Expression of the leukocyte-associated Ig-like receptor-1 on B lymphocytes from systemic lupus erythematosus patients
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Introduction
The leukocyte-associated Ig-like receptor-1 (LAIR-1), now designated as CD305, is an inhibitory receptor expressed on almost all hematopoietic cells, particularly on immune system cells. The known LAIR-1 ligands are extracellular matrix collagen and C1q, the first component of the complement. After the binding of its known ligands [1,2], LAIR-1 inhibits the activation of immune cells using two immunoreceptor tyrosine-based inhibitory motifs located in the cytoplasmic tail of the receptor. LAIR-1 is expressed during B-cell ontogenesis, but is lost on a subset of memory B cells, in plasma blasts, and in plasma cells [3]. From a functional point of view, LAIR-1 cross-linking results in the inhibition of Ca²⁺ mobilization induced by B-cell receptor (BCR)-triggering. On the other hand, prolonged BCR-stimulation or CD40-stimulation induces the downregulation of LAIR-1 on naïve B cells in vitro [3], suggesting an inhibitory role for LAIR-1 on BCR signaling, as for other B-cell inhibitory receptors such as CD22 and Fc gamma receptor IIb (FcγRIIb) [3].

Systemic lupus erythematosus (SLE) is an immune-mediated chronic inflammatory disease characterized by the presence of a wide variety of autoantibodies produced by dysregulated B cells [4–6]. B cells have a central role in the pathogenesis of SLE and exert multiple effects. B cells not only produce pathogenic antibodies, but also contribute to immune dysregulation and tissue injury.

Keywords: B lymphocytes, leukocyte-associated Ig-like receptor-1, serum creatinine, systemic lupus erythematosus

Background
The leukocyte-associated immunoglobulin (Ig)-like receptor-1 (LAIR-1) is a transmembrane molecule belonging to the Ig superfamily. In B cells, LAIR-1 cross-linking leads to downregulation of Ig and cytokine production.

Aim of the work
The aim of the present study was to assess the expression of LAIR-1 on peripheral blood B lymphocyte from systemic lupus erythematosus (SLE) patients, and its correlation with disease manifestations.

Patients and methods
Twenty-two SLE female patients and 16 matched healthy controls were included in the study. Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score was assessed. The expression of LAIR-1 was determined by using flow cytometry.

Results
The 22 SLE patients had a mean age of 25.59±4.22 years and disease duration of 5–24 months. The mean SLEDAI was 8±1.5. The percentage of CD19⁺ B lymphocytes lacking LAIR-1 was markedly increased in SLE patients (27.8±10.9%) compared with healthy controls (16.2±4.4%) (P<0.001). The mean fluorescent intensity ratio (MFIR) of LAIR-1 expression on CD19⁺ B cells was strongly decreased in SLE patients (44.1±12.6) compared with healthy controls (58.9±7.7) (P<0.001). The percentage of CD19⁺ LAIR-1⁻ B cells significantly correlated with the complement (C4) (r=0.45, P=0.03) and the serum creatinine level (r=–0.47, P=0.02), and negatively with the serum albumin level (r=–0.57, P=0.005). The MFIR of LAIR-1 significantly correlated with the serum albumin level (r=0.74, P<0.001) and negatively correlated with the serum creatinine level (r=–0.43, P=0.041). There was no significant association of LAIR⁻ (% or the MFIR with the clinical manifestations of the patients.

Conclusion
This study points out that the lack of LAIR-1 expression on B cells from SLE patients could be a trigger for the dysregulation of antibody production in SLE, and is associated with the degree of renal affection as evidenced by the significant correlation with serum creatinine levels and negative correlation with the levels of serum albumin.

Keywords:
b lymphocytes, leukocyte-associated Ig-like receptor-1, serum creatinine, systemic lupus erythematosus
autoantibodies but have additional pivotal roles in the autoimmune process: they act as antigen-presenting cells, provide costimulatory signals for T-cell activation and differentiation, secrete and respond to cytokines, link innate and acquired immunity by toll-like receptors, affect follicular dendritic cell differentiation, and help shape the architecture of peripheral lymphoid organs. Alteration of these B-cell functions may lead to a breach of tolerance and autoimmune disease [7]. Loss of B-cell tolerance occurs very early in SLE, as shown in a study by Arbuckle et al. [8], who examined the serum samples from the US Defense Serum Repository and found that autoantibodies are typically present many years before the onset of SLE while patients are still asymptomatic. Subsets of B cells are altered in SLE, with an increase in transitional B cells, memory cells, and plasma cells, and an increase in a subset of autoreactive B cells in blood and peripheral lymphoid organs. Autoreactive B cells lead to development of autoreactive memory B cells and plasma cell [7].

The initiating auto-antigen responsible for the disease has not been defined yet, but it is relevant to determine whether B cells in SLE patients may express a particular phenotype and a consequent behavior that may be the reason of the observed dysregulation.

The aim of the present study was to analyze whether B cells of SLE patients may lack LAIR-1 and an inhibiting signal that regulates activating signals, and also its correlation with disease manifestations.

**Patients and methods**

The present study was conducted on 22 female SLE patients attending the outpatient clinics of the Physical Medicine and Rehabilitation department and the Internal Medicine Department at Menoufia University Hospitals. Their ages ranged from 20 to 35 years (mean 25.6±4.2 years), and the disease duration ranged from 5 to 24 months (mean 12.7±5.6 months). Patients were diagnosed according to the modified American College of Rheumatology criteria for the classification of SLE [9]. Sixteen age-matched and sex-matched healthy controls (age 25.5±4.8 years) were also enrolled in this study. Disease activity was assessed by using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score [10]. This study was approved by the ethical committee of the Faculty of Medicine, Menoufia University. All patients signed an informed consent before their blood samples were collected and their medical records reviewed for research purposes.

**LAIR-1 expression on B lymphocytes**

A volume of 100 μl whole blood cells were stained with phycocyanin-conjugated anti-LAIR-1 (eBiosciences, San Diego, California, USA) and fluorescein isothiocyanate-conjugated antihuman CD19 (Immunostep, Salamanca, Spain) for 30 min at 4°C, followed by whole blood lysis. The data were acquired and analyzed by using BD FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, New Jersey, USA). We gated on CD19+ cells and then analyzed the percentage of LAIR-1− cells and LAIR-1 mean fluorescent intensity ratio (MFIR) within this population. Control and patient samples were examined and compared using the same settings and conditions.

**Statistical analysis**

Analysis was performed with SPSS, version 16 statistical software (SPSS Inc., Chicago, Illinois, USA). Continuous variables were presented as mean and SDs. The variances between groups regarding age, number of CD19+ LAIR-1 B cells, and LAIR-1 MFIR were compared using the Student t-test. The association of the CD19’ LAIR-1 B cell percentage and LAIR-1 MFIR with various clinical manifestations of the patients were compared using the Student t-test. Pearson’s correlation analysis was used to show the strength and direction of association between the percentage of CD19’ LAIR-1 B cells and LAIR-1 MFIR with the laboratory data of the studied patients. A value of P less than 0.05 was considered statistically significant.

**Results**

The clinical and laboratory characteristics of SLE patients are summarized in Table 1. The SLEDAI ranged from 6 to 10, with a mean of 8±1.5. All patients were subjected to pulse steroid therapy (methyl prednisone 1 g for 3 days), and maintained on moderate dose steroid therapy (range 30–40 mg/day) and 100 mg azathioprine. Cyclophosphamide 600 mg every 2 weeks was given to one patient who showed transverse myelitis.

The percentages of CD19’ B cells lacking LAIR-1 expression were increased in the SLE patients (27.8±10.9%, n=22) compared with controls (16.2±4.4%, n=16) (P<0.001) (Figs 1 and 2). In addition, the MFIR of LAIR-1 expression on CD19’ B cells was strongly decreased in SLE patients (44.1±10.9%) compared with healthy controls (58.9±7.7) (P<0.001) (Fig. 3).

Correlation between the percentage of CD19’ LAIR-1 B cells and patients’ different laboratory data revealed the
presence of significant correlations with C4 and serum creatinine level ($P=0.03$ and 0.02, respectively) and negative correlation with serum albumin level ($P=0.005$). Correlations with other parameters were not significant (Table 2). When we performed the same correlation using the MFIR of LAIR-1 expression on CD19$^+$ B cells, it showed that the MFIR of LAIR-1 significantly correlated with the serum albumin level ($P<0.001$) and negatively correlated with the serum creatinine level ($P=0.04$) but did not significantly correlate with other parameters (Table 2). There were no significant associations with other clinical manifestations (Table 3).

**Discussion**

Immune cell responses are tightly regulated by the balance of signals from activating and inhibitory receptors that they express. Inhibitory signals tend to predominate over activating signals serving to prevent hyper-responsiveness/autoimmunity against self-antigens that could damage the host. A large number of ITIM-bearing inhibitory molecules with diverse tissue distribution and ligand recognition have been shown to negatively regulate cell activation. ITIM-containing molecules are involved in the control of a large spectrum of immune functions [11]. LAIR-1 is an ITIM inhibitory-bearing receptor expressed by the majority of immune cells, for which cross-linking in-vitro by antibody or collagens delivers an inhibitory signal that can downregulate activating signals [12]. The known LAIR-1 ligands are extracellular matrix collagen and C1q, the first component of the complement [1,2].

SLE is a pleiotropic disease that may hit several organs, and its clinical manifestations are quite varied [13–17]. Each patient may respond to a complex therapy with different immunosuppressant in a characteristic manner [14–17].

In this study, we demonstrated that in patients suffering from SLE, the level of expression of the inhibiting surface receptor LAIR-1 on CD19$^+$ B cells was decreased and the percentage of CD19$^+$ LAIR-1$^-$ cells was increased compared with healthy controls. These findings were in agreement with those obtained by Colombo et al. [18], who studied LAIR-1 expression in peripheral blood B lymphocytes from 54 SLE, 24 mixed connective tissue disease, 20 systemic sclerosis, 14 rheumatoid arthritis patients, and 40 sex-

**Table 1** Demographic, clinical, and laboratory features, as well as disease activity in the systemic lupus erythematosus patients

| Characteristics          | SLE patients ($n=22$) |
|--------------------------|-----------------------|
| Age (years)              | 25.6±4.2              |
| Disease duration (months)| 12.7±5.6              |
| Clinical manifestations  |                       |
| Malar rash               | 22 (100)              |
| Photosensitivity         | 16 (72.7)             |
| Discoid rash             | 0 (0.0)               |
| Arthritis                | 8 (36.4)              |
| Serositis                | 8 (36.4)              |
| Neuropsychiatric         | 1 (4.5)               |
| Oral ulcer               | 20 (90.9)             |
| Hematologic disorder     | 6 (27.3)              |
| Renal disorder           | 22 (100)              |
| Laboratory investigations|                       |
| WBCs ($\times 10^3$/mm$^3$) | 5.80±1.06         |
| Hb (g/dl)                | 9.02±1.24             |
| Platelets ($\times 10^3$/mm$^3$) | 188.9±58.8     |
| ANA positivity           | 22 (100)              |
| DNA positivity           | 22 (100)              |
| C4 (mg/dl)               | 0.03±0.03             |
| C3 (mg/dl)               | 0.08±0.03             |
| ESR (mm/first hour)      | 124.5±17.1            |
| CRP positivity           | 4 (18.2)              |
| Urine protein (g/24h)    | 3.48±2.42             |
| Creatinine (mg/dl)       | 0.9±0.3               |
| BUN (mg/dl)              | 20.9±1.1              |
|Albumin (g/dl)            | 2.5±0.3               |
| SLEDAI                   | 8±1.5                 |

Data are expressed as n (%) or mean±SD. ANA, antinuclear antibody; antId, antidualle stranded antibody; BUN, blood urea nitrogen; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; SLE, systemic lupus erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; WBCs, white blood cells.

**Table 2** Correlation of the percentage of CD19$^+$ LAIR-1$^-$ B cells and LAIR-1 MFIR with the age, laboratory investigations, and disease activity of the studied systemic lupus erythematosus patients

| Parameter                  | CD19$^+$ LAIR-1 B cells (%) | LAIR-1 MFIR |
|---------------------------|-----------------------------|-------------|
|                           | $r$                         | $P$         |
| Age (years)               | 0.23                        | 0.3         |
| WBCs ($\times 10^3$/mm$^3$) | 0.08                      | 0.71        |
| Hb (g/dl)                 | −0.002                      | 0.99        |
| Platelets ($\times 10^5$/mm$^3$) | 0.17                      | 0.44        |
| ESR (mm/first hour)       | −0.23                       | 0.29        |
| C4 (mg/dl)                | 0.45                        | 0.035$^*$   |
| C3 (mg/dl)                | 0.04                        | 0.83        |
| Proteinuria (g/24h)       | 0.11                        | 0.62        |
| Creatinine (mg/dl)        | 0.47                        | 0.025$^*$   |
| BUN (mg/dl)               | −0.103                      | 0.65        |
| Albumin (g/dl)            | −0.57                       | 0.005$^*$   |
| SLEDAI                    | 0.3                         | 0.1         |

BUN, blood urea nitrogen; ESR, erythrocyte sedimentation rate; Hb, haemoglobin; LAIR, leukocyte-associated Ig-like receptor-1; MFIR, mean fluorescent intensity ratio; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; WBCs, white blood cells.

$^*$Significant at $P<0.05$. 

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matched and age-matched healthy donors, and reported markedly reduced LAIR-1 expression in SLE and mixed connective tissue disease but not in systemic sclerosis and rheumatoid arthritis patients.

In the present study, the degree of defect in LAIR-1 expression was found to be associated with the degree of renal affection as evidenced by the significant correlation with the serum creatinine levels and negatively with the levels of serum albumin.

No previous study has discussed such clinical association, but in their study Son et al. [2] have established a functional partnership between LAIR-1 and C1q, which is of particular relevance to SLE and strongly correlate with severe lupus nephritis. They finally proposed that the defects in C1q and LAIR-1 interactions contribute to immune pathology in this disease.

A recent study has declared defective LAIR-1 expression on plasmacytoid dendritic cells (PDC) in SLE pediatric patients compared with that found in healthy donors [19], and concluded that enhancing the expression/inhibitory function of LAIR-1 on pDCs should be included in future immune interventions for controlling jSLE.

This suggests that in SLE, the defective LAIR-1 expression on B cells may leave these cells fully stimulated by still undefined polyclonal stimuli and/or autoantigens, and, then, to proliferate and produce potentially pathogenic Ig, without the negative regulation initiated upon collagen-LAIR-1 interaction.
LAIR-1 has been reported to be downregulated in B cells of chronic lymphocytic leukemia (CLL) [20]. Interestingly, in CLL the engagement of LAIR-1 with specific mAbs blocks the constitutive and BCR-mediated protein kinase Akt phosphorylation and nuclear factor kappa (NF-κB) nuclear translocation, leading to inhibition of B-cell proliferation. Importantly, this inhibiting effect was not evident in LAIR-1 CLL cells [21]. Moreover, LAIR-1 expression was observed to be related to the occurrence of autoimmune phenomena. Both a direct antiglobulin test and autoimmune hemolytic anemia

Table 3 Association of the percentage of CD19+ LAIR-1 B cells and LAIR-1 MFIR with C-reactive protein and clinical manifestations of systemic lupus erythematosus patients

| Manifestation/Parameter | CD19+ B cells LAIR-1(%) | LAIR-1 MFIR |
|-------------------------|-------------------------|--------------|
|                         | Means±SD    t      P   | Means±SD    t      P   |
| Photosensitivity        |             |              |              |
| Yes (16)                | 27.2±10.8   0.42  0.68 | 43.9±13.4   0.11  0.91 |
| No (6)                  | 29.4±12     |              | 44.6±10.9    |
| Arthritis               |             |              |              |
| Yes (8)                 | 32.9±8.3    1.09  0.29 | 45.6±12.2   0.42  0.68 |
| No (14)                 | 27.9±11.4   |              | 43.2±13.1    |
| Serositis               |             |              |              |
| Yes (8)                 | 30.7±10.4   0.93  0.36 | 41.3±11     0.78  0.45 |
| No (14)                 | 26.2±11.2   |              | 45.7±13.5    |
| Hematologic             |             |              |              |
| Yes (6)                 | 26.2±7.3    0.4   0.69 | 51.2±8.2    1.7   0.1  |
| No (16)                 | 28.4±12.1   |              | 41.4±13      |
| Oral ulcer              |             |              |              |
| Yes (20)                | 27.2±11.3   0.79  0.44 | 44.4±13.2   0.34  0.73 |
| No (2)                  | 33.7±0.07   |              | 41.1±10.14   |
| CRP                     |             |              |              |
| Positive (4)            | 23.5±1.7    0.90  0.38 | 45.5±7.3    0.25  0.81 |
| Negative (18)           | 28.8±11.5   |              | 43.8±13.1    |

Malar rash and renal disorders were present in all cases and there was no discoid rash among the studied patients. CRP, C-reactive protein; LAIR, leukocyte-associated Ig-like receptor-1; MFIR, mean fluorescent intensity ratio.
were more frequently observed in LAIR-1− patients, with quite a high percentage of LAIR-1− patients having autoimmune hemolytic anemia at presentation with CLL [20]. It was also reported that LAIR-1 expression on CD4(+) and CD8(+) T cells decreases and serum LAIR-1 level increases in children with idiopathic thrombocytopenic purpura, suggesting that LAIR-1 may play an important role in immune imbalance in these children [22].

In a previous report by Colombo et al.[18], it was shown that the oligomerization of LAIR-1 on the whole peripheral blood B-cell population, but not in LAIR-1+ B cells, can affect BCR-mediated calcium mobilization and NF-κB p65 nuclear translocation. It is known that BCR-induced calcium mobilization and the following activation cascade are dysregulated in B cells from SLE patients; one possible regulating mechanism missing in SLE B lymphocytes might be related to the lower or absent expression of LAIR-1 and of its ITIM domain. Another ITIM-dependent dysregulation of BCR-induced B-cell activation in SLE patients might be related to a deficient FcγR-mediated suppression; indeed, dysfunction of this low affinity Ab receptor, which is equipped with a cytoplasmic ITIM like LAIR-1, is associated with autoimmunity and it has been previously described in SLE patients [23]. In addition, Akt and NF-κB are targets for the action of LAIR-1 in primary myeloid leukemias [24,25], indicating that anywhere LAIR-1 is expressed, its engagement evokes a similar cellular response.

Conclusion
The present study indicated the possibility that the lack of LAIR-1 expression on B cells from SLE patients could be a trigger for the dysregulation of antibody production in SLE patients, and was found to be associated with the degree of renal affection as evidenced by the significant correlation with serum creatinine levels and negatively with the levels of serum albumin. Future studies are recommended to examine the comparison of LAIR-1 expression on B cells from SLE patients with renal lupus as proved by renal biopsy and those without renal affection to reveal its relation to the pathogenesis of lupus nephritis.

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Conflicts of interest
There are no conflicts of interest.

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