# Hemodialysis-Related Lymphomononuclear Release of Interleukin-12 in Patients with End-Stage Renal Disease

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*Abstract.* Interleukin-12 (IL-12) is a cytokine produced by peripheral blood mononuclear cells (PBMC) that causes interferon-γ (IFN-γ) production and enhancement of cell-mediated cytotoxicity. To clarify the role of hemodialysis biocompatibility on IL-12 production and uremic immunodeficiency, we have studied the IL-12 and IFN-γ release by PBMC harvested from 12 patients dialyzed with cuprophan membrane (CU), eight patients dialyzed with polymethylmethacrylate membrane (PMMA), and eight nondialyzed uremic patients (UR). Ten healthy subjects constituted the control group (CON). PBMC were cultured for 48 h with and without nonspecific mitogen stimulation. In unstimulated conditions, CU showed an IL-12 PBMC production higher than CON, UR, and PMMA (46.67 ± 30.13 *versus* 2.56 ± 1.38, 6.16 ± 7.09, and 4.62 ± 4.76 pg/ml, respectively; $P < 0.01$). IL-12 production was correlated with C3a concentration measured at the outlet of hemodialyzer after 15 min of dialysis ($r = 0.69$, $P < 0.01$). IL-12 release in CU remained unchanged under mitogen stimulation (44.34 ± 23.86 pg/ml) and was lower than in CON, UR, and PMMA (66.0 ± 12.41, 68.37 ± 25.78, and 67.75 ± 22.61 pg/ml, respectively; $P < 0.05$). IFN-γ production was similar, in unstimulated conditions, in all groups. Under stimulation, IFN-γ release was lower in CU (13.42 ± 12.04 IU/ml) than in CON, UR, and PMMA (51.84 ± 30.74, 32.16 ± 13.86, and 32.16 ± 13.86 IU/ml, respectively; $P < 0.01$). These results demonstrate that hemodialysis with CU induces monocyte activation with an enhanced release of IL-12. On the contrary, stimulated PBMC production of both IL-12 and IFN-γ is lower in these patients than in CON, UR, and PMMA. The altered release of these cytokines could play a role in cell-mediated immunodeficiency of the uremic patients dialyzed with CU.

Infections are responsible for approximately 20 to 36% of all deaths in patients on chronic maintenance hemodialysis (1,2). This percentage is increased after 6 yr of hemodialysis, being the prevalent cause of death in patients starting regular dialysis treatment (RDT) before 60 yr of age (3). Many comorbid conditions, such as malnutrition and repetitive venipuncture with breaking of the skin integrity, may increase the occurrence of infections. Other important factors, however, are linked to the immunodeficiency state of dialyzed patients in which poor biocompatibility of hemodialysis may play an important role (4). These factors include recurrent activation of complement with degranulation of neutrophils (5), attenuation of phagocytic function (6) and decreased granulocyte-endothelial adhesion (due to reduced expression of neutrophil adhesion molecules) (7), and defective monocyte functions with reduced monocyte-dependent release of interleukin-2 (IL-2) by T lymphocytes (8). Most of these defects may specifically impair cellular immunity of hemodialysis patients. An abnormally low IL-2 production under mitogen stimulation (9,10) with an increased expression and release, in the absence of any stimulation of IL-2 receptor (11,12), may attest to the contradictory coexistence of primarily preactivated T cells that present a functional deficiency state under mitogen challenge (13–15).

Zaoui and Hakim have found a significant decrease of natural killer cytolytic activity in patients dialyzed with cuprophan (CU) membranes (16). Natural killer (NK) cells comprise 10 to 15% of total blood lymphocytes and play an important role in the resistance to viral infections and destruction of autologous tumor cells (16–18).

NK cells constitute the major target of NK cell stimulatory factor or interleukin-12 (IL-12). IL-12 is a cytokine composed of two chains, a heavy chain, or p40, and a light chain, or p35, forming a disulfide-linked heterodimer, or p70 (19–21). IL-12 is produced by peripheral blood mononuclear cells (PBMC) (22). In particular, monocyte/macrophages, B cells, and phagocytic and antigen-presenting cells are the most important physiologic producers of IL-12 (23). In addition to causing proliferation and enhancement of cytotoxic activity of NK cells, IL-12 enhances the production of some cytokines, particularly interferon-γ (IFN-γ), by T cells (24–26). No data are available in the literature regarding *in vivo* production and effects of IL-12 in patients with end-stage renal failure.

This study was performed to clarify the role of hemodialysis biocompatibility and uremic immunodeficiency on IL-12 production. Specifically, the goals of our study were to: (*1*) investigate in IL-12 spontaneous release by PBMC harvested

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from uremic patients undergoing chronic dialysis with new CU membranes; (*2*) compare these results with those obtained in (*a*) healthy control subjects, (*b*) uremic nondialyzed patients, to exclude the potential influence of uremia *per se*, and (*c*) uremic patients dialyzed with a more biocompatible, synthetic membrane; (*3*) evaluate in all groups the effects of a nonspecific mitogen stimulation; and (*4*) evaluate in all groups the T cell IFN-$\gamma$ release under mitogen challenge. Complement activation during dialysis treatment with either CU or polymethylmethacrylate (PMMA) membrane was also investigated to ascertain a possible relationship with IL-12 PBMC release in the absence of any mitogenic stimulation.

## Materials and Methods

### *Patient Selection*

Twelve patients (7 males [M], 5 females [F]; mean age, 46 $\pm$ 14.8 yr) on RDT, regularly dialyzed three times a week for at least 1 yr before the study with new CU membranes (filters manufactured by Bellco, Italy; mean membrane surface: 1.3 mq; thickness: 8 $\mu$m; sterilization: ethylene oxide), were enrolled in the study. As control groups, we studied: (*1*) 10 healthy laboratory staff volunteers (6 M, 4 F; mean age, 33 $\pm$ 7.5) (CON); (*2*) eight patients (6 M, 2 F; mean age, 48.2 $\pm$ 6.4) with advanced chronic renal failure (GFR value, measured as the mean of creatinine and urea clearance: 9.8 $\pm$ 2.5 ml/min) (UR); and (*3*) eight patients (5 M, 3 F; mean age, 38.6 $\pm$ 6.2 yr) on RDT for at least 1 yr with a more biocompatible synthetic membrane (PMMA membrane; filters manufactured by Toray, Japan; model B3.1.3A; mean membrane surface: 1.3 mq; membrane thickness: 20 $\mu$m; sterilization: $\gamma$ ray). With both membranes, dialysate was filtered through hydrophobic membranes, and bicarbonate dry powder cartridges were adopted. In all dialyzed patients (either on CU or PMMA membrane), Kt/V urea, estimated according to the Daugirdas equation (27), was kept between 1.2 to 1.3 to avoid differences in small molecules (blood urea nitrogen and creatinine) concentrations. As suggested by Vanholder *et al.* (28), in fact, leukocyte function deteriorates progressively when serum concentrations of these small molecules increase. Protein catabolic rate was maintained in all patients between 1.0 to 1.3 g/kg per d and albumin concentration was $>$3.5 g/dl. Erythropoietin was given to 80% of patients; 90% of patients were taking vitamin D orally at the end of dialysis treatment. No patients had parathyroid hormone (intact) values $>$250 pg/ml.

No patient had clinical or laboratory evidence of infective, inflammatory, or autoimmune disease, and none was any taking drugs interfering with the immune response. No patient had diabetes mellitus or IgA nephropathy as underlying disease. All subjects gave informed consent before the study.

### *Cell Cultures*

All blood samples (15 ml) for PBMC cultures were collected in the morning in heparinized tubes. In dialyzed patients (both with CU and PMMA membranes), blood samples were drawn before the dialysis session. PBMC were harvested and set up in cultures as described previously (28,29). In brief, PBMC were isolated by a Ficoll-Hypaque (Flow Laboratories, Irvine, United Kingdom) gradient density centrifugation at 400 $\times$ *g* for 30 min. The interphase mononuclear layer was collected and washed twice with RPMI 1640 (Flow Laboratories) at 300 $\times$ *g* for 10 min. The cells were then resuspended in 15 ml of polypropylene round bottom tubes (Falcon; Becton Dickinson, Lincoln Park, NJ) at a concentration of 2 $\times$ $10^6$/ml in an Iscove's culture medium (Flow Laboratories) supplemented with 1% heat-inactivated fetal bovine serum (Sigma, Milan, Italy) and antibiotics (penicillin and streptomycin). The cell suspension contained approximately 95% PBMC with a mean value of 74% lymphocytes (range, 64 to 90%) and 10% monocytes (range, 6 to 15%); more than 98% PBMC were viable, as determined by trypan blue dye exclusion.

PBMC cultures were set up in either the absence or the presence of a stimulation with both 10 $\mu$g/ml lipopolysaccharide (LPS) of *Neisseria meningitidis* (Sigma) and phytohemagglutinin (PHA; Sigma). After 48 h of incubation at 37°C in a humidified atmosphere containing 5% $CO_2$, cell-free supernatants were collected by centrifugation (10 min at 400 $\times$ *g*) and stored at $-$20°C.

### *Cytokine Assays*

All supernatant samples from the different groups of healthy control subjects and patients were analyzed in duplicate at the same time.

**IL-12 Immunoassay.** This assay uses a quantitative sandwich enzyme immunoassay technique (R&D Systems, Minneapolis, MN). In brief, a monoclonal antibody, specific for IL-12, has been precoated onto a microplate. Standards and samples of supernatant from PBMC are pipetted into the wells, and any IL-12 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for IL-12 is added to the wells. After a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-12 bound in the initial step. After 20 min of incubation, the color development is stopped and the intensity of the color is measured. The assay range was 0 to 600 pg/ml; the lower detection limit was $<$3 pg/ml. The coefficient of variation of both interassay and intra-assay was $<$10%.

**IFN-$\gamma$.** Supernatants of both unstimulated and PHA + LPS-activated cultures were assayed for the presence of IFN-$\gamma$ according to the manufacturer's instructions. The test is based on a solid-phase enzyme-amplified sensitivity immunoassay on a microtiter plate. In brief, standards and supernatants containing IFN-$\gamma$ react with a monoclonal antibody coated on the microtiter well and with a monoclonal antibody labeled with horseradish peroxidase. After washing, to remove unbound enzyme-labeled antibodies, bound enzyme-labeled antibodies are measured through a chromogenic reaction. The minimum detectable concentration of IFN-$\gamma$ is estimated to be 0.03 IU/ml. The intra-assay coefficient of variation was $<$5%, whereas the interassay variation was $<$10%.

### *Assay for Complement Activation (C3a)*

C3a concentrations were evaluated in patients dialyzed with either CU or PMMA membranes by using an immunoassay kit from Quidel (San Diego, CA). Blood samples were drawn at 0, 15, and 240 min; at 15 min, blood samples were taken both before and after the hemodialyzer. An equal volume of Quidel stabilizing solution was added to each sample to preserve complement integrity. The kit includes microtiter strips coated with a monoclonal antibody specific for human C3a-desArg. Diluted samples were incubated for 1 h at room temperature. After incubation, a peroxidase-conjugated rabbit anti-C3a was added and the amount of C3a in the samples was quantified, after a washing step, in duplicate by using the peroxidase reaction and a standard curve. The absorbance was measured at 450 nm wavelength. The lower detection limit was $<$20 ng/ml, whereas the coefficient of variation of both interassay and intra-assay was $<$10%.

### *Statistical Analyses*

Statistical analyses were performed using the ANOVA and linear regression analysis. Data are expressed as mean ± SEM. Statistical significance was defined as $P < 0.05$.

## Results

### *IL-12 Production by Unstimulated PBMC*

As shown in Figure 1, the 12 uremic patients dialyzed with CU membrane showed a significantly ($P < 0.01$) higher value of IL-12 production by unstimulated PBMC (46.67 ± 8.70 pg/ml supernatant) compared with IL-12 release in healthy control subjects (2.56 ± 0.44 pg/ml), uremic nondialyzed patients (6.16 ± 2.51 pg/ml), and patients dialyzed with PMMA membranes (4.62 ± 1.68 pg/ml). No difference was observed in basal IL-12 release among CON, UR, and PMMA.

### *IL-12 Production by Stimulated PBMC*

Figure 1 depicts the IL-12 production by PBMC challenged by mitogen (LPS + PHA). When stimulated with mitogens, PBMC harvested from CU released a significantly lower amount of IL-12 (44.34 ± 6.88 pg/ml) than PBMC harvested from CON (66.0 ± 3.93 pg/ml, $P < 0.02$), from UR (68.37 ± 9.12 pg/ml, $P < 0.05$) or from PMMA (67.75 ± 7.99 pg/ml, $P < 0.05$). No difference was observed between unstimulated and stimulated IL-12 production in CU patients; on the contrary, a statistically significant difference (increase) was observed between the unstimulated (basal) and stimulated condition in the other groups (*i.e.*, CON, UR, and PMMA, $P < 0.01$ *versus* basal condition). No difference in IL-12 production was detected among CON, UR, and PMMA after mitogen stimulation.

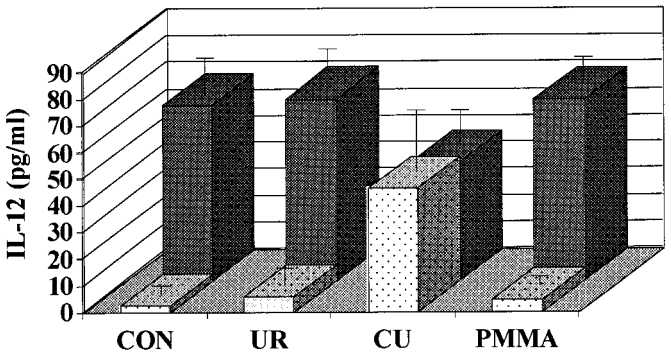


*Figure 1.* Interleukin-12 (IL-12) production after incubating both unstimulated (▨) and stimulated (■) peripheral blood mononuclear cells (PBMC) for 24 h. Results obtained from 10 healthy subjects (CON), eight uremic nondialyzed patients (UR), 12 patients dialyzed with cuprophan (CU) membrane, and eight patients dialyzed with polymethylmethacrylate (PMMA) membrane. In unstimulated PBMC, IL-12 production in CU is significantly greater than in CON, UR, and PMMA ($P < 0.01$). Under mitogen stimulation, IL-12 production is significantly lower in CU compared with CON ($P < 0.02$), UR, and PMMA ($P < 0.05$). In CON, UR, and PMMA, IL-12 release after mitogen stimulation is significantly greater than in basal condition ($P < 0.01$), which is not the case for CU.

### *IFN-γ Production by Unstimulated and Stimulated PBMC*

Figure 2 shows IFN-γ production by PBMC, in the absence and presence of mitogen stimulation, in all groups included in the study. No difference was observed in basal IFN-γ production among all groups (0.89 ± 0.13, 1.12 ± 0.23, 1.24 ± 0.31, and 0.71 ± 0.18 IU/ml in CON, UR, CU, and PMMA, respectively).

IFN-γ production by stimulated PBMC was significantly lower in the CU group (13.42 ± 3.47 IU) compared with the values found in other groups (51.84 ± 9.72, 32.16 ± 4.90, and 39.37 ± 4.40 in CON, UR, and PMMA, respectively; $P < 0.01$). In all groups, the stimulated production of IFN-γ was significantly higher ($P < 0.01$) than in the respective basal condition.

A statistically significant linear relationship was found, under mitogen stimulation, between PBMC IL-12 and IFN-γ productions ($r = 0.404$, $P < 0.01$).

### *Complement Activation*

Table 1 shows C3a (as C3a-desArg) plasma concentrations in both dialysis patient groups (CU and PMMA) before dialysis, after 15 min (both at the inlet and outlet of hemodialyzer), and at the end of the dialysis session. No difference was obtained in predialysis values of C3a between patients dialyzed with CU or PMMA membranes. C3a-desArg concentrations were significantly increased in all patients after 15 min of dialysis, both at inlet and outlet of hemodialyzer. CU patients showed a magnitude of increase, particularly at the outlet of dialyzer, that was greater than in PMMA patients. C3a postdialysis concentrations for PMMA patients were similar to the concentrations for predialysis ones; in contrast, cuprophan patients showed postdialysis values higher than predialysis. C3a-desArg concentrations at 15 min (samples drawn after dialyzer) were significantly correlated with PBMC IL-12 release values under unstimulated conditions ($r = 0.69$, $P < 0.01$).

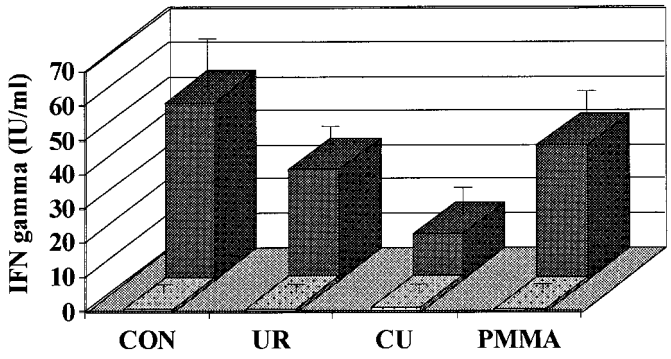


*Figure 2.* Interferon (IFN-γ) production after incubating both unstimulated (▨)and stimulated (■) PBMC for 24 h. Same numbers and abbreviations as in Figure 1. IFN-γ production by stimulated PBMC is significantly lower in CU than in CON, UR, and PMMA ($P < 0.01$). In all groups, the IFN-γ production, after stimulation, is significantly higher compared with respective basal condition ($P < 0.01$).

*Table 1.* C3a-desArg concentrations in 12 patients dialyzed with CU membranes and eight patients dialyzed with PMMA membranes[a]

| Time | C3a-desArg (ng/ml) | |
|---|---|---|
| | Cuprophan | PMMA |
| Before dialysis | 195.25 ± 28.64 | 176.0 ± 22.14 |
| After 15 min at inlet | 1702.92 ± 116.7[b] | 318 ± 51.0[c,d] |
| After 15 min at outlet | 4721 ± 437.0[e] | 644 ± 107.98[e,f] |
| After dialysis | 342.58 ± 37.27[g] | 245.5 ± 26.68[h] |

[a] Blood samples were drawn before, after 15 min (both at the inlet and outlet of hemodialyzer), and at the end of the dialysis session. Results are given as mean ± SEM. CU, cuprophan; PMMA, polymethylmethacrylate membrane.
[b] $P < 0.01$ *versus* before dialysis.
[c] $P < 0.05$ *versus* before dialysis.
[d] $P < 0.01$ *versus* cuprophan 15-min inlet.
[e] $P < 0.01$ *versus* before dialysis and 15-min inlet.
[f] $P < 0.01$ *versus* cuprophan 15-min outlet.
[g] $P < 0.01$ *versus* before dialysis, 15-min inlet and outlet.
[h] $P < 0.01$ *versus* 15-min outlet.

## Discussion

This study demonstrates for the first time that cultured PBMC harvested from uremic patients dialyzed with a CU membrane spontaneously release a greater amount of IL-12 than PBMC from healthy subjects or nondialyzed uremic patients. These findings demonstrate that blood interaction with new CU membranes *per se* may activate PBMC to release IL-12 and seem to exclude a pathogenic role of uremia on this activation. In nondialyzed uremic patients, in fact, a spontaneous PBMC activation was almost absent. In dialysis patients, these results were obtained on samples drawn before the dialytic session, suggesting, in agreement with our previous studies (29,30), that blood interaction with a CU membrane causes a prolonged and/or chronic PBMC activation. When examining IL-12 release from PBMC harvested from another group of patients regularly dialyzed with PMMA membrane, a less complement-activating synthetic membrane, we obtained results similar to those of control healthy subjects as well as of uremic nondialyzed patients. These findings confirm our hypothesis that spontaneous release of IL-12 relates to PBMC activation induced by the CU membrane. The exposure to a more biocompatible dialysis membrane, in fact, normalized IL-12 release by PBMC cultured in the absence of mitogen challenge. The changes of C3a concentration confirm previous results on the different ability of CU and PMMA membranes to recurrently activate complement (31–33). The statistically significant correlation observed between 15 min of C3a concentration and IL-12 spontaneous release suggests that the complement cascade may play a pathogenic role in inducing IL-12 synthesis and release by unstimulated PBMC.

We have also evaluated IL-12 release under mitogen stimulation. IL-12 supernatant levels of PHA + LPS-activated cultures were significantly lower in patients dialyzed with CU membranes, compared with either control healthy subjects or uremic nondialyzed patients. More important, in the patients dialyzed with CU membrane, IL-12 supernatant levels after mitogen stimulation were not different from the values obtained in unstimulated conditions. These findings confirm previous studies regarding release of other cytokines (29,30,34), and suggest a reduction in the ability of PBMC challenged with mitogen to release adequate amounts of this immune-related cytokine in patients dialyzed with CU membranes. Conversely, the chronic use of a more biocompatible, less complement-activating membrane may reverse this serious consequence of poor dialytic biocompatibility. Zaoui and Hakim (34) have suggested several hypotheses to explain this phenomenon, such as a downregulation of these recurrently activated cells to synthesize and release cytokines, or an upregulation to synthesize and release antagonist binding proteins (or soluble receptors) that may bind the cytokines leading to their unavailability.

IL-12 is a cytokine produced very early during infections or immune response that exerts important proinflammatory functions and promotes enhancement of innate resistance by activating both NK and T-helper cells (35–40).

T-helper cells may be differentiated, according to the type of cytokines released, in T-helper type 1 (Th1) and T-helper type 2 (Th2) cells. Th1 cells produce IL-2 and IFN-γ (35–40); Th2 cells produce IL-4, IL-5, and IL-10 (37,38). IL-12 promotes the differentiation of cytokine-producing Th1 cells primarily through its ability to stimulate them for high IFN-γ production. Indeed, IL-12 is a potent acute inducer of the IFN-γ gene expression in both T cells and NK cells (37). In turn, IL-12 synthesis is synergistically downregulated by IL-4 and IL-10 (41,42), which are also potent inhibitors of the production of the proinflammatory cytokines IL-1 and tumor necrosis factor, from lipopolysaccharide-activated PBMC (41). Th1 cell differentiation is associated with cell-mediated immunity and affords protection against intracellular infections. In contrast, the Th2 cells elicit high titer antibody responses, provide poor cell-mediated immunity, and are often correlated with susceptibility to infection. In healthy subjects, the IL-12 produced during the inflammatory phase induces differentiation of Th1 cells while inhibiting the generation of Th2 cells (42,43).

An immunodeficiency state consequent to this cell-mediated immune abnormality has been demonstrated by Kemp *et al.* (44). These authors, in their murine model (infected with *Leishmania*), observed that activation of the IFN-γ-producing Th1 subset resulted in cure and survival, whereas activation of the IL-4-secreting Th2 subset resulted in a progressive disease with fatal outcome. A Th1 to Th2 switch is also proposed as a critical step in the etiology of HIV infection (45).

The role of IL-12 in stimulating IFN-γ release is confirmed, in our study, by the significant correlation between PBMC IL-12 and IFN-γ release. A reduced IL-12 release, following mitogenic stimulation, in uremic patients dialyzed with CU membranes might induce a shift toward a Th2 cell response, contributing to a depressed cell-mediated immune response. This hypothesis is suggested by the lower PBMC IFN-γ production in these patients under mitogen stimulation. Probably the chronic IL-12 release by unstimulated PBMC may induce a chronic stimulation of IL-12 target cells with a downregula-

tion in eliciting IL-12-induced immunologic effects in terms of T cell proliferation and IFN-$\gamma$ production. A downregulation of T cells, chronically activated by IL-12, may be consistent with a downregulation of their IL-12 membrane surface receptors. IL-12 cell receptor (IL-12R) is composed of two proteins designated as IL-12R$\beta$1 and IL-12R$\beta$2. These two proteins individually may bind IL-12 with low affinity while in combination bind IL-12 with high affinity and confer IL-12 responsiveness (46–48). This is consistent with the observations of Zaoui *et al.* (11) on IL-2 receptor (IL-2R). These authors, in fact, have observed that the CU membrane may have an immunomodulatory effect by inducing a persistent upregulation of low-affinity IL-2R subunits on T lymphocytes; this may impair the expression of high-affinity IL-2R and decrease the response to further antigenic or mitogenic stimuli. The switch to PMMA membranes reversed these abnormalities (11).

IFN-$\gamma$, a cytokine released by Th1 and NK cells (49), plays a complex and central role in the resistance to infective agents. The properties of IFN-$\gamma$ include regulation of several aspects of the immune response, such as stimulation of antigen presentation through class I and class II MHC molecules, orchestration of leukocyte-endothelium interactions, and effects on cell proliferation and apoptosis (49–51). The lower IFN-$\gamma$ release, under mitogen stimulation, observed in uremic patients dialyzed with CU membrane in contrast with the values found in patients dialyzed with a more biocompatible membrane may suggest a role of poor dialysis biocompatibility on this response. Indeed, a decrease in IFN-$\gamma$ mRNA inducibility by hemodialysis has been reported by Gerez *et al.* (52), although they did not specify the type of membrane of their hemodialyzer.

As suggested by Descamps-Latscha and Chatenoud (15), immune abnormalities observed in uremic patients might be attributed to a dual reaction of the immunocompetent cells that are activated in their basal state but deficient upon stimulation. Our results regarding IL-12 are in agreement with this hypothesis. The reduction of IFN-$\gamma$ production under mitogen stimulation may be related to the weak, continuous baseline stimulation of T cells by IL-12. This phenomenon may be compatible with a downregulation in IFN-$\gamma$ synthesis and release, as suggested by Gerez *et al.* (52). Similarly, a reduced IL-12 production under stimulation may account for the reduced natural killer cytolytic activity observed by Zaoui and Hakim (16) in patients dialyzed with CU membranes, and elucidate their susceptibility to infection and neoplastic disease (2,53). Additional studies on the role of T-helper cell subpopulations (Th1 and Th2) are required to better define the mechanisms underlying the immune abnormalities of uremic dialyzed patients.

In conclusion, this study demonstrates a spontaneous high production of IL-12 by PBMC harvested from uremic patients regularly dialyzed with single-use CU membranes. The release, as suggested by C3a behavior, is probably secondary to monocyte activation induced by the poor biocompatibility of the membrane since it is absent in uremic nondialyzed patients and in uremic patients dialyzed with less complement-activating membrane. Importantly, the enhanced generation of IL-12 under unstimulated state is associated with the absence of response to mitogenic stimulation, which in turn results in a reduced IFN-$\gamma$ production. It is therefore suggested that the altered release of these cytokines may play a role in cell-mediated immunodeficiency of chronic uremic patients dialyzed with CU membranes and in their susceptibility to infection and neoplastic disease.

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