Artificial Organs
22(2):140–150, Blackwell Science, Inc.

Thoughts and Progress

It is the goal of this section to publish material that provides information regarding specific issues, aspects of artificial organ application, approach, philosophy, suggestions, and/or thoughts for the future.

5-Lipoxygenase Gene Expression in Hemodialysis

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Abstract: Leukotrienes (LTs), the end products of the eicosanoid pathway released during inflammation, are markers of polymorphonuclear cell and monocyte activation. The present study focused on the possibility that 5-lipoxygenase (5-LO), the key enzyme for LT synthesis, was involved in the interaction between blood and the hemodialysis (HD) membrane. 5-LO gene expression was examined by reverse transcriptase polymerase chain reaction (RT-PCR) in samples of mononuclear cells isolated from peripheral blood withdrawn at the start and at 15 min of HD from 10 chronic HD patients, 5 treated with Cuprophan and 5 with polymethylmethacrylate (PMMA) membrane. An increased 5-LO gene expression was detected at 15 min in 4 of 5 patients using the Cuprophan membrane but in none of the 5 PMMA treated patients. Our results showed for the first time that the interaction between blood and the HD membrane upregulates 5-LO messenger ribonucleic acid (mRNA). **Key Words:** 5-lipoxygenase—Leukotrienes—Biocompatibility—Dialysis membranes—Reverse transcriptase polymerase chain reaction.

Leukotrienes (LTs) of the 5-lipoxygenase (5-LO) pathway constitute a class of potent biological lipid mediators released during inflammation and are markers of polymorphonuclear cell and monocyte activation. The key enzyme, 5-LO, catalyzes the oxygenation of arachidonic acid to generate leukotriene (LT)A_4 (LTA_4). This LTA_4 can, in turn, be converted by different enzymes (LTA_4 hydrolase and LTC_4 synthetase) either to LTB_4, which has a strong chemoattractant action, or to LTC_4, which is a powerful bronchoconstrictor. Moreover, leukotrienes affect processes involved in pulmonary margination of selective leukocyte subsets and increase permeability at the postcapillary venule and diapedesis (1).

Dialysis membranes, especially those derived from cellulose, activate the complement cascade (C3a, C5a, and C5b-9) and stimulate leukocytes to produce several inflammatory agents (2–4), including leukotrienes (5–8). While the regulation of 5-LO enzymatic activity has been clarified (10), the mechanisms involved in gene expression upregulation are still incompletely known. The present study focused on the 5-LO gene expression in peripheral blood leukocytes collected from hemodialysis (HD) patients treated with synthetic and cellulose-derived membranes at the time of maximal leukocyte activation.

Materials and methods

The 5-LO gene expression was analyzed in 10 chronical patients (6 men and 4 women, 63.3 years old, range 45–81 years) undergoing 4 h of maintenance bicarbonate HD 3 times a week either with Cuprophan or polymethylmethacrylate (PMMA) membranes. The patients' age was not different in the Cuprophan group (3 men and 2 women) compared to the PMMA group. Clinically detectable infections in the last 4 weeks were excluded with certainty in these patients. The etiology of renal failure was nephroangiosclerosis in 7 cases (4 treated with Cuprophan) and chronic glomerulonephritis in 3. No patients were given steroids, antinflammatory drugs, or angiotensin converting enzyme (ACE) inhibitors.

Five patients were tested with Cuprophan membranes (Spiraflo NT18-08; 1.72 m^2; thickness, 8 μm; and ultrafiltration rate (UFR), 8.6 ml/h; BELLCO, Mirandola, Italy) and 5 with PMMA membranes (Filtryzer B2 1.5H; 1.5 m^2; thickness, 20 μm; and UFR 7.5 ml/h/mm Hg; Toray Industries, Inc., Chuo-ku, Tokyo, Japan).

Mononuclear cells (1–5 × 10^6) were separated from 10 ml of heparinized peripheral blood collected at the start (T0) and at 15 min (T15) of dialysis and at the beginning of the subsequent HD session. The

Received August 1997; revised October 1997.
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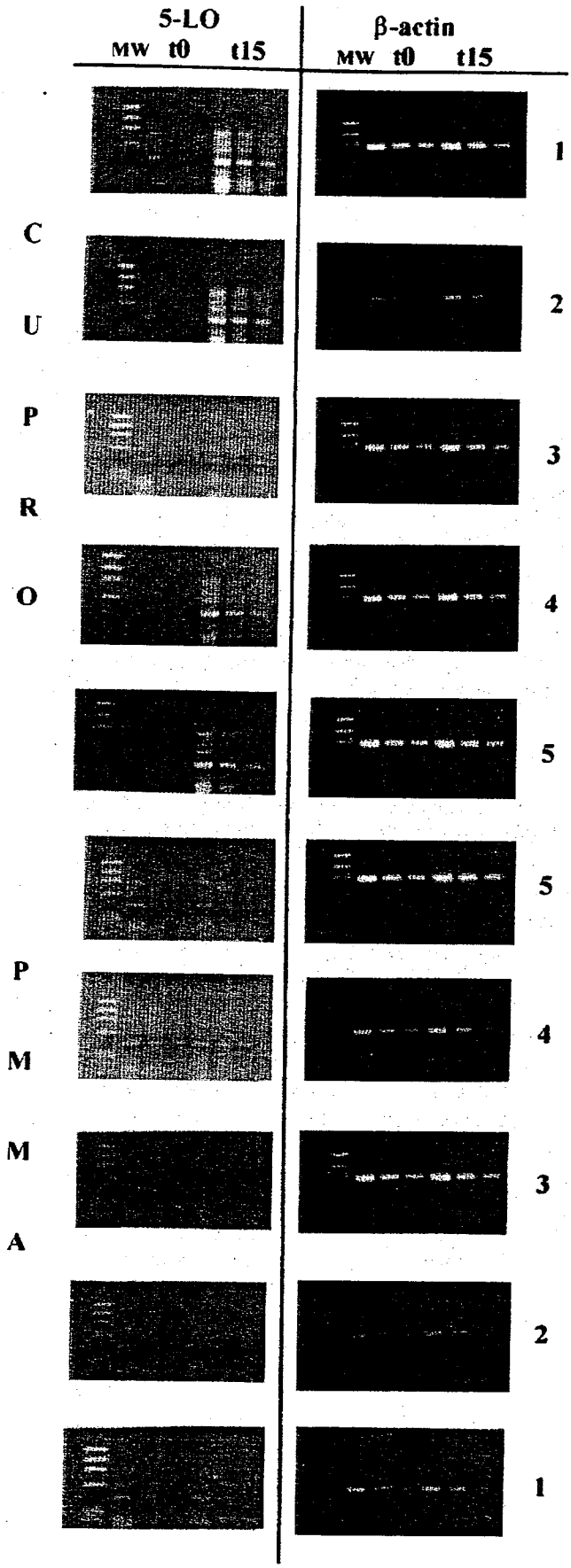

separation was performed by standard density centrifugation on the Ficoll-Paque PLUS (Pharmacia Biotech AB, Uppsala, Sweden). Total RNA was purified using RNAzol reagent (Biotecx Laboratories, Inc., Houston, Texas, U.S.A.), and 2 μg was reverse transcribed into complementary DNA (cDNA). Serial dilutions of cDNA were amplified with specific oligomers for 5-LO (5′-ATC AGG ACG TTC ACG GCC GAG G-3′; 5′-CCA GGA ACA GCT CGT TTT CCT G-3′) for 30 cycles (94°C, 45 s; 61°C, 45 s; and 72°C, 1 min).

A quantitative evaluation of polymerase chain reaction (PCR) targets was performed using the limiting dilutions technique (9) and referring to β-actin as a noninducible gene. Each PCR band, obtained from agarose gel electrophoresis and ethidium bromide staining, was then analyzed by a laser densitometer (LKB 2022 ULTROSCAN, Pharmacia). The integral values of the densitometric signals of the 5-LO mRNA were normalized to those obtained from the β-actin mRNA in the same cDNA sample. This radio in the T0 sample was assumed as a baseline value of 5-LO mRNA and was expressed as a percentage (100%). A ratio value greater than 150% in the 15 min samples was considered to be a significant increase of the 5-LO gene expression.

The Mann-Whitney *U* test and Wilcoxon signed rank test were used for statistical analysis. Differences were considered significant for $p < 0.05$.

Results and Discussion

No difference in 5-LO gene expression was found between samples collected at T0 from Cuprophan and PMMA membrane treated patients (5-LO/β-actin mRNA ratio: 0.417 ± 0.141 (SD), range 0.91–0.108, vs. 0.377 ± 0.166 (SD), range 0.91–0.075). No difference was found by the Wilcoxon test analysis between these values and those obtained at the beginning of the subsequent session (Cuprophan: 0.398 ± 0.167, range 0.856–0.094; PMMA: 0.386 ± 0.158, range 0.894–0.083). Four of the 5 patients treated with Cuprophan membrane had a significant increase in 5-LO gene expression at 15 min (199.4 ± 30 (SD), range 150–287%). The Cuprophan treated patient who did not show any significant variation in 5-LO gene expression did not differ from the others in any of the considered clinical variables. No T15

FIG 1. 5-LO gene expression as evaluated by RT-PCR and quantitated by the limiting dilutions technique in the patients under study. The t0 and t15 are samples collected at the start of HD and after 15 min, respectively. The picture represents 3 cDNA dilutions (from left to right: 1:1, 1:2, 1:4) amplified with specific oligomers for 5-LO (left column) and β-actin (right column) (1–5: Cuprophan membrane patients; 5–1 PMMA membrane patients).

sample collected from PMMA membrane treated patients was found to have a significant increase in the amount of 5-LO mRNA (114.2 ± 6.6 [SD], range 100–135%). The difference between results obtained with the 2 membranes was significant. Results are shown in Fig. 1.

The similar levels of the baseline 5-LO gene expression with the 2 HD membranes suggest that the upregulation of this enzyme mRNA occurring at 15 min of dialysis is an acute event related to leukocyte activation following blood-membrane interaction and limited to the dialysis session.

The exposure of blood to HD membranes leads to the production of several inflammation mediators (including complement fragments) and the activation of leukocytes, which are stimulated to release intragranular proteins and biological active compounds and to synthesize proinflammatory agents de novo, i.e., cytokines IL-1β, tumor necrosis factor alpha (TNFα), IL-6, and eicosanoids (2–4,12,13). It has been reported that one of the possible biologically active end-products of the 5-LO pathway LTB_4 was increased in the plasma of chronically hemodialyzed patients (5–8). Regulation of 5-LO gene expression still needs to be completely clarified. Studies performed in a stimulated HL-60 cell line suggest that 5-LO gene expression is regulated by posttranscriptional mechanisms involving RNA processing reactions (11). These posttranscriptional modifications could lead to an increase in the mRNA stability followed by a higher mRNA amount in the cell. It is likely that, at variance with PMMA membranes, the interaction between blood and Cuprophan membranes triggers not only enzymatic activity (5–8) but also the upregulation of the gene expression machinery.

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Soluble Interleukin 2 Receptor in Dialyzed Patients

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Abstract: Azotemic patients are usually characterized by a state of so-called preactivation resulting in excessive expression of interleukin 2 receptors (IL-2R) on T lymphocytes. The etiological mechanism of this preactivation is, however, still speculative. We studied the serum level of the soluble form of IL-2R (sIL-2R) in azotemic patients on either hemodialysis (HD) (n = 49) or continuous ambulatory peritoneal dialysis (CAPD) (n = 45). Both patient groups had significantly higher sIL-2R levels (1,750 ± 664 U/ml in the HD group and 1,769 ± 647 U/ml in the CAPD group, respectively) $p < 0.00001$ as compared to the normal control group (511 ± 436 U/ml). However, there was no significant difference between the levels of the HD and CAPD group patients. When clinical parameters were studied for their influence on sIL-2R levels, none of the following caused any significant changes: blood transfusion, type of dialyzer used, type of dialysis fluid used, treatment with erythropoietin, hepatitis B infection, or liver function profile. We conclude that chronic renal failure per se is the major cause of the preactivation of T lymphocytes. The modes of treatment and various clinical variables in these patients have no significant influence on sIL-2R levels. **Key Words:** Soluble interleukin 2 receptor—Dialysis—Uremia—Chronic renal failure—Continuous ambulatory peritoneal dialysis.

Patients with chronic renal failure are usually characterized by a state of so-called preactivation of

Received March 1997; revised September 1997.

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immune competent cells involving T cells, B cells, and monocytes (1). The preactivation of T lymphocytes is manifested by an increased expression of interleukin 2 (IL-2) receptors (IL-2R) on T lymphocytes (2). The excessive production of these receptors seems to exhaust IL-2 and results in T-cell dysfunction. Although the elevated soluble form of IL-2R (sIL-2R) in the serum of azotemic patients has been well documented, the exact mechanism is still uncertain. The purpose of this study is to elucidate the clinical parameters that determine sIL-2R in chronic renal failure patients receiving different modes of treatment.

Materials and methods

Uremic patients who were on maintenance dialysis therapy for more than 3 months and followed at our hospital were randomly selected for study. These included 49 hemodialysis (HD) patients and 45 continuous ambulatory peritoneal dialysis (CAPD) patients. Patients excluded were those on immunosuppressive agents or with a recent infection other than hepatitis B or C, evidence of autoimmune disease, evidence of malignancy, or recent operation. All patients in the HD group were dialyzed 4 h per session 3 times a week using one of the following types of dialyzers: cellulose (n = 14), substituted cellulose (n = 25), or synthetic membrane (n = 10). All patients in the CAPD group were treated with 4 to 5 exchanges per day of 2 L Dianeal solution (Baxter, Deerfield, IL, USA) with different glucose concentrations. Medical charts were carefully reviewed for the records of the following parameters checked within 3 months before study: units of blood transfusion, use of erythropoietin, frequency of dialysis, type of dialyzer used, type of dialysis solution used, and status of hepatitis B. Venous samples were collected and centrifuged and serum separated and stored at –70°C until assay for sIL-2R. For the purpose of comparison, serum samples were also collected from a group of healthy volunteers (19 cases). Serum sIL-2R was determined using a commercialized sandwich EIA kit (T Cell Science, Cambridge, MA, U.S.A.). Statistical analysis was performed using the Mann-Whitney *U* test to compare data between the HD and CAPD groups. Stepwise multiple regression was also used to find any correlation between sIL-2R levels and various clinical parameters.

Results

Table 1 shows the patient characteristics and serum sIL-2R levels. Figure 1 shows the distribution of individual points in patients and the control group. Serum sIL-2R levels were significantly higher in both HD (1,750 ± 664 U/ml) and CAPD (1,769 ± 647 U/ml) patients, respectively ($p < 0.00001$), than in the control patients. However, there was no significant difference between the HD and CAPD groups. When clinical parameters were studied for their influences on sIL-2R levels in HD patients, there were no significant differences in sIL-2R with reference to history of blood transfusion, type of dialyzer used, type of dialysis fluid used, usage of erythropoietin, hepatitis B infection, or liver function profile (data not shown). Similar negative results were obtained in the CAPD group.

TABLE 1. *Patient characteristics and sIL-2R levels*

	HD (n = 49)	CAPD (n = 45)	Control (n = 19)
Age (years)	37.1 ± 8.6[b]	51.6 ± 16.4	31.2 ± 7.2
Sex (M/F)	28/21	21/24	14/5
Dur[a] (m)	26.3 ± 22.6	20.3 ± 18.5	
sIL-2R (U/ml)	1,750 ± 664[c]	1,769 ± 647[c]	511 ± 436

[a] Duration of dialysis.
[b] $p < 0.0001$ HD vs. CAPD.
[c] $p < 0.00001$ HD vs. control and CAPD vs. control.
No significant difference between HD and CAPD.

Discussion

Azotemic patients are usually characterized by a state of immune deficiency. No IL-2 activity is detected in culture supernatants of these patients' T cells stimulated by mitogens (2,3). Paradoxically, IL-2R expression on T cells, as well as in serum levels of its soluble form (sIL-2R), is abnormally high in azotemic patients (2,4). It was thought that azotemic T cells are actually in a preactivated state. An increase in IL-2R may lead to increased consumption of IL-2 by means of absorption and inhibit IL-2 dependent cell proliferation (5). The etiologic mechanism of this preactivation is obscure. In dialyzed patients, it may be due to several factors such as blood transfusion, the interaction between the dialysis membrane and lymphocytes, intercurrent infection, the presence of unknown toxins in the serum, and still other unknown mechanisms. The present study shows that

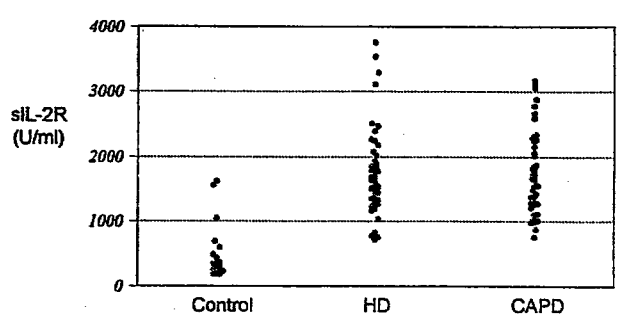

FIG. 1. Shown are the serum sIL-2R levels in the normal control, HD, and CAPD groups.