The system Fas/Fas-L in SLE

NATALIA BELUSHKINA¹, ABDELMAROUF MOHIELDEIN², ULIANA PETROVA³.
¹²Department of Biochemistry, ³Department of Immunology
¹Sechenov Moscow Medical Academy, Research Institute of Molecular Medicine, ²Omdurman Islamic University, ³Speransky Pediatrics’ Hospital
¹Moscow 119881, M.Trubetskaya, 8/2. ²Khartoum 11111; P.O.Box 11414.
¹³RUSSIA, ²SUDAN.

Abstract: - We found significant increase of expression Fas-L on surface lymphocytes from patients with SLE compared to normal controls (2.4* ± 1.3 vs 1.7 ± 1.1 respectively). This was found to be depending on disease activity of SLE. However, there was a tendency to increase Fas expression on a surface of lymphocytes depending on the degree activity of SLE. On all lymphocytes subsets CD3, CD4, CD8, and CD 19, percentages of Fas expression in SLE were significantly higher when compared to normal controls.

There was a slight increase in soluble Fas in patients with SLE when compared to normal healthy donors (1.6±0.2ng/ml versus 1.3±0.1ng/ml respectively). Moreover, we found an inverse correlation between sFas and disease activity in such way that the first stage of disease characterized by the highest level of sFas compared to second or third stages of the disease (1.8±0.1; 1.4±0.1; 1.3±0.02 respectively). Furthermore, our results showed a positive correlation (r =0.5) between plasma sFas and percentage of lymphocytes Fas expression in patients with SLE.

Key-Words: - Fas, Fas ligand, soluble Fas, SLE, lymphocytes subsets.

1. Introduction

The pathogenesis of systemic lupus erythematosus (SLE) is unclear; however, recently much attention has been devoted to the role of apoptosis in the pathogenesis of SLE. A putative role of apoptosis in the pathogenesis of human SLE was especially supported by a report by Emelen et al [3] describing accelerated in vitro apoptosis of SLE lymphocytes in contrast to lymphocytes from normal donors.

In an organism there are protective mechanisms that preventing synthesis of autoantibodies. These are processes of negative and positive selection of lymphocytes in central and peripheral organs of the immune system. The deletion of these autoreactive lymphocytes through negative selection occurs via apoptosis.

One of the major routes which trigger apoptosis is the Fas pathway. The Cross-linking of Fas by Fas ligand (Fas-L) triggers apoptotic cell death with the characteristic cytoplasmic and nuclear condensation and DNA fragmentation [4].

Fas-L is a type II membrane protein i.e. with an amino-terminal cytoplasmic and a carboxyl-terminal extracellular region [1]. It is a member of the TNF family which includes TNF-α, α- and β-chains of lymphotoxin (LT), CD40 ligand, and CD30 ligand [6]. Fas-L is predominantly expressed on the surface of activated T lymphocytes, natural killer (NK) cells [1].

Fas, is type I transmembrane glycoprotein, a member of the tumor necrosis factor (TNF) family that include tumor necrosis factor (TNF) receptor, nerve growth factor receptor, CD40, CD27, CD30, and others [1, 11]. Fas can occur as both a cell-surface and a soluble protein, with the soluble form of Fas (sFas) protein being generated by alternative mRNA splicing [9].

The sFas represents truncated forms of the membrane-bound receptors that can bind ligand in a way similar to that of their membrane-bound counterparts. As a result, these receptors play an important role in regulation of normal receptor activity and autoimmune diseases.
Defect in the mechanism of negative selection of lymphocytes, in particular - occurred in patients with autoimmune diseases who have soluble form Fas (sFas) in blood [2], apparently, may play a role in change of regulation apoptosis through system Fas/Fas-L [5].

2. Material and methods
2.1 Isolation of mononuclear leukocytes from human peripheral blood
Sterile, disposable 15 ml plastic tubes were used for centrifugation. To each test–tube was added Ficoll gradient solution followed by 6 ml heparinised human blood obtained by venepuncture. The tubes were centrifuged at 400 x g for 30 minutes at room temperature. Mononuclear leukocytes concentrated as a ring on the border between plasma band and isolation solution.

2.2 Analysis of lymphocyte subsets
The analysis of lymphocyte subpopulations carried out by flow cytometry - the analyzer and the sorter of cells is EPICS XL-2 (“Beckman coulter”, USA). Suspended lymphocytes incubated with monoclonal antibodies from Sorbent company (Russia). We used the fluorescent label phycoerythrin conjugated monoclonal antibody (CD-3, CD-4, CD-8, and CD-19).

2 µl of the required monoclonal antibodies added to 1x10^6 mononuclear cells suspended in 50µl of culture medium containing 0.1 % sodium azide. Incubated for 30 minutes in darkness at 4 °C. Then twice wash with 200 µl icy solution (PBS with 0.1 % sodium azide). The cells fixed with 0.1 ml of 1 % paraformaldehyde and then analyzed.

2.3 Determination Fas/Fas-L in leukocytes
Primary antibodies to human Fas (clone DX2, mouse IgG1, PharMigen, USA), and to human Fas-L (clone NOK-1, mouse IgG1, PharMigen, USA) were used. Secondary antibodies of a goat to mouse IgG1, conjugated FITC, used in dilution 1:100 with (PBS , 0.5 % gelatin, and 0.1 % sodium azide). The cells fixed with 1 % paraformaldehyde, analyzed by flow cytometry Epics XL (Beckman Coulter).

2.4 Determination soluble Fas (sFas) in plasma by ELISA method
The amounts of sFas in plasma were determined by a sandwich enzyme linked immunosorbent assay (ELISA) using monoclonal antibodies CLB-CD95/2 and CLB-CD95/6. Microtitre plates were coated with 100 µl/well antibodies CLB-CD95/2. The process was blocked by addition 100 µl PBS with addition of 2 % skimmed milk solution, incubated for 30 minutes at room temperature. Samples dissolved 10 times in the ELISA-buffer (HPE - high performance ELISA). Standard samples sFas dissolved in HPE (concentration from 1,000 ng/ml up to 2ng/ml). 100 µl of each samples and a standard solution added 10µl biotinylated antibodies CLB-CD95/6; then add to titre plate wells and incubated 2 hours at room temperature. Followed by reaction with horseradish peroxidase-conjugated streptavidin (100 µl / cell). The analysis of concentration sFas in probes carried out on the color reaction developing after addition of 0.1 mg / ml tetra-methylbenzidine and H2O2 at 0.11 M/L Na-acetate (pH =5.5) for 10 minutes (Which catalyzes the conversion of the chromogenic substrate tetra-methylbenzidine from a colorless to a yellow solution after the addition of a stopping reagent). Development of color reaction stopped by addition 100 µl 2M H2SO4, measurement at wave length 450 nm on «Titertek Multiskan » (USA).

3. Results
3.1 Estimation level expression Fas-L / Fas in leukocytes peripheral blood in patients with SLE
Expression Fas-L on the surface of lymphocytes in patients with SLE was significantly increased in comparison with healthy donors (2.4’ ± 1.3 vs 1.7 ± 1.1 respectively). (Table 1). Typical histograms of distribution Fas and Fas-L on a surface of cells are presented in Fig. 1.

It is known that, the level expression Fas-L on T-lymphocytes is increased during their activation and is stimulated by interleukin-2 and interferon-γ [4]. The high level of expression Fas-L on lymphocytes can indicate activation of lymphocytes. Increased expression Fas-L can commit Fas-expressing cells to apoptosis.
We observed no significant difference in percentage expression Fas on lymphocytes in patients with SLE when compared to healthy donors (30.8 ± 5.6% vs 26.1 ± 4.1). (Table 1).

Table 1. Expression Fas / Fas-L on the surface of freshly lymphocytes and granulocytes peripheral blood.

<table>
<thead>
<tr>
<th>Group investigated</th>
<th>(% )Lymphocytes (X ± SD)</th>
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<tbody>
<tr>
<td>Healthy donors (n=11)</td>
<td>26.1 ± 4.1 1.7 ± 1.1</td>
</tr>
<tr>
<td>Patients with SLE(n=38)</td>
<td>30.8 ± 5.6 2.4 ± 1.3</td>
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* P<0.05 in comparison with values for healthy donors.

Moreover, the high level of expression Fas-L on lymphocytes was found to be depending on activity of SLE disease (Table 2). The third stage activity of SLE characterized by the highest percentages of expression Fas and Fas-L on lymphocytes which can indicate activation of lymphocytes.

Table 2: Dependence of expression Fas/Fas-L on surface lymphocytes.

<table>
<thead>
<tr>
<th>Stage activity of SLE</th>
<th>% lymphocytes (X ± SD)</th>
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<tbody>
<tr>
<td>I (n=18)</td>
<td>26.3 ± 4.9 2.1 ± 1.6</td>
</tr>
<tr>
<td>II (n=6)</td>
<td>27.7± 4.7 2.0 ± 1.8</td>
</tr>
<tr>
<td>III (n=14)</td>
<td>32.8* ± 5.6 4.3* ± 3.3</td>
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* P<0.05 in comparison with values first stage SLE disease activity.

3.2 Expression Fas in various lymphocyte subpopulations

The percentages of Fas expression on all lymphocytes subsets CD3, CD4, CD8, and CD 19 lymphocytes in SLE were significantly higher when compared to normal controls (Table 3).

Table 3. Expression CD95 (Fas) in various lymphocyte subpopulations.

<table>
<thead>
<tr>
<th>Group investigated</th>
<th>lymphocyte subpopulations, %</th>
</tr>
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<tbody>
<tr>
<td>Healthy donor (n=12)</td>
<td>CD3  7.7±1.5  CD4 6.3±1.2  CD8 5.9±1.3  CD19 6.9±2.7</td>
</tr>
<tr>
<td>Patients with SLE,(n=14)</td>
<td>17.3*±3.9  20.4*±7.0 16.2*±3.02 21.0*±5.1</td>
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</tbody>
</table>

* P<0.05 in comparison with values for healthy donors.

3.3 Determination soluble sFas in plasma of patients with SLE

We found out that, there was a slight increase in soluble Fas in patients with SLE (n=46) when compared to normal healthy donors (n=23) (1.6±0.2ng/ml versus 1.3±0.1ng/ml. respectively). (Fig.2).
3.4 Correlation between soluble sFas in plasma of patients with SLE and SLE disease activity

We investigated correlation between level soluble Fas (sFas) and SLE disease activity. There was inverse correlation between sFas and disease activity in such way that the first stage of disease characterized by the highest level of sFas compared to second or third stages of the disease (1.8±0.1; 1.4±0.1; 1.3±0.02 respectively).

3.5 Correlation between expression Fas and soluble sFas in plasma of patients with SLE

Moreover, we found a positive correlation (r =0.5) between plasma sFas and percentage of lymphocytes Fas expression in patients with SLE (Fig.3).

It is possible to assume that, expression Fas increased at the moment of activation lymphocytes. Thus the increased level sFas in patients SLE explained the proteolytic cleavage of transmembrane forms Fas or alternative splicing. This can be the mechanism of regulating function of Fas receptor.

![Graph](image)

Fig.3. Dependence correlation of concentration sFas in plasma patients with SLE upon content activated lymphocytes. X axis- concentration sFas (ng/ml), Y axis-percentage content activated lymphocytes determined by content Fas (X±SD).

4. Conclusion

It is known that, the level expression Fas-L on T-lymphocytes is increased during their activation and is stimulated by interleukin-2 and interferon-γ. In B-lymphocytes expression Fas-L is increased as a result of interaction of B–cells with activated T-lymphocytes or macrophages through system CD40/CD40L. The high level of expression Fas-L on lymphocytes and its dependence on disease activity of SLE indicate activation of lymphocytes, since peripheral resting T cells virtually do not express Fas ligand and express moderate amount of Fas; while activated peripheral T cells express abundant quantities of both Fas and Fas ligand [7]. Increased expression Fas-L can commit Fas-expressing cells to apoptosis which indicate excessive apoptosis in lymphocytes in SLE patients that promoting occurrence of autoantigens in plasma of patients with SLE.

Thus, it was observed the participation of Fas receptor in pathogenesis SLE through activation of apoptosis cells. The increased apoptosis lymphocytes agreed with lymphopenia clinically diagnosed in patients with SLE.

Furthermore, since signaling through Fas can induced apoptosis, this indicates that Fas seems to be functional in SLE patients, in contrast to MRL/lpr mice i.e., it seems that signaling through Fas is normally controlled and regulated in SLE cells [8].

The inverse correlation between sFas and disease activity can be explained by the opportunity of sFas to bind membrane-bound Fas-L, which its expression increased depending upon disease activity at the same time the ELISA method registered the presence of free sFas in plasma after sedimentation of cellular elements of blood. Moreover, renal disease, the characteristic manifestation for the third stage of SLE, can influence circulation of proteins of the small size and their level in plasma. Proteinuria, in turn can result in exit of sFas from circulation [10].

References:


