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Pyrogen Transfer across High- and Low-flux Hemodialysis Membranes

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Abstract: The extent to which bacterial products from contaminated dialysate enter a patient's blood depends upon the type and permeability of the hemodialysis membrane in use. This study was performed to assess the transfer of pyrogenic substances across both high- and low-flux membranes (DIAPES, Fresenius Polysulfone, Helixone, Polyamide S). All experiments were carried out in the saline–saline model. The dialysate pool was contaminated either with purified lipopolysaccharide (LPS) (250 and 500 EU/mL) or with sterile bacterial culture filtrates (20 EU/mL), and in vitro dialysis was performed under diffusive and convective conditions. A significant transfer of endotoxin was observed for both low- and high-flux DIAPES challenged with either LPS or with bacterial culture filtrates. Under identical conditions, no transfer of endotoxins was detectable across Fresenius Polysulfone and Helixone upon challenge with purified LPS. With bacterial culture filtrates, endotoxin concentrations for Polyamide S and Fresenius Polysulfone were about 10% and 1%, respectively, of those measured for DIAPES, whereas no transfer of endotoxin was detectable for Helixone. Using an alternative assay (induction of interleukin-1 receptor antagonist, IL-1Ra, in whole blood), only the DIAPES membrane showed the passage of cytokine-inducing substances. Thus, when saline is present in both the blood and dialysate compartments (i.e., the situation during predialysis priming procedures), dialysis membranes differ profoundly with respect to their permeability to endotoxins. **Key Words:** Hemodialysis membranes—Endotoxins—Cytokines—Inflammation.

End-stage renal disease (ESRD) patients undergoing routine hemodialysis are exposed to large amounts of dialysis fluid during each treatment. For standard dialysis treatments, patients are exposed to 90–120 L daily, equating to approximately 360 L of water per week (1). It has often been assumed, erroneously, that dialysis fluids used in various countries are either sterile or that they comply with appropriate microbiological water quality standards that have been established to ensure safety for the patient. A number of studies have revealed large variations in the microbiological quality of dialysate and water from one center to another, with many centers failing to comply with microbiological standards (1,2).

The improper design and inadequate microbiological monitoring of water treatment systems promotes biofilm growth on various conduits of the hemodialysis machines and the water treatment system (3). The growth and lysis of bacteria (gram-negative strains) at the sites of biofilm formation leads to the release of a variety of substances, including not only endotoxins, but also peptidoglycans and muramyl dipeptides. These substances are collectively termed pyrogens or cytokine-inducing substances (CIS), as they induce the generation of proinflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α) (4). Contaminated dialysis fluids can therefore provoke a series of pyrogenic reactions which, in extreme cases, may even lead to death (5,6). Additionally, even in the absence of an obvious inflammatory response, the persistent subclinical activation of monocytes, macrophages, or neutrophils over prolonged periods by stimuli such as bio-incompatible membranes or endotoxins from contaminated dialysate results in the release of proinflammatory cytokines and acute phase proteins, thereby contributing to chronic inflammatory diseases that are well recognized to be associated with long-term hemodialysis therapy (7–9).

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Despite the availability of elaborate water treatment facilities for many countries and centers, the dialysis membrane should offer an additional, more definitive protection against endotoxins. This issue is of particular relevance for highly permeable membranes used in high-flux dialysis or hemofiltration and hemodiafiltration. The phenomenon of backfiltration (10) causes the transfer of contaminants into the blood compartment as a result of the relative pressure gradient along the length of the dialyzer in the blood and dialysate compartments. It is therefore imperative that high-flux membranes ensure the efficient retention of pyrogenic material from potentially contaminated dialysate. The endotoxin retention properties of membranes are essentially determined by the chemical characteristics and composition of the various polymers used in the manufacturing processes. Although the extent of fluid backtransport (transfer of dialysate components into the blood) is controlled by the membrane structure (i.e., high membrane porosity results in elevated hydraulic permeabilities), adsorption is considered to be the more important mechanism of endotoxin retention (4,9). The evidence is that small endotoxin fragments causing pyrogenic reactions are in the same molecular weight range as uremic substances removed from blood (9). As dialysis is based almost entirely on size-exclusion principles, substances crossing the membrane in one direction are also able to traverse the membrane in the other direction. Thus, membrane chemical composition and surface chemistry, together with permeability characteristics (related mean pore size and more open membrane structure), are involved in the overall endotoxin retention characteristics.

The aim of the present study was to examine and compare the efficacy of diverse high- and low-flux synthetic dialysis membranes to retain endotoxins and CIS under conditions in which saline, instead of whole blood, was present in the blood compartment. Such a situation prevails in the predialysis priming phase, whereby the inner blood compartment is filled with saline while the outer contains (potentially contaminated) dialysate. Several studies that have used blood in the inner compartment have failed to detect the transfer of CIS through highly permeable dialysis membranes (11–13). This may be explained by the fact that plasma components and cells bind lipopolysaccharide (LPS) and influence the detection of CIS (14), thereby providing a misleading estimation of the safety of the membrane being used.

MATERIALS AND METHODS

Filters

The dialyzers used in this study are listed in Table 1.

Experimental set-up

Each dialyzer type was tested in three independent experiments performed at 37°C.

Standard dialysis tubing was connected to dialyzers under sterile conditions. Both the blood compartment (BC) and the dialysate compartment (DC) were filled with pyrogen-free saline. Every in vitro dialysis experiment was preceded by a sterile, pyrogen-free phase of 15 min; this phase represented an important control feature of the experiments in this study. Sampling was carried out from the BC and the DC to confirm the absence of limulus amoebocyte

TABLE 1. *Characteristics of the hemodialyzers used in the study*

Hemodialyzer ultrafiltration coefficient (KUF, mL/h/mm Hg)	Manufacturer	Membrane polymer (sterilization mode)	Membrane designation	Surface area (m^2)
High-flux dialyzers				
BLS814G (KUF = 68)	Bellco, Mirandola, Italy	Polyethersulfone (gamma-sterilized)	DIAPES	1.4
FX60 (KUF = 46)	Fresenius Medical Care, Bad Homburg, Germany	Polysulfone, (inline steam-sterilized)	Helixone	1.4
F60S (KUF = 40)	Fresenius Medical Care, Bad Homburg, Germany	Polysulfone, (inline steam-sterilized)	FMC Polysulfone	1.3
Polyflux 14S (KUF = 50)	Gambro, Hechingen, Germany	Polyarylethersulfone* (steam-sterilized)	Polyamide S	1.4
Low-flux dialyzers				
F6 HPS (KUF = 8.5)	Fresenius Medical Care, Bad Homburg, Germany	Polysulfone, (inline steam-sterilized)	FMC Polysulfone	1.3
BLS 517 SD (KUF = 17)	Bellco, Mirandola, Italy	Polyethersulfone (steam-sterilized)	DIAPES	1.7
BLS 517 G (KUF = 17)	Bellco, Mirandola, Italy	Polyethersulfone (gamma-sterilized)	DIAPES	1.7
Polyflux 14 L (KUF = 9.5)	Gambro, Hechingen, Germany	Polyarylethersulfone* (steam-sterilized)	Polyamide S	1.4

* Polyarylethersulfone is another name for polyethersulfone (17).

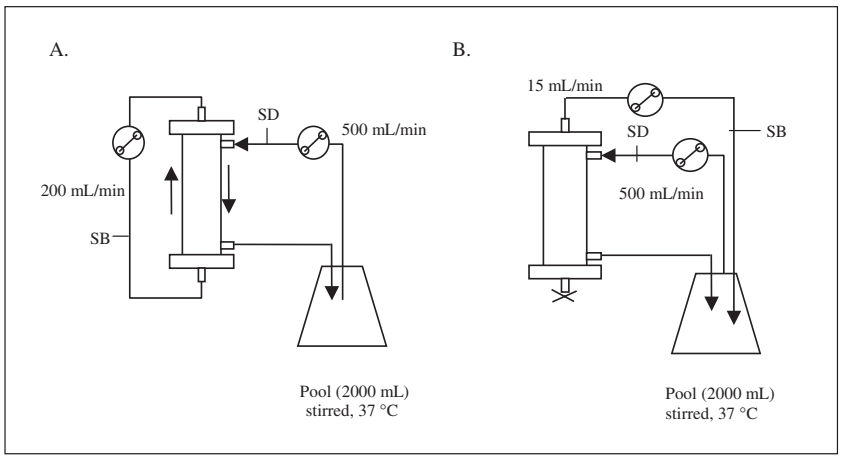

FIG. 1. Experimental set-ups used: panel A, dialysis mode; panel B, filtration mode. S-B, S-D: sampling ports (blood and dialysate compartment, respectively).

lysate (LAL) reactivity and cytokine induction prior to exposure to the challenge solution on the dialysate side.

Following the pyrogen-free control phase, the dialysate pool (2000 mL) was contaminated either with purified endotoxin (250 EU/mL for high-flux dialyzers and 500 EU/mL for low-flux dialyzers), or with a bacterial culture filtrate (20 EU/mL). Contaminated in vitro dialysis was performed for 1 h (2 h for the low-flux filters) at a fluid flow rate of 200 mL/min (BC) and a counter-current flow of 500 mL/min in the DC (Fig. 1, panel A). For high-flux dialyzers, the challenge experiment was continued for another 60 min under convective conditions at a flow rate of 500 mL/min in the DC and a filtration rate of 15 mL/min (Fig. 1, panel B). The 15 mL/min rate was selected as it reflects the estimated in vivo backfiltration rate reported for high-flux dialyzers (15,16). Samples for LAL testing and for the measurement of cytokine-inducing activity were taken from both the BC and the DC after the sterile control phase, at the point of introduction of the challenge material in the DC ($t = 0$), and after 7, 15, 60, 67, 75, and 120 min of each experiment. Nonendotoxin-adsorbing glass tubes (T 100, Coachrom, Vienna, Austria) were used for the storage of the samples.

Challenge material

Lipopolysaccharide from *Pseudomonas aeruginosa* was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). For the preparation of the bacterial culture filtrate, *Pseudomonas aeruginosa* ATCC 27853 and *Stenotrophomonas maltophilia* ATCC 13637 (both from Doenitz ProLab, Augsburg, Germany) were cultured at 37°C in tryptic soy broth (Difco Laboratories, Heidelberg, Germany) until the log-phase of growth. Equal volumes of the cultures were pooled and filtered through 0.45 µm filters (Minisart, Sartorius, Göttingen, Germany). The content of endotoxins in the culture filtrate was quantified by LAL assays (see below). Additionally, the IL-1Ra-inducing activity of the challenge material was assessed by incubating the culture filtrates with human whole blood as described below. Aliquots of the culture filtrate were stored at –70°C until use.

Determination of endotoxin concentration

The endotoxin was quantified using a kinetic chromogenic LAL test (Coatest, Chromogenix, Mölndal, Sweden) with a detection threshold of 0.005 EU(= IU)/mL in saline solution. The assay was performed according to the instructions of the manufacturer. All determinations were performed in duplicate, and a biologic reference preparation (BRP-3) standard (Coachrom, Vienna, Austria) was included in each test as a positive control.

Measurement of cytokine-inducing activity

To avoid donor-dependent variation in cytokine induction, blood from the same healthy donor was used throughout the study. Blood was drawn into Endotubes (Coachrom, Vienna, Austria) using a vacutainer system, gently mixed, and incubated with the collected samples at 37°C for 20 h in a 96-well plate in a humidified atmosphere containing 5% CO_2 (50 µL blood and 200 µL sample). Following centrifugation (3 min, 2000 g), IL-1Ra was quantified in the supernatant by ELISA (Cytoscreen, Biosource Europe, Nivelles, Belgium). Pyrogen-free saline served as a negative control. All samples were assayed in duplicate. IL-1Ra production by human whole blood is expressed as pg/million white blood cells (WBC).

Statistics

A statistical analysis was performed using the SPSS software package (SPSS Inc., Chicago, IL, U.S.A.). Data are expressed as mean ± standard error of the mean. Differences were considered to be significant at $P < 0.05$.

RESULTS

Challenge of high-flux filters with purified LPS

In this first series of experiments, the transfer of LPS through the F60S (Fresenius Polysulfone), FX60 (Helixone), and BLS814G (DIAPES) dialyzers was assessed (Fig. 2). Endotoxin concentrations measured in the dialysate pool after the introduction of the challenge with LPS were 290 ± 13, 281 ± 18, and 266 ± 20 EU/mL, respectively, for each of the three dialyzer test circuits. With the experimental set-up described, no endotoxin transfer was detectable across the membrane into the BC of the FX60 and F60S hemodialyzers. However, for the BLS814G dialyzers, endotoxin concentrations in the blood compartment rose rapidly following the challenge and peaked at 8.7 ± 1.9 EU/mL after 60 min under diffusive conditions. Under the subsequent convective conditions, endotoxin transfer through BLS814G was diminished (0.5 ± 0.1 EU/mL after 60 min), but still highly significant.

Challenge of high-flux filters with filtrates from bacterial cultures

LAL reactivity

The average endotoxin concentration measured in the dialysate pool after challenge with the bacterial culture filtrate was 19.3 ± 1.2 EU/mL. Under diffusive conditions, the endotoxin concentration in the blood compartment rose from less than 0.005 EU/mL (control phase) to a maximum value (at 60 min)

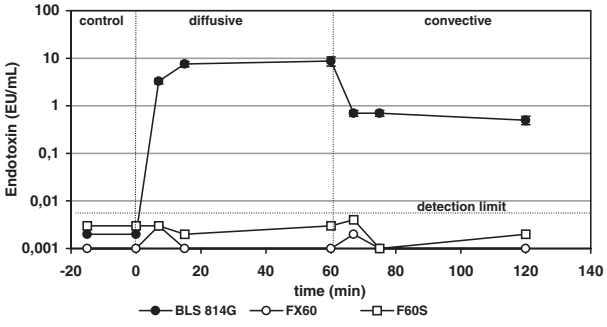

FIG. 2. Endotoxin levels measured in the blood compartment of high-flux filters after challenge with purified LPS from *Pseudomonas aeruginosa*.

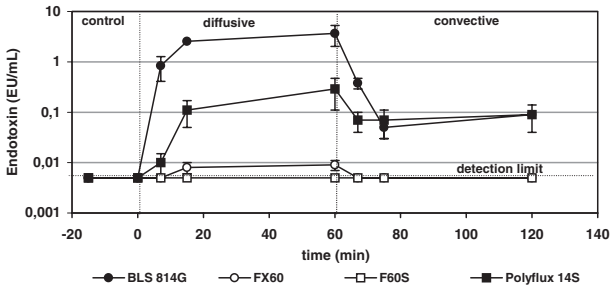

FIG. 3. Endotoxin concentrations measured in the blood compartments of high-flux filters after challenge with bacterial culture filtrates.

of 3.6 ± 1.6 EU/mL for BLS814G, 0.29 ± 0.2 EU/mL for Polyflux 14S (Polyamide S), 0.009 ± 0.002 EU/mL for FX60, and remained below the detection limit for F60S (Fig. 3). During the subsequent filtration phase of the experiment, the transfer of endotoxins was less pronounced with values above the detection limit only for Polyflux 14S and for the BLS814G (both 0.09 ± 0.05 EU/mL after 60 min).

IL-1Ra-inducing activity

The bacterial culture filtrate induced significant amounts of IL-1Ra in whole blood down to a dilution of 10^{-5} (295 ± 48 pg/mL compared to 80 ± 10 pg/mL for the saline control). After challenge of the dialyzers with the culture filtrate (resulting IL-1Ra induction: 1603 ± 227 pg/10^6 WBC), significant pyrogen transfer for BLS814G (809 ± 326 vs. control: 83 ± 6 pg/10^6 WBC) was observed, but no significant passage was observed for Polyflux 14S (92 ± 6 vs. 91 ± 8 pg/10^6 WBC), FX60 (76 ± 5 vs. 80 ± 13 pg/10^6 WBC), and F60S (106 ± 9 vs. 77 ± 10 pg/10^6 WBC) after 60 min of dialysis (Fig. 4). Again, there was reduced transfer of IL-1Ra-inducing material during the filtration phase of the experiment; values above the detection limit were found only for BLS814G (after 60 min: 137 ± 29 pg/10^6 WBC).

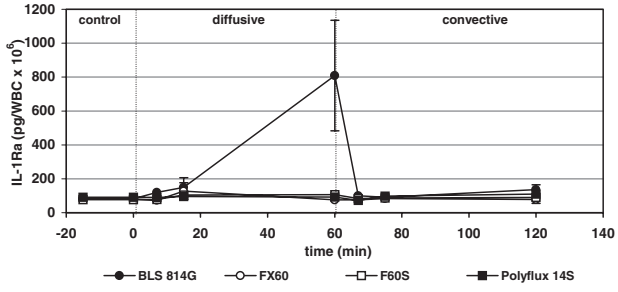

FIG. 4. IL-1Ra-inducing activities in samples from the blood compartments of high-flux filters after challenge with bacterial culture filtrates.

TABLE 2. *Adsorption of endotoxins (EU/cm² membrane surface) to high-flux dialyzers under challenge with bacterial culture filtrates*

Dialyzer	ET concentration/dialysate pool (EU/mL)		
	Preexperiment	Postexperiment	ET adsorption (EU/cm²)
BLS814G	19.3 ± 2.4	3.1 ± 0.8	2.1 ± 0.1
FX60	22.5 ± 3.0	6.7 ± 1.3	2.3 ± 0.3
F60S	17.9 ± 2.9	7.0 ± 3.8	1.6 ± 0.1
Polyflux 14S	19.5 ± 2.4	13.5 ± 1.6	0.7 ± 0.1

Adsorption of endotoxins to the high-flux dialyzer membranes

Endotoxin concentrations in the dialysate decreased with time, indicating the adsorption of endotoxins to the dialyzer membranes. Based upon the endotoxin concentration in the dialysate pool at the beginning (0 min) and at the end (120 min) of each experiment, endotoxin adsorption was estimated for each dialyzer and expressed as EU/cm² membrane surface (Table 2). A blank experiment revealed that the adsorption of LPS to the tubing system was negligible. Upon challenge with bacterial culture filtrates (20 EU/mL), the endotoxin adsorption was in the same range for BLS814G, FX60, and F60S, but considerably lower for the Polyflux 14S.

Challenge of low-flux filters with LPS

F6 HPS (Fresenius Polysulfone), BLS 517SD (DIAPES), BLS 517G (DIAPES), and Polyflux 14 L (Polyamide S) dialyzers were challenged with LPS and tested for 120 min under diffusive conditions. Endotoxin concentrations measured in the dialysate pool after challenge with LPS were 569 ± 82, 559 ± 43, 532 ± 34, and 615 ± 89 EU/mL, respectively, for each of the four dialyzer types tested in this series of experiments. Endotoxin concentrations in the blood compartment remained below the detection threshold for F6 HPS and Polyflux 14 L. For BLS 517SD and BLS 517G, endotoxin concentrations in the blood compartment rose to 0.44 ± 0.13 and 2.11 ± 0.81 EU/mL after 120 min (Fig. 5). The difference in endotoxin concentration measured in the blood compartment of the steam- (BLS 517SD) and gamma-sterilized (BLS 517G) filters over all time points was significant ($P = 0.024$).

DISCUSSION

The transfer of cytokine-inducing bacterial products from potentially contaminated dialysate into the blood compartment during routine hemodialysis has been the subject of considerable debate (13,18,19). The main focus of attention lies on the quality of dialysis water used for the preparation of dialysate, the usage of special endotoxin-adsorbing filters, as well as on the ability of the dialyzer membranes to prevent the passage of bacterial products from the dialysis fluid into the bloodstream. However, while Association for the Advancement of Medical Instrumentation (AAMI) standards (20) attempt to provide guidelines for the microbiological quality of both dialysis water and dialysis fluid, little information or advice is offered in terms of dialyzer integrity involving variations attributed to the membrane

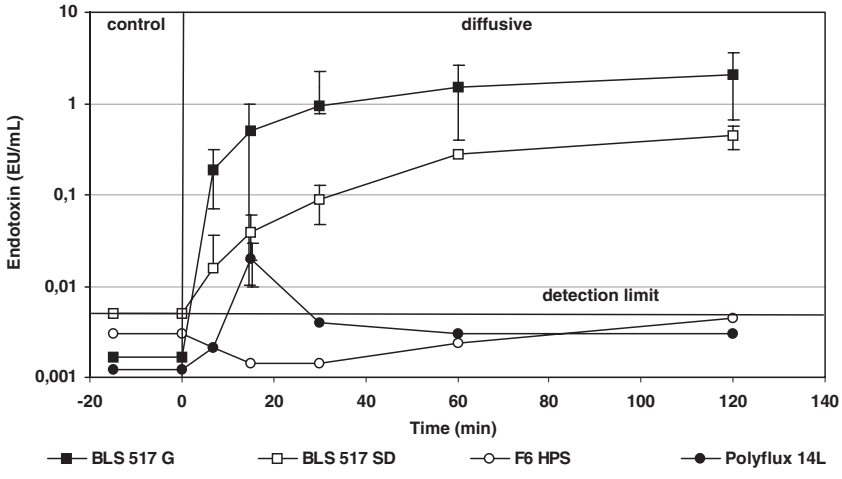

FIG. 5. Endotoxin concentrations measured in the blood compartments of low-flux filters after challenge with purified endotoxin.

materials and the manufacturing processes. Consequently, there is increasing recognition for the need to rigorously evaluate dialyzers that are already in clinical use in in vitro models with respect to their ability to prevent the transfer of bacterial products.

The outcome of such in vitro investigations depends, to a large extent, upon the precise methodology employed to investigate and address specific issues. These considerations have, in the past, led to debate and controversy regarding the interpretation of the reported data, irrespective of the assumption that the passage of even low amounts of proinflammatory substances across dialyzer membranes is regarded as being detrimental to the long-term health of the patient on chronic hemodialysis. Amongst the more critical methodological considerations are the selection of the experimental model, the source and concentration of the bacterial challenge being applied, and the assays used to assess the extent of the transmembrane passage (of CIS) incurred by the membrane.

Schindler et al. (14) have considered in some detail the inconsistencies in the transfer of CIS across a certain membrane; while some investigators report significant transfer, others have been unable to reproduce the findings on the same membrane. The authors regard differences in experimental design between the studies as a major cause for these discrepancies. Crucially, the presence of saline or whole blood in the blood compartment may influence the detection of CIS. The fact that many studies using whole blood failed to demonstrate the transfer of CIS led Schindler et al. to suggest that whole blood may hinder the induction of cytokines. Various plasma components (e.g., lipids, hemoglobin, LPS-binding protein, as well as blood cells) may bind LPS and alter its biological activity.

In view of these deliberations, in this study we selected the saline–saline model as being the more pertinent situation, so as not to obtain a misleading indication of the relative safety of a given membrane. Moreover, during the predialysis priming phase, CIS from the (contaminated) dialysate may penetrate the blood compartment and either bind to the inner surface of the membrane or directly enter the patient's bloodstream during the flush-back procedures.

The challenge material used in most in vitro studies designed to characterize the endotoxin retention characteristics of dialyzers or ultrafilters is prepared from bacterial (e.g., *Pseudomonas aeruginosa*) culture supernatants. This is pertinent, as the genus *Pseudomonas* constitutes more than 50% of all gram-negative bacteria found in water and dialysate in dialysis centers (21). In most studies, the bacterial challenge levels used were around 100-fold higher than those encountered in the majority of clinical situations (21,22), although some authors do not even mention the exact endotoxin concentration chosen for their experimental set-ups. In a recent study, Lonnemann et al. (4) compared the pyrogen permeability of DIAPES and Fresenius Polysulfone using endotoxin challenge concentrations of as high as 20 000 EU/mL and 50 EU/mL from a *Pseudomonas aeruginosa* culture filtrate. In contrast, Di Felice et al. (23) used only 5–48 EU/mL of *Pseudomonas aeruginosa* filtrate to test the endotoxin retention capacity for the evaluation of ultrafilters (as pyrogen filters, which are required to have a much stronger endotoxin retention capability than dialyzers). Thus, in the present study, a concentration of 20 EU/mL was used in the experiments with bacterial culture supernatants to address a clinically relevant situation.

Preparations of *Pseudomonas aeruginosa* culture filtrates contain a variety of endotoxin fragments with different molecular weight and also other non-endotoxin substances, which are not detected by the LAL assay but which have cytokine-inducing activities. LPS, a structural element of the outer cell wall of gram-negative bacteria, is clearly the most important CIS present in contaminated dialysate. Therefore, this study was supplemented by experiments with purified LPS.

IL-1Ra was chosen as an indicator of leukocyte activation for the following reasons. Firstly, IL-1Ra is produced in parallel with IL-1 by leukocytes. Moreover, the half-life of IL-1Ra is much longer than that of IL-1, and, most importantly, IL-1Ra generation correlates with proinflammatory markers of cell activation (24). Consistently, previous studies performed in our laboratory have demonstrated that IL-1Ra production is highly associated with the generation of Il-1β and TNF-α (8). Whole blood assays were used to detect cytokine induction as they are easy to standardize and are associated with few preparation artefacts (14).

In this study, the passage of endotoxins was detectable across DIAPES (both high- as well as low-flux variants) for both challenge situations, i.e., with purified endotoxin (250/500 EU/mL) as well as with bacterial culture filtrates (20 EU/mL). Under the same conditions, no endotoxin transfer was found for Fresenius Polysulfone and Helixone. Analogous results were obtained upon challenge of the high-flux dialyzers with bacterial filtrates. Remarkably, more endotoxin penetrated the DIAPES membrane under diffusive than under convective conditions. The explanation for this observation is, firstly, that

during convection larger endotoxin aggregates form at the outer membrane surface facing the dialysate compartment, which results in decreased endotoxin transfer. Secondly, the phenomenon is almost certainly related to the unique membrane structure of the DIAPES membrane. Most asymmetric dialysis membranes are two-layered, comprising a thin, inner "skin" separating layer followed by a more porous bulk support region. The DIAPES membrane, in contrast, has a three-layered structure in which the outermost third layer (dialysate side) is extremely dense. Of the total wall thickness of 30 μm, as much as one-third (10 μm) is considerably denser than the porous middle region of the membrane (25). Thus, during convection, there is a time-dependent increase in the accumulation of endotoxin aggregates at the outer surface forming a form of barrier that eventually leads to a decreased transfer of endotoxin (in the experimental set-up, the net filtration of fluid from the dialysate to blood compartments exacerbates the situation). This is supported by our finding that there is less transfer at higher filtration rates (additional experiments, data not included): the endotoxin concentration in the blood compartment after challenge of the dialysate pool with 250 EU/mL was 0.1 vs. 0.5 EU/mL after 60 min using filtration rates of 50 and 15 mL/min, respectively.

Thus, the extent of total endotoxin adsorbed by DIAPES (Table 2) as well as the high endotoxin leakage of DIAPES are attributable to the unique structure of this membrane. Because of the large, dense outer structural region of the membrane, endotoxin retention by filtration appears to play a more prominent part in DIAPES for large endotoxin constituents, rather than adsorption mechanisms that are particularly important for the hindrance of small-sized CIS fragments that are able to penetrate most membranes (9).

The findings of our studies suggest that the sterilization procedure may also profoundly affect the endotoxin permeability of the filters, as a significant difference in the endotoxin transfer was seen between the steam-sterilized and the gamma-sterilized low-flux DIAPES membrane. This alteration of the endotoxin permeability characteristics could be attributed to the direct effects of the sterilization procedure on the membrane structure and/or chemistry. Gamma irradiation, although an effective mode of sterilization, is known to affect the stability of polymers, causing, for instance, material stress and inducing changes of the chemical functionality of the polymers (e.g., cross-linking, molecular weight alterations, or liberation of chemical groups from the polymers). Often, the changes due to the effects of sterilization with gamma irradiation are even visually apparent in the form of discoloration of polymeric medical devices.

The study of Lonnemann et al. (4) compared the pyrogen permeability of DIAPES and Fresenius Polysulfone. The fact that we find a much higher transfer of endotoxins than Lonnemann et al. can be explained by differences in the experimental set-up. Firstly, we used saline solution in both compartments. Thus, protein coating, which was shown to reduce the pyrogen permeability of synthetic membranes (26), did not occur in our study. Filters were tested under the most unfavorable conditions, which, nevertheless, are clinically relevant during the priming/rinsing procedure. Secondly, in contrast to our study, endotoxins were not quantified directly in the study performed by Lonnemann et al. However, their finding that cytokine induction in the BC could be partly inhibited by polymyxin B implies that there was transfer of endotoxin in their experimental set-up as well.

In summary, we were able to show that hemodialysis membranes differ vastly with respect to their endotoxin permeability characteristics. Unlike the two polysulfone membranes (Fresenius Polysulfone and Helixone) and Polyamide S, the DIAPES membrane exhibited high permeability to endotoxin under the conditions of investigation in this study. These findings were obtained in experiments with saline in both the blood as well as the dialysate compartments. This experimental design was selected for two reasons. Firstly, the presence of whole blood in the blood compartment may prevent or impair the detection of CIS using commonly-used assays (11); thus, an inability to detect CIS is not necessarily indicative of an absence of transfer of CIS across the membrane. Secondly, by applying the saline–saline model, we addressed the predialysis priming phase situation, whereby the inner blood compartment of dialyzers is filled with saline while the outer compartment is filled with dialysate that may potentially contain contaminated dialysate. Our data clearly demonstrate that the transfer of endotoxins occurs rapidly (within less than 10 min). Thus, during the priming phase, which could last from minutes to several hours in routine clinical practice, the passage of CIS may occur into the blood compartment from the dialysate side. By using dialysis membranes that are not leaky to endotoxic material, the underlying considerations of patient safety and well-being are ensured.

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