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The role of micro-inflammation in the pathogenesis of uraemic pruritus in haemodialysis patients

Martin Kimmel[1,2], Dominik Mark Alscher[1], Robert Dunst[1], Niko Braun[1], Christoph Machleidt[3], Thomas Kiefer[4], Christina Stülten[2], Heiko van der Kuip[2], Christiane Pauli-Magnus[5], Ulrich Raub[6], Ulrich Kuhlmann[1] and Thomas Mettang[7]

[1]Division of General Internal Medicine and Nephrology, Department of Internal Medicine, Robert-Bosch Hospital, [2]Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, [3]Dialysis Center Stuttgart and [4]Dialysis Center Dürrlewang, Stuttgart, Germany, [5]Division of Clinical Pharmacology and Toxicology, Department of Internal Medicine, University Hospital Zuerich, Zuerich, Switzerland, [6]Division of Psychosomatic Medicine, Department of Internal Medicine, Robert-Bosch-Hospital, Stuttgart, Germany and [7]Division of Nephrology, Department of Internal Medicine, Deutsche Klinik für Diagnostik, Wiesbaden, Germany

Abstract

Background. Uraemic pruritus (UP) is still one of the most vexing and disabling symptoms in chronic renal failure. The pathogenesis of UP is obscure and effective therapeutic strategies are elusive. Deduced from partial successful treatment modalities, there is evidence that an alteration of the immune system with a pro-inflammatory pattern along with a deranged T-helper-cell differentiation may be involved in the pathogenesis of UP. We, therefore, investigated whether UP is related to an augmented Th1-differentiation as measured by determination of intracytoplasmatic (i.c.) cytokines and expression of chemokine receptors. Additionally, pro-inflammatory cytokines were determined in serum.

Methods. In a multicentre study, 171 patients on haemodialysis (HD) were screened for UP. Finally, 13 HD patients with and 13 HD patients without UP, as well as 15 healthy controls were enrolled in the study. Peripheral blood mononuclear cells were isolated and the proportion of Th1- and Th2-cells was determined by flow cytometry. The expression of chemokine receptors on CD4 cells (CXCR3 preferentially on Th1 and CCR4 on Th2) and i.c. cytokines (IFNγ for Th1 and IL4 for Th2) were measured after *in vitro* stimulation. Serum cytokine levels (IL6 and TNFα) and CRP were measured by ELISA.

Results. Compared to HD patients without UP, those complaining of UP showed a significantly enhanced proportion of Th1-cells as measured by both techniques. Additionally, serum CRP and IL6 levels were significantly higher in HD patients with UP, compared to HD patients without UP.

Conclusions. These results point to a central role of inflammation in the pathogenesis of UP in HD patients.

Keywords: CRP; cytokines; inflammation; Th1/Th2; uraemic pruritus

Introduction

Uraemic pruritus (UP) remains a frequent and sometimes tormenting problem in patients with end-stage renal failure. Many attempts have been made to relieve patients from this bothersome symptom, with only limited success. The main obstacle in the effort to create effective treatment modalities is the incomplete knowledge of the underlying pathophysiological mechanism. In the last 20 years, the discussion has focused on metabolic abnormalities, such as secondary hyperparathyroidism, precipitated calcium phosphate crystals, histamine secretion by mast cells and μ-receptor alteration as underlying causes of UP. Fundamental questions about each of these pathophysiological concepts were raised by subsequently published conflicting data [1–5].

Based on several observations and results from various trials on UP, there is increasing evidence that UP is a systemic rather than an isolated skin disease and that an alteration of the immune system with an inflammatory pattern resulting in a deranged T helper cell (Th) differentation may be involved in the pathogenesis of UP [5].

Correspondence and offprint requests to: Martin Kimmel, Robert-Bosch-Krankenhaus, Auerbachstr. 110, D-70376 Stuttgart, Germany. Email: vm.kimmel@t-online.de

The balance between different Th-lymphocyte subsets determines the resulting physiological immune response. In a Th1-dominant immune response, a more cytotoxic and inflammatory cytokine pattern is present. It is often associated with an inflammatory state, because the Th1 cytokines recruit and activate inflammatory leucocytes. In contrast, Th2 cells secrete anti-inflammatory cytokines and in this subset the cytokine pattern is associated with antibody and allergic responses [6].

The Th1/Th2 cells can be discriminated by the cytokines they produce or the chemokine receptors they express. The balance of Th1/Th2 cells can be measured on a cellular level [6,7].

We, therefore, investigated whether UP is related to an augmented Th1 differentiation as measured by the determination of intracytoplasmatic (i.c.) cytokines and chemokine receptors in patients on haemodialysis with and without UP. Additionally, the pro-inflammatory cytokines interleukin-6 (IL6) and tumour necrosis factor alpha (TNFα), as well as the inflammatory biomarker C-reactive protein (CRP) were determined in serum.

Material and methods

Patients and controls

171 patients with end-stage renal disease (ESRD) undergoing haemodialysis treatment in three dialysis centres in southern Germany were screened for UP between March 2001 and May 2002. Patients were included if they experienced substantial UP for more than 3 months. Substantial UP was defined as reaching a visual analogue score above 3 (0 = no pruritus to 10 = unbearable pruritus). Furthermore, patients had to be on HD for at least 6 months and had to be well dialysed with a single Kt/V of at least 1.2.

Thirteen age-matched HD patients without UP but fulfilling the remaining selection criteria were recruited as controls.

A secondary control group consisted of 15 healthy individuals who were in a healthy condition, as confirmed by clinical history and basic laboratory parameters.

Written informed consent was obtained from all patients enrolled in this trial.

Exclusion criteria

Patients had to be free of infection or other active inflammatory disease. Patients with a history of dermatologic diseases and those with skin alterations other than the usual cutaneous findings in uraemia such as xerosis or ecchymosis and lesions due to scratching were excluded. Also, patients with systemic disease such as malignancy, liver disease, allergic diathesis or those on immunosuppressive therapy were excluded.

Haemodialysis treatment characteristics

Patients were dialysed 3–4 times a week for 4–5 h. Polysulfone/polyamide membranes (Fresenius F6/F8/F 60/FX 60 or Gambro, Polyflux14S/Polyflux17S) were used in 25 patients; only in one patient without UP was a polyacrylonitrile membrane (Hospal, AN69 membrane, Crystal 3400) used because of polysulfone intolerance. Filters and bloodlines were steam-sterilized.

Pruritus assessment

For screening, patients were asked to estimate their itching intensity by marking a visual analogue scale (VAS) (0 = no pruritus to 10 = unbearable pruritus). In patients entering the study, UP was assessed by a VAS during a 7-day period (marked daily during the week the blood samples were obtained) and a median value was calculated for the VAS scores recorded during this week. Furthermore, a detailed questionnaire (modified Duo-Score [4]), monitored the severity and distribution of pruritus, as well as the frequency of pruritus-related sleep disturbance as follows:

Severity. A slight itching sensation which did not require scratching received 1 point; a sensation which required scratching but without excoriation received 2 points; scratching accompanied by excoriation received 4 points. Pruritus causing total restlessness received 5 points.

Distribution. Itching at less than two locations received 1 point, itching at two or more locations received 2 points and generalized itching received 3 points.

The scores for severity and distribution were recorded and multiplied separately for the morning and the afternoon, so that it was possible to reach a maximum of 30 points.

Sleep disturbances. Each episode of waking up because of itching received 2 points (maximum 10 points) and each scratching episode leading to excoriation during the night received 1 point (maximum 5 points).

The score of sleep disturbances and the severity-distribution product was added to obtain the final score (maximum 45 points).

Blood collection and preparation

In all HD patients, blood samples were obtained after a long dialysis-free weekend interval before starting haemodialysis. In the second control group (healthy controls), blood was drawn after exploring medical history and a short physical examination in the morning. Samples were processed within 2 h of collection. For FACS analysis, peripheral blood mononuclear cells (PBMC) were isolated by standard Ficoll-Paque density-gradient centrifugation (Ficoll-Paque, Amersham Pharmacia Biotech, Sweden).

Standard clinical laboratory investigations

The following parameters were measured automatically in our clinical routine laboratory: leucocytes (G/l), haemoglobin (Hb) (g/dl), haematocrit (Hct) (%), platelets (G/l), ferritin (μg/l), transferrin (mg/dl), albumin (g/dl), total protein (g/dl), creatinine (mg/dl), urea (mg/dl), glucose (mg/dl), sodium (mmol/l), potassium (mmol/l), calcium (mmol/l), phosphate (mmol/l), CRP (mg/dl), iPTH (pmol/l), ALT (U/l), AST (U/l).

CRP was measured in distinct values of 0.1 mg/dl. Values lower than 0.5 mg/dl were merged as <0.5 mg/dl. For data management purposes, values <0.5 mg/dl were set to 0.4 mg/dl.

Table 1. Patient characteristics

Patient characteristics	HD	HD-UP	HD *vs* HD-UP	Correlations with UP (VAS)	Controls
Number	$n=13$	$n=13$			$n=15$
Age (years)	71 (55–84)	70 (56–84)	NS	NC	52 (28–75)
Female	4	1			3
Duration on HD (months)	12 (6–89)	45 (8–192)	$P=0.009$	NC	–
Cause of ESRD					
Glomerulonephritis	3	2			
Polycystic kidney disease	1	1			
Nephrosclerosis	5	3			
Interstitial nephritis	1	1			
Diabetic nephropathy	3	5			
Unknown	–	1			
Kt/V	1.51 (1.30–2.10)	1.42 (1.31–2.60)	NS	NC	

Values are expressed as median (range); NS, not significant; NC, no correlation.

ELISA cytokine measurements

Circulating levels of the inflammatory biomarkers IL6 and TNFα were measured by highly sensitive ELISA kits (Quantikine®, HS R&D Systems, Minneapolis, USA) according to the manufacturer's instructions.

Intracellular cytokine staining

Cytokine production was determined according to Pala *et al.* [8]. In brief, 10^5–10^6 cells/ml were stimulated for 4 h at 37°C with phorbol myristate acetate (5 ng/ml; Sigma-Aldrich, Taufkirchen, Germany) and ionomycin (0.5 μg/ml; Sigma-Aldrich, Taufkirchen, Germany) in order to determine cytokine production. After 2 h, brefeldin A (10 μg/ml; Sigma-Aldrich, Taufkirchen, Germany) was added to increase the sensitivity of cytokine detection because it inhibits intracellular transport of cytokines [8]. After being washed twice in phosphate buffered saline (PBS)/1% bovine serum albumin (BSA, Carl Roth GmbH+Co, Karlsruhe, Germany), 3×10^6 cells were incubated for 30 min at room temperature with FITC-labeled anti-human-CD4 or its isotype-control (Immunotech, Marseille, France). After washing, cells were fixed and permeabilized (Fix&Perm®, An der Grub GmbH, Kaumberg, Austria). These cells were incubated for 45 min with PE-labeled mouse IgG1 anti-human IL4 or its isotype control (the concentration of antibodies was $0.25\,\mu g/5\times10^5$ cells), as well as PE-labeled mouse IgG2b anti-human IFNγ or its isotype control (the concentration of antibodies was $0.021\,\mu g/5\times10^5$ cells). After washing, samples were resuspended in PBS and analysed by flow cytometric analysis.

Chemokine receptor staining

For staining of chemokine receptors, 500 000 of the isolated PBMC were incubated with combinations of the following antibodies for 30 min on ice: FITC-labeled anti-human CD4 was used to identify CD4+ cells (R&D Systems, Minneapolis, USA). PE-labeled mouse IgG1 anti-human CXCR3 (R&D Systems, Minneapolis, USA) or PE-labeled mouse IgG1 anti-CCR4 (BD PharMingen, San Diego, USA) was used for detecting cells positive for CXCR3 and CCR4. After washing in PBS, cells were resuspended in PBS and analysed by flow cytometric analysis.

Table 2. Pruritus assessment

Pruritus assessment	HD-UP ($n=13$)
VAS	4.5 (1.5–8.5)
Duo	16 (4–40)

Values are expressed as median (range).

Flow cytometric analysis

Samples were run on a Becton Dickinson FACScan® using the Cellquest® software (BD Biosciences, San Diego, USA). Between 10 000 and 20 000 events were collected per tube. Thresholds were set on control stains (included for every sample at every time point) to lie on the first percentile.

Statistical analysis

Data management was performed by SPSS® (Version 10.0.7, SPSS, Chicago, Illinois, USA) and Prism® V3.0 statistical software (Graphpad, San Diego, USA).

Non-parametric Mann–Whitney U-test was used to test for significant differences in the mean tendency. The level of significance was set to $P<0.05$. Data are given as median (range) or mean ± standard deviation. Correlations were calculated by Spearman's rank correlation.

Results

Patient characteristics

Out of 171 screened patients with ESRD who were on HD, 51 patients currently had UP; 37 were excluded because they fullfilled at least one exclusion criterion – 1 patient was excluded because the sample preparation failed. The remaining 13 patients aged from 56 to 84 years (12 male, 1 female) were entered in the study. The characteristics of patients and controls are shown in Table 1. For UP assessment, two different scores (Table 2) were used to allow a better and more complete assessment of the various characters of UP – these two scores were well correlated ($r=0.735$).

Table 3. Laboratory examinations

Laboratory exam.	HD ($n=13$)	HD-UP ($n=13$)	HD *vs* HD-UP	Correlations with UP (VAS)	Controls ($n=15$)
Hct (%)	0.36 ± 0.44	0.36 ± 0.05	NS	NC	0.44 ± 0.03
Ferritin (µg/l)	325.8 ± 200.6	337.9 ± 280.1	NS	NC	89.7 ± 90.8 ($n=11$)
Transferrin (mg/dl)	189.3 ± 29.9 ($n=10$)	190.8 ± 25.0	NS	NC	254 ± 27.8 ($n=13$)
Albumin (g/dl)	3.5 ± 0.3	3.4 ± 0.3	$P=0.039$	NC	4.0 ± 0.3
Calcium (mmol/l)	2.30 ± 0.16	2.25 ± 0.19	NS	$r=0.601$	2.34 ± 0.10
Phosphate (mmol/l)	1.6 ± 0.26	1.92 ± 0.64	NS	$r=0.558$	0.96 ± 0.21
iPTH (pmol/l)	8.5 ± 5.9	21.9 ± 19.0	NS	NC	3.3 ± 0.9

Values are expressed as means ± SD, NS, not significant; NC, no correlation.

Table 4. ELISA and FACS results

Cytokines/chemokine receptors	HD ($n=13$)	HD-UP ($n=13$)	Correlations with UP (VAS)	Controls ($n=15$)
ELISA				
CRP (mg/dl)*	0.80 (0.4–1.6)	1.0 (0.5–5.4)	NC	0.4 (0.4–0.8)
IL6 (pg/ml)	7.7 (2.2–41.7)	12.5 (4.0–51.7)	NC	1.3 (0.5–2.6)
TNFα (pg/ml)	2.7 (1.9–4.5)	3.4 (1.5–5.5)	NC	1.5 (1.3–2.0)
FACS				
i.c. IFNγ (%)	18.4 (9.1–36.7)	32.6 (7.0–54.0)	NC	16.0 (6.1–39.4)
i.c. IL4 (%)	5.9 (0.7–13.8)	2.7 (0.4–8.6)	NC	7.5 (0.7–15.4)
CXCR3 (%)	40.3 (24.8–55.7)	53.9 (26.2–74.3)	NC	36.3 (21.0–62.7)
CCR4 (%)	33.4 (18.8–71.7) ($n=12$)	37.7 (24.1–62.3) ($n=12$)	NC	36.2 (21.4–59.2)

Values are expressed as median (range), NC, no correlation.
* < 0.5 was set to 0.4.

HD patients with UP were significantly longer on dialysis therapy compared to patients without UP [45 (8–192) months *vs* 12 (6–89) months, $P=0.009$].

In routine laboratory examinations, the serum albumin was significantly lower in HD patients with UP compared to patients without (3.4 g/dl (2.9–4.0 g/dl) *vs* 3.6 g/dl (2.7–4.0 g/dl), $P=0.039$)). No significant differences between the two groups were observed with regard to haemogram, serum electrolytes, total protein, serum glucose, liver tests, creatinine, urea, ferritin, transferrin and iPTH (Table 3).

Th1/Th2 subset, characterized on a single cell level by i.c. cytokine production of CD4+ cells

The proportion of i.c. IFNγ secreting CD4+ cells was significantly enhanced in patients with UP compared with patients without [32.6% (7.0–54.0%) *vs* 18.4% (9.1–36.7%); $P=0.026$]. Healthy controls yielded comparable results [16.0% (6.1–39.4%) *vs* 18.4% (9.1–36.7%)] to HD patients without UP. The frequencies of the i.c. IL4 secreting CD4+ cells did not significantly vary between the groups [patients with UP 2.7% (0.4–8.6%), patients without UP 5.9% (0.7–13.8%), healthy controls 7.5% (0.7–15.4%)] (Table 4, Figure 1).

Th1/Th2 subset, characterized by chemokine expression on CD4+ cells

CXCR3-expressing CD4+ cells (indicating Th1-cells) were significantly increased in HD patients with UP compared to those without (53.9% (26.2–74.3%) *vs* 40.3% (24.8–55.7%), $P=0.016$). Healthy controls yielded comparable results [36.3% (21.0–62.7%) *vs* 40.3% (24.8–55.7%)] to HD patients without UP (Table 4, Figure 2). This is in line with the findings of an increased Th1-mediated cytokine production (Figure 1). No significant differences were detected in the percentages of CCR4-expressing CD4+ cells (indicating Th2 cells) between the groups [patients with UP 37.7% (24.1–62.3%), patients without UP 33.4% (18.8–71.7%), healthy controls 36.2% (21.4–59.2%)].

Correlation between the two different measuring methods for the Th subsets

A positive correlation was found by comparing the two Th1 measuring methods (CXCR3 and i.c. IFNγ) ($r=0.568$). There was no correlation for the Th2-'markers' (i.c. IL4 and CCR4) ($r=0.286$).

Inflammatory biomarkers in serum

CRP levels were significantly higher in patients with UP compared with that in patients without UP [1.0 mg/dl (0.5–5.4 mg/dl) *vs* 0.80 mg/dl (0.4–1.6 mg/dl), $P=0.010$]. Serum levels of IL6 [12.5 pg/ml (4.0–51.7 pg/ml) *vs* 7.7 pg/ml (2.2–41.7 pg/ml) $P=0.019$] were also significantly elevated in HD patients with UP compared with those without. TNFα showed a non-significant elevation in HD patients with UP

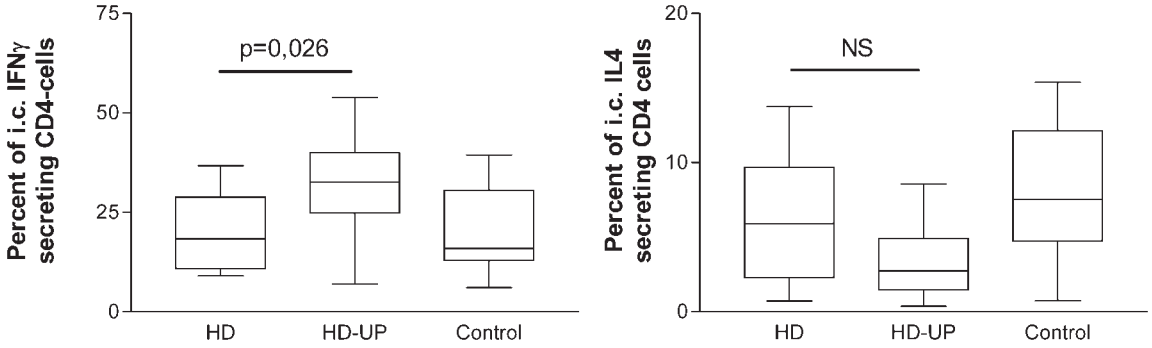

Fig. 1. Percent of i.c. IFNγ and IL4 secreting CD4+ cells in healthy controls, patients on haemodialysis with UP and without UP. (Box-and-whiskers plots: the bottom and the top represents the 25th and 75th percentiles, the line in the box shows the median, NS = not significant.)

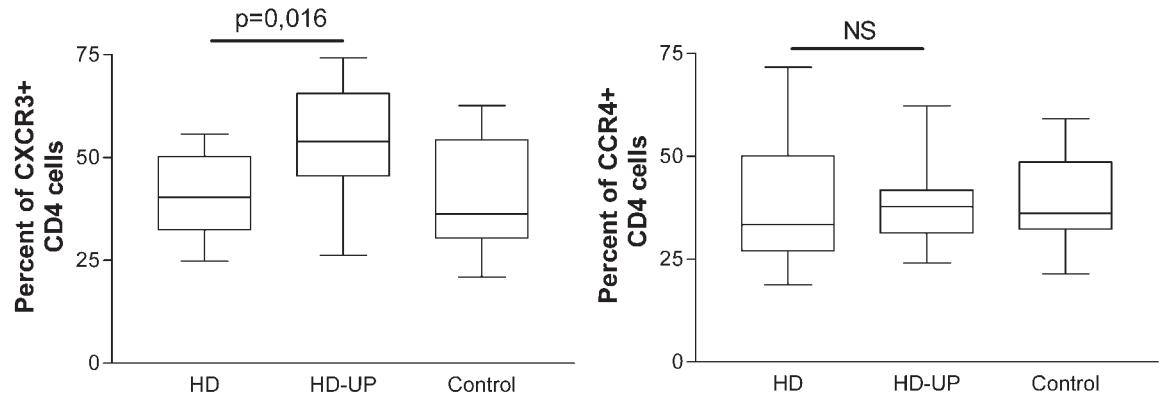

Fig. 2. Percent of CXCR3+ and CCR4+ CD4 cells in healthy controls, patients on haemodialysis with UP and without UP. (Box-and-whiskers plots: the bottom and the top represents the 25th and 75th percentiles, the line in the box shows the median, NS = not significant.)

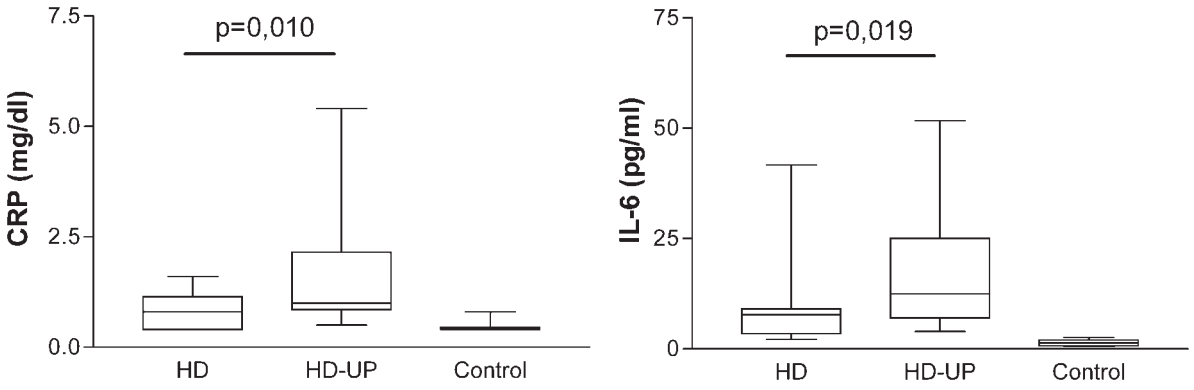

Fig. 3. CRP and IL6 in healthy controls, patients on haemodialysis with UP and without UP. (Box-and-whiskers plots: the bottom and the top represents the 25th and 75th percentiles, the line in the box shows the median.)

compared with those without [3.4 pg/ml (1.5–5.5 pg/ml) *vs* 2.7 pg/ml (1.9–4.5 pg/ml)] (Table 4).

HD patients with UP exhibit an increased level of inflammatory biomarkers (IL6 and CRP) in serum compared with HD patients without UP (Figure 3). CRP-, IL6- and TNFα-levels in the healthy control group were within the normal limits.

There was a positive correlation between CRP and IL6 ($r = 0.786$) Furthermore, there were correlations between parathyroid harmone (PTH) and CRP ($r = 0.522$), PTH and IL6 ($r = 0.652$), PTH and i.c. IFNγ ($r = 0.330$), PTH and TNFα ($r = 0.621$).

Correlations between UP and clinical parameters-/Th1-/Th2-/Inflammatory markers and other serum parameters

There was no correlation between the severity of UP and the Th1-/Th2 or inflammatory markers (Table 4). There was only a correlation between the severity

of UP and the serum calcium ($r = 0.601$) and serum phosphate ($r = 0.558$), but no correlation with serum PTH ($r = 0.039$).

Discussion

The results of this study indicate that an up-regulated inflammatory state is associated with UP in haemodialysis patients. In the absence of clinical signs of inflammation, elevated pro-inflammatory cytokines and serum acute phase proteins as well as the predominant differentiation of Th1 cells in haemodialysis patients with UP support this hypothesis.

As previously stated, evidence that UP is associated with a micro-inflammatory process can be deduced from a series of indirect hints [5]. As UP evolves in haemodialysis patients, the skin appears quite normal without any signs of inflammation, redness, swelling or excoriations, although itching might be extreme. The most important argument in favour of this 'immuno-hypothesis' is the observation that different treatment modalities relieve UP and have a Th1-inhibiting effect. It has been shown that tanning patients with Ultraviolet B (UVB) light was accompanied by relief from UP – even if only half of the body was irradiated [1]. This observation is of special interest because UVB-exposure leads to an attenuated Th1 differentiation [9]. Moreover, immunosuppressive medications like thalidomide (a specifically Th1-inhibiting agent) reduced UP to a substantial extent, [3,10]. Similarly, as shown in a case series [11] and a prospective study [12], tacrolimus led to a rapid relief from UP. It is noteworthy that patients with a kidney transplant independently of the degree of kidney failure experienced, almost never complain of UP as long as the immunosuppressive therapy is continued [13].

A newer approach to evaluate the manner in which the immune system is activated is based on the measurement and proportional differentiation of T helper cells [6]. As Th1 and Th 2 cells produce different cytokines themselves, they can be phenotyped by measuring the intracytoplasmatic cytokine pattern at a single cell level with FACS. This technique allows a more accurate characterization of T helper cells than former methods measuring cytokines in T helper cell culture with ELISA or bioassays [8]. Another way to segregate T helper cells can be achieved by staining them for chemokine receptors. Chemokines are a family of polypeptide mediators. Chemokines and their receptors regulate diverse aspects of immune homeostasis and inflammation; they direct cell trafficking and recruitment and activate inflammatory cells. Recent findings in the *in vitro* polarized Th subsets as well as T cell clones have indicated that chemokine receptors are differentially expressed on Th1 and Th2 effector cells and serve as surface-markers for the Th1- and Th2-mediated immune response. It has been shown that Th1 cells predominantly express CXCR3 and Th2 cells mainly express CCR4 [7]. To strengthen the significance of our results, both techniques (i.c. cytokine and chemokine staining) were applied. Both methods yielded similar results and showed a close positive correlation supporting the validity of the data obtained. In accordance with the above-mentioned up-regulation of Th1 cell differentiation, other serum markers of inflammation such as IL6, CRP and TNFα are also markedly increased and the serum albumin (which can be regarded as a negative acute phase protein) is decreased in HD patients with UP. The association of UP with decreased levels of albumin/transferrin and elevated levels of CRP indicating up-regulated inflammation were recently reported by Virga and coworkers and is in accordance with our results [14]. Nevertheless, increase in serum concentrations of TNFα in patients with UP failed to achieve statistical significance. TNFα as an important pro-inflammatory cytokine probably plays a central role in the pathogenesis of UP, as it has been demonstrated to sensitize the itch-recording nerve endings (c-fibres) in the skin, resulting in a more pronounced signal following a distinct stimulus [15]. Deduced from a mouse model, clearance of TNFα is reduced in renal insufficiency [16]. This observation might explain at least in part the higher TNFα levels in uraemic patients without UP compared to controls. However, highly fluctuating serum levels of this short-lived cytokine may not necessarily reflect conditions within the tissue.

In recent years, a large body of evidence has been accumulated that uraemia itself is a state of more or less pronounced inflammation as reflected by elevation of the classical inflammatory biomarkers, TNFα, IL6 and CRP [17]. It is likely that there are multiple factors that constitute potential causes of the inflammatory state in haemodialysis patients and they can be attributed to dialysis-related factors, uraemia *per se* and the loss of kidney function. They include oxidative stress, uraemic toxicity, non-biocompatible dialyzers, vascular access infection, less-than sterile dialysate, and dialysate back leak [17]. The role of AGEs and parathyroid hormone, both suspected uraemic toxins, in the pathogenesis of uraemia-related micro-inflammation is unclear at present. There are data that PTH might act as a proinflammatory peptide [18]. In line with our hypothesis, serum PTH correlated with some of our inflammatory markers (CRP, IL6, i.c. IFNγ, TNFα), although there was no correlation between the severity of UP and PTH serum concentrations in patients with UP. Further, the difference in PTH levels between HD patients with and without UP failed to reach statistical significance, which is in accordance with our own previous findings and a series of other studies [5]. Hence, the precise impact of elevated PTH levels in UP remains to be elucidated.

Given the marked difference in time on dialysis between the patients with and without UP at a first glance, matching of the study groups seem to be poor, as it was done solely according to age. However, age was a well-documented parameter influencing the proportion of Th1/Th2 cells [19], so it was reasonable to choose it as a selection criterion for the haemodialysis control group. The observation that patients

with UP in our study happened to be on dialysis for a longer time period also fits quite well in the concept of inflammation as a cause of UP. It can be suggested that the longer the uraemia persists, the higher the probability of an uraemia-related stimulation of the immune system. In accordance with this hypothesis, Sethi *et al*. described a significant correlation between CRP values and time on haemodialysis [20]. The quality of dialysis probably plays an important role in this respect, i.e. more intense dialysis may lead to less uraemia-related inflammation and consecutive symptoms. It must be stressed that the condition of inadequately dialysed patients with UP could be improved by intensifying the treatment modalities [2].

In conclusion, UP seems to be associated with an up-regulation of micro-inflammation in uraemia. The precise pathogenesis remains still unclear, but a cytokine-driven sensitization of itch receptors in peripheral c-fibers might be an attractive explanation.

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Conflict of interest statement. None declared.

References

1. Gilchrest BA, Rowe JW, Brown RS, Steinmann TI, Arndt KA. Ultraviolet phototherapy of uremic pruritus. Long-term results and possible mechanisms of action. *Ann Intern Med* 1979; 91: 17–21
2. Hiroshige K, Kabashima N, Takasugi M, Kuroiwa A. Optimal dialysis improves uremic pruritus. *Am J Kidney Dis* 1995; 25: 413–419
3. Silva SR, Viana PC, Lugon NV, Hoette M, Ruzany F, Lugon JR. Thalidomide for the treatment of uremic pruritus: a crossover randomized double-blind trial. *Nephron* 1994; 67: 270–273
4. Pauli-Magnus C, Mikus G, Alscher DM *et al*. Naltrexone does not relieve uremic pruritus: Results of a randomized, double-blind, placebo-controlled crossover study. *J Am Soc Nephrol* 2000; 11: 514–519
5. Mettang T, Pauli-Magnus C, Alscher DM. Uraemic pruritus–new perspectives and insights from recent trials. *Nephrol Dial Transplant* 2002; 17: 1558–1563
6. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. *Nature* 1996; 383: 787–793
7. Yamamoto J, Adachi Y, Onoue Y *et al*. Differential expression of the chemokine receptors by the Th1- and Th2-type effector populations within circulating CD4+ T-cells. *J Leukoc Biol* 2000; 68: 568–574
8. Pala P, Hussell T, Openshaw PJ. Flow cytometric measurement of intracellular cytokines. *J Immunol Methods* 2000; 243: 107–124
9. Garssen J, Vandebriel RJ, DeGruijl FR *et al*. UVB exposure-induced systemic modulation of Th1- and Th2-mediated immune responses. *Immunology* 1999; 97: 506–514
10. Mc Hugh SM, Rifkin IR, Deighton J *et al*. The immunosuppressive drug thalidomide induces T helper cell type 2 (Th2) and concomitantly inhibits Th1 cytokine production in mitogen- and antigen-stimulated human peripheral blood mononuclear cell cultures. *Clin Exp Immunol* 1995; 99: 160–167
11. Pauli-Magnus C, Klumpp S, Alscher DM, Kuhlmann U, Mettang T. Short-term efficacy of tacrolimus ointment in severe uremic pruritus. *Perit Dial Int* 2000; 6: 802–803
12. Kuypers DR, Claes K, Evenepoel P, Maes B, Vanrenterghem Y. A prospective proof of concept study of the efficacy of tacrolimus ointment on uraemic pruritus (UP) in patients on chronic dialysis therapy. *Nephrol Dial Transplant* 2004; 19: 1895–1901
13. Altmeyer P, Kachel HG, Schafer G, Fassbinder W. Normalization of uremic skin changes following kidney transplantation. *Hautarzt* 1986; 37: 217–221
14. Virga G, Visentin I, La Milia V, Bonadonna A. Inflammation and pruritus in hemodialysis patients. *Nephrol Dial Transplant* 2002; 17: 2164–2169
15. Yosipovitch G, Greaves MW, Schmelz M. Itch. *Lancet* 2003; 361: 690–694
16. Bemelmans MH, Gouma DJ, Buurman WA. Influence of nephrectomy on tumor necrosis factor clearance in a murine model. *J Immunol* 1993; 150: 2007–2017
17. Kaysen GA. The microinflammatory state in uremia: causes and potential consequences. *J Am Soc Nephrol* 2001; 12: 1549–1557
18. Martin-Ventura JL, Ortego M, Esbrit P, Hernandez-Presa MA, Ortega L, Egido J. Possible role of parathyroid hormone-related protein as a proinflammatory cytokine in atherosclerosis. *Stroke* 2003; 34: 1783–1789
19. Sakata-Kaneko S, Wakatsuki Y, Matsunaga Y, Usui T, Kita T. Altered Th1/Th2 commitment in human CD4+ T cells with ageing. *Clin Exp Immunol* 2000; 120: 267–273
20. Sethi D, Muller BR, Brown EA, Maini, RN, Gower PE. C-reactive protein in hemodialysis patients with dialysis arthropathy. *Nephrol Dialysis Transplant* 1988; 3: 269–271

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