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Hemofiltration in human sepsis: Evidence for elimination of immunomodulatory substances

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Hemofiltration in human sepsis: Evidence for elimination of immunomodulatory substances. Continuous hemofiltration is widely used for renal replacement therapy in patients with acute renal failure. It has been suggested that hemofiltration may also eliminate toxic mediators thought to be important in the pathophysiology of sepsis. The present study examined whether hemofiltration can activate or eliminate inflammatory mediators in patients with sepsis, and whether ultrafiltrate can alter specific functions of peripheral blood mononuclear leukocytes (PBMC) *in vitro*. Veno-venous hemofiltration was performed in 16 patients and in 5 healthy volunteers. Pre-filter (afferent), post-filter (efferent) and ultrafiltrate concentrations of cytokines (IL-1β, IL-6, IL-8, TNFα) and of complement components (C3, $C3a_{desArg}$, $C5a_{desArg}$, terminal complement complex) were measured after the beginning of hemofiltration (t_0), and 60 minutes later (t_{60}). PBMC, and monocyte and lymphocyte subfractions were incubated with ultrafiltrate, and cytokines were determined in the supernatants. Hemofiltration did not induce significant mediator activation. There was no evidence for significant cytokine elimination. However, pre-filter $C3a_{desArg}$ concentration showed a significant decline during hemofiltration (patients: $t_0 = 676.9 \pm 99.7$ ng/ml, $t_{60} = 545.4 \pm 83.2$, $P < 0.001$; volunteers: $t_0 = 54.8 \pm 13.3$ ng/ml, $t_{60} = 33.9 \pm 10.7$, $P < 0.001$). Ultrafiltrate from septic patients significantly stimulated PBMC and monocyte TNFα release, but suppressed lymphocyte IL-2 and IL-6 production. Ultrafiltrate from volunteers was without effect. Hemofiltration effectively eliminates certain mediators such as $C3a_{desArg}$. Ultrafiltrate contains compounds with significant immunomodulatory qualities. Therefore, hemofiltration may represent a new modality for removal of immunomodulatory mediators.

Multiple organ dysfunction syndrome is currently the leading cause of death in patients with sepsis. To treat individual organ failure, modern technology offers a variety of concepts to be used in intensive care medicine. Besides artificial ventilation and left-heart assisting devices, continuous renal replacement therapy has gained prominence in the treatment of critically ill patients. In recent years, the established procedure, intermittent hemodialysis, is being superseded by various types of continuous renal replacement therapy, including continuous arterio-venous hemofiltration, continuous veno-venous hemofiltration and continuous veno-venous hemodiafiltration. Continuous high volume hemofiltration has proven equally effective in controlling uremia, but found to be superior to hemodialysis, because hemofiltration results in fewer cardiovascular side effects [1]. Furthermore, continuous hemofiltration allows better control of fluid balance and simultaneous continuation of total parenteral nutrition [2].

Recently, a new aspect of isovolemic hemofiltration has emerged, namely, the possibility that it can eliminate inflammatory mediators, considered important in the pathophysiology of human sepsis [3]. In a recent clinical study, we demonstrated a correlation between the daily amount of ultrafiltrate and the survival rate in critically ill patients with sepsis who simultaneously had kidney failure [4]. Several animal studies indicate that isovolemic hemofiltration may improve various organ functions [5–8] or increase survival in sepsis and endotoxinemia [5, 6, 9, 10]. Correspondingly, toxic mediators (cytokines, cyclic endoperoxides) have been identified in ultrafiltrate from septic animals [9] and patients [11]. However, the possibility that hemofiltration also improves specific organ failure in human sepsis, is a point of controversy. Additionally, the detoxifying mechanisms of hemofiltration in sepsis remain unclear. It has been argued that mediators detected in septic ultrafiltrate, were only activated and released into the ultrafiltrate by blood-filter contact. The latter mechanism has been observed with certain dialysis membranes [1].

It was the aim of the present study to examine whether short-term hemofiltration can eliminate or activate established mediators in human sepsis. To do this, we compared and analyzed plasma and ultrafiltrate from patients with sepsis who simultaneously had multiple organ dysfunction, and from healthy controls. We focused on those mediators (IL-1β, IL-6, IL-8, TNFα, and anaphylatoxins $C3a_{desArg}$ and $C5a_{desArg}$) of which plasma concentration had been shown to correlate with patient outcome and which are believed to play a pivotal role in the pathophysiology of human sepsis [12, 13]. We further characterized the specific qualities of ultrafiltrate by exposing different subfractions of mononuclear leukocytes to ultrafiltrates and by measuring several aspects of cell function (cell proliferation and cytokine synthesis).

Methods

The studies were reviewed and approved by the local institutional review board.

This article is dedicated to Prof. Dr. G. Heberer on the occasion of his 75th birthday.

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Table 1. Clinical characteristics of patients with multiple organ dysfunction due to a systemic inflammatory response syndrome

Patient No.	Age	Sex	Score points (a)	Diagnosis	Outcome (b)
1	54	M	23	Esophagectomy, mediastinitis	D
2	82	M	21	Short bowel syndrome, pneumonia	D
3	68	M	21	Lung resection, pneumonia	D
4	58	M	20	Empyema of the gall-bladder, pneumonia	D
5	78	M	21	Abdominal aortic aneurysm repair, pneumonia	D
6	55	M	23	Hernial repair, liver failure	D
7	63	F	20	Lung resection mediastinitis	D
8	77	M	23	Lung resection, mediastinitis	D
9	47	F	22	Colon resection, peritonitis	S
10	32	F	26	Pneumonia, liver failure	D
11	69	M	22	Esophagectomy, aortic rupture, ARDS	D
12	64	M	20	Esophagectomy, ARDS	S
13	61	M	22	Bowel perforation, peritonitis	D
14	81	M	23	Bowel perforation, peritonitis	D
15	37	M	20	Necrotizing pancreatitis	S
16	64	M	23	Nephrectomy, peritonitis	D

[a] Modified Elebute score according to Ref [15]
[b] S = survival; D = death

Subjects

Patients. Biochemical studies were conducted in 16 post-traumatic or postoperative patients (13 males, 3 females; age 60 ± 3.8 years), who were admitted to the surgical intensive care unit at the Munich University Hospital, Klinikum Grosshadern. All patients were suffering from a septic multiple organ dysfunction syndrome, as defined recently [14], and were scored daily by a slightly modified Elebute sepsis score [15]. In addition to the original score, bleeding diathesis from disseminated intravascular coagulation was defined as follows: antithrombin III < 50% of control value, INR < 2, fibrinogen < 1.5 g/liter, platelet count < 100 g/liter. At least three of these criteria must have been fulfilled to achieve points in the absence of clinical bleeding diathesis. All patients were mechanically ventilated and received conventional therapy (crystalloids, vasoactive agents and antibiotics). Three patients had acute renal failure at the time of entry into the study. When the patients reached a score of 20 or more Elebute points (mean Elebute 22 ± 0.4) they entered the study. The ages, diagnoses, Elebute scores and outcomes are listed in Table 1.

Healthy volunteers. After informed consent was obtained, five healthy scientists from our laboratory (4 males and 1 female, mean age 37.4 ± 4.3) received continuous hemofiltration, including vascular access, low dose heparinization and continuous monitoring under intensive care conditions (see below).

Study design

Continuous veno-venous hemofiltration was performed using the Gambro hollow fiber hemofilter FH 66 D which contains a highly permeable polyamide membrane (Gambro, Hechingen, Germany). Vascular access was obtained via percutaneous puncture of the femoral vein or the subclavian vein and by subsequent insertion of a double-lumen Shaldon catheter. Hemofilters were initially rinsed with 2 liters of Ringer's solution, including 5,000 IU of heparin per liter. Then the system was connected to the subject. After 10 minutes, a steady state rate of 2 liters of ultrafiltrate per hour and isovolemic fluid replacement were achieved via a digital balance control system and a flow-controlled blood roller pump (AK 10, Gambro AB, Lund, Sweden). A balanced and isotonic electrolyte solution (HF 03, Fresenius, Bad Homburg, Germany) was infused through the efferent limb of the extracorporeal system to maintain zero fluid balance. Low dose heparinization was performed to prevent filter clotting by adding 300 IU/hr heparin into the afferent line of the hemofilter. Figure 1 shows the arrangement of the hemofiltration system.

Collection of plasma and ultrafiltrate

From patients and volunteers, citrate (0.011 mmol/liter) and EDTA (10 mmol/liter) anticoagulated sterile samples were collected after steady-state conditions of the hemofiltration procedure were reached (t_0), and 60 minutes later (t_{60}). Samples were taken simultaneously from the afferent (pre-hemofilter) line (proximal to the heparin infusion port), from the efferent (post-hemofilter) line (post-dilution, distal to the electrolyte infusion port) and from the ultrafiltrate port (Fig. 1). Immediately after collection on ice, all plasma tubes were centrifuged at 4°C for 15 minutes, aliquoted under a laminar flow hood and stored at −80°C until further use. Native ultrafiltrate samples (without anticoagulant) were used for the cell cultures. These samples had been kept frozen until use.

Assays

In patients and in healthy volunteers, cytokines and complement factors were measured in the pre-hemofilter line, in the post-hemofilter line and in ultrafiltrate at t_0 and t_{60}. One assay was used to measure all samples from an individual patient or volunteer. Sieving coefficients were calculated by the formula: ultrafiltrate concentration/pre-hemofilter concentration. Cytokines were also determined in the supernatants from cell experiments.

Interleukin-6 (IL-6) bioassay. IL-6 activity was determined by the 7TD1-specific, IL-6 dependent cell line as described previously [16] with slight modifications. Briefly, cells at a density of 10^4/ml were cultured in 96-well plates (Falcon, Oxnard, CA, USA). The viability of 7TD1 cells was tested using 0.5% Trypan blue exclusion tests. Serial dilutions of supernatants were incubated in 100 μl RPMI 1640 medium (Gibco, Paisley, UK), supplemented with 10% fetal calf serum (FCS) (Vitromex, Vilshofen, Germany) and 0.213 g/liter gentamicin sulfate (Merck, Darmstadt, Germany) in duplicate for four days at 37°C and in a 6% CO_2 air. Then 10 μl tetrazolium salt solution (MTT) (Sigma, Deisenhofen, Germany) at a concentration of 5 mg/ml were added. After four hours of incubation the test was stopped with 100 μl of 10% sodium dodecylsulfate (SDS) and incubated overnight at room temperature. The day after, optical densities, reflecting the degree of proliferation, were measured using a micro-ELISA plate reader at 620 nm. One unit of IL-6 was defined as half maximum growth of the hybridoma cells. Calculations were done based on a standard curve which had been obtained from serial dilutions with human recombinant IL-6 (Boehringer, Mannheim, Germany). The slope of the sample

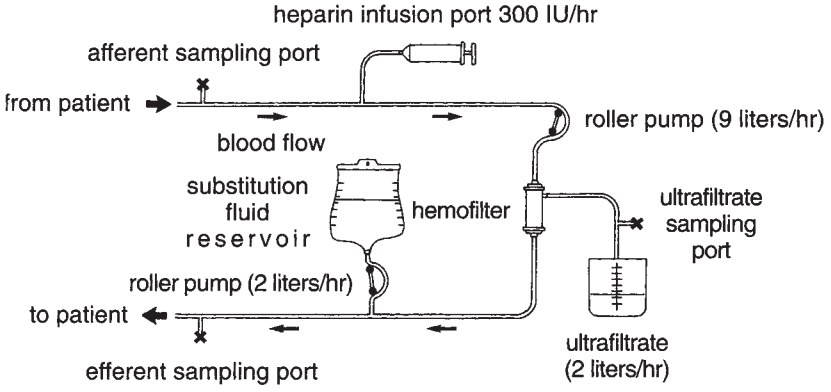

Fig. 1. *Arrangement of hemofiltration devices, lines and ports.* Vascular access is performed via puncture of the femoral or subclavian vein and by subsequent insertion of a double-lumen Shaldon catheter. After continuous addition of heparin (300 IU/hr) into the afferent line of the hemofilter to prevent filter clotting, venous blood passes through a flow-controlled roller pump (pump rate approximately 9 liters/hr) and reaches the hemofilter. There, ultrafiltrate is generated at a rate of 2 liters/hr by adjusting the perfusion pressure of the pump. Subsequently, isovolemic fluid replacement is achieved by adding the corresponding amount of substitution fluid into the efferent line of the hemofilter via a pump-controlled digital balance system. Blood is recycled back into the body via the Shaldon catheter. Samples are taken from the afferent and efferent line of the hemofilter at the Shaldon catheter, and from the ultrafiltrate line.

curve was compared to that of the standard. Repeated intra-assay measurements of one sample gave a coefficient of variation < 5%.

Tumor necrosis factor alpha (TNFα) bioassay. TNFα induced cytotoxicity was measured using the highly specific fibrosarcoma cell line WEHI 164 subclone 13 [17]. Briefly, the cells were cultivated at a concentration of 10^6/ml in RPMI 1640 medium supplemented with 10% FCS and 0.5 μg/ml actinomycin D (Sigma, St. Louis, MO, USA), to increase sensitivity of WEHI cells. After 20 hours incubation the MTT assay was done as described above. The optical density reflected the degree of non-lyzed, and therefore, still proliferating cells. Calculations were done as described above, using serial dilutions of a standard human rTNFα solution (Genzyme, San Diego, CA, USA) with the only exception being that one unit of TNFα was defined as half-maximum cytotoxicity. This bioassay has been shown to be highly sensitive and specific for TNFα [18].

Interleukin-2 (IL-2) bioassay. The assay for detection of IL-2 activity was done as described previously [19] with slight modifications. T-cell cultures from one immunologically tested human volunteer were cultured with Concanavalin A (Sigma) for five days and then stored frozen in liquid nitrogen (Con A blasts). To perform the assay, Con A blasts were warmed up, washed and resuspended with RPMI 1640 medium and tested for viability as described above. To assess IL-2 release, 100 μl aliquots of serially diluted lymphocyte supernatants were placed in microtiter plates and 100 μl of Con A blasts were added to each well. The final concentration was 4×10^4 cells per well. Cells were also incubated for 72 hours at 37°C and in a 6% CO_2 atmosphere with 15% serum, 3% highly purified phytohemagglutinin (PHA; Welcome, Burgwedel, Germany), or 2% Con A as controls. Six hours before harvesting on glass fiber paper with an automated sample harvester, 1 μCi of ^3H thymidine per well was added. The uptake of ^3H thymidine was counted in a beta-counter and reflected the proliferation of IL-2-dependent cells. Comparisons of IL-2 activities were performed using a standard solution of 1 U/ml by definition (Lymphoccult HP, Biotest, München, Germany). Units of IL-2 in supernatants were calculated in comparison with the standard solution. The percentage of the maximum number of counts, obtained from the standard, was plotted against the log 2 of dilution. The dilution, when the Lymphoccult preparation reached 50% of maximum thymidine uptake, was taken considered one unit.

Interleukin-1β (IL-1β), interleukin-8 (IL-8), C3, $C3a_{desArg}$ and $C5a_{desArg}$ assay. Concentrations of IL-1β, IL-8, C3, $C3a_{desArg}$ and $C5a_{desArg}$ in plasma, in ultrafiltrate or in supernatants were determined by using commercially available ELISA kits according to the manufacturers' instructions (IL-1β, Immunotech S.A., Marseilles, France; IL-8, Amersham, Braunschweig, Germany; C3, Boehringer; $C3a_{desArg}$, Progen, Heidelberg, Germany; and $C5a_{desArg}$, Behring, Marburg, Germany). The detection limit reported for IL-1β and IL-8 was 5 pg/ml, for $C3a_{desArg}$, 5 ng/ml and for $C5a_{desArg}$, 0.1 ng/ml.

Assay for the terminal complement complex (TCC). TCC concentrations in the afferent and efferent blood lines of the hemofilter were measured by a sensitive and specific micro-ELISA, using a monoclonal antibody against TCC neoantigen, as described previously [20].

Cell cultures

PBMC, monocyte and lymphocyte culture studies were performed with ultrafiltrate from patients and healthy volunteers. Ultrafiltrate effects were analyzed at t_0 and t_{60}. One cell preparation was always used, to study ultrafiltrate from one patient and one volunteer. Simultaneous analysis of PBMC, monocyte and lymphocyte cultures allows differentiation between the effect of ultrafiltrate on isolated subfractions (monocytes or lymphocytes) and on the combination of both subfractions (PBMC). Thereby one can also study the effect of ultrafiltrate on specific cell-to-cell interactions.

PBMC cultures. Peripheral blood mononuclear cells (PBMC) were prepared by standard laboratory methods [21]. Approximately 200 ml peripheral blood from healthy volunteers was placed in sterile, pyrogen-free and heparinized (20 U/ml) tubes and diluted (1:2) in Hanks balanced salt solution (HBSS; Gibco, St. Louis, MO, USA). Standard ficoll hypaque (Biochrom, Berlin, Germany) density gradient centrifugation (150 g, 30 min, 4°C) was then performed. The cells from the interphase were washed three times with HBSS and resuspended in RPMI. Cell viability was tested, as described above, and always exceeded 95%.

Table 2. IL-1β, IL-6, IL-8, TNFα, $C3a_{desArg}$, C3, $C5a_{desArg}$, and TCC (terminal complement complex) concentrations in 16 septic patients

	IL-1β *pg/ml*	IL-6 *U/ml*	IL-8 *pg/ml*	TNFα *U/ml*	$C3a_{desArg}$ *ng/ml*	C3 *mg/ml*	$C5a_{desArg}$ *ng/ml*	TCC *ng/ml*
Concentrations in the afferent line								
t_0	66.02 ± 28.00	1,091 ± 896	1,439 ± 749	33.14 ± 6.04	676.9 ± 99.7	0.723 ± 0.098	26.58 ± 4.71	2,966 ± 663
t_{60}	63.24 ± 24.90	1,127 ± 989	1,447 ± 731	28.55 ± 6.43	545.4 ± 83.2[b]	0.728 ± 0.097	25.65 ± 4.39	3,362 ± 804
Ultrafiltrate concentrations								
t_0	11.9 ± 3.72	[c]	630 ± 367	[c]	140.9 ± 35.9	ND	0.446 ± 0.08	[c]
t_{60}	ND	[c]	604 ± 292	[c]	103.7 ± 25.9[a]	ND	0.183 ± 0.03[b]	ND

Measurements were performed in plasma samples from the afferent filter line and in ultrafiltrate samples, when a steady state in the hemofiltration procedure was reached (t_0) and 60 minutes later (t_{60}).

[a] $P < 0.01$ vs. t_0

[b] $P < 0.005$ vs. t_0

[c] Not detectable by bioassay; ND, not determined

PBMC cultivation. PBMC were cultivated in 5 ml culture tubes (Falcon, Oxnard, CA, USA) at a final concentration of 5×10^6 cells/ml in RPMI supplemented by 7.5% FCS and 0.213 mg/ml gentamicin sulfate (Merck, Darmstadt, Germany) and stimulated in duplicate in the presence of: (a) ultrafiltrate only, at an end concentration of 50%, (b) ultrafiltrate at an end concentration of 50% combined with 1 μg/ml endotoxin derived from *C. parvum* (LPS) (Welcome, London, UK), and (c) isotonic saline solution (NaCl) at an end concentration of 50% combined with 1 μg/ml LPS (control assay). After 20 hours cell suspensions were centrifuged at 600 g, and supernatants were immediately frozen at −80°C until processing. Supernatants were analyzed for TNFα, IL-6 and IL-1β release by the assays described above.

Proliferation tests. PBMC were seeded at 1×10^5 cells per well in triplicate to flat bottomed, 96-well microtiter plates, and 7.5% FCS and 0.213 mg/ml gentamicin sulfate (Merck) were added. Three test series were performed in presence of: (a) ultrafiltrate only, at an end concentration of 25%, (b) ultrafiltrate at a final concentration of 25% combined with 0.5 μg/ml PHA, and (c) in presence of 25% NaCl combined with 0.5 μg/ml PHA. The plates were incubated at 37°C in 6% CO_2 for 72 hours. Cultures were pulsed with ^3H thymidine and harvested as described above. Activity uptake during four hours was counted on a beta-counter and reflected cell proliferation.

Monocyte cultures. Two ml of PBMC suspension adjusted to a concentration of 5×10^6 cells/ml were seeded out in six-well plates (Greiner, Nürtingen, Germany). Suspensions were incubated for four hours to purify the monocyte population by glass adherence at 37°C and 6% CO_2 in Dulbecco's culture medium (Gibco) supplemented by 10% FCS [22]. Afterwards the supernatants were removed and the plates were gently washed with sterile phosphate-buffered saline (PBS). More than 90% of adherent cells were monocytes as indicated by morphological analysis (non-specific esterase reaction). The monocytes were then cultivated in FCS (10%) enriched Dulbecco's medium for 20 hours. Three incubation series (50% ultrafiltrate only, 50% ultrafiltrate combined with LPS, and 50% NaCl combined with LPS) were performed in triplicate, as described above. After incubation, supernatants were collected and frozen at −80°C until assays were performed. Monocyte supernatants were analyzed for TNFα, IL-6 and IL-1β.

Lymphocyte cultures. The non-adherent cells (lymphocytes) from the monocytes preparation were centrifuged at 200 g for 10 minutes and cultivated after testing of viability by Trypan blue, as described above. Three incubation series (50% ultrafiltrate only, 50% ultrafiltrate combined with PHA at a final concentration of 2.5 μg/ml, and 50% NaCl combined with 2.5 μg/ml PHA) were done as described above. Incubation was performed for 48 hours. Then cell suspensions were centrifuged at 600 g and supernatants were frozen at −80°C until processing. Lymphocyte supernatants were analyzed for IL-2 and IL-6.

Statistics

All data were expressed as mean ± SEM. The differences between different incubation procedures (ultrafiltrate vs. NaCl) and between different time points (t_0 vs. t_{60}) were compared with the Wilcoxon rank test. The multiplicity of comparisons was accounted for by using a significance level of $P = 0.01$ throughout the study.

Results

Concentration of cytokines and complement factors

In septic patients pre-hemofilter (afferent) complement and cytokine levels at t_0 and t_{60} were markedly elevated compared to those of healthy volunteers (Tables 2 and 3). In the latter group, IL-1β, IL-6 and TNFα could not be detected in plasma (Table 3). During hemofiltration pre-filter (afferent) concentrations of cytokines remained constant in patients, as well as in healthy subjects. In both groups, however, we observed a significant fall in afferent $C3a_{desArg}$ concentration (Tables 2 and 3), whereas afferent C3, $C5a_{desArg}$ and TCC did not change during the first 60 minutes of continuous veno-venous hemofiltration.

Septic ultrafiltrate contained IL-1β, IL-8, $C3a_{desArg}$ and $C5a_{desArg}$ (Table 2). Sieving coefficients (SC) of IL-8 and of $C3a_{desArg}$ in patients remained constant during hemofiltration (IL-8, SC at t_0: 0.56 ± 0.065 vs. SC at t_{60}: 0.80 ± 0.173, NS; $C3a_{desArg}$, SC at t_0: 0.204 ± 0.02 vs. SC at t_{60}: 0.211 ± 0.029, NS). IL-6, TNFα and the terminal complement complex could not be detected in septic ultrafiltrate. Ultrafiltrate from volunteers demonstrated small amounts of IL-8, $C3a_{desArg}$ and $C5a_{desArg}$ (Table 3). After 60 minutes of hemofiltration, ultrafiltrate concentrations of $C3a_{desArg}$ declined in patients and healthy subjects (Tables 2 and 3).

Table 3. IL-1β, IL-6, IL-8, TNFα, $C3a_{desArg}$, C3, $C5a_{desArg}$, and TCC (terminal complement complex) concentrations in five healthy volunteers

	IL-1β *pg/ml*	IL-6 *U/ml*	IL-8 *pg/ml*	TNFα *U/ml*	$C3a_{desArg}$ *ng/ml*	C3 *mg/ml*	$C5a_{desArg}$ *ng/ml*	TCC *ng/ml*
Concentrations in the afferent line								
t_0	0	[c]	41.20 ± 6.04	[c]	54.82 ± 13.25	0.487 ± 0.034	9.70 ± 1.60	363 ± 153
t_{60}	0	[c]	31.00 ± 6.04	[c]	33.91 ± 0.68[b]	0.495 ± 0.04	8.82 ± 1.00	769 ± 279
Ultrafiltrate concentrations								
t_0	ND	[c]	30.00 ± 2.16	[c]	18.22 ± 1.63	ND	0.552 ± 0.062	ND
t_{60}	ND	[c]	ND	[c]	7.99 ± 1.29[b]	ND	0.059 ± 0.027[a]	ND

Measurements were performed in plasma samples from the afferent filter line and in ultrafiltrate samples, when a steady state in the hemofiltration procedure was reached (t_0) and 60 minutes later (t_{60}).

[a] $P < 0.01$ vs. t_0

[b] $P < 0.005$ vs. t_0

[c] Not detectable by bioassay; ND, not determined

Table 4. TNFα, IL-6 and IL-1β release of human peripheral blood mononuclear cells (PBMC) *in vitro*

	Septic patients ($N = 16$)			Healthy volunteers ($N = 5$)		
	Ultrafiltrate at t_0	Ultrafiltrate at t_{60}	NaCl controls	Ultrafiltrate at t_0	Ultrafiltrate at t_{60}	NaCl controls
TNFα *U/ml*	41.0 ± 10.0[a]	40.2 ± 10.7[a]	12.8 ± 4.3	26.5 ± 8.6	31.2 ± 12.1	23.8 ± 7.8
IL-6 *U/ml*	2,015 ± 466	1,485 ± 244	2,006 ± 255	1,518 ± 212	1,471 ± 294	1,398 ± 175
IL-1β *pg/ml*	966 ± 256	ND	946 ± 183	935 ± 211	ND	936 ± 173

All PBMC were co-stimulated with endotoxin (LPS). Cells were incubated with septic or healthy ultrafiltrate collected, when a steady state in the hemofiltration procedure was achieved (t_0) and 60 minutes later (t_{60}). Ultrafiltrate and corresponding NaCl incubations were always performed in the same cell preparation.

[a] $P < 0.001$ vs. NaCl

ND is not determined.

Table 5. TNFα, IL-6 and IL-1β release of human monocytes separated by glass adherence *in vitro*

	Septic patients ($N = 16$)			Healthy volunteers ($N = 5$)		
	Ultrafiltrate at t_0	Ultrafiltrate at t_{60}	NaCl controls	Ultrafiltrate at t_0	Ultrafiltrate at t_{60}	NaCl controls
TNFα *U/ml*	23.5 ± 3.9[a]	25.0 ± 5.8[a]	16.3 ± 4.1	18.0 ± 4.9	22.3 ± 4.6	21.4 ± 4.0
IL-6 *U/ml*	709 ± 191[a]	571 ± 149[a]	1,568 ± 331	800 ± 231	918 ± 209	1,079 ± 364
IL-1β *pg/ml*	334 ± 157	ND	407 ± 185	401 ± 182	ND	456 ± 202

All monocytes were co-stimulated with endotoxin (LPS). Cells were incubated with septic or healthy ultrafiltrate collected, when a steady state in the hemofiltration procedure was achieved (t_0) and 60 minutes later (t_{60}). Ultrafiltrate and corresponding NaCl incubations were always performed in the same cell preparation.

[a] $P < 0.001$ vs. NaCl

ND is not determined.

No pre/post-filter concentration differences in cytokines were detected in patients or in volunteers at t_0 or t_{60}. Also pre/post-filter concentrations of the terminal complement complex did not change significantly during hemofiltration in patients (t_0, pre-filter, 2,966 ± 663 ng/ml, and post-filter, 3,429 ± 710, NS; t_{60}, pre-filter 3,362 ± 804 ng/ml, and post-filter, 3,961 ± 678, NS) and in volunteers (t_0, pre-filter, 363 ± 153 ng/ml, and post-filter, 598 ± 133, NS; t_{60}, pre-filter, 769 ± 279 ng/ml, and post-filter, 1,215 ± 442, NS).

Cell culture experiments

Peripheral blood mononuclear cell cultures (Table 4). Septic or healthy ultrafiltrate *per se* did not alter cell function in comparison to NaCl control experiments (data not shown). Reproducible changes in PBMC and monocyte cultures (see below) occurred only when PBMC cultures were incubated with ultrafiltrate and LPS.

Septic ultrafiltrate produced a significant rise at t_0 and t_{60} in TNFα release in LPS-stimulated PBMC when compared to LPS-stimulated NaCl controls. PBMC did not respond to healthy ultrafiltrate. IL-6 and IL-1β release was not affected by either healthy or septic ultrafiltrate in comparison to NaCl controls.

PBMC proliferation. PBMC thymidine uptake was significantly suppressed compared to NaCl when septic ultrafiltrate was incubated with PHA-stimulated PBMC (ultrafiltrate from septic patients, 14,754 ± 1,534 cpm, and NaCl, 37,418 ± 160 cpm, $P < 0.01$; ultrafiltrate from volunteers, 27,744 ± 2,653 cpm, and NaCl, 32,856 ± 228 cpm, NS). Only ultrafiltrate at t_0 was used.

Monocyte cultures stimulated with LPS (Table 5). Septic ultrafiltrates from t_0 and t_{60} significantly accelerated the TNFα release

Table 6. IL-2 and IL-6-release of human lymphocytes separated by glass adherence *in vitro*

	Septic patients ($N = 16$)			Healthy volunteers ($N = 5$)		
	Ultrafiltrate at t_0	Ultrafiltrate at t_{60}	NaCl controls	Ultrafiltrate at t_0	Ultrafiltrate at t_{60}	NaCl controls
IL-2 *U/ml*	0.35 ± 0.07[b]	ND	0.66 ± 0.07	0.48 ± 0.18	ND	0.41 ± 0.12
IL-6 *U/ml*	4,295 ± 896[a]	3,093 ± 741[b]	6,496 ± 873	5,879 ± 2,670	5,316 ± 1,833	5,433 ± 1,497

All lymphocytes were co-stimulated with phytohemagglutinin (PHA). Cells were incubated with septic or healthy ultrafiltrate collected, when a steady state in the hemofiltration procedure was achieved (t_0) and 60 minutes later (t_{60}). Ultrafiltrate and corresponding NaCl incubations were always performed in the same cell preparation.

[a] $P < 0.001$ vs. NaCl

[b] $P < 0.001$ vs. NaCl.

ND is not determined.

from monocytes and suppressed their IL-6 release in comparison to NaCl controls. Monocytes incubated with ultrafiltrate from volunteers showed no response compared to NaCl controls and no change in IL-1β release when incubated with either healthy or septic ultrafiltrate.

Lymphocyte cultures stimulated with PHA (Table 6). IL-2 and IL-6 release from lymphocytes decreased significantly after incubation with septic ultrafiltrate in comparison to NaCl controls. Healthy ultrafiltrate remained without effect on lymphocytes' cytokine release.

Discussion

According to a recent consensus conference, all patients in the present study were suffering from a multiple organ dysfunction syndrome [14]. Multiple organ dysfunction syndrome is thought to be a result of a self-enhancing generalized reaction mediated by several cascade systems. It is likely that the pathogenesis and pathophysiology of various clinical entities that comprise the multiple organ dysfunction syndrome are similar. Several types of mediators, such as cytokines or complement factors, which play a role in the pathophysiology of sepsis were identified in the past [12]. In the present study, we wanted to test the hypothesis that hemofiltration could eliminate some of these mediators, thereby rendering continuous veno-venous hemofiltration useful as an immunomodulatory strategy in critical care medicine, independent of hemofiltration's importance in renal replacement therapy.

We found that, among the commonly known cytokines and complement factors, hemofiltration may eliminate IL-1β, IL-8, $C3a_{desArg}$ and $C5a_{desArg}$, but not TNFα and IL-6, in patients with systemic inflammatory response syndrome. Septic ultrafiltrate contains a mixture of mediators which stimulates TNFα production in PBMC and in monocyte fractions, and which suppresses IL-2 and IL-6 synthesis in lymphocytes.

When hemofiltration is tested as a tool to eliminate mediators of the multiple organ dysfunction syndrome, the possibility that the procedure itself causes substantial mediator activation by blood-hemofiltration membrane contact must be excluded. Our results show this phenomenon to be unlikely.

Firstly, hemofiltration in volunteers produced an ultrafiltrate which contained far lower concentrations of established mediators than did septic ultrafiltrate, and in which certain mediators, such as IL-1β, were absent. Furthermore, healthy ultrafiltrate, unlike septic ultrafiltrate, did not change white blood cell function. Secondly, neither volunteers nor patients with a multiple organ dysfunction syndrome showed signs of an hemofiltration-induced activation of the complement system, a hallmark characteristic of bio-incompatible membranes [1, 23], which can be tested by measuring concentrations of the terminal complement complex [20]. According to recent studies, bio-incompatibility is reflected by a rise in pre- and post-filter (afferent and efferent) concentrations of the terminal complement complex and by a rise in the concentration difference of the terminal complement complex across the filter, even during short term treatment [1, 20]. With the hemofiltration procedure used in our study, we observed no such increases in patients or in volunteers. Substantial activation of the complement system is also made unlikely by the finding that $C3a_{desArg}$ concentrations decreased during hemofiltration, indicating even elimination of complement factors. Besides, concentrations of the terminal complement complex in the plasma from our healthy volunteers did not differ from concentrations in the plasma from healthy non-treated subjects [20].

Therefore, it is safe to conclude that the mediators present in ultrafiltrate are indeed eliminated from the critically ill organism, but their exact nature and quality are unknown. However, a few general comments are possible. The membranes which were developed for hemofiltration, demonstrate high permeability in the so-called middle molecule range, such as for β_2-microglobulin, which has a molecular weight of 11.815 kD [24]. *In vitro* studies, which used dextran and proteins of various sizes, identified a cut-off point of about 30 kD in polyamide membranes such as ours [24]. However, this finding does not necessarily mean that all compounds with molecular weights below 30 kD can be removed via hemofiltration since other variables such as molecular structure, electric charges, hydrophilicity and hydrophobicity, the presence of plasma antagonists, receptor binding of compounds, and filtration modalities (such as transmembrane pressure) have been shown to be important determinants for the efficacy of compound filtration [24]. The complex mechanism of compound removal is illustrated by the fact that IL-6 could not be detected in ultrafiltrate from septic patients, although its biologically active monomer form has a molecular weight of 26 kD [25], and large amounts of active IL-6 were found in septic plasma (Table 2). Presumably, a different mechanism than molecular weight exclusion (such as binding to the plasma protein α2-macroglobulin [26]) must have been responsible for the absent IL-6 filtration.

In contrast, molecular weight explains why IL-1β and IL-8, but not TNFα, appeared in ultrafiltrate from patients. TNFα, in its active form, is a trimer, with an estimated molecular weight of 51 kD [25]. A molecule of this size is not expected to be eliminated via hemofiltration. Our finding corresponds to *in vitro* experiments which used lipopolysaccharide-induced active TNFα, and which did not detect TNFα in the filtrate compartment, even when a

highly permeable polyacrylonitrile membrane was used [27]. Correspondingly, we and others [9, 11] could not measure a change in pre-filter TNFα concentration during hemofiltration. Recent conflicting results, which showed a substantial clearance of TNFα by hemofiltration in clinical sepsis [11, 28] or in *in vitro* hemofiltration [29], may be explained by differences in the TNFα assay used. Thus, ELISA procedures may also measure smaller, biologically inactive, split products (monomeric forms) of the biologically active TNFα molecule.

IL-1β (molecular wt 17 kD) and IL-8 (molecular wt 8 kD) could be detected in ultrafiltrate. Filtration of IL-1β has also been shown during *in vitro* hemofiltration [29]. However, in our studies pre- and post-filter concentrations of these cytokines remained constant during hemofiltration. This corresponds to recent studies performed in septic patients [11, 28] and may be explained by two phenomena. Firstly, the variability of the cytokine assays used may have been too large to allow detection of small concentration differences over time and, secondly, the elimination rate of both cytokines may have been far below their corresponding production rate. If one assumes a rough half life for plasma IL-1β of three minutes [30], a plasma volume of 3 liter and a constant plasma concentration of 60 pg/ml, then approximately 1.8 μg of plasma IL-1β will be produced per hour. On the other hand, with an IL-1β ultrafiltrate concentration of 11.9 pg/ml and a filtration rate of 2 liter/hr, about 24 ng of IL-1β will be eliminated per hour. This is only about 1.5% of the roughly estimated plasma production rate, and thus an elimination rate of IL-1β and presumably also of IL-8 must be considered too small to be of significant biologic importance. Obviously, this statement cannot necessarily be extended to other still unknown cytokines or pro-inflammatory peptides.

In contrast to the examined cytokines, we found evidence of the important elimination of complement factors by hemofiltration, during which pre-filter $C3a_{desArg}$ concentrations decreased significantly in patients, but also in healthy volunteers (Tables 2 and 3). $C3a_{desArg}$ (molecular wt 9 kD) and $C5a_{desArg}$ (molecular wt 11 kD) could both be identified in septic and healthy ultrafiltrate. This observation is in line with results of a recent study which showed $C3a_{desArg}$ elimination after cardiopulmonary bypass by a hemofiltration procedure using a polyamide membrane similar to the membrane we used [31]. $C5a_{desArg}$ concentrations did not change during the first 60 minutes of hemofiltration, but effective elimination of $C5a_{desArg}$ may have been delayed due to its rapid binding to cellular receptors [20]. Preliminary results from our institution show that longer periods of hemofiltration may not only lead to a further decrease of plasma $C3a_{desArg}$, but also of $C5a_{desArg}$ [32]. Reduction of anaphylatoxin concentration could be of clinical importance, since beneficial therapeutic effects in sepsis have been correlated with a fall of anaphylatoxin concentrations [13, 33].

At present, we cannot describe the exact nature of all mediators removed by hemofiltration and the individual biological significance of their removal. However, it appeared feasable to evaluate the biological effects of the ultrafiltrate as a whole by exposing several peripheral mononuclear blood cell subfractions to ultrafiltrate *in vitro*.

A key finding from the incubation experiments was that septic, but not healthy ultrafiltrate, stimulated TNFα release in the PBMC and in the monocyte fraction (Tables 4 and 5). The exact mechanism of ultrafiltrate-stimulated TNFα release cannot be determined by our experiments. Involvement of endotoxin is unlikely, since it will be rejected by the hemofiltration membrane by molecular size exclusion (molecular wt about 100 kD) [34]. However, $C3a_{desArg}$ and possibly also interferon γ (molecular wt 20 kD) could be potential mediators of the TNFα effect, as they may stimulate TNFα production *in vitro* in PBMC and monocyte cell cultures [35, 36].

Besides its stimulating qualities, septic ultrafiltrate also demonstrated a variety of suppressive effects. Thus, ultrafiltrate prevented the secondary rise of IL-1β release in PBMC and monocyte fractions, which is usually observed as a consequence of enhanced TNFα release [37]. The mechanism of this down regulation is unknown. Furthermore, septic ultrafiltrate effectively suppressed IL-6 release in monocytes (see above), and IL-6 and IL-2 release in lymphocytes. This ultrafiltrate-related suppressive effect indicates a significant impairment in lymphocyte function (IL-6 release and IL-2 release are sensitive markers for lymphocyte protein synthesis [38]) and corresponds to findings in critically ill patients whose lymphocytes demonstrated a decreased IL-2 release [38] and proliferation rate [39], and whose monocytes showed a reduced IL-6 production [22]. A fall in IL-2 synthesis of lymphocytes can also explain why septic ultrafiltrate down-regulated the PHA-induced PBMC proliferation.

The mediators of lymphocyte suppression are not completely elucidated yet. A possible candidate could be prostaglandin E_2, which has a molecular weight of < 0.6 kD. Recent data suggest that arachidonic acid derivatives may appear in ultrafiltrate [9]. Prostanoids have been implicated in the suppression of the cell-mediated immune system (including IL-6 and IL-2 release) [38].

In conclusion, short-term hemofiltration with a highly biocompatible, polyamide membrane in patients with septic multiple organ dysfunction syndrome, does not seem to cause additional significant activation of mediators, known to be involved in the pathophysiology of these clinical conditions. This finding contrasts with the use of cellulosic membranes which have been shown to result in a significant activation of inflammatory mediators and in higher mortality rates due to septic complications [40]. Hemofiltration with synthetic membranes (such as polyamide membranes) may even eliminate a variety of mediators from septic plasma. Filtration of the classic cytokines IL-1β and TNFα is presumably of minor importance, but clearance of other components such as complement factors, arachidonic acid derivatives [9], or granulocyte inhibitory peptides [41], could achieve clinical significance, since these compounds are most likely involved in the development of fatal septic complications. The mixture of mediators in septic ultrafiltrate provokes a stimulation of PBMC and monocytes, with respect to TNFα synthesis, and a suppression of lymphocyte function. Therefore, hemofiltration might be of potential benefit for patients with multiple organ dysfunction syndrome in situations calling for attenuation of monocytic TNFα release and stimulation of lymphocyte function. This view is supported by recent investigations in which isovolemic hemofiltration improved specific organ failure in septic animals [6–8] and increased survival rate in critically ill patients with acute renal failure [4]. Removal of certain mediators via hemofiltration may represent a new therapeutic concept for patients with multiple organ dysfunction syndrome.

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References

1. Pertosa G, Tarantino EA, Gesualdo L, Montinaro V, Schena FP: C5b-9 generation and cytokine production in hemodialyzed patients. *Kidney Int* 43(Suppl 41):S221–S225, 1993
2. Bartlett RH, Mault JR, Dechert RE, Palmer J, Swartz RD, Port FK: Continuous arteriovenous hemofiltration: Improved survival in surgical acute renal failure? *Surgery* 100:400–408, 1986
3. Ronco C: Continuous renal replacement therapies for the treatment of acute renal failure in intensive care patients. *Clin Nephrol* 40:187–198, 1993
4. Storck M, Hartl WH, Zimmerer E, Inthorn D: Comparison of pump-driven and spontaneous continuous haemofiltration in postoperative acute renal failure. *Lancet* 337:452–455, 1991
5. Gomez A, Wang R, Unruh H, Light RB, Bose D, Chau T, Correa E, Mink S: Hemofiltration reverses left ventricular dysfunction during sepsis in dogs. *Anesthesiology* 73:671–685, 1990
6. Grootendorst AF, van Bommel EFH, van der Hoven B, van Leengoed LA, van Osta ALM: High volume hemofiltration improves right ventricular function in endotoxin-induced shock in the pig. *Intensive Care Med* 18:235–240, 1992
7. Stein B, Pfenninger E, Grünert A, Schmitz JE, Hudde M: Influence of continuous haemofiltration on haemodynamics and central blood volume in experimental endotoxic shock. *Intensive Care Med* 16:494–499, 1990
8. Stein B, Pfenninger E, Grünert A, Schmitz JE, Deller A, Kocher F: The consequences of continuous hemofiltration on lung mechanics and extravascular lung water in a porcine endotoxin shock model. *Intensive Care Med* 17:293–298, 1991
9. Heidemann S, Ofenstein J, Sarnaik A: Efficacy of continuous arterio-venous hemofiltration (CAVH) in endotoxic shock. (abstract) *Crit Care Med* 21(Suppl):S159, 1993
10. Lee PA, Matson JR, Pryor RW, Hinshaw LB: Continuous arteriovenous hemofiltration therapy for Staphylococcus aureus-induced septicemia in immature swine. *Crit Care Med* 21:914–924, 1993
11. Bellomo R, Tipping P, Boyce N: Continuous veno-venous hemofiltration with dialysis removes cytokines from the circulation of septic patients. *Crit Care Med* 21:522–526, 1993
12. Bone RC: The pathogenesis of sepsis. *Ann Int Med* 115:457–469, 1991
13. Gardinali M, Padalino P, Vesconi S, Calcagno A, Ciappellano S, Conciato L, Chiara O, Agostoni A, Nespoli A: Complement activation and polymorphonuclear neutrophil leukocyte elastase in sepsis. *Arch Surg* 127:1219–1224, 1992
14. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ: The ACCP/SCCM Consensus Conference Committee: Definitions for sepsis and organ failure and guidlines for the use of innovative therapies in sepsis. *Crit Care Med* 20:864–874, 1992
15. Elebute EA, Stoner HB: The grading of sepsis. *Br J Surg* 70:29–31, 1983
16. Van Snick J, Cayphas S, Vink A: Purification and NH2-terminal amino acid sequence of a T-cell derived lymphokine with growth factor activity for B-cell hybridomas. *Proc Natl Acad Sci USA* 83:9679–9683, 1989
17. Espevik T, Nissen-Meyer J: A highly sensitive cell line, WEHI 164 clone 13, for measuring cytotoxic factor/tumor necrosis factor from human monocytes. *J Immunol Meth* 95:99–105, 1986
18. Eskandari MK, Nguyen DT, Kunkel SL, Remick DG: WEHI subclone 13 assay for TNF: Sensitivity, specificity and reliability. *Immunol Invest* 19:69–79, 1990
19. Gillis S, Ferm MM, Ou W, Smith KA: T-cell growth factors: Parameters of production and a quantitative micro-assay for activity. *J Immunol* 120:2027–2032, 1978
20. Deppisch R, Schmitt V, Bommer J, Hänsch GM, Ritz E, Rauterberg EW: Fluid phase generation of terminal complement complex as a novel index of bioincompatibility. *Kidney Int* 37:696–706, 1990
21. Faist E, Mewes A, Baker CC, Strasser T, Alkan SS, Rieber P, Heberer G: Prostaglandin E2 (PGE2)-dependent suppression of interleukin-2 (IL-2) production in patients with minor trauma. *J Trauma* 27:837–848, 1987
22. Munoz C, Carlet J, Fitting C, Misset B, Bleriot JP, Cavaillon JM: Dysregulation of in vitro cytokine production by monocytes during sepsis. *J Clin Invest* 88:1747–1754, 1991
23. Hakim RM, Breillat TJ, Lazarus JM, Port FK: Complement activation and hypersensitivity reactions to dialysis membranes. *N Engl J Med* 311:878–882, 1984
24. Göhl H, Buck R, Strathmann R: Basic features of the polyamide membranes. *Contrib Nephrol* 96:1–25, 1991
25. Lowry SF: Cytokine mediators of immunity and inflammation. *Arch Surg* 128:1235–1241, 1993
26. Lamarre J, Wollenberg GK, Gonias SL, Hayes MA: Biology of disease: Cytokine binding and clearance properties of proteinase-activated alpha 2-macroglobulin. *Lab Invest* 65:3–14, 1991
27. Lonnemann G, Schindler R, Dinarello CA, Koch KM: Removal of circulating cytokines by hemodialysis membranes in vitro, in *Host Defense Dysfunction in Trauma, Shock and Sepsis,* edited by Faist E, Meakins JE, Schildberg FW, Berlin, Heidelberg, New York, Springer Verlag, 1994, pp 613–623
28. Tonnesen E, Hansen MB, Höhndorf K, Diamant M, Bendtzen K, Wanscher M, Toft P: Cytokines in plasma and ultrafiltrate during continuous arteriovenous hemofiltration. *Anaesth Intens Care* 21:752–758, 1993
29. Goldfarb S, Golper T: Proinflammatory cytokines and hemofiltration membranes. *JASN* 5:228–232, 1994
30. Klapproth J, Castell J, Geiger J, Andus T, Heinrich PC: Fate and biological action of human recombinant interleukin 1 beta in the rat in vivo. *Eur J Immunol* 19:1485–1490, 1989
31. Andreasson S, Goethberg S, Berggren H, Bengtsson A, Eriksson E, Risberg B: Hemofiltration modifies complement activation after extracorporeal circulation in infants. *Ann Thorac Surg* 56:1515–1517, 1993
32. Hoffmann JN, Hartl WH, Deppisch R, Faist E, Jochum M, Inthorn D: Effect of high-volume hemofiltration on hemodynamics and systemic concentrations of anaphylatoxins and cytokines in human sepsis. (submitted for publication)
33. Dofferhoff AS, de Jong HJ, Bom VJ, van der meer J, Limburg PC, de vries Hospers HG, Marrink J, Mulder PO, Weits J: Complement activation and the production of inflammatory mediators during the treatment of severe sepsis in humans. *Scand J Infect Dis* 24:197–204, 1992
34. Schindler R, Dinarello CA: A method for removing interleukin-1- and tumor necrosis factor-inducing substances from bacterial cultures by ultrafiltration with polysulfone. *J Immunol Meth* 116:159–165, 1989
35. Haeffner-Cavaillon N, Cavaillon JM, Laude M, Kazatchkine MD: C3a ($C3a_{desArg}$) induces production and release of interleukin 1 by cultured human monocytes. *J Immunol* 139:794–799, 1987
36. Hart PH, Whitty GA, Piccoli DS, Hamilton JA: Control by IFN gamma and PGE_2 of TNF alpha and IL-1 β production by human monocytes. *Immunology* 66:376–383, 1989
37. Waage A, Brandtzaeg P, Halstensen A, Kierulf P, Esperik T: The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. *J Exp Med* 169:333–338, 1989
38. Wood JJ, Rodrick ML, O'Mahoney JB, Palder SP, Saporoschetz I, d'Eon P, Mannick JA: Inadequate interleukin 2 production: A fundamental immunological deficiency in patients with major burns. *Ann Surg* 200:311–320, 1984
39. Faist E, Kupper TS, Baker CC, Chaudry IH, Dwyer J, Baue AE: Depression of cellular immunity after major injury. *Arch Surg* 121: 1000–1013, 1986
40. Schiffl H, Lang SM, König A, Strasser T, Haider MC, Held E: Biocompatible membranes in acute renal failure: Prospective case-controlled study. *Lancet* 344:570–572, 1994
41. Hörl WH, Haag-Weber M, Georgopoulos A, Block LH: Physicochemical characterization of a polypeptide present in uremic serum that inhibits the biological activity of polymorphonuclear cells. *Proc Natl Acad Sci USA* 87:6353–6357, 1990