



Activation of Basophils Is a New and Sensitive Marker of Biocompatibility in Hemodialysis

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Abstract: The hemodialysis procedure involves contact between peripheral blood and the surface of dialyzer membranes, which may lead to alterations in the pathways of innate and adaptive immunity. We aimed to study the effect of blood–membrane interaction on human peripheral basophils and neutrophils in hemodialysis with high- and low-permeability polysulfone dialyzers. The surface expression of CD203c (basophil selection marker) and CD63 (activation marker) after activation by the bacterial peptide formyl-methionyl-leucyl-phenylalanine (fMLP) or anti-Fcε receptor I (FcεRI) antibody and the absolute number of basophils was investigated before and after hemodialysis with each of the dialyzers. Moreover, the expression on neutrophils of CD11b, the CD11b active epitope, and CD88 was analyzed in the same groups of individuals. The expression of CD63 in basophils following activation by fMLP was significantly higher in the patient group compared with that

in healthy controls, but no differences were observed after activation by anti-FcεRI. During the hemodialysis procedure, the low-flux membrane induced up-regulation of CD63 expression on basophils, while passage through the high-flux membrane did not significantly alter the responsiveness. In addition, the absolute number of basophils was unchanged after hemodialysis with either of the dialyzers and compared with healthy controls. We found no significant differences in the expression of the neutrophil activation markers (CD11b, the active epitope of CD11b, and CD88) comparing the two different dialyzers before and after dialysis and healthy controls. Together, these findings suggest that alterations in basophil activity may be a useful marker of membrane biocompatibility in hemodialysis. **Key Words:** Chronic kidney disease—Hemodialysis—Biocompatibility—Basophil activation—Neutrophil activation.

Chronic kidney disease (CKD) is a major medical condition worldwide and leads to significant morbidity and mortality (1,2). CKD patients have a high susceptibility to infections, at least partly originating in immune insufficiency caused by chronic uremia

or by the hemodialysis procedure (3,4). During hemodialysis, accumulated uremic toxins, minerals, and excessive fluid are removed from the blood by diffusion and convection. Interaction of blood with the dialysis membrane may give rise to a number of adverse reactions in circulating cells and plasma proteins as a consequence of bioincompatibility. Alterations in complement activation, adhesion molecule expression, cytokine synthesis, phagocytic efficiency, apoptosis, and production of reactive oxygen species have been observed (5–7).

These aberrations may be due to the surface material of the dialyzer used or the membrane permeability properties (8,9). Classic unmodified

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cellulose membranes were used in the early era of hemodialysis but have long since been substituted by membranes based on modified cellulose (cellulose diacetate and hemophan) and subsequently by membranes manufactured from synthetic polymers (polyacrylonitrile [AN69] or polysulfone). The modified and synthetic materials were introduced to improve the biocompatibility of dialyzers. In previous studies, it has been shown that neutrophils from patients dialyzed with polysulfone membranes have higher functionality compared with cells collected from patients treated with unmodified or modified cellulose membranes (5). The hemodialysis procedure induces complement activation, as evidenced by the increased production of complement factors. These factors contribute to dialysis-associated microinflammation through the release of interleukins (ILs) by monocytes, which exert chemotactic activity on neutrophils and monocytes (10). The intensity of complement generation is closely dependent on the type of dialysis membrane (11). In addition, the permeability of the membrane contributes to the overall biocompatibility properties. High-flux dialyzers (with large pore size and higher ultrafiltration coefficients) have better clearance of large molecules, such as β_2 -microglobulin and toxins with enzymatic and metabolic inhibitory effect, compared with low-flux dialyzers (with smaller pore size and lower ultrafiltration coefficients), but the clinical consequences in terms of morbidity and mortality of long-term use are still debated (12–14).

Basophils constitute a subtype of granulocytes and represent <1% of total circulating leukocytes (15). Involvement of basophils in autoimmune kidney diseases, such as lupus nephritis, has previously been observed. Formation of immune complexes may trigger basophils to secrete IL-4 and initiate T helper type 2 (Th2) differentiation, B-cell proliferation, plasma cell differentiation, and immunoglobulin production, responsible for the ongoing humoral immune response in affected tissues (16,17). It has also been proposed that hemodialysis patients display IgE-mediated anaphylactic reactions and that basophils from patients with autoimmune kidney disease up-regulate the cell surface expression of markers such as CD203c and CD63 upon activation (18,19). Studies have previously shown that neutrophil activity can be used to reflect the biocompatibility properties of different dialyzers. For example, CD11b expression on neutrophils was increased after hemodialysis with unsubstituted and substituted cellulose membranes, while it was similar to that in healthy controls after hemodialysis with synthetic polysulfone membranes (7).

Given the potential impact of the dialysis procedure on granulocyte function, we aimed to investigate the differences in activation of basophils (CD63 expression) and neutrophils (expression of CD11b and the active epitope of CD11b) and in CD88 expression in CKD patients undergoing hemodialysis with high-flux or low-flux polysulfone membranes, compared with that observed in healthy controls.

PATIENTS AND METHODS

Study population

The hemodialysis patients were recruited from the Department of Nephrology at the Karolinska University Hospital in Stockholm, Sweden. The patients had an estimated glomerular filtration rate (eGFR) of <20 mL/min/1.73 m² with a residual GFR of 6–11 mL/min/1.73 m² (median 7). Patients were undergoing hemodialysis with high-flux polysulfone dialyzers three times per week for 4–4.5 h per dialysis before the study ($n = 10$). The dialysis vintage in this group was 4–84 months with median duration of 24 months, and dialysis was done via an arteriovenous fistula ($n = 8$) or a central dialysis catheter ($n = 2$). Patients with cancer, an ongoing infection, or chronic inflammatory disease and those taking immunosuppressive drugs were excluded. Healthy controls were recruited among healthy blood donors ($n = 10$), all sex- and age-matched (± 5 years) with the hemodialysis patients. Demographic characteristics of all participants are shown in Table 1. The patients were treated with diuretics, beta-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, or a combination of these drugs (Table 1). Participants filled out a questionnaire regarding their health status, current comorbidities, medication, and vaccination status against hepatitis and influenza virus prior to the study. Written informed consent was obtained from all participants.

Dialysis procedure

Before the study, the patients were routinely undergoing hemodialysis with a high-flux polysulfone capillary hemodiafilter with an effective surface area of 2.3 m² (K₀A urea: 1421 mL/min; ultrafiltration coefficient: 76 mL/h/mm Hg; sterilization method: inline steam; FX corDiax 1000, Fresenius AG, Bad Homburg, Germany). In one session of treatment, the dialyzer was shifted to a low-flux polysulfone capillary dialyzer with an effective surface area of 1.8 m² (K₀A urea: 976 mL/min; ultrafiltration coefficient: 14 mL/h/mm Hg; sterilization method: inline steam;

TABLE 1. Demographic characteristics of participants

Category	Healthy controls	Hemodialysis
Sex		
Male (<i>n</i>)	5	5
Female (<i>n</i>)	5	5
Age (years), range (median)	33–81 (64.5)	37–76 (62.5)
Comorbidities (<i>n</i>)		
Diabetes	0	2
History of heart disease	0	4
Hypertension	0	9
Family history of kidney disease (<i>n</i>)	0	3
Treatment (<i>n</i>)		
ACE inhibitors/ARB	0	5
ESA	0	9
Intravenous iron	0	8
Vitamin D	0	9
Smoking (<i>n</i>)	0	0
Swedish snuff (<i>n</i>)	0	2

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockers; ESA, erythropoiesis-stimulating agent.

FX 10, Fresenius AG). The dialyzers were not reused, and interdialytic weight gain was less than 5%, indicating similar ultrafiltration volumes. The purity of the dialysate fluid was tested at recommended intervals according to the European best practice guidelines (20) and was found to be pure at all occasions.

Peripheral blood samples were drawn before and after hemodialysis in each session (one session with high flux and one session with low flux).

Determination of CD203c and CD63 expression in basophils

Initially, samples from eight study subjects were analyzed: four samples from healthy controls and four pre-high-flux hemodialysis samples. Whole blood was drawn into heparin tubes (Vacutainer, Becton Dickinson, Oxford, UK) and then incubated with different concentrations of stimulators (formyl-methionyl-leucyl-phenylalanine [fMLP] and anti-Fcε receptor I [FcεRI] antibody) to achieve the optimal concentration for a maximum basophil activation response (21). fMLP was diluted in RPMI 1640 medium (Sigma Aldrich, Taufkirchen, Germany) and concentrations of 10^{-6} to 5×10^{-5} M were prepared. Anti-FcεRI antibody (3 μg/mL) (Bühlmann Laboratories, Schönenbuch, Switzerland) was diluted (1:3) in stimulation buffer (Bühlmann Laboratories) and concentrations of 0.11 μg/mL to 3 μg/mL were prepared accordingly. Whole blood (100 μL) was distributed among polystyrene tubes (Falcon, Becton Dickinson, Franklin Lakes, NJ, USA) and incubated with the relevant concentrations of fMLP and anti-FcεRI antibody at 37°C for 25 min to initiate the degranulation of basophils and histamine release. Subsequently the

cells were stained with 18 μL of anti-CD203c-PE and CD63-FITC antibodies (Beckman Coulter, Paris, France) and incubated for 25 min at 4°C. Following lysis of red blood cells (RBCs) with 2 mL cold isotonic solution (154 mM NH₄Cl, 10 mM KHCO₃ supplemented with 0.1 mM EDTA, pH 7.2), samples were centrifuged for 5 min at 300 × *g* at 4°C; cells were then washed once with phosphate buffered saline (PBS) before being resuspended in 300 μL of cold PBS and subsequently analyzed. The surface expression of CD203c and CD63 on basophils was analyzed by flow cytometry (Navios, Beckman Coulter, Hialeah, FL, USA). Basophils were gated according to their granularity on side scatter and expression of CD203c (Fig. 1a). The percentage of CD63-positive cells within the total basophil population was calculated as shown in Fig. 1b. Data were analyzed with Kaluza analysis software (Beckman Coulter).

After the optimal concentrations of fMLP and anti-FcεRI antibody were found, the same procedure was applied on the rest of the samples after incubation of 100 μL of whole blood with RPMI medium (as negative control), RPMI medium containing fMLP at a concentration of 5×10^{-5} M, and anti-FcεRI antibody at a concentration of 3 μg/mL in separate tubes.

Estimation of absolute number of basophils

The absolute number of basophils was estimated in 100 μL of whole blood by using the ImmunoPrep reagent system (Beckman Coulter) according to manufacturer's instructions. Subsequently, 100 μL of pretreated whole blood was mixed with 100 μL flow-count beads (Beckman Coulter) before flow cytometric analysis.

Flow cytometric analysis of surface CD11b, active CD11b, and CD88 expression on neutrophils

Whole blood was drawn into EDTA tubes (Vacutainer, Becton Dickinson) before and after the hemodialysis procedure with high- and low-flux dialyzers, respectively. Blood (150 μ L) was distributed in polystyrene tubes and mixed with 2 mL cold isotonic solution to lyse the erythrocytes. After 5 min, the tubes were centrifuged for 5 min at $300 \times g$ at 4°C, the supernatant was discarded, and the cells were washed with PBS. The tubes were divided into three groups: the unstimulated cells, the cells stimulated with increased temperature (+37°C), and the cells stimulated with IL-8 (CXCL8) (Research & Diagnostics Systems, Abingdon, UK). RPMI 1640 medium (200 μ L) mixed with 10% fetal calf serum was added to all the tubes. IL-8-stimulated cells were stimulated with a final concentration of 100 ng/mL IL-8 (22). The unstimulated cells were incubated on ice, while the stimulated cells were incubated at 37°C for 30 min. The tubes were washed with PBS, and cells were resuspended in 100 μ L of cold PBS. Antibodies were added to the relevant tubes—anti-CD11b-FITC (Beckman Coulter; 20 μ L) and anti-CD11b-PE (activated form, clone CBRM1/5, BioLegend Inc., San Diego, CA, USA; 20 μ L), as well as corresponding isotype controls (IgG1). The tubes were incubated for 30 min at 4°C. The cells were washed with PBS, resuspended in 350 μ L of cold PBS, and subsequently analyzed. Data acquisition was conducted by flow cytometer, and data were analyzed using the Kaluza analysis software.

Neutrophils were gated according to their size and granularity on forward and side scatter as shown in Fig. 1c. The mean fluorescence intensity (MFI) of CD11b⁺ cells and percentage of active-CD11b⁺ cells were measured within region G, according to the gates set by the respective isotype controls. For detection of CD88 expression, 150 μ L of blood was added to polystyrene tubes, RBCs were lysed, and cells were washed with PBS, resuspended in 100 μ L of PBS, and incubated with 20 μ L anti-CD88 anti-

body (Becton Dickinson) for 30 min, then washed once and, after addition of PBS, analyzed with flow cytometry.

Statistical analysis

Scatter plots were prepared using GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA), representing the range with whiskers and the median as a middle line. Statistical analysis was done in GraphPad Prism 5. As the study population was not normally distributed, comparison between the groups was performed by the nonparametric Kruskal–Wallis test. Significant differences between groups were analyzed using the post hoc Wilcoxon matched-pairs signed-rank test. A *P* value of <0.05 was considered significant.

Ethics statement

The study was approved by the local ethical committee at the Karolinska University Hospital, Stockholm, Sweden (2012/1027/32).

Written informed consent was obtained from all participants.

RESULTS

Laboratory findings

The laboratory data for the study participants are shown in Table 2. There were no significant differences between the groups with regard to total leukocyte and platelet counts. The erythrocyte count was significantly higher in healthy controls compared with patients, but no differences were seen when comparing pre- and postdialysis samples with either of the dialyzers. Serum albumin was significantly lower in hemodialysis patients before the procedure compared with healthy controls.

Absolute number of basophils following dialysis with high-flux and low-flux dialyzers compared with healthy controls

The absolute number of basophils was calculated by flow cytometry to investigate the effect of dialyzer

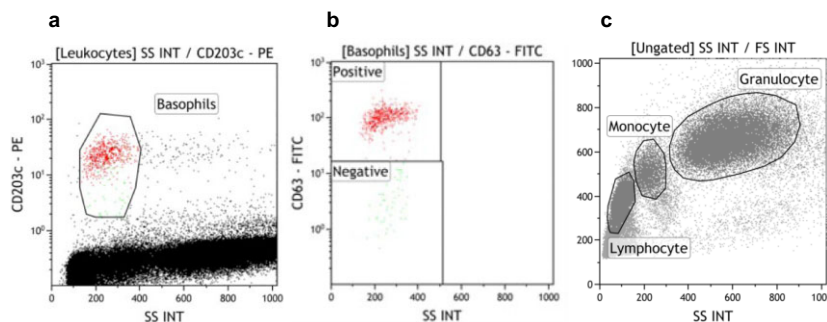


FIG. 1. Flow-cytometric analysis of CD203c and CD63 expression on basophils and CD11b expression on neutrophils. Basophils were gated according to their granularity on side scatter and expression of CD203c (a). The number of CD63-positive cells within the total basophil population was measured (b). Granulocytes were gated according to their size and granularity on forward and side scatter (c).

TABLE 2. Laboratory data for blood parameters (n = 14)

	Leukocyte count ($\times 10^9/L$)	Erythrocyte count ($\times 10^{12}/L$)	Platelet count ($\times 10^9/L$)	Creatinine ($\mu\text{mol}/L$)	Albumin (g/L)	CRP (mg/L)
Healthy	5.3 (4.6–7.3)	4.8 (4.4–5.0)	246 (218–291)	81 (65–83)	39 (36.5–41)	1 (1–3)
Pre-HFD	7.0 (6.6–9.0)	3.7 (3.5–3.8) ^a	215 (165–254)	707 (500–876) ^{e,f}	33 (29.5–37) ⁱ	4 (4–7)
Post-HFD	6.9 (5.5–7.8)	3.9 (3.6–4.3) ^b	192 (164–239)	184 (146–315)	33.5 (31–38)	6 (4–8)
Pre-LFD	6.7 (5.7–7.8)	3.7 (3.5–3.7) ^c	211 (171–271)	651 (516–835) ^{g,h}	33 (30–36) ^j	5 (3–7)
Post-LFD	6.2 (5.4–8.1)	3.9 (3.6–4.1) ^d	212 (178–246)	240 (141–322)	33 (31–38)	5 (3–7)

Values are given as median and interquartile range (25–75%).

^a $P = 0.0006$ compared with healthy controls; ^b $P = 0.04$ compared with healthy controls; ^c $P = 0.0005$ compared with healthy controls;

^d $P = 0.026$ compared with healthy controls; ^e $P = 0.02$ compared with post-HFD; ^f $P = 0.00001$ compared with healthy controls; ^g $P = 0.04$ compared with post-LFD; ^h $P = 0.00001$ compared with healthy controls; ⁱ $P = 0.03$ compared with healthy controls; ^j $P = 0.04$ compared with healthy controls.

CRP, C-reactive protein; HFD, high-flux dialysis; LFD, low-flux dialysis.

membrane on the number of cells. There were no significant differences in the absolute number of basophils following hemodialysis with high-flux or low-flux dialyzers. In addition, the absolute number was similar to that of healthy controls (Table 3).

Optimization of basophil activation response to different concentrations of fMLP and anti-FcεRI antibody (dose–response curve)

Basophils were stimulated by various concentrations of fMLP (bacterial peptide) and anti-FcεRI antibody, and the activation response (CD63 expression) to stimulators was analyzed as shown in Fig. 2. Basophil activation response to fMLP at various concentrations reached a plateau, and therefore the concentration of 5×10^{-5} M was chosen as the optimal concentration (Fig. 2a).

Anti-FcεRI antibodies act by cross-linking the receptors on the surface of basophils and are a stronger stimulator for degranulation. The maximum response of basophils to anti-FcεRI antibody was observed at the concentration of $3 \mu\text{g}/\text{mL}$, and the lowest observed stimulation was seen at a concentration of $0.11 \mu\text{g}/\text{mL}$ (Fig. 2b).

Basophil activation response to fMLP and anti-FcεRI antibody comparing hemodialysis patients with healthy controls

The expression of CD63 in basophils was analyzed following fMLP and anti-FcεRI antibody stimulation. Samples from patients on hemodialysis, obtained before each session of dialysis with high-flux or low-flux dialyzers, were compared with those from healthy controls ($n = 10$ in each group). The

TABLE 3. Absolute number of basophils (cell/ μL) in peripheral whole blood

	Pre-HFD	Post-HFD	Pre-LFD	Post-LFD	Healthy
Number of basophils/ μL blood	16 (12–32)	23 (12–31)	25 (16–36)	28(17–51)	27 (15–39)

The absolute number of basophils (cells/ μL) was calculated in peripheral blood before and after dialysis with high-flux and low-flux membranes. Values are given as median and interquartile range (25–75%).

HFD, high-flux dialysis; LFD, low-flux dialysis.

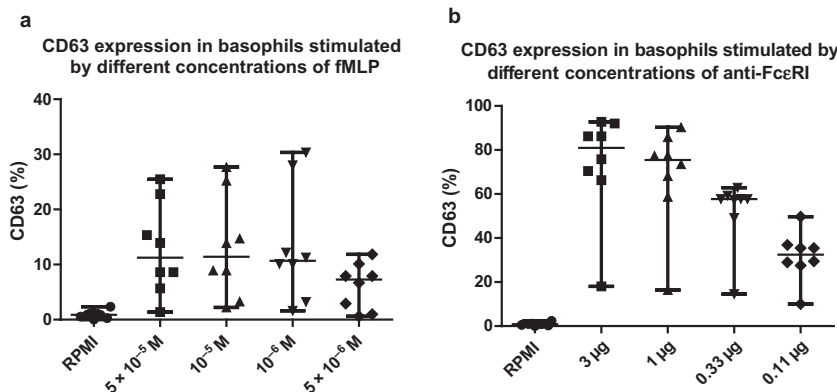


FIG. 2. Basophil activation responses to different concentrations of fMLP and anti-FcεRI antibody (dose–response curve). Scatter plots represent the range with whiskers and the median as the middle line. (a) The level of CD63 expression in basophils following activation by fMLP reached a plateau involving four different concentrations. (b) The maximum expression of CD63 in basophils following activation by anti-FcεRI antibody was observed at a concentration of $3 \mu\text{g}/\text{mL}$ and gradually decreased at lower concentrations of stimulator.

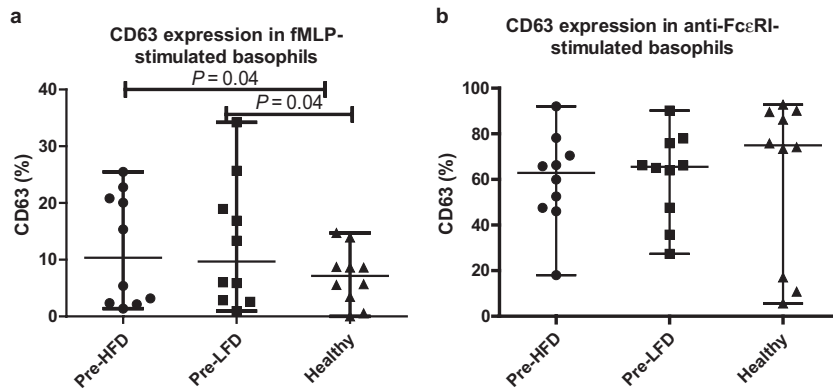


FIG. 3. Basophil activation response to fMLP and anti-FcεRI antibody comparing patients with healthy controls. Scatter plots represent the range with whiskers and the median as the middle line. A P value <0.05 was considered significant. (a) CD63 expression in fMLP-activated basophils was significantly different comparing the samples from patients before dialysis with high-flux dialyzer (HFD) with those of healthy controls. Basophils from patients before dialysis with low-flux dialyzer (LFD) also expressed different levels of CD63 compared with healthy controls. (b) The activation of basophils by anti-FcεRI was not significantly different between the groups.

CD63 expression in fMLP-activated basophils from patients was significantly higher compared with that in healthy controls (Fig. 3a). However, basophil activation following stimulation by anti-FcεRI antibody was not significantly different between the groups (Fig. 3b).

Difference in basophil activation response following hemodialysis with high-flux and low-flux dialyzer membranes

The CD63 expression in fMLP- and anti-FcεRI antibody-activated basophils was analyzed before and after hemodialysis with each type of dialyzer membrane.

Following stimulation with fMLP, we observed no significant differences in CD63 expression comparing predialysis samples with postdialysis samples in any type of dialyzer; however, there was increased cell surface expression of CD63 on basophils after dialysis with the low-flux dialyzer compared with the high-flux dialyzer ($P = 0.01$) (Fig. 4a).

The results demonstrated that anti-FcεRI antibody-activated basophils expressed significantly higher levels of CD63 following hemodialysis with

the low-flux membrane (compared with before dialysis) ($P = 0.002$). This finding was in contrast with that of cells subjected to interaction during high-flux hemodialysis. Moreover, basophil activation response to anti-FcεRI antibody in low-flux membrane-treated cells was significantly higher than in high-flux membrane-treated cells ($P = 0.002$) as shown in Fig. 4b.

Flow cytometric analysis of surface expression of CD11b, active CD11b, and CD88 on neutrophils

No significant differences were observed in terms of CD11b expression (MFI) on neutrophils before stimulation (baseline) when comparing dialysis patients with healthy controls and comparing high-flux dialyzer with low-flux dialyzer (Fig. 5a). In order to evaluate whether the cells had been differently primed, cells were stimulated with IL-8, a major factor regulating the expression of CD11b (22). The surface expression of CD11b (MFI) was increased after stimulation with IL-8, but the expression was similar when comparing cells subjected to high flux with those subjected to low flux and cells from healthy controls (Fig. 5b).

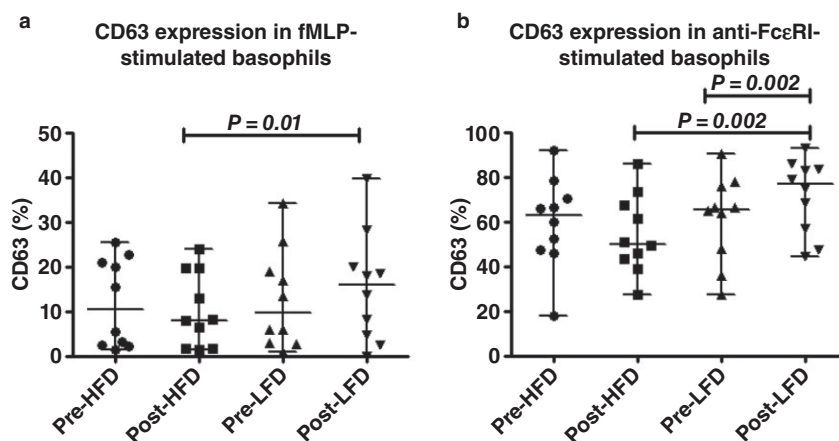


FIG. 4. CD63 expression in activated basophils following dialysis with low-flux and high-flux dialyzers. Scatter plots represent the range with whiskers and the median as the middle line. A P value <0.05 was considered significant. (a) The basophil activation response following stimulation with fMLP was significantly different when comparing samples obtained after dialysis with the low-flux dialyzer (LFD) to those after dialysis with the high-flux dialyzer (HFD). (b) Anti-FcεRI antibody-activated basophils expressed higher levels of CD63 after dialysis with the LFD membrane compared with before dialysis and also compared with those that underwent dialysis with the HFD.

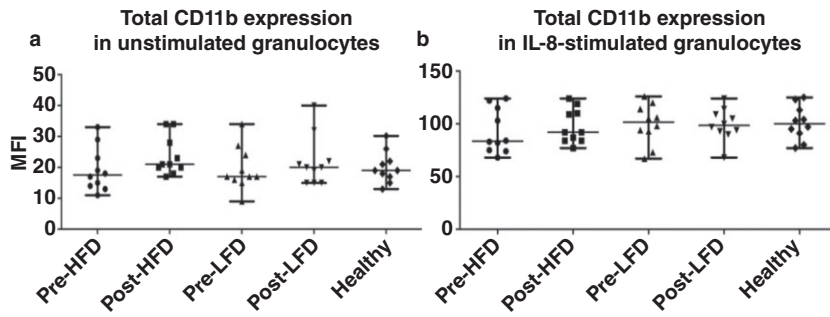


FIG. 5. Total CD11b expression on the surface of neutrophils before (baseline) and after stimulation with IL-8. Scatter plots represent the range with whiskers and the median as the middle line. $P < 0.05$ was considered significant. (a) Total CD11b expression on neutrophils before stimulation with IL-8. (b) Total CD11b expression on neutrophils after stimulation with IL-8. There were no significant differences between the groups.

There were no significant differences in active CD11b expression (percentage) on neutrophils before stimulation (baseline) comparing hemodialysis patients with healthy controls or comparing the effects of high-flux and low-flux dialyzers (Fig. 6a). Furthermore, no significant differences were observed in active CD11b expression (percentage) on neutrophils after stimulation with IL-8 comparing dialysis patients with healthy controls and comparing high-flux and low-flux dialyzers (Fig. 6b).

There were no significant differences in the expression of CD88 on neutrophils after hemodialysis comparing high-flux and low-flux dialyzers (data not shown). The data were similar to those in healthy controls.

DISCUSSION

In the present study, we demonstrate for the first time that blood interaction with dialysis membranes impacts the responsiveness of basophils in patients on maintenance hemodialysis. Moreover, responsiveness of basophils differs according to whether low- or high-flux polysulfone membranes are used. Interestingly, no concomitant changes in neutrophil activation were noticed.

Our data demonstrate that the basophil response to fMLP was higher in the dialysis patient group compared with healthy individuals. However, anti-FcεRI stimulation response did not differ between the patients and healthy subjects. It is not known whether this difference in fMLP response is a conse-

quence of altered expression of fMLP receptors or a consequence of primed intracellular pathways. fMLP binds to receptors expressed on basophils, the formyl peptide receptor (FPR) and formyl peptide receptor-like 1 (FPRL1), which activates intracellular signaling pathways such as the MEK–ERK pathway and leads to chemotaxis and release of mediators such as leukotriene C4 and histamine (23,24). However, better understanding of the mechanisms involved in fMLP activation of basophils in dialysis patients will require further investigations.

We also report a difference in FcεRI-mediated activation when basophils pass through a dialyzer. The basophil response was increased after passage through the low-flux polysulfone dialyzer but unchanged following passage through the high-flux membrane. Cross-linking of Fcε receptors on the basophil surface results in a stronger response than activation by fMLP. It has been shown that patients undergoing hemodialysis may develop anaphylactic or anaphylactoid reactions. The underlying mechanism involved in hemodialysis-associated anaphylactic reactions may be related to activation of basophils through cross-linking of FcεRI on the surface of basophils and rapid release of histamine and heparin and a delayed release of IL-4 (18). IL-4 is regarded as a key mediator in the tuning of the T-cell differentiation, favoring a Th2 response (25,26). The question we addressed in the present study was whether the differences in pore size and ultrafiltration coefficient

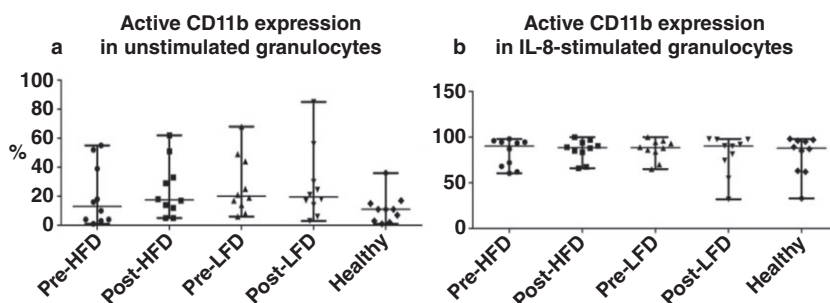


FIG. 6. CD11b active epitope expression on the surface of neutrophils before (baseline) and after stimulation with IL-8. Scatter plots represent the range with whiskers and the median as the middle line. A P value < 0.05 was considered significant. (a) Active CD11b expression in neutrophils before stimulation with IL-8. (b) Active CD11b expression in neutrophils after stimulation with IL-8. There were no significant differences between the groups.

in high- and low-flux polysulfone membranes have an impact on cell activation, as the clinical use of high-flux dialyzers has been suggested to reduce cardiovascular mortality and improve survival (13,14), even though there is an ongoing debate regarding the superiority of high-flux dialyzers over low-flux ones (27). As high-flux dialyzers are more efficient in removing immune modulators generated during the dialysis procedure, this is in line with our finding of increased basophil activation after low-flux hemodialysis.

Previous studies have demonstrated that the hemodialysis procedure may lead to neutropenia, monocytopenia, and thrombocytopenia (28–30). Therefore, we analyzed the absolute number of basophils and did not detect any significant effects on cell number following passage through the different dialyzers. This implies that the variation in the basophil response was due to functional changes and not a consequence of entrapment of low-responding cells in the dialyzer.

The state of neutrophil activation has previously been shown to be a sensitive marker of biocompatibility in hemodialysis. For example, during neutrophil adhesion and transendothelial migration, there is a rapid translocation of CD11b (a member of the integrin family) from an intracellular pool to the cell surface, followed by receptor aggregation and conformational change in the active epitope (31). It is well established that different dialyzer surfaces impact the function of neutrophils. It has been reported that CD11b expression on neutrophils increases at the beginning of hemodialysis with several types of dialyzers but declines to basal level at the end of the procedure with biocompatible dialyzers such as polysulfone membranes, while it remains high after dialysis with bioincompatible membranes (32,33). In the present study, we investigated both the surface expression of CD11b and the expression of the active epitope on neutrophils but found no significant differences in the expression of either marker after one session of hemodialysis between the low-flux membrane and the high-flux polysulfone membrane.

It has been shown that the hemodialysis procedure activates several hemostatic pathways such as generation of C3a and C5a (anaphylatoxins) (34), but it has also been reported that neither C5a levels in plasma nor C5a levels in the dialysate compartment increase after dialysis with high-flux and/or low-flux polysulfone dialyzers (35). As the molecular weight of C5a is similar to that of β_2 -microglobulin, this anaphylatoxin can be cleared by high-flux membranes due to a different kinetic gradient. We therefore investigated the expression of C5a receptor type 1 (CD88) on neutrophils as a cellular marker for

complement activation. Following interaction with the ligand C5a, the receptors internalize within minutes to induce responses such as chemotaxis of neutrophils with up-regulation of CD11b, granule enzyme release, superoxide anion production, and platelet–neutrophil microaggregation (36). In a previous study, a high-flux dialysis membrane induced a decrease in C5a receptor expression on neutrophils in three out of four patients, but internalized C5aR expression showed a nonsignificant change (37). We did not find any significant differences in CD88 expression on neutrophils from patients undergoing high-flux hemodialysis compared with low-flux dialysis, which could be due to the difference in number of study subjects or insensitivity of this molecule for assessment of bioincompatibility.

The hemodialysis procedure induces secretion of several inflammatory mediators besides anaphylatoxins that can impact basophil function, such as IL-1 β and tumor necrosis factor α , from activated monocytes. Of interest is that earlier studies reported a correlation between the cytokine IL-1 and the induction of histamine release from basophils (38). Bradykinin generation has been reported to be increased during hemodialysis with polysulfone membranes, which can be explained by the fact that the membrane surface carries negatively charged groups, which may activate the Hageman factor (coagulation factor XII), known to convert prekallikrein to kallikrein, which will generate bradykinin from high-molecular-weight kininogen (39,40). The bradykinin can trigger the IgE-independent activation of basophils to induce an anaphylactoid reaction during hemodialysis (18). Interestingly, we have also recently shown that the level of eotaxin, a potent chemoattractant to basophils (acting through CCR3 receptors), was increased in patients with CKD (41).

CONCLUSION

This study demonstrates that the responsiveness of basophils to anti-Fc ϵ RI stimulation is affected when the cells pass through a dialyzer. These changes differ between high-flux and low-flux polysulfone dialyzers in patients with chronic kidney disease. Moreover, we also report an increased responsiveness to fMLP in patients on hemodialysis compared with healthy controls. Together, these data demonstrate that basophil activation may be a new and more sensitive marker for evaluating biocompatibility properties of dialyzers.

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