Degranulation of polymorphonuclear leukocytes by dialysis membranes—the mystery clears up?

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Introduction

Degranulation of polymorphonuclear leukocytes (PMN) occurs during extracorporeal circulation. In patients undergoing regular haemodialysis treatment PMN degranulation has been used as one of several markers for membrane bioincompatibility [1]. In the past, it has been thought that the release of components from PMN granules follows the activation of the complement system in a time and membrane material dependent manner. It became clear, however, that complement activation and PMN degranulation observed during haemodialysis therapy are independent activation processes [1,2]. Cheung et al. [3] performed in vitro dialysis treatments in the presence of normal and very low-dose heparinization. Low-dose heparinization caused partial clotting within the filter, resulting in significant complement activation compared to standard heparinization. The release of lactoferrin from PMN during these procedures, however, was identical with both anticoagulation modalities. Complement activation depends not only on the type of dialyser but also on whether it is new or reused. Dialyser reuse causes attenuation of complement activation; PMN lactoferrin or myeloperoxidase release, however, is not affected by reuse [4].

What are the intracellular signals for PMN degranulation during haemodialysis?

Intracellular calcium was suggested as a potential mediator of PMN degranulation. It has been shown that the release of components from neutrophil granules was reduced by pretreatment of haemodialysis patients with calcium-channel blockers [5]. Inhibition of PMN degranulation by calcium-channel blockers was also observed during cardiopulmonary bypass [6]. The concept of the role of intracellular calcium triggering PMN degranulation was further confirmed by two studies depleting intracellular calcium of PMN during extracorporeal circulation by citrate. This mode of anticoagulation abolished lactoferrin [7] or myeloperoxidase [8] release from PMN during haemodialysis treatment. In addition to calcium, other cyotogenic mediators involved in neutrophil degranulation include leukotrienes and prostaglandins [9].

Inhibition of PMN degranulation by newly described proteins

Two degranulation inhibiting proteins identical to angiogenin [10] and complement factor D [11] have been isolated from the ultrafiltrates of end-stage renal disease patients undergoing regular haemodialysis treatment using high-flux dialysers. Both proteins inhibit in vitro dose-dependently the release of lactoferrin and metalloproteinases such as collagenase and gelatinase from PMN [10,11]. Therefore we asked ourselves:

(i) Are plasma levels of the degranulation-inhibiting proteins elevated in end-stage renal disease patients?

(ii) Are plasma levels of these proteins affected by high-flux haemodialyser therapy and/or haemodiafiltration?

(iii) Is degranulation of PMN inhibited not only in vitro by these PMN degranulation inhibiting proteins but also in vivo?

Recent studies have shown that plasma angiogenin [12] and complement factor D [13] levels are markedly elevated in end-stage renal disease patients (up to 15-fold) as compared to healthy subjects. Haemodialysis treatment with high-flux membranes or haemodiafiltration causes a decrease of angiogenin and complement factor D via convection, and particularly via adsorption. Plasma lactoferrin levels were markedly higher during haemodialysis with low-flux cellulose triacetate or polysulphone dialysis than with the high-flux versions of these dialysers, either with the haemodialysis or haemodiafiltration mode of treatment. Low-flux cellulose triacetate or polysulphone dialysers do not reduce plasma angiogenin levels, in contrast to the high-flux versions. These data clearly show that high angiogenin and/or complement factor D levels of chronically uraemic patients protect against lactoferrin release from PMN during extracorporeal circulation. These data also demonstrate that endogenous PMN inhibitors have to be considered if neutrophil activation parameters were measured in order to investigate bioincompatibility of dialyser membrane materials.

In order to further corroborate this novel mechanism of PMN degranulation, we selected chronically uraemic...
patients treated with the polyacrylonitrile (AN69) dialyser. During 4 h of high-flux haemodialysis therapy using this membrane, a marked reduction of plasma angiogenin levels (~66%) was observed, accompanied by an additional significant decrease of complement factor D, both particularly via adsorption [12]. These data explain the even higher PMN lactoferrin release during extracorporeal circulation using polyacrylonitrile (AN69) than using cuprophan dialysers [5]. It should be stressed that cuprophan, but not polyacrylonitrile, causes marked complement activation at the blood–membrane interface. Since specific blockade of factor D activity inhibits complement activation by the alternative pathway [14], reduction of factor D by adsorption and/or convection may be a further mechanism of inhibition of complement activation using biocompatible high-flux dialyser membranes. It has been shown that complement factor D decreased markedly after haemodialysis therapy with polyacrylonitrile, reaching almost normal values [15]. Complement factor D cleaves factor B in to fragments Bb and Ba. Elevated plasma concentrations of fragment Ba might be regarded as an immunosuppressive factor for end-stage renal disease patients. Haemofiltration reduces both complement factor D and the immunosuppressive fragment Ba [16].

**Conclusion**

In order to prefer high-flux over low-flux haemodialysers, one must have strong evidence of superiority of the procedure. Apart from prospective clinical studies on the ultimate end-point, i.e. patient survival (and these are currently not available), appropriate *in vitro* systems may be used as surrogates.

Using PMN degranulation as an *in vitro* test, we obtained information that can be interpreted to either support or reject the hypothesis that high-flux haemodialysers are superior. On the one hand factor Ba with undoubted immunosuppressive properties is lowered (which would be beneficial); on the other hand the degranulation inhibiting factors angiogenin and complement factor D are lowered (which would be undesirable). As a result we do not know whether PMN are deactivated or activated. The net outcome of these two opposing effects on polymorphonuclear cell function *in vivo* is currently unresolved. As a consequence, unfortunately at this point in time, no cogent argument can be derived from such *in vitro* studies for an advantage of high-flux membranes.

**References**