Original Article

Cell-associated adhesion molecules as early markers of bioincompatibility

M. P. Carreno, S. Stuard, M. Bonomini, N. Settefrati, C. Tetta, A. Albertazzi and N. Haeffner-Cavaillon

Institut National de la Santé et de la Recherche Médicale (INSERM U430), Hôpital Broussais, 75674 Paris Cedex, Istituto di Nefrologia, Università G. D’Annunzio, Chieti, Bellco S.p.A. Mirandola, Modena, Italy.

Abstract

Background. Transient nature of adhesive interactions occurring during cell margination is mainly dependent on expression of selectins which are shed by activated cells. This shedding in the circulation may play an important role as anti-inflammatory mediator. Haemodialysis is also associated with P-selectin (CD62P)/sialyl-Lewisx (CD15s) interactions which mediate platelet–leukocyte coaggregation. We further investigated the mechanisms underlying leukocyte margination during haemodialysis.

Methods. CD15s, CD11b and CD61 expression on circulating leukocytes from patients dialysed on synthetic membranes (modified polyacrylonitrile (SPAN), polysulphone (PS), and polyacrylonitrile (AN69) was assessed by cytofluorometry in a prospective crossover trial. We measured plasma levels C3a/C3a desArg, soluble CD62P, and CD62E molecules obtained from patients and healthy individuals.

Results. Expression of CD11b and CD15s was upregulated on neutrophils from patients dialysed with SPAN and PS membranes during the dialysis session. A significant negative correlation was found between the expression of CD11b or CD15s molecules and neutrophil counts as well as between CD15s expression and monocyte counts during haemodialysis. As assessed by CD61 expression on leukocytes, we observed that platelets bound significantly onto both neutrophils and monocytes during dialysis with both membranes. A significant positive correlation was found between the expression of CD11b molecules and the percentage of CD61+ monocytes counts during SPAN and PS dialysis. We found a significant increase of soluble CD62P in plasma samples obtained from haemodialysed patients before the dialysis session as compared to the levels detected in plasma from healthy individuals.

Conclusions. This study documents a major role of CD15s, CD11b, CD61, CD62P molecules in the transient leukocytes activation and margination during haemodialysis on synthetic membranes despite their low complement-activating properties.

Key words: adhesion molecules; biocompatibility; haemodialysis; leukocytes.

Introduction

Cell adhesion processes are activated during haemodialysis procedure and are able to initiate the leukocyte inflammatory response that itself may play an important role in the adverse effects of haemodialysis. Peripheral leukopenia during the early phase of haemodialysis is due in part to an accumulation of leukocytes in the lung vasculature [1–3]. It has been described that during inflammation adhesion molecules are involved in cell margination, activation, and subsequent transendothelial migration [4,5]. During haemodialysis using cellulosic membranes, several studies have shown that leukopenia coincides with complement activation [9–15] and is associated with an increased expression of CD11b molecule on leukocytes [16–19]. The early events of leukocytes margination involve the interaction of selectins with their ligands and we have previously reported that the transient margination observed during the haemodialysis procedure is associated with an upregulation of CD15s, a ligand of selectins, on leukocytes [20]. We have also observed that increased expression of CD15s on leukocytes was not dependent on anaphylatoxins generation. Haemodialysis is also associated with P-selectin (CD62P)/sialyl-Lewisx (CD15s) interactions which mediate platelet–leukocyte coaggregation [21]. Adhesion of activated platelets to leukocytes may induce a reciprocal cell activation which could mediate cellular damage to the endothelium [22–30]. The transient nature of adhesive interactions occurring during cell margination is mainly dependent on the transient expression of selectins which are shed by activated cells [31,32]. This shedding in the circulation may play an
important role as anti-inflammatory mediator [33–37]. Regulation of the inflammatory processes, induced during haemodialysis may be dependent on the ratio between the amount of CD62P expressed on activated platelets and endothelial cells and that of soluble CD62P released in the blood.

The purpose of our study was to further investigate the mechanisms underlying leukocyte margination during haemodialysis. Polycrylonitrile (AN69), a new modified polycrylonitrile (SPAN) and polysulphone (PS) were selected in a prospective crossover trial to reduce individual's variability. We have compared the expression of CD11b and CD15s molecules on circulating leukocytes, the formation of platelet-leukocyte microaggregates and the production of C3a and selectins in plasma. The results indicate that despite the low-complement activating property of all membranes used, PS and SPAN membranes, there was an increased expression of CD15s, CD61, and CD11b on circulating leukocytes from the patients. We also observed, that whatever membrane used, all patients had high levels of circulating soluble P-selectin before starting the dialysis procedure as compared to healthy individuals. The levels were not affected during the dialysis session. The plasma levels of E-selectin remained unchanged in patients dialysed with the three types of membrane and were not statistically different from those observed in healthy individuals.

**Subjects and methods**

**Patient population**

Six stable end-stage renal disease patients (4 men and 2 women between 43 and 67 years old; (mean ± standard deviation 51 ± 9) dialysed for more than 9 months on a stable anticoagulation regimen, and 11 healthy volunteers, were selected for study. Except for renal failure, the patients were in good health. Positive history of first use syndrome, clinical evidence of infection at the time of the study, cardiac and vascular instability, unstabilized erythropoietin dosage and single needle dialysis were exclusion criteria. None of the patients had received any medication known to affect leukocyte, platelet, or endothelial cell function for 2 weeks prior to the study. Informed consent was obtained from each patient.

**Study design**

The study covered 6 weeks of treatment, with patients treated for 2 weeks on each dialyser. The dialyser sequence was randomized. Blood samples were collected for analysis during the fifth treatment. The three membranes tested were polycrylonitrile (Hospal H12-10, 1.3 m², ETO-sterilised), polysulphone (Bellco, BLS 632, 1.9 m², ETO-sterilized), and SPAN a new modified polycrylonitrile membrane with reduced negative charges (Bellco, PA1830, 1.8 m², ETO sterilized).

A bicarbonate-based dialysate was used in all cases. Dialysers were rinsed single-pass with 2.0 litres of 0.9% NaCl solution containing 7500 IU heparin. Heparin was the sole anticoagulant used throughout all treatments with an intermittent infusion. The patient’s individual heparin regimen was maintained during the study. Blood flow rates were between 250 and 300 ml/min. Ultrafiltration rates were among 500 and 1000 ml/h. Venous blood from haemodialysed patients and healthy individuals was collected in K3 EDTA Vacutainers and then immediately stored at 4°C before use.

Blood samples were drawn pre-dialysis (T:0) from the afferent line of the dialyserr and then subsequently from the efferent line of the dialyser at T:8 and T:15 min, and at the end of the dialysis session (T:240).

Dialysates were free of LPS as assessed by LAL.

**Reagents and buffers**

Phosphate-buffered saline (PBS) was from bioMérieux (Marcy l’Etoile, France). Serum Albumin Bovine (BSA) and sodium azide (NaN3) were from Sigma Chemical Co. (St. Louis, MO, USA). Mouse monoclonal anti-human CD61, anti-CD15s, anti-CD11b, anti-CD61, anti-CD62P, FITC-conjugated anti-CD15, PE-conjugated anti-CD14, and isotype Ab controls were purchased from Becton Dickinson (Mountain View, CA, USA) and Immunotech (Marseille, France). FITC-conjugated anti-mouse IgG(ab')2 fragments was obtained from Silenus Laboratories (Australia). Recombinant C5a was from Sigma and recombinant TNFα from R&D Systems (Minnepolis, USA).

**Flow cytometric analysis**

**Neutrophils and monocytes** Whole blood (300 µl) was incubated with decomplemented human AB serum (1.5 ml) for 10 min at room temperature to decrease non-specific binding, and then centrifuged at 4°C. The pellet was incubated with 1 µg of first MoAb diluted in PBS-BSA 1%-sodium azide 0.1% (staining medium) or 10 ml of conjugated mAb for 30 min at 4°C. Samples were washed twice in PBS-BSA 1%. When the first mAb was unconjugated, samples were incubated with a second-affinity isolated FITC-conjugated anti-mouse IgG(ab')2, fragment (50 µl of 1/100 dilution) for 30 min at 4°C. Cells were washed once in staining medium. Erythrocytes were lysed by adding 2 ml of lysing solution (FACS lysing solution, Becton Dickinson) for 5 min at room temperature. This last procedure was repeated until complete lysis of red blood cells occurred. Samples were washed once with the staining medium, once with PBS. Cells were fixed by adding 0.3 ml of cold paraformaldehyde (1%) and stored in the dark at 4°C until analysis. Stained cells were characterized by flow cytometric analysis (EPICS–Elite, Coulter Electronics). The instrument was calibrated twice weekly for fluorescence and light scatter using calibrates beads (Coulter). Fluorescence parameters were collected using 3-decade logarithmic amplification. Neutrophils and monocytes were identified in the linear forward versus side-scatter plots by use of subgroup-specific anti-MoAbs: CD15 and CD14 for neutrophils and monocytes respectively. Mouse isotypic antibodies were used as negative controls.

**Platelets**

CD62P is a specific marker of activated platelets [37] whereas CD61 is a marker of resting and activated platelets [38]. A total of 100 µl EDTA anticoagulated whole blood was incubated for 2 h at 4°C with 1 ml of 1% cold paraformaldehyde. The fixed cells were centrifugated at 1200 g for 5 min at room temperature; the pellets were washed twice in 1 ml PBS with 0.1% azide. Fifty microlitres of fixed blood suspension
were incubated with 20 μl of mAb. After 20 min incubation at room temperature, samples were washed once in 1 ml of PBS 0.1% azide. Samples were then incubated with the second anti-mouse FITC-IgG(ab')2 fragments (50 μl of 1/100 dilution) in the dark, at room temperature, for 20 min. Samples were washed once in 1 ml of PBS 0.1% azide. Platelets were fixed by adding 1 ml of cold 1% paraformaldehyde and stored in the dark at 4°C. Stained platelets were characterized by flow cytometric analysis (EPICS-Elite, Coulter Electronics). The FACScan instrument was set to measure logarithmic FSC, logarithmic SSC. Fluorescence parameters were collected using 3-decade logarithmic amplification. Platelets were identified in the logarithmic FSC versus logarithmic SSC plot by use anti-moAb CD61: the gated platelet population was found to bind >90% the anti-CD61. Mouse IgG1 was used as a negative control.

Platelet–leukocyte interaction

MoAb-anti CD61 was used to evaluate platelet–leukocyte interaction during haemodialysis with SPAN, polysulphone, and polyacrylonitrile (AN69) membranes. The percentage of CD61 expression on neutrophils and monocytes was determined to characterize platelet leukocyte interaction. Neutrophil and monocyte surface staining with MoAb anti-CD61 and their flow cytometric analysis were performed as described above.

Six thousand events were collected in list mode files for neutrophils and monocytes and were analysed on computer equipped with EPICS–Elite software program.

In vitro stimulation of whole blood cells

Human blood (n = 3) was collected from healthy individuals in dry Vacutainers and in K3 EDTA Vacutainers. Whole blood cells were stimulated with rTNFα (100 ng/ml), Neisseria meningitidis LPS (10 μg/ml) or recombinant C5a (rC5a; 250 ng/ml) for 30 min at 37°C. Leukocyte samples were then kept in cold. Neutrophils and monocytes were stained with anti CD 14 and anti-CD 15 to allow cell characterization and with anti-CD15s, anti-CD11b, and anti-CD49d as described above.

Measurement of P-selectin, E-selectin and C3a desArg plasma concentrations

The plasma concentrations of P-selectin and E-selectin were measured by ELISA (Bender Med System, Austria and R & D Systems, UK respectively), and C3a desArg by radioimmunoassay method (Amersham, France).

Analytical methods

Blood cell counts were performed using a Coulter counter (Coultronics, USA).

Statistics

Values are expressed as mean ± SEM. Statistical comparisons were made using Statistica for Windows, version 3.1. Statistical analysis was performed to compare results during the dialysis session with predialysis values for the samples obtained from the patients dialysed with the same membranes. For the analysis of sequential data, a repeated measures analysis of variance was used. Student–Newman–Keuls range test was applied when the F test was significant. Additional comparisons were made between SPAN, polysulphone, and polyacrylonitrile membranes at the same time points by analysis of variance followed by t test for significant differences. Student’s t test was also used to compare patients with healthy individuals (unpaired data) and values obtained at different time points during in vitro studies (paired data).

Results

Leukocytes count

Figure 1 depicts the changes in neutrophil and monocyte counts that occurred in patients (n = 6) haemodialysed using SPAN, polysulphone and polyacrylonitrile (AN69) membranes during the prospective crossover trial. At 8 min after the initiation of dialysis with SPAN membranes, there was a significant decrease in neutrophil and monocyte counts (P < 0.02 and P < 0.02 respectively), whereas at 15 min, only the number of monocytes significantly dropped compared to predialysis values (P < 0.02). At the end of the dialysis session, neutrophil and monocyte counts returned to predialysis levels. During the dialysis session with polysulphone and polyacrylonitrile (AN69) membranes, leukocyte counts also decreased but not significantly, according to the statistical analysis.

A direct time point comparison of neutrophil counts during dialysis session in patients dialysed with polyacrylonitrile (AN69) versus the other membranes revealed statistically significant differences at 8 min with SPAN (P < 0.05) and at 15 min with polysulphone (P < 0.02). Similar comparison of monocyte counts revealed a significant difference between polyacrylonitrile (AN69) and SPAN at 8 min (P < 0.05) and 15 min (P < 0.05).

Expression of CD15s on leukocytes during haemodialysis with SPAN, polysulphone, and polyacrylonitrile (AN69) membranes

Expression of CD15s was assessed by cytofluorometric analysis on neutrophils and monocytes at the same time points as those depicted in Figure 1. Expression of CD15s on neutrophils increased significantly at 8 min during haemodialysis with SPAN membranes (40.4 ± 4.8 T:8 versus 28.4 ± 1.3 T:0, P < 0.03) and decreased to values below those obtained prior to dialysis at the end of the session (17.4 ± 2.1 T:240 versus 28.4 ± 1.3 T:0, P < 0.03) (Figure 2). CD15s antigen expression also increased on monocytes, obtained from the same patients, at 8 min of the dialysis session (14.6 ± 2.2 T:8 versus 8 ± 0.2 T:0, P < 0.03). However, at 15 min after the initiation of haemodialysis, CD15s expression remained elevated as compared to predialysis values (10.5 ± 1.5 T:15 versus 8 ± 0.2 T:0, P < 0.03). Expression of CD15s was slightly increased on neutrophils when patients were dialysed with polysulphone membranes, whereas on monocytes it
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NEUTROPHILS

MONOCYTES

Fig. 1. Time course of neutrophil and monocyte counts during dialysis with (C) SPAN, (B) polysulphone, and (D) polyacrylonitrile (AN69) membranes. Neutrophil and monocyte counts were determined for six patients dialysed either with SPAN, polysulphone, or polyacrylonitrile membranes. Results are expressed as mean ±SEM*. *P<0.03 compared with predialysis (T0). #P<0.05 SPAN versus polyacrylonitrile at the same time. §P<0.02 polysulphone compared with polyacrylonitrile at the same time.

NEUTROPHILS

MONOCYTES

Fig. 2. Modulation of neutrophil and monocyte CD15s expression during dialysis with (C) SPAN, (B) polysulphone, and (D) polyacrylonitrile (AN69) membranes. Expression of CD15s was analysed on neutrophils and monocytes obtained from the same patients at the same time points as those depicted in Figure 1. Results are expressed as mean fluorescence intensity ±SEM of surface CD15s on neutrophils (left panel) and monocytes (right panel). *P<0.03 compared with predialysis (T0). #P<0.04 SPAN versus polyacrylonitrile at the same time.

Expression of CD11b on leucocytes during haemodialysis with SPAN, polysulphone, and polyacrylonitrile (AN69) membranes

Expression of CD11b on neutrophils from patients dialysed with SPAN and polysulphone membranes (Figure 3, left panel) increased significantly at 8 min and remain elevated at 15 min compared to predialysis values (SPAN: 3.5 ±0.6 T:0 versus 8.5 ±0.7 T:8, P<0.005; polysulphone: 3.4 ±0.3 T:0 versus 7.8 ±0.6 T:15, P<0.005).

At the end of dialysis session, CD11b expression returned to predialysis values.

increased significantly at 8 min to reach maximal values at 15 min (7.7±0.7 T:0 versus 11.4±1.4 T:8, P<0.03; 7.7±0.7 T:0 versus 12.1±1 T:15, P<0.03). At the end of dialysis, CD15s expression returned to predialysis values. No changes in CD15s expression occurred on neutrophils and monocytes obtained from patients dialysed with polyacrylonitrile (AN69) membranes.

A direct time point comparison of CD15s expression on monocytes following dialysis with polyacrylonitrile (AN69) versus others membranes revealed a statistically significant difference at 8 min with SPAN (P<0.04); for neutrophils there was no statistical difference between the three membranes.
Fig. 3. Modulation of neutrophil and monocyte CD11b expression during dialysis with (E) SPAN, (B) polysulphone, and (C) polyacrylonitrile (AN69) membranes. Expression of CD11b was analysed on neutrophils and monocytes obtained from the same patients at the same time points as those depicted in Figure 1. Results are expressed as mean fluorescence intensity ±SEM of surface CD15s on neutrophils (left panel) and monocytes (right panel). *P<0.005 compared with predialysis (T0). **P<0.01 compared with predialysis (T0). #P<0.02 SPAN versus polyacrylonitrile at the same time. $P<0.02 SPAN versus polyacrylonitrile at the same time.

At 8 and 15 min, the only significant difference in CD11b expression, as compared to predialysis values occurred with monocytes obtained from patients dialysed with polysulphone membranes (3.2±0.1 T:0 versus 4.6±0.3 T:8, P<0.005; 3.2±0.1 T:0 versus 4.2±0.2 T:15, P<0.01) (Figure 3, right panel). No changes in CD11b expression were observed with leukocytes obtained from patients dialysed with polyacrylonitrile (AN69) membranes.

A direct time point comparison of CD11b expression on neutrophils following dialysis with polyacrylonitrile (AN69) versus SPAN and polysulphone indicated statistical differences at 8 min (AN69 versus SPAN, P<0.02; AN69 versus polysulphone, P<0.02) and at 15 min (AN69 versus SPAN, P<0.02; AN69 versus polysulphone, P<0.02). Similar comparison of CD11b expression on monocytes was performed and showed statistical differences between polyacrylonitrile (AN69), SPAN, and polysulphone at 8 min (AN69 versus SPAN, P<0.02; AN69 versus polysulphone, P<0.02) and at 15 min (AN69 versus SPAN, P<0.02; AN69 versus polysulphone, P<0.02), as well as between polysulphone and SPAN, at the same time points (T:8, polysulphone versus SPAN, P<0.02; T:15, polysulphone versus SPAN, P<0.02). According to the data, we observed a negative correlation between CD15s expression and neutrophil or monocyte counts (Figure 4) indicating that CD15s is directly involved in the margination of circulating neutrophils and monocytes during haemodialysis using SPAN devices.

**Binding of platelets to leukocytes during haemodialysis**

We have investigated the binding of platelets on neutrophils and monocytes during haemodialysis. The results depicted in Figure 5 indicated that platelets bound to neutrophils within 8 min of the dialysis session with SPAN and polysulphone membranes. Relative number of CD61 positive neutrophils reached maximal within 8 min (SPAN T:8, 15±1; polysulphone T:8, 20±2) and remain elevated at 15 min (SPAN, T:15, 12±2; polysulphone T:15, 16±2) then decreased to predialysis values at the end of the dialysis session. Similar results were observed for monocyte–platelet microaggregates during dialysis with SPAN and polysulphone membranes at the same time points (SPAN T:8, 71±4; polysulphone T:8, 74±4), (SPAN T:15, 69±4; polysulphone T:15, 71±5). No changes occurred with cells from patients dialysed with polyacrylonitrile (AN69) membranes.

A direct time point comparison of CD61 positive neutrophils following dialysis with polysulphone versus other membranes revealed statistical differences at 8 min with AN69 (P<0.005), at 15 min with SPAN and AN69 (P<0.05 and P<0.005 respectively) and at the end of dialysis session with SPAN (P<0.05). Similar comparison of CD61 positive monocytes also indicated a statistical difference between polysulphone and SPAN at the beginning and at the end of dialysis session (P<0.005 and P<0.05 respectively).

According to our data, we observed a significant positive correlation between the expression of CD11b on neutrophils and the time course of neutrophil–platelet aggregation during haemodialysis with SPAN (P=0.0001), polysulphone (P=0.0002), and polyacrylonitrile (P=0.002) (Figure 7) as well as between CD11b expression on monocytes and the time course of monocyte–platelet aggregation during dialysis with SPAN (P=0.005) and polysulphone (P=0.0001) (Figure 6).
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**NEUTROPHILS**

**MONOCYTES**

Fig. 4. Correlation curves between time course of neutrophils counts (x axis) and mean fluorescence intensity of CD15s and CD11b (y axis) on neutrophils (upper panels) and monocytes (lower panels) during SPAN dialysis session. A significant negative correlation was found between the expression of CD11b molecules \( (P < 0.004) \) and neutrophil counts, as well as between CD15s molecules and cell counts \( (P < 0.006) \). A significant negative correlation was also found between CD15s \( (P < 0.01) \) and monocyte counts during dialysis.

These data (Figures 6, 7) highlight the fact that CD11b which is one of the fibrinogen receptors, could be directly involved in platelet adhesion to circulating neutrophils and monocytes.

In order to get some insights into the mechanisms involved in upregulation of CD15s and CD11b expression on neutrophils and monocytes, we have incubated whole blood cells with recombinant TNFa, C5a, and LPS for 30 min at 37°C. The results shown in Figure 8 indicated that none of the stimuli tested modified CD15s expression on both cell types, whereas CD11b expression was increased by TNFa, C5a, and LPS. Expression of CD49d was used as negative control.

**Plasma C3a/C3a desArg, soluble P-selectin, and E-selectin levels**

We measured the plasma concentrations of C3a/C3a desArg, P-selectin, and E-selectin obtained from the haemodialysed patients and from healthy individuals. C3a/C3a desArg levels (ng/ml) were determined at T:0, T:8, and T:15, and were 992 ± 237, 1164 ± 245, and 923 ± 115 at T:0 in plasma from patients dialysed on SPAN, PS, and AN69 respectively; 1149 ± 258, 1204 ± 392, and 981 ± 173 at T:8; 1124 ± 272, 1688 ± 346, and 833 ± 174 at T:15. C3a/C3a desArg plasma levels in healthy individuals were 325 ± 30. P-selectin and E-selectin levels were assessed by ELISA in the same samples obtained from the haemodialysed
Fig. 5. Effect of dialysis with (□) SPAN, (■) polysulphone, and (□) polyacrylonitrile (AN69) membranes on platelet–neutrophil and platelet–monocyte aggregation. Platelets bound significantly to neutrophils and monocytes during the dialysis session. Results are expressed as percentage of neutrophils (left panel) and monocytes (right panel) CD61 positive ±SEM. *P < 0.005 compared with predialysis (T0). **P < 0.02 compared with predialysis (T0). †P < 0.002 SPAN versus polyacrylonitrile at the same time. #P < 0.002 polysulphone compared with polyacrylonitrile at the same time. °P < 0.05 SPAN versus polyacrylonitrile at the same time.

Fig. 6. Correlation curves between time course of monocyte-platelet aggregation (percentage monocytes CD61+, x axis) and mean fluorescence intensity of CD11b (y axis) on monocytes, during SPAN, polysulphone, and polyacrylonitrile haemodialysis. Significant positive correlations were found between the expression of CD11b molecules and percentage monocytes CD61+ counts during SPAN (P = 0.005) and polysulphone dialysis sessions (P = 0.0001).

Discussion

We have previously reported that haemodialysis is associated with an increased expression of adhesive molecules involved in cell margination and in the formation of platelet–leukocyte coaggregates [20]. It has been recently demonstrated that molecules bearing Sialyl-Lewis x motifs can mediate cell tethering and rolling under physiological flow conditions of E- and L-selectin-expressing cells [39] and we have shown that the transient margination occurring during haemodialysis is associated with an upregulation of CD15s, a ligand of selectins [20]. Adhesion of activated platelets to leukocytes may induce reciprocal activation, which could mediate damage to the endothelium and there-
fore mediate some of the deleterious effects associated with chronic haemodialysis [22–30]. Selectins have been reported to be involved in the regulation of integrin function. This effect could result from activation of the cells by PAF, which has been shown to induce cell polarization and upregulation of β2 integrins and priming of leukocytes [40, 41].

In the present work we focused on the expression of CD15s, CD11b, CD61 on both neutrophils and monocytes during haemodialysis using three different synthetic membranes, known to have low complement-activating properties in a prospective crossover trial study. It should be pointed out that during the whole of the study particular attention has been taken to prevent the contamination of dialysate with bacterial endotoxins. Despite the fact of low complement-activating property of polysulphone and SPAN membranes, we observed a maximal increase in CD15s

![Fig. 7. Correlation curves between time course of neutrophil-platelet aggregation (% neutrophil CD61 ′+, x axis) and mean fluorescence intensity of CD11b (y axis) on neutrophil, during SPAN, polysulphone, and polyacrylonitrile haemodialysis. Significant positive correlations were found between the expression of CD11b molecules and percentage neutrophils CD61 ′ counts during SPAN (P = 0.0001), polysulphone (P = 0.0002), and polyacrylonitrile haemodialysis sessions (P = 0.002).](image)

![Fig. 8. Stimulation of whole blood from healthy individuals with recombinant TNFα, C5a and N.m LPS. Expression of CD15s and CD11b antigens on neutrophils (left panel) and monocytes (right panel) after incubation of whole blood with TNFα (100 ng/ml), C5a (250 ng/ml) and LPS (10 μg/ml). Neutrophils and monocytes were gated using anti-CD15 and CD14 markers respectively. Results expressed as mean fluorescence intensity are from one representative donor out of three.](image)

| Table 1. Effect of haemodialysis on plasma levels of P-selectin and E-selectin |
|----------------------------------|---------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Soluble selectins | Healthy individuals (n = 11) | Membranes | Haemodialysis patients (n = 6) |
|--------------------|------------------------------|-----------|-----------------------------|-----------------------------|
|                    |                               | T : 0     | T : 8                       | T : 15                      | T : 240                       |
| P-selectin (ng/ml) | 175 ± 28                      | SPAN      | 571 ± 76*                   | 508 ± 72                    | 482 ± 75                     | 560 ± 135                    |
|                    |                               | Polysulphone | 551 ± 83*                  | 448 ± 84                    | 548 ± 101                    | 594 ± 85                     |
|                    |                               | AN69     | 648 ± 56*                   | 621 ± 50                    | 707 ± 97                     | 737 ± 63                     |
| E-selectin (ng/ml) | 96.5 ± 8                      | SPAN      | 67 ± 12                     | 75 ± 14                     | 75 ± 18                      | 71 ± 16                      |
|                    |                               | Polysulphone | 67 ± 13                     | 74 ± 16                     | 69 ± 13                      | 75 ± 16                      |
|                    |                               | AN69     | 76 ± 14                     | 76 ± 13                     | 81 ± 15                      | 82 ± 14                      |

Results are expressed as mean ± SEM. No significant change of soluble P-selectin and E-Selectin with any membrane was observed at different time points of haemodialysis session. *Significantly greater than healthy individuals (P < 0.001).
expression at 8 min of haemodialysis on circulating neutrophils and monocytes from the patients. No significant changes occurred with cells obtained from patients dialysed with polyacrylonitrile (AN69) membranes. Expression of CD11b was upregulated on leucocytes, but to a higher extent on neutrophils. We found, as previously reported [20], a better correlation between the increased expression of CD15s and the leucocyte drop during haemodialysis, rather than CD11b expression and the leucocyte count. Despite the fact that neutrophil margination has been said to result from complement activation, our results strength the fact that modulation of CD15s on leucocytes is independent on anaphylatoxin formation. We observed that dialysis using polysulphone membranes increased C3a generation to a higher degree than dialysis using SPAN membranes, whereas a highest CD15s upregulation was achieved using SPAN membranes as compared to polysulphone devices. Furthermore we observed that recombinant C5a did not upregulate CD15s expression on leucocytes. Preliminary in vitro experiments indicate that CD15s expression can be increased upon stimulation with PAF (data not shown), a lipid mediator known to be generated during haemodialysis [42].

In accordance with the data from Gawaz et al. [43] we did not detect significant changes during dialysis in surface expression of CD62P on platelets, suggesting no change in the functional status of platelets in the systemic circulation. P-selectins are shed by activated platelets and stimulated endothelial cells. We observed that levels of circulating soluble P-selectin were higher in haemodialysed patients as compared to levels present in healthy individuals but were not affected by the dialysis session. In contrast, levels of soluble CD62P increased in patients undergoing cardiopulmonary bypass in which there is a strong complement activation [44]. These observations indicate that shedding of CD62P may be due to activation of the cells through a complement-dependent mechanisms. It was demonstrated that soluble CD62P can act as an anti-inflammatory agent, since the addition of soluble CD62P to TNF-α-activated neutrophils inhibited both their CD18-dependent adhesion to resting endothelial cells and superoxide production [34]. Soluble CD62P could provide a useful marker of platelet activation and vascular endothelial cell perturbation during extracorporeal circulation and contribute as a regulatory factor for cell margination during extracorporeal procedure. It has been described that leukotrienes are generated during neutrophil–platelet interactions [45] and that activated platelets induce superoxide anion release by monocytes and neutrophils through CD62P interaction [35,36]. Preliminary data indicate that the interaction of platelets with neutrophils, which occurs during haemodialysis, induces superoxide anions generation within the cells (unpublished findings).

In the present study we also investigated the expression of CD61 and CD11b as a function of neutrophil and monocyte counts during haemodialysis with the three membranes tested. The data indicated that 60–80% monocytes expressed CD61 antigen and only 15–20% of the circulating neutrophils expressed the molecule. The higher affinity of monocytes for platelets as compared to neutrophils cannot be attributed solely to a higher expression of CD15s on monocytes when compared to neutrophils, since monocytes expressed lower levels of CD15s as compared to neutrophils prior to haemodialysis. It may be due to a different conformation of the CD15s molecule or to the involvement of another monocytic membrane molecule mediating the recognition of platelets. The results also indicate that expression of CD11b was upregulated on neutrophils from patients dialysed with SPAN and polysulphone membranes. We observed a strong correlation between CD11b expression and CD61 expression on neutrophils and monocytes at all time points tested. The CD61 molecule belongs to the gpIIb/IIIa complex with the CD41a, known as the fibrinogen receptor. Fibrinogen has been shown to be involved in the adhesion of activated platelets to neutrophils and a recent work has shown that CD41a is partially involved in the rosette formation of activated platelets to neutrophils [46]. According to these data, it can be proposed that fibrinogen may be involved in the leucocyte–platelets interaction during haemodialysis. We can suggest that the CD15s/CD62P interaction occurs in the first step of leucocyte margination, and stronger firm adhesion involves a CD11b/CD61 and fibrinogen interaction. In conclusion we can postulate that adhesion of platelets to leucocytes is a more complex event that previously anticipated and that several cell receptors or soluble adhesion molecules are involved to control leucocyte margination on endothelium and their activation.

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