PROTEASES II

Potential Role in Health and Disease

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DEFICIENT PHAGOCYTOSIS SECONDARY TO PROTEOLYTIC BREAKDOWN OF OPSONINS IN PERITONITIS EXUDATE

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INTRODUCTION

In peritonitis, proper functioning of the intraabdominal local defence system is crucial for a favourable outcome and survival of the patient. Peritonitis exudate is characterized by the presence of a large number of viable bacteria despite a huge population of intact PMN-leukocytes. Although phagocyte function, the main factor of cellular defence, is intact or even stimulated in peritonitis exudate (1,2), there is no adequate explanation of how bacteria can persist in surroundings rich in PMN-leukocytes.

An adequate sufficient intraabdominal host defence results from a balanced cooperation between cellular and fluid phase components. The humoral immune process of recognizing and labelling a microbe as antigenically foreign is described as opsonization. This can proceed via non-specific or specific mechanisms (3). The latter is immunoglobulin G (IgG) dependent. Both pathways result in complement activation which leads to a liberation of opsonins, mediators of inflammation and microbicidal components. The main factors of opsonization are C3-derived complement components and IgG. Physiological C3 activation results in its breakdown into the fragments C3c and C3d. Unspecific proteolytic breakdown of opsonic factors in pleural empyema has also been described (4).

Phagocytosis leads to cell activation and also results in an extracellular release of lysosomal and oxidative granulocyte enzymes (5). Myeloperoxidase is known to impair opsonization (6). Proteolytic and oxidative destruction can destroy biological activity of protein components without altering their antigenicity. Thus, despite immunologically determined high concentrations, there may be a functional deficit in such factors. In parallel to enzyme release, particle attachment leads to a strong activation of oxygen metabolism in phagocytes, resulting in the generation of oxygen-derived free radicals. These micro-

bicidal and cytotoxic substances are known to destroy α_1-proteinase inhibitor (α_1PI). Using a photometric amplification system, the release of oxygen-derived free radicals can be measured as chemiluminescence (CL) and is assumed to be a quantitative parameter for phagocytic activity. Using a constant number of phagocytes, CL-measurements can be used as a direct parameter for the quality of particle opsonization (7,8).

Little is known about intraabdominal opsonization. We developed a simple CL assay to evaluate opsonic activity (OA) in peritonitis exudates and serum samples of patients with acute and persisting peritonitis. The latter group was treated with Etappenlavage which means planned relaparotomy until clearance of the abdominal cavity. In addition, we investigated opsonin levels as well as released granulocytic proteins.

MATERIAL AND METHODS

Patients

50 abdominal exudates and corresponding blood samples were drawn intraoperatively from 27 patients with diffuse purulent peritonitis. Exudates were centrifugated, while blood was processed into serum and EDTA-plasma.

Chemiluminescence assay for opsonic activity

Zymosan was preopsonized with pooled normal serum, patients' serum or patients' exudate. The final chemiluminescence assay contained 0,05 ml diluted EDTA-blood (1/15) from healthy volunteers, 0,8 ml Veronal buffer and 0,1 ml Luminol solution (9). The reaction was started by adding 0,05 ml of the opsonized zymosan (20 mg/ml). The 30 min. integral of chemiluminescence was calculated. In each assay zymosan opsonized with normal serum, patients' serum and patients' exudate was tested simultaneously. As all other conditions (blood and buffer concentration) were identical, the resulting different chemiluminescence response is due to the quality of opsonization (10). Opsonic activity was expressed as a percentage of the normal serum value.

Opsonin studies

C3 and IgG levels were measured with a standard radial immunodiffusion assay (Behringwerke Marburg, normal values: IgG 1250 mg/dl and C3 82 mg/dl). C3 splitting was demonstrated by crossed immunoelectrophoresis according to Ganroth (11) employing a C3c antibody (Behringwerke Marburg).

Tests for PMN-enzymes

Elastase in complex with α_1-proteinase inhibitor (α_1PI) and myeloperoxidase concentrations were measured by ELISA: plasma reference for complexed elastase: 50-181 µg/l, and for myeloperoxidase: 25-47 µg/l (12, 13). Free elastase activity was measured with a chromogenic substrate (14) or by adding α_1PI and then re-assaying elastase-α_1PI complex.

RESULTS

Opsonization in serum

In acute peritonitis C3 and IgG serum levels were close to the lower limit of the normal range and were increased in patients with persisting peritonitis (Table 1). Opsonic activity in patients' serum was well correlated with a C3/IgG index which results from addition of C3 and IgG concentrations. Computerized correlation analysis resulted in a S-shaped curve, very similar to a dilution curve of normal serum (Fig. 1). In Fig. 1 serum samples of patients (n=23) with acute and persisting peritonitis at the time of sample collection were included.

Table 1. Opsonin levels (IgG, C3) and opsonic activity (OA) in patient serum (% of normal ± standard deviation)

	acute peritonitis (n=13)	persisting peritonitis (n=14)
IgG	62.8 ± 29.3	109.1 ± 30.8
C3	65.3 ± 23.1	83.0 ± 21.6
OA	85.8 ± 33.5	115.4 ± 20.8

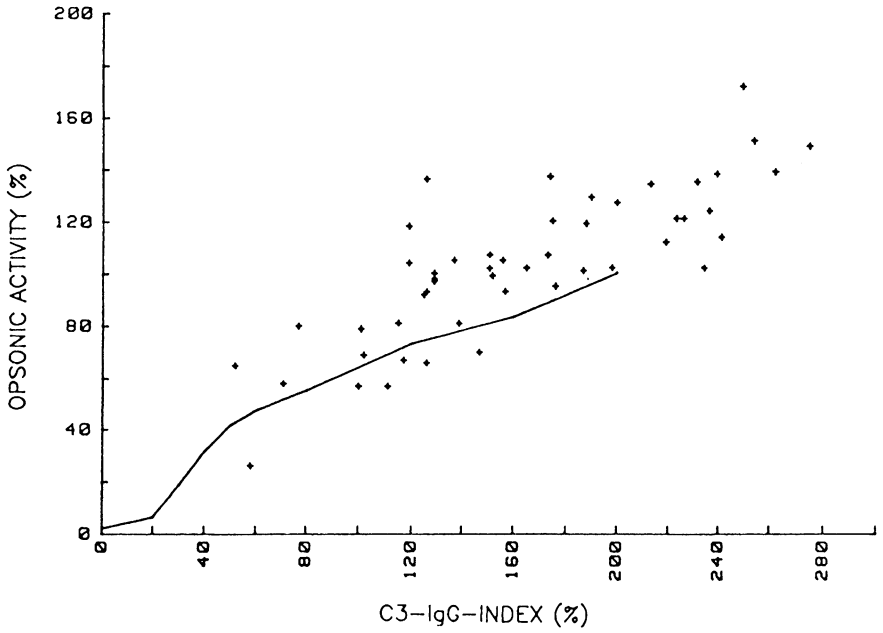

Fig. 1. Correlation of opsonic activity and opsonin concentration in patient serum. The C3-IgG-index results from addition of serum concentrations of both parameters. The curve demonstrates the correlation from serial dilutions of normal serum (1:2 to 1:10).

Opsonization in exudate

In peritonitis exudates, mean protein content was 66 % of

serum levels in acute peritonitis and 62 % of serum levels in Etappenlavage. The electrophoretic protein distribution pattern was similar to serum, indicating peritoneal permeability even for large molecules. Opsonin concentrations are listed in Table 2. According to the serum correlation of opsonin concentration and function, these opsonin levels should result in an opsonic activity of 58 % of normal in acute peritonitis and 56 % of normal in Etappenlavage. The experimental determination of opsonic function, however, showed a much lower activity (8.4 % and 4.6 % of normal, respectively) indicating a pronounced deficit in particle opsonization in peritonitis exudates.

Table 2. Opsonin levels (IgG, C3) and opsonic activity (OA) in peritonitis exudates (% of normal serum value ± standard deviation). OA_{exp} is the expected OA according to the correlation between IgG/C3 and OA in serum, OA_{real} is the actual OA in exudate.

	acute peritonitis (n=13)	Etappenlavage (n=14)
IgG	43.9 ± 21.3	52.9 ± 18.1
C3	35.8 ± 27.8	25.5 ± 6.8
OA_{exp}	58	56
OA_{real}	8.4	4.6

To evaluate opsonin breakdown, crossed immunoelectrophoresis was carried out in 6 patients' serum and exudate samples employing a C3c antibody. In peritonitis serum only a small amount of C3 was fragmented (Fig. 2b). In exudate, however, depending on the leukocyte concentration, a great part (Fig. 3c) or almost all (Fig. 3d) C3 was split into fragments of lower molecular weight. Thus, the opsonic deficit in purulent exudates was accompanied by an extensive breakdown of the complement factor C3.

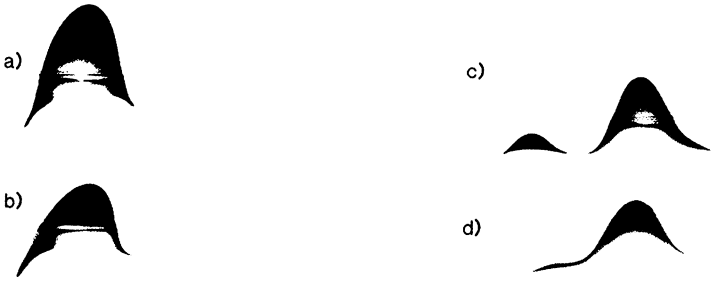

Fig. 2. Crossed immunoelectrophoresis for C3 in serum and exudate.
a) Normal serum, no C3 splitting
b) Patient serum, only trace amounts of C3 breakdown products
c) Exudate (22,000 leukocytes/mm³), pronounced C3 splitting into smaller components (right peak)
d) Purulent peritonitis exudate (110,000) leukocytes/mm³), almost complete breakdown of C3

Unspecific proteolytic and oxidative activity in peritonitis exudate

In 27 exudates, we quantified complexed PMN-elastase and myeloperoxidase levels. Elastase concentrations were elevated up to 250 mg/l, which is 2000 times higher than the normal plasma range. We also found extremely high concentrations for myeloperoxidase, reaching up to 160 mg/l (Table 3).

Table 3. Complexed PMN-elastase and myeloperoxidase levels in peritonitis exudates (mean ± standard deviation, µg/l).

	acute peritonitis (n=13)	Etappenlavage (n=14)
elastase (-α_1PI)	75,972 ± 52,366	89,853 ± 67,570
myeloperoxidase	34,458 ± 42,661	55,402 ± 39,005

In several exudates we could demonstrate free elastase activity, both with a specific chromogenic substrate and an α_1PI-binding assay. In some exudates up to 70 % of the total elastase content was found to be uninhibited free elastase. The concentration for α_1-proteinase inhibitor in these exudates ranged from 99 to 341 mg/dl, which, calculated on the basis of the molar ratio of inhibitor concentration versus proteinase, should be sufficient for complete elastase inhibition. In patients with gastrointestinal perforation, intraabdominal elastase levels varied within a wide range (Table 4).

Table 4. Elastase (in complex or active) and α_1-proteinase inhibitor (α_1PI) in peritonitis exudates.

	α_1PI-elastase complex(mg/l)	free elastase (mg/l)	total elastase (mg/l)	total α_1PI (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

To investigate the possible influence of proteolytic lysosomal enzymes on opsonic activity we compared both parameters. Correlation of the opsonic deficit with elastase levels in exudates revealed that only exudates with a low concentration of complexed elastase (<10 mg//l) reached an almost normal opsonic activity, whereas exudates with high elastase concentrations were deficient in opsonic function (Fig. 3).

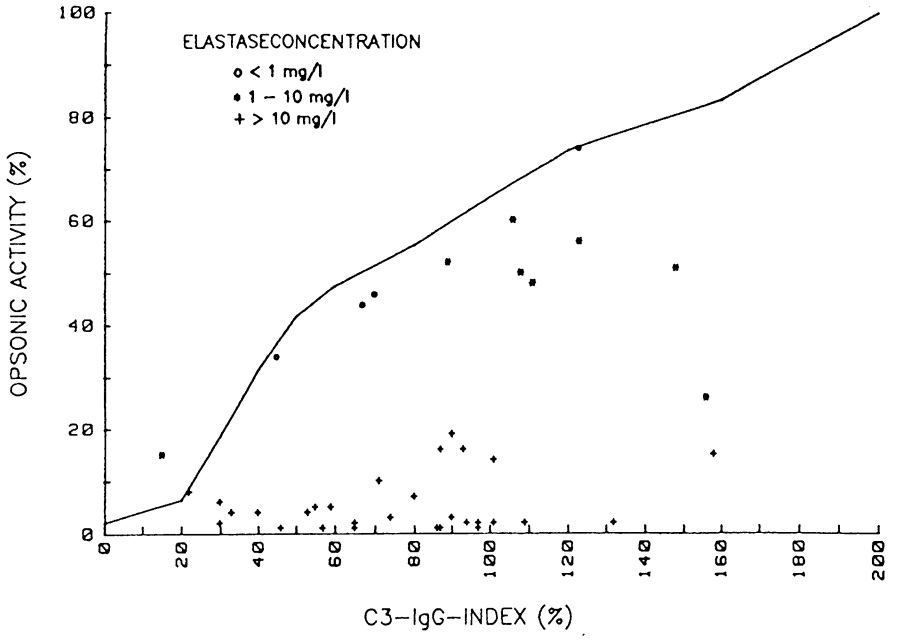

Fig. 3. Correlation of opsonic activity and elastase concentration in peritonitis exudates. The curve demonstrates the relation between opsonin concentration and opsonic activity in normal serum. Only exudates with elastase concentrations $\leq$10 mg/l reveal adequate opsonic activity.

DISCUSSION

The CL-approach described here, provides a rapid,reliable non-destructive method for quantitative analysis of opsonic capacity in serum and exudate. Thereby, in normal as well as in patient serum the key role of IgG and C3 for opsonization could be confirmed.

Little information is available about intraperitoneal fluid-phase defence activity. In pleural empyema, deficient phagocytosis due to breakdown of opsonins has been described (4). We could demonstrate a high peritoneal permeability in peritonitis giving way even for large proteins. Despite sufficient immunologically measurable opsonin levels our results revealed an extended dysfunction of particle opsonization in human peritonitis exudates. Most of the immunologically found opsonins were functionally destroyed. The crossed immunoelectrophoresis gave evidence that purulent peritonitis exudates contained hardly any intact physiologically active C3. The identified C3 fragments seem to be degraded products without opsonic function.

In exudates we found extremely high levels of PMN-elastase (most of it in complex with α_1PI) and myeloperoxidase, indicating the release of a major part of the total phagocytic enzyme content. Due to a slow peritoneal clearance of elastase-α_1PI complexes, enzyme concentrations are further increased. Despite an immunologically sufficient concentration of α_1PI we could demonstrate free elastase activity in some exudate samples. Oxidative impairment of α_1PI has been described (15)

and may be due to the release of myeloperoxidase and highly reactive oxygen products during phagocytosis.

For the first time these data reveal clearly that the dysfunction of the intraabdominal defence system in acute peritonitis results from impaired opsonic capacity. One major underlying pathomechanism may be oxidative inactivation of the α_1-proteinase inhibitor thus allowing unspecific proteolytic opsonin degradation by free lysosomal enzymes. For further improvement in therapy the effect of local proteinase-inhibitor application has to be considered.

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