Nephrol Dial Transplant (1998) 13: 1458–1464

Nephrology
Dialysis
Transplantation

Original Article

Removal of cytokines and activated complement components in an experimental model of continuous plasma filtration coupled with sorbent adsorption

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Abstract

Background. Sepsis is associated with enhanced cytokine production. Here, we examined the *in vitro* removal of plasma cytokines during continuous plasmafiltration coupled with sorbent adsorption.

Methods. Proinflammatory (tumour necrosis factor-α, interleukins-1, −8) and anti-inflammatory (interleukin 1 receptor antagonist, soluble tumour necrosis factor receptor type I and II) cytokines in whole blood spiked with *Escherichia coli* endotoxin were determined during 2-h recirculation in the ultrafiltrate (condition A), plasma filtrate (condition B), before and after different sorbents (of the Amberlite^{R}-, Amberchrome^{R}-Ambersorb^{R} -type and charcoal). We studied the maximal adsorbing capacity, the 1% leakage test for cytokines and C3a des Arg and the adsorption of complement-dependent leukocyte chemiluminescence. Plasma proteins eluted from the resins were examined by sodium dodecyl sulphate polyacrylamide gel electrophoresis and immunoblotting with an anti-human α_2-macroglobulin.

Results. In condition B, we observed a 40- and 121-fold % increase (*vs* condition A) in the removed mass and clearance of tumour necrosis factor-α. For all other cytokines, the removed mass and the clearance increased from 2.3- up to 6-fold. The Amberchrome^{R} but not the Amberlite^{R} or Ambersorb^{R} resins could remove the highest amount of cytokines and could reduce complement-dependent chemiluminescence. Two protein bands of approximately 400 000 D and 200 000 D were eluted only from Amberchrome^{R} resins and immunoprecipitated by anti-human α_2-macroglobulin and anti-human C3c antibodies, respectively.

Conclusions. These studies suggest an efficient removal of cytokines in continuous plasmafiltration with sorbent adsorption. The binding of α_2-macroglobulin, a carrier of cytokines in plasma, might be a additional mechanism in the removal of cytokines from plasma.

Key words: cytokines; resins; haemofiltration; plasma filtration; plasmapheresis; sepsis

Introduction

Sepsis is the leading cause of acute renal failure and mortality in intensive care units [1]. Defined as a 'Systemic Inflammatory Response Syndrome' (SIRS), human sepsis is characterized by widespread endothelial damage caused by persistent inflammation resulting from infectious and non-infectious stimuli [2,3]. Evidence has been accumulated that severe bacterial infections and septic shock are associated with increased levels of plasma cytokines such as tumour necrosis factor-α (TNF-α), interleukins (IL)-1, −6, −8, −10, the unique antagonist for the IL-1 cell receptor (IL-1Ra) and the soluble TNF receptors type I and II (sTNFR I and sTNFR II) [3]. Conventional continuous extracorporeal treatments such as haemofiltration and haemodiafiltration have failed to significantly reduce cytokine plasma levels [4,5].

The aim of the present studies was to examine the *in vitro* removal of cytokines during continuous plasmafiltration coupled with adsorption to different sorbents.

Our results suggest that the removal of cytokines may be remarkably enhanced using continuous plasmafiltration and adsorption with an appropriate sorbent. The binding of α_2-macroglobulin (α_2-M), a recognized plasma cytokine-carrier, might be an additional mech-

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anism common to all sorbents that efficiently remove cytokines from plasma.

Subjects and methods

In vitro *studies*

Commercially available lipopolysaccharide (LPS) from *E. coli* (strain E55:B5, Sigma Chemical Co, St Louis, MO) was added to 400 ml of outdated whole blood (LPS 5 μg/ml of blood). Whole blood was incubated for 210 min in a 37°C water bath. Three different *in vitro* conditions (A through C) were adopted (Figure 1). In condition A (haemofiltration, HT04, 0.4 m^2, Bellco, Mirandola, Italy), recirculation occurred at 100 ml/min by a roller pump. The average ultrafiltration rate was maintained at 20 ml/min. The ultrafiltrate was perfused in a 2.5×3.5 cm polycarbonate cartridge containing 130 g of charcoal (Detoxyl 3, Bellco). Samples (0.5 ml) of whole blood, and ultrafiltrate were drawn from the experimental circuit at 0, 15, 30, 60 and 120 min to measure the blood and the pre-and post- charcoal cartridge ultrafiltrate. In condition B (plasma perfusion coupled with sorbent adsorption) recirculation occurred at 10 ml/min by a roller pump. The plasmafiltrate, obtained with a plasmaseparator (MicroPes, 0.4 m^2, AKZO, Wuppertal, Germany), was perfused into a 2.5×3.5-cm polycarbonate cartridge containing the Amberlite-type resin XAD 1600. The outlet of the cartridges (condition A and B) was connected to the reservoir in order to maintain a constant plasma volume. The percentage of cytokine removed at each time point in condition A and B was calculated using the formula: [C(pg/ml) × V(ml UF or PF]*100/[C(pg/ml) × (V(ml blood) *dilution factor], where C stands for concentration and V for volume. To calculate the total cytokine removed by filtration (=removed mass), the integral plotted area was calculated and the results were expressed as a percentage of the filtered cytokine over the original amount detected in the reservoir. As a non-circulating control, an aliquot of the blood after incubation with LPS was kept on a rotating wheel for the whole duration of the experiment. Levels of cytokines in the samples at different time points were referred to the static control. Circuits with only the plasma separator and blood lines were used as circulating controls (condition C).

TNF-α, IL-1β, IL-1RA, IL-6, IL-8, and sTNF R I and II were assayed by commercially available ELISA kits (R&D Systems, Minneapolis, USA).

Determination of maximal adsorbing capacity of cytokines and complement

Prewashed charcoal and different resins were packed by gravity into 2.5×1.2 cm polystyrene columns. Resins were activated in 50% absolute methanol in water and extensively washed in isotonic saline. Immediately before perfusion, all cartridges were washed with 20 volumes of isotonic saline and conditioned with outdated human plasma (10 ml). Human normal plasma (100 ml), containing 2.5 mCi of ^{125}I-labelled cytokines (TNF-α, IL-1β, C3a des Arg, IL-8, IL-6) (Amersham, Brauschweig, Germany) and C3a (DuPont, Paris, France) were added with the respective cold recombinant molecules at the following concentrations: TNF-α (500 pg/ml) (Genzyme, Milan, Italy), IL-1β (250 pg/ml) (Genzyme), C3a (10 μg/ml) (Sigma), IL-8 (200 pg/ml) (Calbiochem-Novachem Corp., La Jolla, CA, USA) and IL-6 (200 pg/ml, Calbiochem-Novachem Corp.). Each cytokine was perfused in separate experiments using the charcoal or the resin cartridges in single-pass mode. Flow rate was set at 0.4 ml/min (linear velocity: 19 cm/h). Radioactivity (in 0.2 ml) was counted in a gamma scintillation counter in post-cartridge samples. Perfusion was halted when the c.p.m. radioactivity in post-cartridge samples was superimposable to those detected in pre-cartridge samples suggesting that saturation had occurred. The cartridges were disconnected from the circuit, washed in 20 volumes of isotonic saline and opened. Aliquots of each sorbent were heat-dried, weighed and dispensed in tubes for counting. Results were expressed as ng of cytokines bound per gram of sorbent according to the following formula:

$$\frac{\text{c.p.m. bound}}{\text{c.p.m. basal}} \text{mg sorbent} \times 1000$$

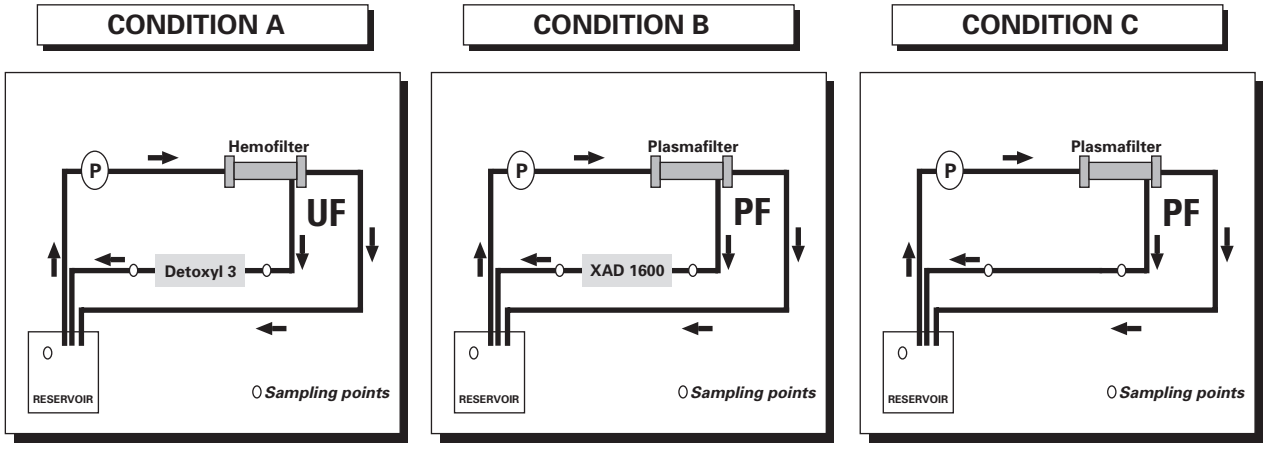

Fig. 1. The three experimental arteriovenous conditions: haemofiltration + natural sorbent (haemofilter + charcoal, condition A), plasma filtration + synthetic sorbent (plasmafilter + Amberlite^{R} XAD 1600, condition B), and control circuit for plasma filtration (only plasma filter, condition C) used in this study. P, pump; UF, ultrafiltrate; PF, plasma filtrate.

Chemiluminescence studies on human polymorphonuclear neutrophils (PMN)

Plasma and PMN were prepared from the same healthy donor. Plasma was obtained by centrifugation (700 *g*, 20 min) of whole blood anticoagulated with the addition of sodium-heparin (Liquemin, La Roche, 1 U/ml of blood). In order to activate the complement system by the alternate pathway, plasma was incubated for 30 min at 37°C with boiled baker's yeast spores (plasma/spore ratio = 2:1). PMN were obtained from whole blood after removing plasma and buffy coat cells. PMN were washed twice with Tris-Tryode's buffer without calcium and magnesium ions. Smears of cells stained with May-Grunwald-Giemsa showed 85–95% PMN. The cells were resuspended at 2×10^6/ml in Tris-Tryode's buffer with calcium and magnesium ions, added with 0.25% bovine serum albumin (Eurobio, St Germain, France). An open circuit was made by connecting the resin- or charcoal-containing cartridges and washed with 60 ml of isotonic sterile saline. Complement-activated plasma was recirculated at 30 ml/h (Perfusor Secura, B.Braun). Samples of 0.5 ml were collected before and after the cartridges at 0 and every 30 s up to 300 s. The oxygen radical production (released by 2×10^5 cells incubated with a constant quantity of plasma) was evaluated by chemiluminescence using a Lumi-Aggregometer (Elvi-Logos, St Gilles, France). The level of PMN activation (i.e. oxygen radical production) in samples collected at time 0 was assumed as 100%.

Determination of the 1% leakage for cytokines and complement

The 1% leakage test was performed in the same conditions as described in the section *'Determination of maximal adsorbing capacities of cytokines and complement'* with the only modification being that the flow rate was increased to 4.2 ml/min and 2.1 ml/min in order to obtain linear velocities in the dialysis range of 200 cm/h and 100 cm/h. Perfusion was halted when 1% c.p.m. radioactivity of the basal c.p.m. radioactivity in 0.2 ml samples was detected in post-cartridge samples.

Studies on the removal of total plasma protein and clotting factor

These studies were performed in the same conditions as described in the section *'Determination of maximal adsorbing capacities of cytokines and complement'* with the only modification being that the flow rate was increased to 4.2 ml/min and that the circuit was used in a closed-loop mode. The biuret method was used for the determination of protein concentration (Merchotest, E. Merck, Darmstadt, Germany). For quantitative determination of the clotting factors, the following tests were used: the Multifibren (Behring, Mannheim, Germany) on the KC10 coagulometer (Amelung, Stuttgart, Germany), for fibrinogen; coagulometry (expressed in % of the reference curve constructed on fresh citrated pooled plasmas from 10 healthy subjects) for Factors II (prothrombin) and VIII.

Western blot analysis of sorbent-bound plasma proteins

After completion of the plasma perfusion, the wet resin (100 µl) was extensively washed in deionized water and centrifuged at 1500 *g* for 10 min at 4°C. The pelleted resin was resuspended in 25 ml of 20 mM Tris-glycine buffer at pH 8.7 dissolved in (1/1, v/v glycerol/water) containing 2% sodium-dodecyl-sulphate (SDS, w/v). The resin was removed by centrifugation and the supernatant was applied to SDS gradient gel (3–20% polyacrylamide) electrophoresis [6]. The separated proteins were then transferred to nitrocellulose membranes (NC-membranes, Schleicher und Schull, Berlin, Germany) by electroblotting according to the method of Towbin *et al.* [7]. The NC membranes were blocked with 1% gelatin (Difco) in phosphate-buffered saline, sequentially incubated with rabbit polyclonal antibody to human α_2-macroglobulin or C3c (Sigma, Munich, Germany), biotinylated antibody to rabbit immunoglobulins (Amersham) and streptavidin-peroxidase (Amersham). The nitrocellulose membranes were then reacted with the peroxidase substrate 4-chloro–1-naphthol and H_2O_2 to detect bound proteins.

Results

In vitro *studies*

Exposure of whole blood to *E. coli* LPS induced the production of high concentrations of TNF-α, IL-1β, IL-8, sTNF RI and II, and IL-1Ra.

As shown in Table 1, the clearance increased fourfold in condition B (*vs* condition A) for IL-1β, IL-8 and IL-1Ra. In condition B, the removed mass of IL-1β and IL-8 increased 2-fold as compared with condition A (see footnote to Table 1). The Amberlite^R XAD 1600 adsorbed 94%, 100% and 100% of IL-Ra, IL-1β, and IL-8 respectively. However, the most striking features of plasma filtration concerned TNF-α. A 40- and 121-fold increase in the removed mass and clearance, respectively, were observed concomitantly with a rapid and significant decline of blood levels (Table 1). TNF-α, at the high concentrations present in the plasmafiltrate, was only partially adsorbed by the Amberlite^R XAD 1600 ($40.1 \pm 2.3\%$).

As shown in Figure 2, using a linear velocity of 200 cm/h, the mean % of TNF-α removed by the Amberlite^R XAD 1600 and the Amberchrome^R CG300md were 22.2% (range 15.3–35.4) and 80.3% (range 69.7–97.6) respectively. At a linear velocity of 259 cm/h, the Amberchrome^R CG300md maintained unaltered its capacity to remove TNF-α (data not shown).

Maximal adsorbing capacity of cytokines and complement

Table 2 summarizes the maximal adsorbing capacities for cytokines and C3a of different sorbents perfused at a very low linear velocity (19 cm/h). Taking charcoal as a reference, the Amberchrome CG300md was the highest adsorbing resin for TNF-α, IL-8 and C3a. The Amberlite^R XAD 1600 showed the highest absorbing capacity only for IL-6.

Chemiluminescence studies on human polymorphonuclear neutrophils (PMN)

As shown in Figure 3, the ability of different sorbents to remove complement-dependent chemiluminescence

Table 1. Adsorption to membranes and sorbents as well as convective clearance in *in vitro* continuous plasma filtration and sorbent perfusion

Cytokines	Conditions	Adsorption to membranes (μg)	Convective Clearance (μg)	Adsorption to sorbent (μg)
TNF-α	A	15.1 ± 2.3*	0.31 ± 0.1	0.31 ± 0.1
	A	(6.04)	(0.15)	(100)
	B	0.01 ± 0.01	37.5 ± 12.2*	8.2 ± 1.1*
	B	(0.004)	(20)	(21)
IL-1β	A	0.04 ± 0.01	10.5 ± 2.3	9.8 ± 2.1
	A	(0.031)	(7.4)	(94)
	B	(0.02 ± 0.01)	24.1 ± 2.1*	24.1 ± 2.1*
	B	(0.014)	(17.2)	(100)
IL-1RA	A	88.2 ± 12.2*	5.5 ± 1.1	2.6 ± 0.51
	A	(56.86)	(3.6)	(47)
	B	15.2 ± 2.3	11.1 ± 3.2*	10.4 ± 0.51*
	B	(9.823)	(3.6)	(94)
IL-8	A	5.7 ± 2.3	8.2 ± 1.5	8.2 ± 1.5
	A	(2.95)	(4.2)	(100)
	B	2.5 ± 1.2	17.2 ± 3.2*	17.2 ± 3.2*
	B	(1.295)	(8.8)	(100)

Condition A. TNF-α, 0.15% of a total mass of 210.65 μg was filtered (clearance, 0.18 ± 0.16 ml/min, mean ± 1 SD; ultrafiltrate concentration, 160.6 ± 158.8 pg/ml, range from 37 to 410 pg/ml). IL-1β, 7.4% of a total mass of 141.56 μg was filtered (clearance, 12.1 ± 3.4 ml/min). IL-1Ra: 3.6% of a total mass of 153.71 μg was filtered (clearance 12.8 ± 2.3 ml/min); IL-8, 4.2% of a total mass of 193.3 μg was filtered (clearance rate of 12.3 ± 1.8 ml/min). sTNF R I and sTNF R II were not detectable in the UF (data not shown). Condition B. TNF-α, 6% of the total mass appeared in the plasmafiltrate (clearance: 21.7 ± 2.5 ml/min). IL-1β, 17.0% was present in the plasma filtrate (clearance, 48.1 ± 9.4 ml/min). IL-1Ra, 21% of a total mass was filtered (clearance 48.3 ± 12.4 ml/min); IL-8, 8.8% was filtered (clearance rate of 49.8 ± 9.8 ml/min). sTNF R I and sTNF R II: removed mass of 7.7 and 6.9% respectively; clearance of 32 ± 13.2 and 23 ± 11.2 ml/min respectively; adsorption to the sorbent: 16 and 12%, respectively.

A variable percent of reduction (15–25%) was applied as a correction factor. The reduction in cytokine level of the non-circulating control was related to the disappearance of detectable native protein due to catabolism or interaction with surface cell membrane specific receptors.

The amount of cytokines adsorbed onto the high permeability (condition A) or plasma filtration (condition B) was calculated as the diffference between the area under the curve of blood and UF concentrations.

The numerals in brackets are percentages over the total mass (calculated as the area under the curve of blood concentrations during the 120-min recirculation). In condition C (see Fig. 1), the following % reductions in plasma levels of each cytokine were determined: for TNF-α (35 ± 12) means ± SD, for IL-1β (33 ± 10), for IL-8 (30 ± 8), for IL-1Ra (30 ± 7), for sTNFR I (10 ± 8), for sTNFR II (13 ± 7) (means ± SD of three experiments performed in the same experimental conditions). *$P < 0.05$ condition A *vs* B. Means ± 1 SD of three experiments performed in triplicate in the same conditions.

showed a remarkable difference among the various sorbents. The Amberchrome^R CG300md was the most effective and rapid one (51 ± 7.6%, at 300 s, $P < 0.000$) while the Amberlite^R XAD 1600 only removed 20.1 ± 6.6% at the same time point of the pre-cartridge chemiluminescence activity. At further time points (from 150 s on), the differences between the Amberchrome^R CG300md and the Amberlite^R XAD 1600 were not significantly ($P > 0.05$) different.

1% leakage test for cytokines and complement

The 1% leakage test was performed in order to test the influence of the linear velocity increasing from 19 to 200 cm/h. As shown in Figure 4, the capacity (ng/g resin) for TNF-α decreased from 325 ± 12 to 8 ± 2 for the Amberchrome^R CG300md and from 170 ± 9 to 0 for the Amberlite^R XAD1600; for IL-8, from 725 to 69 for the Amberchrome^R CG300md and from 280 to 0 for the Amberlite^R XAD1600; for C3a des Arg (in μg/g resin), from 22 to 5 for the Amberchrome^R CG300md and from 3 to 0 for the Amberlite^R XAD1600.

Removal of total plasma protein and clotting factors

Figure 5 shows the results of the time course of total plasma protein and clotting factor removal (expressed in % of post- over pre-values). The end-run concentration in the reservoir diminished to 12.2 ± 2.3% for total plasma protein, to 5.3 ± 1.9% for fibrinogen, 6.2 ± 1.8% for factor II and 7.2 ± 1.6% for Factor VIII. While total plasma protein adsorption was a transient event, occurring only at 5 min (10.4 ± 4.5%), the adsorption of coagulation factors was less predictable and encompassed also the possibility for the coexistence of an adsorption-desorption mechanism (Figure 5).

Western blot analysis of resin-bound plasma proteins

The Amberchrome^R CG300md, but not the Amberlite^R XAD 1600 and the Ambersorb^R XEN 572 and 564, could bind α_2-M and C3c. By Western blot analysis, two protein bands of approximately 400 000 D and 200 000 D were immunoprecipitated by a specific antibody to human α_2-M and C3c respectively.

Discussion

The present studies demonstrated that plasmafiltration allowed the removal of higher amounts of TNF-α, IL-1β, and IL-8 than ultrafiltration. Clearances and sieving coefficients of these cytokines (e.g. namely of

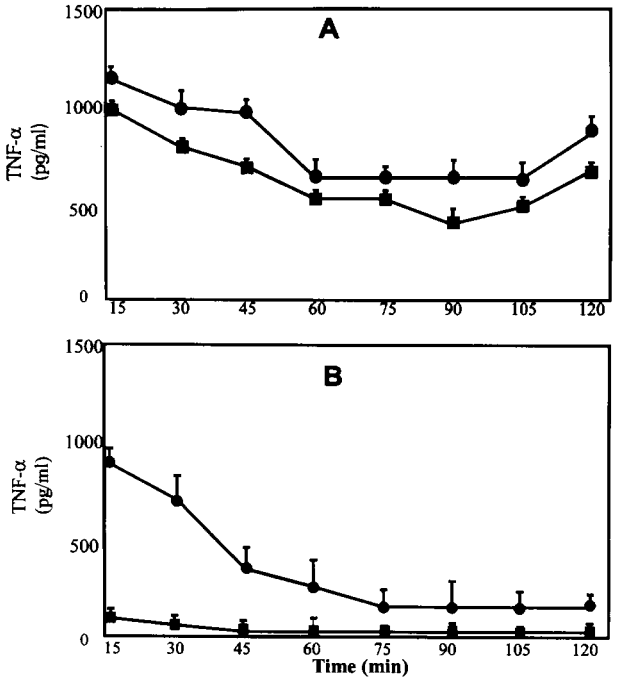

Fig. 2. Time course of plasma filtrate concentrations of TNF-α before (1) and after (n) the cartridge containing using the Amberchrome^R CG300md (A) and the Amberlite^R XAD 1600 (B). Each point is the mean of six experiments performed in duplicate. Bars indicate standard deviation of the mean.

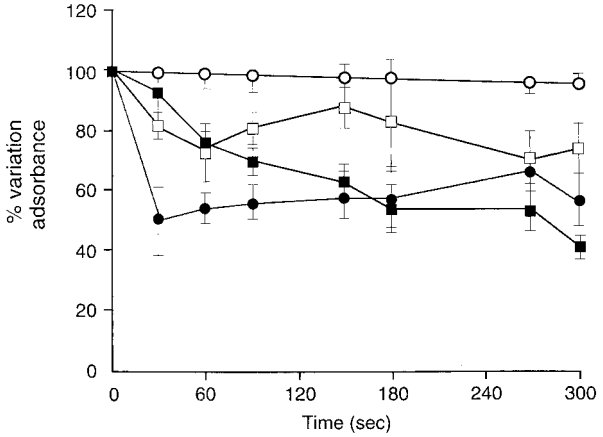

Fig. 3. Removal of PMN chemiluminescence generated in plasma after complement activation before (○) and after passage through cartridges containing charcoal (□), Amberlite^R XAD 1600 (■) and Amberchrome^R CG300md (●). The measurements of cytokines were assessed at 0, 60, 120, 180, 240 and 300 s. PMN activation (control value over time) was tested at each of the time points with control plasmas and referred in percentage with respect to time 0 control values. The other pre- and post-cartridge samples were expressed as percentage *vs* values obtained on PMN exposed with control plasma samples at the same time points. Each point is the mean of three experiments performed in the same experimental condtions as described in the Subjects and methods. Bars indicate standard deviations of the mean.

Table 2. Maximal adsorbitive capacity of different resins and charcoal in respect to various cytokines: *in vitro* experiments

Cytokine	XAD 1600	XAD 1180	CG 300 md	Charcoal
TNF-α (ng/g)	176±10	35±6	395±41	71±5
IL-1β (ng/g)	60±6	231±18	235±20	320±40
IL-8 (ng/g)	69±8	379±15	725±25	100±13
C3a (μg/g)	7.5±0.7	15.6±0.3	25.6±1.1	8.7±1.5

The numbers are means±1 S.D. of three experiments performed in triplicate in the same conditions. The following synthetic resins were used: the Amberlite^R XAD 1600 (particle size, 90% within 0.3–1.2 mm; surface area > 800 m^2/g; porosity, 0.58–0.65 ml/ml); XAD 1180 (particle size, 90% within 1.0–1.6 mm; surface area > 700 m^2/g; porosity, 0.65–0.85 ml/ml); the Amberchrome^R CG300md (particle size, 80% within 50–100 mm; surface area > 650 m^2/g; porosity, 0.55–0.75 ml/ml). The Ambersorb^R resins were XEN 564 (particle size, 20–50 US Sieve series; surface area, 550 m^2/g; porosity, 0.13–0.24 ml/g), and XEN 572 (particle size, 20–50 US Sieve series; surface area, 1100 m^2/g; porosity, 0.19–0.41 ml/g). The amount of cytokines adsorbed onto the different resins and charcoal was experimentally determined using an isotopic dilution method in plasma (see Subjects and methods).

TNF-α) were two to three times higher with 'open' plasmafiltration than with high permeability membranes. The rather low concentrations of cytokines (namely TNF-α) in the ultrafiltrate was in agreement with their reported low concentrations in conventional continuous haemofiltration techniques [8,9].

The application of sorbent to the removal of cytokines has been addressed by others [10–12]. In this paper, we demonstrated the capacity of two different resins, the Amberlite^R XAD 1600 and the Amberchrome^R CG300md to remove TNF-α, IL-1β, and IL-8. However, the Amberchrome^R CG300md was able to remove much higher amounts of TNF-α than the Amberlite^R XAD 1600 at a high linear velocity (200 cm/h). The Amberchrome^R CG300md had the highest maximal adsorbing capacity for the different cytokines. The 1% leakage test allowed us to verify the capacities of each resin for different cytokines and C3a des Arg in relation to increased linear velocities. At variance with previous studies, where very high linear velocities were chosen [10], the range of linear velocities was chosen on the basis of plasma filtrate flow rates used in continuous treatments (20–30 ml/min). A marked reduction of capacity was seen with both resins. However, at a high linear velocity (200 cm/h), the Amberchrome^R CG300md, but not the Amberlite^R XAD 1600, still removed TNF-α, IL-8 and C3a. The adsorbing capacity of Amberchrome^R CG300md at high linear velocities suggested that efficient removal of cytokines and complement-activated products may occur in sepsis. Gardinali and co-workers [13] reported C3a des Arg plasma levels of 2.34±1.7 μg/ml in non-survivors and of 1.27±0.5 μg/ml in survivors. Furthermore, plasma levels of cytokines (in pg/ml) in primary septic shock in humans ranged for TNF-α from <5 to 4875, for IL-1β from <5 to 12 000 and for IL-1Ra from 93 000 to $>3\times10^6$ [13]. The absorbing capacity for the cytokines by the sorbents was therefore quite in excess. Furthermore, although antagonists such as IL-1 Ra would be adsorbed as much as the proinflammatory IL-1β, the pro-inflammatory/

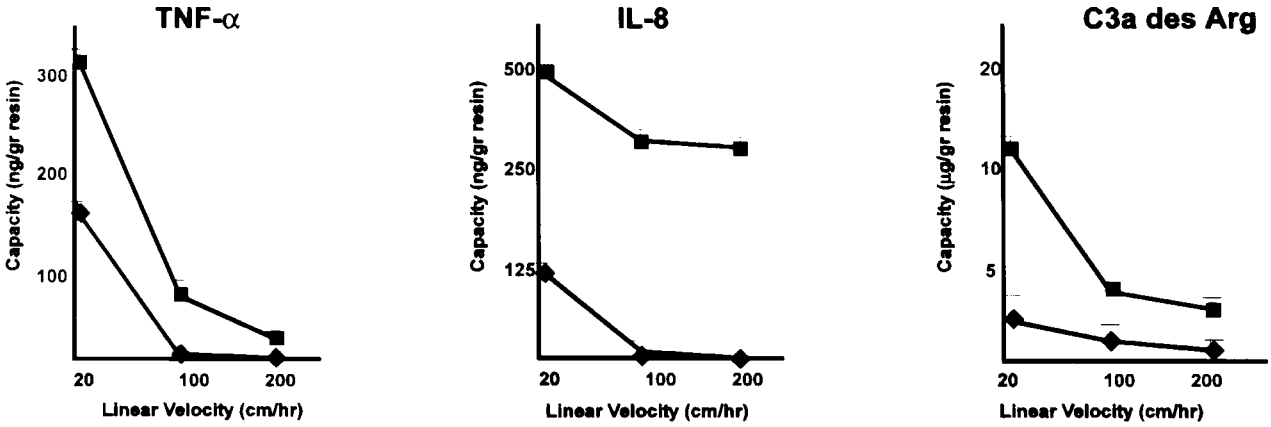

Fig. 4. Assessement of the 1% leakage of various cytokines to different linear velocities using Amberlite^R XAD 1600 (■) and Amberchrome^R CG300md (◆). Each point is the mean of six experiments performed in triplicate. Bars indicate standard deviation of the mean.

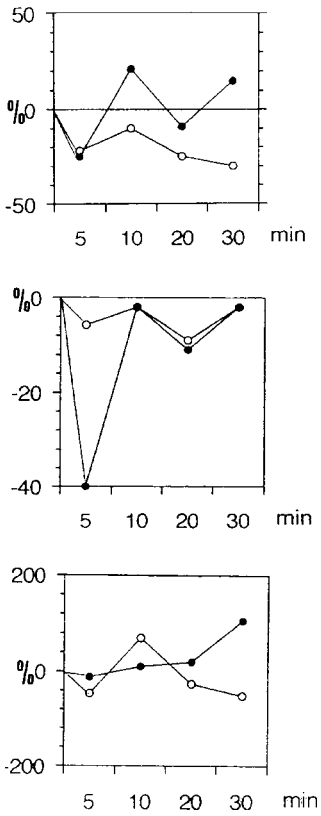

Fig. 5. Time course of Factor II (top panel), Fibrinogen (middle panel), and Factor VIII (bottom panel) removal (expressed as % of post- over pre-cartridge values) with Amberlite^R XAD 1600 (○) and Amberchrome^R CG300md (●) using the experimental condition as described in Subjects and methods. Each point is the mean of two experiments performed in duplicate.

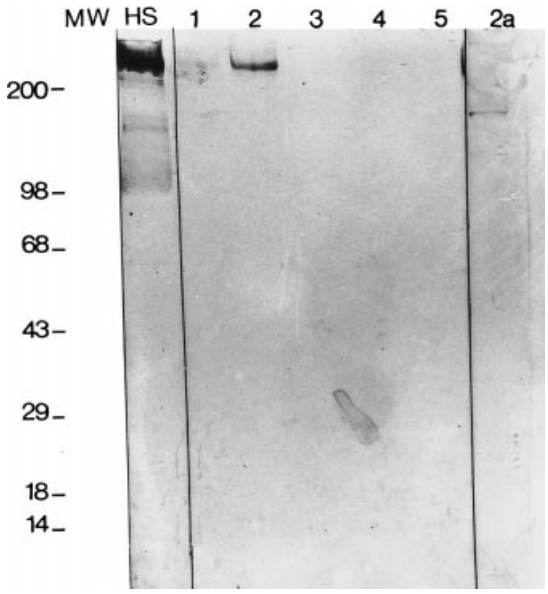

Fig. 6. Analysis by immunoblotting of serum proteins (HS, as control) eluted from different resins (1 and 5, Amberlite^R XAD 1600; 2, Amberchrome^R CG300md; 3, Ambersorb^R XEN 572; 4, XEN 564 using a biotinylated anti-α_2-macroglobulin antiserum). An α_2-macroglobulin band was eluted only with the Amberchrome^R CG300md at the same molecular weight as α_2-macroglobulin from normal serum. Lane 2a, immunoblotting of plasma proteins eluted from the resin using a biotinylated antibody to C3c. A small positive band was evident at about 200 000 D.

anti-inflammatory unbalance would not be modified due to the large excess of IL-1 Ra in plasma.

While adsorption of total plasma proteins ceased after 5 min perfusion, cytokine removal continued for the whole duration of the experiment. By SDS–PAGE electrophoresis, the pattern of proteins bound to the resin differed quite markedly from that of the normal human plasma suggesting that the hydrophobic interaction might involve only some but not all the plasma proteins (data not shown). Amberchrome^R CG300md bound two protein bands of approximately 400 000 D and 200 000 D that were recognized by Western blot analysis by an anti-human α_2-M and anti-human C3c monoclonal antibody respectively. The two protein bands could not be eluted by the other uncharged, hydrophobic resin, the Amberlite^R XAD 1600, that could remove much lower amounts of TNF-α, especially at high linear velocities (i.e. 200 cm/h). Since α_2-M is a carrier for cytokines in plasma [14], it may sustain an additional mechanism in the removal of cytokines by hydrophobic sorbents. Whether the affinity binding

will also involve complement components other than C3c remains to be determined.

In conclusion, sorbent application to plasmafiltration may remove simultaneously high amounts of pro-inflammatory cytokines. The benefit of such a removal in the overall mortality in both experimental and clinical sepsis awaits confirmation. The possibility to regenerate the patient's own plasma rather than infusing fresh plasma, as it would be in conventional plasmapheretic modalities, may eliminate the risk related to the infusion of fresh complement. The latter has been described to be responsible for fatal myocardial depression and circulatory collapse in porcine sepsis [15].

Acknowledgements. These studies were entirely supported by Progetto P 0527 from Istituto Mobiliare Italiano, IMI. M. L. Wratten critically discussed the results of the experiments and reviewed the final text. The excellent technical support of V. Tessore, C. M. Melle, L. Sereni, D. Ognibene and S. Graf is kindly acknowledged.

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Received for publication: 9.6.97
Accepted in revised form: 27.1.98