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DNA Damage in Mononuclear Blood Cells of Patients with Systemic Lupus Erythematosus

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Alkaline electrophoresis of DNA in individual cells (the DNA comet method) showed that the mean DNA damage is increased in blood mononuclear cells and the proportion of cells containing damaged DNA is higher in patients with systemic lupus erythematosus than in healthy donors; the number of hypodiploid cells is increased, indicating intensified apoptosis.

Key Words: *systemic lupus erythematosus; DNA damage; blood cells*

Cytogenetic studies showed that the incidence of spontaneous mutations and *in vitro* sensitivity of blood cells to mutagens in patients with systemic lupus erythematosus (SLE) [9] are increased [1]. The production of potentially mutagenous active oxygen species and oxidative damage to DNA are higher in the patients than in healthy subjects [4,6,14].

On the other hand, the occurrence of apoptosis increases in SLE and in some other autoimmune diseases [5,8,12,17]; apoptosis is a mechanism for elimination of defective aging cells and cells with numerous DNA injuries [13]. Apoptosis in such cases is associated with increased excretion of degraded DNA and histones into the extracellular space, which promotes the development of an autoimmune reaction [8]. Autoimmune antibody attack enhances DNA damage and accelerates the progression of the disease [10,11,13].

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The aim of this study was to assess DNA damage in peripheral blood mononuclear cells of SLE patients by alkaline electrophoresis of individual cell DNA.

MATERIALS AND METHODS

Blood was collected from normal subjects and patients with SLE (ACR diagnostic criteria [16]) - 13 women and 1 man hospitalized at the Institute of Rheumatology routinely treated by prednisolone (daily dose of 5-40 mg) without cytostatics.

Mononuclear cells were isolated from heparinized blood after 2- to 3-fold dilution with RPMI-1640 by centrifuging in Ficoll-Verograffin gradient (1.076-1.079 g/cm^3). Isolated cells were washed and suspended to a concentration of 10^6/ml in RPMI-1640 with 10% bovine serum. According to the Trypan Blue exclusion test, >95% cells were viable.

Cells were immobilized in agarose with a low melting point (agarose concentration 0.67%) used as a thin gel layer on a slide [3]. The final concentration of cells was $50\text{-}100\times10^3$/ml. Immediately after gel hardening, the slides were submerged into lysing

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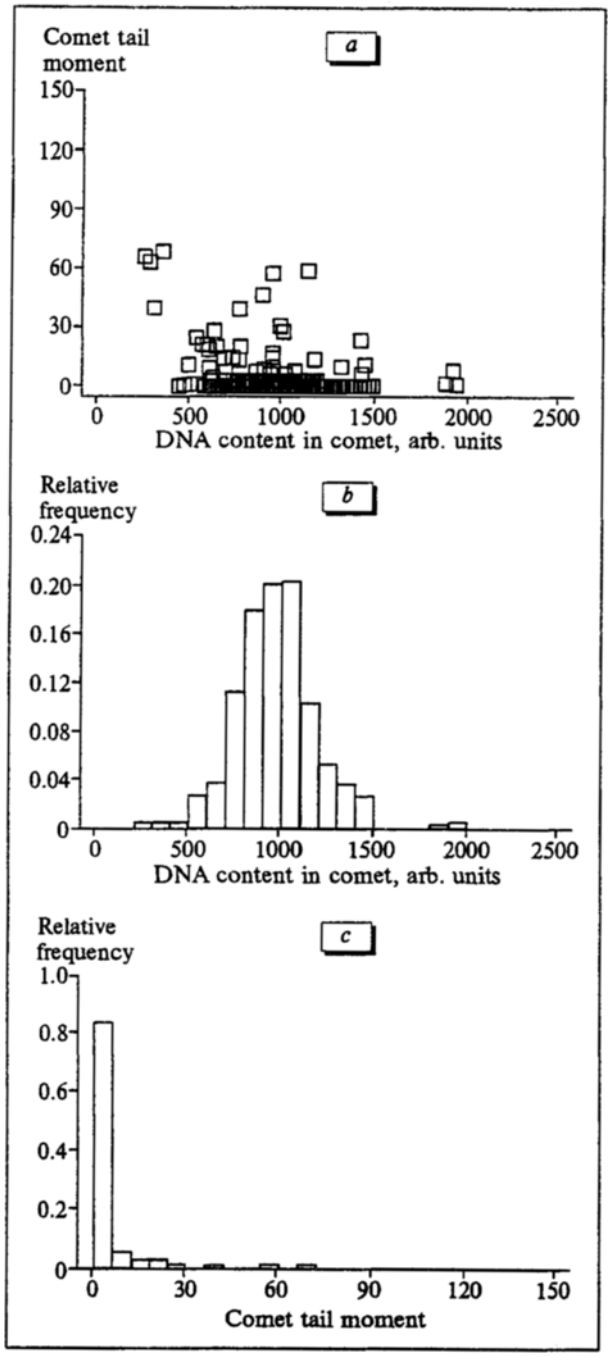

Fig. 1. Bivariant (*a*) and frequency distribution of DNA content (*b*) and comet tail moment (*c*) formed by normal human lymphocytes. Data for 8 donors are presented.

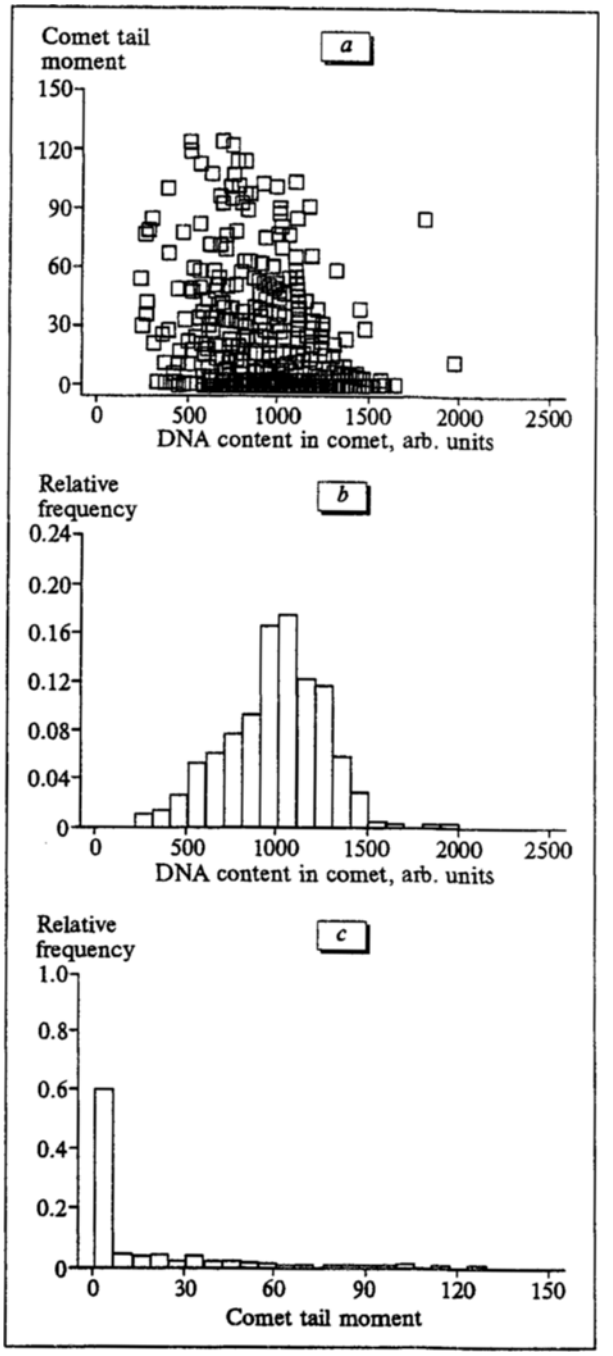

Fig. 2. Bivariant (*a*) and frequency distribution of DNA content (*b*) and comet tail moment (*c*) formed by lymphocytes of patients with systemic lupus erythematosus. Data for 14 patients are presented.

solution containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% lauryl sarcosinate, 1% Triton X-100, and NaOH to attain pH 9.5-10. Lysis was carried out at ambient temperature in the darkness overnight, after which the slide was washed in water and put in denaturing solution (30 mM NaOH+1 mM EDTA) for 1 h to transfer the DNA into a single-strand form. Electrophoresis (1 V/cm, 20 min) was performed in the same solution. After electrophoresis, the slides were neutralized with 0.4 M Tris (pH 7.4), stained with ethidium bromide (10 μg/ml), covered with a coverslip, and examined under a microscope. Photoamplifier was used as a detector of fluorescence. Unidimensional scanning was performed with a rectangular slit probe with optic size of 8×56 μm^2; the fluorescence intensity was auto-

matically recorded every 2 μm. The resultant scanogram helped assess the size of the comet and its parameters: length (*Lc*), DNA content (*Mc*), length of the tail (*Lt*), and comet tail moment (*mt*) [3]. At least 50 comets were scanned for each slide. The results of scanning were presented as distribution of DNA content in the cells (integral fluorescence of the comet, *Mc*), by the moment of comet tail (*mt*), and bivariant distributions of these two independent variables. The former two distributions are presented as frequency histograms; bivariant distributions represent a multitude of points in the *Mc* and *mt* coordinates. Histograms were compared using nonparametrical Kolmogorov-Smirnov statistics. The mean values were compared using Student's *t* test.

RESULTS

As seen from Fig. 1, donor cells are characterized by symmetrical distribution of DNA content (Fig. 1, *b*). The proportion of cells with decreased DNA content (hypodiploid cells) is less than 2%.

After electrophoresis, the majority of donor cells either retained the shape of symmetrical nucleoid spheres or formed slightly asymmetrical comets with short tails. The proportion of the latter cells was no more than 10%. Hypodiploid cells formed the most asymmetric comets with *mt* of more than 40. This indicates a high degree of DNA damage in these cells.

Fidure 2 shows the results of investiggation of cell from 14 SLE patients. In contrast to mononuclear cells of healthy donor, cells of SLE patients were characterized by obvious asymmetry of *Mc* distribution, with the proportion of cells with low DNA content increased more than three-fold: no more than 1.5% for donors and more than 5% for patients (Fig. 2). Comparison of the distributions presented in Figs. 1, *b* and 2, *b* by the Kolmogorov—Smirnov statistics revealed a statistically significant difference ($p<0.01$).

Notable changes were observed in the distribution of cells of SLE patients with regard to *mt* (Fig. 2, *a*, *c*). The proportion of cells forming asymmetric comets with $mt>10$ increased 6-fold in comparison with donors and was 36% ($p<0.001$). The increase of the number of comets was paralleled by increase in the comet tail moment: cells with comets with $mt>80$ appeared (Fig. 2, *a*, *c*).

Figure 3 presents the functions of medium *mt* of comets derived from bivariant distributions (Figs. 1, *a*, 2, *a*). Averaging was carried out for *Mc* bands of 200, 200-400, 400-600, etc. A common feature of this function for both groups was an increase in the mean tail moment with a decrease in *Mc* (this decrease was not always monotonous). The difference

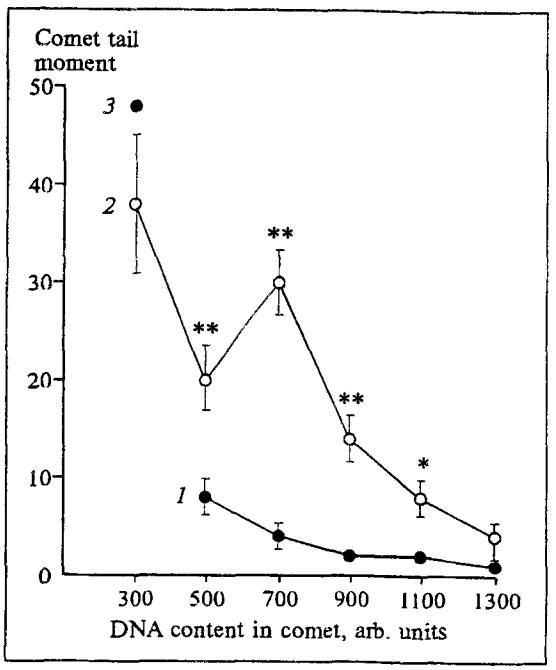

Fig. 3. Relationship between comet tail mean moment and DNA content in the comet for donors (*1*) and patients with systemic lupus erythematosus (*2*). Each point is a result of averaged data presented in Figs. 1, *a*, and 2, *a*, respectively. Point (*3*) representing the mean of 4 values is statistically insignificant. $*p<0.01$, $**p<0.001$ *vs.* donors.

between donors and patients was significant at *Mc* 200-1200 ($p<0.01$).

In many studies by the DNA-comet method the accumulation of defects in DNA (single- and double-strand ruptures) facilitated migration of DNA during electrophoresis.

The comet tail moment (*mt*) is the product of comet tail length and DNA content in it. It means that increased *mt* reflects not only a higher rate of DNA migration in electric field at the expense of its greater damage, but its changed (weaker) association with the nuclear matrix, which leads to an increase of the proportion of DNA migrating to the comet tail.

Thus, our results show that DNA in peripheral mononuclear cells of SLE patients is damaged to a greater extent than in donors. This effect manifests itself as an increased baseline DNA damage and as a statistically significant increase in the count of cells containing damaged DNA.

Another change in the cells of SLE patients is increased count of hypodiploid cells. Their appearance is most often interpreted as the evidence of programmed cells death (apoptosis) [7]. An important feature of apoptosis is internucleosomal DNA degradation and excretion of low-polymeric chromatin fragments into extracellular space [15]. In addition, intracellular proteases are activated, impairing DNA assocation with the nuclear matrix.

In our case these two processes manifest by a much higher electrophoretic mobility of DNA in hypodiploid cells than in cells with normal DNA content (Fig. 3).

Simultaneous increase in the count of cells with damaged DNA and in that of apoptotic cells confirms the current opinion about the relationship between these processes [13]. However, if DNA damage is regarded as undesirable, because it underlies mutagenesis and carcinogenesis [2], apoptosis in SLE patients cannot be regarded so unambiguously. On the one hand, the adaptogenic and compensatory significance of apoptosis is obvious, since apoptosis leads to elimination of genetically defective cells. On the other hand, it is not clear how intensification of apoptosis is related to the course of SLE. Investigation of this problem is important for improving SLE therapy and for a better understanding of the etiology and pathogenesis of this disease.

The use of genome-protecting drugs in combined therapy of SLE [2] may reduce the risk of tumors and have a favorable effect on the disease course by destroying the vicious circle of DNA damage, apoptosis, and autoimmune reaction.

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