Original article

Circulating dendritic cells in pediatric patients with nephrotic syndrome

Background: *Dendritic cells (DCs) represent one of the most extensively studied topics in immunology, because of their central role in the induction and regulation of adaptive immunity, and because of their therapeutic potential for manipulating immune responses.*
Objectives: *To evaluate circulating DC levels in pediatric patients with idiopathic nephrotic syndrome (NS) and its relation to disease activity in these patients.*
Methods*: Fifteen nephrotic patients in relapse (proteinuria>40mg/m^2/hour, hypoalbuminemia, and edema) before initiating steroid therapy (Group I), and another15 nephrotic patients in remission after withdrawal of steroid therapy (Group II) were compared to 15 age- and sex- matched healthy children. Besides clinical evaluation and routine laboratory investigations of nephrotic syndrome, circulating DCs were measured by flowcytometry.*
Results: *Circulating DC count was lower in nephrotic patients in both proteinuria and remission groups [(48.89±13.52) and (64.64±7.69) X106/liter respectively] than in the control group (78.54±9.8) X10^6/liter with highly significant statistical difference (p<0.001), and lower in proteinuria group than the remission group with highly significant statistical difference (p<0.001). There was a positive correlation between DC count and serum albumin (moderate association) (p=0.002) and a negative correlation between DC count and urine protein /creatinine ratio (strong association) (p=0.001).*
Conclusion: *Nephrotic syndrome was associated with decreased number of circulating DCs and the decrease was more apparent in patients with active disease. The positive correlation between DC counts and total protein, and serum albumin, and the negative correlation between DC count and urine protein/creatinine ratio point to the link between the decrease in DC count and the severity of the disease process.*

Key words: Denderitic cells, nephrotic syndrome, immune deficiency.

Ihab Z. El- Hakim, Ahmed A. Mohammed, Hanaa M. Afifi*, Sawsan A. El-Sayed

*Departments of Pediatrics and *Clinical Pathology, Faculty of Medicine, Ain Shams University, Cairo, Egypt*

Correspondence:
Ihab Z. El-Hakim, professor of Pediatrics, Faculty of Medicine, Ain Shams University, Abbassiah, Cairo, Egypt.
E-mail: *ihab.elhakim @gmail.com*

INTRODUCTION

It has been proposed that cell-mediated immunity and T-cell activation are key features in the pathogenesis of idiopathic nephrotic syndrome (NS). Numerous examples of abnormal immune responsiveness have been described in minimal change disease (MCD), and many of these observations have suggested a causal relationship between immune system and renal abnormalities[1]. In 1974, Shalhoub[2] proposed that MCD was a disorder of lymphocyte function with increased levels of a lymphocyte-derived permeability factor. This hypothesis was based on several clinical observations that suggested the involvement of the immune system in the pathogenesis of idiopathic NS[3].

DCs are rare, ubiquitously distributed, migratory antigen presenting cells (APCs), derived from CD34 bone marrow stem cells. In addition to having the unique capacity to prime naive T cells, DCs also regulate various effector cell functions and play central roles in modulating the immune response[4]. DCs are highly mobile cells and their sequential migration between tissues is accompanied by phenotypical and functional changes that are instrumental to their function as sentinels of the immune system[5]. They are being investigated in cancer biology, transplantation, and autoimmunity[6]. However, their role in initiating NS has not yet been studied.

Our study aimed to evaluate circulating DC levels in pediatric patients with idiopathic NS and its relation to disease activity in these patients.

METHODS

Setting

This case-control, cross sectional study was conducted at the Pediatric Nephrology Clinic, Children's Hospital, and the Clinical Pathology Department of Ain Shams University in the period from October 2008 to June 2009.

Study population:

Patients: The study was conducted on 30 patients (19 males and 11 females) with NS followed up regularly in the clinic. They were classified into 2 groups:

Group I (proteinuria group): This group included 15 nephrotic patients in relapse (proteinuria >40 mg/m^2/hour, hypoalbuminemia, and edema) before initiating steroid therapy. They were 7 males and 8 females and their ages ranged from 2 .33 to 16 years with a mean age of 6.11±3.75 years.

Group II (remission group): This group included 15 nephrotic patients in remission after withdrawal of steroid therapy. They were 12 males and 3 females; their ages ranged from 3.5 to 16 years with a mean age of 10.04 ± 3.85 years .

Exclusion criteria:

- Patients with any degree of renal impairment.
- Patients with chronic inflammatory disorders as juvenile rheumatoid arthritis (JRA) and systemic lupus erythematosus (SLE).

Control group: The control group comprised 15 age- and sex-matched healthy children. They were 7 males and 8 females, their ages ranged from 3 to 16 years with a mean age of 8.47± 2.5 years .

An informed verbal consent was obtained from the parents or caregivers of each child before enrollment in the study.

Clinical evaluation

History taking and clinical examination were carried out laying stress on symptoms and signs of NS, underlying etiology of NS, duration of the disease, response to steroid therapy, drug therapy other than steroids, measurement of blood pressure, body weight and height and assessment of body mass index (BMI).

Laboratory investigations

A- Routine investigations for NS including:

- Urine protein/creatinine ratio on Synchrone Cx7 system employing a time end point colorimetric method (GMI, Inc., USA).
- Complete blood count by using automatic Coulter Counter (coulter micro Diff 18, Fullerton CA, USA).
- Serum creatinine, total serum protein and serum albumin by using Hitachi automatic analyzer 917 (Block Scientific, Inc., USA).

B- Assay of circulating DC levels by flowcytometry (Beckman Coulter Epics XL Flow Cytometer, GMI, Inc., USA).

Laboratory analysis:

a- Sample collection and handling:

Peripheral venous blood sample, about 1 cc, was collected from each subject under complete aseptic condition using EDTA as an anticoagulant and processed within 3 hours of collection.

b- Procedure for dendritic cell measurement:

Cells were Fc-blocked by treatment with 1 µg of human IgG/105 cells for 15 minutes at room temperature prior to staining. Then 25 µL of the Fc-blocked cells were transferred to a 5 mL tube. Ten µL of fluorescein-conjugated anti-CD83 reagent were added and incubation was done for 30 - 45 minutes at 2-8°C. Following this incubation, unreacted anti-CD83 reagent was removed by washing the cells twice in 4 mL of the same phosphate buffer saline (PBS). Finally, the cells were resuspended in 200 - 400 µL of PBS buffer for final flow cytometric analysis (Coulter Epics XL, USA). As a control for analysis, cells in a separate tube were treated with fluorescein-labeled mouse IgG1 antibody. Then results of the CD 83% and total leukocyte count were measured. Absolute counts of DCs were then obtained by multiplying the proportion of each DC subset within the total leukocyte population count.

Statistical analysis

The data were coded, entered and processed on computer using SPSS (version 15). Quantitative data were presented as mean ± SD (median and interquartile range for non parametric values). Comparison of the variables of the various groups was done using ANOVA test for normally distributed variables and least significant difference (LSD) post hoc test. Chi-square (χ^2) test was used to compare the frequency of qualitative variables among the different groups. Correlation of various variables was done using Pearson r correlation test. Receiver operating characteristic curve (ROC curve) was drawn to detect diagnostic reliability of circulating DCs in nephrotic syndrome. For all tests a probability (p) less than 0.05 was considered significant and less than 0.001 was considered highly significant.

RESULTS

Circulating DC count was lower in NS patients {both proteinuria (48.89±13.52) and remission (64.64±7.69) groups} than the control group (78.54±9.8) with highly significant statistical difference ($p<0.001$). Circulating DC count was lower in proteinuria group than remission group with highly significant statistical difference ($p<0.001$) (tables 1, 2).

Plots of means and 95% confidence interval for DC count in the three groups revealed that there was no overlap between cases and controls. This reflects the statistical significance and that a sharp cut off value can be defined. There was a minimal overlap between proteinuria group and remission group (figure 1).

ROC (receiver operating characteristic curve) revealed that the area under the curve (AUC) for circulating DC count was 0.89 , i.e. circulating DC count is a very good differentiating parameter between relapse and remission at the cutoff value of 58 with a sensitivity of 80% and a specificity of 80% (figure 2)

Correlation between circulating DC count and other clinical and laboratory parameters among patients revealed that there was positive significant correlation between circulating DC count and serum albumin ($r = 0.54$, $p <0.05$) (figure 3), and a negative significant correlation between circulating DC count and urine protein/creatinine ratio ($r = -0.60$, $p<0.01$) (figure 4).

Comparison among the three groups with respect to clinical and routine laboratory findings revealed comparable mean systolic and diastolic blood pressure, Hb % and TLC ($p>0.05$). However, platelet count was higher in patients (both proteinuria $398.5\pm102.7\times10^9$/L and remission $412.8\pm110.7\times10^9$/L groups) than the control group ($230.6\pm33.9\times10^9$/L) with highly statistically significant difference ($p<0.001$), but there was no difference between both patients' groups ($p>0.05$) (tables 3,4).

There was no significant correlation between platelet count and DC count ($r=0.1$, $p=0.58$).

Table 1. DC count in the studied groups.

Dendritic cells assay	Proteinuria group		Remission group		Control group		F	P	Sig.
	Mean	±SD	Mean	±SD	Mean	±SD			
DC count ($\times10^6$/L)	48.89	±13.52	64.64	±7.69	78.54	±9.80	29.30	<0.0001	**HS**

HS= highly significant, L= liter, Sig. = significance

Table 2. Multiple comparisons of the DC count using LSD test.

			P	Sig.
DC count ($\times10^6$/L)	Proteinuria group	Remission group	<0.0001	**HS**
		Control group	<0.0001	**HS**
	Remission group	Control group	<0.05	**S**

S= significant, HS= highly significant, L= liter, Sig = significance, LSD = least significant difference

Table 3. Comparison of some clinical and laboratory findings of the three groups.

	Proteinuria group		Remission group		Control group		F	P	Sig.
	Mean	±SD	Mean	±SD	Mean	±SD			
Systolic blood pressure	101.33	±8.34	102.00	±14.24	97.00	±11..92	0.80	0.46	NS
Diastolic blood pressure	65.67	±7.29	64.67	±8.34	62.33	±6.23	0.82	0.45	NS
Platelets ($\times10^9$/L)	398.5	±102.7	412.8	±110.7	230.6	±33.9	19.27	<0.0001	**HS**
Hb (g/dl)	11.54	±1.23	11.67	±0.83	11.03	±1.15	1.43	0.25	NS
TLC ($\times10^9$/L)	8.01	±1.48	7.76	±0.89	7.33	±1.70	0.91	0.41	NS

NS= not significant, HS= highly significant, L= liter, Sig. = significance, Hb = hemoglobin, TLC = total leukocyte count

Table 4. Multiple comparisons for platelets using LSD test.

			P	Sig.
Platelets ($\times 10^9$/L)	Proteinuria group	Remission group	0.66	NS
		Control group	<0.0001	**HS**
	Remission group	Control group	<0.0001	**HS**

NS= not significant, HS= highly significant, L= liter, Sig. = significance, LSD = least significant difference

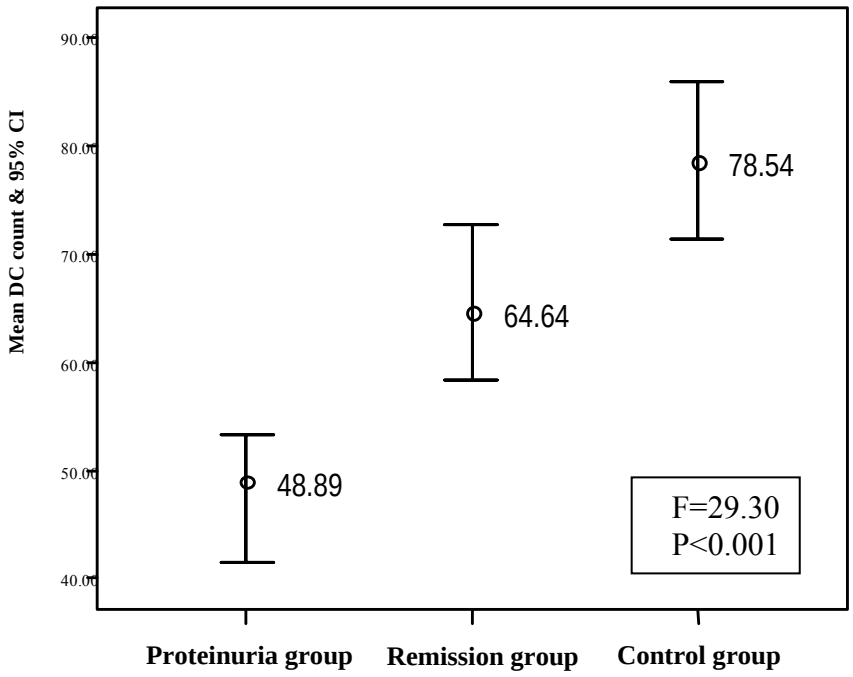


Figure 1. Plots of means and 95% confidence interval for DC count in the studied groups.

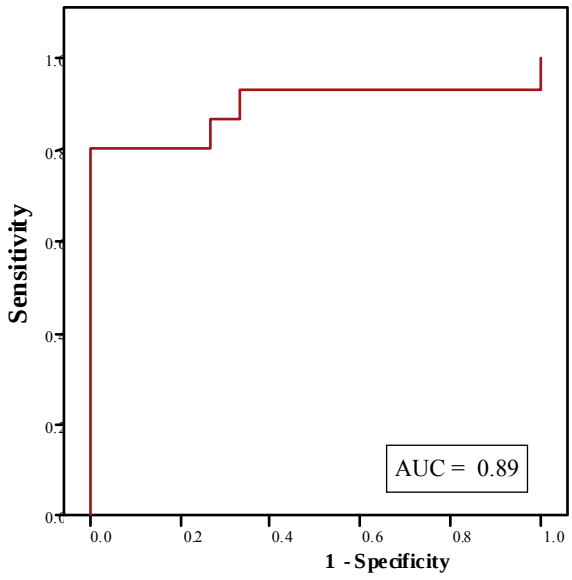
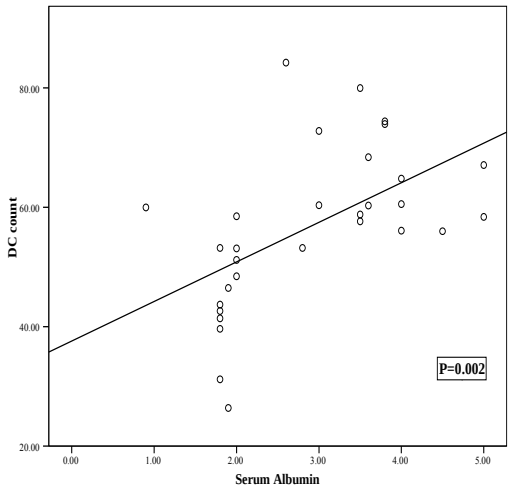
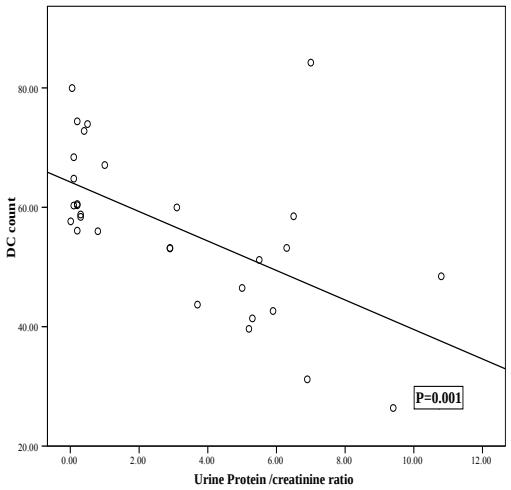


Figure 2. ROC curve (for circulating DC) differentiating proteinuria group and remission group.



r = 0.54, p = 0.002

Figure 3. Correlation between DC count and serum albumin



r = - 0.60, p = 0.001

Figure 4. Correlation between DC count and urine protein /creatinine ratio

DISCUSSION

To date, studies have yet not explored the circulating DCs levels in pediatric patients with NS. As such, our present analysis breaks new ground, providing novel insights into the involvement of DCs in the pathogenesis of NS. Much has been learned on the role of DCs from studies on other diseases such as SLE, lupus nephritis or chronic kidney disease (CKD). In consideration that DCs have entered the field of renal immunity because of their central role in the induction and regulation of adaptive immunity, and because of their therapeutic potential for manipulating immune responses; and the limited data on possible involvement of DCs in human renal diseases, we investigated whether patients with NS had alterations in circulating DCs.

It has been proposed that cell mediated immunity and T-cell activation are key features in the pathogenesis of idiopathic NS[1], as NS is associated with complex disturbances in the immune system, such as atopy and allergy, and a cytokine bias towards Th2 cytokines. Activation of T cells at the initial step of the immune response is dependent on the interaction with DCs through cell-surface receptors[7]. We propose that T-cell disturbances in NS could be induced or promoted, at least in part, by alterations in DCs because these are key regulators of the immune system. We hypothesize that these events could help to initiate and maintain the autoimmune response in NS.

In our study we found that the total number of circulating DCs was lower in patients with NS either those in proteinuria or those in remission than control group. Also we found that the total number of DCs was significantly affected by activity state of the patients with NS being significantly lower in patients with proteinuria than those in remission. This decrease in circulating DCs in NS correlates with specific clinical and serologic manifestations of the disease. There was a positive correlation between DC count and total serum protein, and serum albumin. There was also a negative correlation between DC count and urine protein/creatinine ratio. This may reflect the effect of proteinuria on level of DCs in peripheral blood which may be a part of the immune system affection in patient with NS. These correlations between DC count and other parameters found among patients but not found among control subjects, confirm the relation between NS and DCs .

Considering the DCs biology, it might be possible to hypothesize two mechanisms by which DCs may mediate renal damage in NS. First, as DCs are an important source of several pro-inflammatory factors[4] ; it was suggested that DCs, activated via toll like receptors (TLR) or CD40L might locally release TNF-α and interleukins, enhancing renal inflammation[8]. Second, others' findings in nephrotoxic glomerulonephritis that revealed proglomerular infiltration of renal DCs (rDCs) together with ours that showed a decrease in circulating DCs in NS, we suggest that DCs might migrate to local lymph nodes to present renal auto-antigens to T and B lymphocytes directing an autoimmune response towards self tissue[9].

Our results are in accordance with those observed in other autoimmune diseases such as SLE, lupus nephritis, and AIDS; and in those

observed in allergic diseases such as asthma which may be associated with NS. Also our results are in accordance with those observed in other renal injury diseases e.g. CKD, nephrotoxic glomerulonephritis and IgA nephropathy[10,11,12].

Fiore et al.[10] analyzed circulating DCs in lupus nephritis patients which were compared to healthy controls. Lupus nephritis patients with active disease showed a significant reduction in total circulating DCs compared to patients in the remission state. Also lupus nephritis patients showed a significant reduction in total circulating DCs when compared to healthy controls.

Some studies showed a decreased number of DCs in the peripheral blood of patients with SLE[11,12]. Gill et al.[13] reported that both plasmacytoid DCs (pDCs) and myeloid DCs (mDCs) are decreased in SLE.

In Hesselink et al.[14] study on patients with CKD, they demonstrated that the total number of DCs was lowest among the group of adult patients on regular hemodialysis than those on conservative management and both are lower in comparison with a healthy control group.

In IgA nephropathy, which is the most common biopsy-proven pattern of glomerulonephritis in the world[15], it is most likely that the major cause of IgA deposition in the renal mesangium might be IgA itself[16]. In their study, Eijgenraam et al.[17] showed that DCs of IgA nephropathy patients had a reduced capacity to induce IgA1 and IgA2 production in naïve B cells compared to DCs from control persons.

Studies of asthma in humans as well as its animal models have also highlighted a critical role of DCs, as the most potent APC, that play a central role in initiating the primary immune response. On the basis of the distinct properties of the DC subtypes, mDCs and pDCs were considered to be specialized APC inducing a Th1 and Th2 response, respectively. In asthma, it is suggested that increased pDCs relative to mDCs may be involved in the Th2-biased immune responses[18].

Reduced DC numbers were reported in HIV primary infection and were partially restored by antiretroviral therapy[19]. DC-based vaccines for chronic HIV-1 infection have been shown to be safe and feasible[20,21].

Moreover, our results revealed that there was a significant increase in platelets in NS patients either those in activity state or those who were in remission when compared to control group. This supports the hypothesis that platelets may play a significant role in generating coagulability in NS[22]. In agreement with our finding, Shattil et al.[23] who detected increased platelets, using flow cytometric analysis in NS patients.

In conclusion, DC counts were markedly reduced in the blood of patients with NS compared with healthy volunteers. Moreover, DC counts were markedly reduced in patients with proteinuria in comparison with the remission group. We hypothesized that this decrease could help to initiate and maintain the autoimmune process in NS. The positive correlation between DC counts and total protein, and serum albumin, and the negative correlation between DC count and urine protein/creatinine ratio point to the link between the decrease in DC count and the severity of the disease process .

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