Immune Dysfunction and Cytokine Production in Hemodialysis

Could They Be Lessened by Vitamin E-Coated Dialyzer Membrane?

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Cytokine Pattern in Uremia

Most uremic patients in dialysis therapy develop an immunodeficiency characterized clinically by frequent infectious complications and impaired response to vaccinations [1]. This became evident in the early period of hemodialysis treatment when endemic outbreaks of hepatitis B threatened dialysis centers [2]. Vaccination results against hepatitis B, in dialysis patients, are disappointing: low results are also documented for most other types of vaccinations in these patients, such as influenza [3], tetanus [4] or diphtheria [5]. There is an only exception in using vaccination containing polysaccharide antigens like pneumococcus [6]. Polysaccharide antigens are recognized by B cells on their own, but for recognition of other antigens, B cells need specific T-cell help: these observations indicate that the major defects appear to be in cell-mediated immunity [7–9] and in particular correlated to T-cell rather than B-cell function [10].

In normal conditions, in response to antigens, naive CD4⁺ T cells differentiate into effector T helper (Th) cells. Based on their pattern of cytokine production and their functional responses, Th cells can be subdivided into those that participate in cell-mediated immune responses such as delayed type of hypersensitivity reactions and macrophage activation (Th1 subset) and those releasing

cytokines that induce B cells to secrete antibodies (Th2 subset). Several factors are known to polarize the differentiation of Th cells into either Th1 or Th2 direction, including the co-stimulatory action of antigen presenting cells (APCs), the cytokine environment, altered peptide ligands and the antigen dose. In particular, Th1 are characterized by elevated secretion of interleukin (IL)-2, transforming growth factor- β (TGF- β) and interferon- γ (IFN- γ), thereby activating cytotoxic T lymphocytes and macrophages. Moreover, IFN- γ suppresses Th2 which induces humoral immunity. The Th2 subset of CD4⁺ T cells preferentially secretes IL-4, IL-5, IL-6 and IL-10 [11–13], stimulating the proliferation of mast cells and eosinophils as well as the production of immunoglobulins and IL-10 which may suppress the Th1-induced cell-mediated immunity [14, 15]. In general, Th1 cytokines promote Th1 and inhibit Th2 activities and vice versa. Th1 and Th2 responses should, therefore, be stable [16].

Chronic renal failure induces a clinical state of immunodeficiency in most patients: immune defects may be caused by uraemic state itself and by a direct consequence of dialytic therapy [17, 18].

Dialysis has been associated with acute changes in the complement activation status, granulocyte markers, macrophage function, T-cell activation and the release of various pro-inflammatory cytokines. Although the physiologi-

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ic role of circulating cytokines is unknown, it is conceivable that a proinflammatory milieu characterized by high cytokine plasma levels may influence specific and tightly regulated cellular processes in leukocytes, inducing an inappropriate cellular and/or humoral immune response.

In dialysis patients, both Th lines of differentiation show reduced activation, which is accompanied by the low production of IL-2, a cytokine uniformly involved in the expansion of these lines. However, chronic renal failure induces a bias of differentiation toward the Th1-type cells. This defect most probably results from an abnormality in the regulation of IL-12, the APC-derived cytokine that is primary inducer of the Th1 cells [19]. This mediator is overexpressed by monocytes from hemodialysis patients and this might thus explain the bias toward Th1 differentiation [20].

In addition, another evidence that the primary defect is localized in providing insufficient costimulatory signaling from the APC cells is given by their reduced expression of B7-2 (CD86) in hemodialysis patients that normally binds CD28 on T cells [21]. The expression of B7-1 (CD80) and the primary signaling molecule human lymphocyte antigen class II is not affected. Interestingly, the B7 molecules transmit signals that directly act on the induction of IL-2 [22].

The studies of Daichou et al. [23] and Donati et al. [24] showed that in uremia, T-cell proliferation is associated with the downregulation of IL-2 synthesis by lymphocytes and the induction of an abnormal state of lymphoblast activation that is further enhanced following chronic hemodialysis (HD). In chronic renal failure there is a decreased number of CD4+ [25, 26] and an increased proportion for CD8+ cells [24, 27], but the absolute numbers of CD8+ are supposedly low [28, 29] or unchanged [30, 31]. As T cells produce IL-2, the decreased number of CD4+ cells partly explains the suppressed IL-2 production [32]. A single in vitro study [33] has demonstrated that the production of IL-2 is similar in T cells from patients and controls. However, in vitro mitogen-stimulated human peripheral blood lymphocytes release IL-2R that can bind IL-2 and inhibit IL-2-dependent cell proliferation [34]. In uremic patients, IL-2R plasmatic levels increase [35, 36] and may attenuate the availability of IL-2 by binding to it and may further contribute to the immune system defects associated with uremia in vivo [37, 38].

Another cytokine affected by uremia is IFN- γ but the data are controversial. While some authors confirm non-significant differences in IFN- γ production between healthy controls and HD patients [39, 40], Daichou et al.

Fig. 1. Immune dysfunction and cytokine pattern in uremia.

[23] underline the IFN- γ production is increased in uremia. IFN- γ is produced by Th1 cells and NK cells. IFN- γ selectively inhibits Th2 cell proliferation [41] and IL-10 mRNA [42], while IL-10 inhibits cytokine synthesis by Th1 cells [43]. Moreover, IFN- γ produced by Th1 stimulates CD8+ suppressor/cytotoxic lymphocytes and CD16+ NK cells [44]. IL-12 is capable to stimulate the synthesis of IFN- γ by NK cells: this stimulation leads to macrophage activation which among numerous responses includes increased cytokine synthesis, ultimately leading to even more NK cell IFN- γ production [45]. Moreover, IL-12 plus IFN- γ -inducing factor (IL-18) act on Th1 cells and induce the secretion of IFN- γ [46].

Considering the different behaviours of IL-2 and IFN- γ in uremia, Eszter et al. [47] found that their genes are not coordinately regulated at the post-transcriptional level but the stability of IFN- γ mRNA is modulated by IL-12. This selective control leads to increased IFN- γ production. Sester et al. [20] have demonstrated in vitro that the production of IL-12 per single cell is not altered, thus leaving two main factors contributing to increased levels of IL-12. One factor is that HD patients have increased numbers of circulating monocytes [48]. Secondly, among the uremic patients, a higher percentage is capable of producing IL-12 [24]. Overproduction of IL-12 is a phenome-

non well in line with other observations on dysregulated cytokine production in dialysis patients, such as IL-6, TNF α and IL-1 β .

Moreover, increased IL-6 levels are due to increased percentage of cytokine-producing cells (whereas the expression per single cell equals that of control cells) and to the patient's ability to upregulate IL-10 which controls the overproduction of IL-6 [49]. IL-10 acts as a counter-regulatory mechanism by limiting proinflammatory responses. Secretion of IL-6 is followed by the induction of IL-10 within a few hours. So the inflammation is limited and can be shut down again. The dialysis patients who secrete high amounts of IL-10 can reduce the overproduction of cytokines (fig. 1).

Vitamin E-Coated Dialyzer Membrane

Many factors may influence the immune system in dialysis: type of dialytic treatment, membranes, dialytic age, Kt/V, malnutrition, underlying nephropathy but, among these, the use of more compatible dialyzers or membranes that actively modify immune function such as dialyzers coated with vitamin E seems to give good results [50]. It is well known that hemodialysis membrane materials have been demonstrated to activate the oxidative metabolism of polymorphonuclear (PMN) cells and monocytes/macrophages [51, 52]. Activated leukocytes generate oxygen free radicals (OFR) that react strongly against a wide spectrum of biomolecules [53], resulting in a series of cytotoxic effects commonly described as 'oxidative stress'. In addition, the dialyzer induces, both directly and indirectly, the cytokine production during hemodialysis through complement activation. This control is regulated at a gene level: adherence to dialysis membrane induces selective mRNA expression of monocyte mediators and proto-oncogene [54, 55]. Furthermore, several studies support the hypothesis that terminal complement complex (TCC) generation may have a potential role in activating mononuclear cells [56]. TCC can affect cell metabolism and constitute a potent signal for activation of monocytes to produce inflammatory mediators such as TNF- γ and IL-6 [55].

Cytokine activation may be induced also by bacterial-derived material, for example lipopolysaccharide, contaminating the dialysate and able to cross the membrane [57].

In an attempt to increase the quality of HD filters, vitamin E-coated multilayer hemodialysis filter was recently produced with the two goals of obtaining an improvement

in biocompatibility and a specific protection against OFR [51, 58]. A growing body of evidence points to a defective antioxidant/prooxidant balance, a pathogenic factor in some HD-related side effects such as: anemization, defective immunological and coagulative functions, accelerated atherosclerosis and aging, β_2 -microglobulin amyloid arthropathy and cancer onset.

In this context, the lack of antioxidant protection, and in particular a deficit of vitamin E, has long been a subject of investigation [59]. At the same time, an increased prooxidant stimulus from PMN activated by the bioincompatibility of HD materials has been suggested [60], but is not yet completely understood.

Recent reports evidenced that vitamin E-coated filter can be effective in protecting HD patients against OFR damage by decreasing PMN activation and providing a better control of the blood lipoperoxidation and antioxidant status, apoptosis and respiratory burst on mononuclear cells [51, 54].

In addition, it has also been proposed to lower plasma levels of β_2 -microglobulin by changing its isoelectric point which might influence positively the elimination by dialysis [61]; to have a positive effect on uremic anemia increasing the antioxidant protection and lowering membrane peroxidation in red blood cells [62], and improve endothelial function [63].

Regarding the relationship between vitamin E-coated dialyzers (VE) and immunological status, Girndt et al. [50] have demonstrated that 4 weeks of treatment with VE or PA dialyzers enhanced in vitro proliferation on peripheral blood leukocytes (PBLs) in comparison to treatment with HE used before study entry. Another interesting data is that the acute production of IL-6 was reduced by VE membrane that in part results from a direct inhibitory effect on monocyte activation that is not seen with PA membrane. IL-10 is not inhibited by either membrane. VE membrane seems to have high biocompatibility, besides the parameters of complement activation and cytokine induction. However, the reduced plasma lipid peroxidation may also have a role for cytokine induction in monocytes.

Although PA and VE membranes have similar effects on in vitro parameters of T-cell activation during hemodialysis, these preliminary data indicate that there is an additional direct effect of vitamin E on monokine production that accounts for the reduction of the inflammatory status. In conclusion, in account of vitamin E bound to membrane surface, it displays both biocompatibility and bioactive characteristics that make it better than conventional cellulase membranes.

Conclusions

In uremic patients, affected cytokine network presents a multifactorial genesis; therapeutic interventions might be multiple. Likely, the most important intervention in the present future could be the modifications of dialytic

membranes. Extracorporeal treatment could benefit by vitamin E-coated membrane for its decreasing the inflammatory processes and modify signalling cascades which induce proliferation, differentiation and apoptosis/cell death.

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