Rhinology, 21, 217-222, 1983

Proteinases and their inhibitors in human nasal mucus

K. Hochstraßer, Münich, West-Germany

The defence mechanism of the human nasal mucosa and its secretion depends on the interaction of their physical, cellular, and immunological constituents. An accumulation of neutrophilic and eosinophilic polymorphonuclear (PMN) leucocytes and mononuclear phagocytes is characteristic for this system. Leucocytes carry many digestive enzymes in their compartmental vesicles, the so-called lysosomes. They are responsible for the metabolism of phagocytated particles and materials which are then excreted from the cell by active transport or by cellular lysis. Lysosomal proteinases from PMN-leucocytes are known to be potent pathogens covering reversible or irreversible disorder and destruction of nasal mucous membranes and cells. They also liberate vasoactive peptides locally, thus disturbing the complement system, degrading and inactivating immunoglobulins, and altering the viscosity of the mucus.

Although normal nasal mucus always contains phagocytes, no active lysosomal proteinases have so far been found. Their proteolytic activity is inactivated and compensated by antiproteolytic capacity of normal nasal mucous secretion which is about as high as in serum.

This paper reports on such proteinase inhibitors of human nasal mucus fighting exogenous and endogenous proteinases from PMN-leucocytus, both in vivo and in vitro.

A. Proteinase inhibitors in human nasal mucus

In vitro, proteinase inhibitors are generally detectable with bovine pancreatic trypsin and a synthetic chromogenic substrate. Besides this test for antitryptic activity in nasal mucus other proteinase inhibitors such as α_1-PI (= α_1-antitrypsin), α_2-macroglobulin, α_1-X (= α_1-antichymotrypsin), ITI (= inter-α-trypsin-inhibitor), antithrombin III, and C_1-inactivator, all well characterized from serum, are demonstrable immunologically. Among those only α_1-PI is present in large amounts in nasal mucus. This fact leads to the conclusion that this inhibitor constitutes the main antitryptic capacity of nasal mucus.

Paper presented at the 9th Congress of the European Rhinologic Society and 3rd ISIAN, Stockholm (Sweden), September, 1982.

Acid treatment of nasal mucus irreversible inactivates α_1-PI. The deproteinized supernatant, however, still contains antitryptic activity due to acid resistant antitryptic inhibitors other than derived from serum. Purification by affinity chromatography on immobilized trypsin showed this antiproteolytic activity to inactivate trypsin and chymotrypsin, as well as elastase and cathepsin G from PMN-leucocytes. Similarly purified inhibibtory systems were found also in bronchial secretion, in seminal plasma, and in cervical mucus. They were, therefore, considered to be physiological antagonists for leucocytic proteinases in mucous secretion. More recently, isolation of these inhibitors by different methods revealed distinguishable amino acid compositions, immunological cross reactivity with humoral acid-lable ITI, and also inhibition of pancreatic elastase.
A reinvestigation of these contradictory results finally lead to at least four acid resistant, low-molecular-weight inhibitors. Three of them are specific secretory inhibitors which are synthetized in submucous glands. The fourth one was identified to be HI-14 (= the inhibitorily active part of humoral ITI, m.m. about 14,000) as liberated from its carrier and transsudated from serum.
Although differentiable by their specific inhibitory properties, all inhibitors interact via their methionine residue in their antielastolytic site with elastase from PMN-leucocytes. This was shown by animo acid sequence determination and – indirectly – in experiments for selective inactivation of the antielastolytic property by means of oxidation of this methionine residue. Table 1 lists these inhibitors as differentiated by their specificity against several proteinases.

B. Function of the inhibitory system

An involvement of the identified inhibitors in proteinase neutralization can be elucitated by demonstration of inhibitor – proteinase – complexes in nasal mucus:
Low-molecular-weight proteinase inhibitors are released from such complexes in an active form by acid treatment whereas α_1-PI is not stable against acid in neither free nor complexed form. Thus a quantification of free and complexed secretory inhibitors is only possible when the exact content of free, inhibitorily active α_1-PI is known prior to acidification of the mucus.
Earlier experiments determined the content of α_1-PI immunological methods (Laurell, Ouchterlony) which provided that α_1-PI is present in an inhibitorily active form in any case. This assumption, however, was shown by us to be incorrect since inhibitorily active and inhibitorily inactive α_1-PI as well exhibit identical electrophoretic mobility and immunological antigenicity against α_1-PI-specific antibodies. Immunodiffusion alone does not distinguish between active and inactive α_1- PI. Therefore, α_1-PI determined immunologically in native nasal mucus does not represent totally the antitryptic activity.
Figure 1 shows electroimmunodiffusion patters of serum (a) and nasal mucus (b)

with antibodies against α_1-PI. A second anodic peak in nasal mucus indicates small amounts of α_1-PI complexed already in vivo (b). When serum is titrated with trypsin (c) or elastase (not shown) α_1-PI is transformed and complexed almost completely thus yielding a large anodic peak and indicating inhibitorily ac-

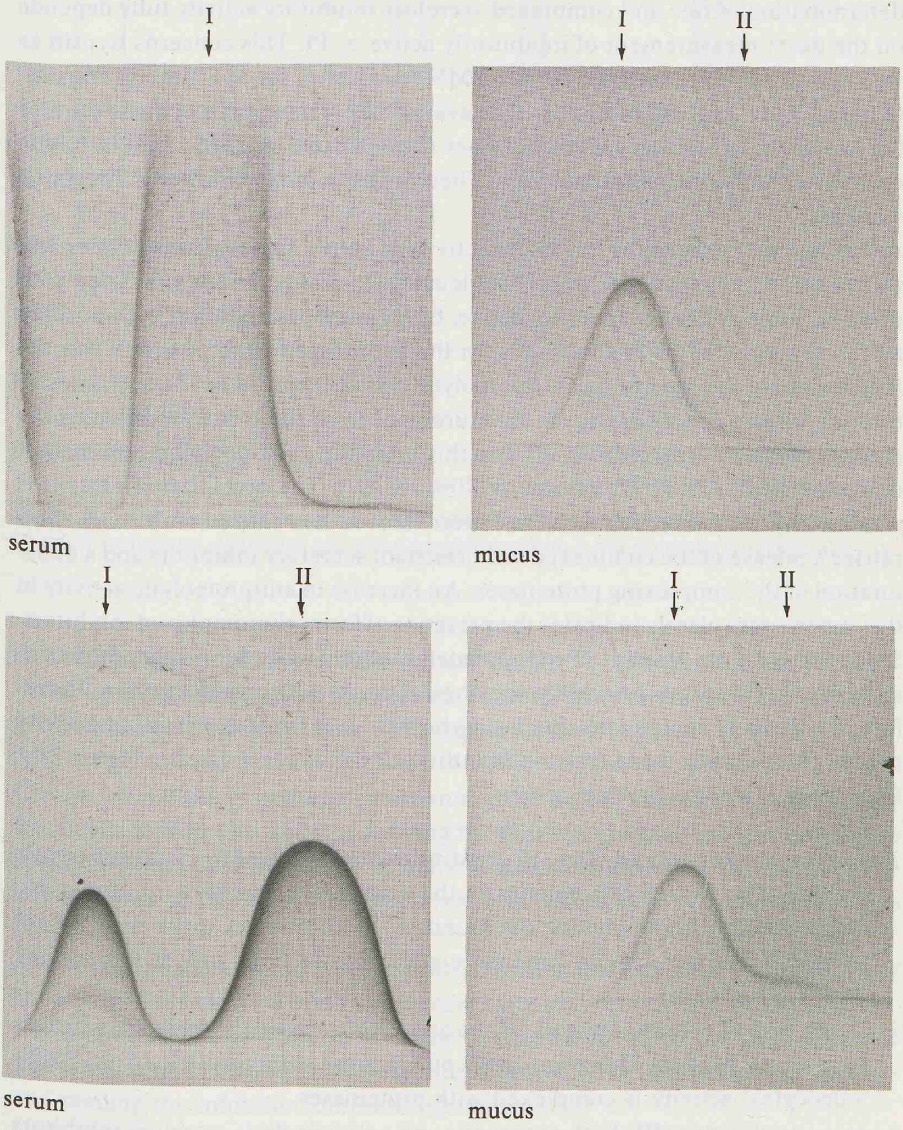

Figure 1. Patterns obtained on crossed immunoelectrophoresis of intravasal and extravasal α_1-PI and after titration with trypsin. I = α_1-PI, II = α_1-PI-trypsin complex. Mucus α_1-PI practically forms no complex.

tive α_1- PI prior to the titration with these enzymes. In contrast, nasal mucus yields only small anodic peaks in this experiment (d) indicating large amounts of inhibitorily inactive α_1-PI in native nasal mucus.

These results show that earlier quantifications of the antitryptic activity in nasal mucus due to α_1-PI were misleading and overvaluated. Furthermore, an exact determination of free and complexed secretory inhibitory activity fully depends on the exact measurement of inhibitorily active α_1-PI. This concerns trypsin as well as elastase and cathepsin G from PMN-leucocytes, the most interesting proteinases in the total inhibitor pool. The availability of new and specific substrates for leucocytic proteinases allow a proper characterization of the antielastolytic capacity in individual nasal secretion. These investigations followed a three-step strategy:

A first step determined the inhibitory activity of native mucus against trypsin and elastase from pancreas and from PMN-leucocytes. Then, the sample is depleted of α_1-PI, inhibitorily active and inactive, by immunoprecipitation with purified antibodies directed to this inhibitor. In the centrifuged supernatant of this depleted sample the remaining antiproteolytic activity represents the active secretory inhibitors. The difference in measurement from these two steps yields the amount of inhibitorily active α_1-PI. In a third step the α_1-PI-depleted supernatant is treated with acid (1/10 volume of 70% $HClO_4$). The precipitated protein is removed by centrifugation and the supernatant re-neutralized with KOH. This causes a release of the complexed, acid resistant secretory inhibitors and a denaturation of the complexing proteinases. An increase in antiproteolytic activity in this supernatant clearly indicates the presence of formerly complexed inhibitors. Since the absolute amount of transsudated humoral and of mucous proteins in nasal secretions varies in a wide range, these data are not very informative. Therefore, the free and complexed antielastolytic as well as the free elastolytic activity based on their molar ratios (related to antitryptic activity) are listed in Figure 2 for four characteristic cases (out of 60):

1. In normal nasal mucus (I) α_1-PI constitutes about 30% of the total antitryptic activity. The rest of 70% belongs to the acid-stable secretory inhibitors. No complexed antitryptic activity is found. α_1-PI constitutes more than 50% of inhibitory activity against pancreatic elastase, but only 30% of this against leucocytic elastase.
2. In the case of chronic sinusitis (II) no antitryptic activity is involved in proteinase neutralization. However, about 60% of total antielastolytic (from PMN-leucocytes) activity is complexed with proteinases.
3. In acute sinusitis (III) both antitryptic and antielastolytic secretory inhibitors are complexed up to 50% of the total inhibitor pool. Characteristically, no inhibitorily active α_1-PI is present in these secretions.

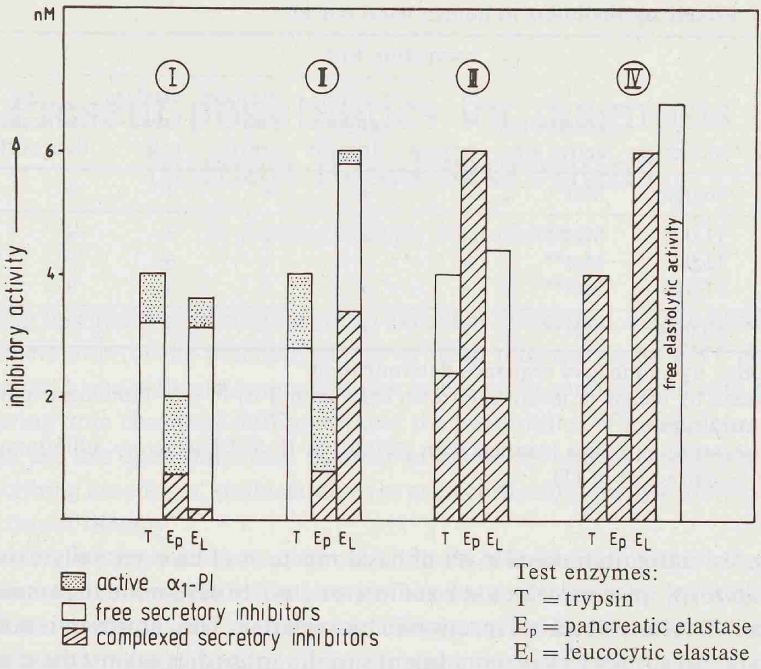

Figure 2. Free and complexed proteinase inhibitors and free elastolytic activity in selected nasal secretions. Activities are given in nM based on 4 nm of total antitryptic activity.

4. Purulent secretions from submaxillary sinus (IV) do not contain any free inhibitors at all; they all are complexed. There is, however, high elastolytic activity, obviously released from PMN-leucocytes.

Clearly, all inhibitors in human nasal mucus are potent antagonists, neutralizing leucocytic proteinases and thus serving the defence mechanism. The amount of free and complexed inhibitors in individual nasal mucus is a measure for the effectiveness of this antiproteolytic system in connection with the proteinases produced.

This model becomes more complicated from the knowledge that the inhibitorily active site of the elastase inhibitors in nasal mucus is represented by methionine. The sulfur in this CH3-amino acid can be oxidized to its sulfoxide and/or sulfonyl derivative which is then incapable of interacting with the catalytic site of elastase and making the inhibitor (inhibitorily) inactive.

Such oxidation of methionine (and inactivation of the inhibitor) can occur by means of peroxidases from myelocytes already in vivo. This is stated by the above mentioned results from immunoelectrophoresis experiments. Intravasal α_1-PI is

Table 1. Proteinase inhibitors in human nasal mucus.

inhibitor	mol.mass	antielastol. active site	competing with				
			trypsin	chymo-trypsin	elastase pan-creatic	elastase leuco-cytic	cathepsin G leucocytic
α_1-PI	56,000	Met*	+	+	+	+	+
SI-I^+	11,000	Met**	+	+	–	+	+
SI-II	11,000	Met**	–	–	+	+	–
SI-III	11,000	Met**	+	+	+	+	+
HI-30	30,000	Met**	+	+	–	+	–

* identified by animo acid sequence determination

** suggested by means of inactivation with chloramin T α_1-PI = α_1-Proteinase inhibitor = α_1-Antitrypsin;
SI-I^+ secretory inhibitor identical with HUSI-I, SI-II, SI-III secretory inhibitors;
HI-30 = active part of ITI.

inhibitorily active, extravasal α_1-PI of nasal mucus is al least partially oxidized and inhibitorily inactive. It would be of interest, too, to determine the amount of secretory inhibitors which are inactivated by oxidation. This, however, is not possible so far because of lack of monovalent specific antibodies against these secretory inhibitors.

There seems to exist a dynamic equilibrium between proteinase inhibitors, proteinases, and peroxidases in nasal mucus. Especially the latter enzymes are released from granulocytes and myelocytes, respectively, which are stimulated by inflammatory agents. In steady state, i.e. normal nasal mucus, the proteinases are compensated by several endogenous inhibitors. The equilibrium may be disturbed by excess of proteinases and/or by oxidative inactivation of elastase inhibitors. A substitution of inactivated inhibitors by exogenous proteinase inhibitors is proposed and should effect such a nasal mucus disorder. They should possess an animo acid other than oxidable methionine in their antielastolytic active site and strongly interact with elastase from PMN-leucocytes.

Prof. Dr. K. Hochstraßer
Univ. HNO-Klinik
Marchioninistrasse 15
D-8000 München
West-Germany