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Interleukin-1β, -6 and Interferon-γ Productions in Patients Undergoing Continuous Ambulatory Peritoneal Dialysis

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In order to investigate cytokine productions in patients undergoing continuous ambulatory peritoneal dialysis (CAPD), we studied the production of interleukin (IL)-1β, -6, and interferon (IFN)-γ by cultured peripheral blood mononuclear cells (PBMC) in peritonitis-free CAPD patients. The correlation of cytokine production with plasma parathyroid hormone (PTH) and albumin levels was also evaluated. While the release of IL-1β was not markedly different from controls, the release of IL-6 from 24-hour cultured PBMCs was significantly greater than that of controls (Mean±S.D., IL-6: 2186.8±1217.9 pg/ml, vs 1516.3±767.9, $p<0.05$). The addition of lipopolysaccahride (LPS, 10 μg/ml) significantly stimulated IL-1β and -6 production of PBMCs in CAPD patients and controls, compared to an unstimulated condition. The LPS-induced IL-1β production was also not markedly different from controls, whereas LPS-induced IL-6 production was significantly higher than controls (IL-6: 13220.7±7177.4 vs 7411.4±1236.9, $p<0.05$). However, the percentage increases of IL-6 production stimulated with LPS in CAPD patients were not significantly different from controls ($p>0.05$). No difference of baseline IFN-γ was detected between CAPD patients and controls, but phytohemagglutinin (PHA, 10 μg/ml)-stimulated IFN-γ release was significantly higher in CAPD patients than controls (2425.9±1565.0 pg/ml vs 1364.0±755.1, $p<0.05$). There was no significant correlation between PTH and, IL-1β, -6, or IFN-γ production. On the other hand, a significant correlation was observed between the serum albumin level and LPS-stimulated IL-6 production ($r=0.54$, $p<0.05$). In conclusion, CAPD seems to partly induce activation of PBMCs with an enhanced release of IL-6 and IFN-γ, and CAPD patients with higher serum albumin levels tend to show higher IL-6 production in immune response.

Key Words: Interleukin-1β, interleukin-6, interferon-γ, CAPD

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The reduction in immune response in uremic patients has been well documented (Raskova and Morrison, 1976; Angflini *et al.* 1994). Explanations for the immunosuppression, the intrinsic defect of uremic T lymphocytes, the abnormal immunoregulation mediated by T lymphocytes or monocytes, and the soluble serum factors have been suggested (Raskova and Morrison, 1976; Raskova and Raska, 1983), but the mechanism underlying the immune defect in uremic patients being treated by dialytic therapy has

not been clarified. A crucial element in immune response is the elaboration of the cytokine network by monocytes, macrophages and activated T lymphocytes (Topley, 1995). The production of cytokines by peripheral blood mononuclear cells (PBMC) seems to be affected by long-term exposure to the continuous ambulatory peritoneal dialysis (CAPD) environment.

Recent studies have indicated that the levels of cytokines such as Interleukin (IL)-1 are indices of biocompatibility in dialytic treatment (Haeffner-Cavaillon *et al.* 1989; Zaoui and Hakim, 1994). Haeffner-Cavaillon *et al.* reported that the production of IL-1 by PBMCs was enhanced in hemodialysis (HD) patients as a consequence of blood/membrane interactions (Haeffner-Cavaillon *et al.* 1989). IL-1 is a mediator of inflammatory reaction inducing symptoms and biochemical changes observed during acute-phase reaction (Pereira *et al.* 1994). IL-6 acts as a mediator of lymphocyte function and is also a major regulator of acute phase protein gene expression and synthesis of albumin in hepatocytes (Ramadori *et al.* 1988). These cytokines appear to be intricately involved in β_2-microglobulin synthesis and osteoclastic bone resorption in uremic patients (Langub *et al.* 1996; Libetta *et al.* 1996). Interferon (IFN)-γ exerts activity on macrophages to induce inhibition of bacterial growth, and phagocytosis as a defense mechanism for bacterial peritonitis in CAPD patients (Ijzermans and Marquet, 1989).

It has been reported that parathyroid hormone (PTH), which was usually high in uremic patients, acts on immune cells and that lymphocytes present PTH-specific receptors (Angflini *et al.* 1994). The alterations of cytokine production according to the level of PTH is suspected. And the serum albumin level is an important nutritional index for CAPD patients (Avram *et al.* 1994). Cytokines such as IL-6 seem to have an influence on albumin synthesis in these patients (Ramadori *et al.* 1988). Therefore, in order to investigate the long-term effect of peritoneal dialysis on the release of cytokines, we studied the production of IL-1β, -6 and IFN-γ by PBMCs in CAPD patients. We also investigated the correlation between these cytokines and the level of PTH, as well as albumin.

MATERIALS AND METHODS

We studied 20 patients on CAPD and 10 healthy renal allograft donors as controls. Patients were treated daily with four exchanges of 2 liters of dialysate (1.5, 4.25% glucose, Baxter, Singapore). None of the patients had clinical evidence of acute infection or autoimmune disease, nor were they taking any drugs interfering with their immune response. No patients had diabetes mellitus and they were all peritonitis-free during the last six months prior to study.

Cell cultures

PBMCs were isolated and set up as primary in vitro cultures as previously described (Libetta *et al.* 1996). Briefly, in order to obtain PBMCs from heparinized blood samples, we used Ficoll-Hypaque (Flow Laboratories, Irvine, Scotland, UK) gradient density centrifugation at $400 \times g$ for 30 minutes at room temperature. The interface layer was washed at $300 \times g$ for 10 minutes with RPMI 1640. The cells were then resuspended in 15 ml polypropylene round-bottom tubes at a concentration of 1×10^6/ml in RPMI 1640 media supplemented with 1% heat-inactivated fetal bovine serum (Sigma St. Louis, MO. USA) and antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). PBMCs were cultured in either the absence or presence of 10 μg/ml of lipopolysaccharide (LPS; Sigma, St. Louis, MO. USA) of E. coli or phytohemagglutinin (PHA; Sigma, St. Louis, MO. USA). The dosage of LPS (10 μg/ml) or PHA (10 μg/ml) was chosen on the basis of preliminary work in our laboratory and previous studies by other groups that demonstrated a maximal release of IL-6 under these conditions (Libetta *et al.* 1996). After 24 hours of incubation with LPS at 37°C in a humidified atmosphere containing 5% CO_2, cell-free supernatants were collected by centrifugation for 10 minutes at 400 g, passed through a millipore filter (0.2 μm), and stored at −20°C. Cell viability was determined by Trypan blue exclusion test and yielded approximately 95% viable cells. PBMCs were stimulated with PHA for 72 hours.

IL-1β, -6 and IFN-γ assay

IL-1β, -6 and IFN-γ were measured in super-

natants from cultured PBMC by a sandwich ELISA (Quantikine, R & D laboratories, Minneapolis, MN, USA) in which a monoclonal antibody to human IL-1β, -6 and IFN-γ was used, a lyophilized horseradish peroxide conjugated goat anti-mouse which binds to the monoclonal portion of the sandwich. The lower detection limit was 3.9 pg/ml for IL-1β or IL-6, and 15.6 pg/ml for IFN-γ. The variation coefficient for intra-assay was 5% and for inter-assay it was 9%.

PTH radioimmunoasay

PTH was measured as intact PTH by a two-site immunoradiometric assay, using a kit (Nichols Institute Diagnostics, San Juan Capistrano, CA., USA), with a sensitivity of 1 pg/ml. Two goat polyclonal antibodies to the C- and N-terminals of human PTH were used. Intact PTH was bound by both immobilized and labeled antibodies to form a sandwich complex. At the end of the assay incubation, the bead was washed to remove unbound components and the radioactivity bound in the solid phase was measured in a γ-counter. The number of lymphocytes was analyzed using NE-8000 (Sysmex, Kobe, Japan), and the serum albumin level was measured by Hitachi 747 (Hitachi, Hitachinaka, Japan).

Statistical analysis

Statistical analyses were performed using the student t-test, Spearman correlation coefficient, and regression analysis. Data are expressed as mean$\pm$S.D. Statistical significance was defined as $p<0.05$.

Table 1. Clinical characteristics of patients undergoing continuous ambulatory peritoneal dialysis

	Controls	Patients
Number	10	20
Age (years)	39.2$\pm$8.5	41.8$\pm$12.4
M/F	2 : 1	1 : 1.5
Duration of CAPD[1] (months)	–	34.1$\pm$16.9
Lymphocytes (/mm^3)	1453.7$\pm$615.4	1633.5$\pm$471.2
PTH[2] (pg/ml)	–	252.9$\pm$189.8
Creatinine (mg/dl)	0.9$\pm$0.3	12.5$\pm$2.7*
Albumin (g/dl)	4.1$\pm$0.4	3.8$\pm$0.3

1: continuous ambulatory peritoneal dialsysis, 2: parathyroid hormone, *: $p<0.05$, vs control

RESULTS

Patients' characteristics

The main characteristics of patients are shown in Table 1. The mean duration of CAPD was 34.1 months and mean age was 41.8 years. The relative number of PBMCs was similar between CAPD patients and controls, being, on average, 32% of the white cells. The underlying causes for end-stage renal disease (ESRD) were chronic glomerulonephritis, hypertension, and unknown, in that order.

IL-1β, -6, and IFN-γ production

There was no significant difference of IL-1β release from unstimulated PBMCs between CAPD patients and controls (1226.3$\pm$361.9 pg/ml vs 1133.1 $\pm$214.7, $p>0.05$, Table 2). Following stimulation with LPS, IL-1β production was significantly increased up to 2192.5$\pm$891.7 pg/ml and 1967.8$\pm$

Table 2. Interleukin-1β, -6 and Interferon-γ production in peripheral blood mononuclear cells harvested from control subjects and patients undergoing CAPD

	Controls	Patients
Interleukin-1β(pg/ml)		
Unstimulated	1133.1$\pm$214.7	1226.3$\pm$361.9
LPS	1967.8$\pm$290.4*	2192.5$\pm$891.7*
% stimulation	78.4$\pm$57.7	82.8$\pm$25.7
Interleukin-6(pg/ml)		
Unstimulated	1516.3$\pm$767.9	2186.8$\pm$1217.9#
LPS	7411.4$\pm$1236.9*	13220.7$\pm$7177.4*#
% stimulation	637.7$\pm$396.8	584.0$\pm$419.5
Interferon-γ(pg/ml)		
Unstimulated	11.0$\pm$5.1	9.5$\pm$1.6
PHA	1364.0$\pm$755.1*	2425.9$\pm$1565.0*#

Mean$\pm$S.D.
LPS: lipopolysaccharides, 10 μg/ml, PHA: phytohemagglutinin, 10 μg/ml, *: $p<0.05$, vs unstimulated, #: $p<0.05$, vs controls

$$\% \text{ stimulation} = \frac{\text{LPS-stimulated release} - \text{unstimulated release}}{\text{unstimulated release}} \times 100$$

290.4, respectively ($p<0.05$, Table 2). The extent of stimulation was similar between CAPD patients and controls (Table 2).

On IL-6 production, the release from unstimulated PBMCs in CAPD patients was significantly higher than controls (2186.8±1217.9 pg/ml vs 1516.3±767.9, $p<0.05$, Table 2, Fig. 1). After stimulation with LPS, IL-6 production was markedly increased in controls and CAPD patients, and the LPS-stimulated IL-6 production in CAPD patients was much greater than in controls (13220.7±7177.4 pg/ml vs 7411.4±1236.9, $p<0.05$, Table 2, Fig. 2). But no significant difference was observed in the percentage increase of LPS-stimulated IL-6 production between CAPD patients and controls (Table 2).

The release of IFN-γ from unstimulated PBMCs in CAPD patients was similar to controls (11.0±5.1 pg/ml vs 9.5±1.6, $p>0.05$, Table 2). However, as depicted in Fig. 3, IFN-γ production following PHA stimulation was significantly higher in CAPD patients than the value measured in controls (2425.9±1565.0 pg/ml vs 1364.0±755.1, $p<0.05$, Table 2).

Correlations of cytokine production with serum PTH and albumin concentrations in CAPD patients

There was no significant correlation between IL-1β production from unstimulated or LPS-stimulated

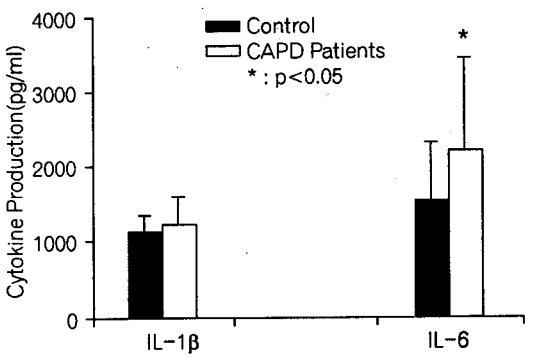

Fig. 1. Comparison of IL-1β and IL-6 production in 24-hour cultured PBMCs harvested from CAPD patients. IL-6 production is significantly greater than controls ($p<0.05$).

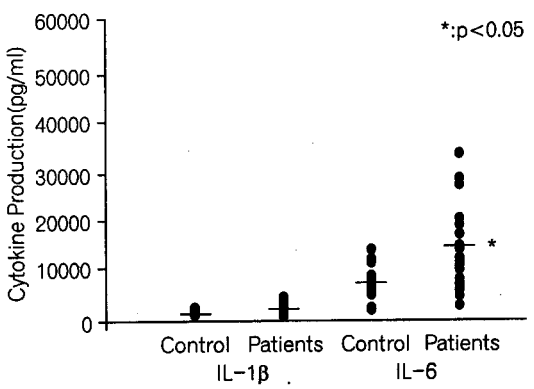

Fig. 2. Comparison of LPS-stimulated IL-1β and -6 production in 24-hour cultured PBMCs harvested from CAPD patients. IL-6 production is significantly greater than controls ($p<0.05$).

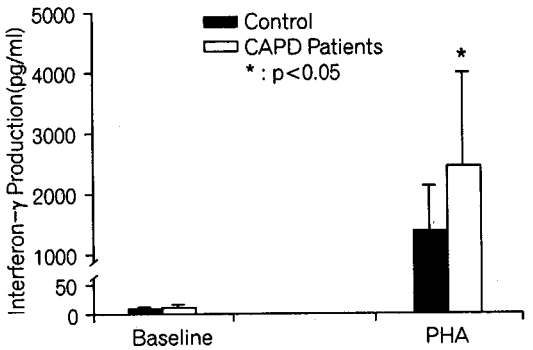

Fig. 3. Comparison of IFN-γ production in 24-hour cultured PBMCs harvested from CAPD patients. PHA-stimulated IFN-γ production is significantly greater than controls ($p<0.05$).

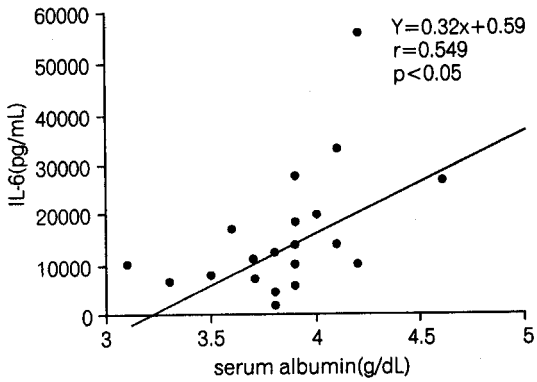

Fig. 4. Correlation between the IL-6 production stimulated by LPS and serum albumin concentrations in CAPD patients. There was a significant correlation between the LPS-stimulated IL-6 production and serum albumin levels.

PBMCs, and PTH or albumin concentrations, respectively. There was also no significant correlation between the baseline release of IL-6 and PTH or albumin concentrations. However, while the correlation between LPS-stimulated IL-6 and PTH was not significant, a significant correlation was obtained between LPS-stimulated IL-6 production and albumin concentrations ($r=0.54$, $p<0.05$, Fig. 4). There was no significant correlation between baseline or PHA-stimulated interferon-γ production and PTH or serum albumin levels, respectively.

DISCUSSION

CAPD has been considered to be more biocompatible than HD. This assumption is essentially based on the absence of the main factors underlying the poor biocompatibility of extracorporeal circulation, such as blood interaction with artificial membranes and the back-filtration/diffusion of endoxotoxin fragments from dialysate (Laude-Sharp *et al.* 1990). Nevertheless, in the past few years, different studies in CAPD patients have demonstrated the presence of chronic sterile inflammation at the level of the peritoneum (Bos *et al.* 1991). Recent studies have shown significant intraperitoneal levels of IL-6 generated by peritoneal macrophages in the absence of peritonitis (Goldman *et al.* 1990). Overall, these data suggest local inflammatory effects strictly dependent on CAPD treatment per se. This study evaluated the changes of IL-1β, -6 and IFN-γ production in a CAPD environment.

In an unstimulated condition, the release of IL-1β by PBMCs from CAPD patients was not significantly different from healthy controls. However, the release of IL-6 in CAPD patients was much higher than controls. This finding suggests that the induction of IL-1β production associated with complement activation or transmembrane passage of bacterial LPS in HD does not occur in CAPD, whereas the production of IL-6 is induced in CAPD. This observation partly supports the hypothesis of a chronic monocyte activation in CAPD patients made by Zaoui and Hakim (1994).

The pathophysiological mechanisms underlying the activation of systemic monocytes reflecting IL-6 production in CAPD patients are not readily apparent. Libetta *et al.* have reported that the release of IL-6 was similarly low in uremic non-dialyzed patients and healthy controls (Libetta *et al.* 1996). From this finding, chronic activation of peripheral monocytes seems to be at least partially attributable to the enhanced cell generation induced by the dialytic treatment per se rather than the retention of uremic solutes. Recent in vitro studies have demonstrated that diethylexylphalate, a "plasticizer" released by storage bags, induces cytokine release from PBMCs (Fracasso *et al.* 1993). Moreover, Dinarello and Krueger have reported the presence in peritoneal dialysis effluent of natural muramyl dipeptides derived from the bacterial cell wall that are capable of activating mononuclear cells with a 10-fold greater potency than endotoxins (Dinarello and Krueger, 1986). It is therefore conceivable to hypothesize that these cells may have been stimulated upon contact with some specific substances in the peritoneum. Another factor potentially contributing to the induction of systemic monocytes is possibly represented by the complement activation which has been reported in peritonitis-free CAPD patients (Young *et al.* 1993). However, considering that IL-1β production is not changed, further studies on other cytokine production are necessary in order to confirm the systemic activation of peripheral monocytes in CAPD patients.

As for IL-6 release in CAPD patients, the increased PBMC production of either IL-6 or β_2 microglobulin was paralleled by a striking increment of serum amyloid A levels, which is the cause of dialysis-induced amyloidosis (Memoli *et al.* 1992; Libetta *et al.* 1996). IL-6 is also considered as the mediator of bone resorption in renal osteodystrophy (Langub *et al.* 1996). So, according to the longer duration of PD, the possibility of amyloidosis and renal osteodystrophy occurring can be increased. A number of biological activities mediated by IL-6 could account for the acute phase responses and some of the adverse effects of HD (Gauldie *et al.* 1990; Memoli *et al.* 1992). IL-6 may play a relevant role in the pathophysiology of biocompatibility in CAPD treatment.

After stimulation with LPS, the amount of IL-1β or -6 released by PBMCs from CAPD patients was comparable to that detected in PBMCs drawn from

healthy controls. Libetta *et al.* observed the reduced response of IL-6 to LPS in CAPD patients, which was possibly dependent on a down-regulation of IL-6 production due to the chronic stimulation of PBMCs (Libetta *et al.* 1996). Compared to the study by Libetta *et al.* our study included more patients with a younger mean age and longer duration of dialysis (Libetta *et al.* 1996). Based on the present study, despite the activation of circulating monocytes with increased production of IL-6, the production of IL-1β and -6 under stimulated conditions seems not to be affected in a CAPD environment.

Angflini *et al.* reported that the elevated levels of PTH affect the lymphocyte function reflected by a reduction in the number of T cells and increases in the levels of IL-2 receptor (Angflini *et al.* 1994). Moreover, a recent report presents evidence that T-cell activation was modified by PTH in uremic patients (Kaneko *et al.* 1997). In this study, we could not observe a significant correlation between PTH levels and IL-1β or -6 production. Based on this result, hyperparathyroidism appears not to have an influence on IL-1β or -6 production of PBMCs in CAPD patients.

Serum albumin level has been well known as a nutritional index and is also considered a major predictive index for mortality and morbidity in dialysis patients (Avram *et al.* 1994). Cytokines including IL-6 may have an inhibitory effect on albumin synthesis on hepatocytes (Ramadori *et al.* 1988). However, in our study, the serum albumin level was significantly correlated only with LPS-induced IL-6 production, suggesting that a CAPD patient with higher levels of albumin has higher IL-6 production when exposed to LPS. It's likely that maintaining the serum albumin level in a relatively high range seems to be an important factor for better IL-6 response in CAPD patients.

IFN-γ is produced by T-helper type 1 cells and natural killer cells (Langub *et al.* 1996). IFN-γ is considered a primary factor required in vivo for the induction of microbial activity of macropahges (Langub *et al.* 1996). In this study, the baseline release of IFN-γ was not significantly different between CAPD patients and controls, whereas CAPD patients showed greater production of IFN-γ to PHA stimulation than controls. This finding seems to provide evidence suggesting the potentiation of IFN-γ production under the CAPD condition. Our study appears to be the first evidence of the potentiation of IFN-γ production by PBMCs in CAPD patients. The possibilities of potentiation by chronic continuous exposure to peritoneal dialysate or subclinical infection during dialysate exchange may be speculated upon, but further studies should be required.

In conclusion, the present study demonstrates that CAPD patients free of peritonitis show an enhancement of IL-6 and an IFN-γrelease from PBMCs, suggestive of partial activation of monocytes in the CAPD environment.

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