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Proteolytic activity and serum protease inhibitors in nasal secretions from adult patients with common colds

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SUMMARY

Proteolytic activity and concentrations of serum protease inhibitors were measured in nasal secretions collected from 14 adult patients (6 males and 8 females) with common colds. Elastase concentration and fibrinolytic activity increased about three days after the onset of the colds, and there was a significant correlation between both values ($p < 0.01$). Trypsin-like protease activity was very low. Of all serum protease inhibitors, inter-α-trypsin inhibitor could not be detected, and α_2-macroglobulin could be detected in only two cases. Variation of α_1-antitrypsin value was very similar to that of α_1-antichymotrypsin, and there was a significant correlation between α_1-antitrypsin and elastase ($p < 0.001$). Phoretic patterns of crossed immuneelectrophoresis revealed the presence of α_1-antitrypsin-protease complex. α-protease inhibitors are major serum protease inhibitors in nasal secretions of persons with colds, and inhibit excess proteolytic activity of serine proteases. This protection is considered to be one of the major factors in preventing irreversible mucosal change.

INTRODUCTION

The common cold is a typical acute infection in the upper respiratory tract. In nasal secretions collected from patients with colds, a number of granulocytes can be detected by cytologic examination. During colds, a large amount of lysosomal proteases are released from infiltrating cells into nasal secretions. Granuloproteases have significant roles in the inflammation, i.e. phagocytosis and breakdown of inflammatory products into micromolecules (Liebermann and Gaward, 1971), and activation of chemical mediators and proenzymes (Johnson et al., 1976). Their proteolytic activities are controlled by saturation of serum protease inhibitors (Heimburger et al., 1971) or low molecular weight trypsin inhibitors, so called antileukoprotease (ALP) (Kueppers and Bromke, 1983).

In this study, proteolytic activity and the concentration of various serum protease

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inhibitors in nasal secretions were measured to assess the relationship between granuloprotease activity and serum protease inhibitors during infections of common colds.

MATERIAL AND METHODS

Nasal secretions were collected from 14 adult patients with colds (6 males and 8 females) by direct aspiration. Diagnoses were established on rhinoscopic findings and clinical manifestations judged by one of the authors (Y.H.). Nasal eosinophilia were examined by nasal smear cytology, using Hansel's staining to exclude patients with nasal allergy from this study. Collected samples were diluted three times with 1/15M phosphate buffered saline, pH 7.2 and stirred continuously for 3 hrs at 4 °C. After centrifugation at 10,000 rpm for 20 min at 4 °C, the supernatant fluids were recovered and passed through filters under slightly positive pressure. Clear filtrates were recovered and stored at − 20 °C until analysis.

Concentration of elastase and cathepsin B

Concentration of neutrophilic elastase and cathepsin B from alveolar macrophage was measured by electroimmunodiffusion (Laurell, 1969), using commercial rabbit antibodies (Serotec Ltd., Oxon), and was expressed by the height (mm) of the precipitin line.

Assay for fibrinolytic activity

Fibrinolytic activity was measured by the fibrin plate method (Astrup and Mullertz, 1952), using plasminogen rich bovine fibrinogen (Daiichi Kagaku, Tokyo). High molecular weight urokinase (Green Cross, Osaka) was used as the standard reference, and fibrinolytic activity was expressed by international units (IU)/ml of urokinase.

Assay for hydrolytic activities of trypsin-like proteases and cathepsin B-like thiol proteases

Hydrolytic activities of trypsin-like proteases and cathepsin B-like proteases were measured by a fluorometric assay, using relatively specific MCA-peptides (Protein Research Foundation, Osaka); Bz-Arg-MCA for trypsin (Kanaoka et al., 1980) and Z-Phe-Arg-MCA for cathepsin B (Barrett, 1980). Hydrolytic activity was expressed by the relative fluoro unit (Hamaguchi et al., 1983).

Concentrations of serum protease inhibitors

Concentrations of serum protease inhibitors (α_1-antitrypsin, α_1-antichymotrypsin, inter-α-trypsin inhibitor and α_2-macroglobulin) were measured by electroimmunodiffusion, using commercial rabbit antibodies (DAKO, Copenhagen). To clarify the presence of α_1-antitrypsin-protease complex, crossed immunoelectrophoresis was performed (Laurell, 1956).

RESULTS

Protease assays

All the results of our protease assays in each case are shown in Figure 1. Elastase concentration increased a few days after the onset of the cold and was relatively constant during the further days. Fibrinolytic activity became apparent about two

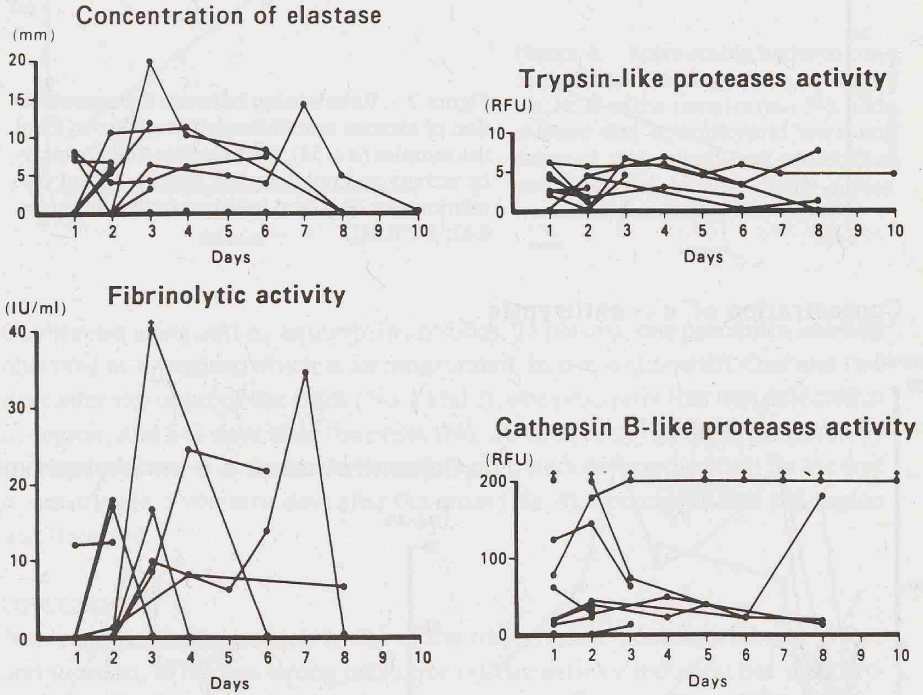

Figure 1. Elastase concentration, fibrinolytic activity and hydrolytic activities of trypsin-like or cathepsin B-like proteases in each case during a cold: elastase concentration and fibrinolytic activity increased a few days after the onset, and elastase concentration was relatively constant during a cold compared to varied fibrinolytic activity. Trypsin-like proteases activity was very low and stable, but cathepsin B-like proteases activity was very varied in individual cases.

days after the onset and was varied in individual cases. In some cases, little elastase and fibrinolytic activity could be detected. Although cathepsin B could not be detected by electro-immunodiffusion in all the samples, hydrolytic activity of cathepsin B-like thiol proteases was detected, and had great individual variations. On the other hand, hydrolytic activity of trypsin-like proteases in all cases was under 10 RFU. Figure 2 shows the relationship between elastase concentration and fibrinolytic activity in nasal secretions of all the cases. Although in 29 of the samples ($n = 54$), both elastase and fibrinolytic activity could not be detected, there was a significant correlation between both values ($r = 0.42$, $p < 0.01$).

Protease inhibitor assays

Concentrations of α_1-antitrypsin and α_1-antichymotrypsin in nasal secretions are shown in Figure 3. Of all protease inhibitors measured in this study, inter-α-tryp-

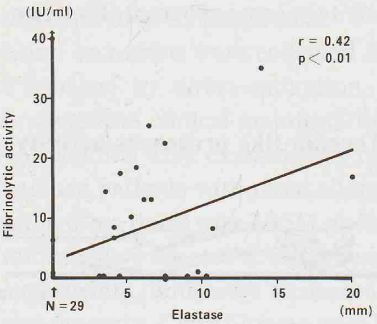

Figure 2. Relationship between the concentration of elastase and fibrinolytic activity: in 29 of the samples ($n = 54$), both elastase and fibrinolytic activity was not detected, but significant correlation was observed between both values ($r = 0.42$, $p < 0.01$).

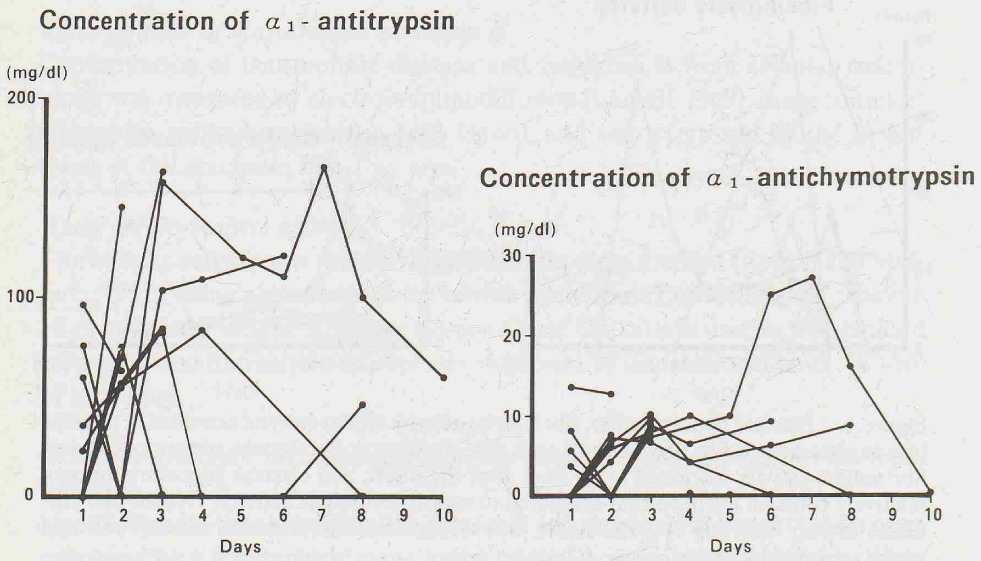

Figure 3. Concentrations of α_1-antitrypsin and α_1-antichymotrypsin in each case during a cold: the values of both inhibitors increased about two days after the onset and both inhibitors showed similar variations.

sin inhibitor could not be detected, and α_2-macroglobulin was detected in only two cases. Variation of α_1-antitrypsin value was very similar to that of α_1-antichymotrypsin. Figure 4 shows the relationship between concentrations of elastase and α_1-antitrypsin. Although both elastase and α_1-antitrypsin could not be detected in 26 of the samples, there was a significant correlation between both values ($r = 0.645$, $p < 0.001$). Figure 5 shows phoretic patterns of crossed immuno-elec-

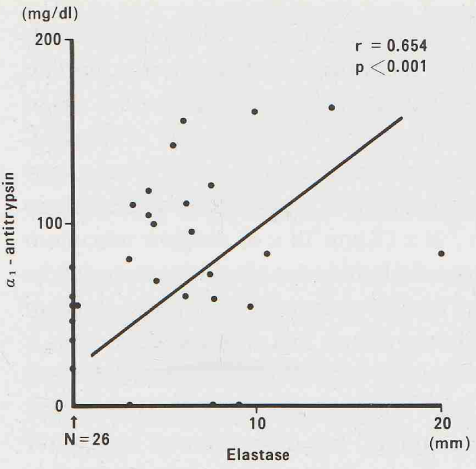

Figure 4. Relationship between concentrations of elastase and α_1-antitrypsin: in 26 of the samples ($n = 54$), both elastase and α_1-antitrypsin were not detected, but significant correlation was observed between both values ($r = 0.654$, $p < 0.001$).

trophoresis using anti-α_1-antitrypsin antibody. In plasma, one precipitin line was observed at α_1-region, which is an unsaturated, free α_1-antitrypsin. One and two days after the onset of the colds (No. 1 and 2), one precipitin line was detected at α_1-region; and five days after the onset (No. 3), two precipitin lines, indicated by short arrows, one at α_2 and the other at β-region, were detected, except for the free α_1-antitrypsin. Even nine days after the onset (No. 4), a precipitin line at β-region was detected.

DISCUSSION

Neutrophilic elastase which is one of the major serine granuloproteases (Werb and Gordon, 1975) has strong elastofibrinolytic activity inducing not only proteolysis of inflammatory products but also tissue damage (Plow, 1980). Unfortunately, we could not measure elastase activity in nasal secretions. But, the close relationship between elastase concentration and fibrinolytic activity suggests that a large amount of active neutrophilic elastase are released from granulocytes into nasal secretions during colds, and that elastase concentration in nasal secretion is an useful index reflecting elastase activity. As polymerized fibrin nets occupy a major portion of the gel-phase of nasal secretions, high fibrinolytic activity at the date with apparent hyperrhinorrhoe is very suitable for destruction and elimination of nasal secretions. But, high elastase concentration in nasal secretions would become a disadvantageous factor leading to mucosal damage, unless elastase activity could be controlled properly by protease inhibitors.

It is interesting that cathepsin B was not detected by the immunological method, while high activity of cathepsin B like thiol proteases was detected, using synthetic substrates. This indicates that little cathepsin B which is widely distributed in alveolar macrophages (Lesser et al., 1983) exists in nasal secretions. High activity

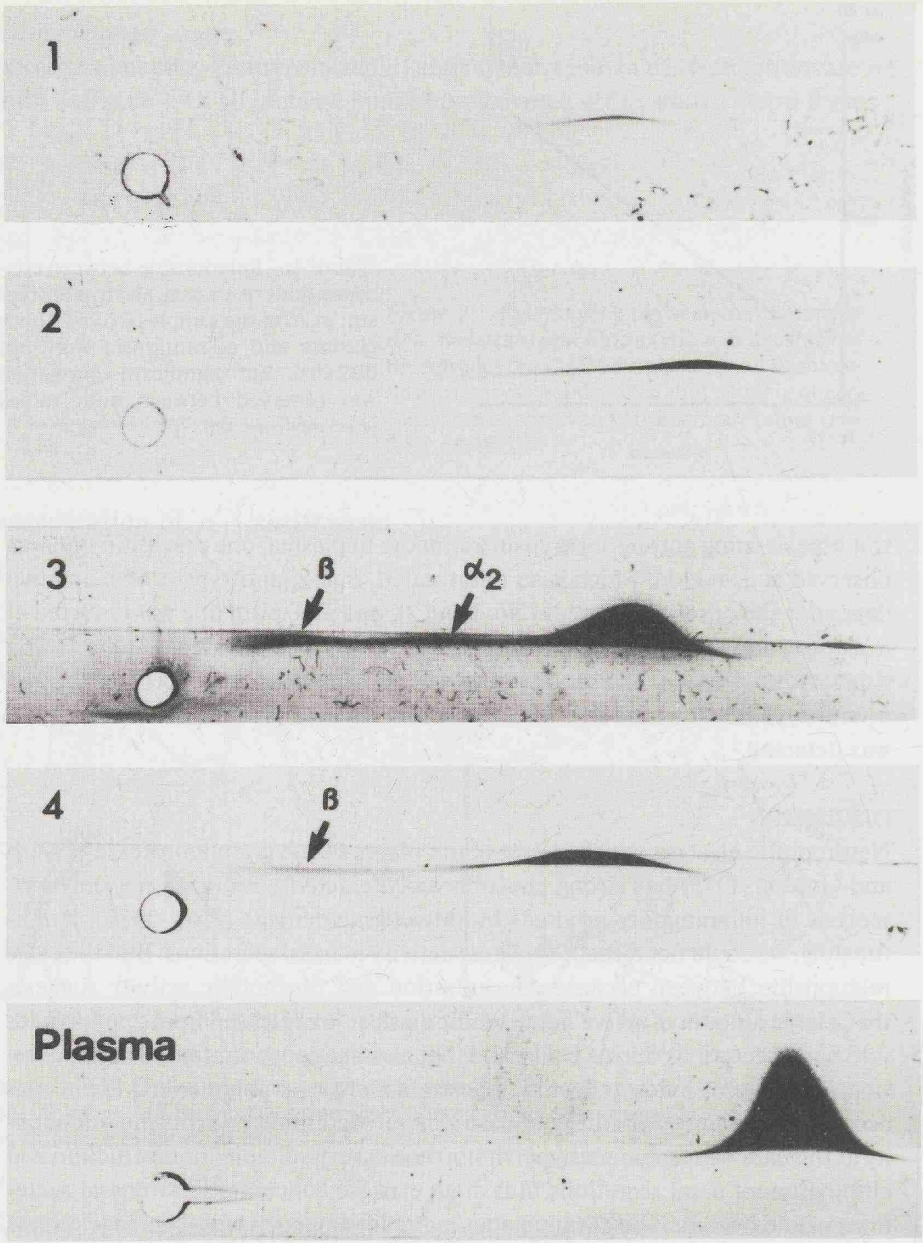

Figure 5. Phoretic patterns of crossed immunoelectrophoresis using anti α_1-antitrypsin antibody: in plasma, single precipitin line could be observed at α_1-region. One or two days after the onset of the cold (no. 1 and 2), single precipitin line could be detected at α_1-region. Five days after the onset (no. 3), two precipitin lines indicated by short arrows could be detected except for free α_1-antitrypsin. Even 9 days after the onset (no. 4), a precipitin line at β-region could be detected.

of cathepsin B-like thiol proteases could be a significant factor in mucosal inflammation, as lysosomal thiol proteases (cathepsin B, H and L) have important roles in the pathogenesis of chronic bronchitis (Orlowski, 1981).

Absence of inter-α-trypsin inhibitor and detection of α_2-macroglobulin in only two cases of which clinical symptoms were the severest of all the cases could be explained by the difficulty of their leakage into nasal secretions due to their high molecular weights, 16×10^4 and 82×10^4, respectively. In the two cases, selectivity of vasopermeability in subepithelial vessels would be damaged remarkably. In almost all cases, both α_1-antitrypsin and α_1-antichymotrypsin were detected. Both α_1-protease inhibitors had similar variations during infections with colds, due to their similar molecular weights (5.4×10^4 and 6.7×10^4). α_1-antichymotrypsin can inhibit chymotrypsin-like protease such as cathepsin G (Mounter and Atiyeh, 1960), and α_1-antitrypsin can inhibit various serine proteases; elastase, trypsin, glandular kallikrein, etc. Moreover, low molecular weight trypsin inhibitors can inhibit granulocytic elastase more effectively than α_1-antitrypsin, and have a significant local protective function (Fryksmark et al., 1984). Significant correlation between concentrations of elastase and α_1-antitrypsin suggests that leakage of α_1- antitrypsin into nasal secretions would increase in accordance with the release of neutrophilic proteases. The presence of α_1-antitrypsin-protease complex in nasal secretions during colds, proven by crossed immunoelectrophoresis means that free α_1-antitrypsin can inhibit proteolytic activity of serine proteases by saturating these proteases until the recovery states of the colds.

It is concluded that high fibrinolytic activity in nasal secretions during colds is attributed mainly to neutrophilic proteases. Besides low molecular weight trypsin inhibitors, α_1-protease inhibitors have an important role in the correlation of "imbalance" between serine proteases and inhibitors. This protection is one of the major factors in preventing mucosal change during colds.

RÉSUMÉ

Les auteurs ont mesuré, dans les secrétions nasales prélevées chez 14 patients adultes (6 hommes et 8 femmes) présentant des rhumes ordinaires, l'activité protéolytique et les concentrations d'inhibiteurs de la sérum-protéase. Environ trois jours après le commencement des rhumes, la concentration d'élastase et l'activité fibrinolytique augmentèrent et il y eut une corrélation significative entre les deux valeurs ($p < 0.01$). L'activité de la protéase, analogue à celle de la trypsine, fut très basse. De tous les inhibiteurs de la sérum-protéase, l'inhibiteur de la trypsine inter-α n'a pu être signalé, la macroglobuline-α_2 a pu être signalée dans deux cas seulement. La variation de la valeur d'antitrypsine-α_1 fut bien similaire à celle de l'antichymotrypsine-α_1 et il y eut une corrélation significative entre l'antitrypsine-α_1 et l'élastase ($p < 0.001$). La présence du complexe de protéase-antitrypsine-α_1 se manifeste par des patrons phorétiques d'immuno-électrophorèse croisée.

Dans les secrétions nasales de personnes présentant des rhumes, les inhibiteurs de la protéase-α_1 sont d'importants inhibiteurs de la sérum-protéase, freinant l'excès d'activité protéolytique de protéases sériques. C'est cette protection qui est considérée comme l'un des facteurs les plus importants dans la prévention de changements irréversibles de la muqueuse.

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