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IMPAIRED PHAGOCYTOSIS IN PERITONITIS EXUDATE SECONDARY TO COMPLEMENT CONSUMPTION‡

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Peritonitis exudates contain numerous viable bacteria despite an intense inflammatory reaction with ingress of a large number of intact, activated phagocytes. Recognition and labelling of foreign particles (opsonization) is an essential prerequisite for efficient phagocytosis. An investigation of opsonin levels and opsonic activity in peritonitis exudates and serum from patients with peritonitis was carried out. Serum opsonic activity was well correlated with levels of the opsonins, IgG and C3 complement. In peritonitis exudates opsonin concentrations were moderately reduced but opsonic activity was almost abolished. Crossed immunoelectrophoresis revealed a pronounced breakdown of C3 complement into smaller fragments. These exudates contained a large number and wide variety of pathogenic bacteria, as well as many phagocytes. In purulent exudates, we found very high concentrations of granulocytes, lysosomal and oxidative enzymes, and free proteolytic activity. It is concluded that specific opsonin consumption and nonspecific cleavage can contribute to the detected C3 breakdown. Local treatment of impaired opsonization by substitution of intact opsonins and protease inhibitors should be considered.

KEY WORDS: Peritonitis, phagocytosis, opsonization, granulocyte enzymes, protease inhibitors.

INTRODUCTION

Purulent peritonitis exudates are characterized by local survival of bacteria despite the presence of large numbers of polymorphonuclear leukocytes (PMNLs). So far, there is no satisfactory explanation for this paradoxical situation. It is generally accepted that complement, antibodies and PMNLs are the essential components in host defence against bacterial infection.[1] Phagocyte function, the main cellular component of the system, seems to be intact in peritonitis exudates.[2,3]

The humoral immune process of recognizing and labelling a microbe as antigenically foreign (opsonization) is a prerequisite for sufficient phagocytosis. Opsonization is mainly achieved by the phagocytosis promoting factors, IgG and C3 complement.[4,5] Thereby complement is activated, resulting in the enhanced liberation of opsonins, other mediators of inflammation and bacteriolytic substances (Figure 1).

Physiological C3 activation eventually results in its breakdown into the fragments, C3c and C3d. Nonspecific proteolytic degradation of opsonins in abscesses and

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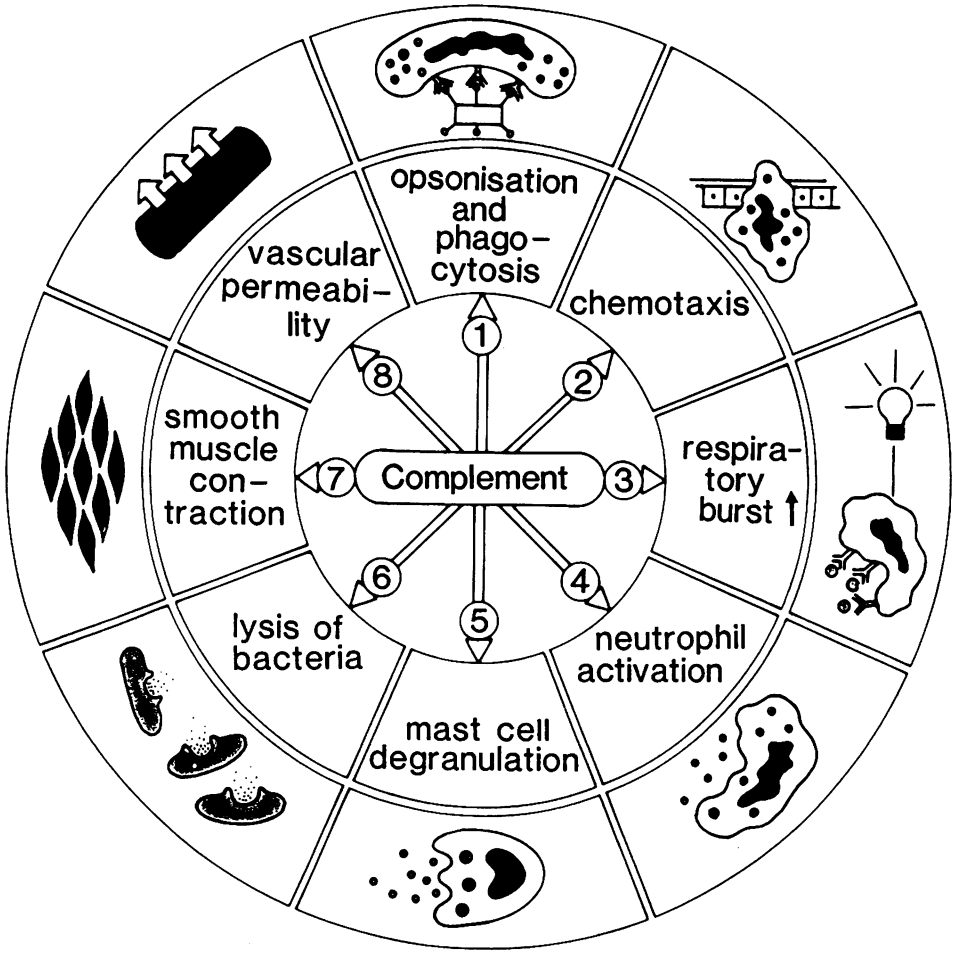

FIGURE 1 The complement system with its key factor C3 playing a central role in inflammation (modif. acc. to[30]).

empyema has also been described.[6,7] These opsonin split products can retain their antigenicity, accounting for high opsonin levels by immunological determination despite the absence of opsonic function. Therefore, both opsonin levels and their activity must be considered in evaluating opsonization.

During phagocytosis PMNL lysosomal enzymes are liberated into the phagosome. However, the process is also accompanied by a major extracellular enzyme release.[8] In sepsis, endogenous proteinase inhibitors can be impaired by oxidative enzymes or oxygen-derived free radicals.[9] Extensive PMNL elastase release in an area of inflammation can overwhelm local inhibitor activity, leading to free proteolytic enzyme activity. Extensive knowledge has accumulated in recent years regarding opsonization and PMNL function in the blood of septic patients. Little, however, is known about intraabdominal fluid levels of complement and immunoglobulins,[10,11] and no data are available about opsonin integrity and opsonic function in peritonitis exudates.

Recently, we found evidence for a marked intraabdominal opsonic dysfunction in peritonitis.[3] Modified chemiluminescence assays provide a quantitative method for rapid determination of opsonic activity in blood.[12,13] Consequently, we developed a similar system to evaluate opsonic function in exudates. Integrity of C3 was investigated by immunoelectrophoresis. The aim of our study was to elucidate the pathophysiology of opsonization in peritonitis exudates. We examined patients with acute and persistent peritonitis. The latter group was treated with Etappenlavage,[14] that is, scheduled abdominal lavage until clearance of the abdominal cavity.

MATERIALS AND METHODS

Sampling Procedure

During 24 operations for acute diffuse purulent peritonitis and 22 operations under Etappenlavage, exudate and blood samples were drawn simultaneously. Exudates collected with disposable plastic syringes were cleared of cells and debris by immediate centrifugation. Blood samples were processed to provide serum and EDTA plasma.

Chemiluminescence Assay for Opsonic Activity

Opsonic activity was determined using a chemiluminescence assay.[15] Briefly, zymosan was preopsonized by incubation with normal serum, patients' serum or patients' exudate for 15 min at 37°C. The final assay solution contained 0.05 ml diluted EDTA blood (1:15 in phosphate buffered saline (PBS)), 0.8 ml Veronal buffer and 0.1 ml Luminol solution (0.7 mM), resulting in a final blood dilution of 1:300. The assay was started by adding 0.05 ml of preopsonized Zymosan (20 mg/ml). Chemiluminescence was measured using a Biolumat (Fa. Berthold, Wildbad, FRG) and integrated over 30 min using a microcomputer (Apple IIe). Opsonic activity of patient serum or exudate was expressed as a percentage of the value obtained using normal serum.

Immunological evaluation of opsonins and related products

Immunological C3 and IgG levels were measured with a standard radial immunodiffusion assay (Behringwerke, Marburg, FRG; normal values = 100%: C3 82 mg/dl and IgG 1250 mg/dl). C3 split products were demonstrated by crossed immunoelectrophoresis according to Ganroth[16] employing the same C3c antibody as used in the radial immunodiffusion. C3a was determined by radioimmunoassay available from Upjohn.[17]

Tests for PMNL-enzymes

Elastase[18] in complex with α_1-proteinase inhibitor (α_1 PI) and myeloperoxidase[19] concentrations were measured by ELISA (test kits from E. Merck, Darmstadt, FRG). The reference range for plasma elastase is 50–181 μg/l, and that for myeloperoxidase 25–47 μg/l. Free elastase activity was measured with a chromogenic peptide substrate[20] or by adding a surplus of α_1 PI and then reassaying the sample for elastase-α_1 PI complex.

Microbiological Investigations

Aerobic and anaerobic bacterial cultures were prepared from all exudates immediately after sampling.

Other Assays

Protein content was determined by the Biuret method.[21] Serum and exudate electrophoresis was performed according to Grabner *et al.*[22] WBC was carried out in a Neubauer chamber after staining with Türks's solution (from E. Merck, Darmstadt, FRG). α_1-PI was measured with a standard radial immunodiffusion assay (Behringwerke, Marburg; normal range: 190–350 mg/l).

Statistics

Computerized correlation analysis was performed for opsonin concentration and opsonic activity selecting the most appropriate correlation out of degree of regression 1 to 4.

RESULTS

In normal serum, there was a close correlation between opsonin levels and opsonic activity and only small amounts of C3 activation products (Table 1). Normal serum dilution results in an S-shaped relationship between opsonic activity and a C3/IgG index. The latter is a simple addition of C3 and IgG concentrations (Figure 2). In acute peritonitis, serum opsonin levels, opsonic activity and protein content were considerably diminished and lay close to the lower limit of the normal range. The C3a level in these samples, however, was 5.3 fold increased. In patients with persistent peritonitis, IgG levels were normal, resulting in a normal opsonic activity despite continuous C3 activation. The relationship between opsonic levels and opsonic activity in the patients' sera was very close to that of normal serum. Computerized statistical analysis for patients' serum values shows a best correlation in the third degree of regression with the variables $y = 10^{-5} \cdot x^3 - 0.006 \cdot x^2 + 1.42 \cdot x - 14.7$ which results in a S-shaped curve, very similar to that of normal serum dilution (Figure 2). Compared to normal, serum opsonins in patients' sera are intact and there is no deficit in opsonin function.

Opsonic activity was investigated in three types of peritonitis exudate:

TABLE I
Serum factors contributing to opsonic activity (OA) (mean ± SEM)

	normal serum (n = 20)	acute peritonitis, serum (n = 30)	persistent peritonitis, serum (n = 30)
protein content (g/dl)	7.08 ± 0.7	5.1 ± 0.1	5.5 ± 0.12
IgG (mg/dl)	1128 ± 31	800 ± 49	1260 ± 92
C3 (mg/dl)	79.9 ± 2.3	62.3 ± 3.86	59.0 ± 3.1
C3a (μg/dl)	12.8 ± 1.24	67.7 ± 10.4	67.3 ± 8.0
OA (% of normal)	96.0 ± 5.7	84.5 ± 6.1	93.0 ± 5.4

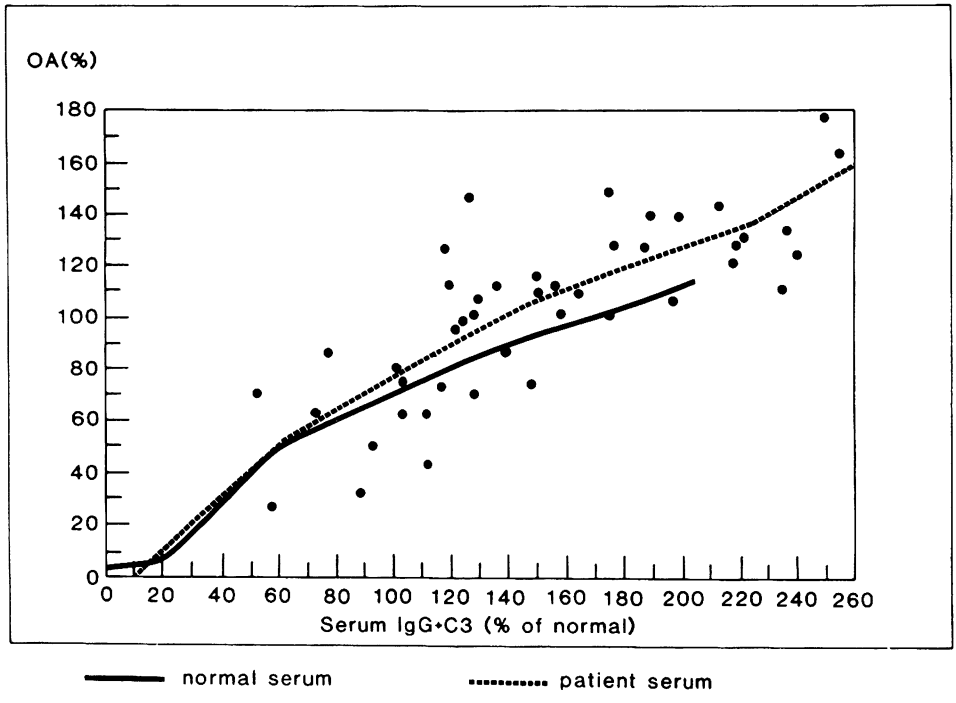

FIGURE 2 Correlation of opsonic activity and opsonin concentration in serum. The C3-IgG-index results from addition of both serum concentrations (in % of normal). The curves represent the relationship obtained by diluting normal serum and in undiluted patients' serum. The points represent patients with acute and persistent peritonitis.

(a) exudates found in the first operation for acute peritonitis;

(b) purulent exudates from patients with persistent peritonitis;

(c) clear exudates found at the successful end of Etappenlavage (EL).

The results are summarized in Table II. Mean exudate protein content was lowest in

TABLE II

Opsonization-related factors and opsonic activity (OA) in peritonitis exudates (mean ± SEM). OA_{exp} is the expected OA according to the correlation between IgG/C3 (concentrations of IgG + C3) and OA in serum, OA_{real} is the actual OA in acute peritonitis and Etappenlavage (EL) exudates

	acute peritonitis exudate (n = 30)	purulent EL exudate (n = 30)	clear El exudate (n = 15)
protein content (g/dl)	3.25 ± 0.23	3.5 ± 0.13	4.1 ± 0.28
IgG (mg/dl)	470 ± 43	690 ± 62	630 ± 70
C3 (mg/dl)	28 ± 2.7	21 ± 1.5	40.1 ± 4.4
C3a (μg/dl)	741.9 ± 327.4	397.8 ± 87.5	198.0 ± 26.0
OA_{exp}(% of normal)	52	56	64
OA_{real}(% of normal)	12.9 ± 2.7	5.9 ± 1.2	53 ± 4.8
leukocytes (1000/mm^3)	73.0 ± 13.7	32.0 ± 14.1	< 0.5

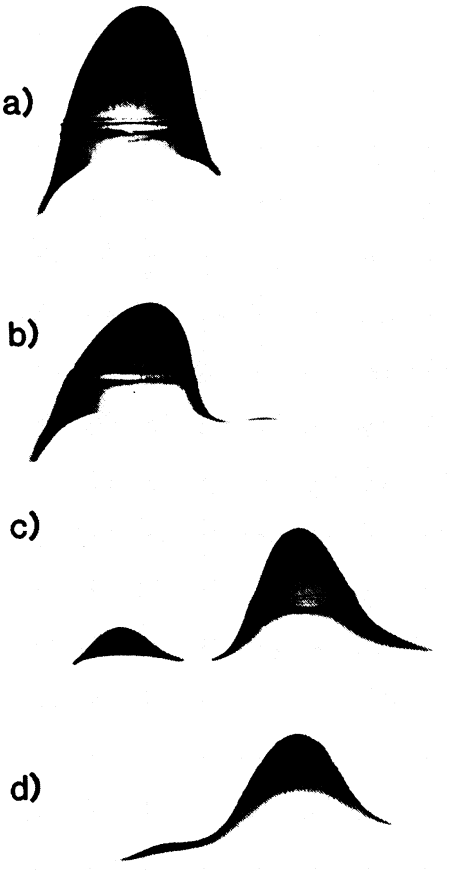

FIGURE 3 C3 splitting in serum and exudate as demonstrated by crossed immunoelectrophoresis. (a) Normal serum without C3 splitting. (b) Patient serum with only trace amounts of C3 degradation products. (c) Exudate (35,000 leukocytes/mm^3) with distinct C3 breakdown into smaller components (right peak). (d) Purulent peritonitis exudate (94,000 leukocytes/mm^3) with extensive splitting of C3 complement.

acute peritonitis and highest in clear exudates of successful EL. The electrophoretic protein distribution pattern was similar to that found in serum (data not shown). Exudates from acute peritonitis contained the highest leukocyte concentrations and also the highest levels of C3 activation products. In purulent EL exudates, both mean leukocyte count and C3a levels were about half of the amount found in acute peritonitis. Clear EL exudates showed the lowest C3a concentrations and only few leukocytes. Based on the serum correlation between opsonin concentration and function (Figure 2) the opsonin levels in the exudate samples should result in an opsonic function of 52% of normal for acute peritonitis, 56% of normal for purulent EL, and 64% of normal for clear EL. Experimentally determined opsonic activities, however, were much lower for acute peritonitis (12.9% of normal) and for purulent

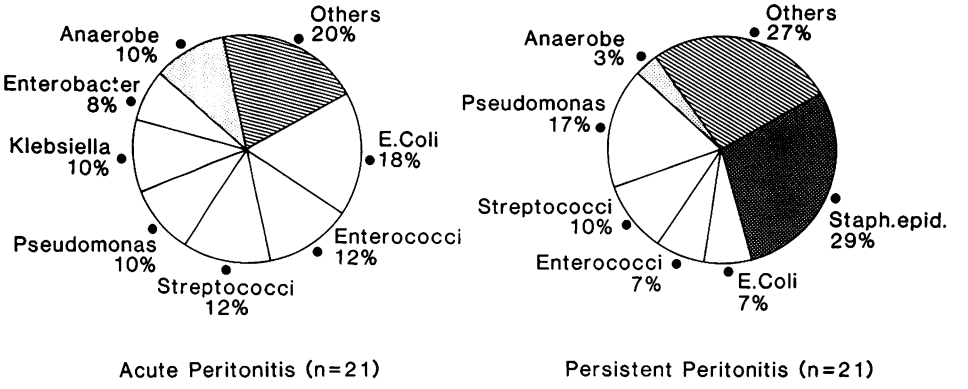

FIGURE 4 Bacterial spectrum in acute peritonitis and Etappenlavage.

EL (5.9% of normal). In contrast the opsonic activity in clear exudate samples from the end of EL was 53% of normal, thus showing only a small reduction of opsonic capacity compared to the immunologically measured concentration (64% of normal).

Obviously, a major part of the immunologically detectable opsonins in purulent exudates are functionally impaired. Opsonin breakdown was examined using crossed immunoelectrophoresis in 6 patients' serum and exudate samples employing a C3c antibody. With this test, no C3 splitting was detectable in normal serum (Figure 3). In peritonitis serum, only a small amount of C3 was fragmented. In contrast, in purulent exudate C3 was split into fragments of lower molecular weight. The degree of splitting appeared to increase with increasing leukocyte concentrations in the exudate.

Cultures of the exudates revealed a wide variety of different bacteria without any single species dominating. In EL, there was a pronounced contamination of the exudates with Staphylococcus epidermidis. Anaerobic infections were also rare in EL (Figure 4). Colonization with multiple bacterial species was increased in purulent EL; no sterile samples were found in these patients (Figure 5).

We quantified PMN-elastase (in complex with α_1-proteinase inhibitor) and myeloperoxidase levels in 67 exudates. Complexed elastase in exudates from acute

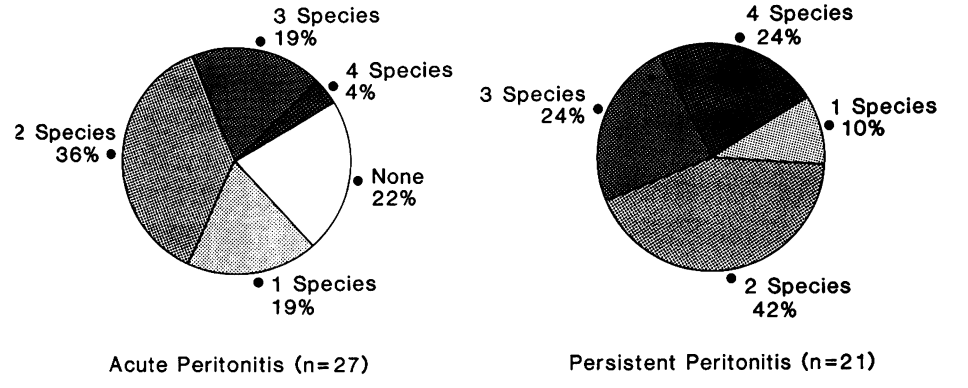

FIGURE 5 Multiple species infection in peritonitis exudate.

TABLE III
Granulocyte enzymes and α_1-proteinase inhibitor (α_1 PI) in peritonitis exudates (means ± SEM)

	acute peritonitis exudates (n = 23)	purulent EL exudates (n = 30)	clear EL exudates (n = 14)
elastase-α_1 PI (μg/ml)	58 163 ± 11 176	80 082 ± 10 380	3 139 ± 967
myeloperoxidase (μg/ml)	52 990 ± 6 317	52 200 ± 14 227	3 770 ± 1 145
α_1 PI (μg/ml)	2 000 ± 159	2 010 ± 187	2 490 ± 300

peritonitis and purulent EL raised up to a mean concentration of 80 mg/l, i.e. 1000 times higher than the normal plasma range. The highest values were found in purulent EL (Table III) with single maximal values up to 160 mg/ml. Moreover we demonstrated extremely high concentrations of myeloperoxidase reaching up to single maximal values of 160 mg/l in acute peritonitis and purulent EL exudates. However, clear EL exudates contained only low concentrations of PMNL enzymes. All exudates showed essentially normal concentrations of immunologically determined total α_1 PI.

Several exudate samples were examined for free elastase activity, both with a specific chromogenic substrate and by adding α_1 PI. In some exudates, up to 70% of the total elastase content was found to be uninhibited, enzymatically active elastase. The concentration for α_1 PI in these exudates ranged from 990 to 3410 mg/l. Calculated on the base of molar ratio, such concentrations should be sufficient for complete elastase inhibition. In these patients (all with gastrointestinal perforation), intraabdominal elastase levels varied within a wide range (Table IV). Plotting opsonin levels versus opsonic activity for these peritonitis exudates, it could be demonstrated that most samples lacked opsonic activity almost completely (Figure 6). A few exudates, however, reached almost normal opsonic activity according to the serum relationship. Analysis of elastase-α_1 PI levels revealed that these were rather clear exudates with only minor elastase contamination (< 10 mg/l), whereas, exudates with high elastase levels (> 10 mg/l) were deficient in opsonic function.

DISCUSSION

Using the chemiluminescence assay, analysis of particle opsonization can be rather easily accomplished in serum and exudate. Our results confirm the key role of IgG and C3 in particle opsonization. In both normal and pathological sera, opsonin concentration and opsonin function was well correlated. Our results also support

TABLE IV
Free and complexed elastase and α_1-proteinase inhibitor (α_1 PI) in 4 patients' exudates after gastrointestinal perforation

	α_1 PI-elastase complex (mg/l)	free elastase (mg/l)	total elastase (mg/l)	total α_1 PI (mg/l)
Pat. 1	120.6	272.3	392.9	2250
Pat. 2	45.7	0.5	46.2	1610
Pat. 3	111.1	1.6	112.7	1060
Pat. 4	54.6	73.1	127.7	990

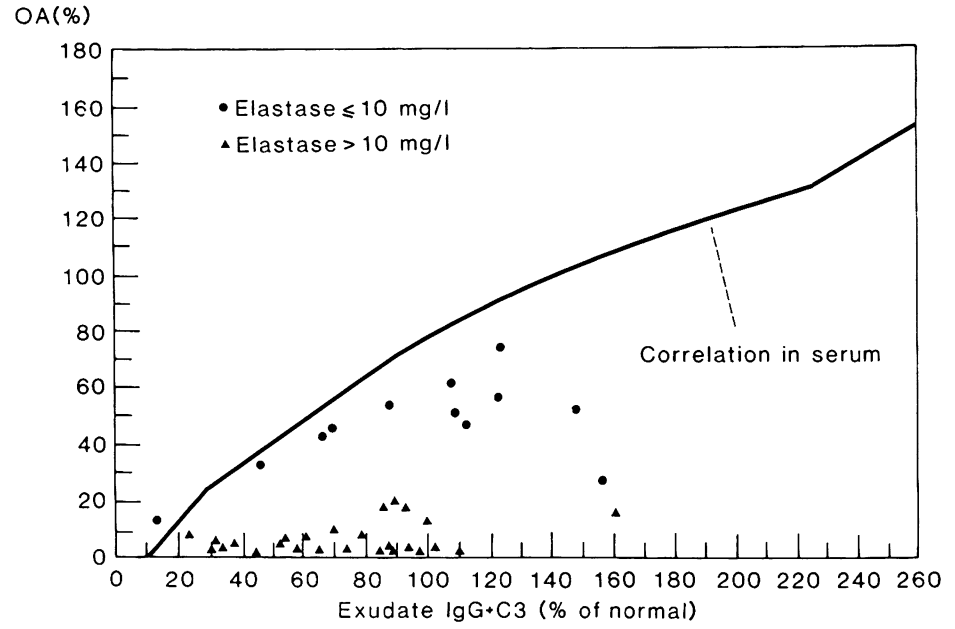

FIGURE 6 Relationship between opsonin levels and opsonic function in peritonitis exudate. The curve demonstrates the relationship obtained in patients' sera.

previous findings that factors of the humoral defence system are diminished in the serum of septic patients.[23,24]

To date, little information has been available about intraperitoneal opsonin concentrations, and no data have been published about particle opsonization in peritonitis exudates. In pleural empyemas, local exhaustion of complement components, with decreased opsonic activity, has been described.[25,26] Since we could demonstrate a normal electrophoretic protein distribution pattern in exudates compared to serum a distinct increase in peritoneal permeability in peritonitis with easy passage even for large proteins can be assumed. Therefore, we conclude, that the reduction of several proteins in peritonitis exudates compared with serum is due to consumption of these specific proteins. Immunologically-determined opsonin levels were 40–50% of serum concentrations. Although this amount should be sufficient for opsonization, we found a marked dysfunction of particle opsonization in human peritonitis exudates. This suggests that most of the opsonins were functionally deficient. Complement C3 is not only one of the main phagocytosis promoting factors but also the central component of the whole complement system.[27] Crossed immunoelectrophoresis for C3 using C3c-antibodies showed an extensive destruction and breakdown of C3 in purulent exudates. The stained, C3c-containing fragments seem to be C3 degrading products without opsonic function. Extensive specific C3 activation is indicated by the high C3a concentrations and might be induced by the contamination of the exudates with multispecies microbes and other foreign particles.

Additionally, C3 degradation can also proceed via a nonspecific pathway of proteolytic and oxidative destruction.[25,26,28] The exudate levels of PMNL enzymes exceed all known pathological blood concentrations even in severe septic shock.[29] This is prob-

ably not only due to a very high number of activated or disintegrated leukocytes but also to a slower clearance of elastase-α_1 PI complexes from the abdominal cavity compared to the circulation. Despite a quantitatively sufficient immunological concentration of α_1 PI we demonstrated uninhibited elastase enzymatic activity. Since oxidative impairment of the inhibitory capacity of α_1 PI has been described *in vitro*[9] such damage might have occurred in the peritoneal cavity due to the release of myeloperoxidase or oxygen derived free radicals. The former has been demonstrated in great quantity in many of the exudates.

Our results support opsonin breakdown being an important mechanism in the impaired intraabdominal defence observed in peritonitis patients. Proteolytic degradation of the essential complement components seems to be due to specific activation of the complement cascades during contact with inflammatory stimuli (microbes, cell debris etc.) as well as to unspecific breakdown by high concentrations of extracellularly released granulocytic proteinases. Although the activity of the latter enzymes is normally well regulated by a sufficient amount of extracellular proteinase inhibitors, the local inhibitory function of these proteins is obviously abolished in the inflammatory focus.

From a therapeutic point of view, clearing of the focus of peritonitis and thorough intraoperative rinsing of the abdominal cavity is essential. However, our results suggest that local substitution of intact opsonins and protease inhibitors may be beneficial. At present, this could most easily be accomplished by intraabdominal application of serum or defibrinated plasma at the end of the peritonitis operation.

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