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.

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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.

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 $\pm$ 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 uncinat 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: $\times 400$. ** $P < 0.01$; *** $P < 0.001$.

Figure 2. d-Dimer levels were decreased in nasal polyp tissue. Measurement of d-dimer in tissue homogenates of uncinat tissue from control subjects, from patients with chronic rhinosinusitis without nasal polyps (CRSsNP), from patients with chronic rhinosinusitis without nasal polyps (CRSwNP), and in nasal polyps using ELISA. d-Dimer concentration was normalized to the concentration of total protein. * $P < 0.05$; ** $P < 0.01$.

u-PA protein levels were significantly lower in UT in comparison with those in IT from control subjects ($P < 0.05$), patients with CRSsNP ($P < 0.001$), or patients with CRSwNP ($P < 0.05$) (Figure 5A). t-PA protein levels were also significantly lower in UT in comparison with those seen in IT from patients with CRSsNP ($P < 0.001$) or patients with CRSwNP ($P < 0.01$) (Figure 5B). Although not statistically significant, t-PA protein levels were also lower in UT from control subjects ($P = 0.068$) compared with IT from control subjects (Figure 5B). These results suggest that the overall fibrinolytic capacity is higher in the inferior turbinate than in the uncinat, and we speculate that low expression of both plasminogen activators in UT might confer susceptibility to fibrin deposition and polyp formation in this region due to reduced capacity for fibrin degradation.

Th2 Cytokines Down-Regulate the t-PA Expression in NHBE Cells

NP from patients with CRSwNP have long been known to be characterized by Th2-dominant eosinophilic inflammation (19). We examined whether levels of plasminogen activators correlated with eosinophilic inflammation in nasal tissues. We assayed the levels of ECP as a marker for the presence of eosinophils in nasal tissue. The concentration of t-PA in UT and NP was significantly negatively correlated with the concentration of ECP ($r = -0.5395$; $P < 0.0001$) (Figure 6A); however, the concentration of u-PA in nasal tissue did not correlate with the concentration of ECP (data not shown). Immunohistochemistry data demonstrated that t-PA staining was mainly observed in glandular and mucosal epithelium in nasal tissue (Figure 4). Therefore, to assess the t-PA mRNA level in epithelium, we used nasal scraping-derived epithelial cells. Although not statistically significant, as shown in immunohistochemistry, t-PA mRNA levels were decreased in epithelial scraping cells from NP ($P = 0.063$) compared with levels in UT from control subjects (Figure 6B). Given that expression of t-PA was reduced in nasal tissue and negatively correlated with ECP, we hypothesized that Th2 cytokines might regulate t-PA expression in airway epithelial cells. To study the regulation of plasminogen activators in airway epithelial cells, primary NHBE cells were stimulated with Th2 cytokines, IL-4, or IL-13 for 24 hours. Although the levels of u-PA mRNA were not altered by Th2 cytokine stimulation (Figure 6C), the levels of t-PA mRNA were significantly down-regulated by both Th2 cytokines in a dose-dependent manner (Figure 6D). To confirm this observation at the protein level, we made cell lysate of NHBE cells and measured the concentration of plasminogen activators using ELISA. Although the levels of u-PA protein were not altered by Th2 cytokine stimulation (Figure 6E), the levels of t-PA protein were significantly down-regulated by both Th2 cytokines (Figure 6F). We also observed that stimulation with Th2 cytokines down-regulated t-PA expression in primary nasal epithelial cells (Figure E4). This result suggests that Th2 cytokines

Figure 3. Expression of plasminogen activators in nasal tissues. Total RNA was extracted from uncinat tissue and nasal polyps, and expression of urokinase plasminogen activator (u-PA) (A) and t-PA (B) was analyzed using real-time PCR. Expression of u-PA (C) and t-PA (D) protein in tissue homogenates of uncinat tissue and nasal polyps was measured using ELISA. The concentration of plasminogen activators was normalized to the concentration of total protein. ** $P < 0.01$; *** $P < 0.001$.

Figure 4. Immunohistochemical staining for tissue plasminogen activator (t-PA) in representative tissue samples from unciniate tissue (UT) and nasal polyps (NPs). (A, B) Negative control of UT from a control subject did not stain. (C–H) t-PA staining of UT from control subject (C, D) showed intense staining in epithelial and glandular tissue, whereas light-to-moderate staining of t-PA was seen in UT from a patient with chronic rhinosinusitis without NPs (E, F) and a patient with chronic rhinosinusitis with NPs (G, H). (I, J) Less staining was seen in NP tissue. Magnification: $\times 400$.

down-regulate expression of t-PA but not u-PA in airway epithelial cells.

DISCUSSION

It is well known that intense edema and pseudocyst formation are major histopathological characteristics of NP tissues, which are infiltrated with plasma proteins, mainly albumin (6). In spite of the presence of considerable albumin in the stroma of NP, the levels of albumin were not increased in nasal lavage from

patients with CRSwNP compared with albumin levels in control subjects or patients with CRSsNP (Figure E3). The mechanism by which NP tissue retains plasma proteins in the stroma has not been explored. The current study demonstrates for the first time that fibrin deposition is profoundly increased in NP from patients with CRSwNP in comparison with that seen in UT from patients with CRS or control subjects (Figure 1). We also found that although there is a great deal of fibrin deposition, d-dimer, a major fibrin degradation product, was significantly decreased in NP compared with UT in the three groups of subjects (Figure 2). These results indicate that excessive fibrin deposition in NP might be caused by a disorder of fibrin degradation. Because fibrin degradation is facilitated by plasmin, which is generated through cleavage of plasminogen by u-PA and t-PA, we examined the levels of these two plasminogen activators. The levels of t-PA, but not u-PA, were significantly decreased in patients with CRSwNP, especially in NP tissue (Figures 3B and 3D). t-PA promotes fibrinolysis by virtue of the presence of t-PA binding sites on fibrin strands, where plasminogen is also localized. It is therefore generally believed that t-PA acts as a central plasminogen activator for fibrinolysis (8). These results suggest that decreased levels of t-PA in NP tissue lead to a deceleration of the rate of conversion of plasminogen to plasmin, reducing fibrinolytic tone. In the face of plasma exudation, reduced degradation of fibrin would in turn facilitate excessive deposition of fibrin in NP. Fibrin deposition might also be involved in retention of albumin in NP stroma. An outline of this hypothetical model is given in Figure 7.

Fibrin, as the final product of the coagulation cascade, plays a major role in blood clotting. In addition, because components of the coagulation cascade reside in, or are transported to, tissues and can stimulate extravascular fibrin formation (20), fibrin deposition in response to inflammation can be integral to normal repair and restoration of tissues. This is believed to play a role in the confinement of microbial or toxic agents to a limited area and in the formation of provisional matrix for the influx of monocytes, fibroblasts, and endothelial cells (21, 22). However, disorder of fibrin turnover facilitates abnormal fibrin deposition and can be deleterious because of its proinflammatory properties (8, 23). Fibrin can directly stimulate expression of IL-1 β and TNF- α in mononuclear cells and can induce production of the chemokines CXCL8 and CCL2 by endothelial cells and fibroblasts, promoting the migration of leukocytes and macrophages (8, 24). Indeed, some evidence suggests that removal of fibrin can diminish disease development and symptoms (8, 25–28).

t-PA converts plasminogen into proteolytically active plasmin, which in turn degrades fibrin and other extracellular matrix proteins (8). In addition, t-PA facilitates the posttranslational activation of several growth factors, such as hepatocyte growth factor or transforming growth factor (TGF)- β via proteolysis, and TGF- β can induce endogenous t-PA expression in an autocrine manner (29, 30). We observed reduced collagen in NP (Figure E1D); other studies have reported that reduced collagen is seen in NP compared with control subjects as a consequence of decreased TGF- β (17). Taken together, the presence of low levels of t-PA and TGF- β provides a milieu for low collagen production in NP (Figure 7). Growing evidence suggests t-PA can act as a cytokine and binds to the cell membrane receptor low-density-lipoprotein receptor-related protein-1 (LRP-1). Independent of its proteolytic capacity, binding by t-PA to LRP-1 induces receptor tyrosine phosphorylation, triggers intracellular signal transduction, and induces collagen production by fibroblasts (30–33). We detected LRP-1 expression in nasal tissue by real-time PCR, and there was no significant difference between UT and NPs from control subjects and patients with CRS (data not shown). In normal wound healing

Figure 5. Comparison of plasminogen activator expression in uncinat tissue (UT) and turbinate tissue (IT). Expression of urokinase plasminogen activator (u-PA) (A) and tissue plasminogen activator (t-PA) (B) protein in tissue homogenates of UT, IT, and nasal polyps was measured using ELISA. The concentration of plasminogen activators was normalized to the concentration of total protein. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. CRSsNP = chronic rhinosinusitis without nasal polyps; CRSwNP = chronic rhinosinusitis with nasal polyps.

processes, the early deposition of fibrin matrix is replaced with collagen produced by fibroblasts, and inadequate removal of fibrin impedes this process (22). In this regard, low levels of t-PA/LRP-1 signaling might hinder fibrin removal and prolong inflammation in NP. In addition, recent studies suggest that

t-PA/LRP-1 pathways induce nitric oxide (NO) production in the central nervous system (34). Because it has been reported that the levels of NO were decreased in NP tissue (35), low levels of t-PA might be involved in down-regulation of NO in NP tissue (Figure 7).

Figure 6. Potential regulation of tissue plasminogen activator (t-PA) expression in epithelial cells by Th2 cytokines. The relationship of t-PA and eosinophilic cationic protein (ECP) in nasal tissue was evaluated using ELISA (open circles, control uncinat tissue [UT]; triangles, chronic rhinosinusitis without nasal polyps [NPs]; open squares, chronic rhinosinusitis with NPs UT; closed circles, NP). None of the individual groups produced a correlation between ECP and t-PA. The correlation shown was assessed using all values with the Spearman rank correlation test (A). Total RNA was extracted from epithelial scraping cells from UT and NPs, and expression of t-PA mRNA was analyzed with real-time PCR. The levels of t-PA were decreased in NPs ($P = 0.063$) compared with levels in UT from control subjects (B). Normal human bronchial epithelial cells were stimulated with 0.01 to 100 ng/ml IL-4 or IL-13 for 24 hours. The levels of urokinase plasminogen activator (u-PA) (C) and t-PA (D) mRNA were determined by real-time PCR. Concentrations of u-PA (E) and t-PA (F) protein in cell lysates from normal human bronchial epithelial cells were measured by ELISA. The concentration of plasminogen activators was normalized to the concentration of total protein. Results shown are mean $\pm$ SEM of six independent experiments (C–F). * $P < 0.05$; ** $P < 0.01$.

Figure 7. Hypothetical model to explain the role of tissue plasminogen activator (t-PA) in excessive fibrin deposition and reduced collagen in nasal polyps. As a protease, t-PA converts plasminogen to plasmin, which promotes fibrin degradation. As a cytokine, t-PA binds to its receptor lipoprotein receptor-related protein-1 (LRP-1), leading to collagen production and nitric oxide (NO) synthesis by fibroblast (A). In the presence of Th2 cytokines, t-PA levels are reduced, promoting fibrinogenesis. Reduced tissue levels of t-PA facilitate abnormal fibrin deposition and diminish collagen expression in nasal polyps (B). FDP = fibrin degradation product.

In the study of CRS, one of the most intriguing questions is “Why do NPs arise only from mucous membranes in and around the middle nasal meatus?” In the current study, we found that protein levels of u-PA and t-PA were lower in UT in comparison with those seen in IT in diseased samples and controls (Figure 5). This suggests that low levels of plasminogen activators might confer an increased susceptibility to excess fibrin deposition in UT and may provide an explanation of why NP arise from mucous membranes in and around the middle nasal meatus but not in the IT. In previous studies, we have found that IT and UT differ dramatically in levels of host defense molecules, so such a regional difference is not unprecedented (36, 37).

It is known that the activation of t-PA is tightly controlled by PAI-1, which directly binds t-PA and inactivates it. We observed that the levels of t-PA protein and the activity of t-PA were decreased in NP in comparison with UT from control subjects and patients with CRS (Figures 3 and E2). However, the levels of PAI-1 protein in NP were not elevated in comparison with control subjects and CRS samples (data not shown), suggesting that PAI-1 is not responsible for inactivation or reduction of t-PA in NP. The regulation of t-PA gene expression is not well described. t-PA is produced by a number of airway cells, including mast cells, macrophages, fibroblasts, endothelial cells, glandular cells, and epithelial cells (38, 39). Our immunohistochemistry data demonstrated that t-PA staining was most prominently

observed in epithelial and glandular cells in UT from control subjects. Recently, it has been reported that tenascin-C down-regulates t-PA expression resulting in abnormal fibrin deposition in a mouse model (40). In the present study, we found an up-regulation of tenascin-C mRNA in NPs in comparison with UT from control subjects and patients with CRS (data not shown), which is consistent with this previous report (41). However, we could not find any correlation between the expression of tenascin-C and t-PA at the mRNA level ($r = 0.182$; $P = \text{NS}$). Further studies are required to determine whether tenascin-C plays a role in the reduced t-PA we have observed in NPs.

Previous studies have demonstrated that NPs exhibit a high degree of tissue eosinophilia as well as T cells, demonstrating skewing toward Th2 cytokine expression (5, 19). We therefore examined the correlation of ECP as a marker of Th2 inflammation with t-PA protein levels in nasal tissue. We found a significant negative correlation between the protein levels of ECP and t-PA (Figure 6A). We have also shown here that NHBE cells constitutively express t-PA and that stimulation with the STAT6-activating Th2 cytokines IL-4 or IL-13 significantly down-regulated t-PA expression while leaving u-PA expression unaltered (Figures 6C–6F). These findings suggest that Th2-related inflammation in NPs might down-regulate the expression of t-PA and play a role in the induction of excessive fibrin deposition through suppression of fibrinolysis (Figure 7). Furthermore, the reduction in levels of t-PA might also be involved in reduction of collagen production in NPs by down-regulation of t-PA/LRP-1 signaling (Figure 7). Th2 immunity, which is generally associated with antiparasite responses, may use fibrin deposition in the pathways designed to impede the movement or growth of parasite worms in tissues.

Our findings suggest potential new strategies for advancing the treatment of NPs. If NP formation is due to excessive fibrin deposition caused by down-regulation of t-PA, it may be feasible to diminish NP formation by administration of t-PA or activators of t-PA or administration of inhibitors of fibrinogenesis. Although in this study we did not assess the coagulation status in NPs, a recent study demonstrated that thrombin, a central component of the coagulation cascade, was up-regulated in NPs (42). Thus, the coagulation cascade might be involved in excessive fibrin deposition in NPs, interacting with the reduced fibrinolytic properties of the tissue that we describe herein. Future studies are required to determine the relationship between coagulation and NP development.

In summary, we report here that excessive fibrin deposition and low levels of d-dimer are observed in NP tissue from patients with CRSwNP. Tissue levels of t-PA were profoundly decreased in NPs, suggesting that down-regulation of t-PA may lead to insufficient fibrin degradation resulting in fibrin deposition. Furthermore, the constitutive levels of protein for both plasminogen activators were very low in UT in comparison with IT, suggesting that low levels of fibrinolysis in UT may lead to a particular susceptibility for fibrin deposition in the ethmoid sinus. This difference of fibrinolytic capacity might be one reason that NPs almost exclusively arise in the proximity of the middle nasal meatus. Our findings indicate that profound fibrin deposition might be involved in the retention of plasma proteins and the formation of the apparent tissue remodeling, intense edema, or pseudocysts in NP tissue and provide potential new targets for novel therapeutic approaches to CRSwNP.

Author disclosures are available with the text of this article at www.atsjournals.org.

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