Imbalance of Antiendotoxin Immunity in Patients with Systemic Lupus Erythematosus (SLE) and its Influence on the Persistent Systemic Inflammation

Denys V. Shaduro, Vladimir A. Beloglazov and Andrei I. Gordienko

***Abstract*— S**ystemic lupus erythematosus (SLE) is a serious autoimmune disease, the etiology and pathogenesis of which are currently undefined. The article reflects the impact of the imbalance of humoral antiendotoxin immunity (antiendotoxin specific immunoglobulin A, M and G) on the formation of systemic inflammation (C-reactive protein, an index of endogenous intoxication and the average mass of the molecules, circulating immune complexes) and the influence on the overall level of immunoglobulin A, M and G in patients with systemic lupus erythematosus as compared to healthy individuals. Our study confirms the relationship of specific antibodies of class G with CRP levels and CIC, so it can be assumed that the disruption of antiendotoxin immunity can be the factor of persistent inflammatory process.

***Keywords*—**Endotoxin, systemic lupus erythematosus, immunoglobulins, endogenous intoxication, CIC.

I. Introduction

SYSTEMIC lupus erythematosus (SLE) is one of the most severe autoimmune inflammatory diseases of the connective tissue which causes mortality. It is known that within 10 years 28% of SLE patients die of vital organs lesion[1]. The occurrence of SLE is increasing with each decade. The obvious increase was seen in the period from 1950 to 1979 when it amounted to 1.51 per 100 thousand people. In the period from 1980 to 1992 - 5.56 per 100 thousand people [2]. At present, the incidence of SLE is 17.1 per 100 thousand people. The last decade has witnessed the surge in cases of SLE which could be due to improved diagnostic facilities, a wider range of laboratory studies and an increase in diagnosing ill-defined forms of the disease [1].

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Nowadays, there are many controversial theories explaining the etiology of this disease. However, modern research confirms the multifactorial etiology and pathogenesis of lupus-specific inflammation [3]. Endotoxin (ET) and autoimmune inducer or lipopolysaccharide (LPS) Gram-negative enteroflora can be a powerful trigger of inflammation as well as processes to enhance its translocation along the axis "mucosal - internal environment of the body." It is widely believed that in pathological conditions ET is a potent proinflammatory factor in the clinical manifestation of its spectrum of action ranging from fever to endotoxic shock [4].

The integrated effect of ET on the body depends on its amount and the capacity of endotoxin to bind humoral and cellular systems of the body.

II. Materials And Methods

A. Research plan

We have examined 48 patients of the rheumatology department of Crimean Republic Clinical Medical Association called "University Hospital" in Simferopol. These patients were diagnosed with SLE I-II degree in the period from 2012 to 2014. The control group consisted of 40 relatively healthy donors. Research material consisted of fresh-frozen serum obtained by centrifugal separation of whole blood, taken with the written permission of patients and donors. The average age of the patients was 36,4 ± 1,8 years, the minimum age equaled 19 years, the maximum one was 72 years old, the average duration of the disease was 8,0 ± 1,4 years, catamnesis from 0.5 years to 25 years. Women predominated in the study and amounted 89.6%. According to the degree of disease activity patients were divided into two groups: I degree- 41,7%, II degree - 58.3%.

B. Examination of antiendotoxin and general immunity system.

The study of blood samples was carried out by means of blood cold chain and was performed in the central research laboratory of the clinical immunology sector "Crimea State Medical University named after SI Georgievskiy ".

Antiendotoxin antibody of classes A, M and G (respectively anti-LPS-IgA, anti-LPS-IgM and anti-LPS-IgG) was determined by means of ELISA. The specimen LPS Escherichia coli K235 (Sigma Chem. Co., USA) was used as an antigen. Levels of anti-LPS-IgA, anti-LPS-IgM and anti-LPS-IgG were expressed in arbitrary absorbance units (ODU) at a wavelength of 492 nm (E492).

The concentration of general immunoglobulins was determined by microturbidimetric method. All along the research, monospecific sheep serum to human immunoglobulins of classes A, M and G (IgA, IgM and IgG) was widely used. It was obtained in the Institute of Epidemiology and Microbiology. NI Gamalei (MoSLEa, Russia). The resulting dimensions were transferred in units mg / ml, multiplying with the corresponding concentration of serum.

The amount of C-reactive protein (CRP) in the blood serum was determined by "sandwich" -Variant ELISA using biotin-streptavidin system of signal amplification. CRP content was expressed in mg / ml. To estimate the amount of endogenous intoxication (EI) we determined the average weight molecules which were evaluated by the ultraviolet absorption spectrum in protein-free fractions of blood plasma or serum. The number of average molecules was evaluated by absorbance at a wavelength of 260 and 280 nm.

Circulating immune complexes (CIC) were determined by precipitation method in 4.2% solution of polyethylene glycol (PEG). The quantity of CIC in the blood serum are expressed in units.

C.Dimension analysis and reporting

Calculations and data analysis were carried out under the licensed software Microsoft Office Excel 2007 and licensed programs MedStat (Kharkov, Ukraine). This involves checking the normal state of samples with subsequent calculations of data reliability with the help of the method according to the parametric Student t-test and Scheffe criterion. Nonparametric methods on T-Wilcoxon test (for related samples) and H-Kruskal-Wallis test (is a generalized U-Mann- Whitney test for unrelated samples). Calculation of correlations and the construction of the linear regression equation were carried out according to the Pearson, Kendall, Spearman coefficient.

III. Results

During the research of the level of antiendotoxin antibodies of specific classes A, M and G the following results were obtained and shown in Table 1. In the course of examining antiendotoxin antibodies of specific classes A, M and G in the peripheral blood we found out that the level of anti-LPS-IgA in SLE patients did not differ from that in the control group and amounted to 0,186 ± 0,011 ODU (p = 0,211). The median level of anti-LPS-IgM in patients with SLE totaled 0,155 ± 0,024 ODU and did not differ significantly from that in the control group (p = 0,072). Thus, obvious differences in antibody levels in patients with SLE compared to the control group were detected only in the study of anti-LPS IgG, the average of which was 0,744 ± 0,041 ODU.

TABLE I
LEVELS OF SPECIFIC ANTIBODIES AND GENERAL ANTIENDOTOXIN IGA, IGM AND IGG ANTIBODIES IN PATIENTS WITH SLE AND CONTROL SUBJECTS

Parameter	Group	The average value / Median	Mean accuracy/ median	Reliability
anti-LPS IgA (ODU)	SLE	0,186 C	± 0,011 C	p=0,211 H
	monitoring	0,195 M	± 0,034 M	
anti-LPS IgM (ODU)	SLE	0,155 M	± 0,024 M	p=0,072 H
	monitoring	0,278 C	± 0,033 C	
anti-LPS IgG (ODU)	SLE	0,744 C	± 0,041 C	p<0,001 H
	monitoring	0,299 M	± 0,07 M	
Общий IgA (g/l)	SLE	2,041 C	± 0,0593 C	p=0,301 S
	monitoring	1,955 C	± 0,0547 C	
Общий IgM (g/l)	SLE	1,927 C	± 0,0874 C	p=0,321 S
	monitoring	1,826 C	± 0,0494 C	
Общий IgG (g/l)	SLE	11,34 C	± 0,171 C	p=0,031 S
	monitoring	10,77 C	± 0,200 C	

Where: C - average value, M - median, H - H-Kruskal-Wallis test, S - Student's t test

The median level of anti-LPS-IgG in patients with SLE was significantly 2.5 times higher than in the comparison group (p <0,001). In determining the levels of total serum immunoglobulins all variational series differed from a normal distribution, and levels of total IgA and IgM were not significantly different from those in the control group. The concentration of IgG was much higher in patients with SLE is only 5%.

While examining the level of endogenous intoxication in SLE patients, an increase in the index of the value of 13.9% with certainty p = 0,002 was determined. The level of the average molecular weight concentration and the index EI are presented in Table 2.

Similarly we found an increase in the number of circulating immune complexes in patients with SLE which was 1.5 times higher as compared with the control group which was 19,29 ± 1,55 cu (Student's t test p <0,001, the criterion Scheffe p <0,01). The average level of CRP in the study group was 6.8 times higher than in the control group where the control median of 16,55 ± 4,51 mg / ml, I quartile 7,75 mg / ml, III quartile 32,79 mg / ml (W-Wilcoxon test: p <0,001, H-Kruskal-Wallis test: p <0,001). Data on CRP levels and circulating immune complexes are presented in Table. 2.

TABLE II
LEVEL AND THE INDEX OF ENDOGENOUS INTOXICATION IN THE SERUM OF PATIENTS WITH SLE AND THE ONES OF CONTROL GROUP

Parameter	Group	The average value / Median	Mean accuracy/ median	Reliability
Endo-intoxication, 260 nm (ODU)	SLE monitoring	0,060^{M} 0,0718^{C}	± 0,004^{M} ± 0,002^{C}	p=0,157^{H}
Endo-intoxication, 280 nm(ODU)	SLE monitoring	0,125^{M} 0,122^{M}	± 0,007^{M} ± 0,006^{M}	p=0,657^{H}
Index of Endointoxication (ODU)	SLE monitoring	2,064^{C} 1,812^{C}	± 0,063^{C} ± 0,047	p=0,002^{S}
CRP (mg/ml)	SLE monitoring	16,55^{M} 2,44^{C}	± 4,52^{M} ± 0,3^{C}	p<0,001^{H}
CIC (c. u.)	SLE monitoring	19,29^{C} 12,67^{C}	± 1,55^{C} ± 1,12^{C}	p<0,001^{S}

Where: C - average value, M - median, H - H-Kruskal-Wallis test, S - Student's t test

Analyzing these findings, we observed an increase in the level of Anti-LPS-IgG, an increase in the level of CRP, endogenous intoxication and CIC, a slight increase in total IgG in patients with SLE as compared to the control group. In examining the correlation between the above parameters, the research showed an inverse correlation between anti-LPS-IgG and CRP, the Pearson correlation coefficient R = -0,341 at the level of statistical significance p = 0,018. Linear relationship can be represented by the formula: CRP = 46,512 - (29,73 * Anti-LPS-IgG), the coefficient of determination was 0.117. And also we managed to define an inverse relationship between anti-LPS-IgG index and endogenous intoxication. Pearson's correlation coefficient R equalled -0,290 at a significance level of p = 0,045. The linear regression model can be represented by the formula: EI = 2,393 - (0,442 * Anti-LPS-IgG), the coefficient of determination was 0.084. Just in SLE we observed a weak positive correlation between the age of the patient and the level of anti-LPS-IgA, Pearson's correlation coefficient was: R = 0,294, at a significance level of p = 0,042. When constructing a linear model (coefficient of determination 0.086) the correlation can be represented by the formula: Anti-LPS-IgA = 0,121+ (0,0018 * Age).

IV. DISCUSSION

Reduction of anti-LPS-IgG leads to an increase in CRP, which is an indicator of systemic inflammation. This relationship indicates that specific antiendotoxin antibodies are significant in the overall picture of lupus inflammation and have a protective function.

It turns out that the relationship between the anti-LPS-IgG and endogenous intoxication display that with an increase of the body EI specific humoral antiendotoxin immunity decreases. Consequently, in the course of SLE, determination of the concentration of peripheral blood anti-LPS-IgG has an important diagnostic value which serves as an additional criterion for laboratory disease severity.

No changes in the concentration of specific IgM against a sharp increase in the titer of anti-LPS-IgG reflect chronic excessive translocation of ET in the internal environment of the body.

The absence of the expected changes of concentration of antiendotoxin antibody of class A that should excessively be formed on the first place in the direction of translocation of endotoxin-intestine mucous-portal and systemic blood flow against overproduction of anti-LPS-IgG in patients with SLE are probably due to several factors. It is known that IgA-dependent plasmocytes primarily respond to anti-inflammatory therapy by decrease of their activity [5], so we believe that the absence of significant changes in serum IgA is an appropriate response to the combined morphological, immune and therapeutic changes in the mucous membranes of the patients. It should also be taken into account that no increase of antiendotoxin IgA and IgM may be the result of their excessive consumption in response to a chronic translocation of ET.

Increasing index of EI negatively affects trophic and functional state of the mucous, glomerular renal microvasculature [6], the tissues of the central and peripheral nervous system [7] –in other words those target organs in the course of SLE. Increased level of anti-LPS-IgG, as described above, confirms that a major cause of EI with SLE patients can be lipopolysaccharide Gram-negative intestinal flora. Therefore, to achieve a controlled remission in patients with SLE, general therapy should not be used solely, but the detoxification and elimination therapy, as well as the restoration of normal intestinal microflora are also recommended.

Increasing the amount of circulating immune complexes in patients with SLE is characteristic of the disease course. We also must keep in mind the fact that the formation of CIC is influenced by gram-negative chronic intestinal infection which can significantly increase the level of CIC and exacerbate the degree of damage to target organs [8].

V. CONCLUSIONS

1. Patients with SLE demonstrated a dramatic increase in titer of specific anti-LPS-IgG index and EI, on the background values of the normative anti-LPS-IgA and IgM, which indicate the chronic endotoxic aggression.
2. The observed correlations between specific anti-LPS-IgG on the one hand and CRP and CIC on the other hand, confirm the association of imbalance of antiendotoxin

immunity to systemic inflammation in patients with SLE. So we can assume that the imbalance of humoral antiendotoxin immunity has a significant role in maintenance of autoimmune lupus inflammation.

3. The high levels of CIC, CRP, molecular average weight (EI) are the combined results of both autoimmune process and endotoxemia which contribute to the target organ damage which worsens the clinical course of SLE. Therefore, elimination of LPS, the normalization of the intestinal bioflora and strengthening the barrier function of the mucous membranes is a new priority and pathogenetically recommended direction in treating SLE.

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