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Original Article

Evaluation of Possible Role of Dendritic Cells in Various Lupus Nephritis

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ABSTRACT

Background & Objectives: **Chronicity of lupus nephritis (LN) should be considered for interaction of cell mediated immunity (CMI) and dendritic cells in glomeruli and tubulointerstitial areas. In this study establishment of immunohistopathological changes of dendritic cells and other immune effector cells in lupus nephritis comparing with non-lupus nephritis was performed.**

Materials & Methods: **Renal needle biopsies of 35 cases of lupus nephritis and 35 cases of other causes of persistent proteinuria were compared for immunohistochemistry for plasmacytoid (CD123), myeloid (CD11c) dendritic cells, macrophages (CD68) and lymphocytes (CD4) markers. Statistical analysis of the data was performed using Spearman and Pearson correlation or ANOVA and t- student test ($P < 0.05$).**

Results: **Significant difference of glomerular and interstitial spaces for presence of myeloid-plasmacytoid dendritic cells and lymphocytes except macrophages between lupus nephritis and other causes of persistent proteinuria have found ($P<0.001$). Positive significant correlations were observed between glomerular presentation of myeloid dendritic cells and chronicity index but not with other markers in lupus nephritis ($P <0.001$). Statistically significant changes between presence of all markers and activity index were not observed ($P >0.05$).**

Conclusions: **The myeloid dendritic cells might have synergistic role with other immune cells in pathogenesis and progression or chronicity of lupus nephritis.**

Keywords: **Lupus Glomerulonephritis, Dendritic Cell**

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Introduction

Systemic lupus erythematosus (SLE) is characterized by defects of the immune system in all manners including innate immunity, antigen presentation, apoptosis, impaired tolerance in T and B cells, and defective release of regulatory cytokines as well as chemokines (1).

Lupus nephritis is a prototype of microvascular injury due to circulating immune complexes (ICs). They are initially deposited in the glomerular mesangium, interstitial tissue and the proximal tubular epithelial cells (PTECs) which result in the release of proinflammatory cytokines and chemokines and then chronic inflammatory process. This infiltration is an innate immune system response with potential of the inflammatory proteases release, consequently endothelial injury and proliferation which induces the adaptive immune system activation secondary to the presence of ICs and dendritic cells (DCs), which these all result in maturation and activation of infiltrating T cells secondary to release of type 1 interferon. These cells were T helper 2 (Th2), T helper 1 (Th1), and T helper 17 (Th17) lymphocytes which could induce B lymphocyte and macrophages response. If this second general response exacerbated due to not longer modulation by regulatory T cells, it finally results in epithelial glomerular proliferation and fibrosis (2).

Dendritic cells were strong antigen presenting cells (APCs) and important in both innate and adaptive immune responses (3). Two subsets of DCs were named myeloid and plasmacytoid according to expression of CD11c/blood dendritic cell antigene1 (BDCA-1) and CD123/BDCA-2 respectively (4). They were the most important cells in production of IL-18 and Th1 lymphocyte responses (3). In SLE, DCs were activated by self-antigens through TLR 3, 7, 8, 9 or Fcγ receptors and released IFN-α, an important molecule in autoimmunity and in LN (5). Only in LN, DCs could infiltrate the glomeruli (4,6).

Here we tried to evaluate the role of dendritic cells with other immune cells and possible effects on pathogenesis and progression of LN.

Materials and Methods

After getting medical counsel and ethic point of view, the name and type of disease was kept secret for each patient.

Kidney needle biopsies from 70 patients (35 cases of lupus nephritis and 35 cases of persistent proteinuria non-lupus nephritis) from Shafa and Afzalipour Hospitals in the city of Kerman (2009-2012) were inspected by two pathologists for definite diagnosis. Histological classification of nephritis and morphologic evaluation on Hematoxylin-Eosin stained slides were done according to the International Society of Nephrology/ Renal Pathology Society 2003 classification. Activity indices (AI) and Chronicity indices (CI) were calculated in proliferative forms too (7).

Immunostaining was based on heat induced epitope retrieval with Novolink polymer detection system which it utilized a novel controlled polymerization technology to prepare polymeric HRP-linker antibody conjugates. Procedure was performed with the following dilutions (Table 1).

Table 1- Immunohistochemistry (antibodies and dilutions)

Antibody	Supplyer	Dilution
CD4	Leica	Ready to use
CD68	Leica	Ready to use
CD123	Leica	1/100
CD11c	Leica	1/100

Immunohistochemical staining procedures

1-Formalin fixed paraffin-embedded block of renal needle biopsy from pathology archives of Afzalipour Hospital were collected.

2-Sections were stained with H & E and also for selected antibodies on sialianized slides which were placed in oven for one hour at 60 °C. Slides without delay and to prevent drying were dehydrated and deparaffinized. The slides were moved immediately to jar containing retrieval buffer which was differed for any of antibody (i.e. Tris-EDTA pH =9 for CD11c and CD68, Tris-EDTA pH=8 for CD4, Citric acid pH=6 for CD123). Slides along with retrieval buffer put into microwave (12 minutes at 850 °C and then 8 minutes at 300 °C).Then it was cooled by water, and left for 10 minutes in running tap water. After10 minutes, we moved slides into the jar containing wash buffer (TBS buffer for 2x5 minutes). Throwed on the slides for 5 minutes from the Peroxidase Block (0.5% H2O2/methanol), Washed in TBS buffer for 2x5 minutes and then incubated with Protein Block for 5 minutes. Washed in TBS buffer for 2x5 minutes. Incubated with optimally diluted primary antibody (30 minutes for Novocastra CD4 and CD68, 60 minutes for Novocastra CD11c and CD123). Washed in TBS buffer for 5 minutes twice. Incubated with Post Primary Block for 30 minutes. Washed in TBS buffer for 2x5 minutes. Incubated with NovoLink Polymer for 30 minutes. Washed in TBS buffer for 2x5 minutes. Developed peroxidase activity with DAB working solution for 30 minutes. Rinsed slides in water. Counterstained with Hematoxylin. Rinsed slides in water for 5 minutes. Dehydrated, clear and mount sections.

For each antibody, positive and negative controls were considered. A positive control for all above mentioned antibodies was tonsil and negative control was cerebellum for CD4 and CD123 whereas skeletal muscle for CD11c. However no negative control was recommended for CD68.

To investigate and scoring immunohistochemistry, cytoplasmic staining for CD68, cell surface staining for CD4 and membranous staining for CD11c and CD123 regardless of their intensity within the context of any non-specific background staining was considered as positive results. Single, countable positive cells which were cytologically compatible with dendritic cells, lymphocytes and macrophages were included. All countable glomeruli and ten field of interstitium were investigated for above mentioned cells (X 400). Slides which had not these criteria were omitted from data collections. For comparison of various classes of LN according to severity and all IHC cell markers due to insufficient number of each group separately we decided to perform the LN patients as three subsets. Therefore we joined some classes of LN as following: class I and II together, class III and IV together and class V and VI together which named subset groups of LN I, II and III respectively.

We used ANOVA and *t*-student test, for comparing quantitative variables that follow normal distribution. For correlation, we used Spearman and Pearson correlation. To display the percentage of nominal data and other data which summarizing the mean and standard deviation (for easier understanding) was used. The *P*-value<0.05 was considered as significant level.

Results

The mean age of lupus nephritis (LN group) was 30.65±1.9 yr and non- lupus nephritis (non LN group) was 38.65±2 yr respectively. The number of patients in both groups was 35. In LN group 20% were male and 80% were female and in non LN group 57.1% were male and 42.9% were female. Frequency of diagnosis in LN group was 2.9% for class I, III, VI and 5.7 for class V, 28.6% for class II and 57.1 % for class IV. 82.9% of LN group had activity index less than 9/24 and 85.7% had chronicity index less than 5/12.

Immunohistochemical findings

Lupus nephritis group showed that the accumulation of CD4+ lymphocytes in the interstitium as focal aggregates especially around the glomeruli, also as individually into the glomeruli with mean of 2-5 cells/ glomerular cross section (gcs) that seemed to be greater than in non LN group. Reticular accumulation of CD11c+ and CD123+ dendritic cells in the interstitium was predominantly in periglomerular and peritubular pattern which seemed to be greater in stage IV than stage II (mean 10-20/10 HPF and 4-9/10 HPF respectively), also in the glomeruli with mean 1-4 cells/gcs which seemed to be greater in higher stage of LN (Fig. 1 and 2).

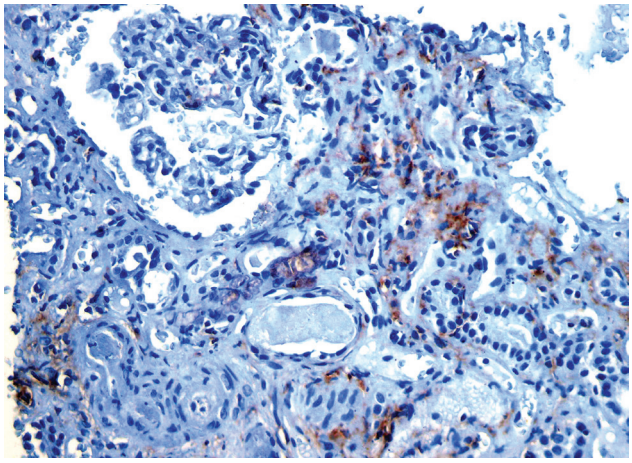

Fig. 1: Increased number of periglomerular & peritubular CD11c + myeloid dendritic cells as reticular formation in a case of LN class IV .

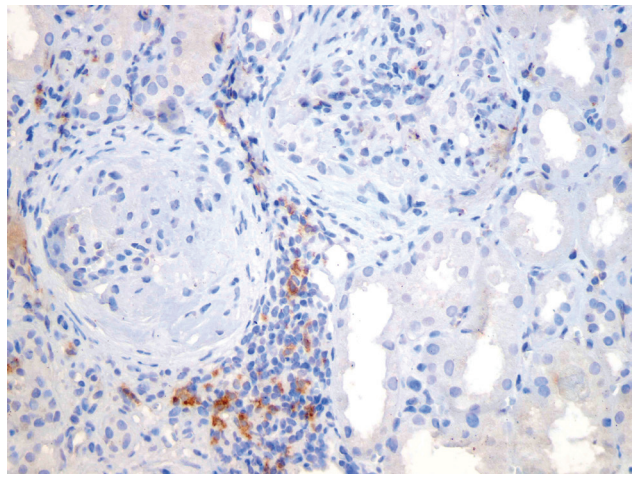

Fig. 2: Increased number of periglomerular& peritubular CD123+ plasmacytoid dendritic cells in a case of LN class VI with complete glomerular sclerosis .

CD68+ macrophages was present as individual cells in the interstitium and glomeruli with mean 3-10 cells/10 HPF and 1-2 cells/ gcs respectively. However it seemed to be greater number of these cells at the site of interstitial fibrosis and glomerular sclerosis.

By analyzing the percentage of all cell markers, the presentation of glomerular and interstitial CD4+ lymphocytes,CD11c+ myeloid and CD123+ plasmacytoid dendritic cells increased in LN group compared with non LN group (P<0.005). In contrast, the percentage of glomerular and interstitial CD68+ macrophages was not statistically significant difference between two the same groups (Table 2).

Table 2- The percent of cell markers in Lupus nephritis and Non Lupus Nephritis groups

Cell marker	Group	
	Lupus nephritis	Non-lupus nephritis
CD4i	14.1±1.2	7.9±1
CD4g	73.1±3.7	52.2±3.7
CD68i	6±0.6	5±0.7
CD68g	29.9±4.3	17.7±4.5
CD11ci	39.1±4.6	4±1.5
CD11cg	9.4±1.2	4.1±0.6
CD123i	41.8±5.1	5.2±1.8
CD123g	8.6±1	3.7±0.5

In addition, we found that statistically significant correlation between glomerular CD11c+ cells presentation and chronicity index (P<0.005) (Fig. 3).

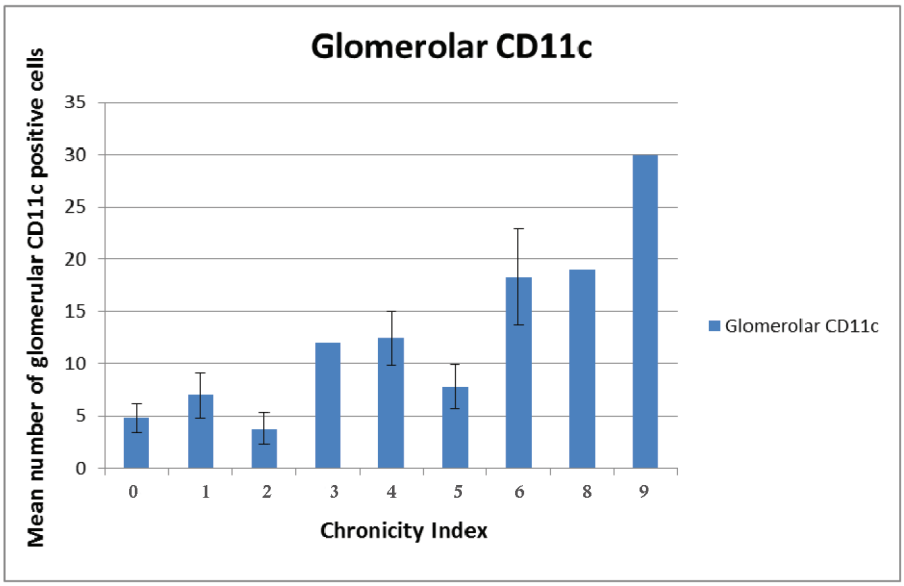

Fig. 3: Glomerular CD11c presentation in different score of chronicity index. The presence of glomerular CD11c was significant between different score of chronicity index.

By categorization of patients in LN groups according to the severity of LN, the higher percentage of glomerular CD11c+ mDCs was demonstrated in subset III (class V and VI of LN) compared with subset I and II (P<0.005) (Fig. 4).

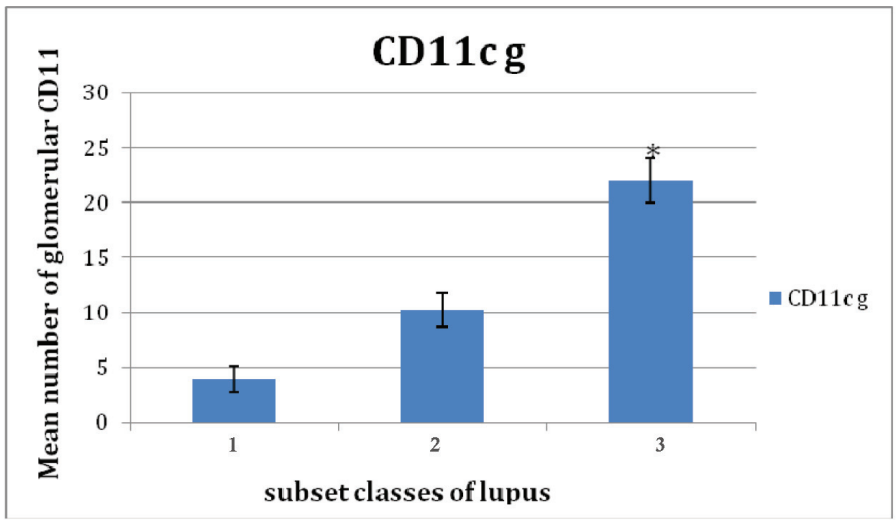

Fig. 4: Glomerular CD11c presentation in subset classes of Lupus Nephritis group. The presence of glomerular CD11c was significant between all groups.

*: significant

We investigated the LN groups and showed that there was greater number of glomerular CD4+ and CD68+ cells, also interstitial CD11c+ and CD123+ cells in class III and IV of LN compared with all other classes although no statistically significance was observed.

However this investigation in non LN groups showed no statistically significance between various diagnosis in this group and percentage of CD4+, CD68+, CD11c +and CD123+ cells.

Discussion

We found that there was significant difference on the presence of myeloid (CD11c+) and plasmacytoid (CD123+) dendritic cells as well as CD4+ lymphocytes between patients with LN and patients with non lupus nephritis, which was higher in LN group but this was not true for CD68+ macrophages.

Tucci *et al*. showed over-expression of glomerular IL-18 and peripheral blood IL-18R (by flow cytometry) and also accumulation of pDCs within the glomeruli in LN (7). Segerer *et al*. showed that DCs in GBM were increased in animal model of lupus nephritis and also in proliferative form of LN. He also found the increased number of CD68+ myeloid/macrophage cells at the glomerular interstitium (8). Fiore and Woltman showed that there was an increase in lymphoid DCs (9, 10).

We demonstrated that the increased number of CD11c+ mDCs were present in higher classes of LN patients. We also found that all of the above mentioned cells increased in number at stages III and IV of LN rather than another stages albeit only glomerular increased CD4+ and CD68+ cells was significant statistically. These findings are supported by following studies:

Fiore and Tucci showed that the proliferative classes had the most cellular infiltration (9, 11). Fiore also highlighted this point that most of these cells in LN were immature in contrast to SLE patients with a marked reduction of mature DCs and lymphoid cells (9) probably due to their migration to the kidneys and other tissues during the activity of the disease (12).

The pDCs were decreased in peripheral blood of patients with LN compared to those without it, whereas the number of mDCs had no changes in healthy individuals. In addition, these cells in patients with active LN had maximum reduction (11).

T cells are either effectors (includes CD4+ Th1, Th2, Th3, cytotoxic CD8+, and Th17) or regulators (FOXP3+ CD25+ T cells and natural killer T cells). These cells have multiple roles in initiation, amplification, and regulation of the immune response in LN as well as migration, destruction, fibrosis, resolution, and exacerbations of the disease (12). In LN, activated CD4, few CD8 T cells, macrophages and DCs infiltrated the renal interstitium, thus worsening renal function (13). T cells also activate proximal tubular cells and consequently parenchymal fibrosis (14).

We did not find any significant correlation between activity index and presence of all cell markers in LN group. Overexpression of IFN-γ, IL-2, IL-12, IL-18, MCP-1, and IL-10 had a significant correlation with the histological activity index of LN (15). Therefore measurement of pro-Th1 in urine can be a biomarker for LN activity especially in proliferative LN class III and IV (16). This finding made worsen the disease and correlated with the histological activity index (17, 18). Diversely, predominant Th2 response was seen in Type V membranous LN (19). In human, the role of T cells in the course of LN was less clear (17, 18). However, in pediatric LN, there was a balance between Th1/Th2 on the basis of IgG subclasses (20, 21). In proliferative LN, there was an overexpression of TNF-related apoptosis-inducing ligand (TRAIL) in the glomerular tubules (22).

We did not study especially about B cells in our experimental but some studies were performed which showed specific role of these cells as following: B cells were also abnormal and hyperactive in SLE (23-26). The role that played by effector B cells was through the synthesis of autoantibodies and also regulators by direct interaction with memory T cells and regulation of DC development (27). In humans, B cells had some degree of organization rather than randomly arranged. Formation of ectopic germinal centers with organized follicles and DCs correlated with the severity of tubulointerstitial disease and deposition of ICs (28).

We thought that the presence of DCs into the glomeruli should have been a reason because routinely these cells must circulate and did not stay in the tissue which was supported by some studies. Shankland *et al*. showed that flattening and sloughing of podocytes were due to release

of cytokines and ICs deposition in glomeruli and tubule-interstitium (29). Moreover Segerer *et al*. showed that the mesangial, endothelial, and epithelial cells were not innocent bystanders but may be involved in proinflammatory mediator release, fibrogenesis, and also APC as signal amplifiers (30).

Active glomerular lesions had abundant inflammatory infiltrates of CD4+ and some CD8+ T cells, monocytes, and plasma cells in the interstitium (18) which was associated with glomerular filtration rate and creatinine levels (31). Some authors have found association between interstitial ICs and serological activity (32). The relation between tubular damage, fibrosis, and atrophy with renal function were less contributed with treatment. These lesions often have seen in Classes III and IV (19).

Conclusion

Due to all revised reasons mentioned previously, we decided to assess the correlation between presence of these cells and choronicity index of LN since we thought that the cell mediated immunity was effective in progression of renal tissue damage although ICs have essential role in initiation of the disease. We found a significant correlation between presence of glomerular CD11c+ cells and chronicity index. Unfortunately, the number of LN specimens (Class I, III, V and VI) was little to evaluate the exact role of dendritic cells in especially progression of LN. We suggest that this study be repeated in large scale with nephropathology units of other Iranian Medical Schools for better revaluation of its role.

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