

Expression of TLR2 and TLR4 on CD14 Monocytes in Female SLE Patients E

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Abstract

Background: Systemic Lupus Erythematosus (SLE) is a prototype autoimmune disease of multifactorial origin mainly assigned to defects in the adaptive immune system. However, evidences supported the crucial role of the innate immune system in its pathogenesis. Toll-like receptors (TLRs) have been proposed as important pathways in disease development. This relatively new idea holds promise for new therapeutic strategies. The aim of this work is to measure surface expression of TLR2 and TLR4 on CD14+ monocytes in SLE patients and compare it with healthy controls, also to find out their relation with disease activity and damage.

Subjects and methods: The current study was carried out on Forty Egyptian female SLE patients and 20 matched control subjects. Disease activity was assessed by the SLE disease activity index (SLEDAI) and damage assessed by the Systemic Lupus International Collaborating Clinics (SLICC) index. Expression of TLR2 and 4 on CD14+ monocytes was studied using flow cytometry.

Results: The age of the patients ranged between 16-56 years with a mean \pm SD of 31.6 ± 9.2 years. Significant increase of TLR2 surface expression and a significant decrease of TLR4 surface expression on CD14+ monocytes in SLE patients compared to the control group ($p=0.006, 0.004$, respectively) were observed. No statistically significant associations were detected with both activity and damage indices.

Conclusion: This study suggests that TLR2 and 4 plays a role in the pathogenesis of SLE but have no impact on disease activity.

Keywords: SLE; Innate immunity; Toll-like receptors; Flow cytometry; SLEDAI; SLICC

Introduction

Systemic lupus erythematosus (SLE) is a multifactorial chronic autoimmune disease of connective tissue with a variety of clinical manifestations that differ from patient to patient and affects multiple organs leading to serious complications. To be noted that it is currently accepted that its onset and development are associated with several genetic, environmental, and hormonal factors [1]. Previously chronic arthritis, which is the hallmark in SLE and other autoimmune diseases, was mainly attributed to deregulations in the adaptive immune system, mainly T-cells. However, evidences supported the important role of the innate immune system in the development of arthritis [2].

Toll-like receptors (TLRs) are a large family of innate immune receptors. They act as a key link between infection, injury and inflammation. TLRs recognize a variety of pathogen and danger-associated molecular patterns (PAMPs and DAMPs) [3]. TLRs expression has been revealed on various immunocompetent cells, such as macrophages and dendritic cells, as well as on non-immune cells and this expression is either constitutive or in an inducible manner [4].

At least eleven TLRs have been identified in humans. All TLRs are synthesized in the endoplasmic reticulum (ER) and are secreted in response to stimulation. Most TLRs reside on the cell surface, however,

there are also intracellular TLRs that are expressed almost exclusively in the endosomal compartments of cells and are specialized in recognition of nucleic acids [5,6]. TLR signals directly regulate the intracellular mechanisms that allow the antigen presenting cells to process an antigen and display it in the context of MHC. TLR engagement also stimulates Dendritic cell (DC) maturation, resulting in induction of expression of co-stimulatory molecules and chemokine receptors and production of cytokines. This allows subsequent antigenic peptide presentation and activation of T lymphocytes. Thus, TLRs play a crucial role in both the activation of innate immune responses and the subsequent development and shaping of adaptive immune responses [7].

The role of TLR mediated inflammation does not only imply host defense, but also is related to the pathogenesis of several autoimmune diseases [8]. Several TLRs have been studied in the development of many autoimmune diseases [4]. Of these are the TLR2 and TLR4. These are cell-surface receptors. TLR4 was the first TLR to be characterized. TLRs 2 and 4 mainly recognize cell wall components of various Gram positive and Gram-negative pathogens, as the lipoteichoic acid and LPS respectively, stress proteins and cell decomposition products [9].

Recent studies have elucidated the role of TLRs in the pathogenesis of autoimmune diseases [10]. Peripheral blood mononuclear cells (PBMCs) and DCs produce pro-inflammatory cytokines such as IL-1 β , TNF- α or IL-6 in response to ligand engagement [11-13].

Hence, the aim of this work is to measure expression of TLR-2 and TLR-4 on the surface of CD14+ monocytes in patients with SLE and compare it with normal controls, and also to find out the relationship between their expression and the disease activity and organ damage.

Patients and Methods

Inclusion criteria

Forty female SLE patients diagnosed according to Systemic Lupus International Collaborating Clinics classification criteria for SLE [14]. Disease activity was assessed with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [15]. Disease damage was recorded according to the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index [16]. Concerning treatment, 27 patients (67.5%) received Azathioprine, all the patients received corticosteroids in a dose of 5-60 mg/day (mean: 25.6 ± 14 mg/day).

Twenty age and sex matched healthy subjects were enrolled as controls. Local institutional research board approval and informed consent were undertaken from all the subjects prior to participation in the study. Patients were excluded if they had any autoimmune disease other than SLE, or if they had fever or any infectious disorders that could affect the white blood cells count.

Assessment of TLR expression in peripheral blood

Blood samples were withdrawn on EDTA anticoagulant. Samples were divided into 3 tubes, In each tube one hundred µL of whole blood sample was mixed with ten µL of PE-conjugated anti-CD14 monoclonal antibody (R&D SYSTEMS, catalog number: FAB3832P, Lot number: LGG04, USA). Ten µL of monoclonal antibodies against TLR2 (R&D SYSTEMS, catalog number: FAB2616F, Lot number: ABCY01, USA) or TLR4 (R&D SYSTEMS, catalog number: FAB6248F, Lot number: ABUN02, USA) conjugated with fluorescein isothiocyanate (FITC) were added. Samples were incubated at 2-8°C for 30 min.

Analysis was performed using Epics XL coulter (Beckman Coulter). Intact monocytes were identified by their size and granularity as assessed by their logarithmic amplification of the FSC and SSC signals and thus they were gated upon for furthermore selection of CD14+ monocytes.

Percentages of expression besides mean fluorescence intensities (MFI) were acquired. Isotype-matched antibody controls were used to detect non-specific staining.

Statistics

All statistical analysis was performed with SPSS Version 20. Results were presented as mean, standard deviation and comparisons of quantitative variables was performed using an independent Student's t-test. Correlation was assessed by Spearman coefficient of correlation. p<0.05 was considered statistically significant.

Results

The current study was carried out on forty Egyptian SLE female patients, as well as 20 age matched healthy females. The age of the patients ranged between 16-56 years with a mean ± SD of 31.6 ± 9.2 years. The age of the controls ranged between 29-38 years with a mean

± SD of 33.4 ± 3.0 years. Clinical and laboratory data of the patients are summarized in Tables 1 and 2.

Clinical data	SLE(n=40)
	n (%)
Pleurisy	19 (47.5%)
Pericarditis	13 (32.5%)
Nephritis	30 (75%)
CNS affection	6 (15%)
Vasculitis	27 (67.5%)
Arthritis	30 (75%)
SLE: systemic lupus erythematosus	

Table 1: Clinical data of SLE patients.

Variable	Patients (n=40)	
	Mean ± SD	Range
Hemoglobin(g/dL)	11.6 ± 1.8	5.9-15.1
TLC (x103/cmm)	7.2 ± 2.7	2.7-12.7
Platelets (103/cmm)	272.4 ± 116.8	66-648
ESR	44.1 ± 29.4	5-115
AST(U/L)	24.1 ± 11.6	8-66
ALT(U/L)	26.2 ± 17.7	6-83
Creatinine (U/L)	0.9 ± 0.6	0.4-3.5

Table 2: Laboratory data of SLE patients. SLE: Systemic Lupus Erythematosus; TLC: Total Leucocytic Count; ESR: Erythrocyte Sedimentation Rate; AST: Aspartate Transaminase; ALT: Alanine Transaminase. Bold values are significant at p<0.05.

All patients (100%) were ANA positive, 31 (77.5%) were anti-dsDNA positive, 22 (55%) were anti-Ro positive, 24 patients (60%) were lupus anticoagulant positive and 13 (32.5%) were anticardiolipin positive. The level of C3 ranged between 16-160 G/L (mean: 88 ± 37), C4 ranged between 2.8-90 G/L (mean: 21.6 ± 17.8) and the SLEDAI ranged between 2-30 (median 14).

A statistically significant increase of TLR2+CD14+ monocytes (p=0.006) and mean fluorescence intensity (MFI) of TLR2+CD14+ monocytes (p=0.03) were found in SLE patients than in control group. A statistically significant decrease of expression as well as the MFI of TLR4+CD14+ monocytes (p=0.004, <0.001 respectively) was also noticed in patients compared to the control group (Table 3) and Figure 1.

Parameter	SLE patients	Controls	P-value
TLR2/CD14 (%)	82.1 ± 13.5	76.9 ± 7	0.006
TLR4/CD14 (%)	7.7 ± 7	12 ± 5.8	0.004
TLR2/CD14 MFI	2.6 ± 0.7	2.2 ± 0.3	0.03

TLR4/CD4 MFI	1.4 ± 0.5	1.8 ± 0.6	<0.001
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Table 3: TLR2 and TLR4 results in SLE Patients. TLR: Toll-like Receptor; CD: Cluster of Differentiation. Bold values are significant at p<0.05.

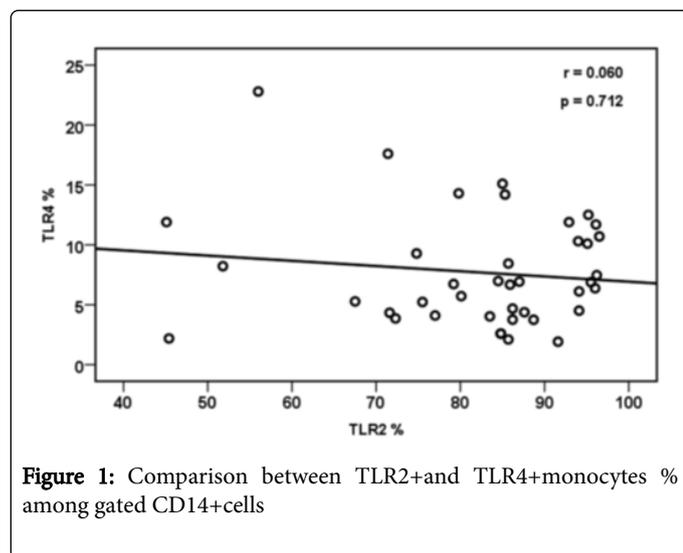


Figure 1: Comparison between TLR2+and TLR4+monocytes % among gated CD14+cells

Within the study group, a tendency towards a decrease of MFI of TLR4+CD14+ monocytes in nephritis subgroup of patients than in non-nephritis was noticed although it did not reach statistical significance (p=0.054). No other statistically significant correlations between the two groups as regards expression of TLR4+CD14+ monocytes, TLR2+CD14+ monocytes and MFI of TLR2+CD14+ monocytes could be detected. Regarding serositis and non-serositis patients, results revealed a significant decrease of TLR4+CD14+monocytes in serositis SLE patients than in non-serositis ones (p =0.017). As in case of nephritis, no other significant differences could be noticed between the two groups.

Correlation of TLR2 and TLR4 results with SLEDAI and SLICC/ACR criteria are summarized in Table 4. Results show a negative correlation between the damage index and MFI of TLR4+CD14+ however it did not reach statistical significance p=0.058. Otherwise no correlations were detected between the expressions and MFI of TLR4+ and TLR2+ with SLEDAI.

	TLR2+CD14+%		TLR2-MFI		TLR4+CD14+%		TLR4-MFI	
	R	p	R	P	R	P	R	P
SLE DAI	0.284	0.075	0.071	0.664	-0.095	0.546	-0.264	0.1
SLI CC	0.265	0.098	0.057	0.728	-0.087	0.595	-0.302	0.058

Table 4: Correlation of TLR Expression with SLEDAI and SLICC. TLR: Toll-like Receptor; CD: Cluster of Differentiation; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index. SLICC/ACR DI Disease damage was recorded according to the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) Damage Index. Bold values are significant at p<0.05.

Discussion

It has been noted that there is a lack of identification of a new FDA-approved lupus treatment for 40 years. This is probably issued to the failure of detection of a “common denominator,” for all lupus patients, owing to the complexity of the disease [17]. The importance of TLRs in the pathogenesis of SLE has been reported in many previous studies [18], and therapeutic interventions targeting these molecules or their signaling pathways warrant high expectation [17].

Our study revealed significant differences between the patients and control subjects regarding the expression and MFI of TLR2+CD14+, TLR4+CD14+ monocytes with significant increase in the levels of the former and significant decrease in those of the latter receptor.

The surface expression of TLR2 and 4 in SLE was not clearly elucidated in previous studies. Our results are in accordance with that of Kirchner et al. who demonstrated a significantly reduced level of cell surface TLR4 expression on CD14+ monocytes compared to that of healthy control subjects. However, their results were different in respect to TLR2 expression where no significant difference was observed between the SLE patients and the control group [3].

In contrast, Migita et al. reported that although TLR4 expressions on CD14+ monocytes were not significantly different between healthy subjects and patients with SLE, TLR2 expression on monocytes was reduced in patients with SLE compared to healthy subjects [19].

Regardless of the discrepancies between results, the significant increase in TLR 2 expression reported in our study goes with the proposed role of TLRs in the pathogenesis of autoimmune diseases [4]. Also it supports the findings reported by Komatsuda et al. which showed that relative TLR2, TLR7, TLR9 mRNA expression levels were significantly higher in SLE patients than in control subjects [20]. However, the decrease in TLR4 expression recorded in our results was in accordance with previous *in vivo* and *in vitro* observations which demonstrate that upon ligand engagement, internalization of TLR4 occurs with subsequent down regulation of surface expression [3].

Hence our study suggests that the association between infection and SLE is often caused by TLR-mediated induction of proinflammatory cytokine and chemokine expression, upregulation of co-stimulatory molecule expression by APCs and production of autoantibodies by hyperactive B cells. Indeed, Lartigue et al. observed that TLR2- and TLR4-deficient B6lpr/lpr mice expressed lower titers of autoantibodies [21]. Also Inhibition of TLRs and their signaling pathways have been shown to be effective in lupus-prone mouse models and successfully inhibit production of IFN by human pDC *in vitro* [17].

From another aspect, the TLR2 and TLR4 genotypes in SLE patients were investigated in a previous study done by Kirchner et al. [3]. The aim of the study was to evaluate whether functional polymorphisms of these TLR genes affect the surface protein expression as reported in other autoimmune diseases as rheumatoid arthritis [22]. However results did not confirm any role of these SNPs on TLR protein expression levels on monocytes in patients with SLE.

In our study, no statistically significant associations were detected with SLEDAI scoring index. It is noteworthy that a novel observation revealed by Houssen et al., is the negative correlation between serum soluble TLR2 (sTLR2) levels and SELDAI score in SLE patients. This negative correlation was attributed to the role of sTLR2 in down regulation of TLR2 signaling through various mechanisms [23].

Conclusion and recommendations

Therefore, we conclude that despite different expression of TLR2 and 4 on the surface of CD14+ monocytes, assertions point to the involvement of the investigated TLRs in the pathogenesis of SLE in our patients. Hence further experiments targeting TLRs and their downstream effectors may hold promise to ascertain new reliable treatment modalities.

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