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Alpha$_2$ macroglobulin state in acute pancreatitis. Raised values of α_2 macroglobulin-protease complexes in severe and mild attacks

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Abstract

Plasma values of C reactive protein, α_1 proteinase inhibitor, α_2 macroglobulin, and complexed α_2 macroglobulin have been determined in serial samples from 27 patients with acute pancreatitis. Complexed α_2 macroglobulin was measured by a novel enzyme linked immunosorbent assay with a monoclonal antibody specific for the complexed form. Patients with severe illness had lower concentrations of total α_2 macroglobulin and higher concentrations of complexed α_2 macroglobulin than those with mild illness, and in the majority of severe attacks the abnormal amounts of complexed α_2 macroglobulin were present throughout the eight days of the study. The proportion of total α_2 macroglobulin in the uncomplexed form, however, was generally >90%, and in 26% of the mild cases completely normal concentrations of uncomplexed α_2 macroglobulin (>99% of total) were found throughout the eight days of the study. This suggests that exhaustion of α_2 macroglobulin in plasma is unlikely to be a major factor in the pathogenesis of acute pancreatitis.

During an attack of acute pancreatitis high concentrations of pancreatic secretory enzymes are found in plasma. Raised concentrations of proteases (trypsin, chymotrypsin, and elastase) can be detected using immunoassay techniques in attacks of all grades of severity, but these are present principally as zymogen (trypsinogen, chymotrypsinogen, and proelastase) with smaller amounts represented by proteases complexed with α_1 proteinase inhibitor, α_2 macroglobulin, and α_1 antichymotrypsin.[1-5] Protease activity cannot be detected in plasma during an attack of acute pancreatitis but evidence that active proteases are released is suggested by a fall in plasma concentrations of α_2 macroglobulin especially in patients with severe attacks.[6-10]

α_2 Macroglobulin is one of the principal proteinase inhibitors found in plasma, to which it is almost exclusively confined. It inhibits a broad spectrum of proteinases including trypsin.[11 12] Uniquely, the proteolytic activity of the bound proteinases is retained, although only towards low molecular weight substrates. This is explained by the 'trap' hypothesis[13] which proposes that the proteinase attacks a 'bait' region in the α_2 macroglobulin molecule producing a conformational change to an electrophoretically 'fast' form,[14] together with physical entrapment of the proteinase molecule, while still permitting access to low molecular weight molecules.

In patients with acute pancreatitis α_2 macroglobulin concentrations are usually mildly depressed at the time of admission but decrease profoundly and for prolonged periods in more severe cases.[6-10] This has been interpreted as being due to consumption of the α_2 macroglobulin by activated proteases and subsequent extraction of the α_2 macroglobulin protease complexes from the intravascular space by cells of the reticuloendothelial system with a reported half life of approximately 10 minutes, compared with several hours for the uncomplexed form.[15] Reports of the amount of complexed α_2 macroglobulin in the circulation in pancreatitis vary. No complexes were detected in plasma using crossed immunoelectrophoresis after isoelectric focusing,[8 16] in keeping with the rapid clearance of the complexed molecule. A decrease, however, in the trypsin binding capacity of plasma α_2 macroglobulin to 70–80% of normal concentrations has been reported during acute attacks,[6] suggesting the presence of circulating enzyme-α_2 macroglobulin complexes. Complexes between α_2 macroglobulin and a pancreatic elastase-like enzyme have also been identified in plasma from patients with acute pancreatitis.[17]

In this study we measured the plasma concentrations of complexed α_2 macroglobulin in serial samples from 27 patients with acute pancreatitis using an enzyme linked immunosorbent assay (ELISA) with a capture antibody specific to the 'fast' or complexed form of α_2 macroglobulin.[18] These results are examined, together with those for total α_2 macroglobulin, α_1 proteinase inhibitor, and C reactive protein.

Methods

The diagnosis of acute pancreatitis was based on a consistent clinical picture and plasma amylase activity exceeding 1200 IU/l (Phadebas, Pharmacia, Milton Keynes, UK). The cause of the attack was classified as (i) biliary, when gall stones were shown by ultrasound, endoscopic retrograde cholangioscopic pancreatography, laparotomy, or necropsy; (ii) alcohol, when average daily consumption exceeded 50 g in the absence of other causes; (iii) other, when an alternative cause was identified; and (iv) idiopathic, when no cause was identified. The severity of the attack was graded on the basis of outcome: a 'mild' attack was uncomplicated or one with only minor complications; a 'severe' attack included death, major organ failure, or a pancreatic complication (pseudocyst, abscess, or necrosis).

Citrated blood samples (10 mM citrate final concentration) were collected on the day of admission to hospital (day 1), usually within 24

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Clinical features of the 27 patients with acute pancreatitis in the study

	Age (years) (range, mean)	*Sex*	*Cause*	*Complications*
Mild (n=19)	30–83 (61)	14M:5F	Gall stones 10 Alcohol 1 After endoscopic retrograde cholangiopancreatography 1 Idiopathic 7	Pre-existing chest problems 1 Minor renal 1
Severe (n=8)	42–96 (67)	4M:4F	Gall stones 3 Alcohol 3 Idiopathic 2	Death 4 Respiratory 2 Pseudocyst 2

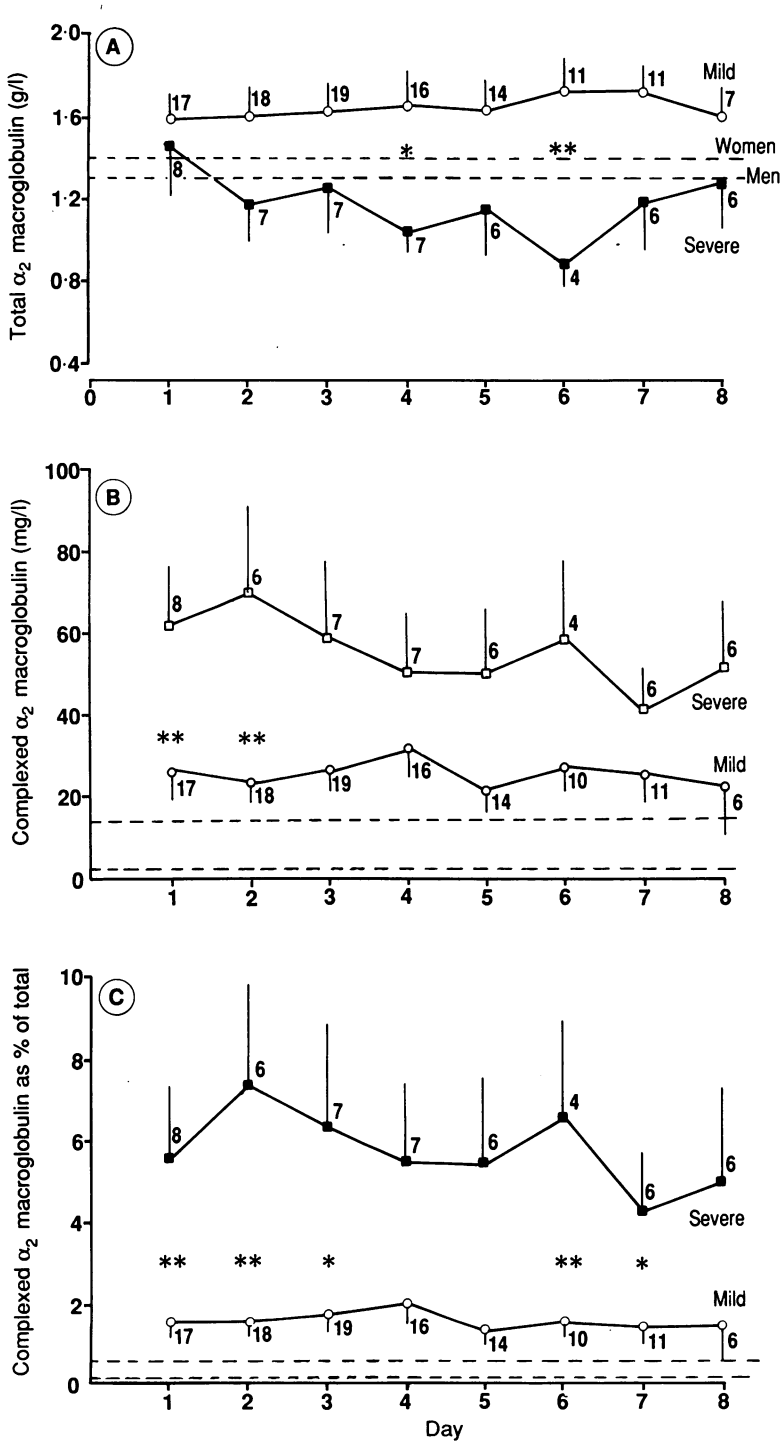

*Figure 1: (A) Total α_2 macroglobulin, (B) complexed α_2 macroglobulin, and (C) complexed α_2 macroglobulin expressed as a percentage of total α_2 macroglobulin concentrations in plasma from 19 patients with 'mild' acute pancreatitis and eight patients with 'severe' acute pancreatitis over eight days. Results are mean (SEM). The number of patient samples per day are shown. The broken lines show the upper and lower limits of normal except in (A) where the lower limits of normal for men and women aged over 40 years are shown. Day 1=day of admission to hospital. *p<0·05, **p<0·02.*

hours of the onset of symptoms. Samples were collected daily until day eight or until discharge from hospital if sooner. Plasma was obtained by centrifugation of the blood samples at 1500 *g* for 10 minutes and stored frozen at −40°C until analysis (within one month of collection).

Complexed α_2 macroglobulin was measured using a sandwich ELISA method previously described.[18] Briefly, a monoclonal antibody F44GA2 was used as the capture antibody. This antibody is specific for the 'fast' or complexed form of α_2 macroglobulin and shows no cross reactivity with native α_2 macroglobulin as shown by reversed dot blotting and rate electrophoresis.[19] The bound antigen was subsequently detected by using a polyclonal antibody directed against α_2 macroglobulin together with a peroxidase-labelled third antibody. Within-batch and interbatch coefficients of variation for the assay ranged from 2·9% to 5·9% and from 6·4% to 8·7% respectively.[18] As previously described[18] storage of biological samples at −40°C results in a gradual increase in concentration of complexed α_2 macroglobulin, although most of the samples examined were stable for at least a month and those which showed an increase during this time remained in the normal range. In the present study where sample storage time varied from <1 week to 1 month in both groups of patients no relation was seen between brevity of storage and concentrations of complexed α_2 macroglobulin.

Total concentrations of α_2 macroglobulin, α_1 proteinase inhibitor, and C reactive protein in the samples were determined using a Behring nephelometric analyser together with nephelometric grade antiserum to α_2 macroglobulin and N-Protein Standard serum (Behring Diagnostics, Hounslow, UK), antiserum to α_1 proteinase inhibitor, and SPS-01 calibrant (Protein Reference Unit, Royal Hallamshire Hospital, Sheffield), and antiserum to C reactive protein and C reactive protein calibrant (Dako, High Wycombe, Bucks) respectively.

Statistical analysis was performed using the two tailed Mann-Whitney U test.

Results

Twenty seven patients were studied and clinical details are given in the Table. The mean daily plasma concentrations of total and complexed α_2 macroglobulins and complexed α_2 macroglobulin expressed as a percentage of total concentrations are shown in Figure 1A-C respectively. Normal concentrations are indicated.[18 20] Sample collection was incomplete for some patients, which in most cases was due to factors such as death or discharge before day 8. The number of samples on which the results are based are given for each time point.

The mean total α_2 macroglobulin of the 'mild' group remained in the normal range, although at its lower limits, whereas the 'severe' group showed a gradual decrease in concentrations, reaching a minimum of 0·89 g/l on day 6 before starting to return towards normal on days 7 and 8 (Fig 1A). Significant differences between the two groups were seen on days 4 and 6. Concentrations of complexed α_2 macroglobulin were higher in

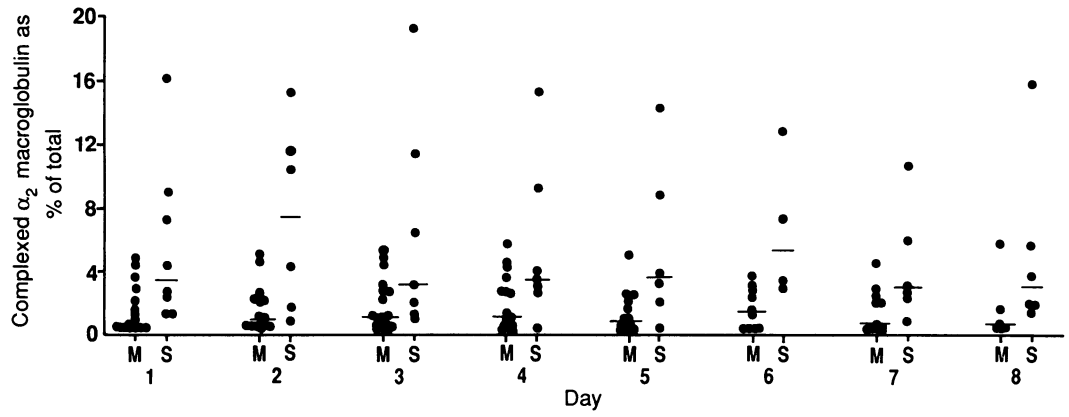

Figure 2: Plasma values of complexed α_2 macroglobulin on days 1 to 8 in patients with 'mild' (M) or 'severe' (S) acute pancreatitis. Lines show the median.

the 'severe' group than the 'mild' group, significantly so on days 1 and 2, but abnormally high values were found in both groups (Fig 1B). When expressed as a percentage of total α_2 macroglobulin the difference between the two groups was more pronounced, with differences being significant on days 1, 2, 3, 6, and 7 (Fig 1C). When individual patient data for complexed α_2 macroglobulin were examined (Fig 2), seven of eight patients classified as 'severe' had values of complexed α_2 macroglobulin (whether expressed as absolute or relative) that were outside the normal range for the entire sampling period compared with seven of 19 patients with mild attacks. Of these last patients, five had values that remained normal throughout the study period.

The mean daily plasma concentrations of α_1 proteinase inhibitor and C reactive protein are shown in Figure 3A and B. Concentrations of α_1 proteinase inhibitor were raised in both groups, being higher in the severe group but not significantly. They peaked in both groups on day 6. Concentrations of C reactive protein were high in both groups with significantly higher values in the severe group on days 1 to 6, the difference being most significant on days 2 and 3. Peak concentrations in both groups occurred on day 3.

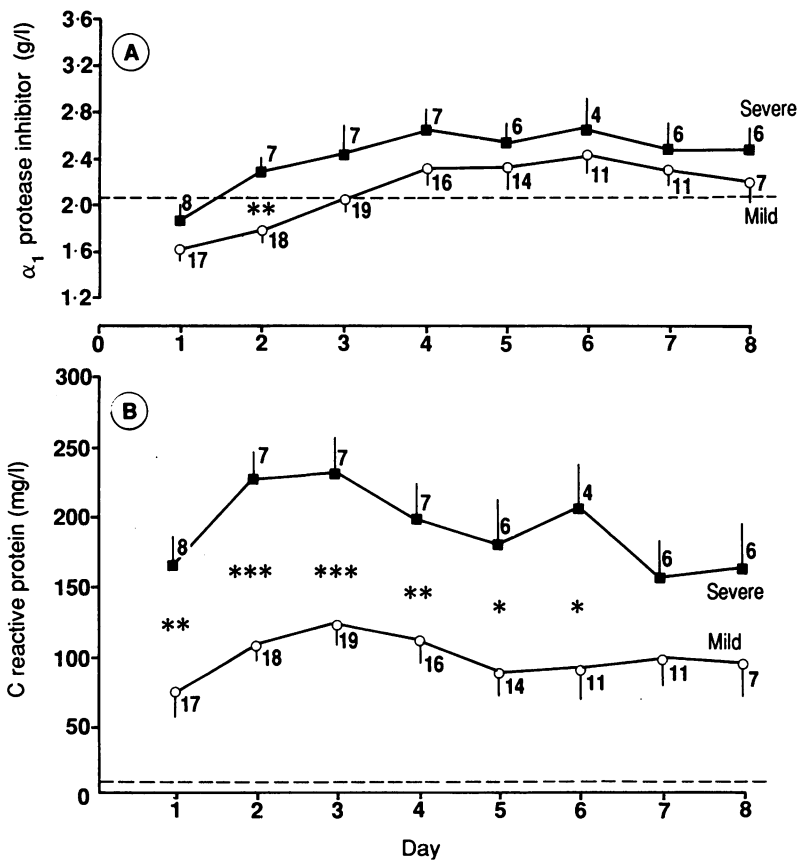

*Figure 3: (A) Plasma α_1 proteinase inhibitor and (B) C reactive protein concentrations in serial samples from eight patients with 'severe' acute pancreatitis and 19 patients with 'mild' acute pancreatitis. Results are mean (SEM). The number of patient samples per day are shown. The broken lines show the upper limits of normal. Day 1=day of admission to hospital. *p<0·05, **p<0·02, ***p<0·002.*

Discussion

We describe the presence of α_2 macroglobulin in the complexed or 'fast' form in plasma from patients with acute pancreatitis, substantiating the earlier findings of Lasson and Ohlsson who found significantly reduced trypsin binding capacity of α_2 macroglobulin during the early stages of attacks of acute pancreatitis.[6] The magnitude of the proposed complex formation is different since we found a maximum of 7·4% (mean) complexed α_2 macroglobulin in the severe group and 2·0% in the mild group compared with the 21–24% of α_2 macroglobulin unable to inhibit trypsin and therefore presumably complexed reported by Lasson and Ohlsson.[6] In this earlier study the values for complexed trypsin were significantly greater during the illness (irrespective of the severity of the attack) than during convalescence (3 to 12 months later) where the trypsin inhibitory capacity still remained at only 95% of that expected. Although the differences between the two studies in the magnitude of the changes may be due to differences in severity of attacks, the finding in the earlier study of significantly raised values of complexed α_2 macroglobulin in all the attacks and after a long convalescence in contrast to our study where many of the patients had normal values throughout the attack or before discharge from hospital, might partly be explained if samples in the former study had been stored before analysis. The spontaneous change of α_2 macroglobulin during storage to the electrophoretically fast form which is incapable of proteinase inhibition has been reported.[14 18 21]

It is impossible to assess the effect of the cause of the attack on the results owing to the small number of patients in each group. The increased concentrations of complexed α_2 macroglobulin in attacks of acute pancreatitis presumably occur as

the result of the release of proteolytic enzymes during the attack. Given the normally rapid removal of the complexed form of α_2 macroglobulin from the circulation[15] the presence of the complexed form must be due to such a massive increase in complex formation that the transport processes responsible for the removal of the complexed material are overwhelmed, resulting in a steady state level of circulating complexes governed by the extent of enzyme liberation. Alternatively, the uptake and removal of the complexes may be impaired in pancreatitis. This would be consistent with an impairment of mononuclear phagocytosis in patients with severe acute pancreatitis (M J McMahon, unpublished observations).

The pancreatic origin of the proteases complexed to α_2 macroglobulin remains unconfirmed. Activation of zymogen is necessary before complex formation, but this might occur in or around the pancreas, in ascitic fluid, or in plasma. Activation of trypsin in the pancreas has been observed in experimental pancreatitis by Ohlsson.[22] Particularly in severe pancreatitis, α_2 macroglobulin in ascitic fluid may be present exclusively in complexes with protease.[16] Moreover, in a proportion of patients with severe acute pancreatitis digestion of fibrin plates by ascitic fluid has been shown,[23] indicating protease activity. It is unlikely that the peritoneal mesothelium would be permeable to α_2 macroglobulin-protease complexes, even during severe attacks of acute pancreatitis, but the opportunity to enter the plasma is provided by the thoracic duct, which may be an important route of transport between ascitic fluid and plasma during attacks.[24] Alternatively, activation of zymogen might occur in plasma, or activated proteases might be transported to the plasma in complexes with α_1 proteinase inhibitor, which can then transfer the enzyme to α_2 macroglobulin.[15]

The lower than normal concentrations of total plasma α_2 macroglobulin found in this study, particularly in patients in the severe group, are in agreement with previously reported results.[7-10 16 25] This presumably reflects the consumption of α_2 macroglobulin by proteinases faster than it can be produced. This is supported by a small but significant inverse correlation ($r=0{\cdot}1861$, $p<0{\cdot}05$) between the complexed α_2 macroglobulin and total α_2 macroglobulin concentrations in the samples. As reflected by the magnitude of the correlation coefficient, however, patients with high values of complexed α_2 macroglobulin were not always those with lowest concentrations of circulating α_2 macroglobulin. This may reflect the wide range of normal α_2 macroglobulin values, and a measure of the magnitude of fall in total α_2 macroglobulin concentrations for each patient may be more appropriate, although impossible to obtain unless concentrations after the illness are used to represent the normal concentration in the individual.

In this study the maximum proportion of complexed α_2 macroglobulin was 19·2% of total α_2 macroglobulin. This suggests that exhaustion of α_2 macroglobulin in plasma is unlikely to be a factor in the pathogenesis of acute pancreatitis and is consistent with the failure of treatment with preparations containing α_2 macroglobulin (fresh frozen plasma) to influence the course of the illness.[26] The importance of the finding of circulating α_2 macroglobulin-protease complexes in relation to the pathogenesis of acute pancreatitis is not clear. Although these complexes may be important because they show peptidase activity and have been shown to hydrolyse such substances as parathyroid hormone[27] and proinsulin,[28] their presence may only be a consequence of impaired reticuloendothelial function which may be a more important factor. In support of this Adham *et al* have shown that in experimental pancreatitis reticuloendothelial stimulation resulted in improved survival.[29] Further investigations are necessary to clarify this.

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