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SHORT COMMUNICATIONS

Humoral Anti-Endotoxin Immunity Imbalance as a Probable Factor in the Pathogenesis of Autoimmune Diseases

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Abstract—The concentrations of serum anti-endotoxin antibodies (LPS-ABs) of different classes and class M and class G autoantibodies (AABs) to native high-molecular-weight DNA and denatured single-stranded DNA were assayed in 28 patients with acantholytic pemphigus. Statistically significant abnormalities of humoral anti-endotoxin immunity (AEI) were observed in the patients and included a significant decrease in class M LPS-ABs with normal levels of class A and class G LPS-ABs; the decrease was not associated with any change in the concentrations of total immunoglobulins of the same classes. Approximately 30% of the patients showed significant increases in class G AABs to denatured single-stranded DNA and native high-molecular-weight DNA. The levels of class M and class G LPS-ABs were in a strong inverse correlation with the levels of class G AABs to native high-molecular-weight DNA in this patient group. A cluster analysis showed that impaired AEI closely correlated with activity of autoimmune processes in the patients with acantholytic pemphigus. The findings were assumed indirectly implicate LPS in the development and progression of autoimmune disorders.

Keywords: anti-endotoxin immunity, endotoxin aggression, autoimmune diseases, acantholytic pemphigus

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Enteric endotoxin, or lipopolysaccharide (LPS), and systemic endotoxinemia (SEE) due to LPS are a mandatory factor of homeostasis [1], primarily because LPS activates innate immunity via interacting with its key receptor TLR4 [2]. SEE is known to play a dual role in human biology. On the one hand, SEE is essential for regulating the activity of adaptive systems, thus playing a favorable role. On the other hand, SEE may exert a harmful or even catastrophic effect because excess LPS present in circulation (the condition is known as endotoxin aggression (EA)) induces systemic inflammation. EA is a basic element in the pathogenesis of various conditions, including allergosis, atherosclerosis, eye inflammation disorders, disseminated intravascular coagulation, postoperative complications in pediatric surgery, female infertility, anorexia nervosa, chronic virus infections (AIDS in particular), type 1 and type 2 diabetes mellitus, and neoplastic processes [1, 3–10]. We therefore assumed that EA may play a role in the induction of autoimmune disorders. Acantholytic pemphigus (AP) occupies a special place as one of the most severe autoimmune disorders [11].

METHODS

A patient group included 28 AP patients (12 males and 16 females, mean age 48.3 ± 4.7 years), who were treated at the Republican Venereal Disease Clinic (Simferopol). A control group included healthy subjects (n = 32), matching the patient group in gender and age compositions. Venous blood serum was collected by a conventional method and stored at −25°C. Serum anti-LPS antibodies (LPS-ABs) of the A, M, and G classes were assayed by ELISA. *Escherichia coli* K30 LPS was isolated from bacterial biomass by water–phenol extraction and used as an antigen [12, 13]. Total immunoglobulins of the A, M, and G classes were measured in the blood by micro-turbidimetric method [14]. Class M and class G serum autoantibodies (AABs) to native high-molecular-weight DNA and denatured single-stranded DNA were assayed by ELISA [15]. Statistical analyses of the results were carried out using STATISTICA 6.0 software (StatSoft, United States). Differences in parameters between independent samples were tested for significance by the Mann–Whitney U test. Differences were considered significant at $p < 0.05$. Spearman's rank correlation coefficient was used as a nonparametric test to detect a correlation between parameters and to estimate its strength. A cluster analysis employed

Table 1. Serum levels of LPS-ABs of different classes in AP patients ($M \pm m$)

Group	LPS-ABs of different classes, conv. units		
	IgA	IgM	IgG
AP patients, $n = 28$	0.456 ± 0.066	0.206 ± 0.027*	0.205 ± 0.032
Healthy subjects, $n = 32$	0.349 ± 0.053	0.402 ± 0.051	0.179 ± 0.019

Here and in Tables 2 and 4, differences from the control group of healthy subjects were significant at (*) $p < 0.05$ or (**) $p < 0.01$.

Table 2. Serum levels of AABs of different classes to native double-stranded DNA and denatured single-stranded DNA in the AP patients ($M \pm m$)

Group	AABs to denatured single-stranded DNA, conv. units		AABs to native double-stranded DNA, conv. units	
	IgM	IgG	IgM	IgG
AP patients, $n = 28$	0.073 ± 0.007	0.091 ± 0.009**	0.075 ± 0.006	0.086 ± 0.008*
Healthy subjects, $n = 32$	0.085 ± 0.005	0.062 ± 0.001	0.086 ± 0.003	0.067 ± 0.003

Ward's hierarchical agglomerative clustering method with the City Block (Manhattan) metric as a distance matrix and the Mac Queen iterative k-means algorithm.

RESULTS AND DISCUSSION

Measurements showed that the class M LPS-AB levels in the blood of the AP patients were, on average, 1.95 times lower than in the healthy controls ($p < 0.01$, Table 1), while their class A and class G LPS-AB levels did not significant differ from their respective normal values. A correlation analysis revealed a significant ($p < 0.05$) moderate ($r = 0.55$) direct correlation between the class A and class G LPS-AB levels in the AP patients.

An increase in serum AABs to native double-stranded DNA and denatured single-stranded DNA is known to provide an important diagnostic and prognostic marker in patients with autoimmune disorders [16]. Because bullous dermatoses, including AP, are classed with autoimmune disorders, antinuclear factors may be expected to appear in the blood of patients and to contribute to the pathogenesis of the main disease. However, only few studies have focused on AABs to DNA in patients with bullous dermatoses in the available literature [17]. Our AP patients were tested for class M and class G AABs to denatured single-stranded DNA and native double-stranded DNA. The results are summarized in Table 2. The class G AABs to denatured single-stranded DNA and native double-stranded DNA in the AP patients were significantly higher than in the healthy subjects; the difference was, respectively, 1.47 fold and 1.28 fold on average ($p < 0.05$). The class M AABs to denatured single-stranded DNA and native double-stranded DNA in the AP patients did not significantly differ from the respective normal levels. A correlation analysis showed a significant strong direct correlation between the individual levels of class G AABs to denatured single-stranded DNA and those to native double-stranded DNA in the AP patients ($r = 0.92$, $p < 0.001$). In addition, the AP patients displayed a significant strong direct correlation between the class G AAB levels to denatured single-stranded DNA and the levels of class M and class G LPS-ABs and a significant strong inverse correlation between the levels of class G AABs to native single-stranded DNA and the levels of class M and class G LPS-ABs (Table 3).

To characterize B-cell immunity in the AP patients, we measured the total contents of IgA, IgM, and IgG in the blood. These parameters are important to examine because high-dose corticosteroids are used to treat AP patients and exert a prominent immunosuppressive effect [18]. The tests showed that the total IgM and total IgG levels in the AP patients were nearly normal ($p > 0.05$), while the total IgA concentration was significantly higher than in the healthy subjects ($p < 0.05$); the difference was 38.4% on average (Table 4). A correlation analysis did not detect significant correlations for the total IgA, total IgM, and total IgG levels; the serum levels LPS-ABs of different classes; and the levels of AABs of different classes to denatured single-stranded DNA and native double-stranded DNA in the AP patients. The finding indicates that the above changes in particular antibodies were not associated with changes in the concentrations of total antibodies of the respective classes.

A cluster analysis showed that our AP patient sample included two clusters, which were conventionally designated AP-1 and AP-2. Within each cluster, we observed certain associations between the serum levels of LPS-ABs of different classes and the levels of class G AABs to denatured single-stranded DNA and native

Table 3. Correlations between the levels of LPS-ABs of different classes and class G AABs to to denatured single-stranded DNA and native double-stranded DNA in the AP patients

Parameter	Rank correlation coefficient *r*		
	LPS-ABs of different classes		
	IgA	IgM	IgG
Class G AABs to denatured single-stranded DNA	–	*0.70*	*0.89*
Class G AABs to native double-stranded DNA	0.78	–0.93	–0.76

(–), significant correlation was not observed ($p > 0.05$).

Table 4. Blood concentrations of total immunoglobulins of various classes in the AP patients ($M \pm m$)

Group	Total immunoglobulins of different classes, g/L		
	IgA	IgM	IgG
AP patients, $n = 28$	2.98 ± 0.23*	1.71 ± 0.10	10.94 ± 0.49
Healthy subjects, $n = 32$	2.21 ± 0.16	1.77 ± 0.15	10.73 ± 0.16

double-stranded DNA (Fig. 1, Table 5). Cluster AP-1 included eight AP patients (28.6% of the total number of AP patients). The average level of class G AABs to denatured single-stranded DNA in the cluster were 1.97 and 2.03 times higher than in cluster AP-2 and the control group, respectively. The average level of class G AABs to native double-stranded DNA in cluster AP-1 was 2.16 and 2.28 times higher than in cluster AP-2 and the control group, respectively. The average class A LPS-AB level in cluster AP-1 was higher than in cluster AP-2 and the control group by a factor of 2.80 ($p < 0.05$) or 2.42 ($p < 0.05$), respectively. The average level of class G LPS-ABs in cluster AP-1 was 2.87 and 2.02 times higher than in cluster AP-2 and the healthy subject group, respectively. At the same time, the average level of class M LPS-ABs in cluster AP-1 was 2.23 times lower than in the control group and did not significantly differ from that in cluster AP-2. Cluster AP-2 included 20 patients (71.4% of the total AP patient number), whose levels of class G AABs to denatured single-stranded DNA and native double-stranded DNA and the levels of class A and class G LPS-ABs did not significantly differ from the respective normal values. At the same time, the average level of class M LPS-ABs in cluster AP-2 was 1.82 times higher than in the control group. Variations in AEI and AAB parameters in the AP patients might reflect an undulating disease course, which has been observed in HIV-infected and AIDS patients [4]. Acute EA periods alternate with "endotoxin insufficiency" (the immune system partly loses its capability of responding to LPS) to form cycles in such cases. A loss of response needs separate investigation with a study protocol expanded to include assays for LPS in circulation and markers of systemic inflammation in the course of the disease.

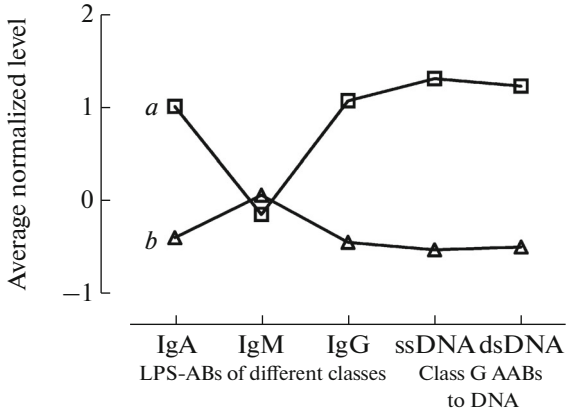

Fig. 1. Associations between the serum levels of LPS-ABs of different classes and the levels of class G AABs to denatured single-stranded DNA (ssDNA) and native double-stranded DNA (dsDNA) in the AP patients (the Mac Queen iterative *k*-means procedure). Clusters: (*a*) AP-1 and (*b*) AP-2.

CONCLUSIONS

Our laboratory study revealed abnormalities of humoral AEI in patients with AP, which is one of the most severe autoimmune diseases. Some of the abnormalities directly correlated with activity of the autoimmune process, indirectly implicating the endotoxin in the pathogenesis of AP. Deficiency of class M LPS-ABs was characteristic of all AP patients and suggested "endotoxin insufficiency", which is a condition where the immune system partly loses its capability of responding to LPS (relative insufficiency of AEI). The variation in the other parameters under study was

Table 5. Serum levels of LPS-ABs of different classes and class G AABs to denatured single-stranded DNA and native double-stranded DNA in the AP patients of the two clusters ($M \pm m$)

Parameter, conv. units	AP patients		Healthy subjects ($n = 32$)
	AP-1 ($n = 8$, 28.6%)	AP-2 ($n = 20$, 71.4%)	
Class A LPS-ABs	0.843 ± 0.121 $p < 0.001$ $p_2 < 0.001$	0.301 ± 0.043 $p > 0.05$	0.349 ± 0.053
Class M LPS-ABs	0.180 ± 0.035 $p < 0.001$ $p_2 > 0.05$	0.221 ± 0.035 $p < 0.001$	0.402 ± 0.051
Class G LPS-ABs	0.361 ± 0.080 $p < 0.001$ $p_2 < 0.01$	0.126 ± 0.008 $p > 0.05$	0.179 ± 0.019
Class G AABs to denatured single-stranded DNA	0.126 ± 0.010 $p < 0.001$ $p_2 < 0.001$	0.064 ± 0.002 $p > 0.05$	0.062 ± 0.001
Class G AABs to native double-stranded DNA	0.153 ± 0.018 $p < 0.001$ $p_2 < 0.001$	0.071 ± 0.003 $p > 0.05$	0.067 ± 0.003

The statistics p and p_2 show the significance of differences in comparisons with the control group of healthy subjects and cluster AP-2 of AP patients, respectively.

observed in AP patients and may reflect an undulating disease course. The finding deserves further investigation with a study protocol expanded to include tests for markers of systemic inflammation and LPS concentration in circulation. The results from such studies will substantially improve the common understanding of the pathogenesis of autoimmune disorders and will help to increase the efficacy of therapy.

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COMPLIANCE WITH ETHICAL STANDARDS

The study was carried out in accordance with the biomedical ethics guidelines of the Declaration of Helsinki (1964) and its later amendments and was approved by the Ethics Committee at the Crimean Federal University (Simferopol).

Informed Consent

All participants voluntarily gave their written informed consent for participation after being informed about potential risks and benefits and the study procedure.

CONFLICT OF INTEREST

The authors declare that they have no real or potential conflict of interest with respect to publication of this article.

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