Interleukin-1 Receptor Antagonist Synthesis by Peripheral Blood Mononuclear Cells: A Novel Predictor of Morbidity among Hemodialysis Patients

VAIDYANATHAPURAM S. BALAKRISHNAN, CHRISTOPHER H. SCHMID, BERTHARD L. JABER, SVETLOZAR N. NATOV, ANDREW J. KING, and BRIAN J. G. PEREIRA
Divisions of Nephrology, New England Medical Center Hospitals and St. Elizabeth’s Medical Center, Boston, Massachusetts.

Abstract. Proinflammatory cytokines have been implicated in the short- and long-term morbidity experienced by hemodialysis (HD) patients. The present study, which is based on long-term follow-up of a cohort of 37 patients, relates peripheral blood mononuclear cell (PBMC) interleukin-1 receptor antagonist (IL-1Ra) synthesis (a reliable marker of IL-1β synthesis in HD patients) and plasma levels of an acute phase reactant, lipopolysaccharide binding protein (LBP), to clinical outcomes. In July 1993, predialysis blood samples from these patients were collected and IL-1Ra synthesis by PBMC and plasma LBP was measured. Hospital records were reviewed and patient follow-up data were obtained until December 1997 (54 mo) or death, whichever occurred earlier. The effect of age, diabetes, endotoxin- and IgG-stimulated IL-1Ra synthesis, and plasma LBP levels on mortality was assessed using the Cox proportional hazard regression model. Poisson regression was used to determine potential relationships between the number of outcome events and each continuous risk factor. Twenty-two patients (59%) died during the follow-up period. Mortality was unrelated to IL-1Ra synthesis but did increase with age (relative risk, 1.05/yr; \( P = 0.01 \)) and diabetes (relative risk, 3.00/yr; \( P = 0.03 \)). Cardiovascular event rates were higher among older individuals and in those with higher endotoxin-stimulated PBMC IL-1Ra synthesis. Cardiovascular events increased with plasma LBP levels in the range of 9,000 to 12,000 pg/ml but then seemed to decrease. In contrast, older age and low IgG-stimulated IL-1Ra synthesis were associated with an increased risk of infectious events. The results of this study demonstrate an interesting link between stimulus-dependent variability in IL-1Ra synthesis by PBMC and clinical outcomes among patients on chronic HD and provide interesting targets for therapeutic interventions in this vulnerable patient population.

During hemodialysis (HD), exposure of blood to various components of the extracorporeal circuit results in activation of a wide variety of circulating cell lines and plasma proteins. In particular, stimulation of peripheral blood mononuclear cells (PBMC) can lead to the synthesis and release of proinflammatory cytokines. Indeed, several studies have shown that plasma levels of proinflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), as well as specific cytokine inhibitors such as interleukin-1 receptor antagonist (IL-1Ra), are elevated among patients who are on HD (1). These cytokines have been incriminated in the short- and long-term morbidity experienced by HD patients (2). Bologa and colleagues (3) found a significant correlation between plasma TNF-α and IL-6 and the degree of hypoalbuminemia and dyslipoproteinemia among HD patients. This study also found that plasma IL-6 levels were a predictor of mortality among these patients. Likewise, Kimmel and colleagues (4) found that elevated plasma levels of IL-1, TNF-α, IL-6, and IL-13 among HD patients were significantly associated with an increased relative risk of death. However, plasma cytokine levels may be affected by several parameters, such as the level of residual renal function, dialyzer clearance, adsorption of cytokines to dialyzer material, variable ultrafiltration rate, and underlying renal and comorbid disease (5,6).

Transcriptional activation of IL-1β in PBMC was observed after a single passage through an unsubstituted cellulose dialyzer (7). Furthermore, studies on PBMC from HD patients have shown that they are “primed” to synthesize increased levels of IL-1β and TNF upon in vitro stimulation (8–10). However, other studies have reported functional defects in these cells after exposure to cellulose membranes, including defective phagocytosis and internalization capacity (11). We recently studied PBMC cytokine synthesis among patients who were dialyzed with unsubstituted cellulose dialyzers and clinical and laboratory characteristics that could influence this process (12). We observed an inverse correlation between IL-1Ra synthesis and duration of dialysis. These findings could...
be interpreted as further evidence of impaired host defense among patients on long-term HD. Alternatively, the lower IL-1Ra synthesis among patients who had been on HD longer could reflect “left truncation,” whereby “high producers” had died early, leaving behind the “low producers.” This latter hypothesis would then support a role for cytokines in dialysis-related morbidity and mortality. To resolve this issue, we undertook a long-term follow-up of the cohort of patients from this study and examined the relationship between PBMC IL-1Ra synthesis and clinical outcomes. We focused on cardiovascular and infectious events as outcome parameters, given that these are the two leading causes of morbidity among patients who are on HD (13). In addition, we took advantage of the fact that plasma levels of lipopolysaccharide binding protein (LBP) had been previously measured and examined the relationship between this acute phase reactant (14) and clinical outcomes.

Materials and Methods

Subjects

This study was based on follow-up of a cohort of patients who had previously been selected to examine the impact of single use versus reuse of cellulose dialyzers on clinical parameters and indices of biocompatibility (12,15). Patients were drawn from the outpatient dialysis unit at St. Elizabeth’s Medical Center (Boston, MA). At the time of entry into the study, all patients were on chronic outpatient HD with unsubstituted cellulose dialyzers (Terumo Corporation, Tokyo, Japan) reprocessed with glutaraldehyde (0.8%) and sodium hypochlorite (<1%) using an automated system (DRS4™, Seratronics Inc., Concord, CA). Clinical records of 107 patients were reviewed by a single observer (S.N.N.). Fifteen patients who were on dialysis and using dialyzers other than unsubstituted cellulose and 5 that were likely to move or undergo renal transplantation in the near future were excluded. Also excluded were 47 patients with conditions that could influence cytokine synthesis, such as acute infection or blood transfusions in the preceding month, chronic infections (hepatitis B, hepatitis C, HIV, osteomyelitis), immunosuppressive therapy, previous transplantation, or history of malignancy. Finally, all patients were screened for cell content of IL-1Ra, and three patients in whom the cell content of IL-1Ra was less than 80 pg/2.5 x 10^6 PBMC were excluded from the study. The remaining 37 patients constituted the study group. In July 1993, predialysis blood samples from these patients were collected before the three dialysis treatments in a single week, and IL-1Ra synthesis by PBMC, cultured under different stimulatory conditions, was measured, as were plasma LBP levels. Patient follow-up data were obtained until December 1997 or death, whichever occurred earlier. Hospital records were reviewed for all hospitalizations during the period of follow-up, and the duration of hospitalizations and principal diagnosis for each admission were recorded. For patients who died, the date of death and primary and secondary causes of death were determined. Each hospitalization in which the primary discharge diagnosis was an infectious or cardiovascular condition was defined as an infectious or a cardiovascular event, respectively.

Laboratory Measurements

Water and tissue culture media used in assays were subjected to ultrafiltration using a polyamide hollow-fiber ultrafilter (U2000, Gambro AB, Hechingen, Germany) to remove cytokine-inducing agents (16). PBMC were harvested as described previously (17,18). Briefly, each 10-ml sample of blood was diluted with 20 ml of sterile pyrogen-free normal saline (Abbott Laboratories, Rockford, IL) and underlayered with 10 ml of Ficoll-Hypaque. The tube was then centrifuged at 450 g for 45 min at room temperature. The PBMC layer was harvested, washed in saline, and centrifuged at 400 g for 10 min. PBMC were washed in saline two additional times and resuspended in ultralfitered tissue culture medium (RPMI 1640 [pH 7.4], Sigma Chemical Co., St. Louis, MO), containing 10 mmol/L L-glutamine, 24 mmol/L NaHCO₃ (Mallinckrodt, Paris, KY), 10 mmol/L HEPES (Sigma), 100 U/ml penicillin, and 100 μg/ml streptomycin (Irvine Scientific, Santa Ana, CA). PBMC were counted using a standard hemocytometer and adjusted to 5 x 10⁶ cells/ml in RPMI. A 0.5-ml suspension of PBMC was aliquoted into each of three 12 x 75-mm polypropylene tubes (Becton Dickinson, NJ). Tube 1 received 0.5 ml RPMI, tube 2 received 0.5 ml RPMI containing 20 ng/ml endotoxin (Escherichia coli, serotype O55:B5, Sigma), and tube 3 received 0.5 ml of RPMI containing 20 μg/ml human IgG (Gammagard, Hyland Laboratories, Duarte, CA). The tubes were incubated upright in a humidified atmosphere at 37°C with 5% CO₂. At the end of 24 h, the cell suspensions were subjected to three freeze-thaw (~70°C) cycles for measurement of total IL-1Ra synthesis (cell-associated and secreted). All PBMC separations were performed by a single individual. Endotoxin-stimulated IL-1Ra synthesis by PBMC is mediated via the CD14 receptor. However, IgG-stimulated IL-1Ra synthesis is mediated via the Fcγ-receptor and is hence an index of Fcγ-receptor function.

Undiluted or diluted samples were assayed in RIA buffer (0.01 mol/L phosphate-buffered saline [pH 7.4], 0.25% bovine serum albumin, and 0.05% sodium azide), and IL-1Ra synthesis was determined by a specific non–cross-reactive RIA (18). The IL-1Ra concentrations were then read from a logit plot of percentage of specific binding versus the log of known concentrations of IL-1Ra from the linear portion of the curve (usually between 35% and 85% specific binding). The lower limit of detection of the RIA for IL-1Ra was 80 pg/ml. To eliminate interassay variability, we tested all samples from each culture condition in a single assay. IL-1Ra synthesis by PBMC is expressed as pg/2.5 x 10⁶ PBMC. Results are expressed as mean ± SEM.

We previously established that there were no significant differences in IL-1Ra synthesis by PBMC harvested before the three different dialysis treatments during the week (12). Therefore, the mean of three IL-1Ra measurements in a single week was used to study the relationship between IL-1Ra synthesis by PBMC and subsequent clinical outcomes. IL-1Ra synthesis by PBMC was selected as the index of cytokine synthesis when the original study was designed (15), because we had shown that IL-1Ra was a more sensitive index of cytokine synthesis than IL-1β (17). IL-1Ra and IL-1β are produced by the same cells (19), and IL-1Ra synthesis is closely correlated with that of IL-1β (20).

The plasma levels of LBP were measured by a sandwich enzyme-linked immunosorbent assay using rabbit polyclonal antibodies against human LBP as primary and secondary antibodies (21). The lower limit of detection for this assay was 160 ng/ml.

Statistical Analyses

Models for Mortality. The effect of age, diabetes, endotoxin- and IgG-stimulated IL-1Ra synthesis, and plasma LBP levels on mortality was assessed using the Cox proportional hazard regression model, computing the relative hazard of mortality for specified changes in the level of risk factors.

Models for Number of Cardiovascular and Infectious Events. To model the number of events that occur in a given time interval, we used a Poisson model that assumes that events within groups of
patients defined by the regression variables occur independently, at random times over the course of the study (22). To determine potential nonlinear functional relationships between the number of outcome events and each continuous risk factor, we used the generalized additive model form of Poisson regression (23,24). Each model was adjusted for duration of dialysis before the study. As the effect of duration was found to be linear, it was adjusted for by a single term in the regression model. This model takes the form

$$\log (\# \text{ events}) = \alpha + \text{offset (time at risk)} + \beta_1 \text{duration} + f(x)$$

where the offset is a fixed constant that is used to adjust the outcome to the logarithm of an event rate per time at risk and $f(x)$ is a smoothing spline with four degrees of freedom. The linear form of this model has $f(x) = \beta x$. The smoothing spline is essentially computed by taking locally weighted averages in which the predicted function at a point $x_0$ is a weighted average of the outcomes corresponding to points with $x$ values near $x_0$. The weights are usually such that the points nearest to $x_0$ get the largest weight and points far from $x_0$ get little or no weight. Because of this weighting, the resulting function can adapt to local behavior and can take a nonlinear shape. The degree of smoothness exhibited by these scatterplot smoothers depends on a smoothing parameter, which determines the weight function to apply to the data. Settings with more smoothness give less weight to the nearest neighbors, thus minimizing the local effects. The use of four degrees of freedom is a common compromise that detects local behavior without becoming data interpolation.

The generalized additive model was calculated using S-Plus statistical software (StatSci Inc., Seattle, WA) by an algorithm that employs backfitting embedded in an iteratively reweighted least squares procedure. The parametric and nonparametric fits were compared using an approximate $\chi^2$ test based on the difference in their deviance functions and degrees of freedom. Noninteger degrees of freedom resulting from the approximation were rounded to the nearest integer. For tests of a nonlinear effect, models with a smoothing spline were compared with the linear model. The resulting test follows a $\chi^2$ distribution with approximately three degrees of freedom. When the nonlinear effect was not significant, we then tested for a linear effect by comparing the model with a linear term to the null model. The resulting test follows a $\chi^2$ distribution with one degree of freedom under the null hypothesis of no effect.

Graphic representations for the effects of each risk factor on the rate of events were plotted as curves depicting the marginal effect of smooth functions and degrees of freedom. Noninteger degrees of freedom resulted from the approximation were rounded to the nearest integer.

Table 1. Demographic characteristics and results of selected laboratory tests

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Mean (± SEM) duration of dialysis before study entry (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (yr)</td>
<td>24–84</td>
</tr>
<tr>
<td>gender</td>
<td>19 male, 18 female</td>
</tr>
<tr>
<td>race (%)</td>
<td></td>
</tr>
<tr>
<td>white</td>
<td>76%</td>
</tr>
<tr>
<td>black</td>
<td>16%</td>
</tr>
<tr>
<td>Asian</td>
<td>5%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>3%</td>
</tr>
<tr>
<td>cause of ESRD (%)</td>
<td></td>
</tr>
<tr>
<td>diabetes mellitus</td>
<td>32%</td>
</tr>
<tr>
<td>hypertension</td>
<td>22%</td>
</tr>
<tr>
<td>glomerulonephritis</td>
<td>14%</td>
</tr>
<tr>
<td>renovascular disease</td>
<td>8%</td>
</tr>
<tr>
<td>chronic interstitial nephritis</td>
<td>5%</td>
</tr>
<tr>
<td>other causes</td>
<td>19%</td>
</tr>
<tr>
<td>Laboratory parameters (Mean ± SEM)</td>
<td></td>
</tr>
<tr>
<td>URR (%)</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.2 ± 0</td>
</tr>
<tr>
<td>plasma albumin (g/dl)</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>PBMC IL-1Ra synthesis (pg/2.5 × 10^6 cells)</td>
<td></td>
</tr>
<tr>
<td>unstimulated</td>
<td>839 ± 64</td>
</tr>
<tr>
<td>endotoxin-stimulated</td>
<td>7498 ± 515</td>
</tr>
<tr>
<td>IgG-stimulated</td>
<td>10,263 ± 531</td>
</tr>
<tr>
<td>plasma LBP (pg/ml)</td>
<td>13,200 ± 618</td>
</tr>
</tbody>
</table>

$^a$ ESRD, end-stage renal disease; URR, urea reduction ratio; Kt/V, “K” represents dialyzer urea clearance, “t” duration of dialysis session, and “V” volume of distribution of urea; PBMC, peripheral blood mononuclear cells; IL-1Ra, interleukin-1 receptor antagonist; LBP, lipopolysaccharide binding protein.

Results

Demographic Characteristics and Laboratory Parameters

The demographic characteristics of patients and results of selected laboratory tests at the time of inclusion into the study are shown in Table 1. These results have been previously reported (15). The mean follow-up was $33 ± 3$ mo.

We previously demonstrated that IL-1Ra synthesis by PBMC under different culture conditions was not significantly associated with age, gender, cause of end-stage renal disease (ESRD), dialysis prescription, or other baseline clinical characteristics (12). In addition, IL-1Ra synthesis by PBMC was not significantly related to baseline laboratory indices (including serum albumin and cholesterol) or medications (12). Dialyzer reuse method did not change during the course of the follow-up period, and careful review of patient records confirmed that all patients remained on unsubstituted cellulose dialyzers for the duration of follow-up.

IL-1Ra Synthesis and Mortality

Twenty-two patients (59%) died during the follow-up period. As shown in Table 2, increasing age and the presence of diabetes were associated with a significantly increased relative risk of death.

IL-1Ra Synthesis and Cardiovascular Events

Cardiovascular events were related to age and endotoxin-stimulated IL-1Ra synthesis but not to diabetes (Table 3) or IgG-stimulated IL-1Ra synthesis (Figure 1C). Event rates were higher among older individuals (Figure 1A) and those with higher endotoxin-stimulated PBMC IL-1Ra synthesis (Figure 1B). Events
increased with plasma LBP levels in the range of 9,000 to 12,000 pg/ml but then seemed to decrease (Figure 1D).

IL-1Ra Synthesis and Infectious Events

Infectious events were related to IgG-stimulated IL-1Ra synthesis by PBMC and age (Figure 2A) but were unrelated to diabetes, endotoxin-stimulated IL-1Ra synthesis (Figure 2B), or plasma LBP (Figure 2D, Table 3). Infectious events decreased with increasing IgG-stimulated IL-1Ra levels up to approximately 10,000 pg/2.5 × 10^6 PBMC and then seemed to level off or increase (Figure 2C). However, this latter increase seemed to result mainly from two subjects with IgG-stimulated IL-1Ra levels near 15,000 pg/2.5 × 10^6 PBMC.

Discussion

The results of this study demonstrate an interesting link between IL-1Ra synthesis by PBMC and clinical outcomes among patients who are on chronic HD. High endotoxin-stimulated PBMC IL-1Ra synthesis was strongly associated with a greater likelihood of cardiovascular events as were high plasma LBP levels. In contrast to cardiovascular events, low IgG-stimulated IL-1Ra synthesis was associated with a greater likelihood of infectious events. Finally, the association between IL-1Ra synthesis and mortality did not reach statistical significance, perhaps because of the small sample size.

There is mounting evidence that inflammation is associated with and probably causally related to atherosclerosis and its sequelae in the form of cardiovascular and cerebrovascular death and that this process possibly is driven by proinflammatory cytokines (25). Cytokines such as TNF-α and IL-1 induce endothelial cell activation, a key and early step in atherogenesis (26). In addition, these cytokines influence multiple aspects of atherogenesis, such as regulation of the expression of adhesion molecules crucial to the recruitment of leukocytes to lesions (27), production of monocyte chemoattractant protein-1 (28), regulation of genes that encode other growth factors and cytokines themselves that influence plaque progression (28), stimulation of smooth muscle cell proliferation (29), and vasomotor tone at the site of lesion (28). This scenario may be particularly relevant among HD patients, who exhibit elevated plasma levels of proinflammatory cytokines (1), increased cytokine synthesis by PBMC (30), and a high prevalence of atherosclerosis and cardiovascular morbidity (31).

The results of this study provide further support for the potential role of proinflammatory cytokines in the increased prevalence of cardiovascular disease among dialysis patients. Higher endotoxin-stimulated PBMC IL-1Ra synthesis was strongly associated with a greater likelihood of cardiovascular events. The mechanisms, however, are yet to be completely elucidated. Nonetheless, our results indicate that PBMC primed by repeated exposure to blood-membrane interactions during HD represent an important source of proinflammatory cytokines that could contribute to the inflammatory milieu that underlies atherogenesis. It is interesting that Nockher and Scherberich (32) demonstrated increased expression of mCD14 receptors by PBMC after HD, as well as increased sCD14

<table>
<thead>
<tr>
<th>Variable</th>
<th>RR</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per yr)</td>
<td>1.05</td>
<td>1.00–1.10</td>
<td>0.01</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3.00</td>
<td>1.14–7.85</td>
<td>0.03</td>
</tr>
<tr>
<td>Endotoxin-stimulated IL-1Ra (per 1000 pg/2.5 × 10^6 PBMC)</td>
<td>1.09</td>
<td>0.96–1.22</td>
<td>0.19</td>
</tr>
<tr>
<td>IgG-stimulated IL-1Ra (per 1000 pg/2.5 × 10^6 PBMC)</td>
<td>0.88</td>
<td>0.74–1.04</td>
<td>0.14</td>
</tr>
<tr>
<td>LBP (per 1000 pg/ml)</td>
<td>1.02</td>
<td>0.92–1.12</td>
<td>0.71</td>
</tr>
</tbody>
</table>

*Relative risk (RR), 95% confidence interval (CI), and P value for testing the linear effect of factors on mortality using a Cox proportional hazards regression model.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Difference in χ² Deviance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cardiovascular Events</td>
</tr>
<tr>
<td></td>
<td>Linear Effect</td>
</tr>
<tr>
<td>Age</td>
<td>12.6***</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.0</td>
</tr>
<tr>
<td>↑ endotoxin-stimulated IL-1Ra</td>
<td>14.0***</td>
</tr>
<tr>
<td>IgG-stimulated IL-1Ra (↓ for infectious and ↑ for cardiovascular events)</td>
<td>0.5</td>
</tr>
<tr>
<td>↑ plasma LBP</td>
<td>0.1</td>
</tr>
</tbody>
</table>

* P < 0.05, ** P < 0.01, *** P < 0.001.
levels, possibly as a result of chronic exposure to trace amounts of endotoxin. The water used for the preparation of dialysate is not sterile (33,34), and the use of bicarbonate dialysate is widely known to facilitate bacterial growth (35,36). Consequently, the enhanced endotoxin-induced PBMC cytokine synthesis in HD patients may be an index of increased mCD14 expression by these cells as a result of chronic exposure to small amounts of endotoxin. Indeed, endotoxin itself has been postulated to initiate atherogenesis by inducing endothelial cell activation and injury both directly via a sCD14-mediated pathway and indirectly through the release of PBMC-derived proinflammatory cytokines such as IL-1 and TNF-α, by its interaction with mCD14 as described below (37).

The cohort of patients involved in the present study were on HD with unsubstituted cellulose dialyzers. Several studies have shown elevated predialysis plasma levels of IL-1 and TNF-α in patients on chronic HD using unsubstituted cellulose membranes (1,9). Furthermore, HD with these “bioincompatible” unsubstituted cellulose membranes leads to a further rise in plasma levels of TNF-α (38,39). In contrast, dialysis with “biocompatible” membranes such as polyacrylonitrile (PAN) was not associated with a further rise in plasma levels of TNF-α (39). Indeed, in some studies, plasma levels of TNF-α declined during dialysis with PAN membranes (40). Also, a single pass through a cellulose dialyzer leads to activation of mRNA transcription for IL-1 and TNF-α in PBMC, whereas passage through a PAN, polysulfone, or polyamide dialyzer does not. Taken together, the preponderance of data suggests that greater cytokine synthesis and release occur upon exposure to cellulose dialyzers. Also, studies based on the United States Renal Data System data have found an increased relative risk of death in general and as a result of cardiovascular events in particular among patients who were dialyzed with unsubstituted cellulose dialyzers compared with synthetic dialyzers (41). The results of our study provide the link among unsubstituted cellulose dialyzers, increased cytokine synthesis, and increased risk of cardiovascular events among patients who are on chronic HD.

Cardiovascular events increased with LBP in the range of 9,000 to 12,000 pg/ml but then seemed to decrease. LBP, an acute phase reactant that binds to endotoxin via its lipid A moiety, is synthesized in hepatocytes as a single polypeptide and secreted as a 60-kD glycosylated protein. CD14 is a 55-kD glycosylphosphatidylinositol-anchored membrane protein (mCD14) of phagocytes, which is also found as a soluble serum protein (sCD14) that lacks a glycosylphosphatidylinositol anchor. Endotoxin activates phagocytes through a LBP-mCD14-dependent pathway, where endotoxin first interacts with LBP to form an endotoxin-LBP complex, and then binds to CD14 on the cell surface of CD14-bearing cells (37). Endotoxin-activated phagocytes secrete inflammatory cytokines such as TNF-α, IL-1, and IL-1Ra, through which endotoxin

![Figure 1. Marginal effects (adjusted for duration of dialysis) of the relationship between cardiovascular events and risk factors. The curves display the fitted functions from the Poisson model with vertical axis measured on the log scale. The vertical lines along the x axis show the distribution of the risk factor. (A) Age and cardiovascular events. (B) Endotoxin-stimulated interleukin-1 receptor antagonist (IL-1Ra) and cardiovascular events. (C) IgG-stimulated IL-1Ra and cardiovascular events. (D) Plasma lipopolysaccharide binding protein (LBP) and cardiovascular events.](image-url)
indirectly induces the activation and injury of endothelial cells (26). Endotoxin also initiates leukocyte adhesion to endothelial cells, oxidative modification of lipoproteins, and activation of the coagulation system (37). These pathophysiologic changes are also important in the initiation and development of atherosclerosis. High plasma LBP levels may, therefore, facilitate the activation of phagocytes through the endotoxin–mCD14 interaction, resulting in the increased synthesis of proinflammatory cytokines. Furthermore, the interaction of LBP with endotoxin facilitates the binding of endotoxin to sCD14, resulting in an endotoxin–sCD14 complex that binds to and activates endothelial cells directly. The apparent decrease in cardiac events with LBP levels above 12,000 pg/ml may be explained by the observation that while potentiating endotoxin-induced cell activation and injury, LBP and sCD14 also synergistically disaggregate and transfer endotoxin to plasma lipoproteins, leading to its inactivation (37).

Infections are a major cause of morbidity and mortality in patients with ESRD, raising the possibility that these patients are immunocompromised. Indeed, investigations of immune function among patients with ESRD have shown depression of cell-mediated immunity, lymphocyte abnormalities, and depression of phagocytosis and chemotaxis by granulocytes (42). Most studies of immune function have focused on lymphocyte function, despite that infections with pyogenic organisms are common. We observed a strong inverse relationship between IgG-stimulated IL-1Ra synthesis and infectious events among HD patients. Because IgG-stimulated IL-1Ra synthesis by PBMC is mediated by IgG–Fcγ receptor interaction, this observation suggests that decreased numbers or function of this receptor is associated with an increased risk of infections. Macrophage Fcγ receptors are important in host defense because they participate in the clearance of IgG-coated microorganisms. Ruiz and colleagues (42) evaluated macrophage Fcγ-receptor function in vivo and in vitro in ESRD patients who were on HD. They demonstrated decreased clearance of IgG-coated (sensitized) autologous red cells as well as impaired recognition of these cells in vitro by FcγRI on blood monocytes. Patients who had relatively more marked FcγRI dysfunction were also observed to experience increased episodes of severe infections during a 2-yr follow-up period. Taken together, impaired macrophage FcγR function, as reflected by diminished IgG-stimulated PBMC cytokine synthesis in our study, probably contributes to the observed immune dysfunction and high prevalence of infection among ESRD patients.

In summary, results from the present study indicate that stimulus-dependent variability in PBMC cytokine synthesis is a new and strong predictor of clinical outcomes among patients who have ESRD and are on HD. Furthermore, our observations provide new and interesting targets for therapeutic interventions. TNF-soluble receptors, which are proteolytic cleavage products of cell surface TNF receptors, have been shown specifically to block the cytotoxic and inflammatory actions of TNF in vitro and in vivo (43,44). The use of these and other
Host defense against infections could be enhanced by the upregulation of Fc receptors for IgG. Granulocyte colony-stimulating factor has been shown to enhance expression of FcyRI by neutrophils in vivo (45). Furthermore, granulocyte colony-stimulating factor treatment when used as adjuvant therapy for the treatment of severe foot infections in diabetic patients was associated with improved clinical outcome (46). The effect of these and other interventions designed to normalize the immunologic status of HD patients on clinical outcomes should form the focus of future studies in this vulnerable patient population. Meanwhile, our observations from this study should be confirmed in a larger and more diverse HD population, involving patients dialyzed with substituted cellulose and synthetic dialyzers, as well as dialyzers processed for reuse with germicides other than the combination of glutaraldehyde and bleach.

### Acknowledgments

This study was supported by National Institutes of Health Grant DK 45609 and the Baxter Extramural Program, McGaw Park, IL. The authors thank the staff of the hemodialysis unit at St. Elizabeth’s Hospital for their help in the conduct of this study.

### References


Access to UpToDate on-line is available for additional clinical information at http://www.jasn.org/