IMMUNOLOGICAL EFFECTS OF ESTROGEN ON CD40 LIGAND EXPRESSION ON T CELLS OF SYSTEMIC LUPUS ERYTHEMATOSUS FEMALE PATIENTS

Hanan Mohammad Farouk, Hekal-Allah Ahmed Al-Shamy*, Eiman Mahmoud Ghanima* and Ghada H. Bashat**

Internal Medicine, Rheumatology & Rehabilitation* and Clinical Pathology** Departments, Ain Shams University Faculty of Medicine

KEY WORDS: IMMUNOPATHOGENESIS OF SLE.

ABSTRACT

Background: Systemic lupus erythematosus (SLE) predominantly affects women during their reproductive years. Its pathogenesis has been postulated to involve T cells hyperactivity that can be induced by over-expression of signaling molecules such as CD40 ligand (CD40L) on T cells. This is supposed to lead to B cells proliferation, differentiation and autoantibodies production.

Objective: To investigate the immunological effects of estrogen on CD40L expression on T cells in vivo as well as its relation to disease activity in SLE female patients.

Subjects: Thirty SLE female patients were included in this study. They are subdivided into two groups: Group Ia: 15 SLE patients during their reproductive years and Group IIa: 15 post menopausal SLE patients. The clinical activity of the disease was assessed with SLE disease activity index (SLEDAI). The results were compared to two control groups: Group Ib: 10 normal females during their reproductive years and Group IIb: 10 normal postmenopausal women.

Methods: Routine investigations were performed. Serum estradiol was assessed with electrochemiluminescence immunoassay. Whole blood was used to determine CD40L expression on T lymphocytes with direct immunofluorescence technique. Renal biopsy was performed for SLE patients only.

Results: CD40L expression on T cells was significantly higher in group Ia than in group IIa (p<0.01). Also it was
significantly higher in group Ia than that in group Ib (p<0.01). There was no significant difference between both groups regarding estrogen level (p>0.05). In spite of that, SLEDAI score was significantly higher in group Ia than that in group IIa (p<0.01). Also 24 hrs urinary protein was significantly elevated in group Ia than that in group IIa (p<0.01) while creatinine clearance and serum C3 level were significantly reduced in group Ia than that in group IIa. Of group Ia 66.7% had WHO class IV and V glomerulonephritis (GN) as compared to only 6.7% of group IIa (p<0.01).

There was a non-significant difference between groups IIa and IIb regarding CD40L expression on T cells (p>0.05). Also, there was a significant correlation between CD40L expression on T cells, estrogen level and SLEDAI score in groups Ia and IIa patients (p<0.01). On the other hand, there was a non-significant correlation between CD40L expression on T cells and estrogen level in groups Ib and IIb (p>0.05).

**Conclusion:** Estrogen plays an important role in the pathogenesis of SLE through increasing the expression of CD40L on T cells in SLE female patients, but not in normal females. This action is dose-dependent as we found that CD40L expression on T cells was significantly higher in SLE female patients during their reproductive years than during their postmenopausal years. Again, its level correlated well with markers of disease activity i.e. SLEDAI.

**INTRODUCTION**

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease of unknown cause that is characterized by abnormal production of autoantibodies and multisystem affections (Ghirardello et al., 2002). It predominantly affects women during their reproductive years (Lahita, 2000). In certain models of lupus, female mice had more aggressive disease than males and ovariectomy of those mice improved their disease symptoms, while estrogen administration aggravated the disease (Roubinian et al., 1978). Although some progress in understanding the role of estrogen in lupus has been reported (Rider et al., 1998), yet the exact role of estrogen in the immuno-pathogenesis of SLE is not completely understood (Tsokos, 1999).

The development of lupus has been postulated to involve abnormal regulation of T cell function (Abdou et al., 1981). T cell hyperactivity in autoimmune disease can be induced by over-expression of signaling
molecules such as CD40 ligand (CD40L) on helper T cells thereby contributing to the pathogenesis of lupus (Datta et al., 1997).

CD40 is a member of the TNF family and is expressed in cells such as T cells, B cells and macrophages. Its ligand (CD40L) is a transmembrane protein and it is also a member of the TNF family, which is expressed mainly on activated T cells (Lederman et al., 1992). Binding of CD40 to its ligand (CD40L) plays an important role in T cell activation (Grewal et al., 1998). Suenaga et al. (1998) reported that, this abnormal regulation of T cell function might be influenced by ligand bound estrogen receptor stimulation of T cell signal transduction molecules.

CD40L binds to CD40 on antigen presenting cells transducing a second signal that is essential for B cell growth and differentiation (Roy et al., 1998). Previous data indicate that CD40L up-regulates the expression of the B7 family of proteins that promote T cell differentiation by interacting with CD28 on T cells and initiating the immune response (Shu et al., 1995). In SLE patients, CD40L has been reported to be over-expressed on T cells and ectopically expressed on B cells and the interaction between CD40 and CD40L is required to sustain the immune response in SLE (Howland et al., 2000).

Recent studies reported that the level of CD40L in the sera of active SLE patients was increased while its level was low in patients in remission, but can be readily induced to active disease level by in vitro stimulation (Vakkalanka et al., 1999). During increasing lupus disease activity CD40L is up-regulated resulting in co-stimulation of CD40 on B cells and excessive production of anti-DNA antibodies of specific idiotypes (Mehta et al., 1999). B cells under the influence of CD40L both proliferate and switch their immunoglobulin Ig from IgM to IgG class (Kosny et al., 2000). B cells from SLE patients but not those from normal controls showed increased production of anti-dsDNA autoantibodies in response to stimulation with recombinant CD40L leucine zipper fusion protein (Harigai et al., 1999). Previous studies on animal murine mice models of lupus showed a predominant female expression of lupus with renal pathology similar to that found in human SLE (Mohan et al., 1995). In those mice, CD40L was expressed early and excessively on activated helper T cells well before the disease manifested itself. Moreover, treatment of those mice with anti-CD40L antibody delayed disease onset and in some cases reversed or prevented the disease process (Yi et al., 2000).

Rider et al. (2001) cultured T cells from SLE female patients with estradiol and reported that, there was over-expression of CD40L on T cells. However, till present no in vivo studies were done to demonstrate the effect of estrogen on CD40L expression on T cells in SLE female patients.
Aim of Study:

The aim of this study was to investigate the in vivo immunological effects of estrogen on CD40 ligand expression on T helper cells, as well as its relation to disease activity in SLE female patients.

**PATIENTS AND METHODS**

**Patients:**

This study included thirty female SL patients who fulfilled the American College of Rheumatology revised criteria for the classification of SLE (Tan et al., 1982). Patients were selected from the Internal Medicine Department and the Rheumatology Outpatient Clinic of Ain Shams University Hospitals. They were divided into two groups:

- **Group Ia:** Including fifteen SLE female patients having regular menstrual cycles, their ages were ranging from 18-38 years (mean 28.6±6.2).
- **Group IIa:** Including fifteen SLE postmenopausal female patients, who had been menopausal at least 6 months before being included in our study, their ages were ranging from 44 to 55 years (mean 47.9±3.1).

Another twenty apparently healthy females were taken as controls and again divided into two groups:

- **Group Ib:** Including ten females with regular menstrual cycles with no history of intake of oral contraceptive pills, their ages were ranging from 18-38 years (mean 26.2±4.3).
- **Group IIb:** Including another ten postmenopausal females, none of them was taking estrogen replacement therapy, their age were ranging from 44-55 years (mean 49.2±2.4).

All the subjects in the control groups were free from known diseases and had normal routine laboratory investigations.

**All Patients Were Subjected To The Following:**

I- Full history taking, including menstrual history.

II-Clinical examination including general, systemic, rheumatological and neuropsychiatric examination. Patients may have organic or non-organic brain syndromes. Organic psychosis is characterized clinically by disturbance of orientation, perception, memory and/or intellectual functions. Non-organic psychosis can manifest as neurosis, mania or depression. Patients might have lupus cerebritis manifested clinically by seizures or focal lesions (Isshi & Hirohata, 1996).
III- Laboratory investigations including, CBC, ESR, liver functions, serum complement C3.

IV- Plain X-rays to chest.

V- ECG and echocardiography if indicated (those with clinical manifestations of cardiac affection).

VI- EEG and CT scan to the brain for those with clinical manifestations of neuropsychiatric lupus.

VII- Kidney function tests including: blood urea, serum creatinine, 24 hours proteins in urine, creatinine clearance and active urinary sediments.

VIII- Renal biopsy was done after taking oral consent from the patients and according to WHO classification they were classified into: Class I: normal, class II: Mesangioproliferative glomerulonephritis (GN), class III: focal proliferative GN, class IV: diffuse proliferative GN, class V: membranous GN and class VI: advanced sclerosing GN (Austin et al., 1994).

Patients were diagnosed as having lupus nephritis if they fulfilled any of the following criteria: (1) active urinary sediments especially RBCs or granular casts and persistent proteinuria (2-4+). (2) Proteinuria more than 1gm/24 hours. (3) 30% decrease in creatinine clearance and (4) renal biopsy showing focal proliferative, diffuse proliferative or membranous glomerulonephritis (Wallace et al., 1997).

Then assessment of the activity of SLE with the systemic lupus erythematosus disease activity index (SLEDAI) was performed for all patients: SLEDAI score (0-5) means inactive disease, score (6-9) means mildly active disease, while score (10-14) means moderately active disease, and score >14 means severely active disease (Bombardier et al., 1992).

Methods:

Blood samples were collected from patients and controls and divided into two parts:

- One part was centrifuged at 3000 rpm for 10 minutes then the serum was separated and stored at -20°C until used for determination of:

1. Antibodies to ds-DNA by indirect immunofluorescent (Inovadiagnostics, USA) (Aarden et al., 1975).
2. Serum complement according to the method of Stites (1982).
3. Serum creatinine with kits obtained from bioMereux (France).
4. Serum estradiol with electrochemiluminescence immunoassay (ECLIA) on the Roche Elecsys 1010/2010 and MODULARANALYTICS E170 (Elecsys module) immunoassay analyzers.
- The other part of the whole blood was used for determination of CD40 ligand. Surface CD40L was done with FCM on coulter EPICS XL (coulter electronics, Hialeah Fl, USA) with direct immunofluorescence technique. Lysis of red blood cells was done by adding 3 ml NH₄Cl (0.83% buffered with KHCO₃ PH7.2) for 5 minutes at 37°C. The CXCR4 is FITC labeled antibodies along with isotypic negative control. The values were expressed in %. The CD40 ligand monoclonal antibody purchased from R and D system* (Fig. 1).

![Flow cytometric histogram showing CD40L +ve expression of lymphocytes in SLE.](image)

Urine analysis was done and 24 hours protein in urine was assayed with the method of Henery et al. (1956). Again, creatinine clearance was assayed according to the method of Wootton (1974).

**Statistics:**

Analysis of data was performed with an IBM computer using SPSS Windows (V 6.2) package. Description of quantitative variables was in the form of mean, standard deviation and range. Chi-square test was used to compare qualitative groups. T test of two independent samples was used to compare two quantitative variables. R-test or correlation coefficient was used to test correlation between two quantitative variables. One way ANOVA test was used to compare more than two quantitative groups with each other and is expressed as F ratio and P value. In all tests, if P value >0.05: non-significant test, if <0.05: significant and if <0.01: highly significant test.
RESULTS

Comparison between groups Ia (SLE females during their reproductive years) and IIa (postmenopausal SLE females) as regards CD40 ligand (CD40L) expression on T lymphocytes revealed a statistically highly significant difference between both groups with a mean of 42.4±19.0 and 12.1±6.8 respectively (t=5.8, p<0.01) (table 1).

Table (1): Comparison between groups Ia and IIa patients as regards CD40L expression on T cells, estrogen level, SLEDAI score and C3 level.

<table>
<thead>
<tr>
<th>Group</th>
<th>CD40L% of expression on T lymphocytes</th>
<th>Estrogen level (pg/dl)</th>
<th>SLE DAI score</th>
<th>C3 level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia (n=15) Mean±SD</td>
<td>42.4±19.0</td>
<td>Mid cycle 242.1±74.1</td>
<td>17.9±3.7</td>
<td>59.6±24.4</td>
</tr>
<tr>
<td>IIa (n=15) Mean±SD</td>
<td>12.1±6.8</td>
<td>Postmenopausal 23.6±9.8</td>
<td>8.5±3.2</td>
<td>94.5±27.0</td>
</tr>
<tr>
<td>t</td>
<td>5.8</td>
<td>11.3</td>
<td>7.2</td>
<td>3.7</td>
</tr>
<tr>
<td>p value (HS)</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

HS: Highly significant, C3: Complement C3, SLEDAI score: SLE disease activity index score.

Table (1) also showed a highly significant difference between groups Ia and IIa regarding estrogen level with a mean of 242.1±74.1 and 23.6±9.8 respectively (t=11.3, p<0.01) (Fig. 2).
A highly significant difference was found on comparing SLEDAI score between groups Ia and IIa patients with a mean of 17.9±3.7 and 8.5±3.2 respectively (t = 7.2, p<0.01) (table 1 & Fig. 3).

Fig. (3): Comparison between groups Ia and IIa patients as regard SLEDAI score.

C3 level was found to be significantly reduced in group Ia (mean 59.6±24.4) more than group IIa (mean 94.5±27.0) (t=3.7, p<0.01) as shown in table (1).

Table (2) showed the comparison between groups Ia and IIa patients as regards the prevalence of nephritis, where there was a significant elevation in the mean level of 24 hours urinary protein of group Ia (mean 1.8±0.9) more than that of group IIa (mean 0.4±0.2) (t = 5.7, p<0.01).

Table (2): Comparison between groups Ia and IIa patients as regards prevalence of nephritis.

<table>
<thead>
<tr>
<th>Screening test</th>
<th>Group Ia (n = 15)</th>
<th>Group IIa (n = 15)</th>
<th>Test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours urinary protein (gm/day)</td>
<td>1.8±0.9</td>
<td>0.4±0.2</td>
<td>t = 5.7</td>
<td>&lt;0.01 (HS)</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>66.4±23.2</td>
<td>93.6±16.4</td>
<td>t = 3.7</td>
<td>&lt;0.01 (HS)</td>
</tr>
<tr>
<td>Active urinary sediments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(+ve)</td>
<td>n = 12 (80%)</td>
<td>n = 3 (20%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(-ve)</td>
<td>n = 3 (20%)</td>
<td>n = 4 (26.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 11 (73.3%)</td>
<td>X^2 = 8.57</td>
<td>&lt;0.01 (HS)</td>
<td></td>
</tr>
<tr>
<td>Renal biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO class II GN</td>
<td>n = 2 (13.3%)</td>
<td>n = 11 (73.3%)</td>
<td>X^2 = 10.05</td>
<td>&lt;0.01 (HS)</td>
</tr>
<tr>
<td>WHO class III GN</td>
<td>n = 3 (20%)</td>
<td>n = 3 (20%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO class IV GN</td>
<td>n = 6 (40%)</td>
<td>n = 1 (6.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO class V GN</td>
<td>n = 4 (26.7%)</td>
<td>Zero</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

GN = glomerulonephritis.
Creatinine clearance was reduced in group Ia patients (mean 66.4±23.2) more than in group IIa patients (mean 93.6±16.4) and this reduction was statistically highly significant (t = 3.7, p<0.01) (table 2).

Table (2) also showed the comparison between groups Ia and IIa patients regarding the results of renal biopsy. It was found that in group Ia 6 out of 15 patients (40%) had WHO class IV GN, 4 patients (26.7%) had WHO class V GN, 3 patients (20%) had WHO class III GN and only 2 patients (13.3%) had WHO class II GN. While in group IIa 11 out 15 patients (73.3%) had WHO class II GN, 3 patients (20%) had WHO class III GN, and only one patient (6.7%) had WHO class IV GN with statistically highly significant difference between both groups (X² = 10.05, p<0.01).

Table (3): Comparison between group Ia patients and group Ib (their controls) as regards CD40L expression on T cells, estrogen level and C3 level.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CD40L% of expression on T lymphocytes</th>
<th>Mid cycle estrogen level (pg/dl)</th>
<th>C3 level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia (n=15) Mean±SD</td>
<td>42.4±19.1</td>
<td>242.1±74.2</td>
<td>59.1±24.4</td>
</tr>
<tr>
<td>Ib (n=10) Mean±SD</td>
<td>11.1±3.6</td>
<td>232.3±76.3</td>
<td>134.1±35.4</td>
</tr>
<tr>
<td>t</td>
<td>5.11</td>
<td>0.33</td>
<td>6.29</td>
</tr>
<tr>
<td>p value</td>
<td>&lt;0.01 (HS)</td>
<td>&gt;0.05 (NS)</td>
<td>&lt;0.01 (HS)</td>
</tr>
</tbody>
</table>

NS: Non significant

Table (3) showed comparison between group Ia (SLE women during their reproductive years) and group Ib (their controls) regarding CD40L expression on T cells. It revealed a statistically highly significant difference between both groups with a mean of 42.4±19.1 and 11.1±3.6 respectively (t = 5.11, p<0.01), while there was no significant difference between both groups as regards estrogen level with a mean of 242.1±74.2 in group Ia and 232.3±76.3 in group Ib (t = 0.33, p>0.05). The mean level of C3 in group Ia patients was significantly reduced more than in group Ib with a mean of 59.1±24.4 and 134.1±35.4 respectively (t = 6.29, p<0.01) (table 3).

Table (4) showed comparison between group IIA (postmenopausal lupus patients) and group IIB (their controls) regarding CD40L expression on T cells. There was a statistically non-significant difference between both groups with a mean of 12.2±6.8 and 10.6±3.1 respectively (t= 0.63, p>0.05). Also, on comparing between both groups as regards postmenopausal estrogen level, there was a non-significant difference with a mean of 23.6±9.8 and 22.5±9.9 respectively, (t = 0.4, p>0.05) (table 4). Also there was non significant difference between both groups as regards C3 level, with
a mean of 94.5±27.1 in group IIa, and 104.2±36.3 in group IIb, (t = 0.8, p>0.05) (table 4).

Table (4): Comparison between group IIa patients and group IIb (their controls) as regards CD40L expression on T cells, estrogen level and C3 level.

<table>
<thead>
<tr>
<th>Groups</th>
<th>CD40L% of expression on T lymphocytes</th>
<th>Postmenopausal estrogen level (pg/dl)</th>
<th>C3 level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IIa (n=15)</td>
<td>Mean±SD 12.2±6.8</td>
<td>23.6±9.8</td>
<td>94.5±27.1</td>
</tr>
<tr>
<td>IIb (n=10)</td>
<td>Mean±SD 10.6±3.1</td>
<td>22.5±9.9</td>
<td>104.2±36.3</td>
</tr>
<tr>
<td>t</td>
<td>0.63</td>
<td>0.41</td>
<td>0.80</td>
</tr>
<tr>
<td>p value</td>
<td>&gt;0.05 (NS)</td>
<td>&gt;0.05 (NS)</td>
<td>&gt;0.05 (NS)</td>
</tr>
</tbody>
</table>

There was a statistically highly significant difference between group Ia patients and all other groups (IIa, Ib, IIb) regarding CD40L expression on T lymphocytes (F = 26.24, p<0.01) (table 5 & Fig. 4).

Table (5): Comparison between group Ia and all other groups as regard CD40L expression on T cells.

<table>
<thead>
<tr>
<th>CD40L% of expression on T lymphocytes</th>
<th>Group Ia (n=15)</th>
<th>Group IIa (n=15)</th>
<th>Group Ib (n=10)</th>
<th>Group IIb (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean±SD</td>
<td>42.4±19.0</td>
<td>12.1±6.8</td>
<td>11.1±3.6</td>
<td>10.6±3.1</td>
</tr>
<tr>
<td>F</td>
<td>26.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01 (HS)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In group Ia (SLE women during their reproductive years), there was statistically highly significant positive correlations between CD40L
expression on T cells, estrogen level, SLEDAI score and 24 hours urinary proteins ($r = 0.87, 0.75, 0.58$ respectively, $p<0.01$) (table 6 and Figs. 5 & 6), while there were statistically highly significant negative correlations between CD40L expression on T cells, $C_3$ level and creatinine clearance in the same group ($r = -0.59, -0.76$ respectively, $p<0.01$) (table 6).

![Correlation between CD40L expression on T cells and estrogen level in both groups](image)

**Fig. (5): Correlation between CD40L expression on T cells and estrogen level in group Ia and IIa patients.**

**Table (6): Correlation of CD40L expression on T cells with estrogen level, SLEDAI score, 24 hours urinary proteins, creatinine clearance and $C_3$ level in groups Ia, Ila.**

<table>
<thead>
<tr>
<th>CD40L% of expression on T lymphocytes</th>
<th>Estrogen level (pg/dl)</th>
<th>SLEDAI score</th>
<th>24 hours urinary proteins (gm/day)</th>
<th>Creatinine clearance (ml/min)</th>
<th>$C_3$ level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Ia</td>
<td>Mid cycle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>0.87</td>
<td>0.75</td>
<td>0.58</td>
<td>-0.76</td>
<td>-0.59</td>
</tr>
<tr>
<td>$p$</td>
<td>$&lt;0.01$ (HS)</td>
<td>$&lt;0.01$ (HS)</td>
<td>$&lt;0.01$ (HS)</td>
<td>$&lt;0.01$ (HS)</td>
<td>$&lt;0.01$ (HS)</td>
</tr>
<tr>
<td>Group Ila</td>
<td>Postmenopausal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>0.63</td>
<td>0.46</td>
<td>0.28</td>
<td>-0.2</td>
<td>-0.3</td>
</tr>
<tr>
<td>$p$</td>
<td>$&lt;0.01$ (HS)</td>
<td>$&lt;0.05$ (S)</td>
<td>$&lt;0.05$ (S)</td>
<td>$&lt;0.05$ (S)</td>
<td>$&lt;0.05$ (S)</td>
</tr>
</tbody>
</table>

S: Significant
Table (6) also showed a significant positive correlation between CD40L expression on T cells, estrogen level, SLEDAI score and 24 hours urinary proteins in group IIa (postmenopausal lupus women) ($r = 0.63$, 0.4, 0.28 respectively ($p<0.05$) (Figs. 5 & 6), while a significant negative correlation was present between CD40L expression on T cells, $C_3$ level and creatinine clearance in the same group ($r= -0.3$, -0.2 respectively, $p<0.05$) (table 6).

Finally, in both group Ib and IIb (control groups) there was non significant correlation between CD40L expression on T cells, estrogen levels and $C_3$ levels ($p>0.05$) as shown in table (7).

Table (7): Correlation of CD40L expression on T cells with estrogen level and $C_3$ level in groups Ib and IIb (controls).

<table>
<thead>
<tr>
<th>CD40L% of expression on T lymphocytes</th>
<th>Estrogen level (pg/dl)</th>
<th>$C_3$ level (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Ib</td>
<td>Mid cycle</td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>0.05</td>
<td>-0.24</td>
</tr>
<tr>
<td>$p$</td>
<td>$&gt;0.05$ (NS)</td>
<td>$&gt;0.05$ (NS)</td>
</tr>
<tr>
<td>Group IIb</td>
<td>Postmenopausal</td>
<td></td>
</tr>
<tr>
<td>$r$</td>
<td>0.01</td>
<td>-0.13</td>
</tr>
<tr>
<td>$p$</td>
<td>$&gt;0.05$ (NS)</td>
<td>$&gt;0.05$ (NS)</td>
</tr>
</tbody>
</table>

Fig. (6): Correlation between CD40L expression on T cells and SLEDAI score in group Ia and IIA patients.
DISCUSSION

An important and characteristic feature of systemic lupus erythematosus (SLE) is that it predominantly affects females especially during their reproductive years (Glinda et al., 1998). Sanchez-Guerrero et al. (1995) reported a decreased risk of developing SLE after menopause, while its risk was increased with long-term use of postmenopausal estrogen therapy (Christoph et al., 1998).

Yellin et al. (2000) suggested that the development of SLE is mainly due to T cell hyperactivity induced by over-expression of signaling molecules such as CD40 ligand (CD40L) on helper T cells, interaction of CD40/CD40L induces B cell proliferation, differentiation and autoantibodies production, also it rescues B cells from apoptosis (Liu et al., 2001). Moreover, Higuchi et al. (2002) proposed that CD40L is ectopically expressed on B cells in SLE patients and may play a crucial role in disease activity. Rider et al. (2001) proposed that T cells from SLE female patients displayed sensitivity to estradiol. In their study, they cultured T cells from female lupus and controls without and with 2-fluroestradiol and found that there was an estrogen dependent increase in CD40L mRNA in SLE T cells.

In our study, we investigated in vivo, the effect of estrogen on CD40L expression on T cells and its relation to the activity of the disease in SLE female patients. We found that, there was a highly significant positive correlation between CD40L expression on T lymphocytes and estrogen levels in SLE patients both during their reproductive years (group Ia) and postmenopausal years (group IIa) with (p<0.01). However, on comparing group Ia (lupus patients with significantly higher estrogen level, mean 242.1±74.1) with group IIa (postmenopausal lupus patients with low estrogen level, mean 23.6±9.8) as regards CD40L expression on T cells, we found a highly significant difference between both groups with a mean of 42.4±19.0 and 12.1±6.8 respectively, (p<0.01). This denotes that the higher the estrogen level, the more the expression of CD40L on T cells in lupus patients.

These results were in agreement with Keltner et al. (2003) who proposed that the estrogen dependent increase in CD40L expression on T cells is correlated with its level and is mediated by the estrogen receptor. Estradiol increases the expression of calcineurin mRNA and phosphatase activity in T lymphocyte. The binding of antigen to the T cell receptor forms a complex that stimulates a signal transduction cascade which is calcium dependent. This leads to activation of protein kinase C leading to a change in the phosphorylation status of regulatory proteins resulting in synthesis and secretion of cytokines including CD40L, through calcineurin. This
calcineurin is a key regulator in this signal transduction pathway as it stimulates the activation of nuclear factor of activated T cells (NFAT), a transcription factor involved in the regulation of CD40L expression on T cells.

On the other hand, when we compared group Ia (lupus patients who had high estrogen levels) with its control group Ib (normal females who had nearly the same estrogen level) regarding CD40L expression on T cells, we found a highly significant difference between both groups with a mean of 42.4±19.1 and 11.1±3.6 respectively (p<0.01). This result was in accordance with Rider et al. (2001) who proposed that the increase in CD40L expression on T cells occurred when estrogen was cultured with SLE T cells but not with T cells from normal women. This was explained by McMurray et al. (2001) who suggested that one difference between normal women and SLE female patients is the hyper-responsiveness of lupus T cells to estradiol, which may be due to certain autoimmune related changes such as aberrant protein phosphorylation that altered the function of estrogen receptors on T cells and provide the opportunity for them to be up-regulated by estrogen.

Rider et al. (2002) reported that estrogen bound to estrogen receptor evoked a direct increase in calcineurin expression in T cells from female lupus patients. Thus, estrogen receptor antagonism by ICI182, 780 would inhibit the increase in calcineurin mRNA, phosphatase activity, reverse transcription and polymerase chain amplification indicate that estrogen receptor alpha and beta are expressed in human T cells.

Also in our study, there were positive correlations between CD40L expression on T cells and SLEDAI score and 24 hours urinary proteins. On the other hand, there were negative correlations between it C3 levels and creatinine clearance. These correlations were highly significant in lupus patients during their reproductive years (group Ia) (p<0.01) and significant in postmenopausal lupus patients (group IIa) (p<0.05).

These results were in agreement with Nagafuchi et al. (2003) who proposed that CD40L molecules are over-expressed in patients with active lupus and their levels are correlated with SLEDAI, as CD40/CD40L pathway is involved in the CD86 over-expression on SLE B cells. This might promote lymphocyte survival, intervening with elimination of autoreactive lymphocytes and render B cells more susceptible to T cells help with polyclonal antibodies production which cause tissue injury.

However, Bijl et al. (2001) reported that CD40L expression was low in inactive SLE patients and controls, and did not increase during active disease. But when isolated blood mononuclear cells were stimulated with
anti CD3, they detected proper increase of CD40L expression in conjunction with T cells activation. Their results might have been due to the small number of patients included in their study (nine patients).

In our study, the highly significant difference in CD40L expression on T cells between groups Ia and IIa resulted in a highly significant differences between both groups as regards SLEDAI score with a mean of 17.9±3.7 and 8.5±3.2 respectively (p<0.01). The same held to serum level of C3 that was reduced in group Ia (mean 59.6±24.4) more than in group IIa (mean 94.5±27.0) (p<0.01). This might occur due to its consumption in the formation of immune complexes that were deposited in various organs resulting in the appearance of the clinical manifestations of the disease activity. Also on comparing both groups regarding the prevalence of nephritis, the 24 hours urinary protein was significantly higher in group Ia patients than group IIa patients with a mean of 1.8±0.9 and 0.4±0.2 respectively (p<0.01). Creatinine clearance was also impaired in group Ia (mean 66.4±23.2) more than group IIa (mean 93.6±16.4) (p<0.01).

As regards the result of renal biopsy, 40% of group Ia patients had class IV and 26.7% had class V lupus nephritis, while only 13.3% had class II lupus nephritis, in contrast to group IIa patients 73.3% had class II lupus nephritis and only one patient (6.7%) had class IV lupus nephritis (p<0.01). Such results were consistent with Desai-Mehta et al. (2001) who demonstrated in their study that active lupus patients had a 22 fold increase in percentage of CD8+ T cells expressing CD40L as compared to lupus patients in remission who had low levels of CD40L + T cells. They also proposed that the more the over-expression of CD40L on T cells, the more the antibodies production and the severest will be the tissue injuries.

In our study, comparing between groups IIa and IIb as regards CD40L expression on T cells, there was a non-significant difference (p>0.05). This may be explained by the low estrogen levels in both groups (mean 23.6±9.8 and 22.5±9.9 respectively). Moreover, there was a highly significant difference between group Ia and all other groups as regards CD40L expression on T cells (F= 26.2, p<0.01). These results were consistent with Crow & Kirou (2002) who proposed that the production of pathogenic autoantibodies from B cells in SLE requires the over-expression of CD40L on T cells. Estrogen through its action on estrogen receptors plays an important role in this mechanism by a direct increase in calcineurin expression in T cells from female lupus patients. He also reported that calcineurin does not respond to estrogen stimulation in T cells from normal females, males or lupus males.
Several studies were performed to evaluate the usage of anti-CD40L antibody to control the disease activity in SLE patients. Huang et al. (2002) and Boumpas et al. (2003) proposed that a short course of BG9588 (a humanized anti-human CD40L antibody) blocked antigen specific IgG responses in patients with proliferative lupus nephritis. This resulted in reduction of anti-ds DNA antibodies, increasing C3 concentrations and decreasing hematuria. They suggested that the drug has an immunomodulatory action, but additional studies will be needed to evaluate its long-term effects.

**Conclusion:**

SLE is more active and aggressive in females especially during their reproductive years due to high levels of estrogen that plays an important role in the over-expression of CD40L on T cells in lupus patients but not in normal women. This action is dose dependent as we found that CD40L expression on T cells was significantly higher in lupus women during their reproductive years than that in lupus women during their postmenopausal years. Its level was well positively correlated with markers of disease activity SLEDAI score.

**Recommendations:**

These data provide increasing evidence that manipulating costimulatory pathways in lupus patients by using anti-CD40L antibody may be a potentially beneficial therapeutic strategy, also the usage of estrogen receptor antagonism would block the estrogen receptors present on lupus women T cells, thus inhibiting the calcineurin mRNA and phosphatase activity resulting in decrease CD40L expression on T cells, B cell inactivation with less autoantibodies production. This may be a novel therapy available for women to control lupus activity in the future.

**REFERENCES**


