

BIOMEDICA BIOCHIMICA ACTA

Volume 50
1991
Number 4–6

Founded as
„Acta biologica
et medica germanica“

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Proteolysis of defensive proteins in peritonitis exudate: Pathobiochemical aspects and therapeutical approach

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Summary

Peritonitis exudate reveals strong proteolytic activity which is paralleled by deficient opsonic capacity and high concentrations of lysosomal proteinases (elastase and cathepsin B). Lysosomal serine and cysteine proteinases (elastase, cathepsins B, L) were shown to degrade immunoglobulin G (IgG) and seem to be at least partially responsible for the observed proteolytic inactivation of IgG in peritonitis exudates. Intraabdominal serum application seems to restore opsonic activity by substitution of opsonins and proteinase inhibitors.

Introduction

Diffuse peritonitis is initially a localized intraabdominal inflammation. The breakdown of local defense mechanisms results in systemic complications like bacteriemia and toxinemia with subsequent sepsis and multi-organ failure. Lethality of peritonitis remains to be as high as 24%–54%. After surgical treatment of the source of peritonitis, the patient's fate depends mainly on the efficiency of the defense system. Peritonitis exudate is characterized by a large spectrum and number of viable bacteria despite the presence of intact phagocytes. In previous work we have been able to demonstrate a pronounced impairment of opsonisation in peritonitis exudate due to proteolytic breakdown of the opsonins immunoglobulin G (IgG) and complement factor C3 [1]. We have now further analysed this proteolytic activity and investigated a therapeutic approach by intraabdominal serum application.

Material and methods

Biochemical studies

Human peritonitis exudate was taken during operations. All factors were measured in cell-free supernatant. Opsonic activity was determined by a special chemiluminescence (CL) assay [1]. Granulocytic elastase (in complex with α_1 -proteinase inhibitor) was measured by ELISA [2], cathepsin B activity with a fluorogenic peptide substrate using the specific cysteine proteinase inhibitor E-64 [3]. Proteolytic activity of exudates was estimated by the fluorescence of non-precipitable fragments released from resorufin-labelled casein (Boehringer, Mannheim).

Human IgG (Sigma) was incubated with 2% (w/w) of papain (Sigma), human cathepsin B, H, L (Medor, D-8036 Herrsching) at pH 5.5 and with human leucocyte elastase (Medor) at pH 7.4 for 18 h at 30 °C. Fluorescein isothiocyanate (FITC)-labelled IgG was added to cell-free exudate (pH about 7.0) and incubated under the same conditions. IgG or FITC-labelled IgG and their proteolytic fragments

were separated by fast gel chromatography on a Superose 12 FPLC column (Pharmacia) and detected by photometry at 280 nm or continuous fluorometry (Excitation: 486 nm, emission: 513 nm) using HPLC detectors. Prior to use, FITC-labelled IgG (Sigma) was purified by FPLC on the same column.

Therapeutic serum application

By the end of the peritonitis operation and after thorough lavage of the whole abdomen with 10 l of Ringer lactate solution, 300 ml of blood bank serum were applied into the abdomen. Controls were without serum application. In both groups, abdominal drainage fluid was collected and pooled from 0–1 h, 1–2 h and 2–8 h after operation.

Results and discussion

Proteolytic activity of exudates

Proteolytic activity of exudates was compared with their opsonic capacity as well as with their content of complexed elastase and their cathepsin B activity (Fig. 1). Exudates with high opsonic deficit (low opsonic capacity) showed high concentrations of lysosomal proteinases and high caseinolytic activity.

Limited proteolysis of human IgG by isolated lysosomal proteinases was followed by gel chromatography (Fig. 2). Catalytic amounts of leucocyte elastase as well as cathepsins B and L cleaved human IgG into (Fab)₂, Fc and/or Fab fragments. The same type of cleavage was observed with FITC-labelled IgG incubated with cell-free exudate. IgG proteolysis was significantly reduced in the presence of E-64, a specific inhibitor of cysteine proteinases.

Clinical therapeutic study

Normal serum contains high amounts of intact opsonins as well as protein inhibitors of serine and cysteine proteinases. Preliminary results indicate an increased opsonic activity of peritoneal exudate for at least eight hours after intraabdominal serum application as compared to the control group without serum (Fig. 3).

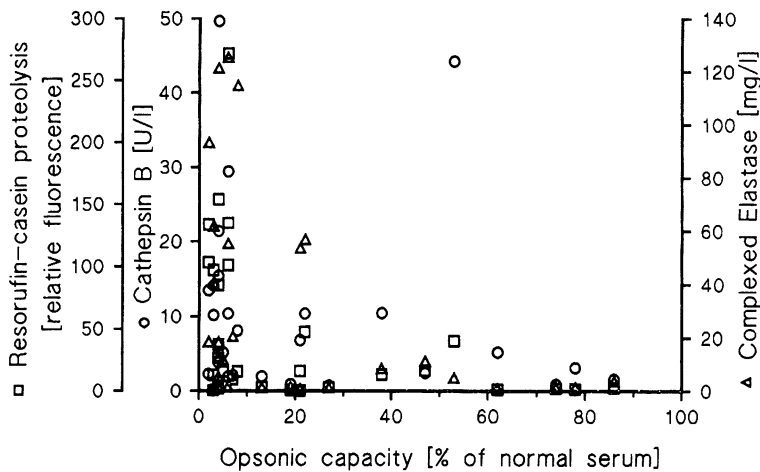


Fig. 1. Proteolytic activity and opsonic capacity of peritonitis exudates ($n = 29$)

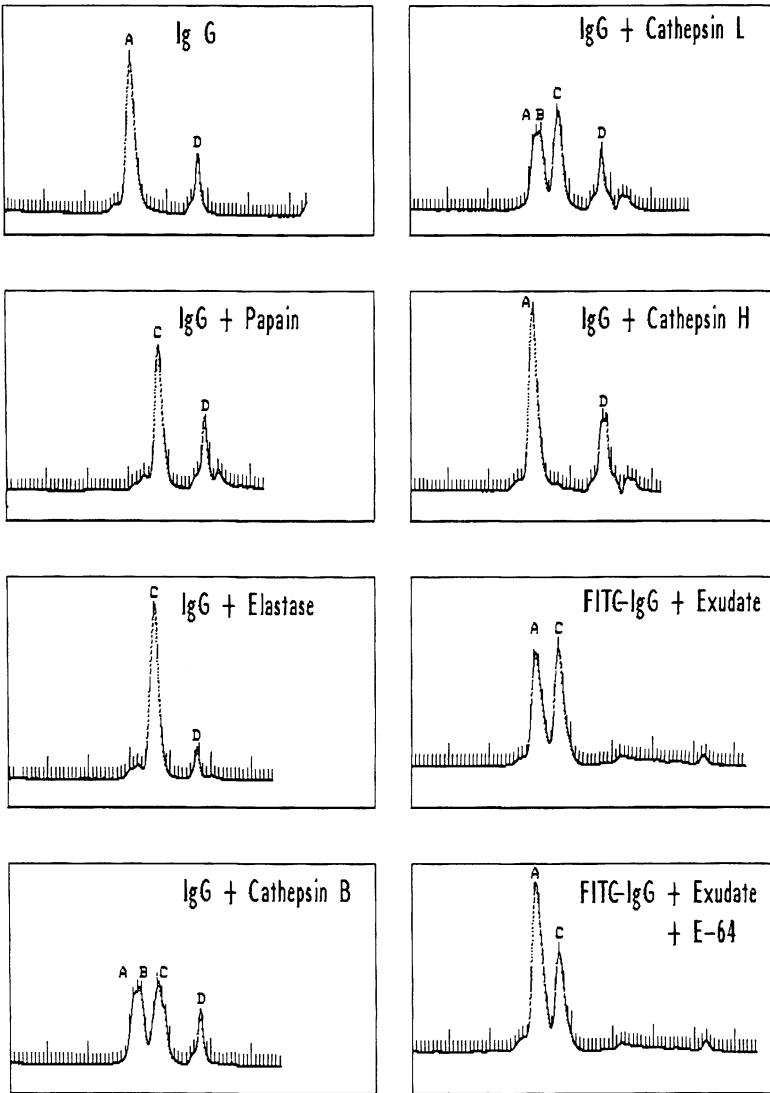


Fig. 2. Limited proteolysis of human immunoglobulin G (IgG) by isolated lysosomal proteinases and by peritonitis exudate. IgG and IgG fragments were separated by fast gel chromatography on a Superose 12 FPLC column. Spikes along the elution profiles indicate fractions of 1.3 min (flow rate 0.2 ml/min). A, intact IgG; B, (Fab)₂ fragments; C, Fc and/or Fab fragments; D, low-M_r buffer peak present in all samples

Conclusions

Peritonitis exudate reveals strong proteolytic activity which is paralleled by high levels of lysosomal serine and cysteine proteinases. Proteolytic breakdown of defensive proteins by phagocytic (and bacterial?) proteinases may be a pathobiochemical key factor in peritonitis.

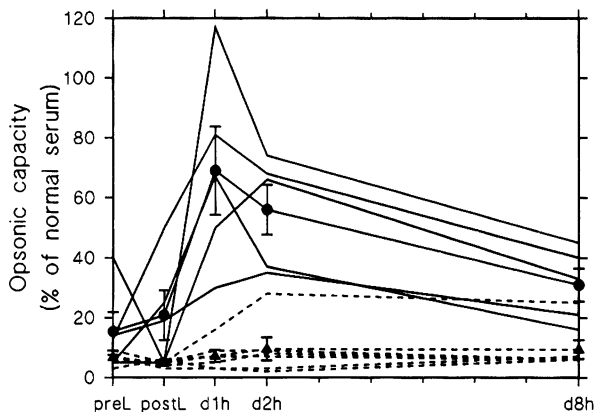


Fig. 3. Effect of intraabdominal serum application on opsonic capacity of peritonitis exudate (—) as compared to controls without serum application (---).

—●—, mean of serum group ($n = 5$) \pm SEM; —▲—, mean of control group ($n = 6$) \pm SEM. Samples: preL, immediately after laparotomy; postL, after lavage with 10 l of Ringer lactate solution; d1 h—d8 h, pooled fractions of postoperative drainage fluid after 1, 2 and 8 h

Local serum application seems to restore opsonic activity during the crucial first hours after peritonitis operation. These preliminary findings will be further investigated in a randomized clinical study.

Acknowledgements

The authors wish to thank HANS FRITZ, Munich, for generous support and stimulating discussion. The skillful technical assistance of GERDA BEHRENS and RITA ZAUNER is greatly appreciated. The work was financially supported by the Sonderforschungsbereich 207 of the University of Munich (projects G1 and G5).

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