Are granulocyte inhibitory proteins contributing to enhanced susceptibility to infections in uraemia?

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Abstract. Infectious complications are still a major cause of morbidity and mortality in patients undergoing regular dialysis treatment. This increased morbidity and mortality of patients with uraemia has been attributed to the dysfunction of polymorphonuclear leukocytes (PMNLs). Uraemia contributes to impaired PMNL function by means of disarrangements in cellular biochemistry and biology. Reduced chemotaxis, adherence, oxidative activity, and glucose consumption in response to phagocytic challenge have been reported. Several compounds present in uraemic serum have been isolated and characterized to inhibit the biological activity of PMNLs. A first granulocyte inhibitory protein (GIP I) responsible for the PMNL dysfunction in uraemia has been isolated and characterized. The polypeptide, with a molecular weight of 28000 Da, inhibits the uptake of deoxyglucose, chemotaxis, oxidative metabolism, and intracellular bacterial killing by PMNLs. A second granulocyte inhibitory protein (GIP II), with a molecular weight of 9500 Da, has been isolated from plasma ultrafiltrates obtained from haemodialysis patients. It interferes with similar functions of PMNLs. GIP I displays homology with light chain proteins and GIP II with β₂-microglobulin, respectively. Both proteins were also isolated from peritoneal dialysis effluents. Isolated kappa and lambda light chain monomers and dimers from haemodialysis and peritoneal dialysis patients also inhibit several PMNL functions. Recently, a PMNL degranulation inhibiting protein (DIP) was purified from plasma ultrafiltrates obtained from patients undergoing regular haemodialysis. DIP shows homology with angiogenin and inhibits spontaneous as well as stimulated PMNL degranulation. These proteins may explain at least in part the enhanced susceptibility to infections in haemodialysis and peritoneal dialysis patients.

Key words: granulocytes; infection; haemodialysis; CAPD; uraemia

Introduction

Patients undergoing regular haemodialysis treatment suffer from increased incidence of infections [1,2]. This is mainly a consequence of diminished function of polymorphonuclear leukocytes (PMNLs) [3,4]. Cellular defence mechanisms in the peritoneal cavity play a primary role in the prevention of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD) [5]. A major component of the local defence during infection is the PMNLs [6].

Vanholder et al. [7] identified p-cresol as a uraemic solute capable of impairing the respiratory burst of PMNLs. Higher molecular weight PMNL inhibitors present in the plasma of uraemic patients and in the peritoneal effluents of CAPD patients include several newly identified proteins. In the following, we summarize our results concerning these proteins accumulating in uraemia and clearly interfering with specific PMNL functions. This may open new perspectives for understanding of impaired cellular host defence in haemodialysis and CAPD patients.

Granulocyte inhibitory protein I

Granulocyte inhibitory protein I (GIP I) was initially isolated from plasma ultrafiltrate obtained from patients undergoing regular dialysis treatment using a polysulphone dialyser [8], then from haemofiltrate of haemofiltration patients [9] and from peritoneal effluents of CAPD patients [9]. This protein has a molecular weight of 28000 Da. In vitro, nanomolar concentrations of GIP I inhibit PMNL deoxyglucose uptake, chemotaxis, and oxidative metabolism stimulated with the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (FMLP). Intracellular killing of Staphylococcus aureus or Escherichia coli is also blocked by comparable low concentrations of GIP I [8,9]. Amino acid sequence analysis of the NH₂-terminus of GIP I shows no homology with factors involved in acute and chronic inflammatory processes [8]. Recent data suggest that GIP I could be a member of the light chain protein family [10].
Immunoglobulin light chains

Kappa and lambda light chains were isolated in their monomeric and dimeric forms from high flux dialyser ultrafiltrates obtained from patients undergoing regular haemodialysis treatment and from peritoneal effluents obtained from CAPD patients. We found that all isolates tested were able to inhibit deoxyglucose uptake and chemotaxis of PMNLs. In contrast, free immunoglobulin light chains have no influence on the phagocytic functions of PMNLs [11]. Up to 5-fold elevated free immunoglobulin light chains were found in sera from patients with severely reduced kidney function by Soiling [12]. Wakasugi et al. [13] observed significantly increased free light chains in sera after the start of haemodialysis therapy. Therefore, free immunoglobulin light chains should be taken into consideration in uraemic patients as one important factor contributing to enhanced risk of infection in this patient population.

Granulocyte inhibitory protein II

A further granulocyte inhibitory protein (GIP II) was isolated from regular haemodialysis patients using the polyamide filter [14], from filtrate of haemofiltration patients and from peritoneal effluents obtained from CAPD patients [9]. The protein has a molecular weight of about 9500 Da. In vitro nanomolar concentrations inhibit PMNL O2- production and glucose uptake stimulated by phorbol-myristate-acetate. The NH2-terminal amino acid sequence of GIP II shows homology to β2-microglobulin. Commercially available intact β2-microglobulin had no effect on PMNL glucose uptake and O2- production. GIP II stimulated significantly in a dose-dependent manner interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumour necrosis factor (TNF) production in cultured human mononuclear cells [15], whereas intact β2-microglobulin had no effect. Miyata et al. [16] have shown that AGE-modified β2-microglobulin increased the secretion of TNF-α, IL-1β and IL-6 [16,17] from macrophages, whereas normal β2-microglobulin was without effect. These data indicate that GIP II not only has homology to β2-microglobulin, but behaves like the AGE-modified protein [15].

Degranulation inhibiting protein

This PMNL inhibitory protein was purified from plasma ultrafiltrates of chronically uraemic patients dialysed with polysulfone or polyamide membranes [18]. Degranulation inhibiting protein (DIP) inhibits release of lactoferrin, collagenase and gelatinase, but not the release of elastase by PMNLs [18]. DIP has a molecular weight of approx. 14 000 Da. The amino acid sequence analysis of the NH2-terminus reveals homology to the angioplastic factor angiogenin. Recombinant angiogenin also inhibits PMNL degranulation in vitro [18]. The same but reduced effect was induced by the tryptic angiogenin fragment. Cellular functions such as chemotaxis, phagocytosis and the oxidative respiratory burst were not obviously affected by angiogenin [18].

Plasma DIP was determined using a highly immunoenzymometric assay according to Bläser et al. [19]. DIP values were significantly elevated in patients undergoing regular haemodialysis treatment and in CAPD patients. Daily peritoneal DIP elimination of CAPD patients markedly exceeded urinary DIP excretion of healthy subjects, suggesting increased DIP production in uraemia [20].

Conclusion

Our data show that specific peptides present in uraemic plasma interfere with PMNL function in dialysed patients. It must be the object of clinical studies to document whether increased concentrations of these peptides relate to infectious complications in dialysed patients and whether this risk can be reduced by removal of such peptides. These peptides may only be the tip of an iceberg. The future will show whether this will lead to revival of a modified middle molecule hypothesis and whether this will have repercussions on the selection of dialysis membranes.

References


