

Role of Fibrinolysis in the Nasal System

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Summary. In this chapter, we show the presence of tissue-type plasminogen activator (t-PA), urokinase-type plasminogen activator (u-PA), and plasminogen activator inhibitor-1 (PAI-1) in nasal mucosa. It is suggested that t-PA synthesized in mucous cells is promptly secreted and modifies the watery nasal discharge in allergic rhinitis and that u-PA activity may help with the passage of large amounts of rhinorrhea by reducing its viscosity. Furthermore, we clarify the relation between fibrinolytic components and the pathology of allergy, particularly during the development of nasal allergy and nasal tissue changes. Wild-type (WT) mice can develop nasal allergy for ovalbumin (OVA) sensitization, but PAI-1-deficient mice (PAI-1^{-/-}) cannot. The production of specific immunoglobulins IgG1 and IgE in the serum and production of interleukins IL-4 and IL-5 in splenocyte culture supernatant increased significantly in WT-OVA mice. In PAI-1^{-/-} mice, these reactions were absent, and specific IgG2a in serum and interferon- γ in splenocyte culture medium increased significantly. Histopathologically, there was marked goblet cell hyperplasia and eosinophil infiltration into the nasal mucosa in WT-OVA mice, but these were absent in PAI-1^{-/-} mice. These results indicate that the immune response in WT-OVA mice can be classified as a dominant Th2 response, which would promote collagen deposition. In contrast, the Th2 response in PAI-1^{-/-} mice was down-regulated and the immune response shifted from Th2-dominant reaction to a Th1-dominant one. Taken together, these findings suggest that PAI-1 plays an important role not only in thrombolysis but also in the immune response.

Key words. PAI-1 · t-PA · u-PA · Nasal allergy · Transgenic/knockout mice

Introduction

The fibrinolytic system is associated with not only intravascular fibrinolysis but also various reactions in tissues, including ovulation, arterial sclerosis, cell migration of keratinocytes and smooth muscle cells, and neovascularization [1–4]. Considering these reports about the actions of fibrinolytic components in various tissues, it is thought that fibrinolytic components act on various kinds of physiological functions related to inflammatory reaction such as cellular infiltration and tissue remodeling.

Although the effect of fibrinolytic components in various tissues has been elucidated, the action and the importance of fibrinolytic components in nasal mucosa have been barely reviewed to date. Oh et al. reported that up-regulation of plasminogen activator inhibitor-1 (PAI-1) synthesis occurs in lung and bronchoalveolar lavage fluids in the ovalbumin (OVA)-challenged murine asthma model and that PAI-1 promotes extracellular matrix (ECM) deposition in the airways and inhibits the activity of matrix metalloproteinase (MMP) and plasmin generation [5]. In addition, Gyetko et al. reported that fibrinolytic components have an influence on cytokine production and play a constant role in the immune response [6, 7]. Based on these reports, it is suggested that fibrinolytic components act on disease formation, and the tissue changes greatly during inflammation of the nasal mucosa, particularly in allergic rhinitis. In late years, an increase of morbidity in individuals with allergic rhinitis, including pollinosis, has become a serious social issue all over the world. Furthermore, it is an important problem clinically in that allergic rhinitis is difficult to cure. The mechanism of allergic rhinitis cannot be explained as a simple type I allergy. It is suggested that allergic rhinitis is a complicated process in which inflammation begins with sensitization, becomes chronic, and causes tissue change (Fig. 1).

In this chapter, we describe the involvement of the fibrinolytic components in serial allergic reactions, especially allergic rhinitis, and present data from the knockout mouse concerning the fibrinolytic component.

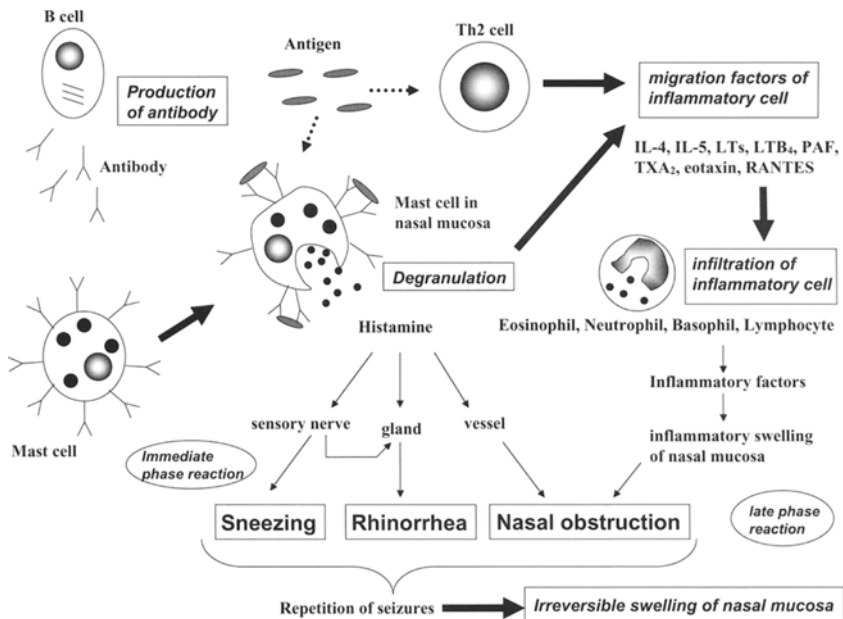


FIG. 1. Mechanism of allergic rhinitis. *IL*, interleukin, *LT*, leukotriene, *PAF*, platelet-activating factor, *TX*, thromboxane, *RANTES*, regulated upon activation of normal T cells expressed and secreted

Localization and Dynamics of Fibrinolytic Components in Nasal Mucosa

The presence of fibrinolytic components in nasal mucosa was identified by reverse transcription polymerase chain reaction (RT-PCR), *in situ* hybridization, and immunohistochemical staining; and it was shown that its expression changed in the presence of allergic inflammation (Table 1). t-PA was constitutively detected in the epithelium of nasal mucosa, and its expression was almost constant or had an attenuation tendency in the allergic state. Particularly, expression of u-PA and PAI-1 mRNA was significantly high in allergic nasal mucosa in comparison with normal mucosa [8]. Larsen et al. determined the t-PA and u-PA activities in nasal polyps and control nasal mucosa and indicated that the shift toward a higher u-PA/t-PA activity ratio and the higher levels of u-PA in nasal polyps suggested an inflammatory process [9]. Therefore, it is suggested that u-PA activity is similar in allergic rhinitis. u-PA is involved in such biological processes as tissue remodeling and cell migration; and tissues with allergic rhinitis or nasal polyps include tissue changes such as remodeling and infiltration of eosinophils and lymphocytes. On the other hand, considering that u-PA is inactivated by forming a complex with PAI-1, the expression of PAI-1 in serous cells may increase to regulate u-PA activity.

In our previous study, immunohistochemical staining for t-PA was negative in submucosal glands [8]. The presence of t-PA mRNA was noted in mucinous cells in the presence of allergic nasal mucosa, whereas it was not detected in mucinous cells of normal nasal mucosa. These results indicated that t-PA produced by mucinous cells is secreted promptly into the nasal cavity during allergic rhinitis. Furthermore, we reported that u-PA expression was noted in normal nasal mucosa, but compared with that in allergic nasal mucosa there was very little of it. In fibrinography of nasal discharge, u-PA was markedly detected in allergic patients [8]. Åkerlund et al. measured fibrinogen in nasal discharge and reported that it was increased in the presence of viral upper respiratory inflammation; they supposed that fibrinolytic peptides were generated to participate in inflammatory and defense processes [10].

TABLE 1. Expression of fibrinolytic components in nasal mucosa (human)

Component	t-PA	u-PA	PAI-1
Control			
Epithelium	+	±	-
Gland (submucosal gland)			
Mucinous cells	-	±	-
Serous cells	-	-	-
Endothelium	+	-	-
Allergic rhinitis			
Epithelium	+	+	-
Gland (submucosal gland)			
Mucinous cells	+	++	-
Serous cells	-	-	++
Endothelium	+	-	-

++, strong expression; +, moderate expression; ±, weak expression; -, no expression

Nasal allergy is characterized by large amounts of serous rhinorrhea. That production of u-PA increases in allergic rhinitis suggests that u-PA activity may help with the passage of large amounts of rhinorrhea by reducing its viscosity. Additionally, it is thought that t-PA acts as an adjunct to treat a large quantity of nasal discharge effectively because of the finding that t-PA has a rapid production turnover and secretion in allergic nasal mucosa. In our study of normal nasal mucosa, t-PA mRNA appeared in mucosal epithelia, and t-PA activity was noted in nasal discharge according to fibrin autography [8]. Thus, t-PA may constitutively adjust the viscosity of the discharge, even in nonallergic tissue. In fact, plasminogen, which is substrate of plasminogen activators (PAs), was present in nasal discharge with or without allergy [8].

In allergic rhinitis, it is interesting that only PAI-1 in fibrinolytic components was produced in serous cells and was not produced in epithelium [8]. PAI-1 has secretory modality and functions distinct from those of the other fibrinolytic components. It is conceivable that PAI-1 functions in circumferential tissue and promotes fibrosis in allergic nasal tissue. Thus, PAI-1 also is secreted into nasal cavity in allergic rhinitis and adjusts the fluidity of nasal discharge. Only u-PA increased in parallel with an increase of PAI-1 in allergic rhinitis, whereas the t-PA concentration did not change. It shows that not t-PA but u-PA acts while competing with PAI-1 in allergic rhinitis. These fibrinolytic components may play an important role in tissue fibrosis, maintaining the fluidity of the nasal discharge. The production of these components is regulated by several cytokines [11, 12], and allergic disease also involves many cytokines. It is possible that the metabolism of fibrinolytic components in allergic tissues is altered under the influence of cytokines.

Tissue Remodeling and Fibrinolytic Components in Allergic Rhinitis

In late years, physiological functions of tissue fibrinolysis, such as cell migration and remodeling, have been investigated regarding the fibrinolytic components. Fibrinolytic components act in the nasal mucosa tissue itself as well as nasal discharge. For allergic disease, it is said that morbid tissue change, or "remodeling," becomes an important factor that makes cure difficult. The phenomenon of remodeling has been studied mainly in bronchial asthma until now. It is characterized by submucosal fibrosis, deposition of ECM, and goblet cell hyperplasia in epithelium [13, 14], and it is thought that the fibrinolytic system is involved in ECM deposition and fibrosis in inflammatory tissues. PAs convert the inactive proenzyme plasminogen to the active form plasmin, a protease of fairly broad substrate specificity. Plasmin degrades fibrin and converts inactive pro-MMP into active MMP. Activated MMP degrades the ECM proteins, including collagen, which is the main protein component of fibrotic tissue in the airway [15]. Previous studies have demonstrated subepithelial depositions of collagen types I and III in bronchial biopsy specimens of asthma patients and allergic nasal mucosa, which correlates with airway hyperresponsiveness [16, 17].

To clarify the relation of fibrinolytic components to the pathology of allergy, particularly the development of nasal allergy and nasal tissue changes, we made a nasal

allergy model with PAI-1^{-/-} mice [18]. In our study, we employed a murine model of allergic rhinitis induced by ovalbumin (OVA). Excess amounts of type I and type III collagen are found in the nasal mucosa obtained from OVA-challenged wild-type (WT) mice in our system. In contrast, collagen deposition in the nasal mucosa from OVA-challenged PAI-1^{-/-} mice appeared less significant than that in the OVA-challenged WT mice. Employing WT and PAI-1^{-/-} mice, Hattori et al. in the bleomycin-induced pulmonary fibrosis model [19] and Oh et al. in the OVA-induced asthma model observed a similar effect of PAI-1 on excess fibrous material accumulation in mouse lung tissues [5].

Protection of PAI-1^{-/-} Mice from Nasal Allergy

In addition, we demonstrated that WT mice can develop nasal allergy for OVA, but the PAI-1^{-/-} mice cannot [18]. In type I allergy, including allergic rhinitis, increased serum immunoglobulin E (IgE) is characteristic of the immune reaction after exposure to an antigen. Based on the type of cytokine helper T-cells secrete, the helper T-cell is classified as Th1 type or Th2 type. Immune response characteristics are prescribed according to the predominant T-cell type. In mice, the Th2 response results in IgG1 and IgE production, whereas the Th1 response leads to IgG2a synthesis [20]. Therefore, allergic rhinitis is thought to be a result of Th2 cell activation. The significantly increased production of IgE and IgG1 and the low levels of IgG2a in WT-OVA mice immunized and challenged with OVA implicated the Th2 response against this allergen. In contrast, high levels of specific IgG1 and IgE were nearly absent in PAI-1^{-/-}-OVA mice. Furthermore, only the PAI-1^{-/-}-OVA group showed a significant increase in the level of specific IgG2a [18]. Thus, these results indicate that down-regulation of the Th2 immune response in PAI-1^{-/-} mice brings about inappropriate overactivation of the Th1 immune response to the antigen, which would otherwise induce the Th2 response. Considering the importance of the Th2 phenotype in the development of fibrotic pulmonary and extrapulmonary complications [21], it is possible that the Th2 phenotype itself would promote collagen deposition in the WT compared to that in PAI-1^{-/-} mice. The change in immune responsiveness in PAI-1^{-/-} mice was also confirmed in the cytokine profiles of the nasal lavage fluid (local) and the supernatant of the cultured lymphocytes of the spleen (systemic) from mice challenged by OVA. The levels of IL-4 and IL-5 in the supernatant of OVA-stimulated lymphocyte cultures from WT-OVA mice were 10–20 times higher than those from PAI-1^{-/-}-OVA mice. OVA-stimulated cells from PAI-1^{-/-}-OVA mice had a 100-fold higher level of interferon- γ (IFN γ) than those from WT-OVA mice [18]. The tendencies of these cytokine profiles were also reflected in the splenocyte proliferation assay from the conditioned mice [18]. These results show that the sensitized group exhibited each immune reaction not only locally but also systemically, explaining the high level of IgE in the WT-OVA mice. The high level of IL-4 would induce high-level antibody production, including IgE as described [22, 23], in the nasal cavity, thereby contributing to the immediate-type allergic reaction after antigen inhalation. On the other hand, IL-5 has highly specific effects on eosinophilic proliferation, migration, activation, and survival [24, 25]. The high level of IL-5 would be responsible for the infiltration of eosinophils into the nasal mucosa. As for the hyperplasia of goblet

cells in sensitized mice, a similar tissue change was observed in human allergic rhinitis [8]. Surprisingly, in PAI-1^{-/-}-OVA mice, however, these mucosal changes were not observed.

Mechanism by which Fibrinolytic Components Control Allergic Inflammation

The histological data and cytokine profiles indicated that mice deficient in PAI-1 fail to generate the Th2 immune response to the OVA challenge. PAI-1 works not only as a serine protease inhibitor to prevent ECM degradation but acts as a de-adhesion molecule to detach cells attached to vitronectin via integrins [26]. In addition, the de-adhesive activity of PAI-1 does not require its interaction with vitronectin. However, there is an absolute requirement for its binding to u-PA. Free u-PA or PAI-1 with vitronectin has only weak detachment activity [27]. The loss of detachment activity in PAI-1^{-/-}-mice might explain the inhibition of cell movement, including eosinophil infiltration. This detachment profile may change the signal transduction of leukocytes through cell-to-cell and cell-to-ligand interactions. Many reports suggest that malignant cells expressing more PAI-1 can metastasize more efficiently than tumors with less PAI-1 production [28]. In addition, there is clinical report that a higher level of PAI-1 in plasma due to the 4G/5G polymorphism of the PAI-1 gene is closely related to allergic disease [29, 30]. These reports, in conjunction with our study, support the idea that PAI-1 is a rather critical regulator of some immune response for antigen stimulation.

Conclusion

We have shown that allergic rhinitis is restrained in OVA sensitization of PAI-1-deficient mice, and the immune response characteristics tend to shift from a Th2-dominant reaction to a Th1-dominant reaction. These findings, with other previous works, demonstrate that fibrinolytic components, including PAI-1, play an important role not only in thrombolysis and proteolysis but also in the immune response by changing the balance between the Th2 reaction and the Th1 reaction.

It was thought that there seemed to be no relation between allergic disease and fibrinolytic components. Therefore, until now, a relation of fibrinolytic components in allergic rhinitis has been little studied. From the standpoints of fibrinolysis, immunology, allergology, and rhinology, a history of the study in this area is short. Thus, there are many points we must elucidate in the future. Figure 2 provides information derived from conventional and current knowledge regarding the mechanism of fibrinolytic factors in the nasal mucosa.

Moreover, from now on we may use fibrinolytic components to intervene in the mechanisms involved in disorders of the nasal mucosa. For example, we may control a local allergic reaction in nasal mucosa by using gene transfection or RNA interference (RNAi). Although the relation between the fibrinolytic system and the immune system is still unclear, it will be useful to study it for new pathological elucidation.

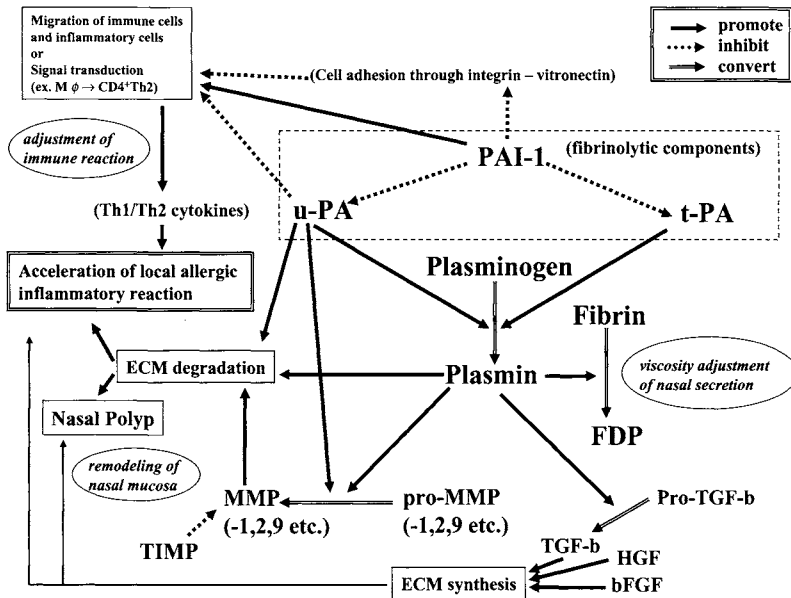


Fig. 2. Hypothesis of the mechanism by which fibrinolytic components influence nasal mucosa. PAI, plasminogen activator inhibitor; u-PA, t-PA, urokinase-type and tissue plasminogen activators; ECM, extracellular matrix; FDP, fibrin degradation product; MMP, metalloproteinase; TGF- β , transforming growth factor- β ; HGF, hepatic growth factor; bFGF, basic fibroblast growth factor; TIMP, tissue inhibitor of MMPs

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