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The Serum Complement System — a Mediator of Acute Pancreatitis

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Summary. The role of the complement system in the initial membrane lesion of acute pancreatitis was investigated. In the experimental sodium-taurocholate pancreatitis of the rat a sudden and steady decline of serum complement was observed. The deposition of C3 component of complement in acute pancreatitis could be demonstrated by immunofluorescence. To rule out mere deposition or activation of complement in the interstitial exsudative fluid, single acinar cells of rat and rabbit pancreatic tissue were prepared and transfered to culture medium. In contrast to heat inactivated serum and C6 deficient serum these cells were lysed by trypsin activated fresh serum. Consequently, an acute pancreatitis could be induced by activating exclusively the complement system by injection of cobra venom factor into the pancreatic duct of rats. The activated complement system is thought to be responsible for initial membrane lesion in exsudative inflammation, as could be shown in acute pancreatitis.

In acute pancreatitis membrane lesion must precede the digestion of a pancreatic cell by pancreatic enzymes. The serum complement system, which was first of all investigated in connection with antigen-antibody reaction, is the only known system to damage viable unaltered cell membranes and to produce lysis of even unsensitized erythrocytes (Kolb, Haxby, Müller-Eberhard, and Arroyave, 1973; Kolb and Müller-Eberhard, 1973). Various cross connections between the complement-, the clotting-, and kallikrein kinin-system enable the stimulation and activation of complement proteins in an inflammatory fluid (Ryan, 1974). The serinproteases trypsin and plasmin are able to enter the complement sequence and to generate cytolytically active complexes of the complement components C5 to C9 (Kolb *et al.*, 1973; Kolb and Müller-Eberhard, 1973). Upon contact with unsensitized membranes (—a previous antigen-antibody reaction is not necessary—) these cytolytically active macromolecular aggregates lead to a membrane alteration which enables the surrounding extracellular fluid to enter the cell and to cause disruption by cell-swelling and, perhaps, by concomitantly activated intracellular enzymes.

Therefore, the role of the serum complement system in the initial membrane lesion of the pancreatic acinar cell in acute pancreatitis was studied.

Method

1. An acute pancreatitis was induced in 14 male rats (Wistar strain, 350–400 g body weight) by intraductal injection of 1 ml sodium desoxycholate (2% in 0.9% NaCl) following the method described by Henning and Heinkel (1952). Nine rats of identical strain and body weight were subjected to laparotomy only and served as control. Blood samples were obtained

by venesectio prior to the operation as well as 10, 60, 180, and 360 minutes postoperatively. The samples were allowed to clot for 60 minutes at $+4°$ C. The serum was held at $-70°$ C until complement titration.

Six hours after injection of sodium desoxycholate all animals were sacrificed and pancreatic tissue was fixed in 10% formalin for histological examinations.

2. An acute pancreatitis was induced in six rats by intraductal injection of 2% Na-Desoxycholate. Pancreatic tissue was obtained 10, 60, and 180 minutes later and snap frozen in isopentan precooled by liquid nitrogen for immunohistological analyses of complement deposits.

3. An acute pancreatitis was induced in four rats by intraductal injection of 2 mg cobra venom factor solubilized in 1 ml buffered saline. The cobra venom factor was isolated from cobra venom by ion-exchange chromatography and gel filtration (method to be published). Pancreatic tissue was obtained 90, 120, 180, and 210 minutes after injection of cobra venom factor, partly fixed in 10% formalin, and partly snap frozen in isopentane.

4. Hemolytic activity of serum complement was determined following the method of Borsos (1970). The decline of complement activity was expressed in percent of the preoperative values.

5. For histological examinations paraffin sections were stained with HE and PAS. For immunohistochemical analyses of complement depositions 3 μ cryostat sections of snap frozen tissue were incubated with anti-rat C3 serum from rabbits. After thorough washing they were incubated with anti-rabbit IgG labeled with FITC (Behringwerke).

Previously, control sections were incubated with normal rabbit serum. Anti-rat C3 serum was obtained by immunization of rabbits with rat C3 which had been isolated following the method of Nilsson and Müller-Eberhard (1965). For detailed immunohistochemical techniques see: Seelig, Seelig and Drescher (1974).

6. Single cell preparations of rat pancreas were obtained in a Potter-S (Braun Melsungen) homogenizator in ice-cold isotonic phosphate buffer pH 7.24. After careful washing in isotonic buffer the cells were transfered to DIFCO 199 medium in a concentration of 150000–200000 cells/mm^3. Cell counts were done in a Neubauer chamber, the viability of the cells was proven by vital dyes ,such as lissamine green and trypan blue.

Five ml of the above cell suspension were incubated at 37° C with 1 mg crystalline trypsin and with 1 ml of fresh autologous serum as well as with 1 ml of heat inactivated C 30′ 56° C) autologous or isologous serum resp. Cell counts were performed every 10 minutes following incubation.

Single cell preparation of rabbit pancreas was obtained in the same way. Five ml of the cell suspension (300000 cells/mm^3) were incubated with 1 mg crystalline trypsin and 2 ml of autologous serum as well as with homologous serum of C6 deficient rabbits. Cell counts were done every 10 minutes.

Results

1. Serum Complement Activity (CH50) in the Experimental Na-Desoxycholate Pancreatitis of the Rat

10 minutes after injection of sodium desoxycholate in the rat pancreatic duct a decline of approx. 20% of serum complement activity was observed. After 60 minutes the rate of decline reached values of about 35%, after 180 minutes average values of 60% were determined. After 360 minutes the average values slightly increased to 65% of the preoperative values.

The control animals showed an immediate decline of about 7% after sham operation. After 60, 180, and 360 minutes the complement activity rose to normal levels. In experimental sodium desoxycholate pancreatitis the decline of hemolytic activity of serum complement components after 60, 180, and 360 minutes was significant ($p < 0.001$) (Fig. 1a).

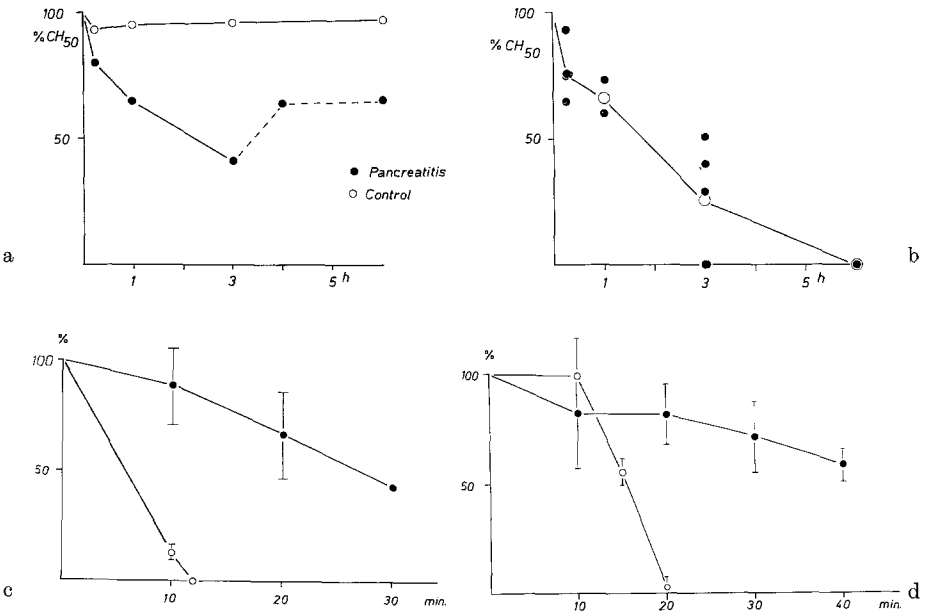

Fig. 1. (a) Decrease of hemolytic activity of serum complement in experimental Na-Desoxycholate pancreatitis of rats. Complement activity (CH50) expressed in percent of preoperative values. (b) Rate of decrease of hemolytic serum complement activity in rats showing more pronounced pancreatitis as judged by histology. (c) Lysis of rat pancreatic acinar cells by fresh autologous serum (○) compared to cell death in heat inactivated serum with equal amounts of trypsin (●); decrease of living cells is expressed in percent of viable cells at the beginning of incubation with serum (150000–200000 cells/mm³). (d) Lysis of rabbit pancreatic acinar cells by fresh autologous trypsin activated serum (○) compared to cell death in fresh trypsin-activated heterologous C6 deficient rabbit serum (●). Viable cells at the beginning of incubation with serum about 300000 cells/mm³

Analysis of single values allows the differentiation of two groups in this series. In the first group, consisting of six animals, a decrease of serum complement activity to 26% of preoperative values was observed after 180 minutes. Five of these six animals did not survive a short ether narcosis for blood letting. The only surviving rat, however, showed a 100% decline of complement activity after 360 minutes (Fig. 1b).

In the second group, consisting of eight animals, the rate of decrease did not fall below 50% of the preoperative values. After reaching a minimum level within three hours, the serum complement activity rose again to 77% of the preoperative values within six hours.

The histological examinations of the pancreas tissues of these two groups showed that parenchymal damage was in some way correlated to the rate of decrease of serum complement activity. Progressive leucocytosis and acinar cell necrosis was mainly observed when the rate of decline of complement components was greater than 50% in three hours.

Immunofluoroscopically detectable deposits of complement around the acinar cells as well as in the interstitial spaces of pancreas tissue could be observed just one hour after injection of sodium desoxycholate. With progressing acinar cell necrosis unspecific fluorescence of less brilliance—as compared to the control section—appeared.

2. Cobra Venom Factor Induced Pancreatitis

Intraductal injection of sterile cobra venom factor (cvf) solution resulted in a marked enlargement of the gland which, in fact, had not been observed to this extent following the injection of equal amounts of sodium desoxycholate solution or desoxycholate trypsin mixture. In contrast to the Na-desoxycholate experiments, the clinical signs of shock appeared immediately. An acute tryptic pancreatitis with acinar cell necrosis, vanishing and disrupting acinar cells, edema, and leucocytosis was seen in the paraffin sections just 90 minutes after the injection of cvf. 120 and 180 minutes later the parenchymal damage had progressed. After 210 minutes fat necrosis of mesenteric tissue and peritoneum could be observed macroscopically.

The immunohistological analysis of complement (C3) deposits showed large amounts of complement within the pancreatic tissue. The spreading out of inflammatory fluid with complement components through the interstitial spaces could be clearly demonstrated by immunofluorescence in the 90 minutes-preparations. Granular deposits of complement along the cell membranes of still intact acinar cells as well as large sheets and plaques of complement components within damaged regions were seen 120, 180, and 210 minutes following intraductal injection of cvf. The control sections showed no specific fluorescence.

3. Influence of Complement on Cell Preparations of Pancreatic Acinar Cells

Rat acinar cell preparations incubated with trypsin lysed within 10 to 12 minutes after the addition of fresh serum. 12 minutes afterwards no acinar cell survived. From time to time swelling with subsequent disruption of the acinar cells could be observed under the microscope. While enlarging, the cells became stainable with vital dyes. The swelling persisted up to approx. 3 times the original size of the cells to the point where they seemed to burst. The disrupted cells were not further stainable.

After the addition of trypsin and heat inactivated autologous or isologous serum a slight and steady decline of cells was observed. 10 minutes after the addition of heat inactivated serum 80% of the cells were still alive (Fig. 1 c).

The manner of cell death in the last preparations differed from cell death caused by fresh serum, in that most dead cells were of normal size and stainable with vital dyes. In contrast to incubation with fresh serum only few disrupted cells could be found. The trypan blue or lissamin green stained cells showed a tendency to build clusters of 3 to about 15 cells.

In rabbit acinar cell preparations incubated with trypsin and fresh autologous serum cytolysis was complete after 15 to 20 minutes. 20 minutes after the addition of trypsin and fresh C6 deficient homologous rabbit serum 82% of the acin were still alive. The experiments were stopped after 40 minutes when 50% cells were still alive (Fig. 1 d).

Discussion

In the experimental sodium desoxycholate pancreatitis of the rat a sudden and steady decline of hemolytic complement activity in serum was observed. These levels reached a minimum after 3 hours. One animal with progressed parenchymal damage was nearly decomplemented after 6 hours. In animals exhibiting more focal necrosis and moderate signs of inflammation the serum complement levels climbed somewhat after having reached a minimum after the first 3 hours. Levels under 50% of the preoperative complement activity seemed to be critical threshold values. Five of six animals which reached these levels after 3 hours did not survive a short ether narcosis for blood letting. Compared to the group with moderate complement decrease, these animals showed a more pronounced leucocytosis and acinar cell necrosis. Control animals which lost equal amounts of blood showed unaltered complement activity, except for a slight decrease of about 7% immediately after laparotomy. This decrease is perhaps the consequence of a laparotomy induced activation of the clotting system with concomitantly activated complement components. An activation and, therefore, consumption of C components in the circulation by pancreatic enzymes is not probable in the early stages of experimental sodium desoxycholate pancreatitis, although later on, when parenchymal damage had progressed, it certainly played an additional role. During the early stages when the complement activity reached values of about 60% of the preoperative levels, no clinical signs of shock could be observed.

The immunofluorescopically detectable deposits of complement in the pancreatic tissue of experimental sodium desoxycholate pancreatitis speak in favour of local consumption of complement components. In order to rule out mere deposition or activation of hemolytically active complement proteins in the interstitial masses of exsudative fluid without causing any membrane lesions, viable single acinar cells of rat pancreatic tissue were prepared, carefully washed from serum constituents, and transfered to culture medium. For the sake of avoiding antigen-antibody reactions with concomitantly activated complement components as far as possible, autologous serum was used. Trypsin, the key enzyme of pancreas which is not able to attack viable undamaged membranes, was used for complement activation and generation of cytolytically active C complexes. Complete cytolysis occurred very quickly within a few minutes. A lag phase of 5 to 7 minutes after the addition of fresh serum was observed. This delay possibly corresponds to the explicit lag phase between the beginning of the C9-binding and the setting in of hemolysis which was noticed in antigen-antibody-coated erythrocytes (Müller-Eberhard and Fjellström, 1965). The higher stability of rabbit acinar cells as compared to rat cells—the former needed 20 minutes, the latter 12 minutes until cytolysis was complete—may be caused by differing membrane behavior of the two species, differing defense mechanisms, and/or by the usually lower complement activity in rabbit serum as compared to rat serum.

The results show that a heat labile trypsin activated system in autologous serum is responsible for cell death. To prove that complement was involved, the rabbit acinar cell suspensions were incubated with trypsin and serum of rabbits genetically lacking the sixth component of complement which is essential for building the cytologically active complex C5 to C9. In complement C6 deficient serum 50% of the acinar cells survived until the experiment was stopped after 40 minutes.

Whereas disrupting cells were typical of fresh trypsin activated serum incubations, immobile trypan blue or lyssamine green stainable cells belonged to the incubation mixtures with heat inactivated or C6 deficient serum. Addition of active serum was followed by complete cytolysis in each incubation experiment, whereas the addition of inactivated or defect serum did not alter the normal decline of cells in an unsatisfactory medium. In this medium the highly specialized acinar cells seemed to die of malnutrition after a certain time. In the first group cell death seemed to come from outside. In the second group, however, the cells stopped metabolizing, the membranes became permeable for vital dyes, and the cells died from inner causes. These observations agree well with the common conception of complement action on biological membranes: The fixation of the cytolytically active macromolecular aggregate C5 to C9 causes membrane alterations, the so-called "Humphrey-Dourmaskin defects" (1965) which allow the extracellular fluid to enter the cell and to initiate cell swelling. Concomitant enlargement of the "holes" is followed by extrusion of cell content and disruption of the cell membranes.

Consequently, we tried to induce an acute pancreatitis by activating exclusively the complement system. A non-toxic protein extractable from crude cobra venom, "the cobra venom factor", is known to activate the C3 component by the C3 bypass (Müller-Eberhard and Fjellström, 1971) and to lead to the consumption of the succeeding components of complement. This factor is commonly used in small amounts to decomplement animals, at higher doses, however, it produces erythrocytolysis. After sterile filtration and proof for nontoxicity in cell culture we injected 1 ml of cobra venom factor solution into the pancreatic duct of the rat. It was supposed that cytolytically active components of serum complement generated by cobra venom factor in mechanical edema would attack the viable acinar cell membrane and initiate acute tryptic pancreatitis. Without previous antigen-antibody reaction this kind of complement activation resulted in an acute tryptic pancreatitis with acinar cell necrosis, large edema, leucocytosis, and fat necrosis after only 90 minutes. The immediate clinical signs of shock were attributed to the liberation of anaphylatoxin and kinin-like substances by activated complement components. The cvf pancreatitis represents a simple model resembling acute tryptic pancreatitis in humans (detailed studies of this model will be published).

These experiments seem to show a way by which general membrane lesions can be caused by exsudative edema.

Because of its special function, namely, the synthesis and secretion of potent proteases, the pancreatic acinar cell—exposed to the attack of activated complement enzymes in an exsudative edema—has a poorer chance to mobilize defense mechanisms than cells of other organs. Further complement activation by floating out of pancreatic enzymes into the circulation, concomitant activation of clotting and the kallikrein-kinin system, as well as the liberation of histamin and kinin-like substances, contribute to the frequent fatal outcome of acute tryptic pancreatitis.

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