Figure 2. d-Dimer levels were decreased in nasal polyp tissue. Measurement of d-dimer in tissue homogenates of uncinate 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 uncinate, 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
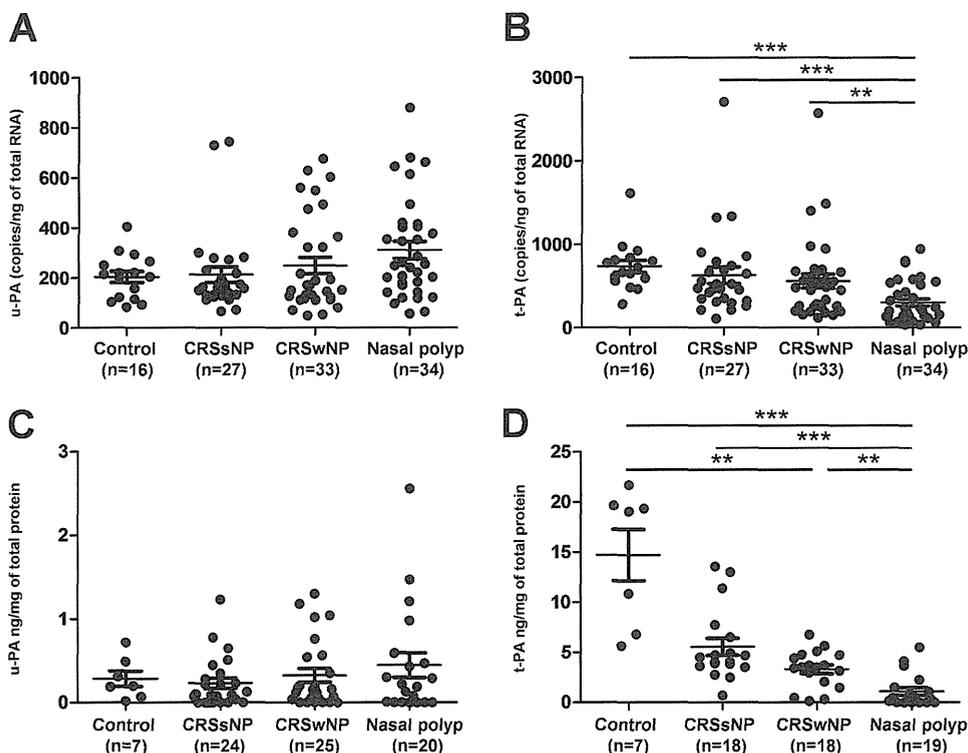

Figure 3. Expression of plasminogen activators in nasal tissues. Total RNA was extracted from uncinate tissue and nasal polyps, and expression of urokinase plasminogen activator (u-PA) (*A*) and t-PA (*B*) was analyzed using real-time PCR. Expression of u-PA (*C*) and t-PA (*D*) protein in tissue homogenates of uncinate tissue and nasal polyps was measured using ELISA. The concentration of plasminogen activators was normalized to the concentration of total protein. **$P < 0.01$; ***$P < 0.001$.
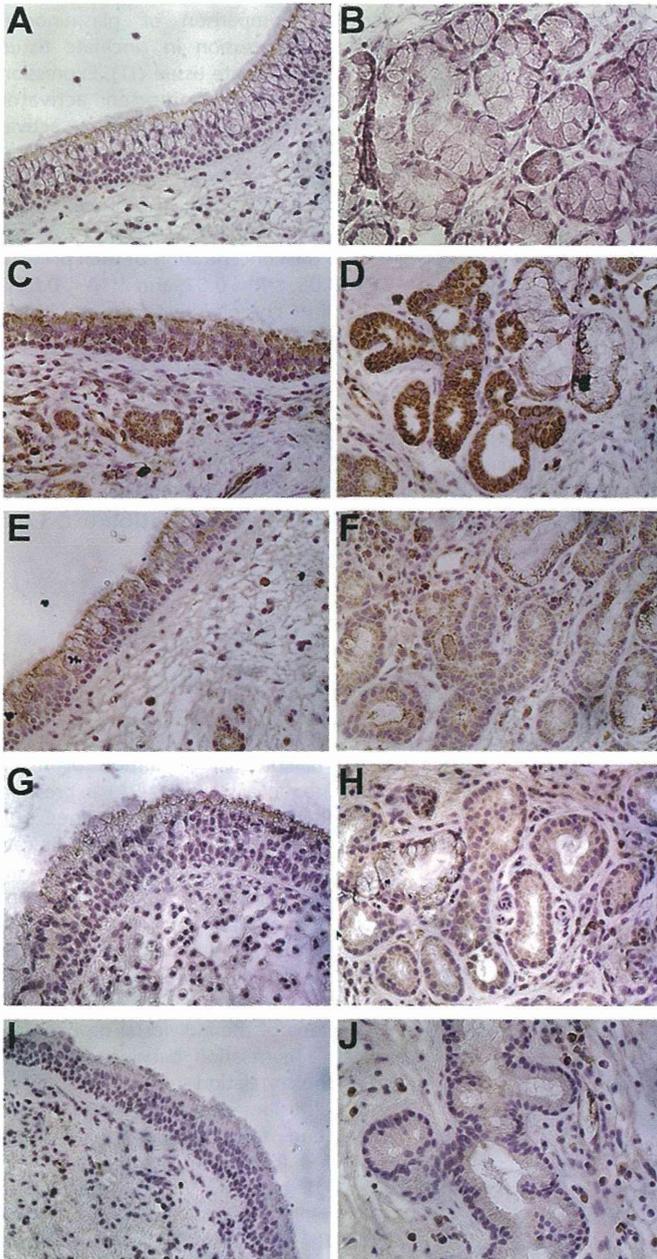
Figure 4. Immunohistochemical staining for tissue plasminogen activator (t-PA) in representative tissue samples from uncinate tissue (UT) and nasal polyps (NPs). (*A, B*) Negative control of UT from a control subject did not stain. (*C–H*) t-PA staining of UT from control subject (*C, D*) showed intense 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: ×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-1β 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
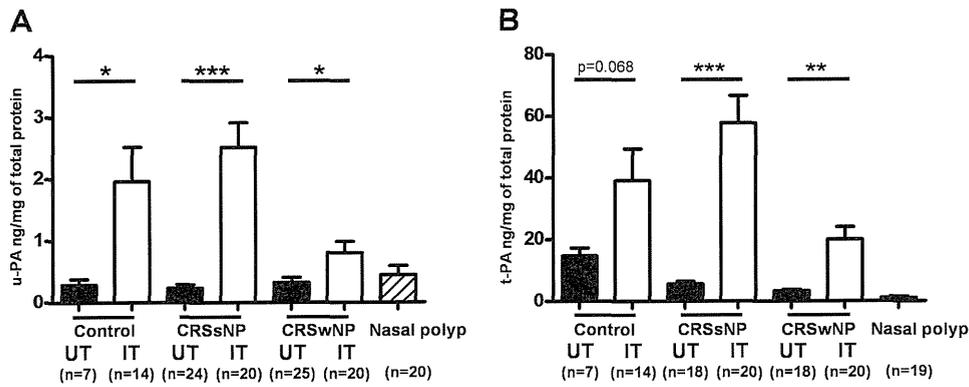
A

B

Figure 5. Comparison of plasminogen activator expression in uncinate 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).
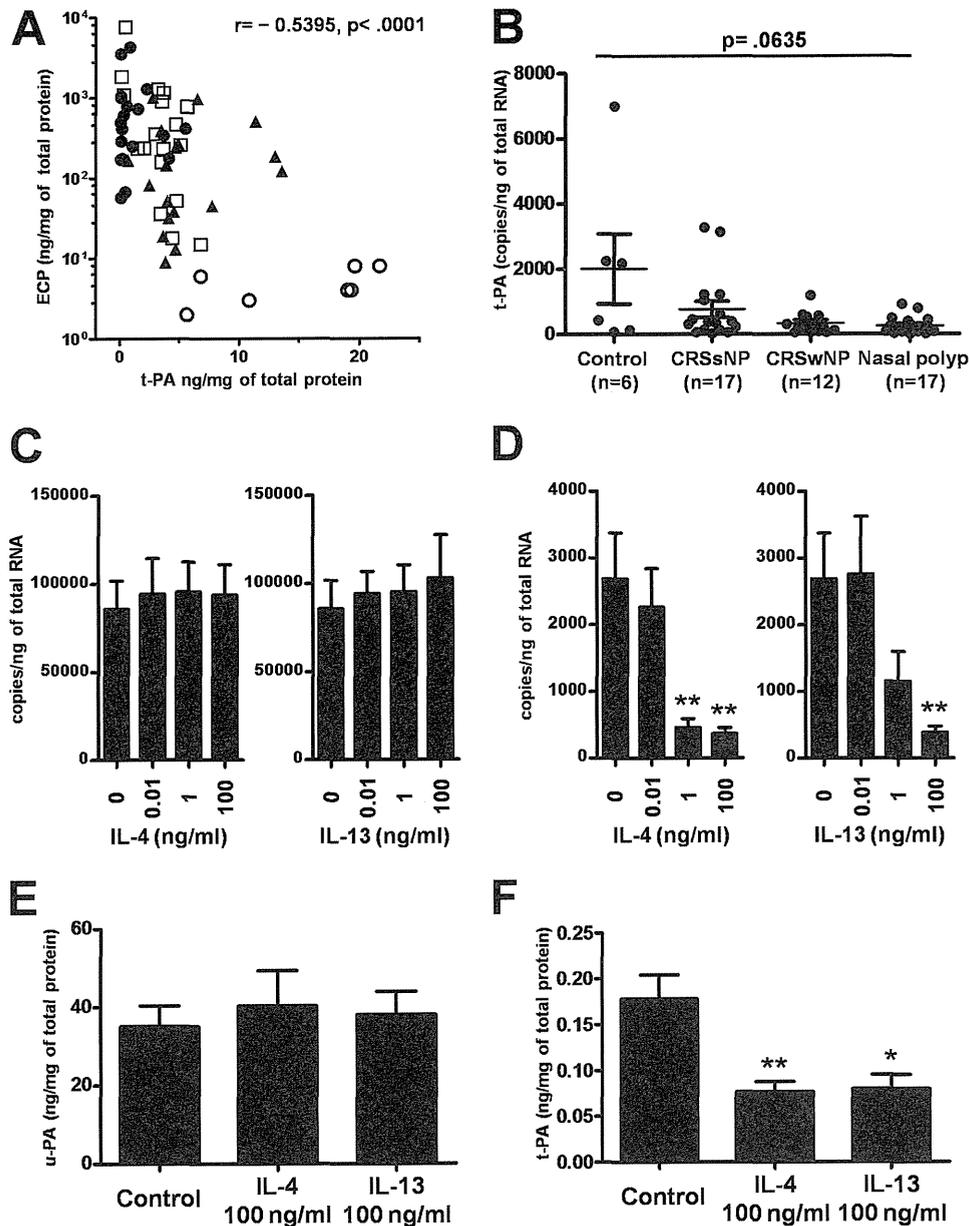
A

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C

D

E

F

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 uncinate tissue [UT]; *triangles*, chronic rhinosinusitis without nasal polyps [NPs] UT; *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 ± SEM of six independent experiments (*C–F*). *P < 0.05; **P < 0.01.
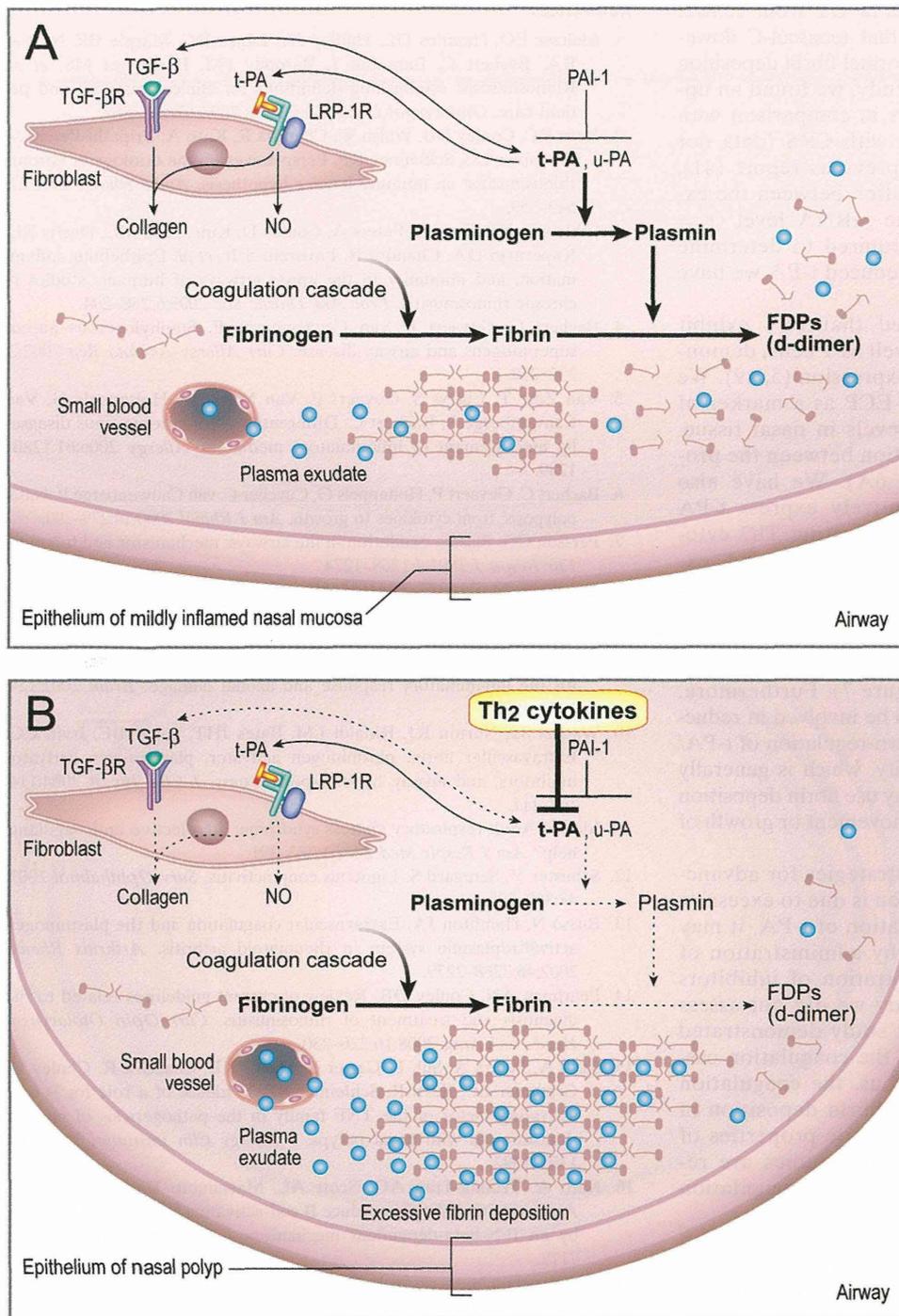
Figure 7. Hypothetical model to explain the role of tissue plasminogen activator (t-PA) in excessive fibrin deposition and reduced collagen in nasal polyps. As a protease, t-PA converts plasminogen to plasmin, which promotes fibrin degradation. As a cytokine, t-PA binds to its receptor lipoprotein receptor–related 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 = $ 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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Increased expression of factor XIII-A in patients with chronic rhinosinusitis with nasal polyps

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Background: Profound edema or formation of a pseudocyst containing plasma proteins is a prominent characteristic of nasal polyps (NP). However, the mechanisms underlying NP retention of plasma proteins in the submucosa remain unclear. Recently, we reported that impairment of fibrinolysis causes excessive fibrin deposition in NP and this might be involved in the retention of plasma proteins. Although the coagulation cascade plays a critical role in fibrin clot formation at extravascular sites, the expression and role of coagulation factors in NP remain unclear.
Objective: The objective of this study was to investigate the expression of coagulation factors in patients with chronic rhinosinusitis (CRS).
Methods: Sinonasal tissues were collected from patients with CRS and control subjects. We assayed mRNA for factor XIII-A (FXIII-A) by using real-time PCR and measured FXIII-A protein by means of ELISA, immunohistochemistry, and immunofluorescence.
Results: FXIII-A mRNA levels were significantly increased in NP tissue from patients with CRS with NP ($P < .001$) compared with uncinate tissue from patients with CRS or control subjects. Similarly, FXIII-A protein levels were increased in NP. Immunofluorescence analysis revealed that FXIII-A expression in inflammatory cells and FXIII-A^{+} cell numbers were

significantly increased in NP. Most FXIII-A staining was observed within CD68^{+}/CD163^{+} M2 macrophages in NP. Levels of FXIII-A correlated with markers of M2 macrophages, suggesting that M2 macrophages are major FXIIIA-producing cells in NP.
Conclusion: Overproduction of FXIII-A by M2 macrophages might contribute to the excessive fibrin deposition in the submucosa of NP, which might contribute to the tissue remodeling and pathogenesis of CRS with NP. (J Allergy Clin Immunol 2013;132:584-92.)

Key words: Chronic rhinosinusitis, nasal polyps, factor XIII-A (FXIII-A), M2 macrophages, fibrin, coagulation cascade

Chronic rhinosinusitis (CRS) is a heterogeneous disease characterized by local inflammation of the upper airways and sinuses, with symptoms lasting longer than 12 weeks despite medical management. CRS is one of the most common chronic diseases in adults in the United States and affects up to 15% of the population.[1-4] Primarily on the basis of physical examination, histology, and clinical course, CRS is typically classified into 2 types: CRS with nasal polyps (CRSwNP) and CRS without nasal polyps (CRSsNP). The etiology and pathogenesis of CRS remain controversial; however, allergy, bacterial and fungal infections, and structural abnormalities have all been theorized to play a role.[5] In general, CRSwNP is associated more closely with clinical complaints of nasal obstruction and olfactory loss, and more frequently linked to comorbidities such as asthma and aspirin hypersensitivity. Sinonasal tissue from patients with CRSsNP displays a predominant infiltration of neutrophils and presence of T_H1 cytokines, whereas CRSwNP tissue is characterized by more intense eosinophilic infiltration and a T_H2-based cytokine profile.[6]

Nasal polyps (NP) usually present as edematous masses originating in and around the middle nasal meatus or paranasal sinuses. Histologically, NP are characterized by an infiltration by inflammatory cells, predominantly eosinophils, intense edematous stroma, and the formation of pseudocysts filled with plasma proteins, mainly albumin.[7] Profound inflammation causes plasma exudation from capillaries; however, the exuded plasma may not only induce edema but also pass through the airway epithelial layer.[8] Antigen stimulation of the nasal cavity of patients with seasonal nasal allergy induces the influx of plasma proteins into the nasal lumen, as detected in nasal lavage.[9] The mechanism responsible for the retention of exuded plasma proteins in NP submucosa is not fully understood. Recently, we reported that

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Abbreviations used
CRS: Chronic rhinosinusitis
CRSsNP: CRS without nasal polyps
CRSwNP: CRS with nasal polyps
FXIII-A: Factor XIII-A
FXIII-B: Factor XIII-B
MMR: Macrophage mannose receptor
NP: Nasal polyps
STAB1: Stabilin 1
t-PA: Tissue plasminogen activator
UT: Uncinate tissue

excessive fibrin deposition and low levels of d-dimer, a fibrin degradation product, were observed in NP tissue from patients with CRSwNP. Our study suggested that downregulation of tissue plasminogen activator (t-PA) in NP may lead to insufficient fibrin degradation by plasmin, because t-PA converts plasminogen to plasmin and low levels of plasmin cause this excessive fibrin deposition, which would be expected to contribute to the retention of plasma proteins in NP tissue.[10]

Activation of the coagulation cascade and deposition of fibrin as a consequence of inflammation is well known, and is thought to play a critical role in host defense and to be involved in containing microbial or toxic agents.[11] However, dysregulation of the coagulation cascade may play an etiologic role in many diseases through excessive fibrin deposition, including rheumatoid arthritis, severe asthma, glomerulonephritis, delayed-type hypersensitivity, and Crohn disease.[12-16]

In this study, we hypothesized that activation of coagulation factors is involved in excessive fibrin deposition in NP, leading to retention of plasma proteins and formation of intense edema and pseudocysts in NP. We sought to investigate the expression of coagulation factors in sinonasal tissue from subjects with CRS. We found that factor XIII-A (FXIII-A) levels were upregulated in patients with CRSwNP and examined the cell types that produce this coagulation factor.

METHODS
Patients and biopsy specimens

Patients with CRS were recruited from the Allergy-Immunology and Otolaryngology Clinics of the Northwestern Medical Faculty Foundation and the Northwestern Sinus Center at the Northwestern Medical Faculty Foundation. Sinonasal and NP tissues were obtained from routine functional endoscopic sinus surgery in patients with CRS. All patients met the criteria for CRS, as defined by the American Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force.[1,17] Patients with an established immunodeficiency, pregnancy, coagulation disorder, diagnosis of classic allergic fungal sinusitis, Samter's triad, Churg-Strauss syndrome, or cystic fibrosis did not participate in the study. Details of subjects' characteristics are included in Table I and in this article's Methods section in the Online Repository at www.jacionline.org. All subjects signed informed consent, and the protocol and consent forms governing procedures for the study were approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine.

Microarray and real-time PCR

Total RNA from sinus tissue was extracted with QIAzol (Qiagen, Valencia, Calif), and the quality of total RNA from sinus tissue was assessed with 2100 Bioanalyzer (Agilent Technologies, Santa Clara, Calif). A comprehensive

microarray was performed as described previously, and gene expression was measured with GeneChip Human U133 Plus 2.0 probe arrays (Affymetrix, Santa Clara, Calif).[18,19] Real-time RT-PCR was performed with a TaqMan method, as described previously.[20] Detailed protocols are found in this article's Methods section in the Online Repository. All microarray data have been deposited to gene expression omnibus: GSE36830.

ELISA

The concentration of FXIII-A in cell-free supernatant was determined by using a specific ELISA kit (HYPHEN BioMed, Neuville-Sur-Oise, France). Details are provided in this article's Methods section in the Online Repository.

Immunohistochemistry

Immunohistochemistry was performed as described previously.[21] Briefly, blocked sections were incubated with anti-human FXIII-A antibody (CELL MARQUE, Rocklin, Calif) at 4°C overnight. After washing, sections were incubated in ABC reagent (Vector Laboratories, Burlingame, Calif) for 1 hour. Sections were rinsed and incubated in DAB reagent (Invitrogen, Carlsbad, Calif) and then counterstained with hematoxylin. Slides were blinded, and 10 photographic fields were randomly taken from each slide. The number of FXIII-A$^+$ cells in the nasal mucosa was counted by a blinded observer. Details of the methods for immunofluorescence and immunohistochemistry are described in this article's Methods section in the Online Repository.

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. Correlations were assessed by using the Spearman rank correlation. A *P* value of less than .05 was considered statistically significant.

RESULTS
Screen of the coagulation factors in sinonasal tissue

We analyzed data from a previously performed microarray analysis to compare coagulation factor gene expression in uncinate tissue (UT) from patients with CRSsNP, patients with CRSwNP, and control subjects, as well as in NP tissue from patients with CRSwNP.[19] We observed that mRNA levels of the FXIII-A were substantially increased in NP tissues from patients with CRSwNP in comparison with levels seen in UT from either patients with CRS or control subjects (see Fig E1 in this article's Online Repository at www.jacionline.org). Interestingly, we found no difference between patients with CRS and control subjects in UT mRNA levels of factor XIII-B (FXIII-B), a subunit that forms a tight tetrameric complex with FXIII-A (FXIII-A$_2$B$_2$) in the plasma (Fig E1).

FXIII-A expression in patients with CRS

Sinonasal and polyp tissues were collected from 56 subjects with CRSsNP, 95 subjects with CRSwNP, and 35 control subjects to determine the presence of FXIII-A expression in patients with CRS. Subjects' characteristics are shown in Table I.

We further assessed the expression of FXIII-A in UT from patients with CRSsNP, patients with CRSwNP, and control subjects, as well as in NP tissue from patients with CRSwNP by using real-time PCR. FXIII-A mRNA levels were significantly increased in NP tissues from patients with CRSwNP ($P < .001$) in comparison with levels seen in UT from either patients with CRS or control subjects (Fig 1, *A*). To confirm this observation at the protein level, we made detergent extracts from homogenates of UT and NP tissues and then measured the concentration

TABLE I. Subjects' characteristics

	Control			CRSsNP			CRSwNP			CRSwNP polyp
Total no. of subjects	n = 35 (14M/21F)			n = 56 (22M/34F)			n = 95 (56M/39F)			—
Age (y), median (range)	49 (16-72)			36 (20-73)			40 (23-72)			—
	Y	N	U	Y	N	U	Y	N	U	–
Atopy	1	32	S	19	29	8	47	26	22	—
Asthma	0	35	0	5	48	3	45	47	3	—
Methodology used:										
Tissue RNA	n = 16 (7M/9F)			n = 27 (8M/19F)			n = 33 (21M/12F)			n = 34 (22M/12F)
Age (y), median (range)	45 (16-62)			35 (20-59)			38 (23-67)			39 (23-67)
Tissue extract	n = 14 (5M/9F)			n = 20 (8M/12F)			n = 19 (10M/9F)			n = 24 (13M/11F)
Age (y), median (range)	46 (35-72)			30 (24-73)			45 (26-68)			44 (28-72)
Immunohistochemistry	n = 10 (3M/7F)			n = 11 (5M/6F)			n = 10 (7M/3F)			n = 12 (8M/4F)
Age (y), median (range)	48 (27-64)			44 (25-67)			36 (28-55)			42 (28-71)

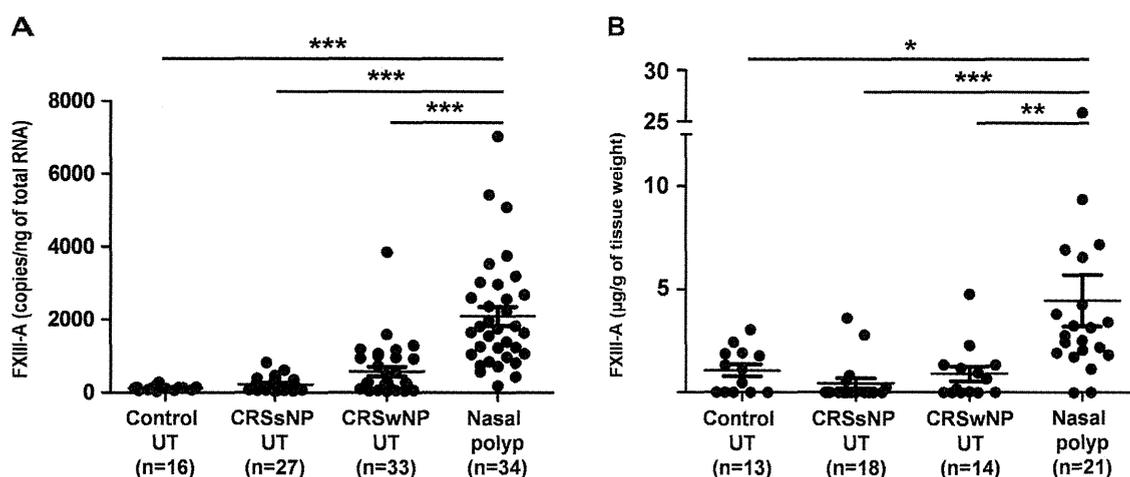*F*, Female; *M*, male; *N*, no; *U*, unknown; *Y*, yes.

FIG 1. Increased expression of FXIII-A in NP tissue. **A,** Total RNA was extracted from UT and NP, and expression of FXIII-A was analyzed by using real-time PCR. **B,** Expression of FXIII-A protein in tissue homogenates of UT and NP from patients with CRSwNP was measured by using ELISA. *$P < .05$, **$P < .01$, and ***$P < .001$.

of FXIII-A by ELISA. In agreement with the mRNA data, FXIII-A protein levels were significantly increased in NP tissue from patients with CRSwNP ($P < .05$) in comparison with those seen in UT from either patients with CRS or control subjects (Fig 1, *B*).

Immunohistochemical analysis of FXIII-A in sinonasal tissue

To further characterize the expression of FXIII-A proteins in patients with CRS, we performed immunohistochemical analysis of surgical samples from control subjects and patients with CRS to determine whether FXIII-A expression could be detected. As shown in Fig 2, we detected FXIII-A staining mainly in submucosal inflammatory cells. We found that FXIII-A[+] inflammatory cell numbers were highly elevated in NP (Fig 2, *D*). We counted the number of FXIII-A^{+} inflammatory cells by using a semiquantitative method and confirmed that FXIII-A[+] inflammatory cell numbers were significantly increased in NP from patients with CRSwNP compared with those seen in UT from patients with either CRS or control subjects ($P < .01$; Fig 2, *F*).

FXIII-A is expressed primarily in cells of bone marrow origin including platelets, megakaryocytes, and macrophages.[22-25] We

therefore focused on macrophages and performed dual-immunofluorescence analysis by using anti-FXIII-A and antibody against markers of macrophages (CD68). We found a high degree of colocalization of FXIII-A with CD68^{+} macrophages in NP (Fig 3, *A*).

Detection of FXIII-A in M2 macrophages

Macrophages are now widely recognized to be polarized by their microenvironment, especially by T-helper cytokines and pathogens.[26-30] Classically activated macrophages (also known as M1 macrophages) develop in response to proinflammatory stimuli, such as T_H1 cytokines (IFN-γ) or bacterial products (LPS). In contrast, alternatively activated macrophages are induced by exposure to T_H2 cytokines, including IL-4 and IL-13, and are therefore called M2 macrophages. Recent studies have suggested that increased expression of FXIII-A is present in M2 macrophages.[25,31] Therefore, we next examined whether M2 macrophages are major FXIII-A-producing cells in NP. We first determined levels of M2 macrophage markers, macrophage mannose receptor (MMR), CD163, and stabilin 1 (STAB1) in UT and NP using real-time PCR. Levels of mRNA for MMR, CD163, and
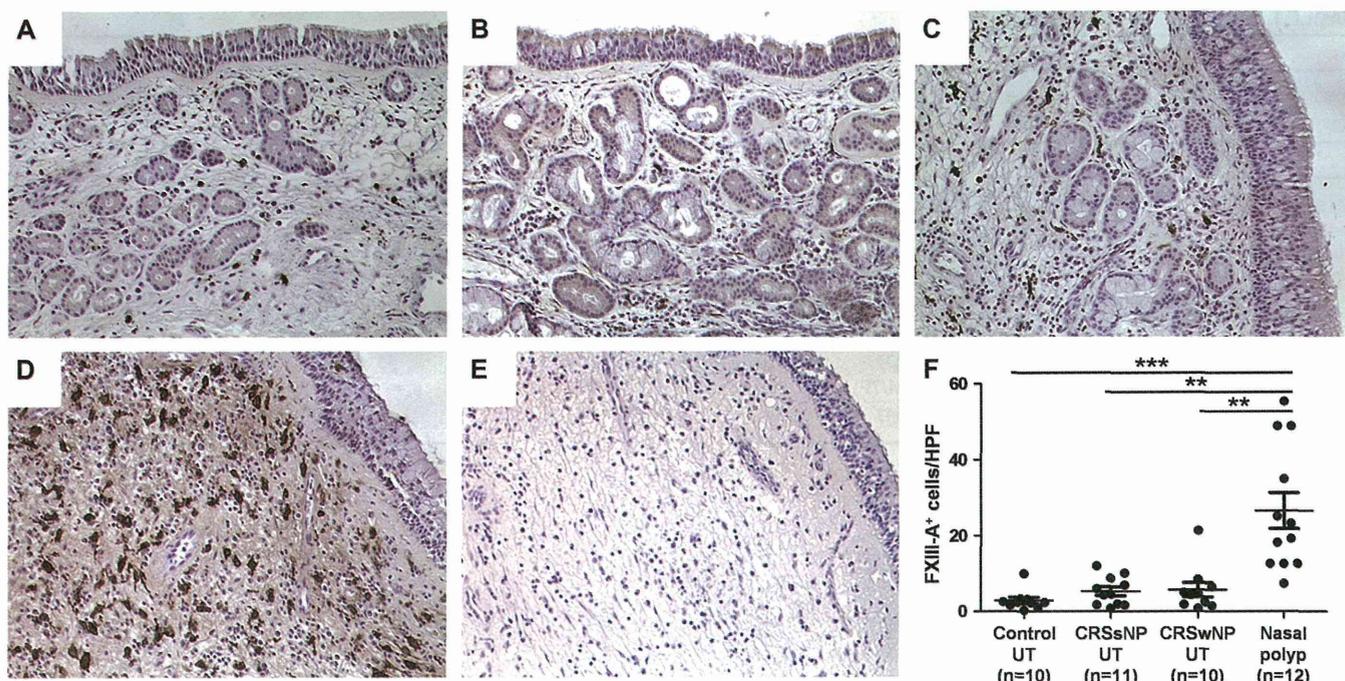
FIG 2. Immunohistochemistry of FXIII-A was performed with anti-human FXIII-A antibody. Representative immunostaining for FXIII-A in UT from control subject **(A)**, a patient with CRSsNP **(B)**, a patient with CRSwNP **(C)**, and NP tissue **(D)**. Negative control antibody staining in NP tissue from a patient with CRSwNP **(E)** is shown. The number of FXIII-A$^+$ cells in UT from control subjects (n = 10), patients with CRSsNP (n = 11), and patients with CRSwNP (n = 10) and in NP (n = 12) was counted by using a semiquantitative method **(F)**. Magnification ×400. **$P < .01$ and ***$P < .001$. *HPF*, High-power field.

STAB1 were significantly upregulated in NP ($P < .01$) in comparison with those seen in UT from either patients with CRS or control subjects (Fig 4, *A*). We also found that the expression of FXIII-A significantly and positive correlated with the expression of MMR ($r = 0.8820$, $P < .0001$), CD163 ($r = 0.7797$, $P < .0001$), and STAB1 ($r = 0.5521$, $P = .0015$; Fig 4, *B*). Levels of MMR also significantly correlated with levels of CD163 ($r = 0.6434$, $P < .0001$) and STAB1 ($r = 0.4741$, $P = .0046$).

To further investigate whether M2 macrophages were the FXIII-A-producing cells in NP, we performed triple-immunofluorescence analysis by using anti-FXIII-A and antibodies against a marker of M2 macrophages, CD163. We detected FXIII-A in CD68$^+$ and CD163$^+$ cells in NP (Fig 5). These results suggest that M2 macrophages are the sole or major FXIII-A-producing cells in NP.

DISCUSSION

Previous studies have demonstrated that NP exhibit a high degree of tissue eosinophilia and mast cell infiltration as well as T-cell cytokines demonstrating skewing toward a T_H2 pattern.[6,32,33] In NP tissue, activation of eosinophils and mast cells facilitates plasma exudation, intense edema, or pseudocyst formation, which are major histopathologic characteristics of NP.[7,34] We observed high levels of albumin, a major constituent of plasma, in NP compared with UT from patients with CRS and control subjects (see Fig E2 in this article's Online Repository at www.jacionline.org), in line with a previous report.[7] However, the mechanism by which NP tissue retains plasma proteins in the stroma has not been explored. Most recently, we showed that excessive fibrin deposition is seen in NP tissue from patients with

CRSwNP and is associated with a reduction of t-PA, which is involved in fibrinolysis by converting plasminogen to plasmin.[10] We hypothesized that profound fibrin deposition is responsible for the retention of exuded plasma proteins and the formation of intense edema and pseudocysts in NP tissue.[10] To further test this hypothesis in the current study, we evaluated the components of the coagulation cascade in control and NP tissues. We demonstrated that FXIII-A levels are increased in NP tissue from patients with CRSwNP (Fig 1). Coagulation factor XIII is a transglutaminase that participates in the final stage of the coagulation cascade. There are 2 forms of FXIII. Plasma FXIII consists of 2 enzymatically active A subunits (FXIII-A) and 2 inhibitory/carrier B subunits (FXIII-B), whereas cellular FXIII is a dimer of FXIII-A, present in platelets, monocytes, and macrophages.[35,36] During the process of plasma FXIII activation, first, thrombin cleaves off an activation peptide from FXIII-A, then FXIII-B dissociates in the presence of Ca^{2+}, and finally, FXIII-A is transformed into an active transglutaminase. In contrast, cellular FXIII, which lacks the inhibitory B subunit, does not require the cleavage of the activation peptide for its activation, and typical levels of Ca^{2+} present in tissue are sufficient to convert this protransglutaminase into an active transglutaminase. Activated FXIII catalyzes the formation of covalent cross-links between γ-glutamyl and ε-lysyl residues on adjacent fibrin chains in polymerized fibrin to yield the mature clot, and also cross-links α2-plasmin inhibitor with fibrin. The cross-linking of fibrin enhances its stiffness and rigidity, which allows it to retain plasma proteins. Cross-linking of α2-plasmin inhibitor to fibrin in the matrix has the predominant role of protecting the newly formed fibrin from degradation by the fibrinolytic enzyme, plasmin.[25,35] We also observed a significant positive correlation between the
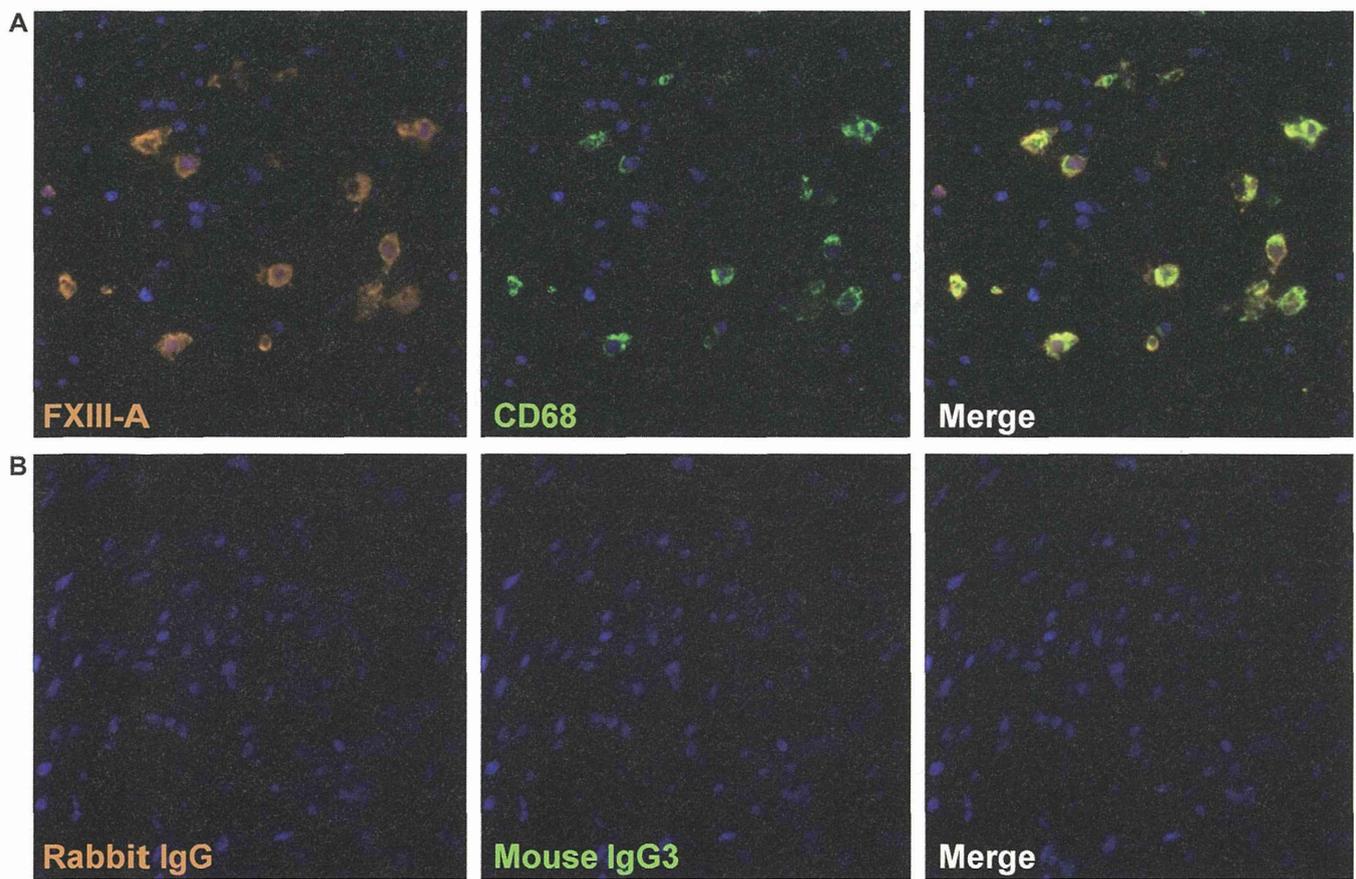
FIG 3. Immunofluorescence of FXIII-A in NP tissue. Immunofluorescence assay was performed with anti-FXIII-A (*orange* fluorescence) and anti-CD68 mAb (*green* fluorescence) for macrophages **(A)**, and control IgG **(B)**. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI; *blue* fluorescence). The results are representative of 4 separate subjects.

protein levels of albumin and FXIII-A in nasal tissues ($r = 0.441$, $P < .0001$; see Fig E3 in this article's Online Repository at www.jacionline.org). Taken together, these data support a hypothesis that the upregulation of FXIII-A plays a critical role in forming excessive fibrin deposition, which is involved in the retention of exuded plasma proteins in NP tissue.

Growing evidence indicates that FXIII-A is a multifunctional protein that plays an important role in a wide variety of physiologic and pathologic process.[25] Using FXIII-A-deficient mice, an essential role of FXIII-A in the wound-healing process was clearly demonstrated, and it was shown that angiogenesis plays a critical role in this process.[37,38] It has been reported that activated FXIII regulates the key steps of angiogenesis by increasing endothelial cell migration, proliferation, and survival, which are found to be dependent on the transglutaminase activity of FXIII-A. These effects of FXIII-A on endothelial cells are accompanied by the downregulation of thrombospondin-1, one of the best characterized antiangiogenic factors.[39] Activated FXIII binding to $\alpha_v\beta_3$ integrin elicited an intracellular signaling cascade leading to c-Jun upregulation, which, in turn, downregulated thrombospondin-1.[40] High levels of angiogenesis are one of the reported characteristics of NP tissue.[41,42] Taken together, the upregulation of FXIII-A in NP may induce fibrin deposition and plasma protein retention as well as angiogenesis, which might also be involved in tissue remodeling in NP tissue. A previous report demonstrated that the induction of fibrin-fibronectin cross-linking by FXIII

also plays a critical role in migration, proliferation, and survival of fibroblasts.[43] Because the activation of fibroblasts is considered to be an important event leading to NP development,[32] the role of FXIII-A in these processes is worthy of further investigation.

Recently, Krysko et al[44] and Peterson et al[45] showed that numbers of MMR⁺ M2 macrophages, but not M1 macrophages, were increased in NP. In the current study, we also confirmed that the M2 macrophage markers MMR, CD163, and STAB1 were significantly upregulated in NP (Fig 4, *A*). It is possible that the increased number of M2 macrophages could be explained by either the T_H2 milieu of CRSwNP or the presence of mast cell and type 2 innate lymphoid cells, as demonstrated in recent reports.[28,46,47] However they are recruited to NP, the role of macrophages in the pathogenesis of CRS remains unclear. Importantly, it has been reported that M2 macrophages express FXIII-A.[48-50] We found that FXIII-A was detected in CD163⁺ macrophages but not in CD163⁻ macrophages in NP (Fig 5 and data not shown). We also showed that levels of M2 macrophage markers correlated well with levels of FXIII-A (Fig 4, *B*). These results indicate that M2 macrophages are the sole or major FXIII-A-producing cell type in NP. Although there is a general agreement on the cytoplasmic localization of FXIII-A in macrophages, FXIII-A lacks an identifiable endoplasmic reticulum signal sequence and it is not clear how it can be released from macrophages to the extracellular milieu.[35] Recent reports demonstrate the appearance of FXIII-A in bronchoalveolar lavage fluid and
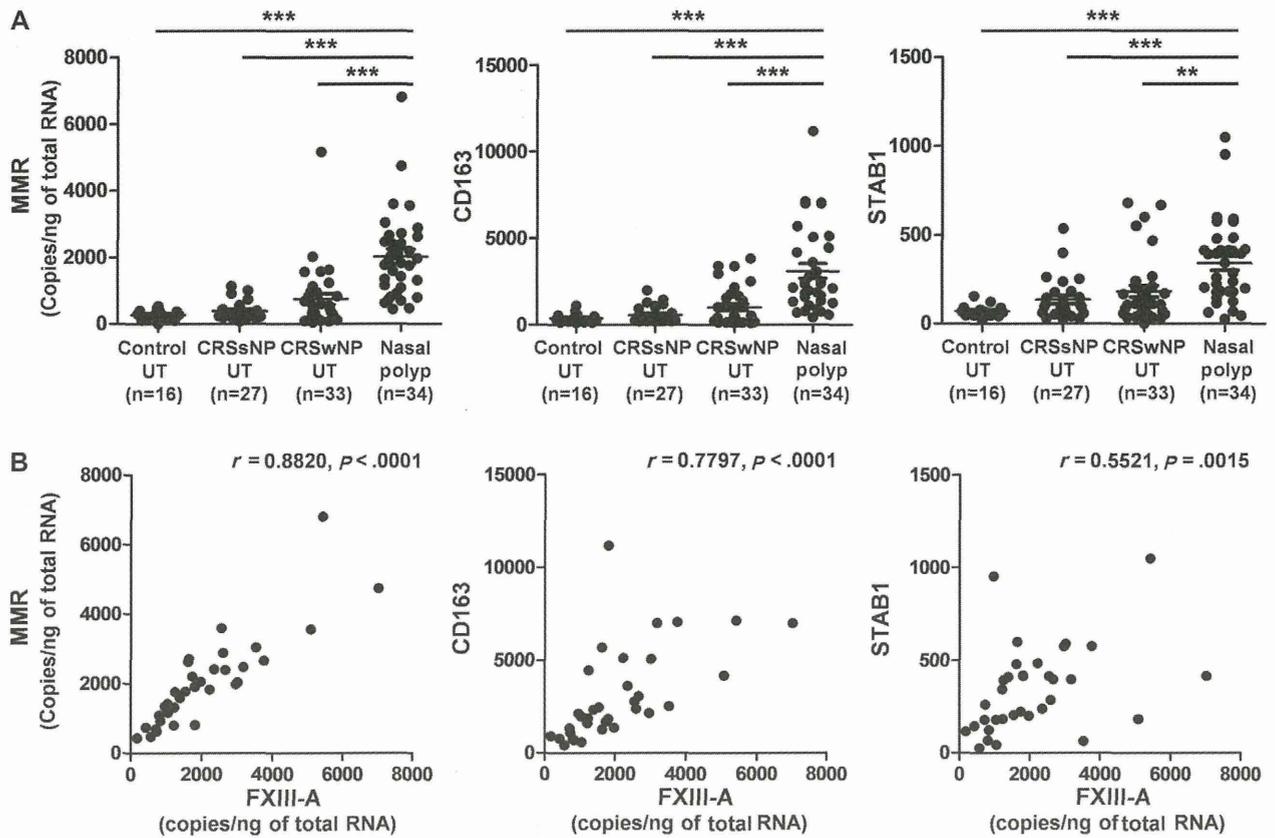
FIG 4. Correlation of FXIII-A with markers of M2 macrophages in NP tissue. **A,** Total RNA was extracted from UT from control subjects (n = 16), patients with CRSsNP (n = 27), and patients with CRSwNP (n = 33) and NP tissue (n = 34). The expression of FXIII-A and M2 macrophage markers MMR, CD163, and STAB1 was analyzed by using real-time PCR. **B,** The correlation in NP tissue was assessed by using a Spearman rank correlation test. **P < .01 and ***P < .001.
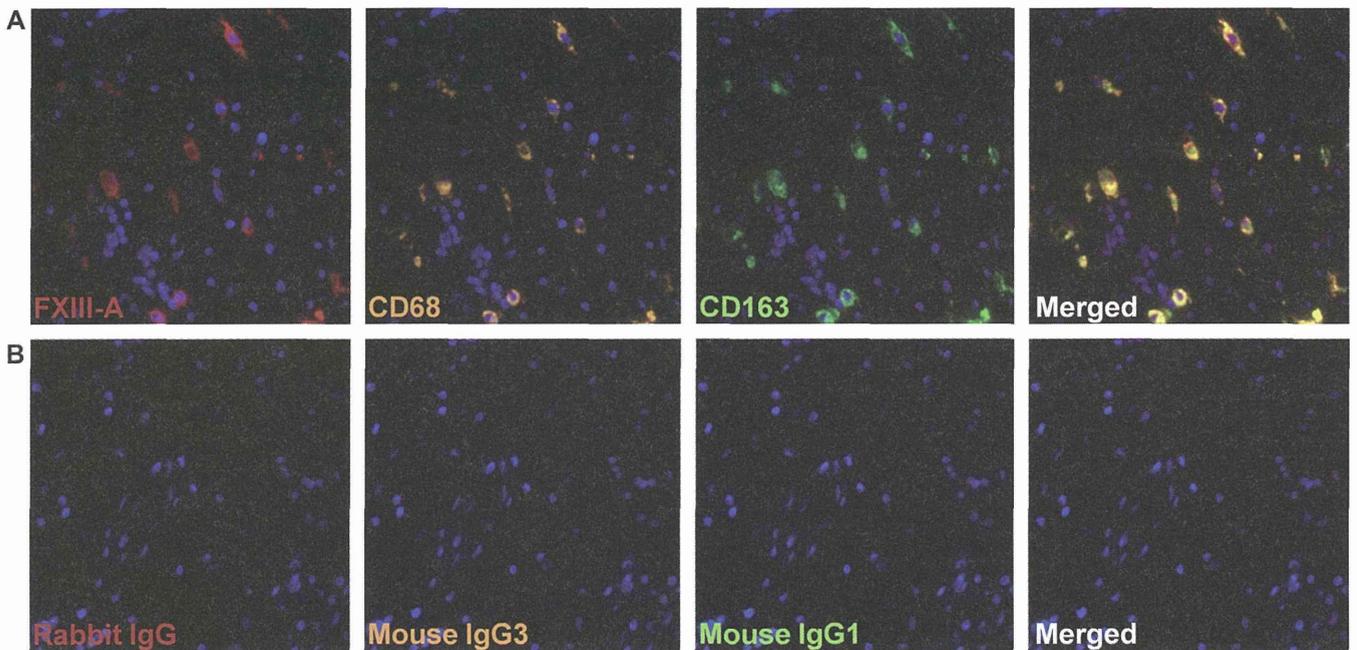

FIG 5. Detection of FXIII-A in M2 macrophages in NP tissue. Immunofluorescence assay was performed with anti-FXIII-A (*red* fluorescence), anti-CD68 mAb (*orange* fluorescence) for macrophages, and anti-CD163 mAb (*green* fluorescence) for M2 macrophage **(A)**, and control IgG **(B)**. Nuclei were counterstained with DAPI (*blue* fluorescence). The results are representative of 4 separate subjects. *DAPI,* 4′,6-diamidino-2-phenylindole.
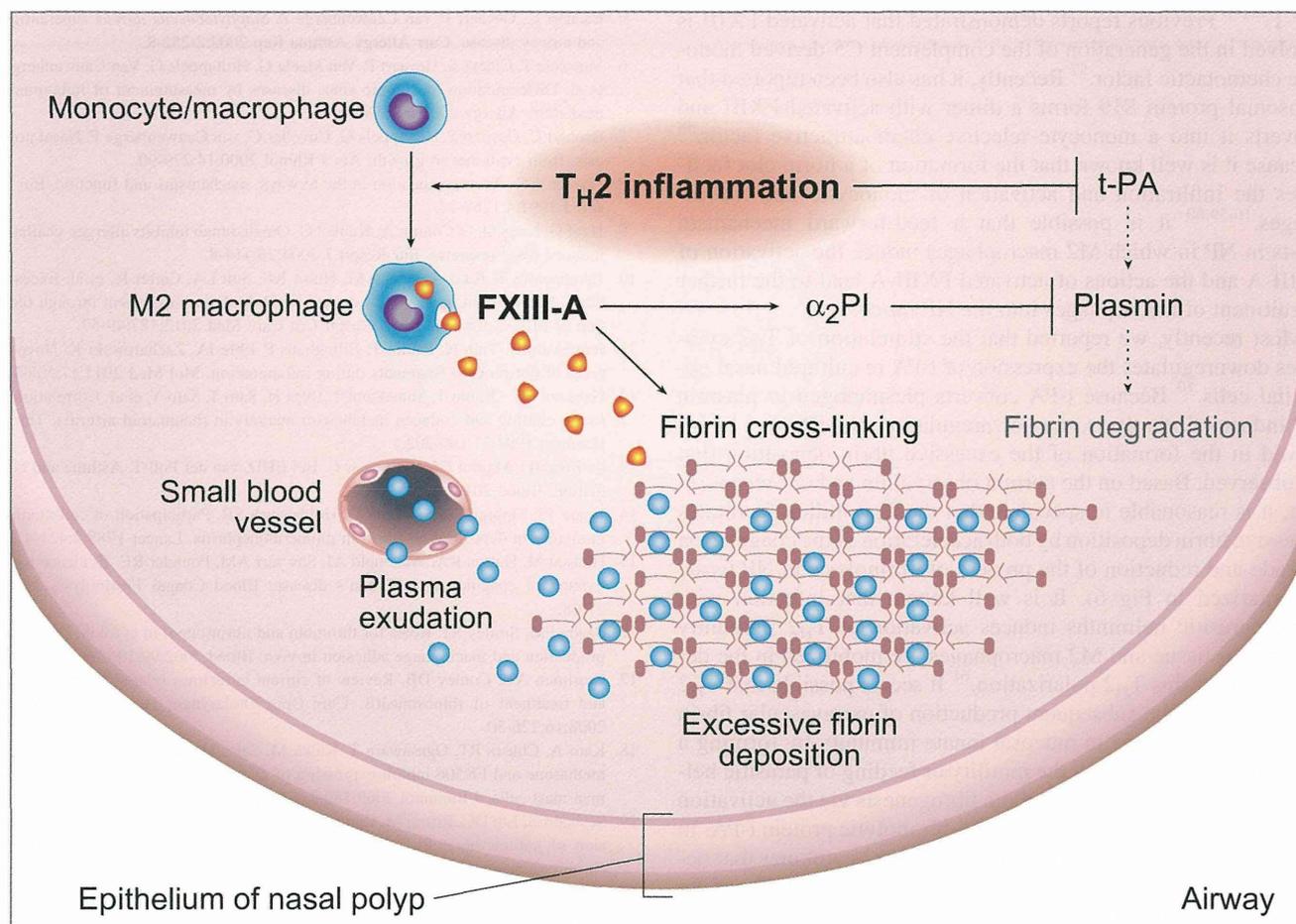
FIG 6. Hypothetical model in which the T_H2 milieu facilitates excessive fibrin deposition in NP tissue. T_H2 inflammation leads to the recruitment of M2 macrophages and the subsequent production of FXIII-A, which induces excessive fibrin deposition by cross-linking of fibrin and via the antifibrinolytic effect through binding α_2PI to fibrin. In the presence of T_H2 cytokines, t-PA levels are reduced, causing impaired plasmin generation, which, in turn, decreases fibrinolysis. α_2PI, α2-Plasmin inhibitor.

in the culture medium of macrophages, and there is evidence that FXIII-A is released by an alternative secretory pathway in human macrophages.[35,49,51] In fact, extravascular fibrin deposition is frequently observed within and around tumor matrix, and tumor-associated macrophages containing profound levels of FXIII-A have been reported.[52,53] It is reasonable to speculate that infiltrating M2 macrophages might contribute to excessive fibrin deposition by secreting FXIII-A in NP tissue, but this will require further experiments to test. In spite of presenting prominent inflammation, NP tissue shows low levels of fibrosis.[4,10] A previous report suggested that the downregulation of TGF-β may partially explain the low levels of collagen detected in NP tissue.[4] It has also been reported that M2 macrophages are involved in the suppression of tissue fibrosis by the production of IL-10, resistin-like molecule alpha, and arginase-1.[28] However, M1 macrophages, which have been identified as key regulators in demyelinating diseases of the central nervous system, produce significant amounts of TGF-β.[54] Thus, predominant infiltration of M2 macrophages might prevent or diminish fibrosis in NP tissue. Phagocytosis is one of the most important functions of macrophages, in which the rearrangement of cell cytoskeleton is deeply involved, and FXIII-A is implicated in phagocytic activities by catalyzing

alterations in certain cytoskeletal components, including actin, myosin, vinculin, small heat shock protein HSP27, and thymosin β4.[24,50] Macrophages from FXIII-A- deficient patients showed an impaired capacity of Fcγ, complement, and lectin-like receptor-mediated phagocytosis.[55] FXIII-A plays a critical intracellular role in receptor-mediated phagocytosis of macrophages. However, a recent study suggested that the phagocytic capacity of M2 macrophages is impaired in NP tissue and facilitated the increased presence of *Staphylococcus aureus* in CRSwNP.[44] Further studies are required to determine how and whether FXIII-A participates in the alteration of phagocytosis in M2 macrophages associated with nasal polyposis.

We found that M2 macrophages were major FXIII-A-expressing cells in NP. However, the regulation of macrophage recruitment in NP is poorly understood. We recently demonstrated that CCL23, known as a chemokine for macrophage, is elevated in NP tissue and CCL23 might play a critical role in macrophage infiltration.[56] It is possible that FXIII-A may play some role. Activated FXIII enhances the proliferation of peripheral blood monocytes, accelerates their migration, and inhibits monocyte apoptosis through the downregulation of thrombospondin-1 and due to the upregulation of c-Jun and

Egr-1.[25,43] Previous reports demonstrated that activated FXIII is involved in the generation of the complement C5-derived monocyte chemotactic factor.[57] Recently, it has also been reported that ribosomal protein S19 forms a dimer with activated FXIII and converts it into a monocyte-selective chemoattractive factor.[58] Because it is well known that the formation of a fibrin clot facilitates the infiltration and activation of monocytes and macrophages,[16,59,60] it is possible that a feed-forward mechanism exists in NP in which M2 macrophages induce the activation of FXIII-A and the actions of activated FXIII-A lead to the further recruitment of macrophages into the NP tissue.

Most recently, we reported that the stimulation of T_H2 cytokines downregulates the expression of t-PA in cultured nasal epithelial cells.[10] Because t-PA converts plasminogen to plasmin and induces fibrinolysis, the downregulation of t-PA may be involved in the formation of the excessive fibrin deposition that we observed. Based on the current observation and our recent report, it is reasonable to speculate that the T_H2 milieu facilitates excessive fibrin deposition by both acceleration of the coagulation cascade and reduction of the process of fibrinolysis in NP tissue (summarized in Fig 6). It is well known that infection with many parasitic helminths induces activation of T_H2 immunity in mucosal tissue and M2 macrophages are mobilized in the development of this T_H2 polarization.[61] It seems possible that T_H2 immunity, and the subsequent production of extravascular fibrin deposition, takes part in mucosal innate immunity by forming a fibrin mesh and impeding the motility or feeding of parasitic helminths. This process may promote fibrogenesis via the activation of FXIII-A and the suppression of the fibrinolytic protein t-PA. In this scenario, the excessive activation of type 2 cytokines that occurs in CRSwNP and may lead to the formation of NP can be viewed as a localized sterile antiparasite response.

We report here that tissue levels of FXIII-A were profoundly increased in NP tissue and that M2 macrophages are the sole or major FXIII-A-producing cell in NP. Overproduction of FXIII-A may lead to the acceleration of the coagulation cascade, resulting in excessive fibrin deposition, which, in turn, retains exuded plasma proteins and participates in tissue remodeling, intense edema, or pseudocyst formation in the submucosa of NP tissue. Our results imply that targeting the local production of FXIII-A from M2 macrophage might therefore be of therapeutic value for treating patients with CRSwNP.

Clinical implications: Overexpression of FXIII-A may have a pathogenic role in CRSwNP and strategies to reduce the activity of the coagulation cascade might have therapeutic value in the treatment of CRSwNP.

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METHODS

Patients and biopsies

Patients with CRS were recruited from the Allergy-Immunology Clinic and Otolaryngology Clinic of the Northwestern Medical Faculty Foundation, the group practice for physician faculty members of Northwestern University, and the Northwestern Sinus Center. 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 the American Academy of Otolaryngology-Head and Neck Surgery Chronic Rhinosinusitis Task Force. The presence of sinusitis or bilateral NP was confirmed by means of office endoscopy and computed tomographic imaging. All patients scheduled for surgery had previously failed to respond to adequate trials of conservative medical therapy (prolonged antibiotic regimens, nasal steroid sprays, oral steroids, saline irrigations, and decongestants) for control of symptoms. Patients with an established immunodeficiency, pregnancy, coagulation disorder, diagnosis of classic allergic fungal sinusitis, Samter's triad, Churg-Strauss syndrome, or cystic fibrosis did not participate in the study. Details of subjects' characteristics are included in Table I. Sinus tissues from disease-free control subjects were obtained during endoscopic skull-base tumor excisions, as well as intranasal procedures for obstructive sleep apnea and facial fracture repairs on patients with a history of CRS or asthma recruited from the otolaryngology clinic at the Northwestern Medical Faculty Foundation.

Subjects underwent skin tests to pollens, dust mites, pets, molds, and cockroaches by using Hollister-Stier (Spokane, Wash) extracts. A positive skin test response was defined as a wheal greater in size than that produced by the saline control by 3 mm or more. Histamine was used as a positive control. Atopic status was assessed in all subjects unless subjects declined or if the history did not suggest atopy.

All subjects signed informed consent, and the protocol and consent forms governing procedures for the study were approved by the Institutional Review Board of Northwestern University Feinberg School of Medicine.

Real-time PCR

Total RNA from sinus tissue was extracted with QIAzol (Qiagen) and was cleaned and treated with DNase I by using NucleoSpin RNA II (MACHEREY-NAGEL, Bethlehem, Pa) according to the manufacturer's instructions. The quality of total RNA from sinus tissue was assessed with a 2100 Bioanalyzer (Agilent Technologies, Carlsbad, Calif) by using an RNA 6000 Nano LabChip (Agilent Technologies). Single-strand cDNA was synthesized with SuperScript II reverse transcriptase (Invitrogen) and random primers. Semiquantitative real-time RT-PCR was performed with a TaqMan method by using an Applied Biosystems 7500 Sequence Detection System (Applied Biosystems, Foster City, Calif) in 15-μL reactions (7.5 μL of 2$\times$ TaqMan Master mix [Applied Biosystems], 400 nmol/L of each primer, and 200 nmol/L of TaqMan probe plus cDNA). Primer and probe sets for β-glucuronidase (human β-Glucuronidase endogenous control, PN; 4326320E), FXIII-A (Hs00173388_m1), MMR (Hs00267207_m1), CD163 (Hs00174705_m1), and STAB1 (Hs01109068_m1) were purchased from Applied Biosystems. A primer and probe set for β-Glucuronidase was chosen as the reference housekeeping gene in sinus tissue because previous studies have demonstrated no difference in the expression of this gene between patients and controls. To determine the exact copy number of the target genes, quantified aliquots of purified PCR fragments of the target genes were serially diluted and used as standards in each experiment. Aliquots of cDNA equivalent to 10 ng of total RNA were used for real-time PCR. The mRNA expression levels were normalized to the median expression of the housekeeping gene β-Glucuronidase.

Measurement of FXIII-A and albumin in tissue homogenates

Freshly obtained tissue specimens were weighed, and 1 mL of PBS supplemented with 0.05% Tween 20 (Sigma-Aldrich, St Louis, Mo) and 1% protease inhibitor cocktail (Sigma-Aldrich) was added for every 100 mg of tissue. The tissue was then homogenized with a Bullet Blender Blue (Next Advance, Averill Park, NY) at setting 7 for 8 minutes at 4°C. After homogenization, the suspension was centrifuged at 4000 rpm for 20 minutes at 4°C, and supernatants were stored at −80°C until analyzed.

The concentrations of FXIII-A (HYPHEN BioMed) and albumin (BETHYL, Montgomery, Tex) in cell-free supernatants were determined by using a specific ELISA kit. The color intensity was measured with a Bio-Rad Spectrophotometer Model 680 Microplate Reader (Bio-Rad, Hercules, Calif). Concentrations of FXIII-A in the tissue homogenate and cell lysate were normalized to the tissue weight.

Immunohistochemistry

Nasal tissue was dehydrated, infiltrated, and embedded in paraffin, and tissue was sectioned at 3 μm by using a Leica RM2245 Cryostat (Leica Microsystems, Inc, Bannockburn, Ill). Sections were rehydrated, and endogenous peroxidase activity was blocked with 3% H_2O_2/methanol. Tissue sections were then boiled in a citrate buffer (Dako, Carpinteria, Calif) for 15 minutes to induce antigen retrieval. After rinsing, nonspecific binding was blocked with 3% goat serum/0.3% Tween-20/PBS. Tissue sections were then incubated with 6.4 ng/mL of rabbit anti-human FXIII-A mAb (EP3372; CELL MARQUE) in blocking buffer overnight at 4°C. In control experiments, sections were incubated with the same concentrations of control rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, Pa). Sections were rinsed and then incubated in biotinylated secondary goat anti-rabbit antibody (Jackson ImmunoResearch Laboratories) at a 1:500 dilution for 1 hour at room temperature. After another rinse, sections were incubated in ABC reagent (avidin–biotin–horseradish peroxidase complex; Vector Laboratories) for 1 hour at room temperature. Sections were rinsed again and incubated in diaminobenzidine reagent (Invitrogen) for 10 minutes at room temperature. They were then rinsed in deionized H_2O, counterstained with hematoxylin, dehydrated, cleared, mounted, and coverslipped by using Cytoseal 60 (Richard-Allan Scientific, Kalamazoo, Mich) in preparation for microscopic analysis. Microscopic analysis was performed with an Olympus IX71 inverted research microscope by using $\times$40 objective lens, and images were collected with SlideBook software (Olympus, Center Valley, Pa). For the quantification of FXIII-A$^+$ cells, slides were blinded, and then 10 pictures were randomly taken from each slide. The number of FXIII-A$^+$ cells in nasal mucosa was counted by a blinded observer. For the immunofluorescence assay, rehydrated sections were blocked with 3% goat serum/0.3% Tween-20/PBS and then were incubated with 6.4 ng/mL of rabbit anti-human FXIII-A mAb (EP3372; CELL MARQUE), 24 ng/mL of mouse anti-human CD68 mAb (clone PG-M1, IgG$_3$, Thermo Fisher Scientific, Fremont, Calif), and 2.9 ng/mL of mouse anti-human CD163 mAb (clone 10D6, IgG$_1$, Thermo Fisher Scientific) in blocking buffer overnight at 4°C. The same concentrations of isotype control IgG were used in control experiments. After washing, sections were incubated with 4 μg/mL of Alexa Fluor 647-conjugated goat anti-rabbit IgG (Invitrogen), 4 μg/mL of Alexa Fluor 568-conjugated goat anti-mouse IgG$_3$ (Invitrogen), and 4 μg/mL of Alexa Fluor 488-conjugated goat anti-mouse IgG$_1$ (Invitrogen) for 1 hour at room temperature in the dark. Images from immunofluorescence slides were obtained with an Olympus IX71 inverted research microscope using $\times$40 objective lens, and images were collected with SlideBook software (Olympus).
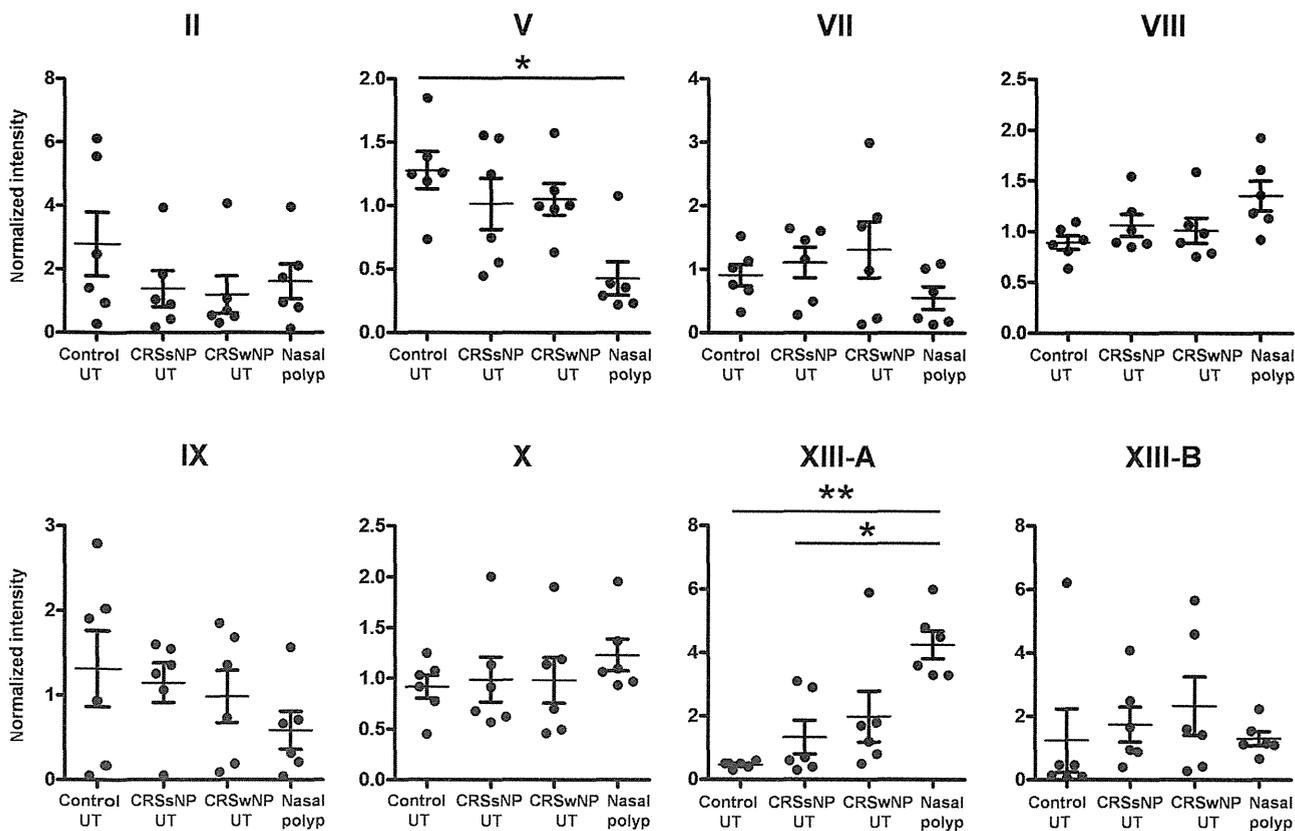
FIG E1. Microarray analysis of coagulation factors in sinonasal tissue. Microarray was used to assess the expression of coagulation factors in UT from control subjects (n = 6), patients with CRSsNP (n = 6), and patients with CRSwNP (n = 6) and in NP from patients with CRSwNP (n = 6). *P < .05 and **P < .01. All microarray data have been deposited to gene expression omnibus: GSE36830.
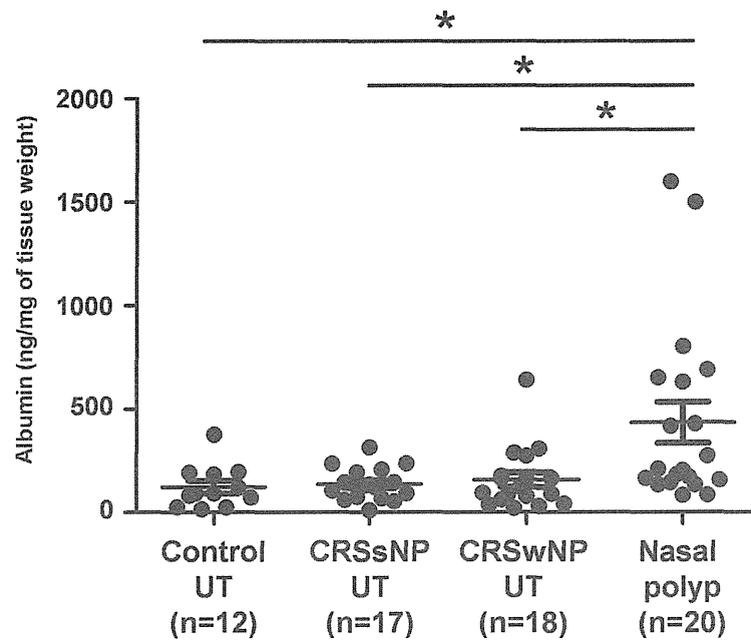
FIG E2. Increased levels of albumin in NP. Measurement of levels of albumin by ELISA in tissue homogenates of UT from control subjects, from patients with CRSsNP and CRSwNP, and in NP. *P < .05.
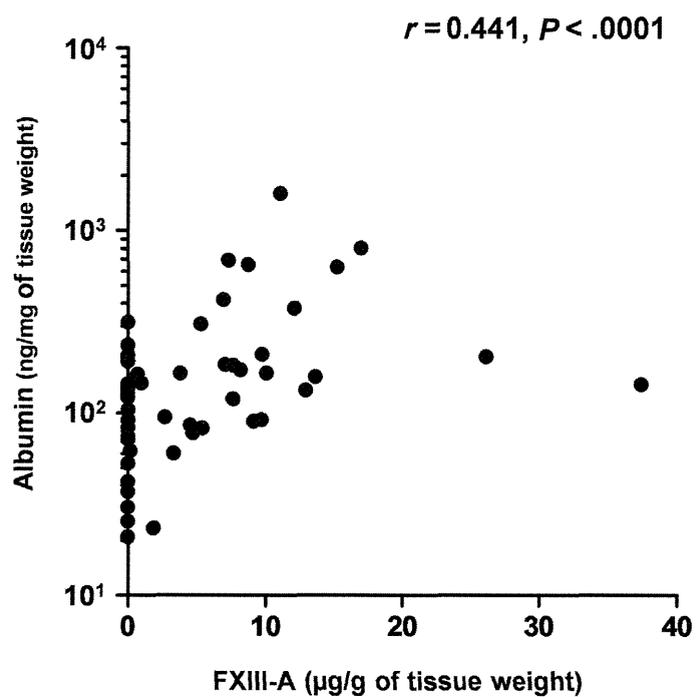
FIG E3. The relationship of FXIII-A and albumin in UT and NP was evaluated by using ELISA. The correlation was assessed by using the Spearman rank correlation test.