Tissue Hypoxia and Intestinal Dysbiosis in Children with Tuberculosis

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We studied the role of autochthonous microflora from body cavities in the development of tissue hypoxia and instability of cell membranes. In children with tuberculosis dysbiosis manifested in nonspecific quantitative changes in the intestinal microflora and the presence of coxsackievirus antigens in the urine. DNA-containing viruses with pronounced immunosuppressive activity (*e.g.*, herpesvirus, measles virus, and rubella virus) were found in most children. Microbiological and virological changes were accompanied by the appearance of laboratory signs for tissue hypoxia, which included inhibition of Krebs cycle dehydrogenases and α-glycerophosphate pathway in blood lymphocytes. Regression analysis revealed a relationship between the content of extraintestinal coxsackieviruses and inactivation of α-glycerophosphate dehydrogenase, succinate dehydrogenase and ratio of facultatively anaerobic bacteria in microbiocenosis, and expression of acid phosphatase and total population of malonate-positive enterobacteria, staphylococci, yeasts, and enterococci.

Key Words: *succinate dehydrogenase; α-glycerophosphate pathway; dysbiosis; coxsackieviruses; tuberculosis*

Studies of polyorgan insufficiency during sepsis indicate that opportunistic bacteria causing endotoxemias play a role in the development of tissue hypoxia. Experiments on animals with endotoxemia showed that endotoxins promote hypoxia and impair blood microcirculation and mitochondrial respiration [7,10]. These data are of considerable interest since autochthonous microflora from body cavities releasing endotoxins and other products into the circulation produces various toxic and infectious effects on the macroorganism [1].

Cytochemical assay of enzymes involved in energy metabolism in mitochondria showed that tissue hypoxia accompanies severe chronic diseases, including pulmonary tuberculosis [5]. We studied the role of intestinal dysbiosis in the development of hypoxia. Enzyme analysis of lymphocytes from children with tuberculosis was performed in parallel with microbiological and virological assays.

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MATERIALS AND METHODS

We examined 15 children (1-7 years) with tuberculosis of thoracic lymph nodes. The patients received standard antituberculosis therapy. The severity of intestinal microbiocenosis was determined by quantitative microbiological analysis of feces. We studied gram-positive obligate anaerobes (bifidobacteria, lactobacilli, and clostridia) and facultative anaerobes (citrate- and malonate-positive enterobacteria, *Escherichia*, protei, staphylococci, enterococci, streptococci, and yeast-like C*andida* fungi). The presence of intestinal viruses (coxsackieviruses 1-5 and B, polioviruses 1-3, and enteroviruses 68-71), rubella viruses, herpesviruses, and cytomegaloviruses in the blood was estimated by direct immunofluorescence assay of the urine precipitate stained with antisera against viral antigens.

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The intensity of fluorescence was expressed in points. Cytochemical analysis of lymphocytes included counting of formazan granules per cell (dehydrogenase activity) and the percentage of cells with active acid phosphatase (AP) [6].

RESULTS

Intensive chemotherapy of patients with tuberculosis is a risk factor for the development of intestinal dysbiosis. Nonspecific changes in the microflora reflected the development of dysbacteriosis: decrease in the count (or disappearance) of lactobacilli (10 patients), bifidobacteria, ($<10^7$ CFU/g, 6 patients), and increase in the content of opportunistic malonate- and citrate-positive enterobacteria ($\geq 10^6$ CFU/g, 9 and 7 patients, respectively), yeast-like *Candida* fungi ($>10^5$ CFU/g, 6 patients), staphylococci, protei, and enterococci. Dysbiotic changes in children were classified as degree II dysbacteriosis.

Virological assay of urine showed that children were infected with human pathogenic RNA-containing viruses of the family *Enteroviridae*. Polyoviruses and enteroviruses were detected in 27 and 49% patients, respectively; coxsackieviruses A and B were found in 100% patients (in 40% patients only group A viruses were found and group B viruses were not detected). The urine contained picornaviruses and viruses with potent immunosuppressive activity, including herpesviruses (80% samples), measles viruses (73% samples), rubella viruses (47% samples), and cytomegaloviruses (23% samples).

Blood lymphocytes are often used for evaluation of energy processes [6]. Low activity of α-glycerophosphate dehydrogenase (GPDH) involved in substrate (shuttle) oxidation of cytoplasmic NADH formed during glycolysis in mitochondria (1.36±0.2 *vs*. 11-13 granules per cell in the control) and succinate dehydrogenase playing a key role in the Krebs cycle (14.6±1.94 and 18.7-19.9 granules per cell in the control) indicate that tuberculosis is accompanied by tissue hypoxia. Similar results were obtained in previous examinations of adult patients [5]. The count of AP-containing cells sharply increased in patients with tuberculosis (71.6±4.8 *vs*. 40-60% in the control).

Regression analysis revealed a strict correlation between GPDH activity and the total content of coxsackieviruses A1, A2, A3, A4, and B antigens in the urine that indirectly reflects the amount of intestinal viruses (r=0.87). The analytic expression of this relationship (R_{sq}=76%, Es=0.08) indicates that the inhibition of GPDH became more pronounced with increasing the content of viruses (Fig. 1). Recent studies showed that coxsackieviruses and their pathogenic effects persist in tissues even in the nonacute phases of infection [11]. The intestine serves as a reservoir for chronic infection [3]. The development of hypoxia depends on the number of coxsackieviruses. In light this the revealed correlation has a biological significance: the α-glycerophosphate pathway, an element of biological oxidation leading to ATP synthesis, serves as a possible site for the effect of intestinal viruses or viral proteins. Published data on viral infections illustrate the toxic and dysmetabolic potentials of viruses providing reversible inhibition (destruction) of the immune system [9]. Our results show that persistence of coxsackieviruses is accompanied by systemic changes: development of tissue hypoxia. The biological benefit of hypoxia for viruses remains unclear. Replication of viruses (*e.g.*, Kaposi sarcoma-associated virus) is intensified under hypoxic conditions [8].

SDH activity depended on the total content of facultative anaerobes in microbiocenosis (r=0.72). In the selected model (R_{sq}=51%, Es=1.32) SDH activity considerably decreases when the number of bacteria attains 10^6-10^7 CFU/g. These changes probably reflect the distant effect of microbiocenosis promoting hypoxia. This effect is related to the release of products from opportunistic microorganisms into the blood. Published data demonstrate a relationship between endotoxemia and pathological changes in intestinal microbiocenosis (dysbacteriosis) [4].

Figure 2 illustrates the third distant effect of microbiocenosis. In the selected model (R_{sq}=68%, Es=0.0007) intensification of AP expression in blood cells was associated with the increase in the total content of staphylococci, yeasts, enterococci, and malonate-positive enterobacteria in microbiocenosis (r=-0.82). The influence of microbiocenosis on blood cells is probably associated with the destabilizing effect of micro-

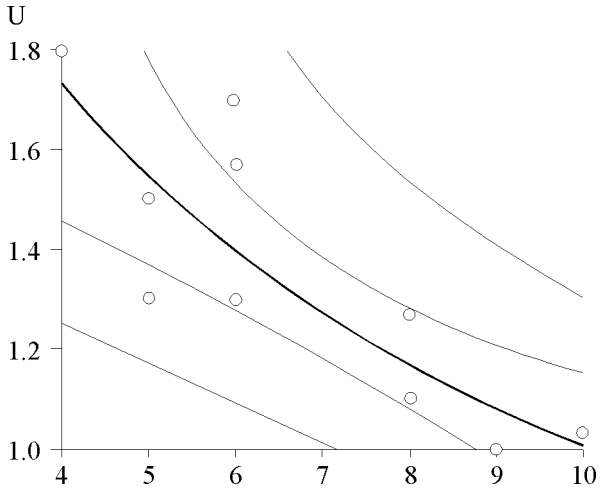

Fig. 1. Dependence of α-glycerophosphate dehydrogenase activity in blood lymphocytes on the entire population of extraintestinal coxsackieviruses A1, A2, A3, A4, and B. Abscise: total fluorescence intensity of urine precipitate (points).

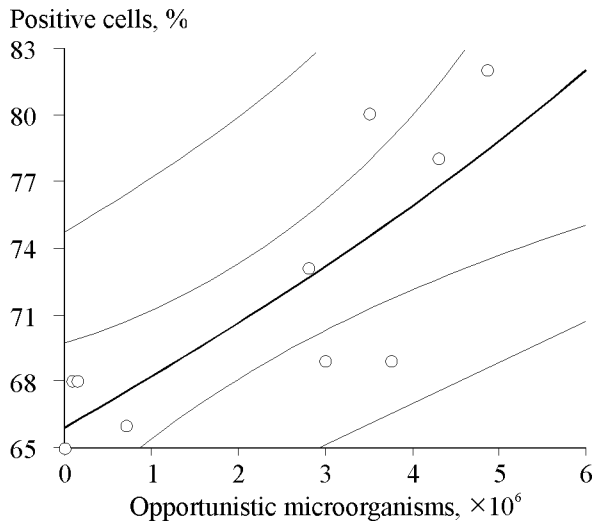

Fig. 2. Expression of acid phosphatase in blood lymphocytes as a function of the total content of opportunistic microorganisms in microbiocenosis (streptococci, enterococci, malonate-positive enterobacteria, and yeast-like *Candida* fungi).

bial products on the lysosomal membrane and release of the enzyme into the cytoplasm [2].

The interaction between macroorganisms and autochthonous microflora is important medical and biological problem. The question arises: whether opportunistic bacteria and viruses present in body cavities determine health and lifetime of the macroorganism and development and course of severe chronic diseases (*e.g.*, atherosclerosis, diabetes mellitus, and psoriasis)? Clinical observations indicate that microflora produces a systemic effect on macroorganisms. Microflora contributes to the development of hypoxia and modifies the state of lysosomes in patients with tuberculosis, which determines the response of macroorganisms to mycobacteria. The role of systemic changes caused by microbiocenoses is often ignored in studying the pathogenesis and therapy of tuberculosis and other human diseases. Previous studies showed that autochthonous microflora produces a positive effect. However, the existence of various microbiocenoses received little attention. Our results and published data [12] indicate that new methods for the therapy of severe human diseases should include correction of dysbiosis [1].

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