New molecular aspects of chronic uraemia and dialysis-related immunocompetent cell activation

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Introduction

Our knowledge of the molecular structure and the role of cell receptors and soluble mediators controlling immune responses has increased tremendously over the last 10 years. Likewise the concept of bioincompatibility resulting from the interaction between extracorporeal blood and the dialysis circuit has been extended far beyond its original definition by complement activation and subsequent pulmonary sequestration of neutrophils. The recent report of the consensus conference on biocompatibility of extracorporeal blood treatment (held in March 1993 in Koenigswinter, Germany), published in a supplement of this journal, has already reviewed in depth the current concepts and knowledge of the mechanisms of bioincompatibility pathways. The major highlights of this report are: a revisit of definitions and terminology used for biocompatibility with particular emphasis on clinical practice, meeting the approval of scientists as well as leading manufacturers; a critical evaluation of the scientific basis and the delimitations of biocompatibility assessment, given the extraordinary complexity of the homeostatic system, the existence of multiple feedback control mechanisms and the interaction networks of these pathways; a series of brief and updated reviews of major fields of biocompatibility, e.g. homeostasis and thrombosis, hypersensitivity, complement activation, stimulation of phagocytic cells, basophils and mast cells, and generation of proinflammatory cytokines; and overviews of experimental systems and in vitro assays for assessment of biocompatibility and of the clinical relevance of bioincompatibility by major specialists in the fields. In their concluding remarks Drs H. Klinkmann and A. M. Davison [1] re-emphasized the major consensus of this conference, i.e. 'the issue of biocompatibility can be considered as a puzzle comprising many different pieces which are slowly being put together, but as yet the complete picture has not emerged'.

In this paper we have focused on the new molecular aspects of the immunocompetent cell activation observed in both undialysed and dialysed uraemic patients [2] (Figure 1), with the aim of gaining further insight into the complex series of events promoting such cellular activation and, for each cell type, of highlighting the respective influence of chronic uraemia per se and of the dialysis procedure.

Neutrophil activation

Since the early demonstration that haemodialysis is associated with a profound transient decrease in the number of circulating neutrophils and monocytes, and the subsequent demonstration that this leukopenia is related to complement activation induced by cellulose dialysis membranes and due to subsequent sequestration of neutrophils in the pulmonary vascular bed, the precise mechanisms of such sequestration have long remained hypothetical. The new concepts of neutrophil activation during dialysis have been recently reviewed [3] and their possible clinical implications are depicted in Figure 1. There is compelling evidence that neutrophil adhesion molecules of the integrin receptor superfamily are involved in the pathogenesis of dialysis-induced leukopenia. Increased granulocyte expression of Mac-1 (CD11b/CD18) during dialysis by cuprophane membranes precedes the development of granulocytopenia and may be involved in increased neutrophil adhesiveness to endothelium. However, at variance with complement activation and neutropenia which are transient, increased expression of CD11b persists throughout the dialysis session despite the return of the neutrophil count to normal [4-6]. The observation of a concomitant decreased expression of L-selectin, probably due to dialysis-induced shedding of this molecule, may explain the rebound granulocytosis despite the persistence of increased expression of CD11b. Indeed, the diminished expression of L-selectin corresponds to the decreased capacity of granulocytes to adhere to cultured endothelial cells [6]. Moreover, the adhesion of leukocytes to endothelial cells relies not only on their expression of adhesion molecules but also on their activation state [7]. Himmelfarb et al. [4] were the first to demonstrate that complement-activating membranes induce over-
expression of Mac-1, while this is not observed with non-complement activating membranes. This was also verified in a recent cross-over study comparing the effect of cuprophone and polyamide membranes on the expression of CD11b and of a novel neutrophil activation specific marker, the MoF11 antigen [6]. It was found that in the dialysis period, with the complement activating cuprophone membrane, the expression of both CD11b and MoF11 molecules was significantly increased, whereas the non-complement activating polyamide membrane did not induce such an effect on these markers.

The effect of the dialysis membrane on the production of reactive oxygen species and proteases has also been widely documented both in vivo and in vitro (reviewed in [2]). More recently, evidence for down-regulation of phagocytic cell function has been reported by several groups and interpreted as a consequence of their relentless activation triggering. Vanholder et al. [8], in a longitudinal study, observed major dysfunction in phagocytic cell oxidative metabolism as measured by glucose consumption, and further suggested that uraemic toxins could be responsible for impaired oxidative responsiveness. These authors also observed that small molecular weight (<2 kDa) HPLC uraemic serum fractions, with both lipophilic and hydrophilic characteristics, depress phagocytosis [9]. As far as neutrophils are concerned, it should be stressed that this is probably the only cell type for which inhibitory soluble factors present in the serum have been directly implicated in the depressed response. Other such factors, that have been mainly individualized by the group of Hörl [10], include the granulocyte-inhibiting protein (GIP) of 28 kDa, which is non-homologous to any other inflammatory protein, and the degranulation-inhibiting protein (DIP) (14 kDa), which shares partial homology with angiogenin and produces the same effects, namely a depression of lactoferrin, collagenase and gelatinase secretion without affecting phagocytosis, oxidative respiratory burst or chemotaxis.

Desensitization of C5a receptors, contrasting with a normal expression of fMLP receptors, has also been reported [11]. It resulted in a defective chemotactic response and was interpreted as the consequence of repeated exposure to activated complement fragments. Another line of evidence comes from our study of the function of CD11b (CR3), CR1 and RFcγ as opsonin receptors, using an ultrasensitive methodology which consists of measuring phagocyte oxidative responses to opsonized zymosan in whole blood, both in the absence (circulating opsonin receptor expression or CORE) and in the presence of priming agents (C5a, PAF or fMLP) (maximal opsonin receptor expression or MORE) [12]. The so-called opsonin receptor reserve given by the CORE/MORE ratio was significantly reduced in dialysis patients compared to controls (article in preparation). The decrease was more pronounced in those treated by a complement activating membrane than in those treated with polyacrylonitrile AN69. Taken together these findings could explain the
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increased susceptibility of these patients to infections (Figure 1).

T cell activation

The presence of increased amounts of the IL-2 receptor both at the surface of circulating T cells and in the plasma, together with the failure to detect circulating IL-2 production, suggested that increased consumption of IL-2 by its receptor leads to inadequate concentration for normal responses to antigens or infectious challenges (Figure 1). The dual role of chronic uraemia and of dialysis at the origin of this preactivation state of T cells has been evidenced by the recent demonstrations that (i) elevated soluble IL-2 receptor levels are present from the incipient stage of chronic uraemia [13] and (ii) dialysis with complement-activating membranes induces an up-regulation of the expression of IL-2 receptor, which can be reversed following dialysis with biocompatible membranes [14]. However an earlier report showing that the non-response to hepatitis B vaccination can be restored by administration of ultrapure IL-2 has not been confirmed by a randomized vs placebo vaccination trial using recombinant IL-2 [15]. Recent knowledge on the mechanisms of antigen recognition by T cells has led to the hypothesis that blunted T cell response to antigens in uraemia is due to down-regulation of the T cell receptor/CD3 antigen receptor complex in a uraemic milieu [16,17]. Such new findings on T cells undoubtedly open new avenues of research that could themselves lead to new immuno-modulating strategies capable of boosting the so-called Janus-faced T cell in uraemia [2]. Finally, it has also become evident that our knowledge of T cell dysfunction would certainly be improved by comparing the function of the Th1 and Th2 cell types in uraemia.

B cell activation

The serum level of the soluble form of CD23, a marker of B cell activation, is raised in uraemic patients, increasing with the progression of chronic renal failure and reaching extremely high levels in dialysis patients. Its further increase during dialysis sessions with cellulosic membranes or high flux polysulfone membranes, but not polyacrylonitrile AN69, could be explained by its adsorption on this latter membrane [18]. The demonstration of a synergistic effect of soluble CD23 on IL-1-induced T cell activation [19] and other cytokine production [20] reinforces the view of soluble CD23 as a multifunctional cytokine which could notably contribute to the immune system dysregulation associated with uraemia and exacerbated by dialysis (Figure 1).

Monocyte activation and pro-inflammatory cytokines

Among monocyte-derived cytokines, IL-1β was first incriminated in acute and chronic inflammatory reac-
tions associated with haemodialysis. This was based on the observations that: (i) both the clinical symptoms observed during dialysis sessions and the sites of complications in long-term haemodialysed patients strikingly reflect the systemic effects and target organs of IL-1; and (ii) numerous haemodialysis-related factors can trigger the production of IL-1 by monocytes. This so-called IL-1 hypothesis has been extended to TNF-α and IL-6, which share most of the biological activities of IL-1β, and verified by numerous studies showing the presence of elevated levels of these cytokines in dialysis patients (reviewed in [2,21]).

Recent findings also stressed the fact that the potentially harmful effects of monocyte-derived pro-inflammatory cytokines are counteracted by specific inhibitors concomitantly synthesized in response to infectious or inflammatory challenge (reviewed in [21]). TNF-α soluble receptors (TNF-sR55 and TNF-sR75), which bind to TNF-α and neutralize its effects, and the IL-1 receptor antagonist (IL-1Ra) which competitively binds to the IL-1 receptor without triggering an activation signal, both deserve special attention in dialysis patients. In our recent study we also found that: (i) both TNF-sR55 and TNF-sR75 are elevated at the incipient stage of chronic renal failure, increase in parallel with the progression of renal failure, and further increase during the course of dialysis sessions; and (ii) IL-1Ra is also elevated at the onset of chronic uraemia and increases further at the advanced stage of renal failure. In contrast to TNF soluble receptors, IL-1Ra decreases during dialysis sessions [13]. Taken together these studies suggest that elevated TNF-sRs are the footprints of TNF in chronic renal failure. They may act as inhibitors or serve as reservoirs of TNF and even augment TNF effects by stabilizing its tertiary structure, prolonging its action [22]. With regard to IL-1Ra, it appears to be a more suitable marker of the severity of inflammation in chronic uraemia than IL-1β, but its concentration in dialysis patients is unlikely to be sufficient to block the tissue effects of IL-1.

According to the classical scheme of the 'interleukin hypothesis', the contact of blood components with the dialysis membrane triggers monocyte activation, leading to the release of the pro-inflammatory cytokines IL-1β, TNF-α and IL-6, through the combined action of activated complement components, adherence and some dialysate components such as acetate and endotoxins (Figure 1). An important issue concerns the respective role of dialysis membrane biocompatibility and of dialysate composition and its possible contamination by endotoxins in the induction of pro-inflammatory cytokine synthesis by monocytes (reviewed in [21]). In vitro dialysis circuit studies have shown that within 2 h following the start of dialysis with a cuprophane membrane, transcription of IL-1 mRNA becomes detectable. However, in the absence of added endotoxins in the dialysate, there is no translation into IL-1 protein [23]. This mRNA is also more rapidly translated when exposed to low concentrations of endotoxin, compared to cells which have
not been primed by dialysis and are exposed to endotoxin only. Similar findings are observed with recombinant C5a as priming agent.

At present, the search for biocompatible membranes comes up against a dilemma: high flux dialysis membranes that are the most compatible in terms of complement-activating potential, adequate extraction and/or adsorption of uraemic toxins favour the passage of cytokine-inducing endotoxins through backfiltration, thus re-emphasizing the need for ultrafiltrate if not sterile dialysate (Table 1).

Preliminary reports have just confirmed that circulating cytokines are significantly less in patients treated with sterile dialysate. However, the search for improving dialysis membrane biocompatibility and minimizing endotoxin in the dialysate is also faced with the necessity to limit the cost of dialysis therapy. In this regard, the reuse of dialysers is developed in some countries and is a matter of great controversy in others. Comparative studies based on cytokine determination in the course of reutilization presently being developed in our laboratory should provide a more rational evaluation of the safety of this lower cost dialysis.

Conclusion

Progress in our knowledge of the immune system in ESRD patients has been outstanding in the past few years. Greater insight into the mediators and cellular actors that could be involved in the uraemia-associated immunodeficiency and its exacerbation by dialysis has shed light on some key factors and suggested some attractive hypotheses that now require further evaluation. The recent challenges posed by the demonstration of the role of uraemia per se in the induction of immune cell activation and dysfunction, and of the unique model of systemic inflammation represented by dialysis, is attracting many basic scientists and clinical researchers to the area. It is to be hoped, therefore, that their joint efforts will lead not only to exciting discoveries but also to optimal dialysis strategies of direct benefit to patients.

References

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