VIROLOGY

Electron Microscopic Characteristics of Peripheral Blood Lymphocytes in Children with Infectious Mononucleosis

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Infectious mononucleosis is associated with pronounced changes in surface architectonics of peripheral blood lymphocytes persisting during convalescence and remote period after the disease. The degree of these changes depends on the disease agent and age-specific characteristics of the body. The most pronounced and sustained disorders in the morphostructural organization of lymphocytes are caused by Epstein—Barr virus (in comparison with agents of other etiology); these disorders are more pronounced in children aged 7-14 years than in those aged 3-6 years.

Key Words: *infectious mononucleosis; leukocytes; electron microscopy*

Herpes virus infections characterized by high geographic prevalence, high incidence of disease and asymptomatic carriership, specifically, infectious mononucleosis (IM), occupy a special place in the structure of human infections today.

It was believed for a long time that IM is caused solely by Epstein—Barr virus (EBV). However, accumulation of clinical experience and improvement of methods for laboratory diagnosis of IM showed a variety of not only clinical manifestations, but also etiological variants of IM. It was revealed for example, that the clinical hematological IM syndrome can be caused by cytomegalovirus, type 1 herpes simplex, varicella, measles, rubella, influenza, and other viruses, as well as by bacteria and protozoa [9,13].

Oro- and nasopharyngeal epithelial cells and lymphoid tissue, circulating T and B lymphocytes, monocytes/macrophages, and neutrophils are the targets for EBV and other herpes viruses. The virus-induced impairment of lymphocytes seems to be the key factor in the pathogenesis of IM, as these cells serve as not only the "reservoir" and "source" of the virus, thus providing the agent dissemination in the body, but as a sort of antiviral "weapons", because they are directly involved in the realization of congenital and adaptive immunity. The cytopathogenic effect of viruses (including EBV) towards lymphocytes can be associated with pronounced disorders of antiinfectious defense in the body, the main manifestations of which are structural metabolic and functional imbalance of target immunocytes [3,13].

The cytoplasmic membrane is a cell structure most sensitive to infection. The membrane plays the key role in determination of normal functioning of individual cell organelles and the cell in general. For example, light and electron microscopy demonstrated an important modifying role of surface membrane in lymphocyte binding to various antigens, in cell-cell interactions of T and B lymphocytes, macrophages, and in phagocytosis reactions [12,14]. The structural characteristics of lymphoid cell membranes were found to be in high correlation with their proliferative and apoptotic activities [11]. It is noteworthy that the data on structural and functional disorganization of membranes in lymphocytes and other blood cells in viral infections are scanty and fragmented. We found no

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published data about pathomorphological changes in cell membranes during IM.

Here we studied the surface architectonics of peripheral blood lymphocytes in children of different age groups suffering from IM caused by EBV and IM of other (herpes virus and unknown) etiology in different periods of the disease and during remote period after it.

MATERIALS AND METHODS

A total of 85 children aged 3-14 years with acute IM of medium severity with smooth (uneventful) course of the disease were observed. The disease was diagnosed on the basis clinical and hematological data [9]. The agent was identified by serological methods (enzyme immunoassay, indirect immunofluorescence) and by PCR. IM caused by EBV (EBV-IM) was diagnosed in 42 children (22 aged 3-6 years and 20 aged 7-14 years), IM of other etiology (OIM) caused by cytomegalovirus, herpes simplex virus, and by unknown agents was detected in 43 children (18 aged 3-6 years and 25 aged 7-14 years). The patients were examined at the peak of IM (on days 4-15 of disease), during early convalescence (days 26-30 after disease onset), and 16-18 months after IM.

The control group consisted of 32 healthy children (17 aged 3-6 years and 15 aged 7-14 years).

The peripheral blood was analyzed. Quantitative characteristics of the peripheral blood were analyzed by common hematological methods. Smears for evaluation of differential blood count were prepared by leukoconcentration of venous blood (with Trilon B) [7].

Surface microrelief of lymphocytic cells was studied under a scanning electron microscope. Lymphocytes isolated from the blood in Ficoll-urograffin density gradient (1.077 g/cm^3) were fixed for 30 min in 2.5% glutaraldehyde solution buffered with 0.2 M cacodylate buffer (pH 7.2) [6]. Then the cells were washed twice from the fixative in cacodylate buffer with 15-min centrifugation at 1000 rpm after each washing. The material was postfixed in 1% OsO_4 for 1 h. The cells were washed from osmium fixative and dehydrated in ascending alcohols (15 min in 30, 50, 70, and 90% and twice (15+15 min) in absolute ethanol). Dehydrated lymphocytes were thinly layered onto degreased aluminum underlayer and dried on air. Dry preparations were sprayed with copper in a JEE-4B vacuum chamber (Jeol) at 10^{-5} atm pressure for 2-3 min and examined under a JEM-100CX II electron microscope with an ASID-4D scanning attachment (Jeol) at a slope angle of 0-30^o and 20 kV accelerating voltage.

The lymphocytes were divided into 5 groups depending on the category of morphological formations on their surface [2,5] (Fig. 1): 1) smooth cells (with completely and more or less smooth surface); 2) cells with thread-like growth (with microvilli); 3) cells with lamellar formations (with folds, ruffles); 4) cells with spheroid (bulbar) protrusions (bubbles); and 5) cells with depressions (presenting as "gullies" and fissures).

The results were statistically processed using Student's t test (for normal distribution of variables) and Wilcoxon and Mann—Whitney's nonparametrical U tests (for data in disagreement with the normal distribution). The differences were considered significant at $p<0.05$. The relationship between the parameters within the studied groups was evaluated using Spearman's ranked correlation analysis. The correlation was considered significant at $p<0.05$.

RESULTS

The study of the quantitative parameters of the white blood revealed increased total leukocyte count in IM patients during the acute period of the disease (*vs.* the norm) and "atypical mononuclears" (AM). Comparative analysis of these parameters in patients with IM of different etiology showed no statistically significant differences (Table 1). The counts of lymphocytes and AM (except the absolute count of lymphocytic cells in children aged 7-14 years with OIM) remained elevated during convalescence and remote period after the disease (Table 1). On the other hand, during the clinical rehabilitation stage the mean count of AM in younger patients with OIM was 1.8 times higher than in patients of the same age with EBV-IM (Table 1). During remote period after disease (16-18 months) the absolute count of AM remained elevated (1.7 times) in patients aged 7-14 years in comparison with patients of the same age with EBV-IM (Table 1).

Electron microscopy of lymphocytes from patients with EBV-IM and OIM during manifest clinical and hematological disease also showed common changes: increased (compared to normal) number of smooth forms of cells and decreased counts of lymphocytes with thread-shaped and lamellar processes on membranes (in children aged 3-6 years; Table 2). Lymphocytes which could not be classified by their surface microrelief in accordance with the above-listed topographic criteria appeared in the blood of IM patients of both age groups. The spherical part of these lymphocytes was covered with microvilli and the elongated part was covered with folds (Fig. 1). No cells of this type were detected in healthy children. These lymphocytes were referred to group 6 (lymphocytes with "transitional" surface).

During clinical convalescence the counts of smooth and lamellar lymphocytic cells returned to normal in younger patients with EBV-IM, while the number of lymphocytes with microvilli remained low (Table 2).

TABLE 1. Quantitative Parameters of Peripheral Blood ($\times 10^9$/liter) in Healthy Children and Patients with IM (numerator: 3-6 years; denominator: 7-14 years; $X \pm m$)

Parameter	Healthy children	Children with EBV-IM			Children with OIM		
		acute period of disease	convalescence	16-18 months after disease	acute period of disease	convalescence	16-18 months after disease
Total leukocyte count	5.96±0.27	10.38±0.67*	8.43±0.84***++	6.73±0.93	10.40±0.74*	7.40±0.80***	8.83±1.30**
	5.87±0.31	10.04±0.25*	5.99±0.64++	5.75±0.36	8.28±0.70*	6.38±0.51++	6.93±0.65
Lymphocytes	3.04±0.23	5.83±0.55*	4.72±0.49**	4.36±0.71***	6.06±0.62**	4.40±0.38**	5.56±0.88**
	2.76±0.24	4.26±0.59**	3.82±0.38***	3.50±0.27***	3.95±0.44***ooo	3.30±0.28	3.53±0.37
AM	0.15±0.03	1.48±0.31*	0.54±0.10*+	0.43±0.09*	1.38±0.19*	0.97±0.21*++	0.64±0.21***
	0.12±0.04++	1.80±0.46*	0.59±0.17*++	0.35±0.06*	1.26±0.28*	0.34±0.07*+oo	0.60±0.11***++

Note. Here and in Table 2: *p<0.001, **p<0.01, ***p<0.05 compared to healthy children; +p<0.01, ++p<0.05 compared to acute period of EBV-IM; xp<0.01, xx$p$<0.05 compared to convalescence after EBV-IM; op<0.001, oo$p$<0.01, ooo$p$<0.05 compared to denominator.

TABLE 2. Distribution of Peripheral Blood Lymphocytes by the Type of Surface Architectonics (%) in Healthy Children and Patients with IM (numerator: 3-6 years; denominator: 7-14 years; $X \pm m$)

Types of cell surface microrelief	Healthy children	Children with EBV-IM			Children with OIM		
		acute period of disease	convalescence	16-18 months after disease	acute period of disease	convalescence	16-18 months after disease
Smooth	13.57±2.20	34.31±4.61*	21.58±5.56++	17.66±4.04	34.98±4.61*	25.07±4.62**++	19.53±5.64
	36.48±3.24o	29.97±8.95	32.20±3.24	33.65±3.18o	35.77±5.55	34.20±6.11	21.28±4.13***++
With processes							
thread-shaped	55.29±3.09	41.30±3.85**	40.13±4.35***	53.86±5.27	39.39±4.53***	45.43±4.49	56.06±7.97
	46.04±3.44	41.78±11.00	31.95±3.94***	37.41±3.75o	37.38±3.84	38.64±8.28	42.09±4.33
lamellar	25.86±2.18	15.91±2.10**	29.09±4.03+	19.94±3.25xx	16.32±2.19**	21.82±4.06	17.02±3.44
	11.65±1.73o	20.19±1.60**	22.50±3.17**	19.78±2.85***	16.59±2.62	15.61±2.61	28.76±3.76*ooxx
spheroid	3.67±0.65	4.01±0.88	3.81±0.78	4.87±1.43	3.36±0.96	3.34±0.83	2.57±0.58
	3.95±0.72	3.76±0.57	6.63±0.77***++ooo	5.22±1.05	4.25±1.09	4.53±1.51	3.96±0.97
Transitional	0	3.34±0.95	1.95±0.76	1.65±0.64	3.76±1.22	2.36±0.40	2.86±1.89
	0	2.85±0.20	1.31±0.41+	2.54±0.59	3.59±0.86	2.44±0.87	1.40±0.56
With depressions	1.61±0.35	1.13±0.45	3.44±1.58	2.02±0.66	2.20±0.76	1.98±0.78	1.96±1.24
	1.88±0.63	1.45±0.65	5.41±1.77**oo	1.40±0.37x	2.42±0.63	4.58±1.69	2.51±1.17

On the other hand, in 7-14 year-old patients convalescing after EBV-IM the decrease in the count of villous cells was paralleled by an increase in the number of lymphocytes with lamellar growth, bubbles, and depressions on the membranes in comparison with the counts of these cells in healthy children of the same age (Table 2). In younger convalescents after OIM the counts of lymphocytes with smooth surface remained increased, while the number of cells with outgrowth (villi, folds, and ruffles) increased to a level observed in normal controls (Table 2). Transitional forms of lymphocytic cells were detected in patients of both age groups with EBV-IM and OIM during convalescence and 16-18 months after IM (Table 2). Moreover, the number of lamellar lymphocytes remained elevated *vs.* the normal in the older group (in children aged 7-14 yars) of convalescents after EBV-IM and OIM. By contrast, the number of smooth forms in older group of convalescents after OIM remained decreased *vs.* the control (Table 2).

The detected changes in the surface architectonics of lymphocytic cells in IM can be caused by changed ratio of circulating T and B cell fractions (increased percentage of T cells, for which smooth type of surface membrane is a differentiation sign [14]), on the one hand, and, which is more probable, by decreased functional activity of infected lymphocytes, on the other. Some scientists claim that the smooth membrane is typical of "silent" functionally inert T and B lymphocytes. The authors also note that the type of lymphocyte surface (smooth or villous) can depend on the degree of cell differentiation [8].

One of the possible causes of decreased counts of lymphocytes with thread-like and lamellar processes in the blood of younger children during the acute period of EBV-IM and OIM is transformation of virus-

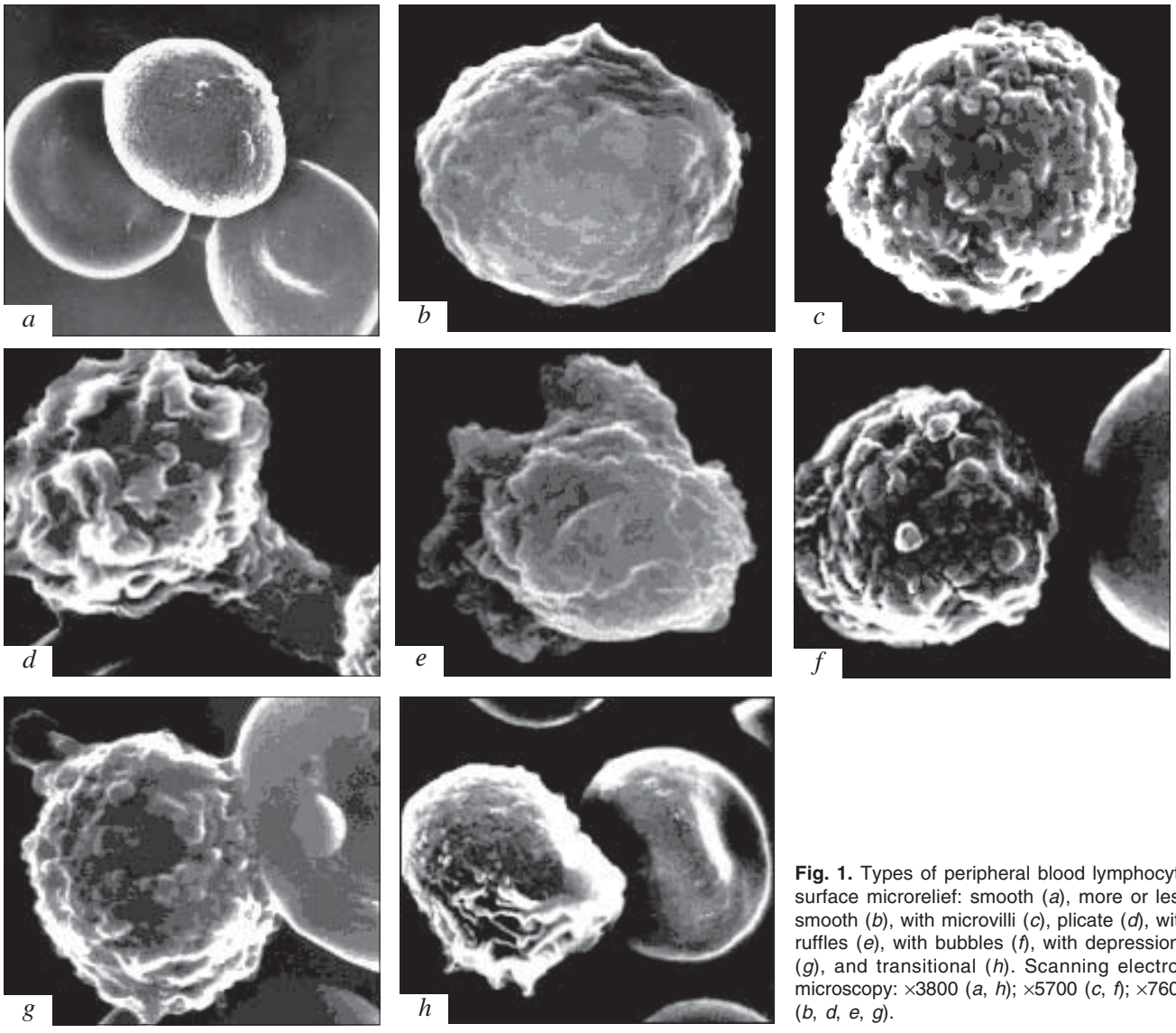

Fig. 1. Types of peripheral blood lymphocyte surface microrelief: smooth (*a*), more or less smooth (*b*), with microvilli (*c*), plicate (*d*), with ruffles (*e*), with bubbles (*f*), with depressions (*g*), and transitional (*h*). Scanning electron microscopy: ×3800 (*a*, *h*); ×5700 (*c*, *f*); ×7600 (*b*, *d*, *e*, *g*).

infected B lymphocytes (these types of surface architectonics are more typical of B lymphocytes than of T cells) into atypical cells, which is confirmed by the results of analysis of correlations. A negative correlation between the absolute count of AM and percentage of lymphocytes with lamellar surface (r=-0.44, p<0.05) was observed during the acute period of EBV-IM. Other scientists reported similar data [4]; they showed that AM were irregularly shaped elongated cells with surface covered by numerous deep wide folds and fissures. In addition, our hypothesis is in line with the data that viruses can disorganize the cytoskeleton system inducing shortening of the villi and decrease in their number on the cell surface [2]. For example, the absolute number of atypical cells in convalescents after EBV-IM was in positive correlation with the number of smooth forms of lymphocytes (r=0.56, p<0.05), while in convalescents after OIM (16-18 months after disease) the correlation between the counts of AM and smooth-membrane lymphocytes was negative (r=-0.69, p<0.01).

The surface ultrastructure of AM remains a disputable point. Lymphocytes with transitional configuration of surface microrelief (with spherical part covered with microvilli and plicated elongated part) present in the blood of patients with EBV-IM and OIM can also be identified as AM (blast-transformed T and B immunocytes). Poor differentiation of transitional lymphocytes was noted previously [10]. In addition, a positive correlation between the content of transitional forms of lymphocytic cells and AM was observed in patients with EBV-IM during clinical recovery (r=0.55, p<0.05).

When interpreting the phenomenon of increased or decreased count of lymphocytes with spheroid formations and ruffles on the membrane in patients with IM of different etiology we took into consideration the fact that bubbles and ruffles are dynamic structures, whose formation and retraction take about 1 min [1]. Therefore speculations about the causes of bubbles and ruffles on lymphocyte surface (whether they are caused by the infection or by structural and functional characteristics of the cells) are dubious. However, there is an opinion that the development of bubbles can be caused by uncontrolled water consumption and disorders in its utilization by the cell [5]. It is also known that "excessive" cell surface (up to 100 μ^2) can be "reserved" in bubbles and ruffles, this promoting the realization of the metabolic functions of the cell [2]. Presumably, folds play the same role as ruffles, but in contrast to ruffles, folds are stable lamellar formations of the cell membrane [2,8].

Hence, changes in the morphology and structure of the peripheral blood lymphocytic cells in EBV-IM and OIM are universally directed and are characterized by increased percentage of smooth cell forms in the population in parallel with decrease in the number of cells with microvilli and lamellar formations on the membrane and appearance of "transitional" forms of lymphocytes. Disorders in the surface architectonics of lymphocytes are observed not only during the acute period of IM, but persist during remission and remote period (16-18 months) after the disease. The severity of these shifts depends on the disease agent and age-specific features of the patient. The most profound and stubborn disorders in the structural organization of lymphocyte membrane are caused by EBV in comparison with other agents of IM; the disorders are more pronounced in children aged 7-14 years than in those aged 3-6 years.

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