



# The Role of Interleukin-6 and of Its Soluble Receptors in the Biocompatibility of Dialysis Treatment

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Proinflammatory cytokines, in addition to their role in host defence, may be considered mediators of disease; a reduction of cytokine synthesis or effects is, therefore, becoming a target of many diseases. IL-6 is a pro-inflammatory cytokine that may play a role in several clinical problems related to dialysis treatment. An enhanced spontaneous production of IL-6 by Peripheral Blood Mononuclear Cells (PBMC) harvested from ESRD patients dialyzed with a poor biocompatible membrane has been first demonstrated by our group. These results were also obtained in patients undergoing continuous peritoneal dialysis, in absence of peritonitis. We have also demonstrated that IL-6 release was inversely correlated with serum albumin changes. Biological activities of IL-6 may be modulated by two soluble circulating receptors, namely sIL-6R and sgp130. sIL-6R may enhance the inflammatory effects of IL-6 and is, therefore, an "agonistically" acting molecule. We have recently studied sIL-6R production in ESRD patients dialyzed with different membranes; the conclusion was that poor biocompatible membranes, via the sIL-6R, might further increase the inflammatory effects of IL-6. On the contrary, sgp130 can efficiently bind the IL-6/sIL-6R complex with "antagonistic" effects. We have evaluated plasma levels of sgp130 in 18 ESRD patients regularly dialyzed with hemophan membranes (HE) and in 15 patients dialyzed with more biocompatible synthetic membranes (BIO). Our results demonstrate that plasma levels of sgp130 in HE are 33% higher than in both healthy controls and BIO. Circulating levels of sgp130 were correlated positively with C-reactive protein ( $r: 0.338, p < 0.05$ ) and negatively with serum albumin ( $r: -0.334, p < 0.05$ ). These results suggest that higher circulating levels of sgp130 are likely associated with higher IL-6 levels. These higher amounts are probably insufficient to control the activity of IL-6 and may be considered only as a marker of PBMC activation.

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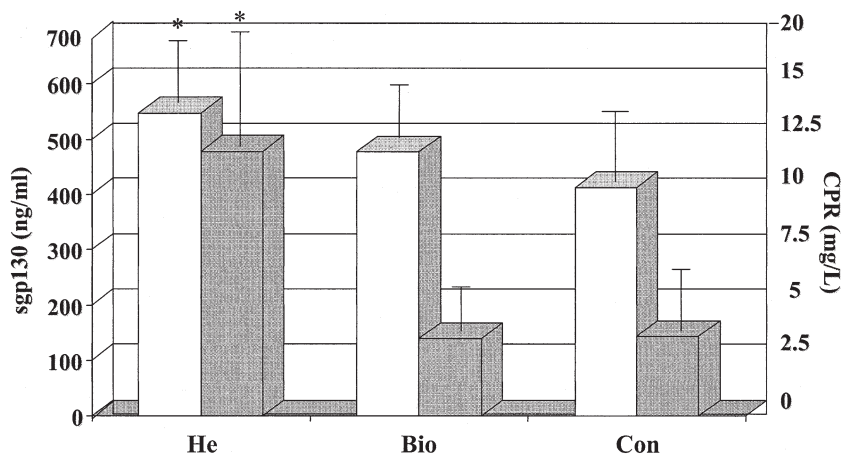
Although a significant decrease in mortality rates has been reported recently in dialysis patients,<sup>1</sup> the survival of young US end-stage renal disease (<40 y) patients is still 20

times lower compared with healthy subjects.<sup>2</sup> Cardiovascular diseases still represent the main cause of mortality in these patients.<sup>1</sup> Several studies have strengthened the hypothesis that atherosclerosis is an inflammatory disease and that, in turn, systemic inflammatory stimuli may play a role in the pathogenesis of vascular atherosclerotic lesions.<sup>3,4</sup> According to this hypothesis, increased circulating levels of C-reactive protein (CRP) have been indicated as strong predictors of mortality in hemodialysis patients.<sup>5,6</sup> CRP is a positive acute-phase protein produced by the liver under systemic inflammatory stimuli.<sup>7</sup> Its synthesis is modulated by interleukin-6 (IL-6); this cytokine, in fact, is the most potent inducer of acute-phase response by the liver with increased production

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**Figure 1** sgp130 (□) and CRP (■50%filled;) concentrations in 18 hemodialysis patients dialyzed with HE membranes, 15 hemodialysis patients dialyzed with Bio synthetic membranes, and 10 healthy controls (Con). \* $P < .05$  versus Bio and Con.

of CRP and serum amyloid A.<sup>8</sup> IL-6 is a proinflammatory cytokine that, in addition to hepatocyte stimulation, plays an important role in the differentiation and proliferation of both T and B lymphocytes.<sup>9</sup>

## IL-6 and Dialysis Treatment

More than 10 years ago we showed that peripheral blood mononuclear cells (PBMCs) harvested from end-stage renal disease patients undergoing regular dialysis treatment with cuprophane membranes, cultured for 24 hours, spontaneously (ie, in the absence of any mitogenic stimulation) released increased amounts of IL-6 compared with both healthy subjects and uremic patients not yet on dialysis treatment.<sup>10</sup> In this study we did not find any significant difference between the samples drawn before and after the dialysis session; these results suggested a chronic PBMC stimulation with a continuous release of proinflammatory cytokines. When the same patients were switched to a more biocompatible polymethylmethacrylate membrane, the IL-6 release decreased, becoming similar to the values of healthy subjects.<sup>10</sup> Our observations suggested that blood interaction with poor biocompatible hemodialysis membranes causes the increase of an inflammatory state, often in the absence of any clinical symptoms. The switch to a more biocompatible dialysis membrane may lead the inflammatory condition within a normal range. PBMC activation is induced by complement activation, with C3a and C5a formation, and/or by endotoxin fragment passage from dialysate.<sup>11,12</sup> Some years later we also showed an increased IL-6 production by PBMCs harvested from end-stage renal disease patients who were treated with continuous ambulatory peritoneal dialysis even in the absence of peritonitis.<sup>13</sup> More recently, many studies have strengthened the crucial role of IL-6 in the pathogenesis of some dialysis-related complications.<sup>14</sup> In particular, negative effects of IL-6 on the nutritional status and serum albumin concentration have been recognized.<sup>15,16</sup> Increased circulat-

ing levels of IL-6, therefore, have been indicated as predictors of mortality.<sup>17</sup> Recently, Stenvinkel et al.<sup>18</sup> defined a clinical syndrome, termed *MIA syndrome* (malnutrition, inflammation, atherosclerosis), in which IL-6 seems to play a crucial role.<sup>19</sup>

## IL-6 Soluble Receptors

IL-6 binds to specific receptors present on cell membrane surface. These receptors include the chain gp80 (or IL-6R) and the 2 gp130 chains. The high binding affinity between IL-6 and the 2 membrane-bound receptors (namely, IL-6R and gp130) causes the transduction of the signal with the activation of the intracellular pathways.<sup>20</sup> Membrane-bound IL-6R and gp130 may produce, by shedding (loss of both transmembrane and intracytoplasmic domains), 2 soluble receptors termed *sIL-6R* and *sgp130*, respectively.<sup>21</sup>

*sIL-6R* is an agonistic circulating receptor of IL-6; this implies that *sIL-6R* may bind IL-6 and this binary complex (IL-6/*sIL-6R*) may activate target cells, even those that do not express gp80 on their surface.<sup>21</sup> We recently studied the behavior of this agonistic receptor in end-stage renal disease patients dialyzed with different membranes.<sup>22</sup> Our results showed that PBMCs harvested from patients dialyzed with a cuprophane membrane showed a higher release by PBMCs and higher circulating levels of *sIL-6R* compared with patients dialyzed with a more biocompatible synthetic membrane. This phenomenon may amplify the inflammatory effects of IL-6.<sup>22</sup>

The other soluble receptor, *sgp130*, is an antagonistic receptor of IL-6. It binds the circulating binary complex IL-6/*sIL-6R*, preventing its binding with target cells.<sup>23</sup> In healthy subjects, normal serum concentrations of *sgp130* ( $\approx 400$  ng/mL) reduces by 55% the effects of IL-6 and IL-6/*sIL-6R*; a 10-fold increase of *sgp130* reduces it by 87%, whereas a 10-fold multiplication of both *sIL-6R* and *sgp130* induces a complete neutralization of the inflammatory effects of IL-6 and IL-6/*sIL-6R*.<sup>24</sup>

We recently evaluated plasma levels of sgp130 in 18 hemodialysis patients regularly dialyzed with hemophan (HE) membranes and in 15 patients dialyzed with more biocompatible (Bio) synthetic membranes (polymethylmethacrylate, polysulfon, polyamide, and so forth); 10 healthy subjects also were enrolled as controls. We assayed sgp130 plasma levels by an enzyme-linked immunosorbent assay procedure, using a specific mouse monoclonal antibody (CD 130, Clone B-R3; Biosource, Camarillo CA); CRP and serum albumin also were measured by nephelometry. The results are reported in Figure 1. Our results show that plasma levels of sgp130 in HE are 33% higher than in both healthy controls and Bio. Circulating levels of sgp130 correlated positively with CRP ( $r = .338$ ,  $P < .05$ ) and negatively with serum albumin ( $r = -.334$ ,  $P < .05$ ) concentrations. These results indicate that higher circulating levels of sgp130 likely are associated with higher IL-6 levels. The increased concentrations of inhibitory receptors, however, are not enough to counterbalance the inflammatory effects of IL-6. They may be considered only as a marker of mononuclear cell activation, as suggested for IL-1Ra.<sup>25,26</sup>

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