Cell Membrane-bound TLR2 and TLR4: Potential Predictors of Active Systemic Lupus Erythematosus and Lupus Nephritis

To the Editor:

Innate immune receptors have been found to be involved in the pathogenesis of systemic lupus erythematosus (SLE)[1]. The binding of nucleic acids to the endosomal Toll-like receptors (TLR) 7 and TLR9 is considered as a triggering mechanism for the production of antinuclear antibodies[2,3]. Also, the cell membrane-bound TLR (mbTLR) might contribute to enhance immune cell responses in SLE. Besides detecting microorganisms, these receptors engage molecules exposed upon apoptosis, such as the DNA-binding high mobility group protein B1, which is thought to facilitate self-DNA antigenicity[4]. The contribution of the mbTLR TLR2 and TLR4 to loss of tolerance and development of nephritis has been consistently found in SLE models conducted in transgenic mice[5,6,7]. However, there is little information about the activation of mbTLR during SLE flares in humans.

We have studied TLR2 and TLR4 protein levels in peripheral blood mononuclear cells from patients with SLE (n = 35) and healthy controls (n = 11) using flow cytometry. Patients were receiving stable medication at the time of the study, and had no signs of active infection. Whereas no global differences in the levels of the mbTLR were noted between the cohorts, the density of TLR4 was significantly increased in the B cells of patients with active (n = 20) compared to inactive (n = 15) SLE (Figure 1A). Levels of TLR4 in these cells were correlated with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) and the British Isles Lupus Assessment Group index (BILAG) activity scores (Figure 1B) and also with the erythrocyte sedimentation rate ($p < 0.001$, not shown). Similarly, the amounts of both mbTLR in T cells and that of TLR4 in monocytes were found to increase in parallel with activity measures (not shown). There was no association between the expression of any of these molecules and anti-DNA antibody titers or complement levels. Dose of corticosteroids was not different in patients with high or low expression levels of the molecules, while T cell TLR were significantly higher in patients taking immunosuppressant drugs.

Interestingly, we further observed that the enhancement of the mbTLR was associated to concurrent active nephritis (aLN). As compared with the rest of the cohort, patients with aLN had higher levels of TLR4 in B cells and monocytes and higher levels of TLR2 in lymphocytes (Figure 2A). In addition, urinary protein excretion rates were associated to both the expression of TLR2 in T lymphocytes and of TLR4 in monocytes (Figure 2B).

Although based on few patients, our data point to a possible involvement of TLR2 and TLR4 in the activation of mononuclear cells during SLE flares and nephritis. Our results are in agreement with a previous study conducted in 16 women with SLE, in whom an association was found between the expression of T lymphocyte TLR4 levels and disease activity[8]. Interestingly, neither study found any association between anti-DNA antibody titers and the expression of mbTLR in mononuclear cells. This fact may indicate that the enhancement of these molecules translates a different activation pathway to the ones acknowledged, which could show up in particular subgroups of patients with SLE. It is tempting to suggest a relationship with the involvement of infections in SLE flares. Mechanistically, pathogens could enhance mbTLR activity and cause tissue injury. Thereby, cooperation of mbTLR with endosomal TLR might boost the reactivity of the latter toward nuclear autoantigens. In this line of thought, data have shown the participation of TLR2 and TLR4 in the recognition and internalization of herpesviridae[9], a family of pathogens closely associated to the disease. Also possible is that the upregulation of mbTLR could be a consequence of the acute-phase response, as elegantly shown in a model of lupus nephritis in which tissue damage is elicited by a cytokine-dependent upregulation of TLR2 and TLR4 in local endothelial cells[10].

Altogether, the results drawn in mice and our current findings suggest that mbTLR could be particularly involved in nephritis. There is an increasing body of evidence that activation of mbTLR also underlies other types of immune-mediated nephritis in humans. As an example, a study showed an enhancement of TLR4 in mononuclear cells from children with Henoch-Schönlein purpura and high-grade proteinuria[11].

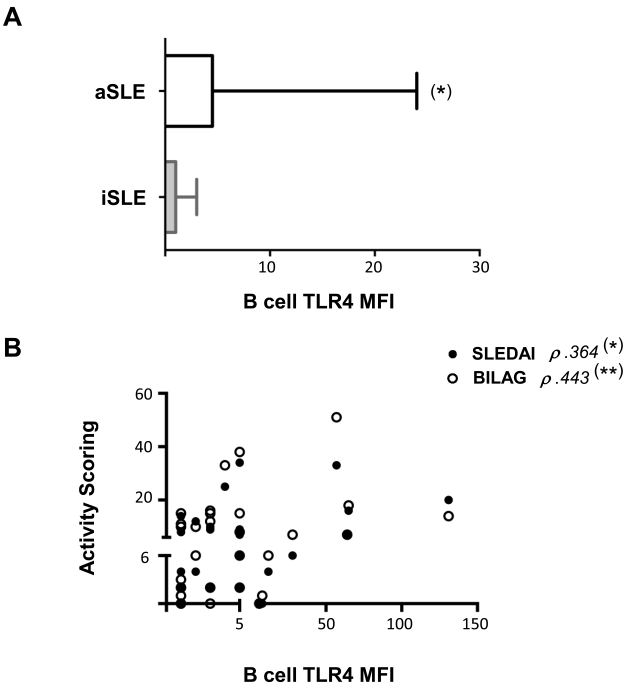

Figure 1. Association of B cell TLR4 levels to disease activity. A. Levels of TLR4 in B cells are expressed as MFI. Data show median (IQR) of patients with aSLE and iSLE. B. Correlation between B cell TLR4 MFI and SLEDAI-2K (filled dots), and BILAG-2004 activity indexes (open dots). The expression of the TLR in circulating mononuclear cells was analyzed with flow cytometry using a FACSCanto II and monoclonal antibodies from BD Biosciences. Each sample was subjected to 3 different reactions (≥ 10^5 leukocytes each) with combinations of the following antibodies: CD3, CD19, CD38, TLR2, CD45, CD123, HLA-DR, TLR2, and TLR4, labeled with different fluorescent dyes. For comparisons, aSLE was defined by any of the following criteria: (1) a ≥ 4 SLEDAI-2K score plus a ≥ 2-point increase in the last 3 months, (2) BILAG categories A or B, or (3) BILAG C plus a ≥ 4 SLEDAI-2K score. Statistical analysis was done with nonparametric tests. * $p < 0.05$. ** $p < 0.01$. TLR: Toll-like receptor; MFI: median fluorescence intensity; IQR: interquartile range; SLE: systemic lupus erythematosus; aSLE: active SLE; iSLE: inactive SLE; SLEDAI-2K: SLE Disease Activity Index 2000; BILAG: British Isles Lupus Assessment Group index.

Our data suggest that the detection of TLR2 and TLR4 in circulating mononuclear cells from patients with SLE might be useful as an additional marker of disease activity, and in particular of active nephritis. Replication of these results in other cohorts is warranted to confirm their relevance.

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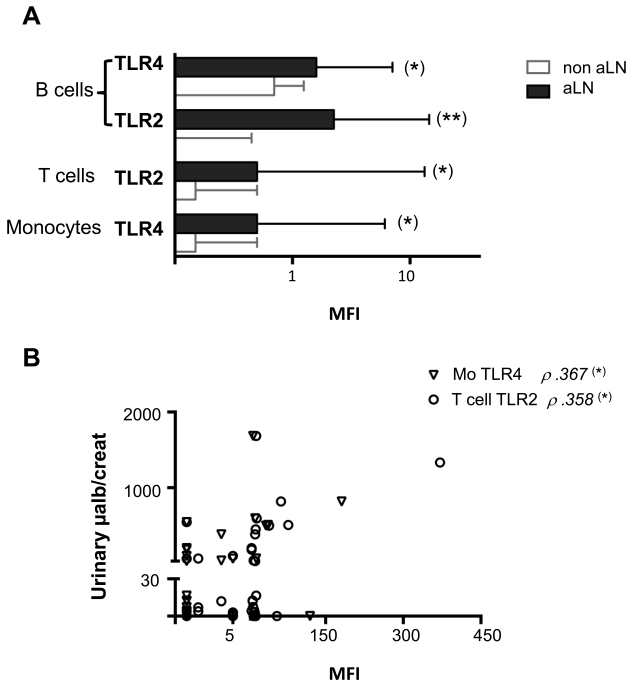

Figure 2. Association of the membrane-bound TLR to activity of LN. A. Levels of each TLR in the different mononuclear cell subsets are expressed as MFI. Data show median (IQR) in patients with aLN and non-aLN. B. Correlation between urinary microalbumin/creatinine ratios and the MFI levels of monocyte TLR4 (circles) and T cell TLR2 (triangles). The expression of TLR was analyzed as specified in Figure 1 caption. Nine of the 35 patients were considered to have aLN according to the following criteria: a recent increase in previous urinary protein > 0.5 g/24 h or > 50 mg microalbumin/g creatinine; an active sediment (presence of casts, leukocytes, and red cells) in absence of infection; history of LN without remission. * $p < 0.05$. ** $p < 0.05$. TLR: Toll-like receptor; LN: lupus nephritis; aLN: active LN; MFI: median fluorescence intensity; IQR: interquartile range.

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