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DEFICIENT PHAGOCYTOSIS IN ABDOMINAL SEPSIS: THE INFLUENCE OF INTRAPERITONEAL SUBSTITUTION OF OPSONINS - FIRST RESULTS

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INTRODUCTION

Peritonitis exudate is characterized by the presence of a large number of bacteria despite a huge population of phagocytes. Cellular phagocyte function seems to be intact in these exudates.[3,4] Recently we have demonstrated impaired particle opsonization in human peritonitis exudate with evidence of a major breakdown of IgG and complement C3.[1] Such exudates contain extremely high concentrations of granulocyte enzymes and free proteolytic activity. Oxygen derived free radicals are liberated from polymorphonuclear leukocytes (PMNL) in this setting and are supposed to destroy functional proteins and protease inhibitors.[8] The intraabdominal invasion of bacteria and foreign particles leads to pronounced opsonin consumption. The current therapy includes surgical treatment for the elimination of the source of peritonitis and a lavage procedure of the abdominal cavity.[6] From the theoretical point of view the intraabdominal application of intact serum at the end of such an operation should be beneficial as to:

- be bactericidal by itself
- restore the pool of intact complement factors, thus repairing particle opsonization
- liberate anaphylatoxins C3a and C5a, leading to phagocyte invasion
- provide a high concentration of broad spectrum proteinase inhibitors
-supply scavengers for free radicals

The present paper describes the effect of serum application on purulent peritonitis exudates *in vitro* and *in vivo*.

MATERIALS AND METHODS

During operations for diffuse peritonitis exudates and blood samples were drawn

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simultaneously exudates collected with disposable plastic syringes were divided and one part cleared of cells and debris by immediate centrifugation. Blood samples were processed to serum and EDTA-plasma. Intraoperative serum application was performed in 3 patients, thereafter drainage fluid was collected over 2.5 hours. For *in vitro* investigations samples of native exudate or cell free exudate supernatant were incubated for 2 hours at 37°C with increasing amounts of normal serum resulting in an exudate:serum ratio from 1:1 to 1:100. Mean levels in normal serum were: IgG = 11.3 g/l, C3 = 79.9 mg/dl, elastase α_1 proteinase inhibitor (elastase α_1 PI) = 71.2 ng/ml, myeloperoxidase (MPO) = 45 ng/ml, opsonic capcity (oc) = 96% of normal serum standard. For *in vivo* serum substitution exudate samples were taken immediately after laparotomy. The source of peritonitis was then treated accoring to surgical standard procedures, the abdomen rinsed with 10 l of Ringer lactate solution and sucked out. One blood-bank serum unit from healthy donors (300 ml) was applied and the abdomen closed with drainage. The drainage fluid was collected and pooled from 0–2 hours and from 2–2.5 hours after operation. Opsonic activity was quantified by a modified chemiluminescence assay as described previously.[2] Opsonin levels (C3 and IgG) were measured with a standard radial immunodiffusion assay (Behringwerke, Marburg FRG). C3 splitting was demonstrated by crossed immunoelectrophoresis[5] employing a C3c antibody (Behringwerke, Marburg FRG). PMNL-elastase (in complex with α_1 PI) and myeloperoxidase (MPO) were measured by ELISA. Bacterial investigations were performed in aerobe and anaerobe cultures employing blood agar and McConkey culture medium.

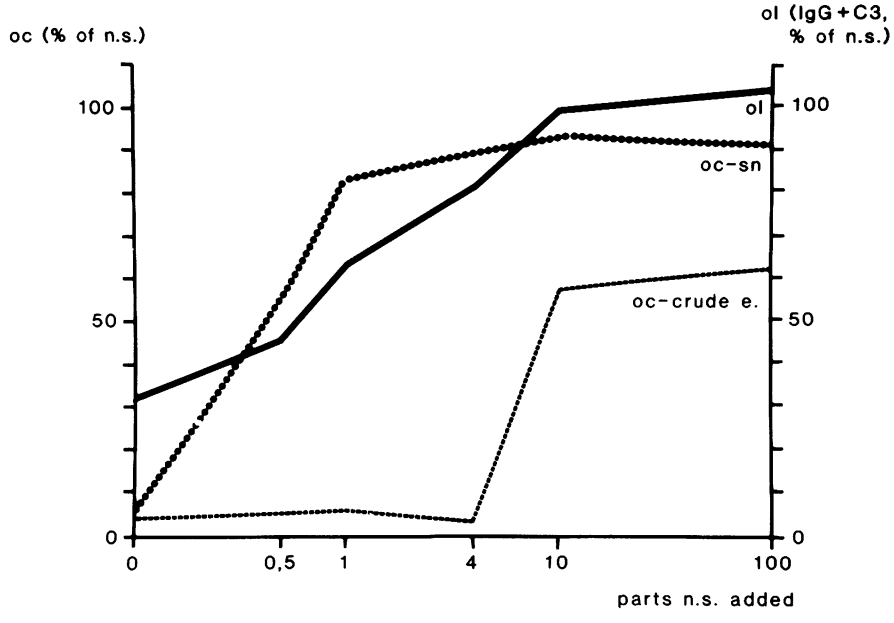

FIGURE 1 *In vitro* serum substitution. Incubation of normal serum with peritonitis exudate can restore opsonin levels (ol) and opsonic capacity (oc). In crude exudate much higher amounts of serum are required to restore oc than in cell free exudate supernatant (sn).

RESULTS

1. In Vitro Incubation of Exudates with Serum

Purulent peritonitis exudate showed virtually no opsonic capacity (oc) and rather low opsonin levels (ol). When cell free exudate samples were incubated with increasing amounts of serum the immunologically measured C3- and IgG-levels in these assays rose according to the substitution effect as expected (Figure 1). The oc in these incubation mixtures recovered according to the rising opsonin concentrations. However, when the same procedure was performed with crude exudate much higher serum concentrations were required to improve opsonization (Figure 1). In two of these mixtures crossed immunoelectrophoresis was performed. Even when a 4-fold serum excess was incubated with crude exudate for 2 hours we found an almost complete breakdown of C3 into smaller components. Only the application of a 100-fold serum excess resulted in an increasing peak of intact C3 but still revealed considerable C3 splitting (Figure 2).

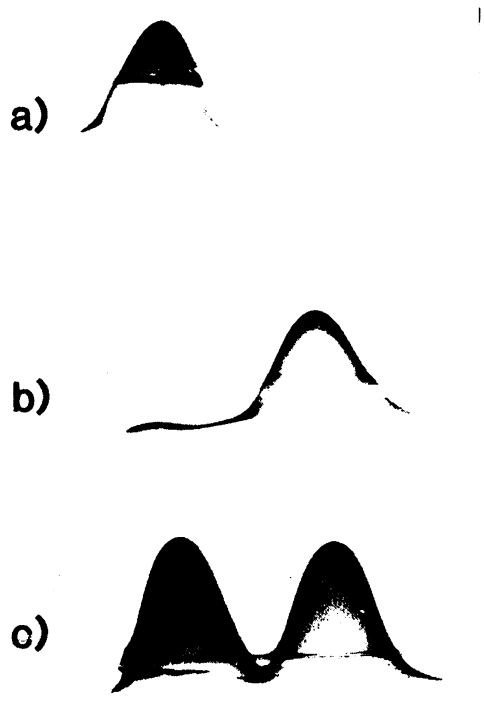

FIGURE 2 Crossed immunoelectrophoresis. Incubation of crude exudate and serum reveal ongoing C3 splitting *in vitro*. a) normal serum: only intact C3; b) exudate:serum = 1:4, the sample contains hardly any intact C3 (left peak); c) exudate:serum = 1:100, even with high serum excess pronounced C3 splitting is evident (right peak).

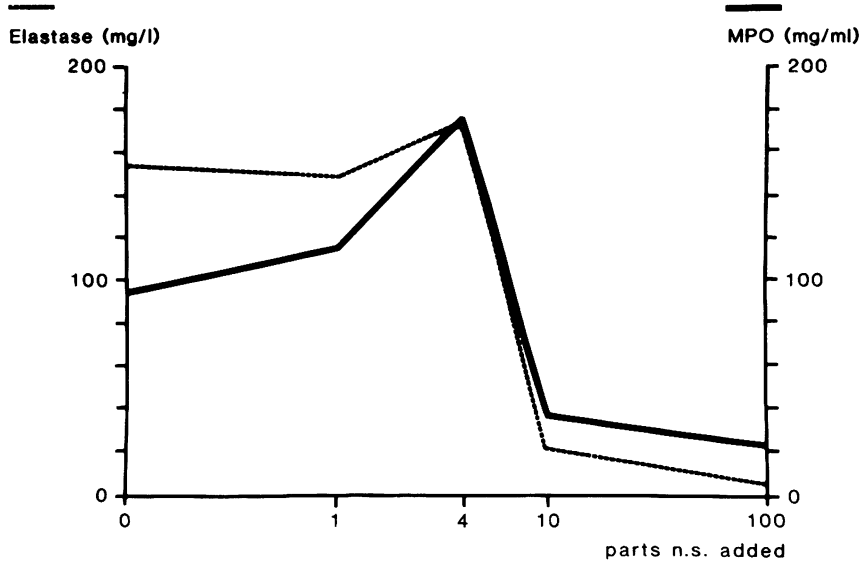

FIGURE 3 *In vitro* serum substitution. Incubation of normal serum with crude peritonitis exudate leads to the extracellular release of further lysosomal enzymes (leukocyte elastase and myeloperoxidase).

Microbiological smear investigation of such incubates revealed decreasing colonization due to increasing serum concentrations.

At the end of the 2 hours incubation period elastase-α_1 PI and myeloperoxidase was measured in each assay. For both enzymes the levels in peritonitis exudate were about 1000-fold those of normal serum. Samples containing crude exudate and serum in 1:4 ratio revealed even higher concentrations for both enzymes than the original exudates despite the dilution of 1:4. Only with increasing serum-excess did the enzyme levels decrease according to the dilution (Figure 3).

2. In Vivo Serum Substitution

The first patient was a 71 years old lady who had diffuse peritonitis 7 days after a large bowel resection. Surgical procedures and serum application were performed as described above. In the first sample of drainage run-off (0–2 hours) we found a pronounced increase of immunological opsonin levels up to 54.5% of normal serum concentration. The oc, however, reached only 29.8% of normal serum (Figure 4). In the second effluate (2–2.5 hours) oc and opsonin levels decreased, but the oc still exceeded the preoperative activity 3.4-fould (Figure 4).

The postoperative concentrations for lysosomal enzymes dropped compared to the original peritonitis exudate but were considerably higher than in the applicated serum. Elastase in the original exudate was 63.5 mg/l, MPO was 29.9 mg/l. In the 2 hours drainage fluid elastase was down to 20.3mg/l, the MPO level was 8.4 mg/l. In the 2.5 hours sample elastase was 14.3 mg/l and MPO 11.4 mg/l.

The second patient was treated for a fresh gastric perforation due to an immunocytome. Here the peritonitis exudate contained only few leukocytes (600/mm^3) and no bacteria. Oc in this exudate was 54% and was increased by serum application

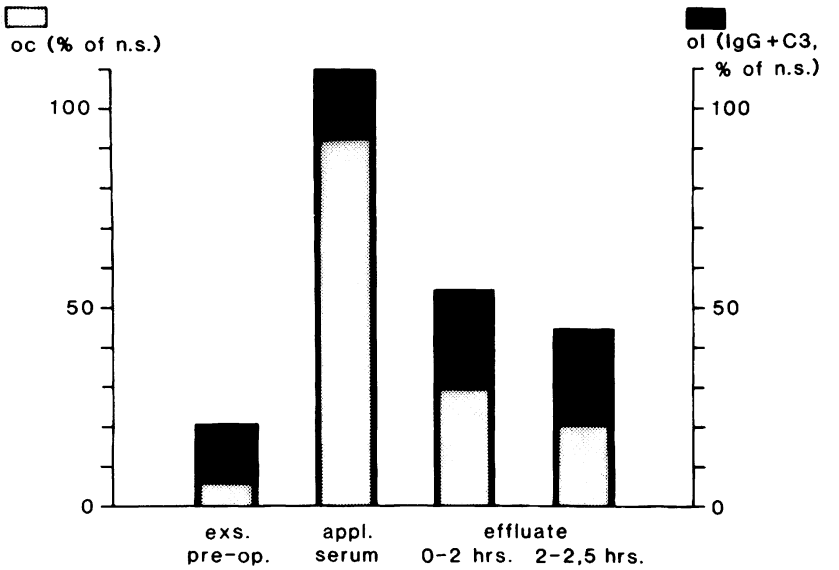

FIGURE 4 *In vitro* serum application. Intraabdominal serum application can restore opsonic capacity (oc) and opsonin levels (ol). The difference between ol and oc is a parameter for opsonin destruction.

to 83% in the first effuate and 75% after 2.5 hours. In the third case (necrotizing pancreatitis) the abdomen was closed for logistical reasons without drainage. The patient was then reoperated after 24 hours. The oc in the original exudate was 8% and in the second exudate (2 days after serum application) 12%. In all 3 patients the septic abdomen was cured. Two survived hospitalization, one died because of an underlying tumor.

DISCUSSION

The above results indicate the possibility of local restoration of opsonin levels by serum application. Interestingly, in purulent crude exudates increase of opsonin concentration due to serum substitution did not result in adequate increasing opsonin function. The fact that more serum was needed to restore oc in crude exudate compared to cell free supernatants suggests that in the former incubation mixture destruction of opsonin function still proceeded. C3-splitting in such samples could be proven by crossed immunoelectrophoresis. Similar consumption of opsonins and production of free proteolytic activity has been described in pleural empyema.[9] We have demonstrated complement splitting and free elastase activity in peritonitis exudates.[2] While these investigations described the final result of destruction *in vivo* the present findings for the first time give evidence for active degradation of substituted opsonins, both *in vivo* and *in vitro*. The difference between opsonin levels and opsonic capacity may be assumed as an indicator for this inactivation process. Therefore, application of a sufficient amount of intact opsonis can for some time restore adequate local opsonization. The increase of oc was less pronounced when the

original exudate provided already good opsonization. 2 days after serum application the effect of substitution has vanished, when the source of peritonitis cannot be cured.

During normal phagocytosis of opsonized particles about one third of the total PMNL enzyme content is released extracellularly.[7] Incubation of crude exudate with minor amounts of normal serum resulted in an increase of the extracellular levels of PMNL lysosomal enzymes. Therefore, it seems to be possible that the addition of intact opsonins to a mixture of viable phagocytes and plenty of foreign particles as well as of bacteria in peritonitis exudate leads to increased phagocytosis with subsequent release of lysosomal enzymes. The high levels of elastase and MPO in drainage effluates following abdominal lavage with serum application indicate that either the abdominal cavity even after surgical treatment of peritonitis and lavage with 10 l of Ringer solution still contains a considerable amount of purulent material or that fresh leukocyte invasion and phagocytic activity occurs very rapidly. A possible increase of PMNL enzyme release by in vivo serum application should prove no disadvantage, as normal serum also provides both a high excess of broad spectrum proteinase inhibitors and radical scavengers to prevent oxidative inhibitor inactivation.

The elimination of bacteria remaining after operation is crucial for the patient's fate as it avoids recurrent abdominal sepsis or abscess formation. We conclude that the intraabdominal application of normal serum following surgical therapy of peritonitis can support the local defence mechanisms and therefore deserves further investigation.

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