鼻腔炎群ポリープ(n=18, 3.3; 0-47.8; $p < .001$)と比較し優位に増多していた。形質細胞数は非好酸球性副鼻腔炎群ポリープ(n=16, 33.1; 11.8-84.2)でコントロール(n=7, 6.0; 2.0-7.4; $p < .001$)、好酸球性副鼻腔炎群ポリープ(n=17, 21.6; 2.4-51.2; $p < .001$)と比較し優位に増多していた。B 細胞数も同様に非好酸球性副鼻腔炎群ポリープ(n=18, 94.6; 16.0-282.3)でコントロール(n=7, 3.3; 2.25-13.5; $p < .001$)、好酸球性副鼻腔炎群ポリープ(n=17, 54.0; 22.7-183.3; $p < .005$)と比較し優位に増多していた。マスト細胞数、T 細胞数については各群で有意差を認めなかった($p > .05$)。

ST2 陽性細胞は全ての群で上皮細胞、血管内皮細胞、腺細胞に分布しており、好酸球性副鼻腔炎群ポリープでは上皮下層の炎症細胞に分布していた。ST2 陽性細胞数は好酸球性副鼻腔炎群ポリープ(n=11, 65.0; 9.6-225.6)でコントロール(n=6, 1.5; 0-5.2; $p < .0001$)、非好酸球性副鼻腔炎群ポリープ(n=13, 10.2; 0.4-34; $p < .0001$)と比較し優位に増多していた。IL-33 は核に分布しており、すべての群において上皮細胞、血管内皮細胞に分布しており、染色パターンは全ての群で同様であった。

IgE 陽性細胞は好酸球性副鼻腔炎群ポリープ、非好酸球性副鼻腔炎群ポリープの上皮下層の炎症細胞に分布していた。IgE 陽性細胞数は好酸球性副鼻腔炎群ポリープ(n=16, 14.8; 4.4-28.8)でコントロール(n=7, 0.0; 0.0-3.8; $p < .001$)、非好酸球性副鼻腔炎群ポリープ(n=18, 3.8; 0.0-20.0;

p<.001)と比較し優位に増多していた。

FcεR1 陽性細胞も IgE 陽性細胞と同様に好酸球性副鼻腔炎群ポリープ、非好酸球性副鼻腔炎群ポリープの上皮下層の炎症細胞に分布していた。Fc ε R1 陽性細胞数は好酸球性副鼻腔炎群ポリープ(n=15, 7.0; 3.2-15.0)、非好酸球性副鼻腔炎群ポリープ(n=17, 5.8; 1.0-17.4)でそれぞれコントロール群(n=7, 1.0; 0.2-4.4; それぞれ p<.001, p<.005)より優位に増多していた。また、上皮下層の FcεR1 陽性細胞数は IgE 陽性細胞と優位に正の相関関係にあった(n = 39, r = 0.9821, p<.005)。CD23 陽性細胞は血管周囲に主に分布しているが、各群とも非常に細胞数が少なかった。

2．Th2 関連サイトカインとその受容体の発現解析（定量 PCR 及び ELISA）

IL-5、IL-13 は mRNA レベルで好酸球性副鼻腔炎群ポリープでコントロール、非好酸球性副鼻腔炎群ポリープと比較し優位に増多していた（図19)。IL-4、IL-33 については mRNA レベルで各群で有意差を認めなかった。また IL-33 については ELISA 法によるタンパク定量も行ったが各群で有意差を認めなかった。

IL-4、IL-13 の共通の受容体である IL-4 レセプターについては mRNA レベルで好酸球性副鼻腔炎群ポリープ、非好酸球性副鼻腔炎群ポリープでコントロールと比較し優位に増多していた。

ST2 は mRNA レベル、蛋白レベル（ELISA による定量）とも好酸球性副鼻腔炎群ポリープでコントロールと比較し優位に増多していた。非好酸球性副鼻腔炎群ポリープとは有意差を認めなかった。

3．ST2 陽性細胞種の同定

ST2 陽性細胞種同定のため酵素抗体法による 2 重染色を行った。ST2 と好酸球を標識する MBP の二重染色において、二重陽性細胞は好酸球性副鼻腔炎群ポリープでは MBP 陽性好酸球中の 14.9-58.7%（中央値 38.3%, n=7）であり、非好酸球性副鼻腔炎群ポリープでは二重陽性率は 0-10.3%（中央値 0%, n=7）であった。また、ST2 陽性細胞中の ST2-MBP 二重陽性率は好酸球性副鼻腔炎群ポリープでは 78.9-97.1%（中央値 89.8% n=7）であったのに対し非好酸球性副鼻腔炎群ポリープでは 0-15.5%（中央値 0% n=7）であった。MBP 陽性細胞中、ST2 陽性細胞中ともに二重陽性率は好酸球性副鼻腔炎群ポリープで非好酸球性副鼻腔炎群ポリープと比較し優位に高かった(p < .0005)。ST2-マスト細胞二重染色では各群とも二重陽性細胞はほとんどなく、ST2-CD3（T 細胞）二重染色では各群で二重陽性細胞を認めなかった。

D．考察

まず IL-33 について、以前の報告では Th2 関連疾患であるアレルギー性鼻炎や喘息で上皮内での IL-33 上昇が報告されている。慢性副鼻腔炎においてもポリープの培養上皮細胞において、治療抵抗性の症例は治療反応性の症例と比較して IL-33 が高発現しているという報告がある。しかし本研究ではタンパクレベル、mRNA レベルとも各群間で差は認めなかった。

一方で、IL-33 のレセプターである ST2 については好酸球性副鼻腔炎症例のポリープでコントロールと比較してタンパク、mRNA とも有意に高発現していた。また、好酸球性副鼻腔炎群のポリ

ープでは非好酸球性副鼻腔炎群ポリープと比較し、有意にST2陽性の好酸球が増多していた。この結果は好酸球性副鼻腔炎において、好酸球のST2発現がupregulateされており、ポリープ中の炎症細胞の中で好酸球が主なIL-33の標的細胞となっていることが示唆される。同じようにアレルギー疾患でのST2発現上昇はアレルギー性鼻炎やアトピー性皮膚炎でも報告されている。またST2陽性T細胞（CD3陽性細胞）は認めなかったことから、副鼻腔粘膜内ではIL-33はT細胞に直接作用しないことが示唆される。

今回の結果ではST2発現は好酸球性、非好酸球性副鼻腔炎の両群間で差を認めなかったが、これはST2が腺細胞に偏在しているため、好酸球での高発現がmRNAや蛋白レベルで反映されなかったからと考えられる。好酸球はIL-33の標的細胞の一つであるとされ、好酸球でのST2発現も報告されているが、好酸球でのST2発現の様式については議論されている段階である。Wongらは Western blot法で好酸球でのST2タンパクの発現を証明しているが、Cherryらは末梢血から分離した好酸球ではST2はタンパクレベルでは発現していないが、mRNAレベルでは発現していると報告している。好酸球でのST2発現は細胞の置かれている環境で左右されると考えられており、好酸球をgranulocyte macrophage colony-stimulating factor存在下に培養すると細胞表面にST2が発現すると報告されている。今回、非好酸球性副鼻腔炎の好酸球で免疫染色においてST2発現を認めなかった理由として、ST2発現レベルが低かったため、免疫染色で検出されなかった可能性もある。

IL-33はアレルギー性鼻炎モデルマウス、ヒトのアレルギー性鼻炎においてアレルゲンの刺激によって粘膜上皮よりの分泌増加が報告されている。副鼻腔炎においても、IL-33分泌刺激因子の検討が必要であり、今後の課題となる。

IL-33は好酸球の遊走や生存延長を促進し、好酸球からのサイトカイン分泌を促進させる。ごく最近の報告では好酸球性肺炎においてIL-33は好酸球の重要な遊走因子として働くことが報告されている。以上より、IL-33は好酸球性副鼻腔炎症例のポリープにおいて、好酸球上のST2発現を介して、好酸球の遊走、活性化因子となり副鼻腔炎症を増悪させる要因となっている可能性が示唆された。

E. 結論

好酸球性副鼻腔炎症例のポリープにおいてIL-33の受容体であるST2が蛋白レベル、mRNAレベルともに上昇しており、ST2陽性好酸球も増多していた。IL-33とST2が好酸球性副鼻腔炎の病態形成に重要な役割を果たしていることが示唆された。

F. 健康危険情報

なし

G. 研究発表

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H．知的財産権の出願・登録状況(予定を含む)

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

Ⅳ. 研究結果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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V. 研究成果の刊行物・別冊

Excessive Fibrin Deposition in Nasal Polyps Caused by Fibrinolytic Impairment through Reduction of Tissue Plasminogen Activator Expression

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Rationale: Nasal polyps (NPs) are characterized by intense edema or formation of pseudocysts filled with plasma proteins, mainly albumin. However, the mechanisms underlying NP retention of plasma proteins in their submucosa remain unclear.

Objectives: We hypothesized that formation of a fibrin mesh retains plasma proteins in NPs. We assessed the fibrin deposition and expression of the components of the fibrinolytic system in patients with chronic rhinosinusitis (CRS).

Methods: We assessed fibrin deposition in nasal tissue from patients with CRS and control subjects by means of immunofluorescence. Fibrinolytic components, d-dimer, and plasminogen activators were measured using ELISA, real-time PCR, and immunohistochemistry. We also performed gene expression and protein quantification analysis in cultured airway epithelial cells.

Measurements and Main Results: Immunofluorescence data showed profound fibrin deposition in NP compared with uncinate tissue (UT) from patients with CRS and control subjects. Levels of the cross-linked fibrin cleavage product protein, d-dimer, were significantly decreased in NP compared with UT from patients with CRS and control subjects, suggesting reduced fibrinolysis ($P < 0.05$). Expression levels of tissue plasminogen activator (t-PA) mRNA and protein were significantly decreased in NP compared with UT from patients with CRS and control subjects ($P < 0.01$). Immunohistochemistry demonstrated clear reduction of t-PA in NP, primarily in the epithelium and glands. Th2 cytokine–stimulated cultured airway epithelial cells showed down-regulation of t-PA, suggesting a potential Th2 mechanism in NP.

Conclusions: A Th2-mediated reduction of t-PA might lead to excessive fibrin deposition in the submucosa of NP, which might contribute to the tissue remodeling and pathogenesis of CRS with nasal polyps.

Keywords: chronic rhinosinusitis; nasal polyps; tissue plasminogen activator; fibrin; fibrinolysis

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Management of patients with chronic rhinosinusitis with nasal polyps (CRSwNP) is unsatisfactory, and frequent recurrences occur despite medical treatment and surgical interventions. It is well known that intense edema and pseudocyst formation are major histopathological characteristics of nasal polyps (NPs), which are infiltrated with plasma proteins. However, the mechanisms by which NPs retain plasma proteins in their stroma remain unclear.

What This Study Adds to the Field

We demonstrate an impairment of fibrin degradation caused by reduction of tissue plasminogen activator and consequent abnormal fibrin deposition in NPs. Abnormal fibrin deposition might be involved in the formation of intense edema or pseudocysts in NPs. Excessive fibrin deposition resulting from reduced fibrinolysis may reflect Th2 inflammatory responses and may have a pathogenic role in CRSwNP. Stimulation of degradation of fibrin might have value as a therapeutic strategy for treating CRSwNP.

Chronic rhinosinusitis (CRS) is characterized by persistent symptomatic inflammation of nasal mucosa and is one of the most common chronic diseases in adults in the United States (1–3). The etiology and pathogenesis of CRS remain controversial; however, allergies, bacterial and fungal infections, and structural abnormalities have all been theorized to play a role (4). CRS is typically classified into CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). Sinonasal tissue from patients with CRSsNP displays a predominant infiltration of neutrophils, whereas CRSwNP tissue is characterized by more intense eosinophilic infiltration and a Th2-based cytokine profile (5). Management of patients with CRSwNP is still unsatisfactory, and symptoms can persist despite medical treatment and surgical intervention (3).

Nasal polyps (NPs) usually present as edematous masses originating in and around the middle nasal meatus or paranasal sinuses. The major histopathological characteristics of NPs are an infiltration by inflammatory cells, intense edematous stroma, and the formation of pseudocysts. It has been reported that the storage of albumin within the edema of pseudocysts determines the growth and size of NPs (6). However, plasma exudation may not readily induce edema but may rather pass through the airway epithelial layer (7). The mechanisms by which NPs retain plasma proteins in their stroma remain unclear.

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Fibrin is the major protein constituent of blood clots as a consequence of activation of the coagulation cascade. In inflamed tissue, vessel permeability is increased, resulting in the leakage of plasma proteins into the extravascular compartment. Much of the extravagated fibrinogen can be rapidly converted to fibrin. Activation of coagulation and fibrin deposition as a consequence of tissue inflammation are fundamental for host defense to confine infections and for repair processes (8). However, the proinflammatory effects of fibrin or the failure to degrade deposited fibrin may play an etiologic role in many diseases, including rheumatoid arthritis, multiple sclerosis, status asthmaticus, adult respiratory distress syndrome, and ligneous conjunctivitis (8–12).

The serine protease plasmin is responsible for the degradation of crosslinked fibrin (i.e., fibrinolysis). Plasmin is generated through cleavage of the proenzyme plasminogen by two physiological plasminogen activators, urokinase plasminogen activator (u-PA) and tissue plasminogen activator (t-PA). The activity of u-PA and t-PA is inhibited by plasminogen activator inhibitor-1 (PAI-1) (13).

We hypothesized that fibrin deposition as a consequence of inflammation retains exuded plasma proteins such as albumin, facilitating formation of intense edema and pseudocysts in NPs. To test this hypothesis, we investigated fibrin deposition and the expression of fibrinolytic components in sinonasal tissue from subjects with CRS. The results provide important new evidence suggesting that excessive fibrin deposition resulting from reduced fibrinolysis occurs in NP tissue. We have also discovered important differences in the fibrinolytic cascade between uncinate tissue (UT) and inferior turbinate tissue (IT).

METHODS

Patients

Patients with CRS were recruited from the Allergy immunology and Otolaryngology Clinics of the Northwestern Medical Faculty Foundation (NMFF) and the Northwestern Sinus Center at NMFF. Sinonasal and NP tissues were obtained from routine functional endoscopic sinus surgery in patients with CRS. All subjects met the criteria for CRS as defined by American Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force (1, 14). Details of the subjects' characteristics are included in Table 1. All subjects gave informed consent, and the protocol and consent forms governing procedures for study have been approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine. Further details are provided in the online supplement.

Immunohistochemistry

Immunohistochemistry was performed as described previously (15). Briefly, blocked sections were incubated with antihuman fibrin antibody (Sekisui Diagnostics, Stamford, CT) or antihuman t-PA antibody (Sigma, St. Louis, MO) at 4°C overnight. Details of the methods for immunofluorescence and immunohistochemistry are provided in the online supplement.

Real-Time PCR

Total RNA was extracted using NucleoSpin RNA II (Macherey-Nagel, Bethlehem, PA) and was treated with DNase I. Single-strand cDNA was synthesized with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). Real-time RT-PCR was performed with a TaqMan method as described previously (16). Further details are provided in the online supplement.

ELISA

The plasminogen activators u-PA and t-PA (Assaypro, St. Charles, MO), eosinophilic cationic protein (ECP) (MBL, Woburn, MA), and d-dimer (Diagnostica Stago, Asnieres-Sur-Seine, France) were assayed with specific ELISA kits as detailed in the online supplement.

Cell Culture

The methods for culture of primary normal human bronchial epithelial (NHBE) cells are detailed in the online supplement.

Statistical Analysis

All data are reported as mean ± SEM unless otherwise noted. Differences between groups were analyzed with the Kruskal-Wallis ANOVA with Dunnett *post hoc* testing and Mann-Whitney U test. Correlations were assessed by using the Spearman rank correlation. A P value of less than 0.05 was considered statistically significant.

RESULTS

Fibrin Deposition in Patients with CRS

Sinonasal and polyp tissues were collected from 126 subjects with CRSsNP, 156 subjects with CRSwNP, and 73 control subjects to

TABLE 1. SUBJECT CHARACTERISTICS

	Control (n = 73)	CRSsNP (n = 126)	CRSwNP (n = 156)	CRSwNP Polyp
Male/female	36/37	50/76	92/64	—
Age, yr, median (range)	43 (16–78)	36 (18–73)	45 (22–74)	—
Atopy				
Yes	4	51	73	—
No	49	55	52	—
Unknown	20	20	31	—
Asthma				
Yes	0	16	66	—
No	67	101	84	—
Unknown	6	9	6	—
Methodology used				
Tissue RNA, n (M/F)	16 (7/9)	27 (8/19)	33 (21/12)	33 (22/12)
Age, yr, median (range)	45 (16–62)	35 (20–59)	38 (23–67)	39 (23–67)
Tissue extract, n (M/F)	31 (16/15)	64 (21/43)	61 (39/22)	55 (34/21)
Age, yr, median (range)	45 (19–72)	36 (18–73)	44 (26–73)	45 (26–73)
Immunohistochemistry, n (M/F)	14 (5/9)	18 (8/10)	16 (9/7)	17 (11/6)
Age, yr, median (range)	43 (19–64)	43 (24–70)	52 (33–64)	50 (27–74)
Nasal lavage, n (M/F)	36 (20/16)	49 (22/27)	48 (35/13)	—
Age, yr, median (range)	42 (18–78)	36 (18–73)	45 (29–72)	—

Definition of abbreviations: CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps; F = female; M = male.

determine the fibrin deposition and the expression of fibrinolytic components in patients with CRS. Subjects' characteristics are shown in Table 1.

To evaluate the fibrin deposition in nasal mucosa, we performed immunofluorescence of surgical samples from control subjects and patients with CRS. Only a small amount of fibrin was seen in UT from control subjects or patients with CRSsNP, and a moderate level of fibrin staining was seen in UT from patients with CRSwNP (Figures 1A–1C); intense staining of fibrin was found in submucosa of NP from patients with CRSwNP (Figure 1D). Cellular staining was graded by blinded observers for intensity, as described in the online supplement. This semiquantitative analysis showed significantly more intense fibrin staining in NP from patients with CRSwNP compared with staining seen in control subjects or in UT from patients with CRSsNP ($P < 0.01$) (Figure 1F). We observed similar results using Masson's Trichrome stain, which highlights fibrin as a pink color (*see* Figure E1 in the online supplement). In addition, NP had much less collagen (blue color), which confirms a previous report (Figure E1) (17).

Extravascular fibrin is ordinarily degraded to fibrin degradation products (FDPs) by plasmin to prevent excessive fibrin deposition (18). To assess the levels of FDPs in nasal tissue, we measured the levels of d-dimer, which is an important FDP. d-Dimer protein levels were significantly decreased in NP from patients with CRSwNP ($P < 0.05$) in comparison with levels in UT from patients with CRS or control subjects (Figure 2). Taken together, these findings suggest the presence of excessive fibrin deposition associated with reduced fibrin degradation in NP.

The Expression of Plasminogen Activators in Patients with CRS

Fibrin is cleaved by plasmin, which is generated from plasminogen by two plasminogen activators, u-PA and t-PA. We therefore assessed the expression of u-PA and t-PA in UT from patients with CRSsNP or CRSwNP and from control subjects as well as in NP from patients with CRSwNP. Although the expression of mRNA for u-PA was not different among the four groups (Figure 3A), t-PA mRNA levels were significantly decreased in NP tissues from patients with CRSwNP ($P < 0.01$) in comparison with UT from patients with CRS or control subjects (Figure 3B). To confirm this observation at the protein level, we made detergent extracts from homogenates of UT and NP tissues and measured the concentration of u-PA and t-PA by ELISA. In agreement with the mRNA data, although u-PA protein levels were not different among the four groups (Figure 3C), t-PA protein levels were significantly decreased in NP from patients with CRSwNP ($P < 0.01$) in comparison with UT from patients with CRS or control subjects (Figure 3D). Tissue plasminogen activator activity was also significantly decreased in NP ($P < 0.01$) (Figure E2). Together, these results show clear reduction of t-PA mRNA, protein, and activity and suggest that the fibrinolytic pathway is severely compromised in NP tissue.

Immunohistochemical Analysis of Plasminogen Activators in Sinonasal Tissue

To further characterize the expression of plasminogen activator proteins in patients with CRS, we performed immunohistochemical analysis of surgical samples from control subjects and patients with CRS to determine whether t-PA expression could be detected. We detected t-PA staining in glands and in mucosal epithelium and endothelium in tissues (Figure 4). Consistent with ELISA data, t-PA staining in glandular and mucosal epithelium of control tissue (Figures 4C and 4D) was more intense when compared with that seen in NP (Figures 4I and 4J and *see* Table E1 in the online supplement) in patients with CRSwNP.

Comparison of Plasminogen Activator Expression between UT and IT

NPs are known to arise from nasal and paranasal sinus mucosa that are mainly situated in the middle nasal meatus but rarely arise from the inferior turbinate (6). We therefore examined the expression level of plasminogen activators between UT and IT from control subjects and patients with CRS using ELISA.

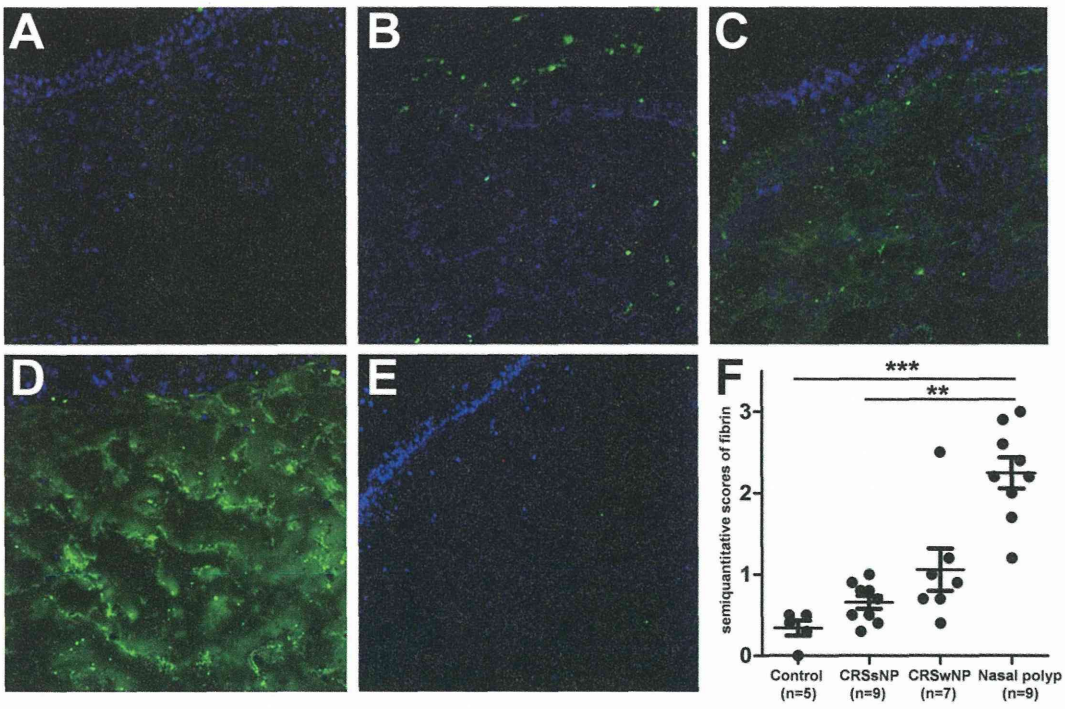

Figure 1. Immunofluorescence of fibrin in nasal tissues. Immunofluorescence was performed with antifibrin (*green fluorescence*). (*A–D*) Representative immunostaining for fibrin in uncinate tissue (UT) from a control subject (*A*), a patient with chronic rhinosinusitis without nasal polyps (CRSsNP) (*B*), a patient with chronic rhinosinusitis with nasal polyps (CRSwNP) (*C*), and nasal polyp (NP) tissue (*D*). (*E*) Negative control antibody staining in NPs from a patient with CRSwNP. (*F*) Semiquantitative analysis of fibrin in UT from control subjects (n = 5), patients with CRSsNP (n = 9), and patients with CRSwNP (n = 7) and NPs (n = 9) was performed. Magnification: ×400. $^{**}P < 0.01$; $^{***}P < 0.001$.