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Effects of dialysis membranes on beta$_2$-microglobulin production and cellular expression

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Effects of dialysis membranes on beta$_2$-microglobulin production and cellular expression. We investigated the effects of different dialysis membranes on the production of beta$_2$-microglobulin (β_2m) in peripheral blood mononuclear cell cultures (PBMNC) obtained from hemodialysis patients in a prospective cross-over design study. Chronic dialysis with cuprophane membrane leads to an increase in β_2m production from 129 ± 11 ng/ml to 192 ± 23 ng/ml ($P < 0.002$). This increase is reversed by the use of a non-complement activating membrane polymethylmethacrylate. In addition, during chronic dialysis with cuprophane membrane, an increasing proportion of these cells display low β_2m expression on their surface (from 6.1 ± 0.8% of PBMNC to 16.9 ± 3.4%, $P < 0.001$), concomitant with the emergence of cells with low density of HLA on their surface (from 4.9 ± 1.2% of cells to 32.9 ± 7.8% of cells, $P < 0.001$). The total content of cell-associated β_2m is also decreased in dialysis patients in general, and in particular in patients chronically dialyzed with new cuprophane membrane. These effects can be reproduced by incubation of PBMNC with cuprophane membranes, and with the addition of C_{5a}, IL-1 and TNF in vitro. Thus, chronic dialysis with cuprophane membrane may be a factor in the genesis of high β_2m levels and causes changes in β_2m and HLA expression on cell surfaces.

Beta$_2$-microglobulin (β_2m) is present on the surfaces of nucleated cells as an integral part of the HLA class I antigen complex (30% of all β_2m expressed on cell surfaces) as well as in a non-HLA associated form [1, 2]. The rate of β_2m synthesis has been estimated to be 150 to 200 mg/day [3]. This middle molecule (molecular wt 11,800) is freely filtered by the glomerulus and metabolized by the proximal tubule. In patients with normal renal function, the plasma concentration of β_2m is stable and in the range of 1 mg/liter; however, plasma β_2m concentration increases markedly with renal failure [4].

Several studies have documented the presence of polymerized β_2m in amyloid deposits in the carpal tunnel and other musculoskeletal sites of long-term hemodialysis patients [5–7]. The incidence of carpal tunnel syndrome (CTS) increases with years on dialysis and reaches 80% in patients dialyzed for greater than 15 years [8, 9]. More recent evidence suggests that synthetic dialysis membranes, such as the polyacrylonitrile or polysulfone which combine improved biocompatibility as well as convective and adsorptive clearance of β_2m, can delay the development of carpal tunnel syndrome in these patients [9–13].

The role that the biocompatibility of dialysis membrane plays in the generation of β_2m has been controversial; intradialytic hemoconcentration, dialyzer-associated convective or adsorptive clearance, and the multicompartment distribution of β_2m make the in vivo study of this role difficult [14, 15].

To investigate this issue at the cellular level, without these confounding variables, we measured the in vitro production of β_2m by peripheral blood mononuclear cells (PBMNC) harvested from chronic hemodialysis patients before and after acute and chronic dialysis with different membranes. In a randomized cross-over study, patients were dialyzed with two different membranes: cuprophane, the most widely used membrane in clinical dialysis, which is known to activate the complement cascade as well as cellular elements and which has no measurable clearance for β_2m [16]; and the polymethylmethacrylate (PMMA) dialyzer, a biocompatible hollow-fiber membrane that has no appreciable convective or adsorptive removal of β_2m. Our results indicate that the biocompatibility of dialysis membrane plays an important role in the production of β_2m by peripheral mononuclear cells. In addition, recurrent use of cuprophane membrane leads to the simultaneous loss of β_2m expression as well as HLA antigens from the cell surface, and may thus play a role in the immune competence of hemodialysis patients.

Methods

Patients

Eight patients receiving chronic hemodialysis for 44 ± 24 months were selected for participation in this study. All patients were previously dialyzed thrice weekly with reused cuprophane membranes. Reuse involved formaldehyde but not bleach; this reuse procedure significantly attenuates the complement activating ability of cuprophane membranes [16]. On average, the number of reuses for each dialyzer was 12; thus, prior to their participation in the protocol, these patients were exposed to a new cuprophane membrane once every month. The mean age of the patients was 69 years (range 51 to 76). None had clinical evidence for any inflammatory disease process or malignancy and none were diabetic. None were on steroid or immunosuppressive therapy and none had clinical or radiological evidence of β_2m amyloid deposition or carpal tunnel syndrome. Informed consent, approved by the Vanderbilt University Medical Center

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and the Nashville VA Hospital IRB, was signed by all participants.

The design of the study was a cross over design. During the first phase of the study all patients were dialyzed each time with a new cuprophane membrane for a period of two weeks (GAMBRO GF-120) (phase I). After two weeks, the patients were switched to two weeks of new PMMA membrane (phase II) and then were returned to a second period of new cuprophane membranes for two additional weeks (phase III). There were no other changes in dialysis parameters. Blood samples were drawn pre- and post-dialysis of the first and sixth dialysis (that is, at the beginning and end of two weeks) with each type of membrane. Control samples were obtained from 18 uremic, non-dialyzed patients (average serum creatinine 7.2 ± 1 mg/dl) seen in the chronic renal failure clinic and from 12 normal volunteers without known medical problems.

Methods

Culture of peripheral blood mononuclear cells (PBMNC). Thirty milliliters of blood were mixed with equal amounts of Hank's balanced salt solution (HBSS, Gibco, New York, New York, USA) and carefully layered over Histopaque (Sigma Chemical Co., St. Louis, Missouri, USA) media allowing gradient density centrifugation. The mononuclear cell layer was collected and washed twice with HBSS and then resuspended in RPMI 1640 supplemented with 2 mM L-glutamine (Gibco), 100 U/ml penicillin G (Sigma), 10 μg/ml Streptomycin (Sigma) and 10% de-complemented, sterile-filtered certified fetal bovine serum (FBS) (Gibco). On average, PBMNC contained 80% of lymphocytes and 20% of monocytes.

PBMNC ($5 \cdot 10^5$ cells/ml) were placed in 24 well plates (Nunc, California, USA) and incubated for four days at 37°C in a 5% CO_2 saturated humidity incubator without additional stimuli (baseline). In addition, the effect of lectin stimulation (PHA-M, Gibco; 1% vol/vol) was investigated in parallel experiments. Viability by Trypan blue dye exclusion was always greater than 99% at the beginning of the culture and greater than 90% at the end of the four-day culture period.

Additional experiments were performed on cells harvested at the end of phase III (6th dialysis with new cuprophane membranes); cells harvested pre- and post-dialysis were incubated under the same conditions and, in parallel experiments, with the addition of cytokines: interleukin-1 (IL-1β) 100 pg/ml (Cistron, New Jersey, USA), Tumor necrosis factor (TNFα) 100 pg/ml (Cistron) or C_{5a} 50 ng/ml (Amersham, Arlington Heights, Illinois, USA). These doses correspond to clinical levels of cytokines that have been reported during dialysis procedures [17, 18].

Radioimmunoassay (RIA) for β_2m production in vitro. The quantity of β_2m released by PBMNC after four days of culture was measured in the culture supernatants [19, 20] using a commercial solid-phase RIA kit (Abbott, North Chicago, Illinois, USA) with the same anti-β_2m antibody as the one used for the cell staining procedure. The titration curve was linear from 50 to 4,000 ng/ml with an interassay variation of less than 3%. Mean counts per minute (CPM) were calculated using a point-to-point data reduction software (Abbott).

Cytofluorometric studies. Cell pellets ($2 \cdot 10^6$ cells) were harvested on the fourth day of culture and incubated for 30 minutes in 200 μl phosphate-buffered saline (PBS) plus 1% FBS, 0.1% sodium azide, 1% mouse IgG (modified PBS), and 0.1 μg of anti-β_2m monoclonal antibody obtained from the mouse hybridoma 4559 (IgG2a; Abbott). Cells were then washed and tagged with goat anti-mouse FITC (Fab'$_2$; Becton Dickinson, California, USA). The cells were then washed twice with 2 ml of PBS, and kept in 500 μl of modified PBS at 4°C until analysis, generally less than two hours after harvesting. HLA staining [21] was performed with similar techniques using either anti-HLA monomorphic determinant antibody from the mouse hybridoma W6/32 (Accurate) by adding 20 μl of this antibody to $2 \cdot 10^6$ cells and indirect tagging with the same goat anti-mouse (GAM) FITC (Fab')$_2$ mentioned above. Alternatively, biotinylated anti-HLA-A, B, C (Olympus, New York, USA) was used. In this case, 5 μl of this antibody was added to $2 \cdot 10^6$ cells followed by 20 μl of streptavidin-phycoerythrin solution (Vector Laboratories, Incline Village, California, USA) for tagging.

Green and red fluorescence analyses were performed on a flow cytometer EPICS 753 with Argon laser (Coulter Electronics, Hialeah, Florida, USA). Fluorescence was standardized each day by fluorescent microbeads and voltage, amplification and compensation kept constant for all procedures except for the need to increase the amplification for analyses using the phycoerythrin fluorescence.

Analysis was performed on 5,000 viable lymphocytes according to size and granularity. The percentage of β_2m and HLA positive cells and the mean log fluorescence channel (MLFC) were recorded on a Coulter computer using Immuno™ (Diagnostics) software. Background autofluorescence of the same unstained cells and non-specific fluorescence with irrelevant mouse IgG and GAM-FITC was digitally subtracted.

Cell associated β_2m. To determine the total content of cell-associated β_2m, 10^6 cells were harvested from each of the cultures and incubated in 1 ml of PBS, 1% Triton X-100 (Sigma), 25 mM Tris-HCl for 60 minutes at 37°C in a shaking water bath. The supernatants were then tested for β_2m release. Control experiments with identical number of cells but without Triton detergent were also carried out and had no detectable levels of solubilized β_2m.

Role of monocytes. To investigate the potential role of monocytes in these interactions, we purified normal PBMNC using an affinity chromatography column (Human T-cell column kit, Beckman Instruments, Fullerton, California, USA). In brief, the column was equilibrated with PBS and anti-B-cell antibody (IgS); 10^7 mononuclear cells, isolated as described previously were then applied to this column and the effluent collected and resuspended in RPMI 1640 with 10% FBS. Staining with CD_2 (pan T-cell marker) indicated greater than 95% T cell purity. This solution was then incubated in different mini-dialyzers made from the same hollow fibers as used commercially with or without the addition of C_{5a}.

Statistical analysis

All cultures of PBMNC from dialysis patients were carried out concurrently with uremic, non-dialyzed patients as well as PBMNC from normal volunteers.

Comparisons between periods were done by multivariate analysis using ANOVA (BMDP software). Comparisons within the same period was done using *t*-test for paired data, whereas comparison between patients and controls was done using non-paired *t*-test.

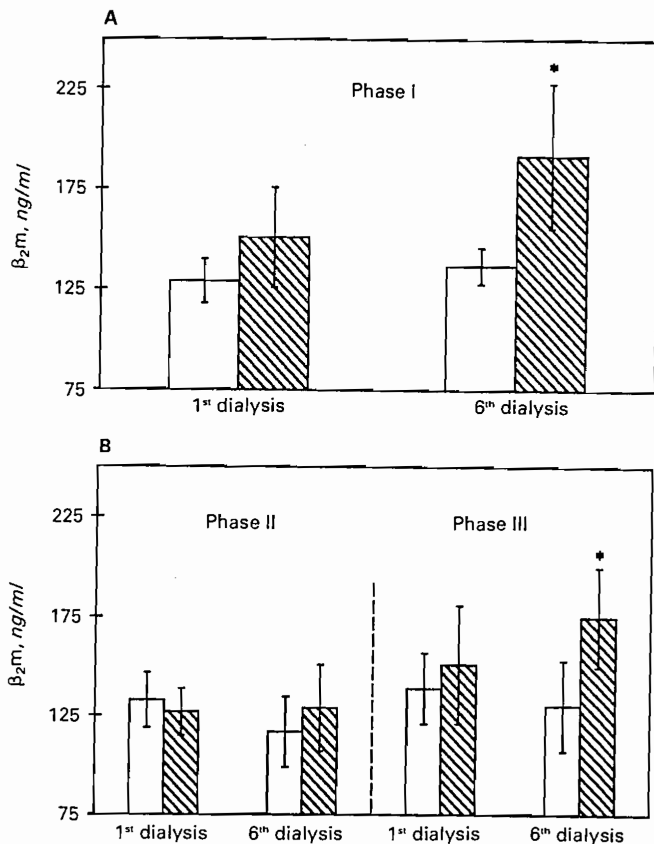

Fig. 1. *Production of β_2m by PBMNC in culture.* **A.** The production of β_2m pre- (white bar) and post- (shaded bar) dialysis during six consecutive dialyses with new cuprophane membrane. **B.** β_2m production during phase II (PMMA dialyzers) and phase III (new cuprophane dialyzers). There was a statistical increase in β_2m production at the end of each of the cuprophane phases.

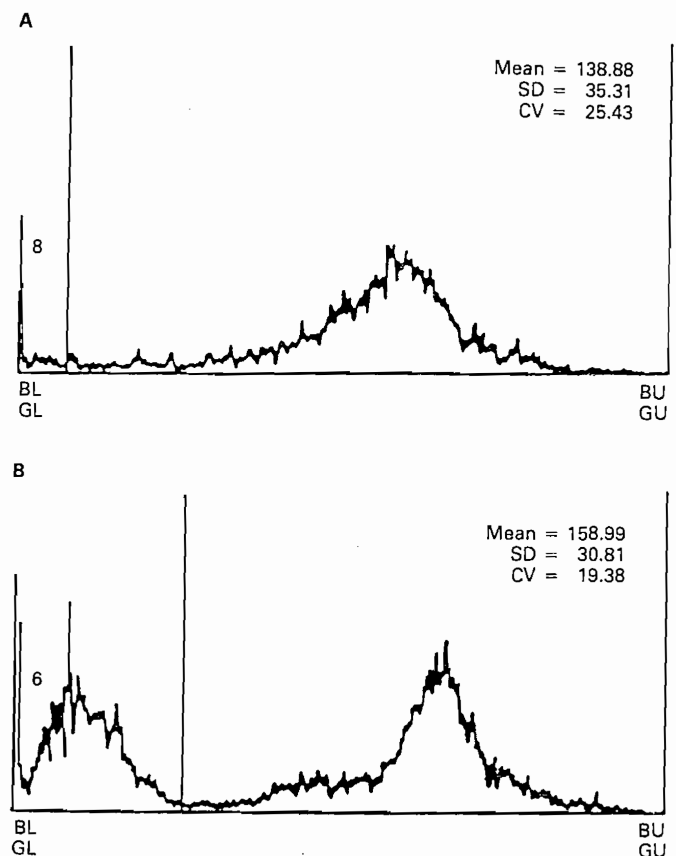

Fig. 2. *Emergence of a subpopulation of cells with low β_2m staining.* **A.** The distribution of β_2m on PBMNC in controls, and in dialysis patients prior to their initiation on recurrent new cuprophane dialysis. **B.** Two distributions of β_2m are seen after 2 weeks of cuprophane dialysis: cells with *low* β_2m staining (left-hand peak) and cells with *high* β_2m staining (right-hand peak).

Results

Production of β_2m in supernatant

PBMNC harvested from patients after two weeks of cuprophane dialysis had a statistically significant increase in their production of β_2m in culture after dialysis (Fig. 1A). Thus at the beginning of the two weeks of new cuprophane dialysis, the concentration of β_2m in the supernatants of cells obtained from these patients was 129 ± 11 ng/ml, similar to values reported in the literature [19, 20, 22]. Although this increased slightly in the supernatants of cells obtained post-dialysis after the first exposure to new cuprophane membrane (151 ± 9 ng/ml), this was not statistically significant. However, samples taken post-dialysis after the two weeks of dialysis with cuprophane membranes showed a significant increase of production to 192 ± 23 ng/ml ($P < 0.002$ compared to initial values) in the supernatant of the PBMNC after four days of culture. This increase over baseline was no longer evident after initiation of the same patients on the non-complement activating dialysis membrane PMMA (Fig. 1B), and β_2m production decreased to 117 ± 9 ng/ml in samples obtained post-sixth dialysis with PMMA membrane. However, as seen in Figure 1B, when the patients were crossed over back to the new cuprophane membrane during phase III, the production of β_2m by PBMNC in culture increased at the end of the sixth dialysis to 179 ± 36 ng/ml ($P < 0.01$).

Cellular expression of β_2m

The increased production of β_2m by PBMNC was also associated with the development of a bi-modal peak in the cytofluorographic analysis. An example is seen in Figure 2. The upper panel shows the typically Gaussian distribution of β_2m staining in PBMNC of cells of normal controls and uremic non-dialyzed patients, whereas the lower panel shows a typical bi-modal distribution of β_2m seen after initiation of dialysis with new cuprophane dialyzers, with the emergence of a percentage of cells with low β_2m staining (left-sided peak).

As seen in Figure 3 and Table 1, in pre-dialysis samples prior to the initiation of phase I (new cuprophane membrane dialysis), the percent of cells with low staining for β_2m was 6.1 ± 0.8%. This was significantly higher than the proportion of low β_2m staining cells in both uremics and normals which was at the detection limit of the cytofluorometric technique (1.9 ± 0.2%). After the first dialysis with the new cuprophane membrane, there was a non-significant increase in the proportion of cells

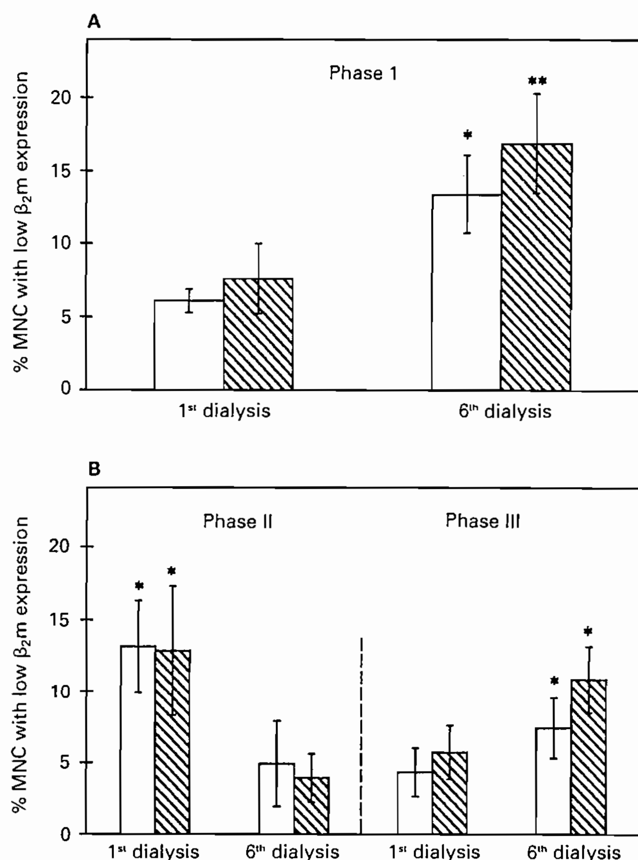

Fig. 3. *Changes in the percent of cells with low β_2m staining during the study.* **A.** During phase I (cuprophane), there is an increase in the proportion of cells with low β_2m staining, particularly after 2 weeks. **B.** This is reversed with the use of a non-complement activating membrane and in a cross over study, the proportion of low staining β_2m cells increases again, both pre-dialysis (white bars) and post-dialysis (hatched bars) after cuprophane re-introduction (phase III).

with low β_2m expression to 7.6 ± 2.4% (P = NS); however, at the end of two weeks of dialysis with a new cuprophane membrane, the number of cells with low β_2m staining was higher: 13.4 ± 2.7% pre-dialysis and 16.9 ± 3.4% post-dialysis, both significantly higher than corresponding values in the 1st dialysis with new cuprophane membrane ($P < 0.001$). The percent of low β_2m staining cells remained high during the first day of the cross over to the PMMA membrane (Table 1) but returned to baseline (3.9 ± 1.7%) levels following two weeks of PMMA. Rechallenge with new cuprophane dialysis resulted in a pattern identical to the first phase, with increase in the percent of low β_2m staining cells to 10.2 ± 4.3%. Thus, chronic dialysis with new cuprophane membrane results in the increased production of β_2m by the PBMNC harvested from these patients and the development of a significant percentage of cells with low β_2m expression.

The changes in the distribution or density of β_2m on cells expressing high levels of β_2m (right-sided peak in Fig. 2) can be assessed from the mean log fluorescence channel number (MLFC) of the high staining population peak [21]. It can be seen from Table 2 that the MLFC increased during the cuprophane period from 117 ± 3 log-fluorescence unit at the first dialysis with a new cuprophane membrane to 158 ± 2 log-fluorescence unit at the end of the new cuprophane period. By the end of the second phase of this trial, that is, after two weeks of PMMA dialysis, the MLFC decreased to 126 ± 4 consistent with the decrease in the density of β_2m back to baseline in cells expressing high levels of β_2m. Thus, chronic dialysis with cuprophane membrane results in the development of two sets of mononuclear cells, one with low expression of β_2m (approximately 15% of cells) and another with increased density of β_2m. Dialysis with a non-complement activating membrane results in loss of these different cell populations.

Effect of PHA stimulation on cellular β_2m expression

PHA stimulation also results in an increase in the number or proportion of low β_2m staining cells as shown in Table 1. However, the effect of PHA was not uniform for all phases of the study. While PHA (1% vol/vol) incubated with PBMNC cultures increased the percent of low β_2m staining of cells harvested prior to the initiation of phase I dialysis to 19.7 ± 4.3%, a further increase was noted when PHA was added to cells harvested after the first cuprophane dialysis. At the end of phase II (PMMA dialyzers), the effect of PHA on β_2m expression was similar to those of normal controls (10.4 ± 2.6).

Cell associated β_2m

Cellular β_2m extracted by 1% Triton X-100 confirmed the loss of cellular β_2m in dialysis cells. As seen in Table 3, β_2m deterged from dialysis patients at the beginning of the study (364 ± 65 ng/ml) was significantly lower than β_2m deterged from controls and was further reduced in cells harvested at the end of phase I and III (new cuprophane dialysis; 224 ± 57 ng/ml, $P < 0.001$ compared to controls and 1st dialysis).

Cellular expression of HLA monomorphic determinant

Similar to the changes in the cellular expression of β_2m, there was also an increase in the proportion of cells with low HLA expression (Table 4). Whereas prior to the initiation of phase III, the patients had less than 6% of the cells with low HLA expression, this percentage was significantly increased pre-dialysis at the two weeks of new cuprophane membrane sampling period (16.7 ± 4.3%) and there was a further increase in the percentage of PBMNC with low HLA expression post-dialysis (32.9 ± 7.8% at the end of the study).

Double staining with specific T-cell subsets (CD_3, CD_4, and CD_8) did not isolate a particular subpopulation that was more susceptible to low β_2m or HLA staining during dialysis with cuprophane membrane. Nevertheless, the crossover design of the study allows us to conclude that the increase in the percentage of these cells is clearly associated with chronic dialysis with cuprophane membrane.

Effect of cytokines on β_2m expression and release

To investigate the potential pathophysiology of these cellular changes, cells harvested prior to the sixth cuprophane dialysis (at the end of phase III) were incubated with either 100 pg/ml of IL-1, 100 pg/ml of TNFα, 50 ng/ml of C_{5a} or without additional stimuli (baseline). Whereas both cytokines as well as C_{5a} resulted in a significant increase in the number of PBMNC with low β_2m expression in samples harvested pre-dialysis, these agonists had no further effect on cells harvested post-dialysis

Table 1. Proportion of cell population with low β_2m expression during chronic dialysis

	Control	Phase I Cuprophane 1st Dialysis pre/post	Phase I Cuprophane 6th Dialysis pre/post	Phase II PMMA 1st Dialysis pre/post	Phase II PMMA 6th Dialysis pre/post
Baseline					
Mean	1.9	$6.1^{b}/7.6^{b}$	$13.4^{a,b}/16.9^{a,b}$	$13.1^{a,b}/12.8^{a,b}$	$4.9^{a,b}/3.9^{a,b}$
SEM	0.2	0.8/2.4	2.7/3.4	3.2/2.6	3.0/1.7
PHA cultures					
Mean	10.4	$19.7^{b}/26.6^{b}$	$24.1^{b}/25.3^{b}$	$17.1^{b}/18.2^{b}$	8.4/8.2
SEM	2.6	4.3/6.1	5.2/6.9	6.2/6.5	3.1/3.3

[a] $P < 0.001$ compared to corresponding 1st dialysis
[b] $P < 0.01$ compared to control

Table 2. MLFC units of high β_2m staining cells during the cross over study

Sampling time	Cuprophane periods 1st Dialysis pre/post	Cuprophane periods 6th Dialysis pre/post	PMMA period 1st Dialysis pre/post	PMMA period 6th Dialysis pre/post
Mean log fluorescence	117/121	$158^{a}/157^{a}$	137/131	133/126
SEM	3/3	2/3	4/5	4/4

[a] $P < 0.05$ vs. 1st dialysis

(Fig. 4). Similarly, β_2m production was significantly increased following incubation of pre-dialysis PBMNC with IL-1β and C_{5a} and to a lesser extent when cells were harvested post-dialysis (Fig. 5). Note however, that β_2m production, either baseline or stimulated were significantly higher than corresponding pre-dialysis samples. These results suggest that β_2m synthesis can be modulated by cytokines and in particular by C_{5a} and that cells harvested post-cuprophane dialysis are either maximally stimulated or have down-regulated their receptors for these cytokines and for C_{5a}.

In vitro incubation of PBMNC with dialysis membranes

β_2m production was assessed after incubation of the PBMNC inside hollow fibers dialyzer membranes identical to those used in clinical practice. As can be seen (Table 5), the production of β_2m by these PBMNC in culture was significantly higher when incubated with cuprophane membrane (in the absence of any exogenous complement) than in control or cells incubated with either of the biocompatible PMMA or AN69 membranes. The decrease in the synthesis of β_2m following incubation with AN69 may reflect the adsorption of β_2m on this surface [10–12]. Nevertheless, these results suggest that increased production of β_2m by PBMNC may not necessarily require specific cytokines.

Similarly, in vitro incubation with cuprophane membranes resulted in a significant increase in the number of PBMNC with low β_2m expression (2.4 ± 0.2% control, 7.3 ± 1.8% after incubation with cuprophane membrane; Table 6). However, the addition of exogenous C_{5a} resulted in a further increase in the percentage of cells with low β_2m expression particularly in cells previously incubated with cuprophane membrane (22.1 ± 7.9%; $P < 0.001$).

When the same experiments were repeated using affinity-purified T cells, with little or no monocyte contamination, there was no change in the β_2m cellular distribution after incubation with any of the membranes or following the addition of C_{5a}. These results therefore suggest that the participation of monocytes or their products of activation (IL-1 or TNF) are important in the observed changes in β_2m distribution on cell surfaces.

Discussion

The contribution of the dialysis membrane to the endogenous production of β_2m in dialysis patients has been controversial [9, 13]. When measured by intradialytic changes in the concentration of β_2m the results have been variable, depending on the extent of hemoconcentration as well as the characteristics of the membrane, specifically its convective and adsorptive capacities for β_2m. This study which investigated the production of β_2m by PBMNC in in vitro cultures supports the hypothesis that the dialysis membrane plays a modulating role on the production of β_2m at the cellular level. Specifically, chronic dialysis with new cuprophane membrane leads to an increased production of β_2m which is reversed by the use of more biocompatible membranes.

The results of in vitro incubation of these cells with C_{5a} and cytokines indicate a possible pathophysiological pathway linked to recurrent complement activation by the cuprophane membrane. Thus, C_{5a} as well as products of activated monocytes such as IL-1 and TNFα have been shown in this study to increase the production of β_2m in vitro. This effect is clearly more marked when the cells are harvested before the initiation of dialysis with cuprophane membranes than post-dialysis, suggesting that these post-dialysis samples may already be maximally stimulated. Incubation of purified T cells with cuprophane membrane and/or recombinant C_{5a} did not show an increase in β_2m production nor changes in cellular β_2m expression, suggesting that the changes observed in PBMNC were initially mediated by monocyte activation [22, 23].

Thus, on the basis of the above results, we postulate that dialysis with cuprophane membrane results in the formation of C_{5a}, which in turn can activate monocytes via their C_{5a} receptors. Since T lymphocytes have minimal direct interactions with the anaphylatoxins (C_{3a} and C_{5a}) [24, 25], the observed changes with cuprophane membranes both in vivo and in vitro are likely to be secondary to such monocyte activation and elaboration of cytokines such as IL-1 and TNFα. Although this sequence of activation fits well with our data, and is confirmed by in vitro data of monocyte activation [22] it requires further validation by the specific demonstration of monocyte activation and elaboration of their products of activation during clinical dialysis.

Table 3. Cellular β_2m content recovered after triton X-100 treatment

		Cuprophane period (Phase I)		PMMA period (Phase II)		Cuprophane period (Phase III)	
	Control	1st Dialysis pre/post	6th Dialysis pre/post	1st Dialysis pre/post	6th Dialysis pre/post	1st Dialysis pre/post	6th Dialysis pre/post
(ng β_2m/10^6 cells) (mean)	642	$364^b/372^b$	$237^{a,b}/254^{a,b}$	$259^{a,b}/302^{a,b}$	$351^b/322^b$	$325^b/321^b$	$221^{a,b}/224^{a,b}$
SEM	24	65/69	48/51	71/63	43/37	67/73	48/57

[a] $P < 0.001$ compared to controls and corresponding 1st dialysis values
[b] $P < 0.005$ compared to normal controls

Table 4. Changes in HLA expression during phase III (cuprophane dialysis

	1st Dialysis pre/post	6th Dialysis pre/post
% Mononuclear cells with low HLA expression	4.9/5.3	$16.7^a/32.9^b$
SEM	1.2/1.7	4.3/7.8

[a] $P < 0.01$ compared to 1st dialysis
[b] $P < 0.001$ compared to 1st dialysis

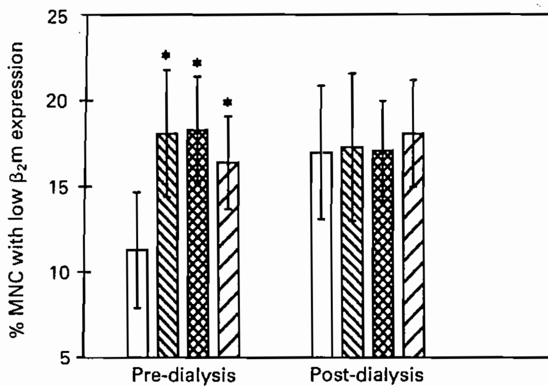

Fig. 4. *Effects of IL-1 (▧), TNF (▩) and C_{5a} (▨) on the development of a sub-population of low β_2m staining cells.* For all pre-dialysis samples, there was a statistically significant increase in these low β_2m staining cells in stimulated cultures over baseline (□). There was no significant increase in post dialysis samples at the end of two weeks of cuprophane dialysis with any of the agonists.

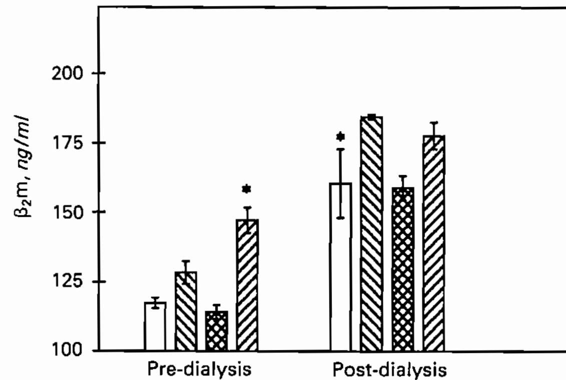

Fig. 5. *Effects of IL-1 (▧), TNF (▩) and C_{5a} (▨) on β_2m production by PBMNC in culture.* Cytokines, and in particular C_{5a}, cause a large increase in the production of β_2m by PBMNC over baseline (□).

Table 5. β_2m production in vitro after incubation of normal cells with different membranes

	Control $N = 12$	PMMA $N = 7$	AN69 $N = 7$	Cuprophane $N = 7$
β_2m *ng/ml* Mean ± SEM	162 ± 19	170 ± 17	139 ± 19	221 ± 37[a]

[a] $P < 0.05$ compared to controls, PMMA and AN69

Table 6. Low expression of β_2m in normal mononuclear cells incubated with different dialysis membranes and C_{5a}

% MNC with low β_2m expression (mean ± SEM)	Control $N = 17$	AN69 $N = 6$	PMMA $N = 6$	Cuprophane $N = 6$
Baseline	2.4 ± 0.2	1.7 ± 0.9	4.1 ± 1.8	7.3 ± 1.8[a]
C_{5a} *50 ng/ml*	2.7 ± 1.1	3.6 ± 1.4	5.2 ± 2.3	22.1 ± 7.9[b]

[a] $P < 0.05$ vs. control
[b] $P < 0.001$ vs. control

An equally important finding in this study is that the expression of HLA is attenuated in a subpopulation of PBMNC after chronic exposure to the cuprophane membrane. The expression of HLA and β_2m is important in the recognition of "self" by immune competent cells [26–28] and their disappearance from the cell surface may be a factor in the immunodeficiency of dialysis patients [29, 30].

Changes in cellular expression of β_2m during cuprophane dialysis are similar to those observed during PHA stimulation [19] (that is, PHA stimulation also leads to the increased release of β_2m and the development of a subpopulation of cells with low β_2m expression). These results are therefore consistent with the process of activation of PBMNC during the dialysis procedure with new cuprophane membranes. Indeed, in vitro incubation of PBMNC with cuprophane membrane and subsequent stimulation with C_{5a} results in percent of cells with low β_2m expression similar to those obtained by PHA stimulation.

In summary, chronic dialysis with cuprophane membrane results in increased production of β_2m and the emergence of a substantial population of PBMNC that have low staining for β_2m and HLA. These changes are reproduced in vitro when mononuclear cells are incubated with cytokines and C_{5a}, but not when these preparations are depleted of monocytes. Non-

complement activating membranes lead to a reversal of these findings. The clinical significance of these findings will require long term clinical studies to detect differences in the incidences of amyloid bone disease and to elucidate the separate effects of the dialysate base [31], endotoxin content [32], back filtration [33] and monocyte activation in their pathogenesis.

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