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Differentiation of Monocyte Derived Dendritic Cells in End Stage Renal Disease is Skewed Towards Accelerated Maturation

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Dendritic cells (DC) play an important role in the induction of immune responses. Patients with end stage renal disease (ESRD) suffer from chronic inflammation, leading to a secondary, uremic immunodeficiency associated with alterations in monocyte subpopulations with increased proinflammatory capacities.
Objectives. The aim of this study was to examine, under isolated conditions, whether alterations in monocyte subpopulations may affect *in vitro* maturation of dendritic cells (DC) in patients with ESRD, thus allowing us to draw conclusions for the situation *in vivo*.
Material and Methods. Monocytes from 30 patients undergoing hemodialysis (HD) and 15 healthy volunteers were enriched from peripheral blood leukocytes, differentiated into immature DC (iDC) in medium containing IL-4 and GM-CSF, and were induced with LPS to differentiate into mature DC (mDC). Monocyte subpopulations and DC maturation stages were phenotypically characterized using flow-cytometry.
Results. Although phenotypically indistinguishable, the number of both iDC and mDC that were generated from uremic monocytes was significantly higher compared to those from healthy controls ($p = 0.02$ and $p = 0.03$, respectively). This was associated with an increased number of CD14+ CD16+ monocytes ($p = 0.02$) and by a higher maturation efficiency of mDC in patients ($p = 0.04$).
Conclusions. A high percentage of CD14+ CD16+ monocytes in patients with ESRD is associated with an increased propensity to differentiate into DC. This indicates that chronic inflammation may substantiate the biased consistence of monocyte subpopulations leading to profound alteration in DC generation and maturation in ESRD (**Adv Clin Exp Med 2015, 24, 2, 257–266**).

Key words: dendritic cells, hemodialysis, monocytes, end stage renal disease.

Patients with end stage renal disease (ESRD) frequently suffer from infectious complications that account for increased morbidity, hospitalization and mortality. According to the 2010 U.S. Renal Data System Report, infections are still among the top three causes of mortality in ESRD patients, next to cardiovascular events and cancer. Nevertheless, the overall 5-year survival rate for ESRD patients has improved since the early 1990s due to improved treatment and disease management [1].

An increased incidence of infectious complications in patients with ESRD arises from

a secondary immune defect [2] that is frequently observed as non-responsiveness to standard vaccinations [3–5]. On the cellular level, this immune defect is at least partly related to an impaired activation of T-lymphocytes and reduced secretion of the T-cell growth factor interleukin-2 (IL-2) [6]. Interestingly, the immune defect is rather localized to antigen presenting cells (APC) than on T-cells, as an impaired T-cell function may be restored in the presence of APC from healthy donors [7, 8]. Moreover, we have previously shown that the defect seems to be associated with an impaired costimulatory signaling of B7 molecules (CD80 and CD86), as anti-CD28 mediated supplementation of B7/CD28 signals *in vitro* led to a normalization of T-cell functions [7]. In line with these findings, we indeed found significantly lower levels of the costimulatory molecule CD86 on uremic monocytes as compared to healthy controls [9]. Moreover, chronic inflammation in uremic patients was shown to be associated with an increased percentage of monocytes that are characterised by a high expression of CD16 and increased proinflammatory activity [10]. Although these CD14+CD16+ cells have been characterised as a more mature subpopulation of monocytes [11] with an increased migratory capacity and ability to differentiate into DC in healthy individuals [12, 13], the maturation potential of monocytes from uremic patients is less well studied. This may be of particular importance, as deficiencies in APC function are among the key contributors to uremic immunodeficiency in patients with ESRD.

When analyzing costimulatory defects, monocytes were chosen as model system for APC, although dendritic cells (DC) represent the most potent APC *in vivo*. DC are of myeloid origin and can be derived either from CD34+ mononuclear progenitor cells or directly from monocytes, especially from the proinflammatory CD14 low CD16 + + monocyte subpopulation [14–16]. They are believed to play a crucial role in the regulation of both the adaptive and the innate immune system. DC are in general divided into immature (iDC) and mature DC (mDC). iDC are mainly located in the peripheral tissues proceeding with antigen-uptake and – processing. In response to inflammatory signals, iDC migrate to the peripheral regional lymphoid organs while differentiating into mDC. When reaching the lymphoid tissue, mDC have lost the ability of antigen-uptake and are mainly specialized in antigen-presentation to naïve T-cells, which takes place in the T-cell areas of the lymphoid organs [17]. These distinct stages of DC may be diagnostically separated by their expression of functionally relevant cell surface molecules such as HLA-DR, CD1a, CD80 and CD86.

Based on the impaired function and alterations in subpopulations of uremic monocytes, this study was carried out to analyse both the maturation efficiency and the phenotypical properties of monocyte derived DC from ESRD patients undergoing hemodialysis.

Material and Methods

Patients

Thirty chronic hemodialysis patients from our outpatient dialysis clinic and 15 healthy control persons from our clinical and laboratory personnel were included in this study after giving informed consent. The mean age of the hemodialysis patients was 61.9 ± 11.4 years, and the mean age of controls was 34.4 ± 11.2 years. No aged matched controls were recruited for the study, as it was difficult to recruit older subjects not suffering from any cardiovascular disease or risk factor, as well as not taking any necessary medication in this regard, potentially influencing the analysed expression pattern on monocytes or DC. Exclusion criteria were clinical signs for the presence of an acute inflammatory process, current immunosuppressive therapy or evidence of malignancy. Patients were examined for present infections, and were excluded if leukocyte count exceeded 10.000 per µL or if CRP levels were more than 10% above mean values of the last 10 months. Patients were dialysed 3 times weekly, undergoing dialysis for 38.9 ± 27.5 months in average, with a weekly Kt/V 3.67 ± 0.74 and a Kt/V of 1.17 ± 0.27 per dialysis session (calculated according to Daugirdas and Ing; double-pool urea kinetics) [18]. Dialysis quality was tested monthly and met the criteria of the association for the Advancement of Medical Instrumentation (AAMI) [19]. Renal failure occurred because of the following diseases: diabetic nephropathy (n = 13), chronic glomerulonephritis (n = 7), nephrosclerosis (n = 2), cystic disease (n = 2), renal tuberculosis (n = 2), systemic lupus erythematosus (n = 1), analgesic nephropathy (n = 1) and chronic pyelonephritis (n = 1). The cause of the renal disease was unknown in 1 case (n = 1). The investigation conforms to the principles outlined in the Declaration of Helsinki and was approved by the local ethics committee.

Blood Sampling and Generation of Immature and Mature DC from Isolated Monocytes

Blood samples were drawn from the arterial line before start of hemodialysis treatment or by venipuncture in healthy individuals. The blood was collected into heparinized syringes (200 IU sodium heparin per

20 mL blood). All specimens were placed on ice immediately, and further proceedings were performed at 4°C. Peripheral blood mononuclear cells (PBMC) were prepared by density gradient centrifugation (Ficoll; Linaris, Bettingen, Germany) and washed twice with phosphate buffered saline (PBS)-EDTA. PBMC yield was assessed by microscopical counting and their monocyte content was determined by flow cytometry. For this an aliquot of PBMC was stained immediately and processed for analysis. For DC generation the PBMC were subjected to plastic adherence for monocyte enrichment. This was performed by plating up to 3×10^6 PBMC/well into 6-well culture plates at a density of 1 x 10^6/mL and incubating for 1 h at 37°C and 6% CO_2. During this time monocytes adhered to the bottom of the well and non-adherent cells were washed off with PBS thereafter.

The *in vitro* generation of immature and mature DC was performed as previously described [20]. In brief, iDC were generated for 6 days in complete DC medium (RPMI 1640 containing 5% fetal calf serum (FCS, low endotoxine grade) supplemented with 100 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF) and 500U/mL IL-4 (both Strathmann Biotech, Hannover, Germany). On day 6 cells were divided in half and washed on time with PBS and resuspended in complete DC medium. Then, one half of the culture was stimulated with 100 ng/mL lipopolysaccharid (LPS, Sigma, Deisenhofen, Germany) to differentiate into mDC, whereas the other half was mock-treated and remained immature. iDC and mDC were then further cultivated for 2 days. On day 8, iDC and mDC were harvested. Cell yield was assessed by counting DC numbers microscopically, and phenotypical properties as well as maturation efficiency were analysed using flow cytometry.

Cell Counts

The number of PBMC and DC were determined microscopically. To achieve the absolute monocyte count/mL among PBMC, monocytes were gated using flow cytometry based on scatter properties (FSC/SSC dotplot). Subsequently, monocytes were further identified by gating on CD14+ cells, including both cells showing high and low expression of CD14. The percentage of gated monocytes was finally calculated in relation to the total PBMC count.

Phenotypical Characterisation of Monocyte Subpopulations and DC

Phenotypical characterisation of monocytes, iDC and mDC was carried out using flow-cytometry. Monoclonal antibodies conjugated to fluorescent dyes were used to detect the following cell surface antigens: CD1a (clone HI149), CD40 (clone 5C3), CD86 (clone IT2.2, all from BD, Heidelberg, Germany), CD14 (clone RM052), HLA-DR (clone B8.12.2, all from Coulter-Immunotech, Krefeld, Germany), CD80 (clone BB-1, Cymbus Biotechnology LTD, Hamburg, Germany) and CD16 (clone DJ130c, DAKO, Hamburg, Germany). Cells were washed twice with PBS containing 0.75% bovine serum albumin, 5% FCS, 1 mM $CaCl_2$ and 1 mM $MgCl_2$, and finally resuspended in 200 µL of PBS containing 1% of paraformaldehyde. Flow-cytometric analysis was performed on a FACScan using the CellQuest® software system (BD).

Statistical Analysis

For data management and statistical analysis GraphPad Prism V4.03 statistical software package was used (Graphpad, San Diego, CA, USA). All data was given as means ± SD. Significance of differences between the groups was calculated using the Mann-Whitney test. Correlation of continuous data among the general study population was performed using Spearman correlation coefficients (r).

Results

Correlation of Age and Time Under Dialysis Treatment

To evaluate the influence of age and hemodialysis we performed a correlation analysis with relative and absolute cell numbers, as well as with major stimulatory and co-stimulatory surface molecules on DC.

For the correlation with age we found no correlation with the analysed study parameters. Solely, for the relative amount of the CD14+/CD16+ monocyte subpopulation and iDC population after 8 days of cultivation a trend for a direct correlation was observed (Table 1).

In contrast, for the time under hemodialysis treatment, we observed a significant direct correlation for the CD14+/CD16+ monocyte subpopulation, as well as for the absolute and relative numbers of iDC and mDC and with CRP levels. PBMC numbers correlated inversely with dialysis time, as did the expression for CD1a on iDC and mDC. Similar results were obtained for the correlation with the weekly kt/V, except for the correlation with the CD14+/CD16 monocyte subpopulation, CD1a on iDC and mDC and CRP (Table 1).

Table 1. Correlation of age, time under dialysis treatment and weekly kt/V

	Age	Time under dialysis (months)	Weekly kt/V
CD14+/CD16+ monocytes [%]	r = 0.3224 p = 0.08	r = 0.4215 p = **0.02**	r = 0.2609 p = 0.19
Spontaneous maturation [– LPS%]	r = –0.0370 p = 0.82	r = –0.0252 p = 0.87	r = 0.0507 p = 0.77
Induced maturation [+ LPS%]	r = 0.2293 p = 0.15	r = 0.3468 p = **0.03**	r = 0.3960 p = **0.02**
PBMC/mL	r = –0.1934 p = 0.20	r = –0.3653 p = **0.01**	r = –0.3351 p = **0.03**
Monocytes/mL	r = 0.055 p = 0.72	r = 0.0293 p = 0.85	r = 0.0473 p = 0.77
Monocytes [%]	r = 0.1595 p = 0.29	r = 0.2675 p = 0.07	r = 0.2817 p = 0.07
iDC/mL	r = 0.2265 p = 0.13	r = 0.3228 p = **0.03**	r = 0.4254 p < **0.01**
mDC/mL	r = 0.2089 p = 0.17	r = 0.3579 p = **0.02**	r = 0.4448 p < **0.01**
iDC [%]	r = 0.2699 p = 0.07	r = 0.294 p = **0.04**	r = 0.3574 p = **0.02**
mDC [%]	r = 0.2422 p = 0.11	r = 0.3605 p = **0.01**	r = 0.3757 p = **0.02**
HLA-DR iDC	r = –0.0487 p = 0.77	r = 0.0487 p = 0.51	r = 0.0157 p = 0.93
HLA-DR mDC	r = –0.1505 p = 0.39	r = 0.0842 p = 0.63	r = –0.0171 p = 0.93
CD1a iDC	r = –0.1930 p = 0.21	r = –0.3673 p = **0.01**	r = –0.1628 p = 0.32
CD1a mDC	r = –0.2050 p = 0.18	r = –0.2994 p = **0.04**	r = –0.2010 p = 0.21
CD40 iDC	r = –0.0105 p = 0.96	r = 0.2572 p = 0.18	r = 0.3055 p = 0.13
CD40 mDC	r = –0.0077 p = 0.97	r = 0.2198 p = 0.24	r = 0.1712 p = 0.39
CD80 iDC	r = 0.021 p = 0.89	r = 0.2529 p = 0.11	r = 0.1326 p = 0.43
CD80 mDC	r = –0.1591 p = 0.32	r = 0.1067 p = 0.51	r = 0.0247 p = 0.88
CD86 iDC	r = –0.2249 p = 0.14	r = –0.1581 p = 0.31	r = –0.1095 p = 0.50
CD86 mDC	r = –0.0780 p = 0.62	r = –0.0213 p = 0.89	r = –0.0262 p = 0.87
CRP [mg/dL]	r = 0.1929 p = 0.20	r = 0.4091 p < **0.01**	r = 0.2823 p = 0.07

Analysis of the correlation of continuous risk factors for the general study population. Correlation coefficients are adjusted for case/control status. Significant correlations are highlighted in bold.

Phenotypical Analysis of iDC and mDC

After 8 days of culture, typical surface molecules on DC were analysed that are characteristic for DC (CD1a), and are necessary and inevitable for antigen presentation (HLA-DR) and costimulation (CD40, CD80, CD86). When comparing ESRD patients and healthy individuals, the expression of the major co-stimulatory molecules CD40, CD80, and CD86 did not differ on either iDCs or mDCs (Table 2). Although monocytes from ESRD patients were previously shown to express less CD86, CD86 expression did not differ on iDC of both ESRD patients and controls after 6 days of cell culture (Table 2). Likewise, there was no difference in CD86 expression on mDC from patients and controls. Further, when analysing HLA-DR, we could not detect a significant difference between patients and controls, neither on iDC nor mDC (Table 2). Finally, the non-classical MHC molecule CD1a was analysed as a marker for DC generation and maturation. In general, mDC show a lower expression of CD1a as compared to iDC, which is in line with previously published results [21]. Analyzing CD1a expression in respect to ESRD patients and controls, iDC did not show any difference in CD1a expression levels between both groups ($p = 0.19$), whereas CD1a expression on mDC of ESRD patients was significantly lower ($p = 0.02$, Table 2).

Taken together, while mDC from ESRD patients show a significantly lower expression of the DC marker CD1a, all other molecules relevant for antigen presentation (HLA-DR) or co-stimulation (CD40, CD80, CD86) were similar in both groups.

Analysis of Maturation Efficiency of DC from Controls and ESRD Patients

To assess the efficiency of monocytes differentiating into DC in patients and controls, respective cells numbers were determined before and after the maturation procedure. To normalise the DC yield to the number of monocytes that was put into culture, the percentage of monocytes was determined using flow cytometry and related to the number of iDC and mDC harvested at day 8.

We observed a trend towards a reduced yield of PBMC, which could be isolated from whole blood in ESRD patients compared to healthy controls (Table 3). However, due to a relative monocytosis in ESRD patients, approximately equal numbers of CD14 positive monocytes were put into cell culture to differentiate into iDC and mDC (Table 3). Interestingly, analysis of the total number of iDC and mDC after 8 days of culture revealed a significantly higher number of both iDC and mDC in patients undergoing hemodialysis compared to healthy individuals (Table 3). Since equal monocyte numbers where put into cell culture in both groups, this was not due to monocytosis, but rather due to an increased percentage of monocytes differentiating into DC after 8 days (Table 3). Together this indicates that monocytes of patients undergoing hemodialysis show an increased capability to differentiate into DC compared to controls.

Table 2. Cell surface expression of stimulatory and co-stimulatory molecules on iDC and mDC

		ESRD	Controls	P-value
Immature DC	HLA-DR	82.48 ± 28.5	92.5 ± 40.56	0.73
	CD1a	171.5 ± 96.76	196.7 ± 77.70	0.19
	CD40	65.72 ± 15.78	62.31 ± 15.66	0.65
	B7-1(CD80)	24.73 ± 10.5	22.82 ± 7.35	0.60
	B7-2 (CD86)	152.6 ± 111.6	209.3 ± 127.8	0.17
Mature DC	HLA-DR	142.7 ± 43.68	154.6 ± 59.06	0.56
	CD1a	98.91 ± 96.73	136.3 ± 66.59	0.02
	CD40	89.24 ± 16.33	87.53 ± 29.55	0.57
	B7-1 (CD80)	39.06 ± 10.64	41.44 ± 11.32	0.70
	B7-2 (CD86)	1105 ± 454.7	882.4 ± 299.5	0.19

Mean fluorescence intensity (MFI) is given as mean ± SD.

Table 3. Cell yield and maturation efficiency of iDC and mDC

	ESRD patients	Control	P-value
PBMC/mL whole blood	$4.02 \pm 1.4 \times 10^7$	$2.41 \pm 0.76 \times 10^7$	0.08
Monocytes [%]	10.65 ± 5.60	8.86 ± 5.75	0.1
Monocytes/mL whole blood	$4.26 \pm 2.50 \times 10^6$	$4.59 \pm 3.59 \times 10^6$	0.66
iDC/mL whole blood day 8	$8.3 \pm 3.8 \times 10^5$	$5.6 \pm 3.2 \times 10^5$	0.02
iDC from monocytes [%]	14.2 ± 15.2	8.4 ± 7.5	0.02
mDC/mL whole blood day 8	$7.9 \pm 4.1 \times 10^5$	$5 \pm 3.2 \times 10^5$	0.03
mDC from monocytes [%]	13.2 ± 12.9	7.1 ± 5.8	0.01

Cell counts for PBMC, monocytes, iDC, and mDC isolated and/or generated per mL whole blood, and relative amount of monocytes put into culture and which successfully differentiated into iDC or mDC after 8 days of cultivation.

DC of ESRD Patients Show Enhanced Maturation After Stimulation with LPS

To more precisely analyse the maturation efficiency from iDC towards mDC, the two DC types were analysed using flow cytometry, where the LPS-induced maturation from iDC to mDC may best be characterised by an increase in CD86 expression and a concomitant decrease in CD1a expression (Fig. 1). When analysing CD1a/CD86 expression with and without LPS, iDC cultures [-LPS] may partially contain cells characteristic of mDC (Fig. 1, left panel, gate R3), whereas some mDC cultures in the presence of LPS may still contain cells that remained in an immature state (Fig. 1, right panel, gate R2). The profile of CD1a and CD86 was then used to comparatively analyse the spontaneous maturation (proportion of mDCs in [–LPS] culture) to the induced maturation (proportion of mDC in [+LPS] culture) between ESRD patients and healthy individuals. The extent of spontaneous maturation was similarly low in both groups (Fig. 2A: 21.03 ± 10.03% in ESRD patients vs 25.27 ± 12.50% in controls, p = 0.88). In contrast, however, induction with LPS led to a significantly enhanced proportion of mDC in ESRD patients compared to controls (Fig. 2B; 83.82 ± 15.87% in ESRD patients vs: 73.84 ± 17.39% in controls, p = 0.04).

Analysis of Monocyte Subpopulations of ESRD Patients and Healthy Controls

An increased maturation efficiency and DC yield may be due to the presence of a larger fraction of proinflammatory monocytes in ESRD patients. As CD16 positive monocytes were shown to have an increased migratory capacity and ability to differentiate into DC in healthy individuals [12, 13], we elucidated whether the increased efficiency to generate DC from monocytes in ESRD patients was based on alterations in monocyte subpopulations

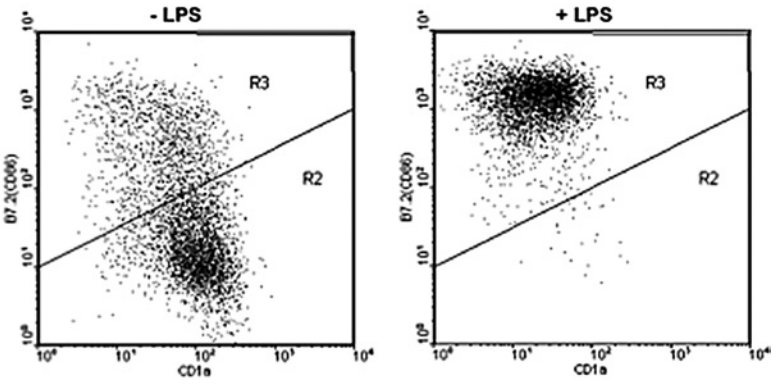

Fig. 1. Characterization of iDC and mDC by flow cytometry. iDC (gate R2) are characterized by a strong expression of CD1a and a weak expression of CD86, whereas mDC (gate R3) show a low expression of CD1a and a strong expression of CD86. Culture of DC without LPS (left panel) may contain spontaneously maturated DC (R3), while culture of DC stimulated with LPS (right panel) may still contain immature DC (R2) at day 8. The proportion of mDC was used to analyse the maturation efficiency of DC

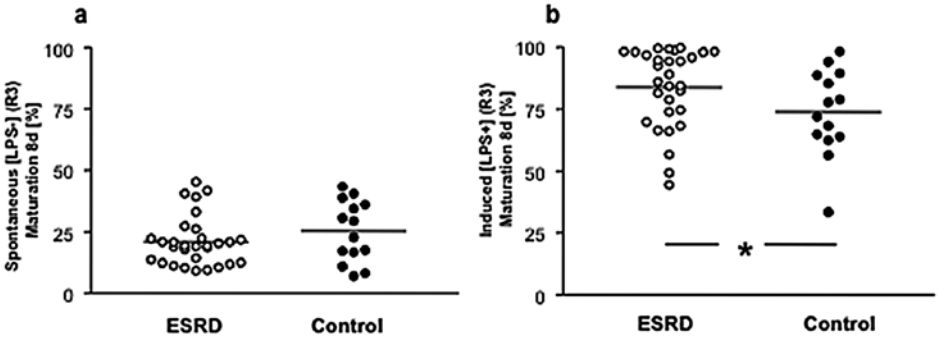

Fig. 2. Increased maturation efficiency in ESRD patients. Maturation efficiency was assessed using flow cytometric staining of CD86 and CD1a to identify iDC and mDC in ESRD patients (n = 28) and healthy individuals (n = 13). (A) The extent of spontaneous maturation towards mDC in the absence of LPS was similarly low in ESRD patients (21.03 ± 10.03% of mDC) and controls (25.27 ± 12.50% of mDC, p = 0.88). (B) Induction of maturation with LPS revealed a significantly higher maturation efficiency in ESRD patients (83.82 ± 15.87% of mDC) compared to controls (73.84 ± 17.39% of mDC, p = 0.04)

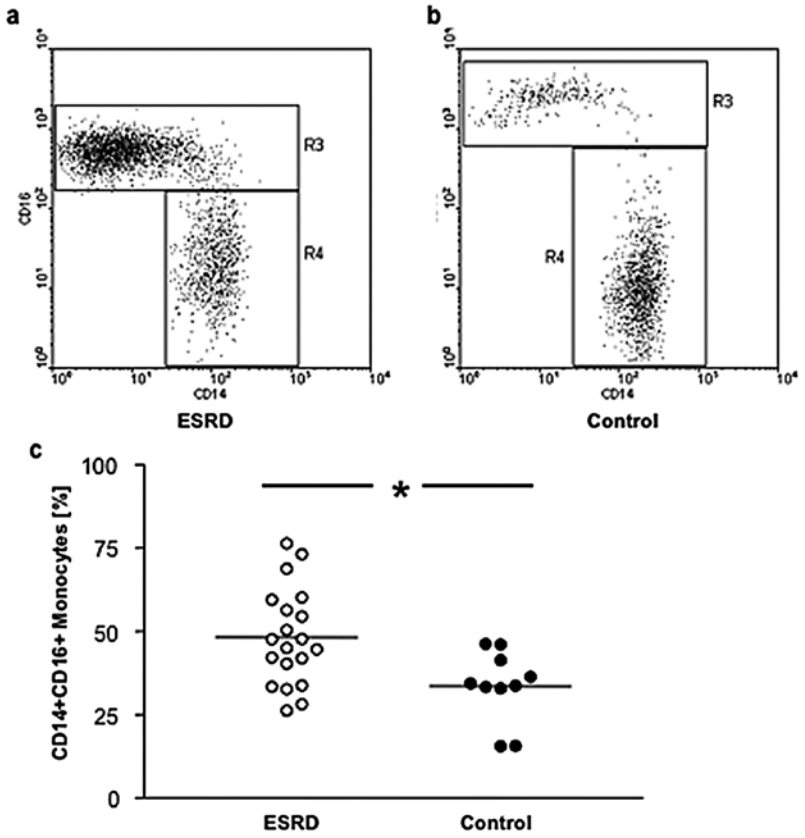

Fig. 3. Increased percentage of CD14+ CD16+ monocytes in ESRD patients. Representative examples of monocytes subpopulations in (A) a healthy control and (B) an ESRD-patient as analysed using co-staining of CD14 and CD16 on monocytes. (C) ESRD patients (n = 20) showed an increased proportion of CD14+/CD16+ monocytes compared to healthy individuals (n = 10) (48.3 ± 14.4% vs controls 33.6 ± 10.7%; p = 0.02)

that were put into the culture. As illustrated in Fig. 3A and 3B, the percentage of CD14 and CD16 positive monocytes was determined in both patients and controls using flow-cytometry. Indeed, our cohort of patients showed an increased proportion of CD14+/CD16+ monocytes as compared to healthy controls (Fig. 3C, 48.25 ± 14.4% in ESRD patients vs 33.62 ± 10.71% in controls, p = 0.02). Thus, the more efficient generation of DC in ESRD patients may at least in part be due to an increased proportion of CD14+/CD16+ monocytes.

Discussion

The immune defect in ESRD patients is associated with a reduced T-cell stimulation, leading to a high susceptibility for infectious complications [2–5, 22]. This impaired T-cell activation is related to an impaired co-stimulation by APC. It was shown that activation of T-cells *in vitro* remained low in the presence of autologous APCs, but the proliferative response was restored to normal in the presence of monocytes from healthy

donors [7, 8]. Furthermore, the impaired co-stimulatory capacity of the monocytes of ESRD patients was shown to be related to a decreased expression of CD86 on monocytes [9]. The present study extends the characterisation of monocytes to DC, since monocytes are not considered to be the most effective APCs. Thus, we reasoned, that the chronic inflammatory state in ESRD patients might account for the generation of highly efficient DC, which might be more potent and effective T-cell activators than those of healthy individuals.

ESRD patients suffer from a persistent systemic inflammatory response. The uremic state of ESRD is accompanied by an impaired immune response coupled with persistent immune stimulation, which leads to a low-grade systemic inflammation and altered cytokine balance; this may clinically translate into increased risk for vascular disease, involving several interrelated processes, such as oxidative stress, endothelial dysfunction, and vascular calcification. This milieu of constant low-grade inflammation further promotes impaired function of neutrophils and T cells, as well as a dysregulated cytokine network. In this respect, the imbalance between anti-inflammatory cytokines such as IL-10 and proinflammatory cytokines such as IL-6 and TNF-α, has been shown to play an important role in the development of T-helper imbalance, coronary artery disease (CAD) and wasting in the uremic milieu [23]. We believe that this chronic inflammatory state promotes a milieu that favors a biased differentiation of monocytes towards more mature subpopulations and towards DC. We found that, despite the reduced PBMC count, a relative monocytosis in ESRD patients allowed us to culture equal amounts of monocytes, which further led to a significantly higher amount of generated iDC and mDC.

The constant inflammation favors alterations in the composition of different monocyte subpopulations, which could further explain the increased generation of iDC and mDC. We found a significantly higher proportion of CD14+CD16+ monocytes, a monocyte subpopulation which is considered to be increased in a proinflammatory setting [10]. They represent a more mature type of monocytes [11] and show an increased migratory capacity and ability to differentiate into DC [12, 13]. An increase in the CD14+CD16+ monocyte subpopulation is present in many different inflammatory diseases, as well as in coronary artery disease (CAD) [24], which may account for a favourable proinflammatory phenotype of DC in CAD [25]. We previously described a temporary activation and sequestration of CD14+CD16+ monocytes during hemodialysis, which might contribute to the state of chronic microinflammation [26]. Thus, the increased proportion of proinflammatory CD14+ CD16+ monocytes in ESRD patients, as a consequence of a chronic inflammation, may more rapidly induce maturation of iDC, thereby favouring a more proinflammatory type of DC. In this regard, we found a stronger tendency of iDC of ESRD patients towards spontaneous maturation to mDC, thus supporting the idea of a more proinflammatory immune system in a uremic environment, which might be therapeutically ameliorated by renal replacement therapy.

This hypothesis was recently supported in patients undergoing renal transplantation, where the afore existing deficiencies in incidence and function of precursor DC were reversed [27]. Moreover, serum from uremic patients, when added to culture medium, impaired DC-function *in vitro*, especially endocytosis and maturation [28]. Furthermore, patients undergoing hemodialysis showed increased serum levels of IL-12p70. Upon LPS stimulation cultured DC in combination with uremia toxin enriched medium showed a further enhanced production of IL-12p70 [29]. In this regard, we previously described an IL-12 overproduction of monocytes of ESRD patients, as a sign of chronic inflammation in ESRD [30]. The uremic milieu is generally thought to be immunosuppressive, not only in regard to maturation or function [28, 29, 31], but also in regard to decreased numbers of circulating DC during ESRD, further enhanced by the inflammatory stimulus during hemodialysis [32]. This led us and others [28, 29] to the conclusion that the removal of uremic toxins by a more effective dialysis treatment might ameliorate DC function, thus providing a better outcome of patients undergoing hemodialysis.

In the present patient group, we did not observe the afore described decreased expression of CD86 on monocytes in ESRD patients [9] (data not shown). Most likely, this is due to an improvement of the hemodialysis therapy in last decade. The patient cohort, where we described the costimulatory immune defect on monocytes [9], underwent hemodialysis therapy with a mean weekly kt/V of 0.87, whereas in the present study a mean weekly kt/V of 1.17 was achieved. The improved dialysis quality might therefore have ameliorated the immune defect on monocytes and consequently on DC, despite the still present chronic inflammation.

Limitations

The small number of patients and controls is a clear limitation of our study. Furthermore, another limitation is the fact not having an age matched control cohort, which gives rise to the speculation, that age might have influenced our findings in the

first place. This however, was possible to rule out after the correlation analysis, where we found the time under hemodialysis treatment to be of bigger impact on the results, although, age probably has still contributed to our findings to some extend.

The authors have concluded that despite an improved dialysis quality, ESRD patients still show an increased pro-inflammatory subset of monocytes and in consequence a more effective maturation of iDC to mDC, even spontaneously. This bias towards a more proinflammatory immune system might therefore favor the observed chronic inflammation in patients with ESRD, leading to the clinically observed accelerated atherosclerosis. In addition, the rapid maturation of DC may hinder an effective uptake of antigens in the periphery, which is the main task of iDC and can explain the observed altered immune function of the adaptive immune system in ESRD. A novel therapeutic strategy to ameliorate the chronic inflammation caused by uremia might, therefore, be profitable for ESRD patients until renal transplantation – as the ultimate treatment of chronic inflammation – can be performed.

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