VIROLOGY

Chromosome Aberrations, Apoptosis, and DNA Repair in Peripheral Blood Lymphocytes in Children with Infectious Mononucleosis

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Infectious mononucleosis is associated with suppression of lymphocyte apoptosis and accumulation of cells with chromosome aberrations in the peripheral blood. The type and severity of these aberrations do not depend on the etiological variant of infection and patient age. At the same time in 3-6-year-old children with infectious mononucleosis caused by Epstein—Barr virus we observed activation of DNA repair system in lymphoid cells.

Key Words: *infectious mononucleosis; lymphocytes; DNA repair; apoptosis*

Infectious mononucleosis (IM) is a typical persistent infection caused in 90% cases by Epstein—Barr virus (EBV) (type 4 herpes virus). Other infectious agents (viruses, bacteria, protozoa) can cause the mononucleosis syndrome [7,10]. There is still a gap between potentialities of modern laboratory diagnosis of etiological variants of IM and its practical use in clinical and experimental studies. It is time to revise acknowledged notions on the nature and mechanisms of disorders in the blood system in IM.

Disorders in systems maintaining genetic homeostasis in the organism at the cellular and molecular levels resulting from damaging effects of the infectious agent, lymphocyte hyperproliferation in IM, and physiological hypersensitivity to mutagenic factors determine high risk of chromosome aberrations and malignant cell transformation [1,8,10,14]. There are numerous reports on mutagenic effects of EBV [4,9], but little is known about the incidence and type of cytogenetic disorders in relation to the apoptosis processes and DNA repair in various etiological variants of IM in patients of different age.

We studied age-associated characteristics of chromosome aberrations, apoptosis, and activity of DNA repair system in peripheral blood lymphocytes of children with IM caused by EBV and other agents.

MATERIALS AND METHODS

We examined 98 children with IM of moderate severity with acute onset and smooth (uneventful) course of the disease. IM was caused by EBV in 47 children (27 aged 3-6 years and 20 aged 7-14 years), by cytomegalovirus, herpes simplex virus, and unknown agents in 51 children (27 children aged 3-6 years and 24 ones aged 7-14 years). The patients were examined during the acute period of disease, during convalescence, and 18 months after the disease. IM was diagnosed on the basis of clinical and hematological symptoms [7]. The agent was identified by serological methods (enzyme immunoassay, indirect immunofluorescence) and PCR.

Control group consisted of 34 healthy children (11 aged 3-6 years and 23 aged 7-14 years).

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TABLE 1. Cytogenetic Status of Peripheral Blood Lymphocytes in Healthy Children and Patients with IM ($M \pm m$)

Parameter	Healthy children	IM caused by EBV			IM of other etiology		
		I	II	III	I	II	III
Percentage of cells with chromosome aberrations							
3-6 years	5.56±0.65	15.17±1.00*	11.33±0.70*$^{+}$	8.33±0.95***^{ooo}	14.00±1.45*	10.91±0.78*	9.25±1.31***
7-14 years	3.85±0.58	16.00±1.69*	11.00±0.45*$^{++}$	7.65±0.82*^{oo}	13.08±1.15*	10.40±0.27*$^{+}$	7.88±0.61*^{o}
with abnormal number of chromosomes							
3-6 years	0	0.33±0.22	0.11±0.11	0	0.57±0.37	0	0
7-14 years	0	1.00±0.38	0.67±0.42	0.24±0.16	0.62±0.27	0.40±0.27	0
Index of DNA repair system stimulation							
3-6 years	0.97±0.23	1.51±0.30***	1.75±0.37***	1.45±0.19***	1.17±0.13	1.13±0.15	1.01±0.14
7-14 years	1.21±0.21	1.26±0.12	1.09±0.07	1.06±0.05	0.99±0.15	1.01±0.14	1.27±0.17
Content of fragmented DNA							
3-6 years	43.02±3.85	15.14±2.49*	27.79±2.86**$^{+}$	33.75±8.74	21.02±4.73*	24.20±3.92**	25.25±5.43***
7-14 years	51.10±8.20	24.17±4.12**	26.15±2.63***	41.94±2.78^{oo}	23.98±4.06**	24.09±1.33**	35.08±4.40^{oox}

Note. *I*) acute period; *II*) convalescence; *III*) 16-18 months after disease. *$p<0.001$, **$p<0.01$, ***$p<0.05$ *vs.* healthy children; $^{+}p<0.01$, $^{++}p<0.05$ *vs.* acute period; $^{o}p<0.001$, $^{oo}p<0.01$, $^{ooo}p<0.05$ *vs.* convalescence; $^{x}p<0.05$ *vs.* IM caused by EBV in the same age group during the same period.

Peripheral blood was analyzed. Blood cells were cultured by the method of E. D. Gol'dberg *et al.* [2], the cultures were treated and the preparations for chromosome analysis of lymphocytes were made by the method of P. S. Moorhead *et al.* [13].

Suspension of cultured cells was prepared by mixing (1:4) heparin-treated (25 U/ml) blood with nutrient medium containing 90% RPMI-1640 (Sigma), 10% ETC (ICN Biomedicals) inactivated by heating at 56°C for 30 min, 10 mmol/liter HEPES (Flow), 280 ml/liter L-glutamine, and 50 mg/liter gentamicin. After addition of phytohemagglutinin (0.01 mg/ml, Difco) the suspension was incubated in flasks (5 ml per flask) at 37°C for 52 h. Colchicine (Fluka, 0.01%, 0.25 ml) was added after 50 h incubation. At least 100 metaphase plates from each patient were analyzed. The percentage of cells with aberrations (chromosome and chromatide breaks and exchanges) and abnormal chromosome number (hyper- and polyploid cells) was determined. Gaps and hypoploid cells were not counted.

Activity of excision DNA repair in lymphoid cells induced by 4-nitroquinoline oxide (2.5 μmol/ml for 30 min) under conditions hydroxyurea-suppressed replicative synthesis (10 mmol/ml for 30 min) was studied analyzed. Radioactivity was measured on a Mark III β-counter. Index of stimulation of DNA repair system was calculated [3].

Lymphocyte apoptosis was induced *in vitro* using Ca^{2+},Mg^{2+}-free Dulbecco saline (Sigma). The samples were incubated at 37°C for 6 h [11]. Irreversible damage to cell plasma membrane was detected using 0.5% trypan blue (Serva) [2].

DNA was isolated from lymphocytes as described previously [15]. DNA electrophoresis for identification of internucleosomal DNA fragmentation was carried out in 1.5% agarose gel with 1 μg/ml ethidium bromide at 20 V/cm for 30 min [12]. DNA content was evaluated by the diphenylamine method [6].

The results were statistically processed using Student's *t* test and nonparametric Wilcoxon and Mann—Whitney tests.

RESULTS

A pronounced increase in the number of cells with chromosome aberrations was detected in all children with IM caused by EBV and other factors (Table 1); single fragments predominated among destroyed chromosomes. These changes persisted during convalescence and in delayed periods (Table 1). This can be explained by integration of DNA-containing viruses, specifically herpes viruses, in cell DNA and their long-term persistence in the nuclei of damaged cells [1]. Blood lymphocytes with abnormal number of chromosomes (mainly hyperploid cells) were also detected (Table 1). In patients with IM caused by EBV these changes persisted longer than in children of the same age with IM of other etiology (Table 1).

On the other hand, decreased level of DNA fragmentation in peripheral blood lymphocytes observed in all patients with IM (Table 1) indicated inhibition of apoptosis. During convalescence the content of fragmented DNA in lymphoid cells remained low and returned to normal only 18 months after the disease. This can contribute to accumulation of cells with chromosome aberrations.

Analysis of functional activity of DNA repair system showed that EBV-induced IM in 3-6-year-old children was associated with stimulation of DNA repair processes in lymphocytes, while in patients of other groups this parameter virtually did not differ from normal (Table 1). This activation of DNA repair system can be due to EBV-induced interferon production by lymphoid cells, as interferon is a potent stimulator of DNA synthesis [4]. In addition, EBV contains genes neutralizing activity of antiviral cytokines (in particular, interferon), inhibiting interferon production and binding to cell receptors. This determines the possibility of virus persistence in cells and development of latent infection [1,5]. Primary infection with EBV occurs usually at the age of 2-5 years. Older children develop IM after previous EBV infection [7,10]. Therefore, defects in the interferon system in 7-14-year-old patients with IM is more serious than in 3-6-year-old children and this defect is responsible for insufficient production of the cytokine for stimulation of cell genome repair under conditions of manifest infection.

We hypothyesize that age factor, *i. e.* the development of specific antiviral immunity in elder children (EBV carriers), is also responsible the same level of cytogenetic disorders in lymphoid cells of 7-14-year-old and young patients with IM caused by EBV, despite activation of DNA repair system in this latter group.

Hence, IM is associated with suppression of lymphocyte apoptosis and accumulation of cells with chromosome aberrations in the peripheral blood. The type and severity of these aberrations do not depend on age and etiology of the infection. This can be a result of cytopathic processes typical of viral infections: specific features of these processes are determined by infected cell, rather than the virus [1]. On the other hand, activation of the DNA repair system in lymphoid cells is observed in 3-6-year-old patients with IM.

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