Abstract

Background. Dendritic cells (DCs) are antigen-presenting cells that are pivotal for the initiation of the primary immune response. Patients with chronic kidney disease (CKD) with or without chronic intermittent haemodialysis (CIHD) show an impaired immune response. Dysfunction of DCs may underlie this phenomenon.

Methods. In this study, several different functions of monocyte-derived DCs (moDC) of patients with CKD class IV–V (glomerular filtration rate <30 ml/min) and patients on CIHD were studied in vitro and compared with age- and sex-matched healthy volunteers.

Results. We demonstrate that, independent of the maturation stimulus used, mature moDC from both groups of patients did not acquire the same level of terminal differentiation as moDC from controls, as shown by analysis of cell surface markers and the relative high macropinocytosis activity of moDC. The stimulation of allogeneic T-cells by immature moDC and mature moDC did not differ between patients and controls. However, in the presence of immature moDC or antigen-loaded matured moDC from patients, less proliferation of autologous T-cells was observed in response to recall antigens. There was no difference between moDC from controls and patients in their ability to activate naive T-cells and to differentiate them into Th1 and Th2 cells.

Conclusions. These results show that the terminal differentiation of moDC in patients with severe CKD is impaired. This impairment is not restricted to one maturation stimulus and is independent of treatment with haemodialysis.

Keywords: chronic kidney disease; dendritic cells; immune deficiency; terminal differentiation

Introduction

Patients with chronic kidney disease (CKD) have an impaired immune response [1]. Clinically, this is apparent by the increased incidence of infectious complications and high infection-related morbidity and mortality among patients with CKD as compared with the general population [2,3]. In addition, loss of glomerular filtration rate (GFR) is associated with a decreased vaccination response [4,5]. Only the responses to T-cell-dependent vaccinations are negatively affected, which is indicative of a uraemia-associated T-cell defect. However, data from in vitro studies have shown normal proliferative T-cell responses upon stimulation with cytokines and lectins [6,7]. Instead, several lines of evidence suggest an underlying problem with the function of antigen-presenting cells, which seems to hamper an adequate activation of T-cells. For instance, a decreased T-cell proliferation was only observed when T-cells from patients on chronic intermittent haemodialysis (CIHD) were stimulated with an antigen-presenting cell-dependent T-cell stimulus [8]. In agreement with these observations, human leukocyte antigen (HLA)-identical monocytes from healthy individuals were able to overcome the defective T-cell proliferation of patients on CIHD in response to tetanus toxoid [9].

Both in vitro and in vivo dendritic cells (DCs) are the superior antigen-presenting cells [10]. However, there are no data available on the function of DCs obtained from patients with CKD. Monocytes can differentiate in vivo into DCs, the so-called monocyte-derived DCs (moDC). This differentiation process is directed by local inflammatory signals, which can be mimicked in vitro by allowing monocytes to phagocytose zymosan, after migration across an endothelial barrier [11–13]. In vitro, immature moDC can be generated by culturing monocytes in the presence of interleukin (IL)-4 and granulocyte macrophage colony-stimulating factor (GM-CSF). These immature DC are specialized in antigen uptake and processing. As immature DCs reach their final stage of differentiation, they lose
their ability to internalize antigens and simultaneously increase their capacity to stimulate T-cells by up-regulating co-stimulatory and major histo compatibility complex (MHC) molecules. This transition of DCs from an immature to a mature stage is called terminal differentiation, and is triggered by various factors, e.g. pro-inflammatory cytokines, products of microbial pathogens, and ligation of CD40.

We hypothesized that the immunodeficiency of CKD is associated with an impaired function of the moDC. In the current study, we therefore investigated the terminal differentiation and the function of in vitro-generated moDC from patients with severe CKD, either with or without renal replacement therapy (RRT). We demonstrate that CKD is associated with an impaired terminal differentiation of moDC. This leads to a decreased antigen-presenting capacity of this cell, which is not reversed once RRT is started.

**Subjects and methods**

**Subjects**

Adult patients (≥18 years) treated at our haemodialysis ward were asked to participate in the study. Patients had to be on CIHD for at least 3 months and were all in a stable clinical condition. Patients with known autoimmune disease, malignancy, clinical signs of active infection or taking immunosuppressive medication were not included. All patients on CIHD were dialysed three weekly sessions for 4 h by use of haemofilter dialyser membranes (MA-12H; Kawasumi Laboratory Inc., Tokyo, Japan). Water for dialysis was prepared by the use of reverse osmosis according to European guidelines. The bacteriological quality of the dialysate was measured at regular intervals according to European guidelines (endotoxin levels <0.25 IU/ml and <100 colony forming units per ml). Studies were first conducted with 17 patients on CIHD (sex ratio M/F = 10/7; median age 64 years, range 34–74 years) and 11 healthy volunteers (M/F = 7/4; median age 30 years, range 24–53 years). The renal pathology within this group was as follows: diabetic nephropathy (n = 3), renovascular disease (n = 6), glomerulonephritis (n = 5) and other or unknown (n = 3).

In addition, age- and sex-matched studies, 10 patients on CIHD (6/4; median age 59 years, range 36–69 years), eight healthy volunteers (6/2; median age 54 years, range 36–64 years), and nine patients with CKD stage IV and V (GFR <30 ml/min) not receiving RRT (7/2; median age 54 years, range 36–64 years; median GFR 20 ml/min, and range 10–30 ml/min) were included. All patients received treatment with recombinant erythropoietin in a dosage to maintain haemoglobin levels above 7.0 mmol/l. All were treated with 1α,25-dihydroxy vitamin D₃, and gave informed consent.

**Isolation and culture of cells**

Fifty millilitres of peripheral venous blood was collected in standard lithium-heparinized tubes and processed within 2 h of collection. Blood from patients undergoing CIHD was always collected immediately before the start of a dialysis session. Next, peripheral blood mononuclear cells (PBMC) were isolated by use of density gradient centrifugation (Ficoll-Paque™ plus; Amersham Biosciences AB, Uppsala, Sweden). Monocytes were isolated by positive selection using an automatic magnetic cell sorter (autoMACS; Miltenyi Biotec, Bergisch Gladbach, Germany) and CD14 coupled magnetic beads according to the manufacturer’s instructions (10 μl beads for maximal 6 × 10⁶ PBMC; Miltenyi Biotec). The CD14 positive fraction (purity always >95%) was seeded in 6-well plates (Costar, Cambridge, MA) in a density of 1.5 × 10⁶ cells per well and cultured for a total of 11 days at 37°C in humidified 5% CO₂. As culture medium we used RPMI 1640 (Cambrex, Verviers, Belgium) supplemented with 100 IU/ml penicillin, 100 μg/ml streptomycin and t-glutamin (all from Cambrex) at 2 mM final concentration and 10% heat-inactivated AB+ pooled human serum. For the cultivation procedure of moDC, 50 ng/ml recombinant human GM-CSF (Leucomax, Sandoz, Germany) and 40 ng/ml recombinant human IL-4 (Tebu-bio, Heerhugowaard, The Netherlands) were used. Fresh medium and cytokines were added at days 3, 6 and 8. At day 8 of culture a cytokine cocktail containing IL-1β (10 ng/ml), IL-6 (10 ng/ml), TNFα (20 ng/ml) (Tebu-bio) and PGE₂ (1 μg/ml) (Sigma-Aldrich, Zwijndrecht, The Netherlands) was added to GM-CSF and IL-4 as a maturation stimulus. As alternative maturation stimuli, we used CD40L-transfected L-cells in a ratio of moDC to L-cell of 4:1 [15] (kindly provided by Dr C. van Kooten, Leiden, The Netherlands) and lipopolysaccharide (LPS; 1 μg/ml). Cell viability was determined by Trypan blue exclusion and was always >95%.

**Immunofluorescence staining and flow cytometric analysis**

Antibodies used for cell surface staining included CD14, CD40, CD86, anti-HLA-ABC (DPC, Oxford, UK), CD83, anti-CCR5 (Pharmingen, San Diego, USA), anti-HLA-DR (Becton-Dickinson Biosciences, San Jose, CA, USA) and anti-CCR7 (R&D Systems Europe, Abingdon, UK). All antibodies were mouse monoclonal antibodies conjugated to either fluorescein isothiocyanate (FITC) or phycoerythrin (PE). Isotype controls were used at the same protein concentration as the test antibody. The staining intensity of anti-CCR7 showed a remarkably low background, which was consistently less than the isotype control (Figure 1). Data acquisition and analysis were performed on a FACSCalibur (Becton-Dickinson) using CellQuest Pro software (Becton-Dickinson). Results are expressed as geometric mean fluorescence intensity. For monocytes, the mean fluorescence intensity was measured in a semi-quantitative way. The flow cytometer was calibrated with specific calibration beads in the range of 500 to 50 000 molecular equivalent of soluble fluorochrome (MESF) (Calibration Beads Quantum 1000, RPE medium level; Bangs Labs Inc., Fisher, IN, USA). The intensity of the fluorescence was converted to a standard curve using Quick Cal program for Quantum beads (Bangs Labs Inc.). The geometric mean fluorescence level is used to calculate MESF.

**FITC-labelled albumin internalization**

A total of 50 μg/ml FITC-labelled bovine serum albumin (Sigma-Aldrich) was added to 1 × 10⁵ moDC, which were
incubated for 60 min in the dark at 37°C. As a negative control, we incubated moDC at 0°C with the same concentration of FITC-labelled albumin. After incubation, cells were washed twice in ice-cold FACSflow (Becton Dickinson), and the fluorescence intensity was measured on a FACSCalibur (Becton Dickinson). Results are presented as the difference in fluorescence intensity (expressed as geometric mean) between moDC incubated at 37°C and those at 0°C.

### Allogeneic mixed leucocyte reaction

The T-cell stimulatory capacity of moDC was assessed in an allogeneic mixed leucocyte reaction (alloMLR). As responder cells, we used the CD14-negative fraction from one healthy volunteer in all assays. These cells were obtained during the isolation of monocytes by positive selection on an autoMACS, and were resuspended in RPMI 1640 containing 10% pooled human serum and 10% dimethyl sulfoxide (Sigma-Aldrich). Aliquots were stored at −80°C for a maximum of 14 days. Responder cells were thawed immediately before use, washed and resuspended in RPMI 1640 containing 10% pooled human serum and plated in a density of 5 × 10^5 cells per well. Decreasing numbers of moDC were mixed with the responder cells. As control for background proliferation, responder cells were cultured in the absence of moDC. As a positive control, T-cells were stimulated with phytohaemagglutinin (PHA; 2 μg/ml of purified PHA-HA16; Murex Biotech Ltd., Kent, UK) or phorbol myristate acetate (PMA; 100 ng/ml; Calbiochem, Darmstadt, Germany) with ionomycin (10 μg/ml; Calbiochem). Cells were cultured in triplicate for 6 days in a 96-well round-bottom plate (Nunc, Roskilde, Denmark). Proliferation was assessed by adding [3H]thymidine (0.5 μCi/well, Amerham Pharmacia, Biotech, Little Chalfont, UK) during the last 8 h of culture. [3H]thymidine incorporation was measured on a liquid scintillation spectrophotometer (Betaplate 1205; LKB Wallac, Perkin Elmer, Finland) and is expressed in counts per minute (cpm).

### Antigen-specific T-cell proliferation

Antigen-specific T-cell responses were assessed in two different ways. One, immature moDC were harvested...
and directly mixed with responder cells and tetanus toxoid (SVM, Bilthoven, The Netherlands) or *Candida albicans* antigen (ARTU, Biological N.V., Lelystad, The Netherlands). Two, immature moDC were pulsed for 4 h with either tetanus toxoid or *Candida albicans* antigen, whereafter a cocktail of pro-inflammatory cytokines was added as a maturation stimulus for 3 days. Then, the antigen-pulsed mature moDC were harvested (day 11) and subsequently mixed with responder cells. The final concentration of the recall antigens was done both ways: 35 limit of flocculation units (Lf)/ml for tetanus toxoid, and 5 μg/ml for *Candida albicans*. As responder cells, CD14-negative cells were used and were plated in a density of 1 × 10^5 cells per well with decreasing numbers of moDC. As control for background proliferation, responder cells were cultured with the antigen in the absence of moDC. As control for background proliferation, responder cells were cultured with the antigen in the absence of moDC. In both ways, cells were cultured for 5 days in triplicate in a 96-well round bottom plate (Nunc), and subsequently incubated with 0.5 μCi [3H]thymidine per well for the last 8 h of culture.

**Cytokine production by moDC**

Supernatants of moDC cultures were collected 24 h after the addition of the cytokine cocktail and stored at −20°C. The supernatants were collectively thawed, and cytokine levels were assessed by use of ELISA for IL-10, IL-12p70 (U-Cytech, Utrecht, The Netherlands) and IL-15 (Biosource, Nivelles, Belgium) according to the manufacturer’s instructions.

**Assessment of T-helper cell polarization by moDC**

To analyse whether moDC-induced T-helper (Th) type 1 or Th type 2 responses, mature moDC were harvested and co-cultured at a density of 5 × 10^6 with 2.5 × 10^6 allogeneic CD14-negative cells per well. On day 11, the CD14-negative cells were harvested and subsequently cultured in 96-well flat-bottom plates (1 × 10^5/well) and restimulated with monoclonal antibodies directed against CD3 (RIV9) and
CD28 (Serotec) at a final concentration of 1 μg/ml or medium alone. Supernatants were collected after 24 h and stored at −20°C. The supernatants were collectively thawed, and the concentrations of IL-2, IL-4, IL-6, IL-10, TNFα and IFNγ were measured using a cytometric bead array kit (BD Pharmingen, San Diego, USA) according to the manufacturer’s instructions.

**Statistical analysis**

The results of the experiments were analysed with the unpaired Mann–Whitney *U*-test. A *P*-value at *P* < 0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA).

**Results**

**Chronic intermittent haemodialysis influences monocyte immunophenotype**

There was no difference in the viability of monocytes derived from patients on CIHD and those from healthy volunteers (mean ± SD: 96 ± 3% vs 97 ± 2%; *P* = 0.2). The cell surface expression of CD14 on monocytes derived from patients on CIHD was lower compared with monocytes from healthy individuals (24 ± 104 ± 18 × 10^3 vs 31 ± 10^4 ± 29 × 10^3 MESF; *P* = 0.03), but the monocyte yields after isolation were similar. In line with previous results [16], there was no statistically significant difference between the two groups in the expression of MHC class I or II, or any of the co-stimulatory molecules.

**Immunophenotypic terminal differentiation of moDC is impaired in CKD and CIHD**

During 8 days of culture, monocytes derived from healthy volunteers and patients on CIHD differentiated into immature moDC that were indistinguishable as assessed by their immunophenotype and light microscopic appearance (data not shown). After the addition of the cytokine cocktail, all immature moDC acquired a mature immunophenotype with the expression of HLA class I and II, CD40, CD83, CD86 and CCR7 (Figure 1A). In addition, the terminally differentiated moDC displayed a veiled morphology. However, in comparison with control subjects, the expression of HLA class I [CIHD: median 782 (252–1200); controls: 1300 (464–1797); *P* = 0.01] and II [CIHD: 1179 (672–1735); controls: 1984 (1233–2285); *P* = 0.001], CD83 [CIHD: 24 (4–52); controls: 42 (21–46); *P* = 0.01], CD86 [CIHD: 380 (312–436); controls: 459 (396–536), *P* = 0.001] and CCR7 [CIHD: 18 (1–41); controls: 52 (13–75); *P* = 0.002] were significantly lower on mature moDC from patients on CIHD (Figure 1B). To investigate whether the lower expression of these cell surface...
markers was the result of CIHD or resulted from the loss of GFR, we conducted an additional set of experiments. In these experiments, we compared the immunophenotype of moDC after stimulation with the cytokine cocktail, between age- and sex-matched volunteers, patients on CIHD and a group of patients with CKD class IV and V not receiving RRT. MoDC from patients with CKD class IV and V had a lower expression of HLA class II [CKD: median 1573 (1153–1993); controls: 1906 (1393–2162); \( P = 0.02 \)], CD83 [CKD: 4 (3–12); controls: 19 (13–45); \( P = 0.0001 \)] and CD86 [CKD: 350 (290–409); controls: 435 (336–575); \( P = 0.006 \)] compared with healthy volunteers, whereas CCR7 expression was numerically but not statistically significantly lower compared with control subjects [CKD: 23 (14–41); controls: 34 (25–52); \( P = 0.11 \)] (Figure 1C). The data on CCR7 and CD83 expression of mature moDC within these matched groups differ from our initial experiments, which may indicate an influence of age and/or gender on the expression of these molecules.

**Table 1. Decreased expression of maturation markers after stimulation with L-CD40 L-cells**

<table>
<thead>
<tr>
<th>Cell surface molecule</th>
<th>Stimulus</th>
<th>Healthy controls</th>
<th>CHID patients</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median MESF Range</td>
<td>Median MESF Range</td>
<td></td>
</tr>
<tr>
<td>CD86</td>
<td>CK</td>
<td>539</td>
<td>396–660</td>
<td>404</td>
</tr>
<tr>
<td></td>
<td>LCD40L</td>
<td>425</td>
<td>308–587</td>
<td>275</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>217</td>
<td>145–482</td>
<td>286</td>
</tr>
<tr>
<td>HLA-II</td>
<td>CK</td>
<td>2124</td>
<td>1754–2258</td>
<td>1316</td>
</tr>
<tr>
<td></td>
<td>LCD40L</td>
<td>1304</td>
<td>1076–1802</td>
<td>791</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>1011</td>
<td>764–1571</td>
<td>859</td>
</tr>
<tr>
<td>CCR7</td>
<td>CK</td>
<td>51</td>
<td>32–94</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>LCD40L</td>
<td>23</td>
<td>9–75</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>0.25</td>
<td>0–11</td>
<td>0</td>
</tr>
<tr>
<td>CD83</td>
<td>CK</td>
<td>34</td>
<td>9–45</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>LCD40L</td>
<td>18</td>
<td>8–29</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>3</td>
<td>2–7</td>
<td>4</td>
</tr>
</tbody>
</table>

Expression of cell surface antigens on moDC from patients on CIHD and healthy volunteers after stimulation with the cytokine cocktail, L-CD40 L-cells and LPS (controls \( N = 6 \); patients \( N = 7 \)).

CK, cytokine cocktail; LCD40L, CD40 ligand; LPS, lipopolysaccharide; data are shown as molecular equivalent of soluble fluorochrome.

\( P \)-values shown in italics are below 0.05 and therefore considered statistically significant.

**MoDC from patients on CIHD partly keep their capability for macropinocytosis upon maturation**

The uptake of albumin by moDC occurs via macropinocytosis [17]. When immature moDC differentiate into mature moDC, they lose their ability to take up antigen, and therefore determination of albumin uptake is used to monitor terminal differentiation [17,18]. Immature moDC from healthy volunteers and patients on CIHD were equally capable of taking up albumin [CIHD: median 778 (452–1258); controls: 795 (664–878); \( P = 0.98 \); Figure 2]. However, after inducing terminal differentiation by adding the cytokine cocktail, we observed a difference between patients and controls in FITC-labelled albumin uptake. Mature moDC from CIHD patients captured more FITC-labelled albumin compared with mature moDC from controls [CIHD: 250 (86–352); controls: 122 (88–233); \( P = 0.01 \); Figure 2]. This finding is in accordance with our data derived from FACS analysis and suggests that the terminal differentiation of moDC from patients on CIHD is impaired.

**MoDC-induced allogeneic T-cell and autologous antigen-specific T-cell proliferation**

Immature moDC had a roughly 3-fold lower allosstimulatory capacity than mature moDC (Figure 3A). Interestingly, there was no difference between healthy volunteers and patients on CIHD in the capacity of
immature moDC or mature moDC to stimulate allogeneic T-cells (Figure 3A). Next, we studied the capacity of moDC to stimulate autologous T-cells in response to tetanus toxoid and Candida albicans antigen. The proliferation of autologous T-cells from patients on CIHD or with CKD class IV and V in response to immature moDC in the presence of tetanus toxoid was statistically significantly lower compared with controls (Figure 3B). Using the antigen-pulsed mature moDC, we observed a higher T-cell proliferation in response to tetanus toxoid, but the differences between the patients and controls remained (Figure 3C). This difference was less pronounced for moDC from patients with CKD in the presence of Candida albicans (data not shown). To assess whether there existed a difference in T-cell proliferative capacity between patients and control subjects, we stimulated the CD14-negative responder fraction of both groups with PHA and PMA. No significant differences in proliferation after PHA and PMA stimulation were observed (data not shown).

Cytokine production by moDC

To investigate whether the difference in T-cell proliferation between healthy volunteers and patients on CIHD resulted from a difference in cytokine production by moDC, we measured the levels of IL-10, IL-12p70 and IL-15 in the supernatant of moDC stimulated with the cytokine cocktail. No significant differences in the concentration of IL-10 [mean (SEM); controls: 91(35); haemodialysis: 46(9); P = 0.09] and IL-12p70 [controls: 55(34); haemodialysis 17(3); P = 0.18] were found to exist between both groups (Figure 4). However, IL-15 production by moDC from CIHD patients tended to be higher than of moDC derived from healthy volunteers [controls: 298(75); haemodialysis: 605(99); P = 0.06].

No Th cell polarization induced by moDC

We addressed the question whether the impaired terminal differentiation of moDC from patients on CIHD and CKD class IV and V resulted in a Th cell polarization. In patients on CIHD evidence has been found for a dominance of Th1 cells over Th2 cells [19]. However, compared with healthy volunteers, moDC from both patients on CIHD and CKD class IV did not cause a skewing of Th cells as determined by the concentration of Th1 and Th2 cytokines after re-stimulation of primed T-cells (Figure 5).

Discussion

In the present study, we demonstrate that the terminal differentiation of moDC from patients with CKD class IV–V and from patients treated with CIHD is impaired. The evidence for this is 2-fold. First, stimulation of immature moDC from patients resulted in an immunophenotype that, measured by cell surface expression of HLA, CD83, CD86 and the chemokine receptor CCR7, was less mature compared with healthy volunteers. This was most clearly observed using a cocktail of inflammatory cytokines, which proved to be the most potent stimulus to maximize the terminal differentiation of moDC. However, similar effects were observed when terminal differentiation was induced by CD40L, which act by ligation of CD40.

Second, after stimulation with the cytokine cocktail, the uptake of FITC-labelled albumin was higher in moDC from patients on CIHD compared with healthy volunteers. Under physiologic conditions, when immature moDC advance towards their terminal stage of differentiation, they lose their ability to endocytose antigen [20,17]. Albumin is taken up by endocytosis, and therefore measurement of the amount of internalized albumin indicates the stage of moDC differentiation.
differentiation [18]. Our observation that mature moDC from patients on CIHD took up more FITC-labelled albumin may, therefore, also be interpreted as an indication of a deficient terminal differentiation. Of interest was that immature moDC of patients were similar to healthy controls with respect to their phenotype and macropinocytosis capacity. This indicates that only the maturation capacity of DCs
is negatively affected in patients with end-stage renal disease. RRT by haemodialysis did not reverse this difference. This is in line with the clinical observation that unresponsiveness to vaccination in patients with end-stage renal disease is not improved by haemodialysis [4].

One must assume that the impaired maturation of moDC from patients with CKD is determined by a difference, which is already present at the level of monocytes. Indications for monocyte activation have consistently been described in relation to CKD with or without RRT [9,21,22]. This may be in part mediated by an increased plasma concentration of advanced glycated end-products and increased oxidative stress [23–25]. Whether such pre-activated monocytes are indeed less susceptible for differentiation into mature DC is not known. However, it has been shown that by activating monocytes with LPS it was possible to hamper moDC differentiation in vitro [26]. Levels of glyco-oxidative stress proteins are partially restored [27,28] and DC function reverted to normal [29] after renal transplantation, which emphasizes that renal failure by itself—possible by glyco-oxidative stress proteins—induces immunodeficiency.

Toll-like receptors (TLR) are key regulators of DC function and maturation [10,14]. Decreased expression or altered function of these receptors on moDC from CKD patients could, therefore, be a putative cause of impaired maturation. Our data on LPS-induced maturation of moDC, which did not show a difference between patients and controls, are not in support of such a hypothesis. However, to dismiss this possibility a more detailed study, using several TLR ligands, would be necessary.

The functional consequences of the impaired maturation of moDC in patients with severe CKD were only appreciable in the recall antigen T-cell proliferation assay using tetanus toxoid and Candida albicans antigens. Immature moDC in the presence of antigen show maturation after encountering the antigen-specific T-cells, a process in which CD40–CD40L interaction plays a key role [30]. The decreased antigen-specific T-cell proliferation using immature moDC from patients may, therefore, be interpreted as a functional consequence of suboptimal terminal differentiation. The cytokine cocktail was added as an external stimulus to the antigen-pulsed moDC to test whether a superior maturation stimulus could nullify the difference in antigen-specific T-cell proliferation. This procedure resulted in increased T-cell proliferative responses, but again a significantly lower response in patients with severe CKD and in patients on CIHD was observed, indicating a decreased function of mature moDC. Stimulation with PHA or PMA resulted in a similar high T-cell proliferation for both patients and healthy controls, thereby ruling...
out a difference in the proliferative capacity of T-cells. Because central memory T-cells are decreased in patients with CKD [31], we cannot rule out that differences in the amount of antigen-specific T-cells influenced our results. However, our findings are in accordance with the early observations on impaired antigen presentation in uraemic patients [8,9]. In these studies normal T-cell proliferation, including tetanus toxoid-specific T-cell proliferation, was observed when antigen-presenting cells of a healthy donor replaced the antigen-presenting cells of the patient.

In contrast to the decreased recall T-cell response, there was no difference between patients and volunteers with regard to the allostimulatory capacity of both immature and mature moDC. In a previous study in which PBMC, rather than purified moDC, were used as stimulator cells, the recall antigen tests also proved to be more sensitive than the alloresponse to the effects of subtle changes in DC numbers [16]. Although this observation is not readily explained, an allogenic response is fundamentally different from a memory T-cell response. Alloantigenic stimulation will result in the activation of large numbers of T-cells, whereas stimulation with a nominal antigen such as tetanus toxoid or Candida albicans will result in the activation of far fewer (memory) T-cells [32]. This quantitative difference in responder T-cells may very well overcome the effects of a relatively impaired peripheral maturation of dendritic cells.

In patients on haemodialysis, a bias towards Th1 has been described [19]. We therefore investigated the possibility that the impaired terminal differentiation of moDC derived from patients would lead to skewing of the Th1/Th2 ratio. However, no such difference for both groups of patients and healthy volunteers could be detected. In agreement with this finding, we were also unable to find a difference in the profile of cytokine production by mature moDC in patients with CKD when compared with healthy controls.

In conclusion, we present data that indicate an impaired terminal differentiation of moDC from patients with CKD class IV-V that is not reversed once patients are on CIHD. The functional consequence of the impaired terminal differentiation is reflected by decreased in vitro memory T-cell responses in both groups of patients, and may at least partly explain the impaired cellular immune response observed in patients with severe renal failure with or without RRT.

Acknowledgement. The authors are indebted to Dr C. van Kooten for kindly providing the CD40L transfected L-cells.

Conflict of interest statement. None declared.

References


Received for publication: 23.6.06
Accepted in revised form: 7.8.06