The HSP72 stress response of monocytes from patients on haemodialysis is impaired

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Abstract
Background. Induction of heat shock proteins (HSP), i.e. of the major family member HSP70, is an important cytoprotective-resistance mechanism for monocytes/macrophages (Mφ). Patients on haemodialysis present with a high infectious morbidity and enhanced carcinoma incidence. Renal insufficiency-related alteration of microbicidal and tumoricidal functions of Mφ, major effectors of the immune system, might promote these diseases.

Methods. Freshly isolated Mφ from Sprague–Dawley rats 2 weeks after 5/6-nephrectomy and from patients on intermittent haemodialysis (IHD) were stimulated by heat shock (HS) and compared to stimulated Mφ of control rats or healthy volunteers (CTR). Expression of HSP72 (inducible HSP70) was assessed by RT-PCR, and/or flow cytometry. Apoptosis of Mφ was detected by flow cytometry (CD14/annexin V-labelling).

Results. In rat Mφ, baseline HSP72 expression was similar in both groups, but its induction was significantly impaired in renal insufficiency (214 ± 68% less HSP70-mRNA versus CTR, n = 6). In patients, HSF-1-mRNA and HSP72-mRNA/protein response to HS was significantly lower, but not affected by dialysis session itself. In parallel, apoptosis of Mφ of patients was enhanced (+83 ± 29% constitutive apoptotic Mφ versus CTR, n = 8), and HS-dependent protection from apoptosis with and without serum depletion (48 h depletion: HS, +275 ± 37% apoptotic Mφ versus CTR, n = 6; full medium: +166 ± 62% versus CTR, n = 8, P < 0.05) was inferior.

Conclusions. Impaired HSP72 stress response of Mφ in patients on haemodialysis might contribute to the observed immune dysfunction and, therefore, to the increased susceptibility to infection and malignancy. Stress impairment is not restricted to uraemia but is already present in a rat model of chronic kidney disease.

Keywords: apoptosis; haemodialysis; HSP72; human monocytes

Introduction
Patients on haemodialysis present with a high infectious morbidity, enhanced carcinoma incidence, impaired wound healing and reduced effect of vaccination—diseases that are directly related to the function of the immune system [1–4]. Immunological abnormalities of leukocytes have been described in patients on dialysis [5]. Mφ (monocytes/macrophages) are major effectors of the immune system with widespread microbicidal and tumoricidal functions that are altered in uraemia [6–8]. Uraemic immunodeficiency is a complication of renal failure reflecting stress, e.g. exerted by as yet poorly defined uraemic toxins,
imbalances of antioxidants and pro-oxidants, malnutrition or iron overload, membrane bio-incompatibility and inflammation [9–11]. Therefore, patients with end-stage renal disease (ESRD) suffer from increased oxidative stress, which can only be ineffectively treated by haemodialysis [12–14]. The response of human cells to stress, e.g. heat shock (HS), is a highly conserved adaptive cell response found in all cells in all life forms conferring resistance to different stressors, i.e. increased temperature (fever), nutritional deficiency, oxidative stress or hypoxia [15–17]. The corresponding heat shock proteins (HSPs), like the major stress-inducible family member HSP72 (70-kDa HSP, inducible HSP70, HSPA1A, HSP70-1) [18], can also be found in cells under normal physiological conditions controlling the folding of proteins. Constitutively expressed HSP70 (Hsc70) binds to newly translated immature proteins and prevents premature and improper binding and folding [19–21]. This is vital, since HSP72-deficient animals are susceptible to stress or even not viable [22]. In response to stress, housekeeping activities of HSPs, e.g. degradation of unstable and misfolded proteins, control of regulatory proteins or transport of proteins, switch towards reaction against stress to increase the cell’s chance of survival. The cellular injury decreases, either by induction of cytosorrection or by enhancement of repair mechanisms [18]. Thus, HSP72 induction confers protection even against sublethal or damage causing apoptosis and is, therefore, referred to as a cellular lifeguard. We and others showed HSP72-dependent injury protection of monocytes [23,24]. Of note, housekeeping functions of HSPs are critically involved, e.g. as potent immune adjuvants, into the innate and adaptive immune response in inflammation, infection and tumour defence [25–30]. In the case of infection, HSP72 induction is even crucial keeping Mφ alive and, therefore, capable for anti-hazardous actions [31–33]. Several factors and pathways have been identified as responsible triggers causing immunodeficiency in patients with ESRD [8,34,35]. But still, the underlying mechanisms determining Mφ’s dysfunction are complex and remain, to a certain extent, unknown. Thus, they are addressed in this study. Having identified a reduced stress-related HSP72 response in Mφ of rats with chronic kidney disease, we used HS, as a simple model of fever/stress, to investigate the capacity of HSP72 induction in Mφ of patients on intermittent haemodialysis (IHD).

Subjects and methods

Subjects

We enrolled 37 stable patients (20 males) on IHD with mean age of 38 (range 18–76) years chosen at random from the Dialysis Unit of the University Hospital Münster (Münster, Germany). Patients’ Mφ were compared to those of 37 apparently healthy volunteers (23 males) on no medication, who presented no complaints or active pathology with a mean age of 32 (CTR, range 22–59) years. There was no statistically significant difference in age among the groups studied. Patients had haemoglobin levels between 11.5 and 13.5 g/dl, treated with erythropoietin (epoetin alpha: 0–24 000 I.E., median 10 000 I.E./week i.v.) and intravenous iron, and standard heparin anticoagulation. Blood pressure was controlled, whereas 23 patients received a dihydropyridine calcium channel blocker and/or an angiotensin-converting enzyme inhibitor, respectively, to reach normotension. Patients received haemodialysis for 4 h on three occasions each week for more than 12 months, using polysulfone membranes (F8, FMC, Bad Homburg, Germany) and bicarbonate dialysate (32 mmol/l) containing

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<th>Table 1. Real-time PCR primers and Antibodies</th>
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<td>Primer</td>
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<tr>
<td>Human</td>
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<tr>
<td>GAPDH sense</td>
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<tr>
<td>HSP72 sense</td>
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Antibody

Annexin V | FITC-labelled, Bender Medsystems, Vienna, Austria |
Anti-mouse FITC | FITC-labelled F(ab)2 fragments of goat anti-mouse IgG, Dianova, Hamburg, Germany |
Anti-rabbit HRP, HRP | DakoCytomation, Glostrup, Denmark |
CD14 | PE-labelled, clone P9, IgG2b, Becton Dickinson, Palo Alto, USA |
GAPDH | a99848, abcam, Cambridge, UK |
HSP70 | Inducible HSP70, SPA-810, Stressgen, Victoria, Canada |
Ig isotypes | PE- and FITC-conjugated murine IgG mAb of unrelated specificities, Becton Dickinson |

138 mM Na+, 3.0 mM K+, 1.5 mM Cu2+, 0.5 mM Mg2+, 111 mM Cl− and glucose 1 g/l (SK-F 313/1, FMC). Dialysate flow rate was 500 ml/min and blood flow >250 ml/min. Patients had primary arteriovenous fistula (radiocephalic or brachiocephalic), and two needles were used for each dialysis session. The renal diagnoses were nephrosclerosis (n = 10), inactive glomerulonephritis (n = 7), interstitial nephritis (n = 5), polycystic kidney disease (n = 3), renal failure related to chronic heart failure (n = 3) and diabetic nephropathy (n = 2), and in seven patients the reason remained undefined. In both groups, the exclusion criteria were as follows: haemodialysis duration <12 months, PTH >400 pg/ml, evidence of infection, neoplasia or liver disease. Patients on antibiotic, antiinflammatory or immunosuppressive drugs were also excluded. Informed consent was obtained from all participants, and the study was approved by the ethics committee of the Münster University Hospital, Münster, Germany.

Animal model

Eight-week-old male Sprague-Dawley rats (Charles River, Sulzfeld, Germany) with free access to standard rat chow (Sniff, Soest, Germany) and tap water were randomly assigned to surgical 5/6-nephrectomy (5/6-NX, n = 6) or control group (CTR, n = 6). Experiments were approved by a governmental committee on animal welfare and were performed in accordance with National Animal Protection guidelines.

In short, the 5/6-NX involved midline incision, removal of the right kidney and selective ligation of 2–3 branches of the left renal artery. Surgery was performed under general anaesthesia [ketamine (100 mg/kg)/xylazine (10 mg/kg)]. Body weight (BW) was measured before surgery and 2 weeks after surgery. Serum parameters, blood pressure (tail cuff plethysmography) and total kidney function were recorded after 2 weeks of disease progression. Twenty-four hours before kidney recovery, animals were housed in metabolic cages. Urine and blood samples were analysed for creatinine (enzymatic assay, Creatinine-Pap, Roche Diagnostics, Mannheim, Germany), BUN (urease-GLDH method) and protein (Bradford Blue, BioRad Laboratories, München, Germany).

Antibodies and reagents

Antibodies were obtained as described in Table 1. Saponin and D-mannitol were purchased from Merck (Darmstadt, Germany). Unless otherwise...
indicated, all reagents were purchased from Sigma-Aldrich (Taufkirchen, Germany).

Histology
Portions of kidneys were snap-frozen and fixed in 4% formaldehyde in PBS. Histological changes (e.g. glomerular sclerosis, vascular walls, tubular casts and infiltration) were examined by light microscopy in paraffin-embedded tissue with periodic acid-Schiff staining.

Isolation and culture of mononuclear cells from peripheral blood
Blood samples were obtained under sterile conditions in all patients just before or before-and-after the first weekly dialysis session (long interval), respectively. In rats, trunk blood was collected after decapitation 2 weeks after 5/6-NX. Mphi were purified as published before [36]. In short, Mphi were separated from heparinized whole blood by density gradient separation (Ficoll/Hypaque; Biochrom Seromed, Berlin, Germany). Monocytes were further purified by adherence to the culture dishes, resulting in a final purity of >85%. Cells were washed and seeded in 24-well plates (Greiner, Nürtingen, Germany) at 37°C (5% CO2/95% air atmosphere) in RPMI 1640 culture medium (CM, PAA Laboratories, Pasching, Austria) supplemented with 2 mM l-glutamine, 50 µg/ml penicillin/streptomycin, 5 mM HEPES and 10 µM mercapto-ethanol. For HS, Mphi (5 × 10^6/ml) were seeded in preheated CM (temperature as indicated) containing 5% inactivated fetal calf serum (FCS; endotoxin content <0.01 ng/ml) and chemicals as indicated. After 40-min HS, heated CM was replaced by CM (37°C) and cells were further incubated at 37°C until measurement. For experiments with D-mannitol and urea, Mphi were incubated with CM, CM + 150 mg/dl mannitol or CM + 150 mg/dl urea. HS was performed after 60 h (40 min, 47°C), and all cells (±HS) were harvested for real-time PCR analysis after 72 h. Differing incubation times or temperatures are indicated, all reagents were purchased from Sigma-Aldrich (Taufkirchen, Germany). Histological changes (e.g. glomerular sclerosis, vascular walls, tubular casts and infiltration) were examined by light microscopy in paraffin-embedded tissue with periodic acid-Schiff staining.

Flow cytometric detection of inducible HSP70 (HSP72)
After fixation with paraformaldehyde (4%, 15 min, 4°C) and washing, cells (5 × 10^6/ml) were incubated in a staining buffer (1% FCS in a 50 mM HEPES buffer, saponin 0.1%, pH 7.4) for 30 min at 4°C and labelled with mouse anti-human HSP70. After washing, the cells were incubated for 30 min with the secondary FITC-labelled goat anti-mouse-IgG1. Cytofluorometric analysis was performed with a FACScan cytometer (BD Life Science Research) for a total of 10 000 events.

Western Blot
For western blot analyses, proteins were separated by SDS-polyacrylamide (4–20%) electrophoresis, and transferred to a PVDF membrane incubated with blocking agent (Amersham, Freiburg, Germany). After incubation with the primary antibodies anti-HSF-1, or anti-HSP70 and anti-GAPDH for 90 min at room temperature, the secondary HRP antibodies goat anti-rabbit and anti-mouse (1:10 000, DakoCytomation, Glostrup, Denmark) were incubated for another 45 min. For signal development, the blots were covered with chemiluminescent substrate (SuperSignal, Pierce, Bonn, Germany) before exposure.

Flow cytometric quantification of apoptosis
Forty-eight hour serum depletion (0.2% FCS) was used to render Mphi apoptotic. Simultaneous detection of CD14 expression and annexin V-binding identifies apoptotic monocytes [37]. Mphi were deemed to be apoptotic when CD14 expression was low and annexin V-binding was high. Mphi prepared and treated as described above were double-labelled with PE-conjugated CD14 and annexin V-FITC in a staining buffer (PBS, containing 0.05% FCS and 0.05% sodium azide, pH 7.4) for 15 min on ice. PE- and FITC-conjugated murine IgG mAb of unrelated specificities were used as controls. The cells were washed after staining and flow cytometry was applied for a total of 10 000 events. Necrosis was excluded by trypan blue staining.

RT-PCR
Messengar RNA expression profiles for selected genes were validated by real-time PCR. Total RNA (10 µg) from freshly isolated monocytes was used for cDNA synthesis with the high capacity cDNA reverse transcription kit. Real-time PCR was performed using SYBR Green PCR Master Mix on an ABI PRISM 7700 sequence detection system. The specific primer pairs used for amplification are listed in Table 1 (MWG-Biotech, Ebersberg, Germany). Relative gene expression values were evaluated with the 2^−ΔΔCt method using GAPDH as a housekeeping gene [38].

Statistical analyses
Data are presented as means ± SD. Data were compared with Student’s t-test or ANOVA variance analysis for multiple comparisons using GraphPad Prism 4.0 (GraphPad Software, La Jolla, CA, USA). Significance was inferred at P < 0.05.

Results
Animal model
Two weeks after 5/6-NX, subtotal renal ablation induced adaptations in remnant nephrons, whereas kidneys from controls presented with physiological histology (Figure 1A). Kidneys of 5/6-NX showed glomerular hypertrophy, fibrosis, a mild tubulo-interstitial infiltrate and thickening of the vessels’ muscular layer. Atrophy and detachment of epithelial cells, in addition to luminal proteinaceous casts, were found in proximal tubules (Figure 1B).

Functional data from measurements performed 2 weeks after 5/6-NX are given in Table 2. Abnormal renal histology in 5/6-NX was accompanied by elevated blood pressure and functional features of renal insufficiency. Creatinine clearance decreased and occurring polyuria was paralleled by significant proteinuria and weight loss. Retention parameters, i.e. serum creatinine and blood urea nitrogen, were increased significantly.

HS induces HSP72-mRNA in rat Mphi
Twelve hours after HS, HSP72-mRNA expression of Mphi from rats with renal insufficiency (5/6-NX, n = 6) was compared to those of control rats (CTR, n = 6). Basal mRNA expression showed no difference between Mphi of CTR and 5/6-NX (Figure 2). HS significantly increased monocyte HSP72 in both CTR and 5/6-NX, but to a significantly lower extent in Mphi of 5/6-NX (214 ± 68% less than HS-CTR, n = 6).
Temperature- and time-dependent induction of HSP72 protein in human Mφi

To evaluate the most efficient setting for HSP72 induction (Figure 3A), Mφi isolates from six healthy subjects were exposed (40 min) to different temperatures and then allowed to recover at 37°C for 12 h. Induction was most striking after exposure to 47°C (79 ± 14% HSP72-positive cells versus 3 ± 1% basal, n = 27). To estimate to what extent induced HSP72 remains detectable after HS, samples were exposed to 47°C for 40 min and allowed to recover at 37°C during various time periods. The level of HSP72-positive Mφi increased starting 1 h after HS, with maximal values apparent after 9–12 h of recovery (Figure 3B). As shown in Figure 7 and by trypan blue staining (data not shown), HS even in the applied sublethal range did not evoke injury or apoptosis. Therefore, monocyte viability was maintained most likely by HSP induction.

Impaired HSP72 response of Mφi of patients on haemodialysis

Having defined optimal HS-stimulation conditions for human Mφi (Figure 3), we tested our initial hypothesis, that the HS response of Mφi from patients on haemodialysis is impaired. Analogously to Mφi from rat with or without renal failure, no differences were found between basal mRNA-expression level of HSP72 from patients and controls (Figure 4A). Nevertheless, after stimulation with HS, we observed significantly increased HSP72 mRNA in Mφi of CTR and patients. In the next step, we analysed the expression of the responsible transcription factor HSF-1 and found a significantly lower expression in HS-stimulated Mφi of patients then in Mφi of CTR (Figure 4B and C). Consistent with mRNA data, the impaired HSP72-HS response of patients was evident as well, when HSP protein expression was assessed (Figures 4C, 5A and B). Again, no difference was found when basal expression was compared (Figure 5A–C).

To exclude additional posttranscriptional mechanisms possibly involved in the impairment of HSP70 expression of Mφi of patients on haemodialysis, Mφi were incubated either with the transcription inhibitor actinomycin D (1 µg/ml) with or without HS or with the translation inhibitor cycloheximide (15 µg/ml) with or without HS for 1 hour starting at time of HS. Treatment with inhibitors abolished HSP72 induction usually observed 12 h after

Fig. 1. PAS staining of a representative kidney from control and from 5/6-nephrectomized rats (5/6-NX) 14 days after surgery. Whereas kidneys of control rats showed physiological histology (A), interstitial fibrosis and infiltration, glomerular sclerosis, tubular atrophy and casts were extensively present in kidneys of 5/6-NX rats (B). Original magnification ×400.

Fig. 2. Impaired HSP70 response of Mφi from 5/6-NX. HSP70-mRNA response of freshly isolated monocytes from rats 12 h after heat shock (HS, 40 min, 47°C) with (5/6-nephrectomy, 5/6-NX, n = 6) and without (control, n = 6) renal insufficiency. mRNA expression was quantified by real-time PCR. No significant difference was found between Mφi of CTR and 5/6-NX without HS (5/6-NX HSP72-mRNA: 0.83 ± 0.26). HS significantly increased HSP72 (4.56 ± 0.62 fold) in both, Mφi of controls and of 5/6-NX rats (HSP72-mRNA: 2.13 ± 0.68 fold). Results are mean values ± SD. *Statistical difference to controls (basal, P < 0.05).

Table 2. Effects of 5/6-nephrectomy on whole animal functional data (Day 14 after surgery)

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<tr>
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<th>CTR</th>
<th>5/6-NX</th>
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<tr>
<td>Weight change post-operation (% BW)</td>
<td>43 ± 4</td>
<td>−5 ± 11*</td>
</tr>
<tr>
<td>Urinary volume (ml/24 h)</td>
<td>16 ± 3</td>
<td>39 ± 7*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>124 ± 4</td>
<td>167 ± 10*</td>
</tr>
<tr>
<td>Protein excretion (mg/mg creatinine)</td>
<td>0.35 ± 0.12</td>
<td>2.64 ± 1.30*</td>
</tr>
<tr>
<td>Creatinine in serum (mg/dl)</td>
<td>0.19 ± 0.02</td>
<td>0.34 ± 0.03*</td>
</tr>
<tr>
<td>Urea nitrogen in serum (mg/dl)</td>
<td>16 ± 3</td>
<td>31 ± 14*</td>
</tr>
<tr>
<td>CrCl (ml/min/100 g BW)</td>
<td>1.15 ± 0.19</td>
<td>0.56 ± 0.08*</td>
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Mean values ± SD.
CTR: control; 5/6-NX: 5/6 nephrectomy; BW: body weight; CrCl: creatinine clearance; BUN: blood urea nitrogen. n = 5.
*Significantly different to CTR (P < 0.05).
HS in both groups to the same extent excluding such mechanisms.

**Urea does not impair HSP72-HS response of Mphi**

In the next step, we investigated if urea is the responsible trigger for the impaired HS response. To our surprise, incubation with urea significantly increased HSP72 mRNA response to HS, while the osmotic control substance mannitol did not (Figure 6).

**Impaired HS-dependent protection from apoptosis in Mphi of patients on haemodialysis**

To assess the impact of the observed impaired HS response of patients' Mphi on cellular susceptibility, apoptosis was analysed (Figure 7). First, baseline apoptosis rates were quantified. The level of constitutive apoptotic Mphi (after 48-h cultivation in CM containing 5% FCS) was rather low but doubled nearly when Mphi of patients were analysed. Nevertheless, induction of the cytoprotective, anti-apoptotic HS response by HS reduced the...
Impaired HSP72 response of patients on haemodialysis

Fig. 5. Impaired HSP72 response of Mphi of patients on haemodialysis. One exemplary experiment out of 27 independent experiments is shown in (A, B). No difference of HSP72 protein expression was observed between basal level in Mphi from controls (A, 2.1% HSP72-positive cells) and patients (B, 1.7% HSP72-positive cells). Twelve hours after heat shock (HS, 40 min, 47°C), the HSP72 protein expression is significant lower in Mphi from patients on haemodialysis (B, 60.7%) than in Mphi from controls (A, 94.7%). A summary of HSP72 protein expression measured before and after HS is presented in (C). Basal HSP72 protein expression in Mphi of CTR (2 ± 1% HSP72-positive cells, n = 27) was similar to Mphi of patients (3 ± 1%, n = 27). In contrast, HS led to a significant stronger HSP72 induction in Mphi of CTR than in Mphi of patients (79 ± 3% HSP72-positive Mphi versus 65 ± 3% in patients, n = 27, P < 0.05). To elucidate if there is a posttranscriptional difference in the HSP72 response of CTR and patients, monocytes were incubated with the transcription inhibitor actinomycin D (1 µg/ml) ± HS or the translation inhibitor cycloheximide (15 µg/ml) ± HS for 1 h starting at time of HS. Treatment abolished HSP72 induction usually observed 12 h after HS in both groups (actinomycin D: patients: 2 ± 1% HSP72-positive monocytes versus CTR: 3 ± 1%, n = 6, cycloheximide: patients: 13 ± 7% HSP72-positive monocytes versus CTR: 10 ± 5%, n = 3).

Fig. 6. Urea increased HSP72 mRNA response of Mphi to heat shock (HS). Mphi from six healthy controls were incubated with culture medium (CM), CM + 150 mg/dl mannitol or CM + 150 mg/dl urea. HS was performed after 60 h (40 min, 47°C) and all cells (± HS) were harvested for real-time PCR analysis after 72 h. There was no significant difference in baseline HSP70 expression between CTR, mannitol [1.09 ± 0.05 fold change in times of control (fold)] and urea [1.11 ± 0.09 fold] treated Mphi. HS significantly induced HSP72 in all groups with the highest expression level measured in urea-treated Mphi (CTR 9.08 ± 2.5, mannitol 4.53 ± 1.33, urea 52.64 ± 10.63). CTR: control; M: mannitol. Results are mean values ± SD. * Statistical difference to controls (basal) (P < 0.05). # Statistical difference to stimulated controls (P < 0.05).

Influence of haemodialysis session on HSP72-protein expression

To clarify if haemodialysis session-related stress possibly translates into HSP72 stimulation in patients’ Mphi, HSP72 expression of Mphi freshly isolated from patients before and after haemodialysis was investigated but no difference was observed (Figure 8).

Discussion

Uraemic stress-related dysfunction of Mphi is an important contributory factor for the increased incidence of infections and tumours, or the impaired wound healing of patients on haemodialysis [1–4,39]. We herein show that in Mphi of rats with renal insufficiency and in Mphi of patients on IHD, the stress-related HSP72 induction, an important cytoprotective, anti-apoptotic mechanism of cells, is impaired.
HSP acted strongly anti-apoptotic and allowed survival of later enhanced [23]. The rapid and massive induction of Figure 3B) but is still, as previously demonstrated, even 60 h Expression of HSP72 declined with time (peak 9–12 h, cause lower stimulation was less effective (Figure 3A). We have chosen a high and rather long thermal stress, be- specific detection of the inducible form of HSP70. We shortly after HS (Figure 3B) and, therefore, confirmed pro-apoptotic stress.

Upregulation of HSP72 in human monocytes started shortly after HS (Figure 3B) and, therefore, confirmed specific detection of the inducible form of HSP70. We have chosen a high and rather long thermal stress, because lower stimulation was less effective (Figure 3A). Expression of HSP72 declined with time (peak 9–12 h, Figure 3B) but is still, as previously demonstrated, even 60 h later enhanced [23]. The rapid and massive induction of HSP acted strongly anti-apoptotic and allowed survival of monocytes, even when thermal stress with sublethal temperature or stress by serum starvation was applied (low apoptosis and necrosis rates, Figure 7). These results are consistent with previous studies mostly analysing cell lines, which confirmed the role of HSP conferring resistance to physical stress or pro-apoptotic triggers [18,21,41].

Several attempts were made, to clarify the mechanisms causing immunodeficiency in patients with ESRD. What is known so far is that the mechanism cannot be broken down to a single effector. The pathways are rather complex and not yet fully understood. Interestingly, HSP72 induction acts not only anti-apoptotic but influences Mphis’ phagocytosis capacity and resistance to intracellular infection, too [26,29,42,43]. Memoli et al. stated that the impaired immunocompetence observed in Mphi of patients on IHD results from ‘chronic over-stimulation’ caused by pro-inflammatory cytokines, complement activation and toxins, conducting a decreased resonance against further stimulation (fatigue) [35]. Nevertheless, we could neither detect dialysis session-dependent HSP72 induction (stimulation) nor find elevated baseline HSP72 in patients (Figures 4 and 8) or rats with renal insufficiency (Figure 2). Recently, and in contrast to our data, Calabrese et al. measured elevated HSP70 level in lymphocytes of patients with diabetic nephropathy [44]. As diabetes-related stress and patient’s metabolism considerably differ between diabetic and non-diabetic patients and the measurements were performed on EDTA-treated lymphocytes (leading via calcium efflux to pre- and de-activation of cellular pathways), the results may vary. Further, monocytes of patients on peritoneal dialysis exhibit significant HS response after contact with peritoneal dialysis fluid meaning that they are not over- stimulated [45]. Unfortunately, this study lacks the healthy controls.
HSP70 proteins are potent immune adjuvants released e.g. upon tissue damage regulating the immune-response [26–28,30,33,46]. HSP70 also increases the expression of Toll-like receptor 4 (TLR4) [33,46]. Toll-like receptors are critical for activation of immunocompetent cells [47,48]. Ando et al. described reduced expression of TLR4 in monocytes of uraemic patients contributing to the impaired cytokine response [49]. Taken together, a reduced HSP72 response to stress might cause less effective recognition of harmful antigens and less activation of TLR4-dependent pathways.

Urea in clinically relevant concentrations (40–200 mg/dl) induces HS response in the short run (0.5–10 h) in cultured neuroblastoma cells. Maddock and Westenfelder charged protein carbamylation for being the trigger [50]. Interestingly, the HS response returned to zero by 48 h assuming some kind of cellular adaptation in the long run. This is in agreement with our data, showing the same baseline amounts of HSP72 in Mphi of healthy rats and those with increased serum BUN (Figure 2), in urea-treated Mphi (Figure 6), as well as in patients and controls (Figures 4 and 5). Consistent with Marzeck et al. [45], monocytes from rats with renal insufficiency and from patients on IHD responded to HS but, in the case of HSP72, to a significantly lower extent than the ones from controls (Figures 2, 4, and 5). In contrast, skeletal muscle of chronic dialysis patients in the steady state contains increased amounts of protective HS proteins [51]. Notably, HSP72 expression in long-term urea-incubated Mphi responded to a significant higher extent to HS than controls (Figure 3). A possible explanation for urea-induced HSP72 expression in heated Mphi is that urea-mediated protein destabilization may be enhanced by increased temperature. Since disruption of the protein tertiary structure is the final common pathway of HSP induction, this mechanism could also account for urea-induced HSP72 expression at high temperature [52].

Malnutrition is frequently present in patients on IHD. Uraemia changes plasma and intracellular amino acid levels further whereas IHD alters protein turnover and amino acid transport kinetics [53]. In critically ill patients, a decreased plasma glutamine level causes an impaired monocyte function leading to substantial immunosuppression [54,55]. Glutamine deprivation increases Mphi’s susceptibility to stress and apoptosis, as well as it reduces their responsiveness to stimuli [55]. Glutamine starving reduces thermoresistance (to fever) of Mphi that is, according to Pollheimer et al., caused by impaired HSP70 response [54]. Thus, one can assume that glutamine depletion contributes to the reduced HSP72–HS response and favours apoptosis as demonstrated in patients’ Mphi (Figure 7). Nevertheless, accelerated efflux of amino acids from the muscle (protein catabolism) helps to stabilize the intracellular concentrations of amino acids during IHD [53]. Further, we performed all experiments in CM containing 2 mM L-glutamine excluding undersupply and effects were already present in the rat model of chronic kidney disease. Hence, glutamine starvation is probably not involved.

Treatment with erythropoietin induces HSP70 in organs, like heart and kidney, exhibiting anti-apoptotic, cytoprotective effects [56,57]. Recently, Ribell et al. identified an important mechanism leading to differentiation of human erythrocytes based on HSP70 expression [8]. They showed caspase-3 activation during both terminal differentiation and (erythropoietin-starvation-induced) apoptosis. Only in the presence of erythropoietin, HSP70 protected erythroid precursors from caspase-mediated proteolysis [58]. As erythropoietin is an integral part of the pharmacological therapy in ESRD, one can speculate that in untreated ESRD patients the HSP70 response might be worse and apoptosis even elevated. In addition, the HSP72 response was already impaired in the rat model excluding medications being the main underlying trigger altering Mphi’s stress response.

Our results demonstrate an impaired HSP72-stress response of Mphi from patients on IHD that is accompanied by a decreased HSF-1 response to HS. It is alarming that this impaired stress response is not necessarily bound to uraemia but is already present in chronic kidney disease as demonstrated in a rat model. Patient’s Mphi show enhanced susceptibility to stress, e.g. serum starvation, as shown by increased cellular apoptosis values. Impaired HSP72 response of Mphi might explain in part, why patients on IHD are challenged by higher incidences of infections and tumours or impaired wound healing.

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Conflict of interest statement. None declared.

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