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SHORT COMMUNICATIONS

Intestinal Endotoxin as a Trigger of Type 1 Diabetes Mellitus

P. L. Okorokov[a], I. A. Anikhovskaya[a], I. E. Volkov[b], and M. Yu. Yakovlev[a,c]

[a] *Institute of General and Clinical Pathology, Clinical-Diagnostic Department, Russian Academy of Natural Sciences, Moscow, Russia*

[b] *State Institution Russian Children's Clinical Hospital, Moscow, Russia*

[c] *Russian State Medical University, Moscow, Russia*

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Abstract—The blood serum level of intestinal flora endotoxin was studied in 45 children and adolescents aged 3–17 years with type 1 diabetes mellitus. In all the patients the endotoxin levels 2.89 ± 0.33 *EU*/ml) were significantly elevated compared to the control group (0.4 ± 0.03 *EU*/ml). A higher level of endotoxin at the disease onset (3.93 ± 0.79 *EU*/ml) compared to that in children afflicted with diabetes for more than two years (2.37 ± 0.27 *EU*/ml) gives evidence of the involvement of endotoxin in the initiation of the disease.

Keywords: endotoxin, innate immunity, type 1 diabetes mellitus.

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Selective autoimmune damage to the β cells of the pancreatic islets of Langerhans underlies the development of type 1 diabetes melltus (DM1). Genetic factors play in important role in the pathogenesis of DM1. However, genetic predisposition can result in the development of DM1 only when the body is influenced by the trigger factor. As predicted 20 years ago, it could be intestinal flora endotoxin (ET) [1], a lipopolysaccharide (LPS), which is an obligate component of the cell membrane of all gram-negative bacteria.

Recently, the first reports about the involvement of the gut flora in the initiation of DM1 began to appear in the foreign press [2]. The very possibility of the intestinal factor being involved in the initiation of DM1 was predetermined both by the endotoxin theory [3, 4] and the well-known ability of the LPS to decrease 10-fold the diabetogenic dose of streptozotocin in the experimental animals [5].

LPS is one of the best-studied pathogen-associated molecular structures, which is recognized by the *Toll*-receptors of innate immunity and activates the synthesis of a wide spectrum of proinflammatory cytokines, chemokines, and adhesins [6]. The role of innate immunity in the pathogenesis of DM1 and specific complications is well-studied [7–9]. In this connection, the fact of the ability of ET to determine by the system of innate immunity the activity of adaptive immunity [10] and, hence, to initiate the development of DM1 and vascular complications is extremely interesting.

Thus, the aim of this work was to determine a possible role of the excess of intestinal flora LPS (endotoxin aggression) in the initiation of DM1.

To solve the tasks set, we determined the integral serum values of the intestinal ET levels in DM1-afflicted children and adolescents at the disease onset (group 1) and in those with a more than two-year experience of diabetes (group 2). The LPS concentration was determined using the micro-LAL test, the author's modification of the LAL test, based on the ET capacity for forming protein fractals. This method was adapted to the clinical conditions by researchers from the Institute of General and Clinical Pathology, Clinical-Diagnostic Department, Russian Academy of Natural Sciences, and allows the integral serum values of all the LPS concentrations to be determined, because it interacts exclusively with *Re*-glycolipid, a shared ET molecule fragment. The assessment of the degree of compensation of carbohydrate metabolism was made using the routine methods for determining the levels of glycosylated hemoglobin and the requirement for insulin on conversion to kilograms of body mass.

Forty-five DM1 patients (24 boys and 21 girls) with ages varying between three and seventeen years (10.46 ± 0.72 years) with no hereditary DM load and disease duration from 0 to 13 years (4.94 ± 0.59 years) were examined and followed up at the Diabetology and Endocrinology Department of the Russian Children's Clinical Hospital. The control group including 50 healthy children without any carbohydrate metabolism disorders was also examined. A detailed characteristic of the groups is given in the table.

In all the patients examined, the plasma level of intestinal ET (2.89 ± 0.33 *EU*/ml) was significantly ($p < 0.0001$) elevated compared to the control group values (0.4 ± 0.03 *EU*/ml). The intestinal flora LPS levels in each of the study groups also differed signifi-

Characteristic of the groups studied

Parameter	Groups of patients		Control group
	Group 1	Group 2	
The number of those examined	15	30	50
Average age, years	6.6 ± 1.12	12.3 ± 0.71	11.14 ± 0.57
The gender structure of those examined	M – 9 (60%) F – 6 (40%)	M – 15 (50%) F – 15 (50%)	M – 29 (58%) F – 15 (42%)
The level of glycosylated hemoglobin, %	8.1 ± 0.59	8.73 ± 0.33	4.56 ± 0.04
The level of intestinal endotoxin, *EU*/ml	3.93 ± 0.79	2.37 ± 0.27	0.4 ± 0.03
Duration of diabetes, years	0.83 ± 0.19	6.99 ± 0.6	–
The presence of complications, %	0	86.6	–
The structure of complications		Diabetic polyneuropathy – 100%. Diabetic nephropathy – 19%. Diabetic retinopathy – 3.8%	–
The requirement for insulin, u/kg	0.45 ± 0.07	0.94 ± 0.04	–

cantly (p <0.0001) compared to the control group. It is important to note that the LPS concentrations at the disease onset were significantly higher (p < 0.05) in children and adolescents than in patients with an experience of diabetes of more than two years (Fig. 1).

Group 1 children revealed a lower requirement for insulin (0.45 ± 0.47 u/kg compared to children whose duration of diabetes exceeded two years (0.94 ± 0.04 u/kg). The glycosylated hemoglobin level was also lower in the group of children with a less than two-year experience of diabetes (8.1 ± 0.59% at the disease onset against 8.73 ± 0.33% in patients with the experience). The intergroup differences in the glycosylated hemoglobin level and the requirement for insulin were expected and explainable. After the disease's manifestation and the institution of adequate doses of insulin therapy, the honeymoon period characterized by low requirements for insulin and a decrease in the average glycemic values and, consequently, the glycosylated hemoglobin level was noted in many children and adolescents. The high requirement for insulin and the glycosylated hemoglobin level in the group of patients who experienced diabetes for more than two years conform to consistent rules, because adolescents are characterized by pubertal insulin resistance and a reduced quality of self-control, which leads to the unsatisfactory compensation of carbohydrate metabolism.

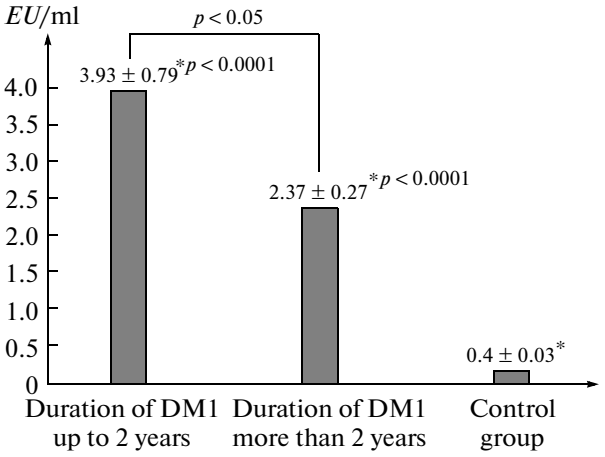

Fig. 1. Relationship between the integral intestinal endotoxin values and the duration of type 1 diabetes mellitus (DM1) in children and adolescents.

Higher ET concentrations at the disease onset may be indicative of the endotoxin aggression of intestinal origin being involved in the initiation of DM1, and the pathogenesis of diabetes itself may be represented from the positions of the endocrine theory of human physiology and pathology in the form shown in Fig. 2.

Thus, the scientific facts obtained by us allow the endotoxin aggression of intestinal origin to be considered as the most probable cause of the development of DM1 (in the presence of genetic predisposition in children), which opens up new possibilities in the treatment and prevention of the disease.

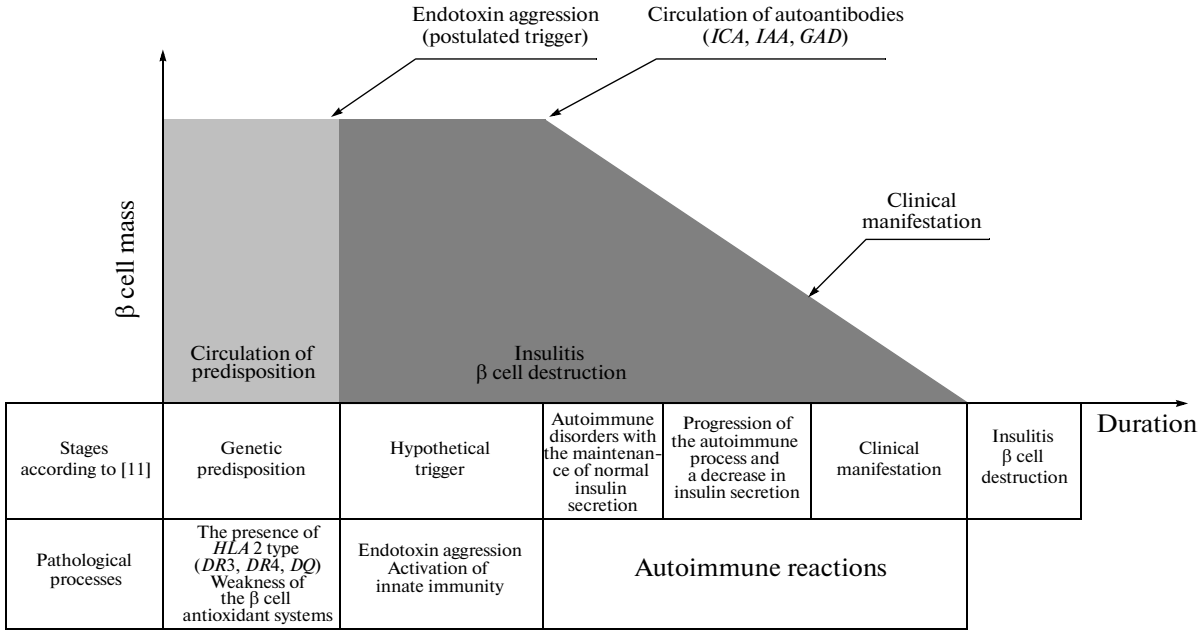

Fig. 2. Stages of diabetes mellitus development (cited according to [11], modification).

CONCLUSIONS

(1) In children genetically predisposed to the development of type 1 diabetes mellitus, endotoxin aggression of intestinal origin may be the only factor inducing the disease.

(2) Further studies of the role of intestinal endotoxin and innate immunity in the pathogenesis of type 1 diabetes mellitus will enable us to develop principally new approaches for the treatment and prevention of diabetes.

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