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Heinz Redl

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Part A: Pathophysiological Role of Mediators and
Mediator Inhibitors in Shock

First Vienna Shock Forum
Part B: Monitoring and Treatment of Shock

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BREAKDOWN OF C3 COMPLEMENT AND IgG IN PERITONITIS EXUDATE - PATHOPHYSIOLOGICAL ASPECTS AND THERAPEUTIC APPROACH

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INTRODUCTION

The breakdown of local defense mechanisms in peritonitis is one of the major causes of systemic complications - sepsis and multiple organ failure. In peritonitis exudate we could demonstrate a marked dysfunction of particle opsonisation and strong proteolytic activity (Billing et al., 1988). The underlying pathomechanisms for these findings were investigated. The current therapy for patients with diffuse purulent peritonitis includes surgical elimination of the source of peritonitis and a lavage procedure of the abdominal cavity (Guenther et al., 1987). If the local source of peritonitis cannot be cured for technical reasons chances for survival of the patient are poor (Weiser et al., 1986). Even in patients with primarily successful surgical treatment abscess formation and recurrent abdominal sepsis occur frequently. For the latter group postoperative support of local intraabdominal defense mechanisms might be crucial. We investigated the effect of intraperitoneal application of normal serum at the end of the peritonitis operation. Thereby intact opsonins should be substituted, scavengers of free radicals supplied and a broad spectrum of proteinase inhibitors provided. Moreover, serum being bactericidal by itself can liberate anaphylatoxins thus inducing phagocyte invasion.
MATERIALS AND METHODS

Exudates and blood samples were drawn simultaneously during operations for diffuse peritonitis. Exudates collected with disposable plastic syringes were divided and one part was cleared of cells and debris by immediate centrifugation. Blood samples were processed to serum or EDTA-plasma. Opsonic activity was determined by a chemiluminescence assay and C3 and IgG levels were measured by radial immunodiffusion as described previously (Billing et al., 1988). C3- and IgG-splitting was demonstrated by crossed immunoelectrophoresis (Ganroth 1972) employing the same C3c and IgG antibodies as used in the immunodiffusion assay. Protein content was determined by the Biuret method. Serum and exudate protein distribution patterns were studied by electrophoresis according to Grabner et al. For in vitro investigation of serum application samples of native peritonitis exudate or cell free exudate supernatants were incubated with different amounts of normal donor serum for two hours at 37°C. For in vivo serum substitution one bloodbank-serum-unit (300 ml) was applied intrabdominally at the end of the peritonitis operation. Drainage fluid was collected and pooled from 0-2 hrs. and from 2-2.5 hrs. after operation and studied for opsonin levels and opsonic activity.

RESULTS

1. Opsonin dysfunction

To quantify the peritoneal permeability for larger proteins, e.g. opsonins, the protein distribution patterns were determined in serum and exudate from 11 patients (Tab.1). In the same group opsonin levels and opsonic activity were evaluated (Tab.2).

TABLE 1. Amount and electrophoretic pattern of proteins in peritonitis serum and exudate (n=11, mean ± standard deviation)

<table>
<thead>
<tr>
<th>Protein amount (g/l)</th>
<th>Serum</th>
<th>Exudate</th>
<th>(= % of patient serum value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (rel.%)</td>
<td>53 ± 7.0</td>
<td>35 ± 7.0</td>
<td>66</td>
</tr>
<tr>
<td>(rel.%)</td>
<td>57.6 ± 8.2</td>
<td>53.9 ± 8.5</td>
<td>93</td>
</tr>
<tr>
<td>(rel.%)</td>
<td>4.7 ± 1.3</td>
<td>6.3 ± 3.1</td>
<td>134</td>
</tr>
<tr>
<td>(rel.%)</td>
<td>8.6 ± 1.7</td>
<td>9.7 ± 3.6</td>
<td>113</td>
</tr>
<tr>
<td>(rel.%)</td>
<td>7.7 ± 1.6</td>
<td>8.7 ± 2.5</td>
<td>113</td>
</tr>
<tr>
<td>(rel.%)</td>
<td>21.3 ± 7.9</td>
<td>21.5 ± 7.6</td>
<td>100</td>
</tr>
</tbody>
</table>
TABLE 2. Opsonin levels and opsonic activity (OA) in acute peritonitis (mean ± standard deviation, % of normal serum, n=13)

<table>
<thead>
<tr>
<th></th>
<th>patient serum</th>
<th>exudate</th>
<th>(=% of patient's serum value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>62.8 ± 29.3</td>
<td>43.9 ± 21.3</td>
<td>69.9</td>
</tr>
<tr>
<td>C3</td>
<td>65.3 ± 23.1</td>
<td>35.8 ± 27.8</td>
<td>54.8</td>
</tr>
<tr>
<td>OA</td>
<td>85.8 ± 33.5</td>
<td>8.4 ± 4.3</td>
<td>9.7</td>
</tr>
</tbody>
</table>

For further investigation of the apparent dysfunction of opsonins in peritonitis exudates the immunological integrity of IgG and C3 was studied by crossed immunoelectrophoresis (Fig.1).

Figure 3. Crossed immunoelectrophoresis for IgG and C3
a) patient's serum
b) moderate peritonitis exudate
c) severe purulent peritonitis exudate
The peaks to the right reveal split products of lower molecular weight.
2. Serum substitution

Case report 1 (64 yrs male patient with colon perforation).

In vitro incubation of increasing amounts of normal serum (N.S.) with the patient's exudate or exudate supernatant (SN) resulted in increasing amounts of immunologically measurable C3- and IgG-levels. In the mixtures containing cell free exudate SN the opsonic capacity (OC) recovered according to the rising opsonin concentration. When the incubation was performed with crude exudate, however, much higher serum concentrations were required to improve opsonisation (Fig.2).

Figure 2. In vitro incubation of peritonitis exudate with normal serum (N.S.) = opsonin levels, OL (C3 + IgG) in the mixtures, □ = opsonic capacity (OC) in exudate supernatant dilution, \( \equiv \) = OC in crude exudate dilution.

In two patients with gastric perforation the same incubation procedure was performed. In these mixtures OC increased according to the increase of OL both in exudate/serum dilution and in exudate-SN/serum dilution.
Case report 2 (71 yrs male pat., colon perforation)

In vivo serum application was performed as described above at the end of the operation. Peritonitis exudate's OC was 6 % of NS. Serum treatment resulted in a marked increase of OL and OC. In the second effluate sample (2-2.5 hrs.) OC still exceeded the preoperative activity up to 3.5-fold (Fig.3).

So far 6 patients have been treated with serum application. In all of them exudate OC was definitely enhanced and the procedure was well tolerated.

DISCUSSION

The above pattern of protein amount and distribution in patients' blood and exudate indicated the pronounced increase of peritoneal permeability in peritonitis giving way even for the passage of larger proteins, e.g. opsonins. The local (intraabdominal) concentration of IgG and C3 was in accordance with this finding. Although about 60 % of each protein was found in the exudates, the functional activity of both factors was almost abolished.
Immunoelectrophoresis demonstrated a marked breakdown of these proteins. Similar results have been published in other infected exudates (Waldvogel et al., 1984) and were assigned to proteolytic destruction. As to the C3 splitting the above results might partly be due to physiological activation with release of C3a, whereas the destruction of IgG gives evidence for unspecific proteolysis.

The incubation experiment in patient 1 reveals ongoing destruction of supplied opsonins still in vitro. Addition of specific proteinase inhibitors to such mixtures might be helpful to differentiate the underlying mechanisms. The results of intraabdominal serum substitution demonstrate the general possibility of therapeutic improvement of local opsonisation.

The proper recognition and labelling of foreign particles is generally understood as a main prerequisite for sufficient phagocytosis. The postoperative support of the impaired intraabdominal opsonisation deserves further investigation as it might be a key factor to prevent recurrent abdominal sepsis or abscess formation.

ACKNOWLEDGEMENT

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