

Endotoxaemia and complement activation in acute pancreatitis in man

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SUMMARY Twenty-four patients who experienced 26 attacks of acute pancreatitis were studied. Endotoxaemia, as measured by the limulus lysate assay, was present in 13 of the attacks. Six out of seven patients with systemic complications of the disease had endotoxaemia. C3 catabolism was increased in all 26 attacks of pancreatitis, and a falling level of C3 during attacks of severe pancreatitis was associated with a fatal outcome. There was statistical evidence of more complement activation in serum samples taken when patients had positive limulus lysate tests than when endotoxin was not detected in their blood.

In 1974 it was reported that three patients with acute pancreatitis had associated peritonitis and endotoxaemia, both of which disappeared when the disease resolved.¹ Cuevas *et al* showed that in experimental sterile peritonitis in rabbits there was release of endotoxin from the gut into the systemic circulation² and it has been proposed that this was the source of endotoxin in patients with pancreatitis.^{1,3} There is evidence of increased complement catabolism in acute pancreatitis in man⁴ and studies of experimentally induced pancreatitis showed that complement activation may play a pathogenetic role, as the severity of the pancreatitis was reduced in complement-depleted animals.⁵ As endotoxin activates the complement system it is possible that endotoxaemia is responsible for at least some of the complement activation that occurs in this disease.

Patients with pancreatitis are prone to develop hypoxia, hypotension, renal failure, hypocalcaemia, and disseminated intravascular coagulation as complications.⁶ All of these changes can be produced experimentally by endotoxaemia.^{7,8} Patients who die of pancreatitis commonly have evidence of shock lung and, recently, complement activation has been associated with the development of this syndrome.⁹

The present study was designed to assess the incidence of endotoxaemia and complement activation in pancreatitis, to see if they were related,

and to correlate the presence of each with the development of complications.

Methods

PATIENTS

Patients with a diagnosis of acute pancreatitis substantiated by a serum amylase ≥ 1200 IU/l were included in the study. Patients with postoperative or traumatic pancreatitis were excluded. The severity of the attack was assessed using Imrie's criteria.¹⁰ Patients were considered to have severe acute pancreatitis if, within the first 48 hours of admission, three or more of the following nine prognostic factors were present: serum albumin < 32 g/l (3.2 g/100 ml); serum calcium < 2.0 mmol/l (8 mg/100 ml); white cell count $> 15 \times 10^9/l$ (15,000/mm³); transaminase enzymes > 100 U/l; LDH > 600 U/l; plasma glucose in excess of 10 mmol/l (180 mg/100 ml) (in the absence of known diabetes); blood urea > 16 mmol/l (100 mg/100 ml) and not responding to intravenous therapy; age > 55 years; Pa O₂ < 8 K Pa (< 60 mmHg). Pa O₂ was not always measured in patients who otherwise had a factor count of zero or one.

The patients were managed conservatively by nasogastric drainage and intravenous fluids with the exception of one patient who had a partial pancreatectomy 12 hours before death. Imrie's factors were measured daily for a minimum of two days and a maximum of five days. A daily haematological screen included measurement of haemoglobin, white blood count, packed cell volume, and platelet

count. Fibrin degradation products (FDP) were measured on day 1 of the study. Daily blood samples were also taken for blood culture, endotoxin assay, and complement studies.

The presence of endotoxin in the patients' plasma was assessed by the limulus lysate technique¹¹ and reactions were graded from zero (no endotoxin) to three (≥ 5 ng endotoxin per ml). In order to minimise false positives caused by contamination, either during venesection, or during the test, a patient was deemed to have endotoxaemia only if endotoxin was detected on two consecutive days.

Serum concentrations of C3, C4, factor B, and plasma concentrations of C3d were measured immunochemically.¹¹ The functional activity of the classical pathway was measured by total haemolytic complement (CH50) titration.¹² The functional activity of the alternative pathway (AP-CH50) was measured by a modification (in preparation) of the method described by Nelson and Ruddy.¹³ The normal ranges for C3, C4, and factor B were established by measuring their concentrations in the sera of 50 normal blood donors.¹⁴ The normal ranges for the CH50 and AP-CH50 assays and plasma C3d were established by measuring their levels in 25 healthy laboratory personnel.

STATISTICAL ANALYSIS

The concentrations of complement components and haemolytic complement activity were analysed for their relationship to the presence or absence of endotoxaemia and the severity of the attack by Student's *t* test.

Results

PATIENTS

Twenty-four patients (16 men and eight women) had 26 attacks of acute pancreatitis, two patients having two attacks. The age range of the patients was 21–91 years with a mean of 53 years. Ten patients were shown to have gallstones, 10 gave a history of alcohol abuse, one patient had hyperpara-

thyroidism, and in three patients the aetiology of the pancreatitis remains obscure. Four patients died, two within 48 hours of admission, one on the third day, and one on the fourth day of the illness. A partial pancreatectomy was performed on one patient 12 hours before death. By Imrie's criteria there were eight attacks of severe pancreatitis (factor count ≥ 3), including the four fatalities.

ENDOTOXAEMIA

Thirteen of the 26 attacks of pancreatitis were associated with endotoxaemia (Table 1). There was no statistically significant correlation between the presence of endotoxaemia and the severity of the disease, as judged by factor count. Three out of the four patients who died had endotoxaemia.

Bacteriological evidence for a possible source of endotoxaemia was sought in all patients: one patient who died had a positive blood culture with a mixed growth of *Clostridium welchii* and *Escherichia coli*, and another patient had a *Proteus sp.* isolated from urine. Screening of all other patients with endotoxaemia failed to demonstrate a possible bacterial source. Urethral catheterisation did not appear to be related to endotoxaemia. Thirteen patients had an indwelling urinary catheter and five of them had significant endotoxaemia. Eight patients had significant endotoxaemia in the absence of a catheter.

COMPLEMENT ACTIVATION: ITS RELATION TO SEVERITY AND MORTALITY

All patients had a plasma C3d level raised above the normal range (given in Table 3) at some time during their illness, indicating increased C3 catabolism in acute pancreatitis. Samples taken during the first day after admission from patients with mild disease showed rises of mean C4 (mean \pm SEM = 534 ± 31 μ g/ml), factor B (274 ± 18 μ g/ml), C3d (11.01 ± 0.93 μ g/ml) and CH50 (246 ± 11.1 units) relative to normal controls ($p < 0.001$ for each). Mean levels of C3 (1214 ± 69 μ g/ml) and AP-CH50 (270 ± 18 units) did not differ from the norm. The findings on the

Table 1 Endotoxaemia and its relationships to complications and complement activation in acute pancreatitis

Patients	No.	Severe, factor count ≥ 3	Deaths	Intra-vascular coagulation	Hypoxia, $pa O_2 < 8$ kPa*	Renal failure	Hypotension (systolic BP < 100 mmHg for > 6 h)	Normal range			
								C3 below	C3d above	AP-CH50 below	CH50 below
Endotoxaemia	13	5	3	4	4	2	2	5	13	8	4
No endotoxaemia	13	3	1	0	1	1	1	1	13	1	1

* Partial pressure in kPa $\times 7.52$ = partial pressure in mmHg.

second day were similar. Samples taken during the first 24 hours after admission in patients with severe pancreatitis had a reduced mean C3 ($974 \pm 108 \mu\text{g/ml}$, $p < 0.01$) and a raised mean C3d ($17.1 \pm 3.6 \mu\text{g/ml}$, $p < 0.01$) relative to controls. Mean levels of C4 ($414 \pm 37 \mu\text{g/ml}$), factor B ($254 \pm 32 \mu\text{g/ml}$), CH50 (187 ± 22 units), and AP-CH50 (221 ± 40 units) were normal. Findings during the next 24 hours in these patients were similar apart from a raised mean factor B ($267 \pm 32 \mu\text{g/ml}$, $p < 0.05$). Comparison between mild and severe patients within 24 hours of admission showed reduced mean C3 ($p < 0.05$), C4 ($p < 0.05$), and CH50 ($p < 0.05$) in the severe group. In addition to these differences, mean C3d was greater in the severe group ($18.6 \pm 3.1 \mu\text{g/ml}$) than the mild group ($12.2 \pm 1.3 \mu\text{g/ml}$) at 48 hours ($p < 0.05$).

The greatest degree of hypocomplementaemia was seen in the four patients who died (Table 2). Within 24 hours of admission mean C3, CH50, and AP-CH50 were lower in non-survivors than survivors. Of the criteria used by Imrie to establish the severity of the attack of pancreatitis only the urea level was significantly different between the two groups at this time ($p < 0.01$). Levels of C3, C4, and CH50 fell between the first and second sample in all the patients who died, whereas these values did not alter significantly in the survivors. At the time the second blood sample was taken all complement determinations were different between the two groups (Table 2), the best discriminating features being C3 and CH50 (Fig. 1).

COMPLEMENT ACTIVATION: ITS RELATIONSHIP TO ENDOTOXAEMIA

The mean levels of C3, factor B, CH50 and AP-CH50 were significantly reduced in the serum samples taken when patients had positive limulus lysate tests than when endotoxin was not detected in their blood (Table 3). Levels of C3d were raised

Table 2 Complement measurements in survivors and non-survivors (mean \pm SEM)

	Survivors (22)	Non-survivors (4)	
<i>Day 1</i>			
C3 ($\mu\text{g/ml}$)	1184 \pm 67	900 \pm 84	<0.01
CH50 (units)	250 \pm 13	161 \pm 31	<0.01
AP-CH50 (units)	274 \pm 18	176 \pm 51	<0.05
<i>Day 2</i>			
C3 ($\mu\text{g/ml}$)	1223 \pm 73	623 \pm 134	<0.001
C4 ($\mu\text{g/ml}$)	515 \pm 26	357 \pm 61	<0.05
Fac B ($\mu\text{g/ml}$)	309 \pm 20	235 \pm 30	<0.05
C3d ($\mu\text{g/ml}$)	12.8 \pm 1.2	22.1 \pm 5.3	<0.05
CH50 (units)	257 \pm 14	134 \pm 23	<0.001
AP-CH50 (units)	265 \pm 18	132 \pm 36	<0.01

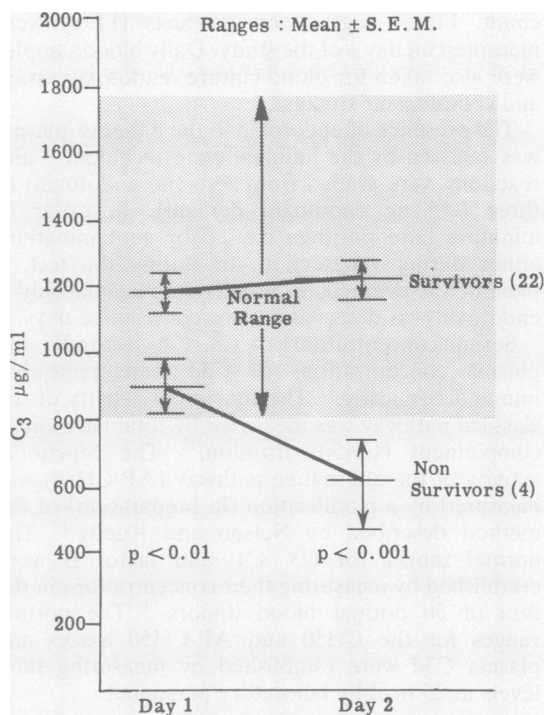


Figure Comparison of C3 on days 1 and 2 in survivors and non-survivors.

significantly in the endotoxin positive samples and mean C4 was the same in both groups. Similarly, patients with endotoxaemia were more likely to have abnormally low C3, AP-CH50 or CH50 than patients with no endotoxaemia (Table 1).

SYSTEMIC COMPLICATIONS (Table 1)

One patient who died was shocked, hypoxic, and developed acute renal failure. This patient did not have endotoxaemia. The three other patients who died did have endotoxaemia. Shock and renal failure were present in two of these patients one of whom also had necropsy evidence of disseminated intravascular coagulation. The third died of respiratory failure and also had disseminated intravascular coagulation at necropsy. Three survivors were shown to have complications, all of whom had endotoxaemia; two of these had evidence of intravascular coagulation (FDP level $>40 \mu\text{g/ml}$ on day 1) and one of these patients was also hypoxic. The third survivor was hypoxic.

When blood samples containing endotoxin were compared with blood samples in which endotoxin was not demonstrated the mean albumin corrected

Table 3 Comparison of complement measurements in endotoxin positive and negative blood samples (mean \pm SEM)

	Normal range	Mean \pm SEM for normal controls	Endotoxin +ve (n=39)	Endotoxin -ve (n=47)	p
C3	769–1794 μ g/ml	1282 \pm 37	1152 \pm 67	1284 \pm 41	<0.05
C4	149–550 μ g/ml	350 \pm 14	527 \pm 24	493 \pm 19	NS
Factor B	111–303 μ g/ml	207 \pm 7	287 \pm 17	349 \pm 15	<0.01
C3d	4.7–7.7 μ g/ml	6.2 \pm 0.15	15.9 \pm 2.2	11.5 \pm 0.7	<0.05
CH50	150–250 units	200 \pm 5	234 \pm 11	261 \pm 10	<0.05
AP-CH50	185–307 units	246 \pm 6	245 \pm 17	302 \pm 15	<0.01

calcium level was the same in both groups. There was no significant correlation between any complement determinations and the development of specific complications.

Discussion

Half of the patients studied had endotoxaemia but only two of these patients had evidence of a Gram-negative bacterial source of endotoxin. Indwelling urinary catheters, associated with bacterial colonisation, have been reported to cause endotoxaemia¹⁵ but catheterisation did not appear to be related to its development in our patients. Thus it remains possible that the gut is the main source of endotoxin in pancreatitis.

One patient who died and showed evidence of respiratory and renal failure did not have endotoxaemia. Apart from this patient the six other patients either with respiratory or renal failure, or intravascular coagulation, all had endotoxaemia and in all a three-plus degree of endotoxaemia was detected. Liehr *et al*¹⁶ have reported an increased incidence of endotoxaemia in patients with complications of pancreatitis, and all nine of their patients who died had endotoxaemia. Hypocalcaemia, however, was not related to endotoxaemia in the present study.

Mean levels of C4 and factor B were raised in patients with mild pancreatitis, and only the raised level of C3d in all these patients indicated that increased complement catabolism was present. Within 24 hours of admission the mean C3 level in patients with severe pancreatitis was reduced. In all four patients who died the level of C3 declined further during their illness. Levels of C3 rose during convalescence in the four patients with severe pancreatitis who survived. Thus, during an attack of severe pancreatitis, falling C3 levels are associated with a poor prognosis.

In any situation serum complement levels are determined by a balance between component synthesis and degradation. C3, C4, and factor B behave as acute phase reactants and tend to be raised in inflammatory diseases as a result of

increased synthesis.¹⁷ The same factors are subject to increased consumption in acute pancreatitis as evidenced by raised C3d levels. Thus, in acute pancreatitis, synthesis and degradation are both increased and caution must be observed when interpreting results from a single blood sample, which may not show any abnormality other than an increased C3d level. Serial samples will give an indication of the balance that exists between synthesis and consumption. In patients with mild pancreatitis the balance is in favour of synthesis, resulting in increasing levels of C3, C4, and factor B during the acute phase of the illness. In fatal pancreatitis the balance is in favour of consumption.

In this study endotoxaemia appeared to be related to increased complement activation. Endotoxin can activate complement by at least three different mechanisms. First, the polysaccharide portion of the endotoxin molecule can activate the alternative pathway.¹⁸ Second, the same polysaccharide is a potent T-independent antigen and antibody binding with antigen will activate the classical pathway,¹⁹ and third, the lipid A portion of the endotoxin combines directly with C1 to initiate classical pathway complement activation by an antibody-independent mechanism.¹⁸

In the bacteraemic phase preceding the development of septic shock in man there is evidence that complement activation is primarily by the alternative pathway.²⁰ In our patients with endotoxaemia, activation by the alternative pathway was found more commonly (Table 1), but there was evidence of activation by the classical pathway too, particularly in patients with severe disease.

The finding of increased C3d levels in patients who were not shown to have endotoxaemia suggests that agents other than endotoxin also activate the complement system in acute pancreatitis. In experimental pancreatitis in dogs, free trypsin was found in the pancreatic exudate in the early stages of the disease²¹ and trypsin is known to cleave C3, C4, and C5 to yield biologically active fragments.^{22–24} However, attempts to demonstrate free proteolytic enzymes, unbound to plasma protease inhibitors, in either the circulation or in peritoneal exudates from

patients with pancreatitis have failed.^{4 25} Activation of the complement system within the pancreas by free trypsin remains a theoretical possibility. Although intrapancreatic C3 deposition has been reported in bile salt induced pancreatitis in rats,⁵ it was not observed in choline deficiency pancreatitis in mice.²⁶

Most patients who die of pancreatitis do so within the first 10 days of the illness and they die of a shock syndrome characterised by cardiovascular and respiratory failure.²⁷ It is possible that the chemical peritonitis resulting from acute pancreatitis causes increased release of endotoxin from the gut lumen resulting in endotoxaemia and that endotoxaemia is at least partly responsible for the development of shock and other complications. If this is so, then treatment of the peritonitis may be of value. In animals, endotoxaemia caused by an ischaemic loop of bowel could be prevented by peritoneal lavage when lavage was started within 15 minutes of the onset of ischaemia. Endotoxaemia occurred in this model only when lavage was stopped.²⁸ Therapeutic peritoneal lavage has been tried in the treatment of acute pancreatitis and has been shown to prevent deaths due to shock in the first 10 days.²⁷ However, lavage does not appear to affect the inflammation in the pancreas. Other possible ways of preventing release of endotoxin from the gut include intraluminal cholestyramine which binds endotoxin,²⁹ or gut sterilisation to eradicate the source of endotoxin.² These therapeutic manoeuvres await clinical evaluation.

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