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Plasma α_1-antichymotrypsin Activity in Renal Diseases of Childhood

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Summary. **To clarify the changes in plasma proteinase inhibitors in renal diseases, we measured the activity of plasma α_1-antichymotrypsin (α_1-Achy) under such conditions. The level of plasma α_1-Achy activity was found to be significantly lower in idiopathic nephrotic syndrome (INS) at relapse than in INS during remission, as well as in patients with chance proteinuria and/or hematuria (CPH) and in normal control subjects. The α_1-Achy activity in CPH was significantly elevated compared with that in INS ($p<0.001$) and normal controls ($p<0.001$). A significant inverse correlation was noted between the plasma α_1-Achy activity and the inhibitory effect of plasma on lymphocyte blastogenesis induced by Concanavalin A. Although the mechanism is unclear, the elevation of α_1-Achy in CPH (implicated in various types of glomerulonephritis) may be one of the responses of the body to counteract inflammatory processes by inactivating proteinase(s) released from infiltrating and/or proliferating cells in the glomeruli. The decreased plasma α_1-Achy level in INS may contribute to the depressed cellular immunity seen in INS.**

INTRODUCTION

There are at least seven major proteinase inhibitors in human plasma.[1] Of these, α_1-antichymotrypsin (α_1-Achy) is specific proteinase inhibitor of chymotrypsin-like proteinases, forming stable complexes with bovine and human chymotrypsin[2-4] as well as with human neutrophil cathepsin G.[1,5] In general, α_1-Achy has been seen as one of the acute phase reactants, and has been reported to be elevated in Crohn's disease, ulcerative colits,[6] and some types of cancer,[1] suggesting a wide variety of roles for this inhibitor.

On the basis of studies on the association rate of the inhibitor with chymotrypsin and cathepsin G, control of the activity of cathepsin G in neutrophils is now considered to be a more important function of α_1-Achy than to inactivate pancreatic chymotrypsin.[2,7] In addition, α_1-Achy has been reported to have a role in controlling chromatin through its ability to bind to DNA[8] and to take part in the immunoregulatory system through its action on the natural killing and antibody-dependent cytotoxic activity of lymphocytes.[9] These reports have prompted interest in the association of the inhibitor with various diseases. The purpose of this study was to clarify the changes in plasma α_1-Achy in renal disease and to discuss their clinical significance.

SUBJECTS AND METHODS

Twenty-two pediatric patients with idiopathic nephrotic syndrome (INS), 23 patients with chance proteinuria and/or hematuria (CPH), and 25 normal control subjects seen on routine examination were included in this study. The patients with CPH had been discovered during routine school screening for renal disease, and the abnormal urinary findings of hematuria and/or proteinuria had persisted for more than one year. Ten of the 23 patients had undergone renal biopsy, and the biopsy-proven diagnoses were as follows: membranoproliferative glomerulonephritis (2 cases), mild to moderately proliferative glomerulonephritis (6 cases), and IgA nephropathy (2 cases). Although the remaining 13 patients had not undergone renal biopsy, they were thought to have

varying degrees of proliferative glomerulonephritis on the basis of their normal complement levels and their clinical courses. Plasma samples were prepared from heparinized blood and stored at −80℃ until assay.

The α_1-Achy activity was determined to be the inhibitory activity on boving chymotrypsin using the method by Travis and Morii[1] with some modifications. Since plasma contains other inhibitors such as α_2-macroglobulin (MW: 725,000) or α_1-proteinase inhibitor (MW: 54,000) that can inactivate chymotrypsin, we examined plasma fractions for α_1-Achy activity. A sample of 1.0 ml of plasma was applied to a Sephadex G-200 column, and the fractions were examined for their inhibitory activity on chymotrypsin. The relationship between the inhibitory activity of plasma on chymotrypsin and the plasma concentrations of α_2-macroglobulin, α_1-proteinase inhibitor were examined using a single radial immunodiffusion method.

The inhibitory activity of plasma on lymphocyte transformation induced by Concanavalin A was determined by the method by Thurman et al.[10] as follows: Plasma was obtained from the heparinized venous blood of patients and normal volunteers. Lymphocytes of normal volunteers were collected from buffy-coat cells on a Ficoll/Hypaque gradient. The blastogenic response to Concanavalin A was determined by the incorporation of ^{3}H-thymidine in lymphocytes cultured in microtiter plates. An amount of 40 μl of plasma from either a patient or a normal volunteer was added to the culture medium and incubation was performed at 37℃ for 96 h. The inhibitory activity of the patients' plasma was calculated as

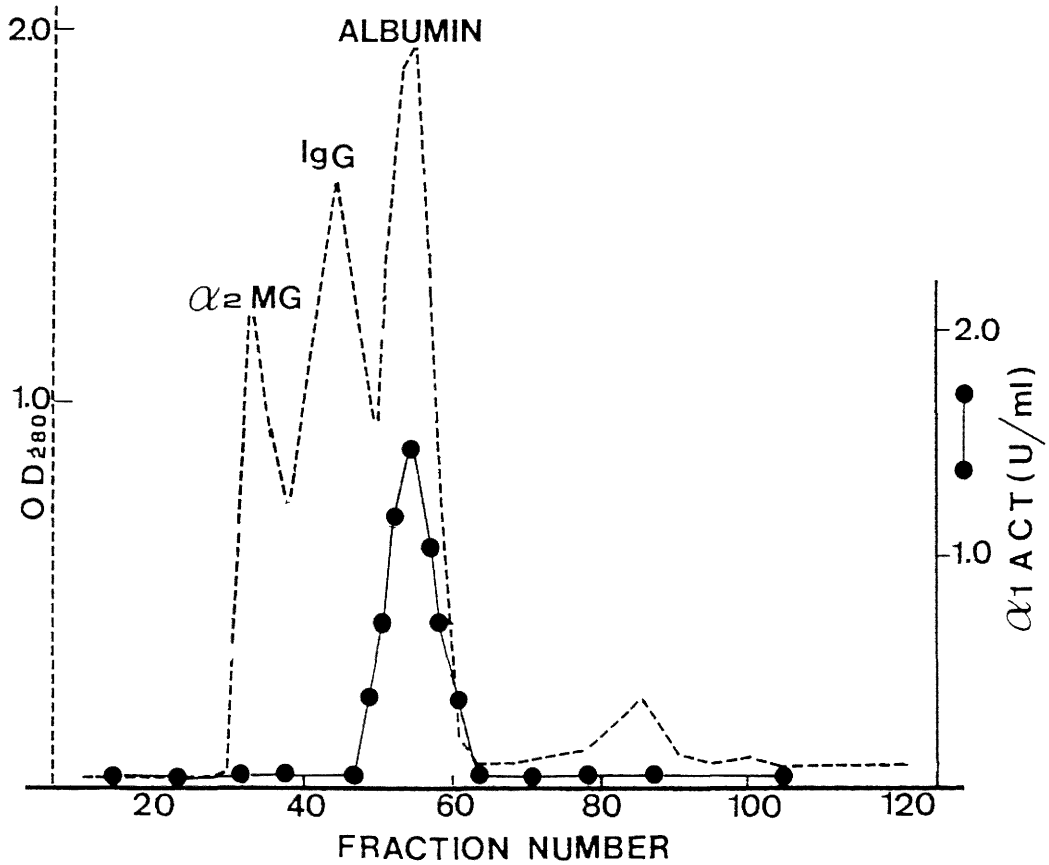

Fig. 1. Sephadex G-200 gel-chromatography of plasma. A sample of 1.0 ml plasma was applied to a Sephadex G-200 column eluted with 0.01 M sodium phosphate buffer, pH 7.4. The dashed line represents protein concentration expressed by OD280, and closed circle shows the inhibitory activity of the plasma fractions on chymotrypsin. A single peak of the inhibitory activity was revealed only in the fraction No. 48-62 coinciding with α_1-Achy. The void volume fractions containing α_2-macroglobuln did not inhibit chymotrypsin activity.

follows: A=DPM in the culture medium with each patient's plasma; B=CPM in the culture medium with control plasma; Inhibitory activity of plasma on lymphocyte blastogenesis=[(A−B)/B]%. Statistical analysis was performed using the unpaired Student's t-test.

RESULTS

1. *Sephadex G-200 gel chromatography of plasma*

A single peak of inhibitory activity on chymotrypsin was demonstrated in the plasma fractions (No. 46-62) coinciding with the molecular weights of α_1-Achy (MW: 68,000) and albumin (MW: 66,000), but not with the α_1-proteinase inhibitor (MW: 54,000) (Fig. 1). The void volume fractions (No. 30-38), containing α_2-macroglobulin, did not cause any inhibition of chymotrypsin activity (Fig. 1).

2. *α_1-Achy activity in renal disease*

Patients with CPH had significantly elevated plasma α_1-Achy levels as compared with those in INS and control subjects (Fig. 2). In contrast to CPH, the mean plasma α_1-Achy was significantly decreased in patients with INS at relapse (Fig. 2).

3. *Relationship between the inhibition of chymotrypsin activity and various plasma factors*

There was no significant correlation between the inhibitiion of chymotrypsin activity and the plasma α_2-macroglobulin concentration. A significant correlation was noted between chymotrypsin activity inhibition and the α_1-proteinase inhibitor concentration ($r=0.584$, $p<0.01$, $n=20$). Of interest was a significant inverse correlation between α_1-Achy levels and the inhibitory activity of plasma on lymphocyte blastogenesis induced by Concanavalin A ($r=-0.37$, $p<0.01$, $n=50$).

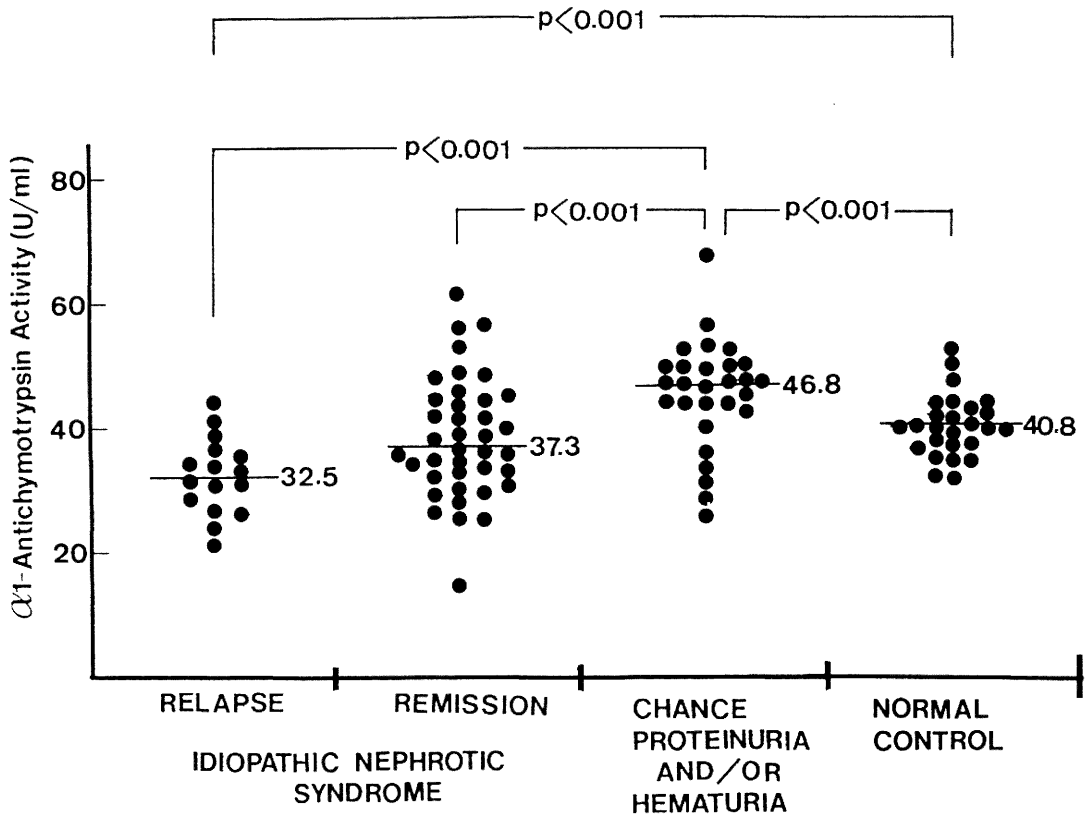

Fig. 2. α_1-antichymotrypsin activity in renal diseases. α_1-Achy activity activity was 32.5±5.9 U/ml in indiopathic nephrotic syndrome (INS) at relapse, 37.3±9.5 U/ml in INS in remission, 46.8±6.0 U/ml in chance proteinuria and/or hematuria (CPH), and 40.8±5.0 U/ml in normal controls.

DISCUSSION

Recently, the role of proteinases and proteinase inhibitors has been studied in relation to the pathogenesis of renal disease.[11-15] It is well known that proteinases are involved in the pathogenesis of inflammatory diseases by participating in the activation of mediator systems and by producing tissue injury.[11] Furthermore, an attempt to treat renal disease with synthesized proteinase inhibitor has been made successfully in animal experiments[11] and in a clinical trial.[16] This has promoted interest in the plasma proteinase inhibitor levels in various renal diseases. Several studies on plasma proteinase inhibitors have been performed,[17-19] but little is known about plasma α_1-Achy levels in renal disease.

In the present study we demonstrated a significant elevation of α_1-Achy levels in patients with CPH compared with INS patients and control subjects. In contrast to CPH, in which a variable degree of proliferative change was shown or thought to be occurring in glomerular tissue, the patients with INS at relapse had a significantly low plasma level of α_1-Achy. Despite its name, the main function of α_1-Achy is now considered to be the control of cathepsin G in human neutrophils rather than chymotrypsin.[2-7] Proteinases can be direct effectors of tissue injury upon release from leukocytes[11] or proliferating mesangial cells;[12,14] the elevation of plasma inhibitor level may therefore be considered to indicate that an inflammatory process is still occurring in glomerular tissue.

For the determination of α_1-Achy, we used the method by Travis and Morii[7] with some modifications. Since the assay was based on the inhibition of chymotrypsin activity resulting from complex formation during preincubation with the inhibitor, we examined plasma fractions for their inhibitory effect on chymotrypsin activity. Gel chromatography (Sephadex G-200) revealed that inhibitory activity for chymotrypsin was present only in the fractions coinciding with α_1-Achy. Although α_2-macroglobulin has a wide range of activity against various proteinases including chymotrypsin, inhibition of chymotryspin[1] was not demonstrated with the void volume fractions, and no significant relation was shown between the inhibitory activity and plasma concentrations of α_2-macroglobulin. These observations support the contention that the measured inhibition of chymotrypsin activity is attributable to α_1-Achy.

One problem was the significant correlation between chymotrypsin inhibition and the plasma concentration of α_1-proteinase inhibitor. A possible explanation is that the α_1-proteinase inhibitor reacted similarly to α_1-Achy, because the amino-terminal sequence of α_1-Achy shows a great deal of homology with the amino-terminal sequence of α_1-proteinase inhibitor, suggesting a common origin for both plasma proteinase inhibitors.[1-3]

Another important observation was the inverse correlation between α_1-Achy and the inhibition of lymphocyte blastogenesis induced by Concanavalin A, indicating that the lower the plasma α_1-Achy level, the higher the inhibition of lymphocyte blastogenesis. These findings are consistent with the depression cellular immunity seen in INS and the normal or hyperimmune state seen in proliferative glomeronephritis. Nephrotic plasma has been reported to inhibit the normal lymphocyte response to mitogens,[20,21] indicating the presence of inhibitor(s) of the lymphocyte function in nephrotic plasma. It has been shown that α_2-macroglobulin is one of the plasma inhibitors,[22,23] but complete explanation of the mechanism of the depression of cellular immunity in INS is difficult on the basis of the high plasma α_2-macroglobulin levels alone, and the possibility of the presence of and/or deficiency of other plasma factors may need to be taken into account. The decreased plasma α_1-Achy level may simply be a secondary change to massive urinary loss in INS because of the small molecular weight of this inhibitor.

In contrast to INS, the elevation of plasma α_1-Achy activity in CPH is obviously different from that described above. One possible explanation is overproduction by the liver by a mechanism not fully understood. This increase may be one of responses of the body to counteract the inflammatory processes by inactivating proteinases released from infiltrating or proliferatin cells in the glomeruli.

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