Haemodialysis-induced pulmonary granulocyte sequestration in rabbits is organ specific

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Abstract

Background. Haemodialysis (HD) with cuprophan (CU) dialysers leads to a severe transient granulocytopenia. In the present study, we challenge the hypothesis that granulocytes sequester within the pulmonary vasculature simply because this is the first vascular bed encountered. This hypothesis is based upon experiments in which activated plasma or complement fragments were infused into animals, and may not pertain to the more complex HD situation.

Methods. We used a rabbit model of HD, and returned the blood into the caval vein (v-HD) or aorta (a-HD). The mesentery was continuously monitored by intravital video microscopy, whereas other tissues were collected at the nadir of granulocytopenia and analysed immunohistochemically.

Results. Compared with controls, the number of granulocytes within alveolar walls was almost 2-fold higher following HD, with no difference between venous and arterial blood return. In addition, both v-HD and a-HD induced granulocyte accumulation within part of the larger pulmonary microvessels, though the amount of granulocytes found was 2-fold higher after v-HD. At no time did a-HD induce granulocyte sequestration within the mesenteric microcirculation. Neither did arterial return increase their number in other first-pass tissues like skeletal muscle or renal glomeruli, but it did so in the liver. In the heart, granulocyte content decreased during HD.

Conclusions. Pulmonary sequestration of granulocytes during CU HD is not simply a first-pass effect, but is organ specific to a great extent. The accumulation within larger microvessels suggests an important role for adhesion molecules, whereas cellular stiffening may be involved in granulocyte retention within alveolar capillaries.

Keywords: animal; heart; immunohistochemistry; intravital video microscopy; leukopenia; lungs

Introduction

Haemodialysis (HD) with cellulosic membranes induces a severe transient granulocytopenia that is associated with activation of the complement system [1–3]. The lungs have been identified as a site of granulocyte sequestration in man by means of radiolabelled granulocytes combined with scintigraphy as well as in animals by measuring differences in granulocyte counts before and after the lungs [4,5]. Infusion of cellophane-exposed plasma into rabbits, which may be considered as a simple model for HD-induced granulocytopenia, results in the entrapment of granulocytes within small pulmonary vessels, including arterioles, venules and capillaries [2,6]. To date, histological examination of the lungs of animals that have been subjected to HD and were killed at the nadir of granulocytopenia has not been reported.

Despite the efforts to elucidate the factors responsible for HD-induced granulocytopenia, the principal mechanism remains obscure. Granulocyte aggregation and/or adhesion to endothelial cells have been proposed [2,7]. Suggestive for such mechanisms is the upregulation of the integrin CD11b as found during HD [8]. However, upregulation itself is inadequate. Integrins have to undergo a conformational change to become active, and upregulation is not necessarily paralleled by an increase in their functionality [8,9]. Alternatively, cellular stiffening due to actin assembly has been suggested [10]. While the latter mechanism will exclusively result in sequestration of granulocytes...
within capillaries, aggregation and/or adhesion may cause accumulation in larger vessels as well.

Besides the mechanisms involved, the question as to whether the lungs play a specific or non-specific role in HD-induced granulocytopenia remains to be answered. According to current opinion, granulocytes sequester in the lungs, because the pulmonary circulation is the first vascular bed encountered by the blood after leaving the dialyser. This assumption is mainly based upon the finding that venous infusion of activated plasma and/or isolated complement fragments into animals predominantly induced pulmonary sequestration of granulocytes, while arterial infusion resulted in systemic accumulation [6,7]. However, clinical HD is obviously more complex. Infusion of activated plasma or isolated factors may therefore not adequately reflect the clinical situation.

In the present study, we assessed a possible organ specificity of granulocyte sequestration during HD-induced granulocytopenia with a rabbit model of HD that allows the use of clinical equipment [11]. As for the pulmonary vasculature, the vessel type in which the granulocytes sequester was studied. Various tissues were analysed for granulocyte content at the nadir of granulocytopenia using immunohistochemistry. Moreover, intravital video microscopy was employed to monitor granulocyte behaviour within the mesenteric microcirculation continuously. Our results show that the granulocytes do not simply sequester in the first vascular bed the activated blood encounters after leaving the dialysis circuit, but that they are rather specifically retained within the lungs.

**Subjects and methods**

**Animals and anaesthesia**

Conventional New Zealand White rabbits (3.2–4.2 kg) were used, that had access to acidified water and pelleted food ad libitum. The institutional animal care and use committee approved all procedures in accordance with NIH guidelines. The rabbits were prepared for HD as described previously [11].

Briefly, the animals were anaesthetized with ketamin (50 mg/kg, intramuscularly; Kombivet, Etten-Leur, the Netherlands) and pentobarbital (20 mg/kg, intravenously; Sanofi Santé B.V., Maassluis, the Netherlands). Anaesthesia was maintained by intravenous administration of ketamin (50 mg/kg/h) and pentobarbital (10 mg/kg/h). After intubation, the rabbits were ventilated at a rate of 40 times per minute. Tidal volume and the air–oxygen mixture were set to keep arterial pO2 above 100 mmHg (yielding 126 ± 4.5 mmHg, mean ± SEM) and blood pH between 7.3 and 7.4 (yielding pH 7.35 ± 0.01, mean ± SEM); pCO2 was 37.9 ± 2.9 mmHg. The rabbits were laid on a heating pad that was servo-controlled at 38 ± 0.2 °C.

**HD circuit and experimental groups**

Cuprophan (CU) dialysers (Hemoflow E1, ethylene oxide sterilized; Fresenius AG Bad Homburg, Germany) were adapted for use in rabbits [11]. Approximately 87% of the capillaries were closed by insertion of a sterile silicone ring with an opening of 9.6 mm in diameter, leaving ~4 cm² of dialyser membrane area available per millilitre of the animal’s blood volume. The adapted dialyser was connected to paediatric bloodlines (A-883 and V-884; Gambro Dialysatoren GmbH and Co. KG, Hechingen, Germany), that were extended using polyethylene infusion lines (VYGON, Ecouen, France). The dialysate compartment of the CU dialysers was filled with sterile saline (Baxter B.V., Uden, the Netherlands) and closed. The blood compartment of the extracorporeal circuit was rinsed with 11 of sterile saline containing heparin (LEO pharmaceutical products B.V., Weesp, the Netherlands; 5 IU/ml), after which the dialysers were immersed in a water bath (38–39 °C).

Rabbits were divided into three groups, two of which were subjected to HD. The third served as a time-matched control. All rabbits were anticoagulated with heparin (300 IU/kg, intravenously). In both HD groups blood was pumped for 110 s from the right carotid artery into the extracorporeal circuit at a rate of 12.5 ml/min using an AK-10 dialysis machine (Gambro Dialysatoren GmbH and Co. KG), whereas in the control group the blood was wasted. Simultaneously, hypovolaemia was prevented by administration of gelofusine (Vifor Medical SA, Crissier, Switzerland; 23 ml) to all animals via the jugular vein. In one of the HD groups the efferent bloodline was then connected to the jugular vein (venous return, v-HD group). In the other, it was connected to a catheter inserted in the left carotid artery with its tip facing the aorta (arterial return, a-HD group). Thereafter, in both HD groups extracorporeal circulation was started and maintained for 10 min with a blood flow of 12.5 ml/min, i.e. 3–5% of the cardiac output of the animal. Anticoagulation was maintained by infusion of heparin (100 IU/kg/h); controls, which were not subjected to extracorporeal circulation, did receive the same maintenance dose.

**Intravital video microscopy**

Leukocyte behaviour within the mesenteric microcirculation was continuously monitored using intravital video microscopy in a subset of the experiments (n=3 for each group). Hereto, a small midline abdominal incision was made, through which the distal ileum was exteriorized. The exteriorized tissue was spread over a siliconized glass plate mounted in the microscope stage (Axiotech vario 100 HD; Zeiss, Oberkochen, Germany). The preparation was continuously superfused with a bicarbonate-buffered solution (25 mM NaHCO3, 130 mM NaCl, 5.6 mM KCl, 2.9 mM CaCl2, 0.6 mM MgCl2; all components from Merck, Darmstadt, Germany) equilibrated with 5% CO2 in nitrogen and kept at 37–38 °C. The bowels were overlaid with wet gauze. After stabilization of the tissue for 20 min and another 10 min after the maintenance administration of heparin had started, circulating leukocytes and vascular endothelial cells were in vivo labelled by intravenous infusion of acridine orange (2 ml; 5 mg/kg; Chroma Technology Corp., Rockingham, VT, USA). Fluorescent cells were made visible by stroboscopic (Chadwick Helmuth, El Monte, CA, USA) epifluorescence microscopy in a subset of the experiments (XBO 75; Zeiss) through a FITC filter set using a salt-water immersion lens (SW25X/0.60; Leitz, Wetzlar, Germany). Images were recorded on videotape by a CCD camera (7020/20; Philips, Eindhoven, the Netherlands);
final magnification at the monitor (LDH 2135/10; Philips) was 870 times. In two additional experiments in the a-HD group, acridine orange was not administered and mesenteric microvessels were visualized by transillumination with a 150 W halogen lamp (Xenophot HLX, Osram, München, Germany).

Granulocyte counts
Blood samples for the assessment of granulocyte concentrations during HD were drawn from the carotid artery or the afferent blood line. Granulocyte counts were determined either with a Helios cell counter (ABX Diagnostics, Montpellier, France) or using a Bürker counting chamber combined with a May–Grünwald–Giemsa stained blood smear as described previously [11].

Tissue collection and immunohistochemistry
After 10 min of HD, rabbits were euthanized with an overdose of pentobarbital. The thorax and pericardial sac were rapidly opened, and a ligature was placed around the base of the heart. Subsequently, the heart and lungs were removed en bloc, and placed on ice. A small upper lung lobe was ligated and then the rest of the lungs was inflated by instilling a 4% gelatin solution (35 ml; Merck). The gelatin was allowed to solidify for ~30 min after which the ligated lung lobe, as well as biopsies from the inflated large lung lobes and the left ventricle wall were collected and snap-frozen in liquid nitrogen. Also from the liver, the right kidney and skeletal muscle (semi-membranosus), biopsies were taken, snap-frozen and stored at ~80°C.

Sections were cut with a thickness measuring 10 μm for lung and 8 μm for other tissues, and collected on microscopic slides precoated with vectabond (Vector laboratories, Burlingame, CA, USA). They were air-dried and stored at ~80°C until further processing.

Sections were fixed in acetone, and stained for CD11b using indirect immunohistochemistry with streptavidin-biotin alkaline phosphatase (AP). They were incubated for 45 min with successively the primary antibody M1/70 (IgG2b, rat anti mouse CD11b, dilution 1:100, kindly provided by Dr R. H. J. Beelen), 1% normal rabbit serum (Sigma-Aldrich, London, UK), the secondary antibody affinity purified biotinylated rabbit anti rat IgG (H+L) (1:100; Vector laboratories), and the streptavidin-AP conjugate (1:100; Vector laboratories). Tris-buffered saline (TBS) at pH 7.6 containing 0.1% bovine serum albumin (BSA; Sigma-Aldrich) was used for preparation of working dilutions as well as for the triple washings in between two incubation periods. AP was visualized by incubation of the sections for 10 min with a TBS solution with pH 8.7 containing 3.6 mM NaNO2 (Merck), 0.22 mM of new fuchsin (Sigma-Aldrich), 0.66 mM naphthol AS-BI phosphate (Sigma-Aldrich) and 1.0 mM levamisol (3.1 mM for kidney and liver sections; Sigma-Aldrich). After washing in tap water, the sections were counterstained with Mayer’s hemalum solution (Merck). The sections were washed and mounted in glycerin-gelatin (Merck).

Sections were analysed as follows. Granulocytes were identified based upon CD11b-positivity and upon morphological characteristics, such as cell size and nuclear shape. Blood monocytes are also CD11b+, but large resident macrophage populations like alveolar macrophages and hepatic Kupffer cells were not stained with the anti-CD11b monoclonal antibody. Although the integrin CD11b is not an exclusive marker for granulocytes, and a small part of the cells that were designated as granulocytes may actually have been monotypic, the positive cells will be referred to as granulocytes. The number of granulocytes per 750 CD11b-negative cells within the alveolar walls was assessed at a magnification of 1000×, being a measure for the number of granulocytes in and/or around alveolar capillaries. The total number of cells being designated (CD11b+ or CD11b−) was always 1000, and these cells were randomly chosen from alveolar areas in two inflated pulmonary sections. Non-capillary pulmonary microvessels were analysed in non-inflated sections. The number of positive cells within 50 of these randomly chosen larger microvessels was determined and the vessel’s circumference was measured using the NIH image software package. Based upon the counted number of positive cells, known depth of each section (10 μm) and vessel circumference, the granulocyte cell concentration in each non-capillary pulmonary vessel was calculated. For the kidney, the number of granulocytes within 50 glomerular cross-sections, which were randomly chosen within two renal sections, was determined at a magnification of 400×. For liver, left ventricular tissue and skeletal muscle, respectively, 50, 200 and 100 high power fields (1000× magnification) within two sections were analysed for the presence of granulocytes.

Statistics
All data are expressed as mean ± SEM values. Group variances were tested for equality using Levene’s test. If the variances were equal, data were analysed for differences between groups using a one-way ANOVA, except for the pulmonary microvessels (see below), followed by a t-test with Bonferroni correction for multiple comparisons. If the variances were not equal, differences between groups were analysed using the non-parametric Kruskal–Wallis test followed, if appropriate, by the Mann–Whitney U-test. An unpaired two-tailed t-test was used to detect differences between controls and the pooled HD data. Data for the larger, i.e. non-capillary, pulmonary microvessels were analysed as follows. Each vessel was put into a discrete class of vessels based upon the calculated concentration of granulocytes. This way, a frequency distribution of vessels containing different concentrations of granulocytes was obtained for each group. Median frequency distributions were tested for between group differences according to the χ2 method. Differences were considered statistically significant if P < 0.05.

Results
Leukocyte behaviour in the mesentery during the progression of granulocytopenia
Both the free flowing white blood cells as well as leukocytes interacting with the mesenteric vascular wall could be observed after fluorescent labelling with acridine orange using intravital fluorescence video microscopy. While prior to HD part of the leukocytes rolled along the venular endothelium, rolling almost disappeared during the 10 min of HD, irrespective of the site of blood return. Also, the total number of leukocytes passing the monitored vessel segments
clearly decreased reflecting the systemic granulocytopenia induced by v-HD and a-HD (Table 1). At no time was firm leukocyte adhesion observed in the mesenteric microcirculation during either of these HD modalities; neither were leukocyte aggregates nor capillary plugging detected. Such potential effects of HD were also absent in two additional a-HD experiments, in which intravital transillumination microscopy was used to monitor the mesenteric microcirculation without fluorescent labelling of the leukocytes.

**Granulocyte content in the lungs**

Irrespective of the way of blood return, HD increased the number of granulocytes within the lungs. Figure 1 shows that the ratio of CD11b+ granulocytes to CD11b− cells within the alveolar walls of rabbits subjected to HD was 2-fold higher than for controls. Arterial return of the blood did not decrease the level of granulocyte sequestration within alveolar walls compared with venous return.

Accumulation of granulocytes was not limited to the alveolar walls. Figure 2 depicts for each group the percentage of larger pulmonary microvessels containing a normal, a significantly higher and a much higher concentration of granulocytes as compared with blood concentration (see Table 1). The diameters of the vessels ranged from 19 to 1380 μm. Median vessel diameters did on average not differ between controls (87 ± 9 μm), v-HD (84 ± 5 μm) and a-HD (82 ± 4 μm). Compared with controls, the percentage of larger microvessels with a granulocyte concentration clearly exceeding that of physiological blood concentrations was twice as high in both HD groups (Figure 2). This effect of HD was more pronounced in the v-HD group than in the a-HD group, inasmuch that those vessels containing much higher numbers of granulocytes contained an average concentration (x10⁶ cells/ml) of 124 ± 22 following v-HD and 73 ± 6 after a-HD (P < 0.05).

**Granulocyte content in first-pass extrapulmonary tissues on arterial return**

When the blood from the HD circuit is returned into the aorta, most of it has to pass other tissues before reaching the pulmonary circulation, and neutrophils may sequester in those tissues. The kidney, which receives a substantial amount of the cardiac output, is one of those. However, neither a-HD nor v-HD caused a significant increase in the number of granulocytes per glomerular section (Figure 3). In controls, 2.1 ± 0.14 granulocytes were observed per section compared with 2.7 ± 0.40 for HD-treated animals with no difference between a-HD and v-HD.

Another tissue that the blood encounters before reaching the lungs upon arterial return is skeletal muscle, which like the kidney contains a large capillary network. Granulocytes were scarcely observed within the muscle tissue, and their low number was not significantly affected by HD (Figure 4). Importantly, arterial blood return did not even tend to increase the number of granulocytes within skeletal muscle as compared with venous return. Neither in the kidney nor in skeletal muscle did we observe engorgement of larger arterioles or venules with granulocytes.

In contrast to the findings in skeletal muscle and glomeruli, the site of blood return did affect the number

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**Table 1. Effect of HD with a CU dialyser on blood granulocyte concentrations (x10⁶/ml)**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control (n=6)</th>
<th>v-HD (n=6)</th>
<th>a-HD (n=7)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.0 ± 0.38</td>
<td>3.3 ± 0.32</td>
<td>4.0 ± 0.61</td>
<td>0.483</td>
</tr>
<tr>
<td>5</td>
<td>4.3 ± 0.57</td>
<td>0.9 ± 0.31</td>
<td>1.0 ± 0.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>10</td>
<td>4.4 ± 0.68</td>
<td>0.2 ± 0.03</td>
<td>0.5 ± 0.37</td>
<td>&lt;0.001</td>
</tr>
</tbody>
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**Fig. 1.** Accumulation of granulocytes within the alveolar wall of the lungs after 10 min of HD with a CU dialyser. The ratio of the number of CD11b⁺ granulocytes to the number of CD11b⁻ cells within the alveolar wall was determined in HD rabbits in which the blood was either returned in the venous system (v-HD, n=6) or the arterial system (a-HD, n=7), and in time-matched controls (n=6). Data are expressed as mean ± SEM values, P < 0.01 for a-HD vs controls.

**Fig. 2.** Accumulation of granulocytes within non-capillary pulmonary microvessels after 10 min of HD with a CU dialyser. Depicted is the percentage of vessels containing a normal, significantly higher and much higher concentration of granulocytes as compared with blood concentration. In HD rabbits the blood was either returned in the venous system (solid bars, n=6) or the arterial system (dashed bars, n=7). Time-matched controls (n=6) are indicated by open bars. Data are expressed as mean ± SEM values, P < 0.05 for a-HD vs controls and for v-HD vs controls.
of granulocytes found within the hepatic sinusoids. Significantly more granulocytes were observed per microscopic field of liver tissue following a-HD than after v-HD (Figure 5). Compared with controls, v-HD tended to slightly decrease the number of granulocytes within hepatic sinusoids, while a-HD strongly tended to increase this number. Granulocyte accumulation within the portal triads and central veins was not observed.

**Granulocyte content in the heart: a second- or third-pass organ**

For both ways of blood return, the coronary circulation of the left ventricle is never the first vascular bed the blood encounters after leaving the HD circuit. In left ventricular tissue of controls an average number of 0.26 ± 0.027 granulocytes per high power field was observed, which is significantly more than the 0.004 ± 0.004 found in skeletal muscle. Following 10 min of HD with a CU dialyser the number of granulocytes per high power field had decreased by ~25% to a value of 0.19 ± 0.015 (P < 0.05); no difference was observed between a-HD and v-HD (Figure 6).

**Discussion**

The present study shows that HD with CU membranes in anaesthetized rabbits induces sequestration of granulocytes within the pulmonary vasculature irrespective of the way of blood return, i.e. either venously or arterially. Granulocytes were found to accumulate not only in alveolar capillaries, but also in larger pulmonary microvessels, albeit more pronounced with venous return. Arterial blood return did induce sequestration of granulocytes within hepatic sinusoids, but not within mesenteric microvessels, glomeruli, skeletal muscle or left ventricle. These findings indicate that granulocyte sequestration does not simply occur in
the first microvascular bed the blood encounters after leaving the HD circuit, suggesting marked lung specificity in the HD-induced granulocytopenia.

The specific nature of pulmonary granulocyte sequestration during HD may be related to the expression of C5a receptors within the lungs. While once thought to be restricted to cells of myeloid origin, evidence has been presented for their expression on other cell types including pulmonary endothelial cells [12]. The rapid accumulation of intravenously injected C5a in rabbit lungs, even in animals devoid of circulating granulocytes, is suggestive for a high C5a binding capacity of the pulmonary vascular wall [13]. In accordance, despite the infusion of C5a from the dialysis circuit into the venous system, arterial levels of C5a do not rise during HD with CU dialysers, not even at a time when circulating granulocytes are scarce [3,11]. Contrary to the pulmonary vasculature, the C5a binding capacity within the rabbit hindlimb vasculature appears to be negligible [13], which may explain the absence of granulocyte sequestration within skeletal muscle capillaries upon arterial blood return in our study.

Our results contradict findings obtained with zymosan-activated plasma (ZAP) as a source of C5a [14]. In the latter study, arterially injected granulocytes were found to sequester in all organs containing capillary networks, including the kidney and skeletal muscles. The discrepancy with our data may be related to a difference in arterial concentrations of C5a or other factors as well as to the time delay of several minutes between the infusion of ZAP and granulocytes [14], during which extrapulmonary vascular beds may have altered their properties. Of clinical interest is that our data are also in contrast with an assumed glomerular accumulation of granulocytes due to HD, as based upon findings following infusion of CU-incubated blood in a rat model of acute renal failure (ARF) [15]. The latter observation has been interpreted as a possible deleterious effect of HD on renal recovery from ARF, a matter that is subject of an ongoing debate [16]. However, a more recent study in rats suffering from ARF shows that actual HD does not affect renal recovery, not even when complement activating CU dialysers are applied [17]. Our data are supportive of this study, as well as of clinical studies that failed to show a benefit of biocompatible dialysers over unmodified cellulosic membranes with respect to the recovery of ARF [16]. In addition, they indicate that infusion of CU-incubated plasma may not adequately reflect clinical HD.

The various tissues under investigation had not the same architecture, which necessitated different ways for expressing their granulocyte content. Therefore, our data do not allow a direct quantitative comparison of the contribution of liver and lungs to the development of granulocytopenia upon arterial blood return. Nevertheless, the capacity of hepatic sinusoids to retain granulocytes was clearly demonstrated. In this respect, it is likely that the rapid removal of granulocytes and/or C5a from the circulation by the lungs prevented granulocyte sequestration within the liver at the nadir of granulocytopenia. Interestingly, granulocytes released from the bone marrow during ZAP infusion in rabbits accumulated within the liver after initial sequestration within the lungs [18], suggesting, in line with our finding, that during clinical HD hepatic sequestration of granulocytes may eventually still take place. Sequestration of granulocytes within the liver is likely to induce the release of liver-derived acute phase proteins, like C-reactive protein (CRP). Plasma levels of CRP are elevated in HD patients, which in turn has been implicated with their long-term risk of cardiovascular events [19].

The proportion of larger pulmonary microvessels containing high granulocyte numbers did not differ between both HD groups. However, with venous blood return individual vessels within this subgroup contained more granulocytes than with arterial return. Especially upon venous return, granulocytes within larger pulmonary microvessels were partly clustered, indicating granulocyte aggregation. In this respect, it is interesting to note that deficiency of CD18, the β-chain of an integrin involved in granulocyte aggregation, seems to reduce the extent of granulocytopenia in a bovine HD model [20]. Granulocyte expression of this integrin, i.e. CD11b/CD18, is upregulated during clinical HD [8], as well as in our rabbit model (unpublished observations). The nadir of granulocytopenia is reached at a time at which only part of the granulocytes will have passed the HD circuit. In particular, these granulocytes may be able to form aggregates, because they have been exposed to higher circulating levels of activating factors. With arterial blood return part of these cells may have lost the ability to form aggregates before reaching the pulmonary circulation, while another part may have been lodged within the hepatic sinusoids. Sequestration within the liver is probably also dependent on adhesion molecules and not on cellular stiffening, because the dimensions of hepatic sinusoids are much larger than those of granulocytes.

We did not observe granulocyte aggregation or adhesion to the endothelium in the mesenteric microcirculation upon arterial return of the blood. Nor did arterial blood return result in engorgement of larger vessels after 10 min of HD in skeletal muscle, kidney and liver. This suggests that in our rabbit model the granulocyte aggregates were formed within and/or just before the larger pulmonary microvessels. Nevertheless, aggregation of ex vivo fluorescently labelled granulocytes within mesenteric and other microvascular beds has been observed upon arterial injection of ZAP or C5a into rats [7]. With regard to this discrepancy with our results, it is highly unlikely that our in vivo labelling with acridine orange prevented granulocyte aggregation within the arterial blood, because also without fluorescent labelling we did not observe leukocyte aggregation or adhesion upon arterial blood return.

In conclusion, pulmonary granulocyte sequestration during HD is organ specific. Of the various non-pulmonary tissues studied, the liver was shown to retain
granulocytes upon arterial return of the blood, but the others, including the glomeruli of the kidney, did not. Our findings refute the long-held hypothesis that the pulmonary sequestration is simply a first-pass effect, and emphasize that studies using infusion of ZAP or isolated factors like C5a may not adequately reflect the more complex situation of clinical HD.

Conflict of interest statement. None declared.

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