Monocyte adhesion molecule expression in interstitial inflammation in patients with renal failure

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Abstract

Background. Patients with renal failure have an increased susceptibility to infections. We therefore studied the recruitment of monocytes and their expression of adhesion molecules CD11b and CD62L at the site of interstitial inflammation in patients with renal failure. Furthermore, we studied if the capacity of monocytes to up-regulate CD11b in interstitial inflammation was determined by the interstitial concentration of chemotactic factors.

Methods. Three intensities of interstitial inflammation (0, intermediate and intense) were established in skin blister chambers. Leukocyte count, CD11b/CD62L expression, monocyte chemotactic protein-1 (MCP-1) and blister activity in terms of CD11b mobilization were determined.

Results. The CD62L expression on monocytes was lower in the peripheral circulation in patients with renal failure compared with healthy subjects (P < 0.005 and P < 0.001). At the site of interstitial inflammation patients had a higher expression of CD62L (intermediate, P < 0.05; intense, P < 0.005). Furthermore, monocytes from patients had an impaired capacity to mobilize CD11b both in the peripheral circulation (P < 0.005) and at the intermediate and intense sites of interstitial inflammation (P < 0.005 and P < 0.001, respectively) compared with cells collected from healthy subjects. We incubated monocytes in blister exudates, in order to explore whether this phenomenon is caused by cellular factors and/or to the interstitial concentration of chemotactic mediators. The expression of CD11b on monocytes from healthy blood donors incubated in blister exudates from either patients or healthy subjects in vitro was similar. The interstitial concentration of MCP-1 at the site of intermediate inflammation was significantly lower in patients with renal failure compared with the corresponding blister exudate collected from healthy subjects (P < 0.05), but no differences were observed at the site of intense inflammation. Furthermore, neutralizing the action of MCP-1 in blister exudates with monoclonal antibodies did not have any impact on monocyte CD11b expression following incubation in blister exudates.

Conclusion. These studies indicate that the impaired capacity of monocytes to mobilize CD11b at the site of inflammation in patients with renal failure is more dependent on constitutive cellular factors than the concentration of CD11b mobilizing factors in the interstitium.

Keywords: adhesion molecules; anti-MCP-1; CD11b; CD62L; inflammation; interstitium; MCP-1; monocyte; renal failure; renal insufficiency; skin blister; skin chamber technique; transmigration

Introduction

Patients with renal failure reveal a number of signs of an impaired humoral and cellular immune response [1]. These patients are more susceptible to infectious diseases, display extended survival of skin allografts and a low response rate to vaccinations, which indicate that both polymorphonuclear leukocyte, lymphocyte and monocyte functions are defect in patients with uraemia [2].

Monocytes play a central role in immune regulation and host defense against immunopathogenic organisms and are activated through molecular signals provided by structures of the infective agent, or inflammatory mediators and chemotactic factors released by adjacent cells. The recruitment of leukocytes under inflammatory conditions involves a multi-step process where adhesion molecules on leukocytes and endothelial cells
play an important role. The currently held concept is that adhered leukocytes undergo migration into the tissue through a series of adherence-detachment events mediated by adhesion molecules on leukocytes and endothelial cells. Attachment and rolling are promoted by vasodilatation and by expression of selectins while firm adhesion is mediated by integrins. The selectin CD62L (L-selectin) is expressed on leukocytes and is involved in the initial attachment between leukocytes and endothelial cells. Following cell activation CD62L is widely shed off. The adhesion molecule CD11b, a member of the \( \beta_2 \)-integrin family, strengthens the initial contact induced by CD62L and is involved in the migration into the inflamed tissue [3]. CD11b is mobilized from intracellular pools upon activation and plays an important role in the innate immunity towards invading microbes [4]. The movement of cells into the tissue is mediated via chemotactic gradients, with the highest concentration at the site of inflammation. Specific chemotactic factors have been isolated and classified into a superfamilly of chemotactic cytokines (chemokines). Monocyte chemoattractant protein-1 (MCP-1) is primarily chemotactic for mononuclear leukocytes while interleukin-8 is primarily chemotactic for neutrophils [5].

The modulation of leukocyte adhesion molecules on inflammatory cells in patients with renal failure or on dialysis has been studied previously almost exclusively on cells in the peripheral circulation [6]. To extend these observations, we have recently applied a skin suction chamber technique, which enables studies on the ability of monocytes and granulocytes to transmigrate into induced inflammatory foci in patients on haemodialysis [7].

The present investigation focuses on the ability of monocytes to transmigrate and to be recruited into three different intensities of inflammation in the interstitium in patients with renal failure who do not require haemodialysis. We specifically addressed the question whether the modulation of monocyte CD11b expression at the site of inflammation differs between patients with renal failure and healthy subjects. In addition, we sought to determine whether adhesion molecule modulation in the interstitium is determined by the local concentration of chemotactic factors in the interstitium or whether it is merely dependent on cellular determinants.

### Subjects and methods

#### Study population

We studied 10 patients (seven males, three females) with a median age of 59 (50–72) years with impaired renal function, a serum creatinine of 453 (236–694 \( \mu \)mol/l) and an estimated GFR level (according to the Cockcroft and Gault equation) of 11.7 (7.8–25.1) ml/min. The renal diagnoses were the following: four patients had inactive glomerulonephritis, four had nephrosclerosis and two had polycystic kidney disease. Nineteen healthy subjects (five men, 14 women), age 32 (28–40) years with an estimated GFR level of 94.7 (89.4–95.1) ml/min were also examined. None of the patients or healthy subjects had any clinical signs of active inflammation. All patients suffering from infectious diseases, diabetes mellitus and active inflammatory diseases as well as those receiving antibiotics, corticosteroids or non-steroidal anti-inflammatory agents were excluded. None of the healthy subjects were on any medication at the time of examination. Informed consent was obtained from all participants and the study was approved by the ethics committee of the Karolinska Hospital.

#### Collection of blood samples

Blood samples were collected in the morning when the first pool of blister exudate was collected (see below) and 10 h later in tubes containing EDTA (Vacutainer, 5 ml, with 50 ml of 21% EDTA, Terumo, Leuven, Belgium). The samples were kept on ice to prevent further complement activation and adhesion molecule receptor modulation.

#### Skin suction chambers and collection of blister fluid

A cutaneous inflammation was induced by use of a skin suction chamber technique in both patients and healthy subjects and the monocyte response with regard to number of transmigrating cells and adhesion molecule modulation at the local site of inflammation in the interstitium was determined. The establishment of skin suction chambers has been described previously in detail [7]. Briefly, two skin blisters were raised on the volar surface of one of the forearms. A constant vacuum (300 mmHg) was applied by continuous gentle suction and blister formation was promoted by heating until the blisters were sufficiently developed. Two 9 mm blisters were formed in 2–3 h. The vacuum was then released and the suction chamber was removed. The blisters were covered overnight with a plastic eye chamber and in the next morning, 12–14 h after the formation of blisters, the roofs of the blisters were carefully removed after the blister fluid had been aspirated, pooled and saved for further analysis. This pool of exudate was designated 0 h and in the group of patients it represented the condition in the state of renal insufficiency. To protect the intact skin from irritation, a transparent sterile adhesive plastic film was applied around the exposed blister floor. Skin bond cement was then applied around the bottom edge of sterilized open-bottom plastic skin chambers with a volume of 1 ml, which were placed over the unroofed blisters and secured. In the proximal chamber, 1 ml of heparinized autologous serum was added (6 \( \mu \)l heparin added to 1 ml serum). In the distal chamber 1 ml phosphate-buffered saline (PBS) and 6 \( \mu \)l heparin were inserted. The administration of serum and buffer in the respective chambers was done in order to induce a difference in the intensity of inflammation in the blisters: an intense in the chamber with serum and an intermediate in the chamber containing buffer. The autologous serum was collected the day before, centrifuged for 15 min at 4 °C and immediately frozen at −70 °C. After 10 h of incubation the fluid was aspirated from each chamber and placed on ice. The blister fluid was centrifuged at 300 g for 5 min at 4 °C and the pellets were resuspended in 500 \( \mu \)l PBS, pH 7.4, supplemented with 0.1 mM EDTA and 0.02% NaN₃.
Preparation of peripheral leukocytes

Peripheral blood from healthy subjects and patients was collected in tubes containing EDTA (Vacutainer, 5 ml, with 50 μl of 21% EDTA; Terumo, Leuven, Belgium). Erythrocytes were haemolysed by addition of 2 ml 4°C isotonic NH₄Cl-EDTA ‘lysing solution’ (containing 154 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA, pH 7.2) to 100 ml blood. The solution was incubated at 4°C for 5 min; the cells were then centrifuged at 300 g for 5 min at 4°C.

The leukocytes were washed in 2 ml 4°C PBS–EDTA. The pellet was resuspended in 100 μl cold PBS–EDTA before immunostaining.

Number of monocytes in the blisters and peripheral circulation

In the blister exudates (one pooled sample aspirated from overnight intact blisters and two samples drawn after 10 h from chambers stimulated with serum and buffer, respectively) monocytes were counted using a flow cytometer (Epics Elite, Beckman Coulter Inc., Hialeah, FL, USA). This instrument gives the actual number of cells and the mean fluorescence intensity (MFI), which represents the density of the antigens of the cell population within a chosen field. The monocytes were distinguished by their different light scattering properties. The forward scatter signal reflecting the cell size was expressed on the x-axis, and the scatter signal representing the granularity was expressed on the y-axis. The monocytes were gated in a cluster and a minimum of 300 cells were collected during analysis. To determine the amount of monocytes in the peripheral circulation, 100 μl blood was haemolysed, stabilized and fixed according to the Multi-Q-prep, ImmunoPrep technique (Beckman Coulter) and the cells were counted by flow cytometry (Beckman Coulter).

Analyses of CD11b and CD62L expression on cells in skin blister exudate and in the peripheral circulation

Expression of adhesion molecules CD11b and CD62L on monocytes was analysed by addition of 5 μl of phycoerythrin-conjugated monoclonal anti-CD11b (Dako AS, Glostrup, Denmark) and 10 μl FITC-conjugated anti Leu-8 (Becton Dickinson Immunocytometry Systems, Mountain View, CA, USA) respectively. A comparable concentration of FITC-conjugated IgG2 (Becton Dickinson Immunocytometry Systems), an isotype control matched antibody was used as background control to determine the non-specific binding. The cells were incubated for 30 min, washed once in 3 ml cold PBS–EDTA and finally resuspended in 0.5 ml cold PBS–EDTA before analysis by flow cytometry.

In vitro mobilization of CD11b on monocytes from healthy blood donors following incubation in blister fluid from patients with renal failure and healthy subjects

Monocyte preparations from eight healthy blood donors were incubated in vitro in 100 μl cell free blister fluid from patients and healthy subjects obtained from the two types of skin blister exudates (10 h blister stimulated with buffer representing intermediate inflammation and 10 h blister stimulated with serum representing intense inflammation). Preparations were diluted 1:2 in 1640 RPMI medium, supplemented with 0.01 mmol/l HEPES (Gibco Ltd., Paisley, Renfrewshire, UK) and 10% heat-activated calf serum (RPMI–HEPES) and were incubated at 37°C for 15 min. Monocytes activated in the presence of 5 × 10⁻⁷ mol/l formyl-metionyl-leucyl-phenylalanine (Sigma Chemical Co., Dorset, UK) for 15 min at 37°C, served as a control and monocytes incubated in RPMI–HEPES alone for 15 min at 4 and 37°C, respectively, served as controls. After incubation, the cell suspensions were washed once in PBS–EDTA (300 g for 5 min, 4°C) and resuspended in 100 μl PBS–EDTA and kept at 4°C until immunostaining and flow cytometric analysis.

Analysis of MCP-1

MCP-1 levels in serum and in blister exudates were measured in 11 of 19 of the healthy subjects (due to shortage of blister fluid) and in 10 patients with renal failure with a commercially available immunoassay (the Quantikine human MCP-1 immunoassay; R&D Systems Inc., Minneapolis, MN, USA). The assay was done according to the manufacturer’s instructions and the minimum detectable concentration of MCP-1 was 5 pg/ml. The volume of blister exudate is small, which limits the number of cytokines that can be studied. We decided to study MCP-1, since it is one of the key chemotactic factors for monocytes.

Inhibition of MCP-1 in blister exudates and its relation to CD11b mobilization in vitro

Blister exudates from healthy subjects representing intermediate or intense inflammation were pre-incubated for 30 min in room temperature in RPMI with 5% fetal calf serum (FCS) supplemented with an anti-human MCP-1 antibody (R&D Systems Inc.) at a final concentration of 0.2 μg/ml. Blister exudates incubated with RPMI with 5% FCS only served as controls. Monocyte preparations (as described above) from four patients and four healthy subjects were incubated with 100 μl blister exudates in 37°C for 15 min. The cell suspensions were washed once in PBS–EDTA (300 g for 5 min, 4°C) and resuspended in 100 μl PBS–EDTA and kept on ice for immunostaining and flow cytometric analysis.

Statistical analysis

Results are expressed as median and interquartile range. The figures are presented in bars representing median and interquartile range or box plots representing median, interquartile range and minimum and maximum values. Statistical
comparisons were made using Wilcoxon matched pairs test and Mann-Whitney U-test. The correlation analysis was done using Spearman Rank test. NS = Not significant.

Results

Expression of CD11b and CD62L on monocytes in the peripheral circulation

The expression of CD11b on monocytes in the peripheral circulation at times 0 and 10 h was significantly lower ($P < 0.005$ and $P < 0.005$) in patients with renal failure [3.1 (2.9–5.2) MFI and 3.8 (3.2–5.1) MFI, respectively] compared with in healthy subjects [6.3 (5.5–7.8) MFI and 6.8 (4.8–8.2) MFI, respectively; Figure 1]. The expression of CD62L on monocytes was also lower in patients with renal failure as compared with healthy subjects, both at time 0 h [19.4 (15.2–22.7) MFI vs 26.3 (21–29) MFI, $P < 0.005$] and at 10 h [17.3 (10.6–20.7) MFI vs 25.7 (21.8–29.4) MFI, $P < 0.001$; Figure 2]. There were no significant differences in the number of monocytes in the peripheral circulation between patients and healthy subjects at the different time points.

Number of transmigrating monocytes into skin blister exudate

The numbers of monocytes transmigrating into the three different blisters were similar in patients with renal failure and healthy subjects. The number of cells transmigrating into the blister at time 0 h, representing the state of renal insufficiency in patients, was $20 \times 10^3$ (15–50 $\times 10^3$) compared with $20 \times 10^3$ (13–37 $\times 10^3$) in healthy subjects (NS). The number of cells recruited to the blister stimulated with buffer was $40 \times 10^3$ (12–80 $\times 10^3$) in patients and $37 \times 10^3$ (13–100 $\times 10^3$) in healthy subjects (NS) and the number of cells transmigrating into the blister stimulated with serum was $100 \times 10^3$ (60–250 $\times 10^3$) in patients and $104 \times 10^3$ (62–209 $\times 10^3$) in healthy subjects (NS).

Expression of CD11b and CD62L on transmigrating monocytes in skin blister exudate

In Figures 1 and 2 the expression of CD11b on monocytes in peripheral circulation and in the three different skin blister chambers is presented. The expression of CD11b was 3–4-fold higher on transmigrated monocytes in the interstitium compared with blood (Figure 1). The expression of CD11b on monocytes collected from the unstimulated blister of patients was lower (but no statistical significance $P = 0.053$) than on corresponding monocytes collected from healthy subjects at time 0 h. However, the expression of CD11b on monocytes in the blister stimulated with buffer and that stimulated with serum was significantly higher in healthy subjects as compared with in patients ($P < 0.005$ and $P < 0.001$, respectively, Figure 1). Thus, monocytes from patients with renal failure have a lower CD11b expression at the three different sites of

Fig. 1. Expression of CD11b on monocytes in the peripheral circulation and on monocytes in skin blister exudates from healthy subjects ($n = 17$) and patients with renal failure ($n = 10$). The data are presented as median ± interquartile range. MFI = Mean fluorescence intensity.
interstitial inflammation compared with the corresponding monocytes collected from healthy subjects.

Figure 2 shows the modulation of CD62L on monocytes transmigrating into the sites of inflammation in the interstitium. The expression was two to five times lower in skin blister exudates compared with in blood. As opposed to the situation in the peripheral circulation, the CD62L expression on monocytes at the sites of intermediate and intense inflammation was significantly higher in patients compared with healthy subjects \((P<0.05\) and \(P<0.005\), respectively; Figure 2).

No correlations were observed between the expression of CD11b or CD62L on transmigrated monocytes and the number of cells in the respective skin blister exudate. The CD11b/CD62L ratio indicates the capacity of monocytes to switch into a CD11b\(^{\text{high}}\)/CD62L\(^{\text{low}}\) phenotype in response to inflammation. This ratio was significantly lower on monocytes from patients at the sites of intermediate and intense interstitial inflammation as compared with corresponding cells from healthy subjects (Figure 3).

The mean age of the patients was higher than in healthy subjects. We therefore studied if there was any correlation between age and the expression of CD11b or CD62L on monocytes in the peripheral circulation or at the three sites of interstitial inflammation. No correlation between age and the expression of CD11b in the interstitium \((0\ \text{h}, \ R=0.21, \ P=0.43; \ \text{intermediate}, \ R=0.11, \ P=1.0; \ \text{intensive}, \ R=0.14, \ P=0.82)\) or CD62L expression \((0\ \text{h}, \ R=0.2, \ P=0.91; \ \text{intermediate}, \ R=0.46, \ P=0.39; \ \text{intensive} \ R=0.26, \ P=0.33)\) were observed. In addition, there were no
correlations between serum creatinine and the cell surface expression of adhesion molecules (data not shown).

In vitro incubation of monocytes from healthy blood donors in skin blister exudates

Monocytes collected from healthy blood donors were incubated in the intermediate and intense pools of skin blister exudates from patients and healthy subjects in order to determine the local biological activity in terms of CD11b-mobilizing factors. Blister exudates from patients had a similar capacity to mobilize CD11b on monocytes from blood donors as exudates from the respective blister in healthy subjects. The expression of CD11b on monocytes following incubation in blister exudates stimulated with buffer (representing intermediate inflammation) was 28.3 (20.8–31.8) vs 24.8 (22.2–28.9) MFI (exudates from patients vs healthy subjects, NS); in the blister stimulated with serum (representing intense inflammation) 30.6 (25.3–42.1) vs 28.3 (21.8–34.8) MFI (NS). In patients, the exudates collected from the blister stimulated with serum had a higher capacity to mobilize CD11b on monocytes from healthy blood donors as compared with cells incubated in the blister stimulated with buffer ($P < 0.05$). In healthy subjects however, the biological activity in terms of CD11b mobilization on monocytes was similar in the blisters representing intermediate and intense inflammation. Thus, despite that monocytes recruited to the sites of inflammation in the interstitium of patients had an impaired capacity to mobilize CD11b compared with monocytes from healthy subjects, the biological activity in terms of CD11b-mobilizing factors at the local sites of inflammation in the interstitium was similar in blister exudates.

Concentration of MCP-1 in skin blister exudates and the effect of monoclonal antibodies directed towards MCP-1

The concentration of MCP-1 was 10–20 times higher in the blisters stimulated with serum as compared with those stimulated with buffer in both patients and healthy subjects ($P < 0.01$ and $P < 0.01$, respectively; Figure 4). In patients, the concentration of MCP-1 in the blister stimulated with buffer was significantly lower as compared with the corresponding blister exudate collected from healthy subjects [1827 (1753–2908) vs 4903 (4082–7048) pg/ml, $P < 0.05$; Figure 4]. There were no correlations between the concentrations of MCP-1 in the skin blisters and the number of transmigrating monocytes. Furthermore, there were no significant correlations between the expression of CD11b on monocytes in the blister exudates and the local concentration of MCP-1 in the respective blister in patients or healthy subjects. In addition, inhibition of MCP-1 activity in the blister exudates, with monoclonal antibody, showed no changes in the CD11b expression in patients or healthy subjects (Table 1).

Discussion

The alteration of monocyte adhesion molecule expression and their migration into induced inflammatory sites in patients with renal failure were investigated in the present study by use of a skin suction chamber

Table 1. Expression of CD11b on monocytes from patients with renal failure and healthy subjects following incubation in blister exudates with RPMI or with RPMI and monoclonal antibodies against MCP-1

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<thead>
<tr>
<th></th>
<th>Intermediate inflammation</th>
<th></th>
<th>Intense inflammation</th>
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<tr>
<td></td>
<td>RPMI</td>
<td>RPMI + anti-MCP-1</td>
<td>$P^a$</td>
<td>RPMI</td>
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<tr>
<td>Patients with renal failure</td>
<td>27.6 (24.9–36.7)</td>
<td>26.4 (22.8–30.4)</td>
<td>NS</td>
<td>31.9 (27–39.9)</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>29.1 (24–29.8)</td>
<td>30.7 (22.2–33.9)</td>
<td>NS</td>
<td>38.8 (33.2–41.6)</td>
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$^a$Mann–Whitney $U$-test.
of CD11b in the interstitium. These issues were not addressed in the present study and need to be further investigated.

Another possible explanation is that the interstitial milieu differs between patients and healthy subjects. In order to explore whether the impaired CD11b expression observed on monocytes from patients with renal failure is dependent on the interstitial milieu, MCP-1 was determined in blister exudates. MCP-1 is a potent monocyte activator, which induces the up-regulation of CD11b and CD11c rapidly and facilitates monocyte adhesion to endothelial cells. The rapid CD11b up-regulation in monocytes is mainly mediated through mobilization from intracellular pools and re-sequestration rather than transcriptional regulation [12]. These experiments showed that the highest concentration of MCP-1 was observed in the skin chamber stimulated with serum, representing an intense inflammation, in both patients and healthy subjects. The concentration of MCP-1 was significantly lower at the site of intermediate inflammation in patients as compared with in healthy subjects. However, no significant difference between patients and healthy subjects was observed at the site of intense inflammation. One possible reason for the lower concentration of MCP-1 at the sites of inflammation in patients with renal failure might be that inflammatory cells have a reduced capacity to synthesize and secrete chemokines in response to inflammation in the interstitium. This issue was beyond the focus of this investigation and needs additional experimental studies to explore. Furthermore, no correlations between the concentration of MCP-1 and the expression of CD11b were observed at the sites of interstitial inflammation in patients or healthy subjects. There were no significant differences in the number of cells transmigrated to the inflammatory sites although the cells had a reduced activity to express CD11b. This is in accordance with one recent experimental study in which we did not observe a strict relationship between leukocyte transmigration in vitro and CD11b expression [13]. The process of monocyte transmigration is complex and not fully elucidated and probably influenced by several mediators and pathways.

We also performed in vitro incubation experiments to study the effect of the interstitial milieu. In these experiments, monocytes from healthy blood donors were incubated in blister exudates from patients and healthy subjects, respectively, in order to determine the biological activity in terms of CD11b-mobilizing factors in blister exudates. In subsequent in vitro experiments, the action of MCP-1 was inhibited by monoclonal antibodies. The biological activity in terms of CD11b mobilization was similar in blister exudates from patients and healthy subjects. Finally, addition of monoclonal neutralizing antibodies towards MCP-1 had no impact on the expression of CD11b on monocytes following incubation in blister exudates in vitro, neither in patients nor in healthy subjects. Thus, monocytes that have transmigrated and been recruited to inflammatory sites in the interstitium in...
patients with renal failure have an impaired ability to mobilize the CD11b receptor to the cell surface despite the fact that cells migrate in a milieu with a similar biological capacity to mobilize the receptor. These findings suggest that determinants other than chemotactic factors and the interstitial milieu determine the expression of CD11b on monocytes in the interstitium, and we hypothesize that factors related to constitutive cellular function may play an important role.

The expression of CD62L on monocytes was significantly lower in the peripheral circulation in patients with renal failure as compared with healthy subjects. During the transmigration and recruitment phase the CD62L receptor was shed. The expression of CD62L in the unstimulated blister was similar in patients and healthy subjects, but higher in patients at the sites of intermediate and intense inflammation as compared with healthy subjects. The data indicate that the physiological alteration of the CD62L expression that occurs during recruitment is dysregulated in patients with renal failure. The monocyte modulation of CD62L in the interstitium of patients with renal failure has not been studied before.

The skin suction chamber technique is well documented and has been used by a number of other investigators to study transmigration and recruitment of leukocytes in both human and experimental models to elucidate different disease mechanisms [14–16]. In a previous study of haemodialysis patients treated with cuprophan dialysis membranes using a similar experimental model, we found that the expression of CD11b on monocytes was similar in serum and in the blister representing the uraemic state. However, it is important to remember that there are fundamental differences between patients with renal failure not yet on dialysis and those treated with bioincompatible cuprophane dialysis three times a week [7]. In the latter group of patients, leukocyte activation occurs regularly and results in aberrations of adhesion molecule phenotypes on inflammatory cells both during and after haemodialysis, as has been shown in a number of previous studies.

One factor that might influence the expression of adhesion molecules in the interstitium is the concentration of leukocyte inhibitory proteins [17]. These proteins have been reported to inhibit chemotactic movements of leukocytes towards chemotacticants during bacterial infections [1]. They might also bind to the CD11b receptors and thereby limit their function. Furthermore, immunoglobulin light chains inhibit chemotactic actions of polymorphonuclear cells [1,18]. Another possibility is that monocytes in patients with renal failure are more refractory to further stimulation due to an altered modulation of CD11b on cells in the peripheral circulation. Further clinical and experimental studies are needed to determine the molecular mechanisms underlying the disturbed modulation of adhesion molecule expression in the interstitium. The monocyte expression of CD11b may also be influenced by either renal function or the underlying renal disease. We did not observe any differences in the behaviour of monocytes in patients with different underlying renal diseases in the present investigation and also no correlation between renal function and monocyte CD11b modulation in the interstitium was observed.

The biological consequences of the impaired ability of monocytes to up-regulate CD11b in the interstitium may be important. To be able to perform cytotoxic actions cells have to adhere to the target cells mainly through up-regulation of CD11b molecules [19]. This receptor also plays a fundamental role in the process of phagocytosis [4]. Consequently, host defense against invading microorganisms might be impaired due to this monocyte defect induced by the impairment of renal function. The altered cellular behaviour at the sites of interstitial inflammation may also have important implications in the development of diseases in which inflammatory processes play a major part, e.g. atherosclerosis and amyloidosis.

We conclude that monocytes in patients with renal failure had a lower expression of CD11b and a higher expression of CD62L at the sites of interstitial inflammation compared with cells from healthy subjects, despite the fact that the biological activity in terms of CD11b-mobilizing factors in the interstitium was similar. In addition, inhibition of the action of MCP-1 in blisters did not have an impact on monocyte expression of CD11b. We therefore propose that the impaired capacity to express CD11b at the interstitial site is more dependent on constitutive cellular factors than the concentration of soluble mediators.

Acknowledgements. The authors want to thank Anette Bygden and Titti Nieminen for skilful technical assistance. The research on this topic was supported by an unrestricted grants from the Karolinska Institutet, the Swedish National Federation of Kidney Patients, the Swedish Society of Nephrology, the Lisa and Johan Grönnbergs Stiftelse and TERUMO EUROPE N.V., dedicated to well being.

Conflict of interest statement. None declared.

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Received for publication: 8.1.03
Accepted in revised form: 18.9.03