Dendritic cell depletion in ESRD


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Effects of end-stage renal disease and haemodialysis on dendritic cell subsets and basal and LPS-stimulated cytokine production

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Abstract

Background. Although bacterial infections have dramatically declined as a cause of death in the general population, they remain a major cause of mortality in patients with end-stage renal disease (ESRD). Moreover, the response to vaccination is profoundly impaired in this population. Dendritic cells (DC) are the major antigen-presenting cells that bridge the innate and adaptive immune responses. Activation of DC by pathogens results in secretion of inflammatory cytokines and up-regulation of co-stimulatory molecules. The activated DC prime naïve T and B cells to the captured antigens.

Methods. Using flow cytometry, the number and phenotype of circulating DC [myeloid DC (mDC) and plasmacytoid DC (pDC)] were quantified in pre- and post-dialysis blood samples from 20 ESRD patients maintained on haemodialysis. Ten normal individuals served as controls. In addition, the level of DC activation and their response to lipopolysaccharide (LPS) stimulation were determined by assessing expression of co-stimulatory molecule, CD86, and antigen-presenting molecule, HLA-DR, as well as production of TNFα, IFNα and IL-6.

Results. Compared to the control group, the circulating dendritic cell count was significantly reduced in the ESRD patients before dialysis and declined further after dialysis. The reduction in pDC numbers was more striking than mDC. The magnitude of the LPS-induced up-regulation of CD86 was comparable among the study groups as well as pre- and post-dialysis samples. However, LPS-induced
TNFα production was significantly reduced in the post-dialysis samples with no significant difference in IL-6 and IFNα productions among the study groups and in pre- and post-dialysis samples.

**Conclusions.** ESRD results in significant DC depletion which is largely due to diminished plasmacytoid DC subset. Haemodialysis procedure intensifies DC depletion and impairs LPS-induced TNFα production.

**Keywords:** CKD; immune deficiency; infection; inflammation

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**Introduction**

Patients with end-stage renal disease (ESRD) are prone to bacterial, viral and fungal infections and suboptimal response to vaccinations [1–4]. The immune system has evolved to recognize and eliminate invading microbial pathogens and simultaneously avoid responding to innocuous and self antigens. Dendritic cells (DC), which are derived from bone marrow progenitor cells, serve as antigen-presenting cells that play a major role in the initiation and maintenance of innate and adaptive immunity. They serve as the sentinel cells for adaptive immunity by regulating immune responses in T cells, B cells and natural killer cells and thereby play a critical part in tolerance to self antigens, tumour surveillance and defence against microbial pathogens [5]. DC continuously survey the antigenic milieu and act as the sensors of microbial invasion or tissue damage. After their release, immature DC settle in the lymphoid and non-lymphoid tissues. Following uptake of antigen and in presence of inflammatory stimuli, they undergo terminal differentiation and transformation into an immuno-stimulatory mode. This transformation is marked by increased surface expression of specific chemokine receptors, major histocompatibility complex (MHC) classes I and II and various adhesion and co-stimulatory molecules as well as the loss of ability to capture antigens. Following transformation, DC travel to secondary lymphoid tissue to stimulate effector cells. [5]. DC can be activated directly via pathogen recognition receptors such as toll-like receptors (TLRs) or indirectly through exposure to specific cytokines. Once activated, DC secrete a wide array of cytokines and other inflammatory mediators with which they communicate amongst themselves and other immune cells and profoundly influence the immune system. Consequently, the inherent elevation of the pro-inflammatory cytokines and other inflammatory mediators in the course of non-infectious systemic inflammatory states can recruit dendritic cell to amplify the underlying inflammation. This is exemplified by the reported changes in the DC function in the elderly with age-associated inflammation [6].

In humans, DC are composed of myeloid (mDC) and plasmacytoid (pDC) subsets. These DC subsets perform different functions in both innate and adaptive immune responses. At the same time, both DC subsets have functional plasticity to induce appropriate T-cell responses depending on the types of stimuli.

Myeloid DC play a critical role in directing different effector T-cell responses. They have the capacity to produce IL-12 in response to the microbial stimuli and, thereby, induce Th1 development [7,8]. However, this capacity varies with the type of signals they receive. For example, lipopolysaccharides (LPS) derived from Escherichia coli [9–11], mycobacterium tuberculosis and double-stranded viral RNA [8,9] stimulate mDC to produce IL-12 and to induce Th1 development. In contrast, LPS from Porphyromonas gingivalis [10] activate mDC to induce Th2 development, and yeast zymosan induces regulatory actions [12], which lead to different types of Th responses to eliminate the given pathogens.

pDC are main mediators of antiviral immunity by virtue of their ability to produce large amounts of type I interferons such as IFNα in response to viral infections. This process is mediated by activation of the intracellular TLRs, namely TLR7 and TLR9, which are constitutively expressed in pDC and are capable of sensing viral nucleic acids. In certain autoimmune diseases, self nucleic acids gain entry to the pDC endosomes, where they activate TLR signalling and type I interferon production. By contrast, mDCs produce type I interferons and IL-12 in response to TLR3 and TLR4 agonists but not to CpG-containing DNA which are TLR7 and TLR 9 agonists [13].

Several earlier studies have shown significant reduction of the circulating DC in patients with ESRD [14–17]. The present study was undertaken to determine the effect of ESRD and haemodialysis procedure on the circulating dendritic cell subsets and their functional capacity by determining the expression of surface activation markers, CD86 and HLA-DR and cytokine-induction capacity, events that are critical in shaping the adaptive immunity.

**Materials and methods**

**Patients**

Twenty patients with ESRD maintained on haemodialysis for a minimum of 3 months were recruited for the study. Individuals with evidence of acute or chronic infection, acute intercurrent illnesses and those with autoimmune disorders and individuals receiving immunosuppressive drugs were excluded. Medical history, systolic and diastolic blood pressures, body weight, routine monthly laboratory data and medications were recorded. Haemodialysis therapy was performed for 3 h thrice weekly using cellulose triacetate dialyzers. A group of 10 normal age-matched control subjects served as controls. Individuals exhibiting acute or chronic infection, acute intercurrent illnesses, chronic illnesses such as hypertension, diabetes, malignancy, psychiatric disorders or those requiring chronic medications were excluded. All participants provided informed consent prior to enrolment in the study. The study protocol was approved by the Human Subjects Institutional Review Board of the University of California, Irvine, and completed with the assistance of the University of California General Clinical Research Center.

**Blood collection**

In all ESRD patients, whole blood was collected from the vascular access immediately before and after haemodialysis treatment. The blood samples were obtained by a syringe, applying gentle aspiration to minimize shear stress. Blood samples from the control individuals were collected from a peripheral vein in the same manner.

**Endotoxin assay**

Endotoxin level in the dialysate samples was measured using the Limulus amoebocyte lysis method. The assays were performed by Satellite Laboratory Services (Redwood City, CA).
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**Antibodies and reagents**

The following anti-human immunoglobulins were used: Lineage FITC (a mixture of CD3 FITC, CD19 FITC, CD56 FITC, CD14 FITC), CD123 PE, CD11c APC, CD80 PE, CD86 PE, HLA-DR PerCR IL-6 PE, TNFα PE and IFNα PE, all from BD Parmingen (San Jose, CA). TLR-4 agonist (E. coli LPS) was obtained from InvivoGen (San Jose, CA).

**Immunostaining**

Dendritic subsets were analysed by four-colour flow cytometry using PerCP-conjugated anti-HLA-DR monoclonal antibodies (mAb), FITC-conjugated anti-lineage mAb and APC-conjugated anti-CD11c, PE-conjugated anti-CD123, CD80, CD86 or isotype control mAb. One hundred microlitres of whole blood were incubated with 10 μl of each of the above antibodies for 25 min at room temperature in the dark. The cells were then incubated in 2 mL of fluorescence-activated cell sorting (FACS) lysing solution (Becton Dickinson, San Jose, CA, USA) for 15 min to lyse red blood cells. The cells were then washed twice with phosphate-buffered saline (1× PBS) to remove red blood cell remnants, and lymphocytes were resuspended in 0.5 mL of 1% paraformaldehyde and used for flow cytometry.

**Flow cytometry**

Dendritic cell phenotyping was carried out by four-colour analysis using a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA, USA). Forward and side scatters were used to gate and exclude cellular debris, and FL3 channels were used to gate HLA-DR-positive cells and FL1 channel used for lineage FITC. Five thousand to 10 000 events of lineage-negative and HLA-DR+ cells gated dendritic cells. The incidence of circulating precursor DC subsets relative to total peripheral blood mononuclear cells was analysed in healthy controls and ESRD patients pre- and post-dialysis. DC subsets were identified and characterized phenotypically by multicolour flow cytometric analysis. Acquired cells were analysed using Flowjo software (Treestar).

**Cytokine detection by intracellular staining**

One hundred microlitres of whole blood were stimulated with LPS (1μg/ml) for 4 h. The secretion of extracellular cytokines was blocked by adding Brefeldin A at 37°C. After 4 h incubation, blood was stained for dendritic cells as above, and after 25 min, erythrocytes were lysed and fixed by paraformaldehyde (PFA). The WBCs were permeabilized by Perm buffer (BD biosciences) for 30 min; the excess Perm buffer was washed, and cells were stained for intracellular cytokines IL-6, TNFα and IFNα and isotype. Cells were acquired and analysed as above.

**Table 1. Blood haemoglobin concentration, total and differential leukocyte counts in normal control subjects and in ESRD patients obtained before and after haemodialysis (HD) procedure**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pre-HD</th>
<th>Post-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>14.6 ± 1.3</td>
<td>11.8 ± 1.1†</td>
<td>12.8 ± 1.2*</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>42.5 ± 3.6</td>
<td>34.7 ± 3.7†</td>
<td>37.1 ± 4.8*</td>
</tr>
<tr>
<td>Total leukocytes (per μl)</td>
<td>6800 ± 1300</td>
<td>7300 ± 1900</td>
<td>6700 ± 2000</td>
</tr>
<tr>
<td>Neutrophils (per μl)</td>
<td>4100 ± 870</td>
<td>5000 ± 1200†</td>
<td>4500 ± 1500</td>
</tr>
<tr>
<td>Monocytes (per μl)</td>
<td>520 ± 180</td>
<td>550 ± 240</td>
<td>430 ± 140</td>
</tr>
<tr>
<td>Lymphocytes (per μl)</td>
<td>1900 ± 500</td>
<td>1500 ± 540†</td>
<td>1400 ± 400</td>
</tr>
</tbody>
</table>

For Control vs. Pre-HD: †p <0.05. For Pre vs. Post-HD: *p <0.05.

(BD biosciences) for 30 min; the excess Perm buffer was washed, and cells were stained for intracellular cytokines IL-6, TNFα and IFNα and isotype. Cells were acquired and analysed as above.

**Measurements of plasma cytokine concentrations**

Plasma concentration of IL-6, IL-8 and TNF-α were determined using Millipore 13-plex inflammatory cytokine panel (Billerica, MA, USA) run on Luminex 100 IS system. Data were analysed using MiraiBio MasterPlex QT version: 0.1.171 software (San Francisco, CA, USA).

**Data analysis**

Percentage of cell expression and the intensity of the measured analytes were quantified by flow cytometry, and data were analysed by GraphPad Prism (GraphPad Software; La Jolla, CA). Paired t-test was used to compare pre- and post-dialysis values, whereas Student’s t-test was used for comparison of data obtained from ESRD patients and control individuals.

**Results**

**General data**

The underlying cause of renal disease in the ESRD group included diabetic nephropathy in 10 patients, hypertension in five and chronic glomerulonephritis, polycystic kidney disease, cystinosis, interstitial nephritis, ischaemic nephropathy and an ESRD of unknown aetiology in one patient each. The types of vascular access included A-V
fistulas in 11, A-V grafts in four and tunnelled central catheters in four of the patients. As expected, serum creatinine (8.7 ± 3.2 mg/dl) and urea nitrogen (69.6 ± 14.9 mg/dl) concentrations were significantly elevated, and blood haemoglobin concentration was significantly reduced in the ESRD patients. The mean Kt/V value in the ESRD patients was 1.46, reflecting adequacy of dialysis regimen in the study participants. Endotoxin concentration in the dialysate samples was consistently below ≤ 0.1 E.U.

**Peripheral blood leukocyte data**

Data are shown in Table 1. Compared to the normal control group, the ESRD group exhibited significant elevation of the number of neutrophils and a significant reduction of the number of lymphocytes. No significant difference was found in the total leukocyte count or the number of circulating monocyte between the two groups.

**Dendritic cell count and subset data**

In human peripheral blood, two DC subsets have been recognized: (i) myeloid DC, characterized as lineage negative and HLA-DR+ and CD11c+, and (ii) plasmacytoid DC (pDC) based on its plasmacytoid morphology at the DC precursor stage and characterized based on lineage negative, HLA-DR+ and CD123+ (Figure 1). We investigated whether the numbers and phenotype of DC were altered by ESRD or haemodialysis procedure.

Total DC count in pre-dialysis blood was significantly lower in the ESRD patients compared with that in the control group and declined further after dialysis (Figure 2A). The number of myeloid DC in the ESRD patients was comparable to that found in the control group and remained unchanged after dialysis (Figure 2B). However, the number of plasmacytoid DC subset was significantly reduced in the ESRD patients before dialysis and decreased further after dialysis. Accordingly, the difference in total DC count between control and ESRD patients was primarily due to reduction of plasmacytoid DC subset (Figure 2C).

**Dendritic cell function data**

In order to evaluate the effects of ESRD and haemodialysis on DC function, we studied the expression of the activation markers including co-stimulatory molecules, CD80 and CD86, and maturation marker CD83 on these cells at baseline and after stimulation with TLR4 agonist, LPS. No significant difference was found in the percentage DC expressing the co-stimulatory molecule, CD86, among the ESRD and control groups. Likewise, the magnitude of basal CD86 expression measured as mean fluorescence intensity was similar in the two groups. Stimulation with LPS resulted in significant up-regulation of CD86 expression and significant rise in the percentage of CD86-expressing DC in the blood samples obtained from the control subjects and the pre-dialysis samples from the ESRD group. Likewise, the magnitude of response to LPS was greater in the pre-dialysis blood samples from the ESRD patients than in the blood samples obtained from the controls. Haemodialysis attenuated the magnitude of LPS-mediated activation/up-regulation of CD86 in DC (Figure 3C). CD80 and CD83 expressions were minimal in both groups (data not shown).

In contrast to CD86, the percentage of DC expressing HLA-DR (MHC class II) was lower in ESRD patients than in the control group (Figure 4A, B). This expression was
not affected by dialysis treatment (Figure 4A, B). Stimulation with LPS resulted in significant reduction ($P < 0.05$) in the percentage of DC expressing HLA-DR between pre- and post-dialysis ESRD patients (Figure 4C). However, LPS stimulation led to significantly greater ($P < 0.04$) up-regulation of HLA-DR expression on dendritic cells in ESRD patients than that found in the control group (Figure 4D).

To further explore the state of dendritic cell activity, we measured production of TNFα, IL-6 and IFNα by intracellular cytokine detection. The study revealed a significant increase in the number of TNFα-expressing dendritic cells in the pre-dialysis blood samples of ESRD patients as compared to the control group. Haemodialysis procedure resulted in a significant reduction of TNFα-expressing cells ($P < 0.015$) (Figure 5A). Stimulation with LPS resulted in an equally significant up-regulation of TNFα expression and a significant increase in the number of TNFα-expressing dendritic cells in the blood samples obtained from the control group and ESRD patients before and after dialysis. No significant difference was found in either prevalence or intensity of IL-6 expression by the dendritic cells in the blood samples obtained from control subject and ESRD patients before and after dialysis. Likewise, LPS-stimulated IL-6 expression was comparable among dendritic cells contained in the blood samples obtained from control subject and ESRD patients before and after dialysis (Figure 6).

Upon stimulation with LPS, DC also produces IFNα, a potent anti-viral cytokine, via a MyD88-independent pathway. To explore the effect of ESRD on IFNα production capacity of the DC, we measured its intracellular contents at baseline and after stimulation with LPS. No significant difference was found in either prevalence or intensity of IFNα expression by the resting dendritic cells in the blood samples obtained from control subject and ESRD patients before and after dialysis (Figure 7A, B). However, activation with LPS resulted in significant increase in intensity and prevalence of IFNα expression in the dendritic cells before dialysis. Haemodialysis procedure significantly reduced the prevalence of the LPS-stimulated IFNα expression in the dendritic cells in post-dialysis blood samples.

**Fig. 3.** Expression of co-stimulatory molecule, CD86 on DC of pre-haemodialysis and post-haemodialysis patients and control. **A** Percentage of DC expressing CD86. **B** Percentage of DC expressing CD86 after stimulation with LPS. **C** Bar diagram depicting the mean fluorescence intensity (MFI) of CD86 on DC. **D** Bar diagram depicting the mean fluorescence intensity (MFI) of CD86 on DC. Pre-dialysis and post-dialysis stages ($n = 20$) and control ($n = 10$). Bars represent mean ± SEM values. $P$ values indicate the difference between patients on pre-dialysis and post-dialysis stages (*$P \leq 0.05$).
However, the intensity of IFNα expression in response to LPS stimulation remained high in the dendritic cells contained in post-dialysis blood samples (Figure 7C, D).

**Plasma cytokine data**

The ESRD group exhibited significant elevation of plasma TNFα, IL-6 and IL-8 as compared to that found in the control group. Likewise, plasma concentrations of the classical Th2 type cytokines including IL-4, IL-5 and IL-13 were higher in ESRD patients than those found in the control group. Similarly, plasma level of the anti-inflammatory cytokine, IL-10, was elevated in the ESRD patients (Table 2).

**Discussion**

In confirmation of earlier studies [14–17], we found a significant reduction of circulating DC in the ESRD patients included in the present study. Haemodialysis procedure resulted in a further decline in the circulating DC count in our ESRD patients confirming the findings of an earlier study reported by Womer et al. [19]. Given the critical role of DC in initiation and maintenance of the adaptive immunity, DC depletion shown here and in the previous investigations must contribute to the increased propensity of the ESRD population to microbial infections and impaired response to vaccination. The reduction in the circulating DC count in our ESRD patients was mainly due to diminished number of pDC subset. As noted earlier, the pDC are a major source of type 1 interferons including IFNα, which are critical for defence against viral infections [13,20]. The mechanism(s) responsible for the reduction in DC population in ESRD patients is presently unknown. Flt3L is a haematopoietic growth factor that markedly expands both mDC and pDC subsets in nonhuman primates, and granulocyte-macrophage colony-stimulating factor (GM-CSF) is essential for survival of mDC. An earlier study by Lim et al. [17] showed elevated plasma levels of Flt3L and GM-CSF in ESRD patients, thus excluding their deficiency as a potential cause of DC depletion in this population.

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![Fig. 4. Expression of HLA-DR on DC of pre-haemodialysis and post-haemodialysis patients and control. A Percentage of DC expressing HLA-DR. B Percentage of DC expressing HLA-DR after stimulation with LPS. C Bar diagram depicting the mean fluorescence intensity (MFI) of HLA-DR on DC. D Bar diagram depicting the mean fluorescence intensity (MFI) of HLA-DR on DC. Pre-dialysis and post-dialysis stages (n = 20) and control (n = 10). Bars represent mean ± SEM values. P values indicate the difference between patients on pre-dialysis and post-dialysis stages (*P ≤ 0.05).](image-url)
The underlying mechanism responsible for the acute fall in the circulating DC count following haemodialysis procedure is unclear. Potential causes include migration to tissues and adherence to and entrapment in the dialyzer and tubing system. Further studies are needed to discern the mechanism of ESRD- and haemodialysis-induced DC depletion.

Exposure to pathogens or products of the damaged tissue and consequent capture of antigens at the site of infection leads to activation/maturation of immature DC. This results in secretion of cytokines and chemokines and expression of co-stimulatory molecules including CD80, CD86 and CD83 and migration of the activated/mature DC to the draining lymph nodes. In the lymph node, activated DC stimulate differentiation of naïve T cells into functionally competent effector T cells. It is of note that the naïve CD4+ T cells can differentiate into different effector T helper (Th) cells producing different panels of cytokines. The prototypes of these divergent Th cell responses include: Th1 (IFN gamma production), Th2 (IL-4, IL-5 and IL-13 production), T regulatory (TGFβ and IL-10 production) and Th17 [21]. Together, these processes culminate in elimination of a variety of microbial pathogens, avoidance of response to self or innocuous antigens, expression of allergic reactions as well as transplant rejection and tolerance [22,23].

In order to examine the effects of ESRD and haemodialysis procedure on the functional status/capacity of DC, we determined the expression of key co-stimulatory molecules and TNFα production at baseline and after stimulation with TLR-4 ligand, LPS. The study revealed no significant difference in the percentage of DC expressing the co-stimulatory molecule, CD86, or the intensity of basal CD86 expression among the ESRD and control groups. In contrast, expression of HLA-DR which is an MHC class II molecule and an activation marker was significantly reduced, and percentage of HLA-DR-positive dendritic cells was diminished in ESRD patients when compared with the control group. However, stimulation with LPS resulted in a greater up-regulation of both CD86 and HLA-DR expression on DC in the ESRD patients than in the control group. Haemodialysis did not change HLA-DR expres-

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**Fig. 5.** Intracellular cytokine staining of TNFα in DC of pre-haemodialysis and post-haemodialysis patients and control. A Percentage of DC expressing TNFα. B Percentage of DC expressing TNFα after stimulation with LPS. C Bar diagram depicting the mean fluorescence intensity (MFI) of TNFα in DC. D Bar diagram depicting the mean fluorescence intensity (MFI) of TNFα in DC. Pre-dialysis and post-dialysis stages (n = 20) and control (n = 10). Bars represent mean ± SEM values. *P values indicate the difference between patients on pre-dialysis and post-dialysis stages (*P ≤ 0.05).
sion but attenuated the magnitude of LPS-mediated up-regulation of CD86 and HLA-DR expression on dendritic cells.

To further explore the state of dendritic cell function, we measured production of TNFα, IL-6 and IFNα by intracellular detection. The study revealed a significant increase in the number of TNFα-expressing dendritic cells in the predialysis blood samples of ESRD patients as compared to the control group. Haemodialysis procedure resulted in a significant reduction of TNFα-expressing cells. Stimulation with LPS resulted in an equally robust up-regulation of TNFα expression and a significant increase in the TNFα-expressing dendritic cells in the blood samples obtained from the control group and ESRD patients before and after dialysis. However, no significant difference was found in either prevalence or intensity of IL-6 expression by the dendritic cell in the control subject and ESRD patients before and after dialysis. Likewise, LPS-stimulated IL-6 expression in dendritic cells was comparable among control subjects and ESRD patients before and after dialysis.

Upon stimulation with LPS, the myeloid DC also produce IFNα, a potent antiviral cytokine to a lesser extent than plasmacytoid DC, via a MyD88-independent Toll/IL-1 receptor-domain-containing adapter-inducing interferon-β (TRIF) pathway. To explore the effect of ESRD on IFNα production capacity of the DC, we measured intracellular IFNα contents at baseline and after stimulation with LPS. No significant difference was found in either prevalence or intensity of IFNα expression by the resting dendritic cells among the control subjects and ESRD patients before and after dialysis. However, activation with LPS resulted in a significantly greater increase in the intensity and prevalence of IFNα expression in the dendritic cells in ESRD patients. Haemodialysis procedure significantly reduced the prevalence of the LPS-stimulated IFNα expression in the dendritic cells without changing the intensity of IFNα expression in response to LPS stimulation.

The precise reason for alteration of DC function in ESRD is not known. However, exposure to the uraemic milieu is a likely candidate. In support of this supposition, Lim et al. [24] recently demonstrated that, when cultured in presence of either normal or uraemic sera, monocyte-derived DC from ESRD patients exhibited depressed endocytosis and impaired maturation but enhanced IL-12p70 production and allogeneic T-cell proliferation only when cultured with uraemic sera. They further showed that when cultured in presence of uraemic sera, monocyte-derived DC from normal subjects had decreased endocytosis and impaired mat-
Intracellular IFNα

While the number of circulating DC was significantly lower, the proportion of DCs exhibiting increased IFNα and TNFα production and CD86 expression in response to LPS stimulation was greater among the ESRD patients than in the healthy control individuals. The available data do not allow definitive conclusions as to the mechanisms by which uraemia confers these abnormalities. However, it is tempting to speculate that heightened sensitivity of DC to LPS may be caused by the priming effect of the systemic inflammation which is a constant feature of advanced CKD. It is well known that activation of DC results in their migration to lymphoid tissues and their accelerated death within 3–4 days after interaction with the activated T cells. While uncertain, the priming state imposed by the inflammatory milieu may also shorten the DC life span [25]. If true, this phenomenon can, in part, account for uraemia-associated DC depletion. Alternatively, the reduction of DC in patients with advanced renal disease may be due to

**Table 2.** Plasma concentrations of a panel of cytokines measured in the normal control group and the ESRD patients before and after haemodialysis (HD) procedure

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Control</th>
<th>Pre-HD</th>
<th>Post-HD</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β (pg/mL)</td>
<td>0.17 ± 0.04</td>
<td>0.17 ± 0.05</td>
<td>0.18 ± 0.08</td>
</tr>
<tr>
<td>IL-2 (pg/mL)</td>
<td>7.68 ± 4.23</td>
<td>3.31 ± 1.29</td>
<td>4.11 ± 1.79</td>
</tr>
<tr>
<td>IL-4 (pg/mL)</td>
<td>0.61 ± 0.31</td>
<td>46.4 ± 27.1</td>
<td>46.6 ± 25.9</td>
</tr>
<tr>
<td>IL-5 (pg/mL)</td>
<td>0.08 ± 0.01</td>
<td>0.35 ± 0.18</td>
<td>0.29 ± 0.18***</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>1.43 ± 0.34</td>
<td>8.66 ± 3.3*</td>
<td>8.12 ± 2.6</td>
</tr>
<tr>
<td>IL-7 (pg/mL)</td>
<td>0.18 ± 0.03</td>
<td>0.86 ± 0.33</td>
<td>0.81 ± 0.53</td>
</tr>
<tr>
<td>IL-8 (pg/mL)</td>
<td>1.87 ± 0.35</td>
<td>5.70 ± 0.34**</td>
<td>4.92 ± 0.12***</td>
</tr>
<tr>
<td>IL-10 (pg/mL)</td>
<td>4.48 ± 1.43</td>
<td>19.86 ± 10.8</td>
<td>20.37 ± 13.2</td>
</tr>
<tr>
<td>IL-12 (pg/mL)</td>
<td>0.71 ± 0.28</td>
<td>1.29 ± 1.1</td>
<td>1.14 ± 0.94</td>
</tr>
<tr>
<td>IL-13 (pg/mL)</td>
<td>1.81 ± 0.94</td>
<td>16.5 ± 7.9</td>
<td>16.1 ± 6.6</td>
</tr>
<tr>
<td>IFN-γ (pg/mL)</td>
<td>2.42 ± 1.6</td>
<td>2.57 ± 1.6</td>
<td>3.75 ± 2.4</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>2.01 ± 0.31</td>
<td>10.64 ± 1.4**</td>
<td>5.75 ± 0.73***</td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.005, control vs. pre-HD***P < 0.05, ****P < 0.005, pre- vs. post-HD.
their diminished production in the face of elevated levels of GM-CSF and Flt3L [17,25], which are potent DC growth and differentiation factors. Finally, cumulative losses of DC, if any, incurred by haemodialysis procedures over extended periods may potentially contribute to diminished circulating DC in ESRD patients.

Examination of the serum cytokine profile of ESRD patients showed a Th2-type profile marked by elevations of IL-4, IL-5 and IL-13; the chemokine IL-8; inflammatory cytokines TNFα and IL-6; and anti-inflammatory cytokine IL-10. It is of note that the state of dendritic cell activity and the abundance/profile of various cytokines and chemokines are tightly interconnected as each can profoundly modify the other. Dendritic cells have enormous capacity to produce inflammatory cytokines including TNFα approximating 1000-fold greater than other leukocytes. Thus, the observed increase in the circulating TNFα level shown here and numerous other studies may be, in part, derived from DC.

In conclusion, ESRD results in dendritic cell depletion, which is primarily due to the reduction of plasmacytoid subset and is transiently exacerbated by haemodialysis procedure. Dendritic cell depletion in ESRD is associated with their increased basal and LPS-induced production of TNFα. The exaggerated DC response to LPS in ESRD patients may be due to the priming phenomenon occasioned by recurrent influx of endotoxin fragments from dialysate compartment during haemodialysis treatments.

Conflict of interest statement. None declared.

References


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