Costimulatory Molecules CD80 and CD134 Are Associated with Lupus Nephritis, Skin Lesions and Disease Activity in Systemic Lupus Erythematosus

Neveen S. Seif Eldin, Maha S. Hamdy, Sheren B. El Sayed, Sherin Saad

Departments of 1 Dermatology, Venereology and Andrology, 2 Medical Microbiology and Immunology and 3 Pediatric, Faculty of Medicine, Ain Shams University, Cairo- Egypt.

Abstract: This study aimed at studying the co stimulatory molecules (CD80, CD134) and soluble CD134 ligand (CD134L) in SLE, and their correlation with disease activity and renal involvement, also correlation between the expression of these costimulatory molecules with skin lesions and SLE activity. Forty patients with SLE (Twenty of them were having biopsy-proven lupus nephritis) and 40 healthy controls were included in this the study. Clinical disease activity was assessed according to systemic lupus erythematosus disease index (SLEDAI). CD4+ T cell populations in the peripheral blood were analyzed for the expression of co-stimulatory markers CD134 and CD80. CD134L level was also measured in serum of SLE patients and controls. SLE patients showed an increased soluble CD134L concentration in the serum and increased frequency of peripheral CD4+ T cells expressing high levels of CD80 and CD134 compared to healthy controls. These costimulatory molecules were significantly higher in SLE patients with lupus nephritis compared to patients without nephritis and they were significantly correlated with SLEDAI. Our study revealed also that the presence of skin lesions specific for LE was associated with a milder disease, also patients with only LE-non-specific skin lesions showed an increased frequency of peripheral CD4+ T cells expressing high levels of CD80 and CD134 and soluble CD134L concentration in the serum compared to those with only LE-specific skin lesions. In conclusion, increased expression of CD80 and CD134 on CD4+ T cells and increased serum concentration of soluble CD134L are associated with increased incidence of renal disease, disease activity and more serious skin lesions in SLE.

Key words: CD80, CD134, CD134L, SLE, Skin lesions, lupus nephritis.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterized by loss of immune tolerance, increased antigenic load, excess T cell help, defective B cell suppression, and shifting of T-helper 1 (Th1) to Th2 immune response resulting in B cell hyperactivity and production of a variety of pathogenic antibodies (Tseng et al, 2007). It has been shown that the activation of autoantibody-producing B cells is dependent on T-cell help through cytokines and costimulatory molecules.

The initiation of T-cell activation requires a primary signal delivered by the antigenic peptide presented by major histocompatibility complex (MHC) molecules and a non-specific signal generated by the interaction of costimulatory molecules (Greenfield et al, 1998 and Dolff et al, 2007). In addition to the well-characterized T cell co-stimulatory molecules CD28 and CD152 which bind to CD80 and CD86 on antigen presenting cells (APCs), several other molecules as CD134 (OX40), CD137 (4-1BB) and CD154 have been shown to induce co-stimulatory signals upon binding to their cognate receptors (Finney et al ;2004)

CD134 (OX40) and its binding partner CD134L (OX40L) are members of the tumor necrosis factor receptor (TNFR) superfamilies that appear to be crucial for many types of immune reactions mediated by T cells. CD134 is induced on T cell surface several hours or days after antigen (Ag) recognition, and its expression coincides with the appearance of CD134L on several (APCs) such as, B cells, macrophages and dendritic cells when activated. Interaction between CD134 and its ligand (CD134L) is involved in costimulation of T and B lymphocyte activation, and in T cell adhesion to endothelium (Croft et al; 2007, Valzasina et al;2005)
Signals from CD134, increase expression of anti-apoptotic Bel-2 family members and negatively affect the expression or activity of the pro-apoptotic molecules, thus conferring resistance to spontaneous apoptosis and enhancing T cell survival. This ultimately results in greater number of T cells surviving the primary immune response and developing into memory T cells (Rogers et al; 2001 and Evans et al; 2001). CD134 can be expressed on a T cell at a low level in the absence of inflammatory signals. Thus, co-stimulatory signals provided through binding of CD134L to CD134 may reverse tolerance and may lead to autoimmunity (Bansal-Pakala et al; 2001). Therefore, in certain conditions that promote prolonged expression of both CD134 and CD134L, tolerant self-reactive T cells may gain normal function when they encounter their specific antigen (Ag) and lead to development of late-onset autoimmune diseases (Bansal-Pakala et al; 2001 and Yuan et al; 2003).

The relevance of co-stimulatory molecules for immune-mediated disease such as SLE was investigated in several studies. Expression of CD134L has been shown to be upregulated in proliferative lupus nephritis, suggesting a role for the CD134/CD134L pathway in its pathogenesis (Aten et al; 2000 and Patschan et al; 2006). Furthermore, high levels of co-stimulatory molecules, especially CD80 and CD86 on B cells, were also found in human SLE and the expression of these markers correlated with disease activity as assessed by the SLEDAI score (Patschan et al; 2006 and Yi-qun et al; 1996).

The costimulatory signal results from the interaction of CD28 on T cells with the B7 family B7-1 (CD80) and B7-2 (CD86) on antigen-presenting cells. Resting APCs are negative for CD80 and CD86 expression but monocytes and dendritic cells constitutively express CD86. Expression of CD80 is mainly activation induced (Sfikakis and Via; 1997 and Wong et al, 2005). Curiously, CD80 and CD86, that are usually found on APCs, were also found on T cells of patients with SLE, but the meaning of this finding for disease development and activity remained unclear (Patschan et al; 2006 and Abe et al; 1999).

It is evident that dysregulation of the T- and B-cell costimulatory pathways are related to the development of SLE.

Aim of the Work:

The main goal of our study was to elucidate the clinical importance of CD80, CD134 and CD134L in SLE pathogenesis, and to look for a correlation of these co-stimulatory molecules with disease activity and renal involvement in patients with SLE. Considering a lack of reliable data on the links between skin lesions and SLE disease activity, this study aimed also at answering the question whether skin lesions may help predict the activity of SLE and their correlation with costimulatory molecules expression.

MATERIAL AND METHODS

Fourty patients (37 females and 3 males, mean age 15.45 ± 2.50 years, range 10-18 years) fulfilling at least four of the American college of rheumatology (ACR) revised Criteria for diagnoses of SLE (Hochberg, 1997) were included in this study. The mean disease duration was 3.95 ± 2.42 years, range 1-10 years. These patients were collected from the Pediatric Allergy and Immunology Clinic, Children's Hospital and the outpatient clinic of the dermatology, venereology and Andrology department, Ain Shams University, Cairo, Egypt. For all patients the following was done: Full history especially age, duration of the disease, urinary symptoms, SLE manifestations as joint and muscle pains, skin rash, photosensitivity, hair loss, Raynaud's phenomenon, CNS symptoms including seizures, symptoms of hypertension as vomiting, headache, blurred vision and history of medication received by the patient.

Thorough clinical examination was performed to each patient including: blood pressure measurement, joint affection, chest and heart examination, abdominal examination for hepatosplenomegaly and CNS examination especially consciousness level, and motor and sensory systems. Thorough dermatological examination was done also to determine malar rash, any skin rash, oral ulcers, hair and nail changes, pigmentary changes and others. According to the cutaneous findings, patients were categorized into 3 Groups according to Zecevic et al, 2001:

**Group (A):** Those having only LE-specific lesions as acute cutaneous lupus erythematosus (ACLE), subacute cutaneous lupus erythematosus (SCLE), discoid lupus erythematosus (DLE), and mucus membrane ulcers. They were 4 patients (20%) of cases.

**Group (B):** Those having only LE-nonspecific lesions as cutaneous vasculitis, photosensitivity, urticaria, Raynaud's phenomenon, livedo reticularis, thrombophlebitis, alopecia, hyper/hypopigmentation, and rheumatoid nodules. They were 13 patients (65%) of cases

**Group (C):** Those having both types of lesions and they were only 3 patients (15%) of cases.

4150
SLE patients were categorized according to renal involvement as reported by Weening et al; 2004 into 2 groups:

Group (A): patients having clinical manifestations and biopsy proven lupus nephritis. They were 20 patients; 17 females and 3 males, mean age 15.30±2.21 years, range 11-18 years, and interquartile range = 14.25-16.75 years. They were having clinical evidence of renal involvement in the form of persistent proteinuria (more than 0.5 gm/day or more than 3+ by dipstick test) and/or cellular casts in urine (red blood cells, haemoglobin, granular, tubular or mixed) and were divided into classes according to the WHO. WHO class II (mesangial proliferative) (n=6), class III (focal) (n=4), Class IV (diffuse) (n=6), and class VI (membranous) (n=4).

Group (B): Patients with no clinical or laboratory evidence of renal involvement. They were 20 female patients, mean age 15.60±2.88 years, range 10-18 years, and interquartile range 13.5-18 years. These patients had normal urine analysis, serum creatinine and creatinine clearance.

Clinical disease activity at the time of the study was assessed according to the systemic lupus erythematosus disease activity index (SLEDAI) (Bombardier et al., 1992).

All patients were receiving oral prednisone (0.5-2 mg/kg/day) either alone or in combination with other immunosuppressive drugs, such as intravenous pulsed cyclophosphamide (dose 500 mg/m²/mo for 7 doses), or oral azathioprine (dose 2mg/kg/day). The cumulative dose of steroid therapy during the whole duration of the disease was calculated based on data obtained from the patient's records. The use of intravenous pulsed dose of steroids was also given if needed. The average dose of steroids used per square meter body surface area was calculated according to Mosteller's formula (Lee et al; 2008).Eight patients were receiving antihypertensive drugs and one patient was receiving anticonvulsant therapy, in the form of phenytoin in a dose of 5mg/kg/day) for controlling seizures.

Fourty age and sex matched apparently healthy controls; 30 females and 10 males, mean age 15.77±2.07 years, range 10-18 years, and interquartile range 15-17 years were also included in this study as controls. These patients had no evidence of renal, skin or other organ affection

Study protocol was approved by the institutional review board and all studied subjects gave informed consent before participation in this study.

Samples:

1-Blood:Four milliliters of venous blood were collected. Two milliliters were transferred into clean dry tube and left to clot. Prompt separation of serum was carried and used for direct assay of urea, creatinine, antinuclear antibody (ANA), C3, anti-dsDNA, and soluble CD134 ligand. The other two milliliters were collected into tubes containing K- EDTA and analyzed as will be discussed later.

2-Twenty four hours urine was collected to test for the presence of protein and for doing routine urine analysis.

Laboratory Tests:

For every patient and control the following laboratory tests were done:

A) Routine laboratory investigations including: Complete blood picture by Coulter counter (Coulter Microdiff 18, Fullerton, CA, USA), erythrocyte sedimentation rate estimation by Westergren method, serum Anti nuclear antibody (ANA) assessment by indirect immunofluorescence supplied by the IMMCO Diagnostics (USA), serum anti double stranded DNA (dsDNA) antibodies were measured using ELISA technique (ORGENTEC) (Carl-Zeiss-StraBe 49, 55129 Mainz, Postfach 100352 55134 Mainz), and complement level assessment using the turbimeter supplied by Behring Diagnostics (Germany) was assessed. Renal function tests were also done including serum creatinine, blood urea, creatinine clearance and routine microscopic urine analysis for presence of pyuria, hematuria and casts, albuminurea by Dipsticks. Twenty four hours urine was used to test for the presence of proteins in urine.

B) Measurement of soluble CD134 ligand in serum by ELISA using Quantikine® human OX40 Ligand kit(R&D systems, Minneapolis, MN,USA), as recommended by the manufacturer. CD134L concentrations were expressed in pg/ml according to a standard curve.

C) Flowcytometry: Tubes containing K- EDTA blood (1.2mg/ml) were analyzed within 6 hours to analyze peripheral blood lymphocytes of the patients and control groups and to measure CD80 and CD134 expression on peripheral blood CD4+ve T lymphocytes by flow cytometry (Coulter EPICS XL) as follows: One hundred μl of each sample was stained (triple-color surface staining) using 10μl of each of FITC (Green)(fluorescin isothiocyanate) conjugated mouse monoclonal antihuman CD80 antibodies (Caltag Laboratories, CA,

4151
Burlingame), PC5(purple) (phycoerythrin-cyanin) conjugated mouse monoclonal antihuman CD134 antibodies (Caltag Laboratories, CA, Burlingame), and Phycoerythrin (PE) (orange) conjugated mouse monoclonal antihuman CD4 antibodies (R&D systems, Minneapolis, MN, USA). The tubes were then incubated in dark at room temperature for 15 minutes, then erythrocytes were lysed using ammonium chloride lysing solution (Al-Gomhoreya CA, Egypt). After two washes with phosphate buffered saline (PBS), the cells were resuspended in PBS for flow cytometric analysis. Negative isotype matched controls (IgG mouse monoclonal antibodies) were included with each sample to determine the non specific binding of the monoclonal antibodies. The results were expressed as the percentage of the positive cells relative to the isotypic control(%).

Statistical Analysis:
Analysis of data was done by IBM computer using SPSS, version 12.

RESULTS AND DISCUSSION

Lymphocyte Subsets:

Table 1: Lymphocyte subset distribution in the peripheral blood of SLE patients and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>Patients (n: 40) Mean ± SD</th>
<th>Controls (n: 40) Mean ± SD</th>
<th>t</th>
<th>p</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>720.95 ± 164.41</td>
<td>1226.3 ± 230.17</td>
<td>11.299</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>CD4</td>
<td>434.25 ± 134.7</td>
<td>859.65 ± 155.63</td>
<td>13.071</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>CD8</td>
<td>219.25 ± 37.73</td>
<td>274.55 ± 62.38</td>
<td>4.798</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
</tbody>
</table>

n: number of patients; SD: standard deviation; sig: significance; HS: highly significant

Lymphocyte subset distribution in the peripheral blood of all SLE patients and healthy controls revealed that SLE patients had a statistically highly significant (HS) lower number of circulating lymphocytes (CD3, CD4, and CD8+ cells) compared with healthy controls (P<0.001).

Table 2: Lymphocyte subset distribution in the peripheral blood of SLE patients with and without nephritis.

<table>
<thead>
<tr>
<th></th>
<th>SLE with nephritis (n: 20) Mean ± SD</th>
<th>SLE without nephritis (n: 20) Mean ± SD</th>
<th>t</th>
<th>p</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>598.9 ± 82.15</td>
<td>843 ± 131.82</td>
<td>7.028</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>CD4</td>
<td>313.9 ± 21.61</td>
<td>554.6 ± 79.28</td>
<td>13.1</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>CD8</td>
<td>214.1 ± 32.52</td>
<td>224.4 ± 42.52</td>
<td>0.86</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

n: number of patients; SD: standard deviation; sig: significance; HS: highly significant

Comparison between SLE patients with and without nephritis revealed that patients with SLE nephritis had statistically highly significant lower number of circulating CD3 and CD4 cells (P<0.001) compared with those without nephritis, while the difference was non significant (NS) regarding the number of CD8 cells (P>0.05).

Fig. 1: Expression of Co-stimulatory molecules (CD80 and CD134) on CD4+ T cells in the peripheral blood of SLE patients.

SLE patients showed an increased frequency of both peripheral CD4+ T cells expressing high levels of CD80 and CD134 compared to healthy controls (CD80: 8.42 ± 4.78 versus 1.09 ± 0.45 %(P <0.001); CD134: 18.68 ± 6.99 versus 6.65 ± 1.38 %; (P < 0.001).
Table 3: Expression of CD80 and CD134 on CD4+ T cells of SLE patients with and without renal disease:

<table>
<thead>
<tr>
<th></th>
<th>SLE with nephritis (n: 20) Mean ± SD</th>
<th>SLE without nephritis (n: 20) Mean ± SD</th>
<th>t</th>
<th>p</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of CD80</td>
<td>12.98 ± 1.62</td>
<td>3.85 ± 0.62</td>
<td>23.58</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td>Expression of CD134</td>
<td>24.9 ± 4.01</td>
<td>12.46 ± 1.69</td>
<td>12.78</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
</tbody>
</table>

n: number of patients; SD: standard deviation; sig: significance; HS: highly significant

All SLE patients with biopsy-proven lupus nephritis (n=20) had higher levels of CD80 on CD4+ T cells and a marked increase in the presence of CD134 on CD4+ T cells in the peripheral blood compared to patients without renal involvement and to healthy controls. This difference was statistically highly significant (P <0.001). Meanwhile there were no significant differences between patients with WHO class IV and with other WHO classes of nephritis concerning the expression of CD80 and CD134 on CD4+ T cells (CD80: 12.1± 1.47 versus 11.08± 1.51 %; P = 0.4; CD134: 23.9 ±3.91 versus 24.1 ±3.89 %; P = 0.4) (Table 3, Fig. 2).

Disease activity and expression of co-stimulatory molecules:

Disease activity as assessed by SLEDAI was correlated with the percentage of co-stimulatory molecules on CD4+ T cells; CD80 (r=0.977, P=0.001) as well as CD134 expression (r= 0.985, P <0.001) correlated highly significantly with the SLEDAI (Fig. 3).

Measurement of Soluble CD134 Ligand (CD134L) in the Serum of SLE Patients and Controls:

Soluble CD134L concentration in the serum of SLE patients was significantly higher compared to healthy controls (31.66 ± 3.93 versus 29.21 ± 2.74 pg/ml P<0.05), furthermore SLE patients with renal disease had a statistically significantly higher concentration compared to patients without renal disease (34.06 ± 3.85 versus 29.26 ± 2.19 pg/ml, P<0.05). In addition, soluble CD134L was significantly correlated with increased disease activity index (SLEDAI) score (r=0.665, p=0.001) (Fig.4).

Table 4: Correlation between the type of skin lesions and disease activity measured by SLEDAI

<table>
<thead>
<tr>
<th>SLE categories</th>
<th>n</th>
<th>%</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only LE-specific lesions</td>
<td>19</td>
<td>47.5</td>
<td>6.40±2.25</td>
</tr>
<tr>
<td>Only LE-nonspecific lesions</td>
<td>15</td>
<td>37.5</td>
<td>17.35±3.60</td>
</tr>
<tr>
<td>Both types</td>
<td>6</td>
<td>15</td>
<td>19.40±7.05</td>
</tr>
</tbody>
</table>

n: number of patients; SD: standard deviation
Fig. 3: Correlation between the percentage of CD134 (a) and CD80 (b) expressing CD4+ T cells and disease activity as measured by systemic lupus erythematosus disease activity index score.

Fig. 4: Correlation between the Soluble CD134L concentration in the serum and disease activity as measured by systemic lupus erythematosus disease activity index score.
Out of 40 SLE patients 19 (47.5%) had only LE-specific lesions, 15 (37.5%) had only LE-nonspecific lesions, and 6 (15%) had both types of lesions. The mean value of SLEDAI score in those with only LE-nonspecific lesions was significantly higher compared to those with only LE-specific lesions (P<0.001). On comparing those with only LE-specific lesions and those having both types of lesions, SLEDAI was significantly higher in those having both types of lesions. No statistical difference was found between those with LE-nonspecific and those having both types of lesions (P>0.05).

Fig. 5: Expression of CD80 and CD134 on CD4+ T cells and soluble CD134L concentration in the serum of SLE patients according to different lesions categories.

Table 5: Expression of CD80+ and CD134+ T cells and soluble CD134L concentration in the serum of SLE patients according to different lesions categories.

| Patients with only Patients with only Both types (n=6) t |
|-----------------|-----------------|-----------------|---|
| LE-specific skin | LE-nonspecific skin | (n: 19) Mean ± SD | Mean ± SD |
| Expression of CD80 | 3.6 ± 0.39 | 11.63 ± 3.43 | 12.31 ± 3.75 | 9.289 |
| Expression of CD134 | 12.03 ± 1.61 | 23.12 ± 5.47 | 24.13 ± 5.75 | 7.861 |
| Level of CD134L | 29.61 ± 2.32 | 41.03 ± 5.52 | 42.33 ± 5.05 | 2.943 |

SLE patients with only LE-nonspecific skin lesions showed an increased frequency of peripheral CD4+ T cells expressing high levels of CD80, CD134 and soluble CD134L concentration in the serum compared to those with only LE-specific skin lesions (11.63 ± 3.43 and 23.12 ± 5.47 and 41.03 ± 5.52 versus 3.6 ± 0.39 and 12.03 ± 1.61 and 29.61 ± 2.32 respectively), and the difference was highly significant (P<0.001). On comparing the expression of CD80, CD134 and soluble CD134L concentration in the serum between those having both types of lesions and patients with only LE-specific lesions (12.31±3.75 and 24.1±5.75 and 42.33±5.05 versus 3.6±0.39 and 12.03±1.61 and 29.61±2.32 respectively), the difference was highly significant (P<0.001). On comparing those having both types of lesions with those with LE-nonspecific lesions, the difference was non-significant (12.31±3.75 and 24.1±5.75 and 42.33±5.05 versus 11.63±3.43 and 23.12±5.47 and 41.03±5.52 respectively) (P>0.05).

Discussion:

Numerous abnormalities in T cell function have been described in SLE patients and one common feature appears to be a reduced threshold for T cells activation. Provision of co-stimulatory signals may be responsible for the heightened sensitivity or prolonged response to T cell receptor (TCR) activation seen in T lymphocytes from patients with SLE. Studies of Patschan and his colleagues in 2006 in lupus prone mice indicate that co-stimulatory molecules play an essential part in the interaction between B and T cells (Patschan et al; 2006). In lupus patients, it can be speculated that aberrant expression of co-stimulatory molecules provides a condition in which autoantibody production is facilitated, possibly because autoreactive memory B cells and T cells can expand in the absence of adequate apoptotic signaling (Bijl et al, 2001).

This study showed that SLE patients had a statistically highly significant lower number of circulating lymphocytes (CD3, CD4 and CD8+ cells) compared with healthy controls. Also SLE patients with nephritis had a statistically significant lower number of circulating CD3 and CD4 cells compared with those without nephritis, while the difference was non-significant regarding the number of CD8 cells. Our results were similar.
to those of Wang et al, who studied the apoptosis of lymphocyte subpopulations in SLE (Wang et al, 2005). They revealed that the apoptosis of CD3, CD4 and CD8+ T cells was increased as compared with healthy controls and this may be related to the high levels of IL-10 in SLE serum. IL-10 may induce the abnormally activated T cells to trigger apoptosis via the Fas-Fas-L pathway, and this is increased in active SLE patients. Furthermore, Lahita reported that anti-T lymphocyte antibodies have been found in SLE sera and may be responsible for the elimination of T-cells; this decrease was more relevant in active SLE (Wang et al, 2005).

A highly significant increase in the expression of CD80 and CD134 was found on CD4+ T lymphocytes in the peripheral blood of SLE patients compared with controls. Moreover, there was a highly significant correlation between the expression of these two co-stimulatory molecules and both lupus nephritis and disease activity as assessed by SLEDAI score. Our results were in agreement with those of Wang et al; 2005 and Patschan et al; 2006 who suggested that the aberrant production of soluble T-cell costimulatory molecules is important in the immunopathogenesis of SLE, which occurs by the dysregulation of T-lymphocyte costimulation. Abe et al; 1999 detected increased levels of CD80 and CD86 on T cells, but did not show an association with disease activity or renal manifestation. It is possible that CD80 and CD86-positive T cells could become independent from co-stimulation of antigen-presenting cells leading to a self-enhancing loop of T cell activation (Patschan et al; 2006).

It has been reported that CD28-CD80 pathways is necessary for activation of autoreactive T cells and for polyclonal B cells activation, and the percentage of CD80+ Cells in the activated B cells subset in SLE was significantly higher than in controls and correlated with the anti-ds DNA level and SLEDAI scores (Tokunaga, 2005)

This study revealed also a higher significant concentration of soluble CD134L in the serum of SLE patients compared to healthy control subjects, and in patients of lupus nephritis compared to those without lupus nephritis. To the best of our knowledge, one study done by patschan et al; 2006 investigated the soluble CD134L concentration in the serum of SLE patients, but there was no significant correlation with the disease activity or renal affection. They stated that secretion of soluble CD134L by glomerular endothelial cells or antigen-presenting cells (APCs) with subsequent activation of T cells via CD134 is a possibility that need further investigation.

A functional role for the interaction of CD134 with its ligand (CD134L) has been demonstrated in maturation of dendritic cells, in costimulation of T lymphocyte proliferation and cytokine production, in B lymphocyte proliferation and immunoglobulin (Ig) production, and in T lymphocyte adhesion to endothelial cells (Aten et al;2000). The stimulatory effect of CD134 ligation on T cell function in SLE patients and its role in lupus was investigated in many studies. CD134 CD4+ T cells could be involved in the inflammatory process of lupus nephritis by their engagement with CD134L, which is present in glomerular endothelium in almost all cases of proliferative lupus nephritis (Aten et al; 2000)

Patschan et al, 2006 also demonstrated an up-regulation of CD134 on T cells in SLE patients with more clustering in (as shown by confocal microscopy) those with active renal disease. There are two hypothetical ways in which CD134 CD4+ T cells could mediate lupus nephritis. Firstly, these cells could provide help to B cells producing anti-ds DNA antibodies and also to other antibodies that may contribute to the kidney lesions (Puttermann, 2004 and Zhao et al, 2005) . Secondary, it is also possible that these T cells subpopulations infiltrate glomerular endothelial cells after ligation with CD134L and cause direct damage (Patschan et al, 2006).

Cutaneous changes occupy a prominent position within the ACR diagnostic criteria for SLE, and few studies have investigated their relationship to SLE activity i.e. their prognostic significance, for this reason another aim of our study was answering the question whether particular skin lesions may help to predict the activity of SLE. According to other studies, about 85% of SLE patients have had LE-specific lesions on their skin and about 25% of patients have cutaneous manifestations as a presenting sign (Jablonska et al, 1993 and Yell et al, 1996)

Our study revealed that the presence of skin lesions specific for LE was associated with a milder disease as assessed by SLEDAI index (table 5). Our findings confirm those of Zecevic et al, 2001 which can be explained by the fact that differences in the basic pathophysiologic mechanisms implicated in the development of LE-specific (T-cell mediated immune response) and LE-nonspecific (mostly immune complex- mediated damage) cutaneous lesions might explain the results.

According to our results and those of Zecevic et al the type of lupus skin lesion may serve as a useful and reliable predictor of disease activity and its prognosis, Zecevic and his co-workers also realized that the number of different skin lesion types also correlated with disease activity. It was significantly increased in a group with three different types of lesion, either specific or nonspecific. Patients with only one type of lesion
had mild disease. An intermediate disease activity was found in the group with two different lesion types. (Zecevic et al, 2001)

Many authors have tried to demonstrate a positive correlation between some laboratory tests and disease activity measured by SLEDAI, however, no clear-cut correlation has been established in these studies (Provost 1994) and (Vanderlugt and Miller, 1996)

In this study we investigated the expression of CD80+, CD134+ T cells, and soluble CD134L concentration in the serum of SLE patients according to different lesions categories. Our results revealed that SLE patients with only LE-nonspecific skin lesions showed an increased frequency of peripheral CD4+ T cells expressing high levels of CD80 and CD134 and soluble CD134L concentration in the serum compared to those with only LE-specific skin lesions and the difference was highly significant. On comparing those having both types of lesions with those with LE-non specific lesions, the difference was non-significant.

These results reveal that increased co-stimulatory molecules expression is associated with more disease activity and more serious skin lesions in SLE patients. To our knowledge no studies have made a correlation between skin lesions and costimulatory molecules expression in SLE patients. The results of this study and that of Zecevic et al, emphasize a need for a thorough and expert dermatological examination in SLE patients (Zecevic et al, 2001). In addition, recognition and differentiation of cutaneous changes in SLE patients may contribute to the management of the patients; nonspecific skin lesions mostly are a sign of vasculitis, which may also be found in internal organs. These patients have a significantly more active disease compared to those with only LE-specific skin lesions. Patients with LE-nonspecific skin lesions might be candidates for more intensive treatment and disease monitoring. We emphasize more studies to be done to confirm our results.

Blocking co-stimulatory molecules such as CD28-CD80/CD86 interaction is an established therapeutic approach that may result in a decrease in Ig secretion, autoantibody production and disease activity in SLE patients through decreasing B cell activity and T cells function (Patschan et al;2006). Meanwhile blocking CD134/CD134L pathway has to be discussed as a possible therapeutic strategy for SLE in the future, as anti-CD134L Abs or CD134-Ig fusion proteins that bind specifically to CD134L have been used successfully to abrogate some Th2 induced autoimmune diseases as inflammatory bowel diseases and collagen-induced arthritis (Zhou et al;2009). Furthermore, measurements of CD80, CD134 and soluble CD134L could be useful for predicting disease activity or recurrence of disease. Other markers, such as ds-DNA titers or complement measurements, are not very reliable tools for this purpose. This issue, however, needs to be investigated in a longitudinal study with a larger patient population.

In conclusion, our data indicate that both increased expression of co-stimulatory molecules (CD80 and CD134) on CD4+ T cells and the increased serum concentration of soluble CD134L are associated with increased incidence of renal disease and disease activity and more serious skin lesions in patients with SLE. Targeting these co-stimulatory molecules could be a new therapeutic approach in patients with lupus nephritis.

REFERENCES


