Serum Soluble CD14 Levels and Ultrafiltered Dialysate in Hemodialysis Patients

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Dear Sir

Endotoxin (lipopolysaccharide, LPS) exists in the dialysate and is closely associated with the pathophysiology of dialysis-related amyloidosis [1]. LPS binds to LPS-binding protein (LBP), and the complex binds to CD14 on the surface of mature monocytes. CD14, the receptor of this LPS-LBP complex, is released into the blood and becomes soluble CD14 (sCD14). sCD14 inhibits binding of the LPS-LBP complex to membrane-bound CD14, thereby neutralizing LPS. sCD14 has a role in the LPS-dependent activation of endothelial and epithelial cells [2]. We measured serum sCD14, hyaluronic acid, and dialysate LPS in hemodialysis patients in order to evaluate their association with dialysis-related amyloidosis. One hundred and three patients (57 men and 46 women, mean age 60.4 years) with chronic renal failure participated in the study. They were undergoing maintenance hemodialysis for more than 3 months (mean length of dialysis: 84.8 months, range 3-266) thrice weekly, 4 h/session. Cuprophane (n = 54), cellulose acetate (n = 16), ethylene/vinyl alcohol (n = 8), polymethyl methacrylate (n = 11) or high-flux (n = 14) membranes were used prior to the study. Nine continuous ambulatory peritoneal dialysis (CAPD) patients were also enrolled in the study. The dialysate was either untreated or ultrafiltered using 1 or 2 polyethersulfone filters for 15 months. The subjects were divided into groups as follows: group 1, 23 patients, dialysed with ultrafilters after sessions on a reverse-osmosis unit and a central supply unit; group 2, 26 patients, dialysed with an ultrafilter after sessions on a reverse-osmosis unit, and group 3, 54 patients, treated with regular dialysate. For bacteriological testing, a 50-ml sample of the dialysate was cultured on nutrient agar plates at 30°C for 3-7 days. Endotoxin levels of the dialysate were measured using Endospecy, an endotoxin-specific chromogenic limulus test reagent [3]. sCD14 levels were determined by enzyme immunoassay [4]. Hyaluronic acid was measured using a sandwich binding protein assay [5].

The predialysis sCD14 level was significantly higher in the dialysis patients, and in 79.6% of the cases it exceeded 6 µg/ml. No correlation was observed between the sCD14 level and the level of hyaluronic acid or the duration of hemodialysis. No significant difference of sCD14 was seen between groups with and without dialysis-related amyloidosis. The high levels of

sCD14 in dialysis patients were not influenced by the dialysis session or the type of hemodialysis membrane. There was no bacterial growth, and < 0.5 pg/ml endotoxin resulted with two filters. The medians for bacterial counts and endotoxin with a filter were 0.3 CFU/ml and 3.6 pg/ml, respectively, and 3.2 CFU/ml and 6.9 pg/ml without a filter, respectively. In group 1, with ultrafilters, the sCD14 level was significantly lower than with regular dialysate, group 3 (fig. 1).

The normal value for serum sCD14 has been reported as 1-6 μ g/ml [6]. The source of sCD14 has yet to be determined. The

increase in sCD14 levels is considered the consequence of the activation of cells expressing CD14. Labeta et al. [7] demonstrated the release of two different soluble forms of CD14 (α , 48 kD, and β , 56 kD). Moreover, sCD14 may also originate from nonmyeloid cells [8]. The addition of LPS and TNF- α led to an increase in sCD14 levels, IFN- γ and IL-4 down-regulate sCD14 release in monocytes and macrophages. LPS-sCD14 complexes interact with an as yet unknown receptor on the endothelial surfaces. sCD14 mediates activation of transcription factor NF- κ B by LPS in human endothelial cells independent of LBP [9]. sCD14 is involved in the induction by LPS of HUVEC IL-6 and IL-8 biosynthesis, as well as ICAM-1 and VCAM-1 expression [10].

It has been shown that high concentrations of LPS down-modulated the expression of CD14 on monocytes through a shedding process. CD14 was partially down-modulated in monocytes during PAN or cuprophane dialysis. sCD14 was increased in CAPD and hemodialysis patients compared to controls. We suggested that up-regulated expression of inflammatory cytokines and adhesion molecules promotes activation and infiltration of macrophages causing dialysis-related amyloidosis in maintenance hemodialysis patients [11]. The changes in the sCD14 levels may reflect the monocyte activation because of a slight influence by dialyzer clearance. The use of sterile dialysates may decrease the monocyte activation of

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Controls

CAPD

Group 1 with

2 filters n=23

Group 2 Group 3

with without

1 filter filter

n=26 n=54

n=9

$\pi=10$

long-term hemodialysis. The limulus amoebocyte lysate assay only detects species of LPS with an apparent molecular weight above 8,000 D [12]. We have demonstrated that the LPS in the dialysate had a low molecular weight of approximately 4,000 D and that LPS may transfer across the dialysis membrane [13].

These results suggest that bacterial contamination of the dialysate increases mono-cytic sCD14 in dialysis patients. Serum levels of sCD14 can be diminished by the use of ultrafiltered dialysate. The sCD14 level may be a marker for the state of monocyte activation by LPS.

Fig. 1. Serum sCD14 levels in $\mu\text{g/ml}$, in the control, CAPD and HD groups. Results are expressed as mean $\pm$ SD. * $p < 0.01$ versus controls. The difference between group 1, with ultrafilters, and group 3, without ultrafilters, was significant ($p < 0.01$).

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