RELATIONSHIP BETWEEN SPONTANEOUS INSTABILITY OF CHROMOSOMES AND SISTER CHROMATID EXCHANGES IN FANCONI'S ANEMIA

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The study of the principles governing the onset of spontaneous instability of chromosomes in certain rare hereditary human diseases with disturbance of individual stages of the DNA repair process and, in particular, in Fanconi's anemia (FA) is essential not only for an understanding of the etiology of these diseases, but also to explain the mechanisms of formation of chromosomal aberrations (CA). Much information on the frequency of both spontaneous and induced CA and sister chromatid exchanges (SCE) in the cells of patients with FA has accumulated in the literature. However, there have been few investigations into the dynamics of spontaneous instability of chromosomes in cell generations with attempts to determine a possible relationship between CA and SCE in these cells, and their results are ambiguous [3-6].

The aim of this investigation was to study the frequency and spectrum of spontaneous CA in patients with FA in lymphocytes of different mitotic divisions and the connection between CA and the locations of SCE.

EXPERIMENTAL METHOD

Chromosomes of peripheral blood lymphocytes of a 6-year-old girl with FA and of four normal subjects were studied. This case of FA corresponded clinically to the following diagnostic criteria: small birth weight, pancytopenia of the bone marrow, marked anemia, delayed growth and development, hypoplasia of the right thumb, anomalies of pigmentation of the skin, and a high frequency of spontaneous instability of chromosomes. Whole peripheral blood, in a volume of 0.5 ml, was cultured for 72 h in the presence of 5-bromodeoxyuridine (BUdR) in Eagle's medium containing 20% bovine serum and 0.015 ml of phytohemagglutinin (PHA, from Difco, USA). The final concentration of BUdR in the medium was 10 μg/ml. The cultures were incubated in darkness. Cells were fixed and chromosome preparations obtained by the usual method. Sister chromatids were differentially stained by the method described in [2]. The experiments were conducted in three repetitions. The following cytogenetic parameters were analyzed: 1) the serial number of mitosis of the cell; 2) chromosome breaks, structural changes, and endoreduplications; 3) the number of SCE per cell; 4) if a chromatid break was presented in a cell at the second mitosis the location of the injury was recorded – at the site of the SCE, in the parlely or darkly stained chromatid. The results were subjected to statistical analysis by the chi-square and Student's t tests.

EXPERIMENTAL RESULTS

Table 1 summarizes the results of three repetitions of experiments to study the frequency of spontaneous instability of chromosomes in a patient with FA and in normal subjects. The spontaneous level of CA was approximately 20 times higher in FA. The predominant types of CA were single breaks of chromosomes, and less frequently paired fragments, tri- and quadriradials, and endoreduplications were found. Addition of BUdR to the culture had no effect on the frequency and spectrum of CA, as is clear from a comparison of the pooled data on the frequency of CA in the first and second mitoses in cultures with and without BUdR ($P > 0.5$). The frequency of aberrant metaphases fell from 29.6 to 22.6% ($P < 0.05$) and the total number of chromosome breaks fell by 32% from their level in the first mitosis ($P < 0.01$). These dynamics could be due to selection of cells

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TABLE 1. Frequency of Spontaneous Instability of Chromosomes in Peripheral Blood Lymphocytes of First Two Mitotic Divisions in Patient with Fanconi's Anemia and Normal Individuals

Donor of cells	BUdR	No. of mitosis	Number of cells analyzed	Frequency of aberrant metaphases	Percentage of aberrant metaphases	Percentage of endoreduplications	Number of chromosome breaks per 100 cells			
							single	paired	in exchanges	total
Patient with FA	+	1	351	104	29,6	0	28,5	8,8	6,8	44,1
		2	318	72	22,6	1,9	23,3	5,0	1,9	30,2
		Σ	669	176	26,3	0,9	26,0	7,0	4,5	37,5
	—	Σ	300	77	25,7	0,7	22,0	9,0	6,0	37,0
Control	—	Σ	1200	17	1,4	0	0,9	0,5	0	1,4

TABLE 2. Efficiency of Lymphocyte Proliferation in Patient with FA and Normal Individuals

No. of mitosis	FA		Control	
	number of cells	%	number of cells	%
1	377	53,2	198	39,0
2	329	46,4	291	57,3
3	3	0,4	19	3,7

most severely affected by structural chromosomal aberrations. However, spontaneous instability of chromosomes in the second mitosis in cells from the patient with FA was considerably higher than the control level, indicating a high frequency of reappearance of CA in this disease.

The efficiency of lymphocyte proliferation in FA (Table 2) was appreciably lower than in normal individuals ($P < 0.001$). Most cells in lymphocyte cultures from the patient with FA were in the first mitotic division, and the frequency of metaphases of the third mitosis was only 0.4%. In control cultures of normal individuals cells in the second mitosis constituted the modal class, and the frequency of metaphases of the third mitosis was almost an order of magnitude higher than in the patient with FA.

The mean number of SCE per cell in FA was 1.7 times greater than in the control (11.2 and 6.6 respectively; $P < 0.01$). Analysis of the frequency of appearance of spontaneous single chromosome breaks at the site of SCE, and also in palely and darkly stained, i.e., with double and single BUdR substituted chromatids, demonstrated their unequal vulnerability (Table 3). The ratio of the number of chromatid deletions in darkly obtained chromatids to the number of breaks at the site of SCE and to their number of palely stained chromatids was 0.5:1:2 ($P > 0.7$). Since there was no difference in the frequency of CA in cultures with and without BUdR, the more frequent injury to the palely stained chromatids is difficult to explain on the basis of the sensitizing effect of the presence of BUdR in two DNA chains. The more frequent injury to the daughter DNA chains in FA was most probably due to the actual mechanism of formation of CA in this disease. The high frequency of appearance of CA in FA at the site of SCE, which was 33.8%, is noteworthy. These results are in agreement with existing data [3]. Considering the finite resolving power of the light microscope, as a result of which exchange between sister chromatids can be recorded in a certain region of the chromosome for a definite length which amounts to about 0.0018 of the total length of the human karyotype [1], it can be calculated that on average 33.8% of all single chromosome breaks occur on 2% of the length of all the chromosomes, which is occupied by SCE. Hence it follows that in FA chromosomes at sites of SCE are injured about 25 times more often than regions of palely and darkly stained chromatids.

The nonrandomness of appearance of CA at SCE sites raises the question of the mechanism of this interrelationship. Despite the generally accepted view that CA and SCE are the end result of different pathways of realization of primary injuries to DNA, it can be postulated that in some cases, especially in FA, the mechanism of formation of CA is closely linked with the process of SCE formation. The results also suggest that the mechanism of formation of spontaneous CA in diseases with disturbance of DNA repair differs significantly from the mechanisms of formation of induced CA in normal cells. For instance, x-rays induced a very small

TABLE 3. Number of Spontaneous Single Breaks in Single and Double BUdR Substituted Chromatids and at Sites of SCE in Fancoli's Anemia

Number of single chromosome breaks	Chromatids		
	darkly stained	in exchange	palely stained
Absolute	10	25	39
%	13,5	33,8	52,7

number of chromatid breaks in Chinese hamster cells at sites of SCE [8], whereas in FA, according to our data, a considerable proportion of chromatid deletions (33.8%) arises in precisely these sites. This value is close to the frequency of appearance of CA in SCE sites predicted to Revell's exchange hypothesis of CA formation, which would be 40% [8]. Deficiency of one enzyme of the recombination system in FA, responsible for repair of spontaneous DNA injuries of the type of cross-linkages between strands, arising in the cell, and SCE formation, may perhaps lead to the formation of chromosome breaks. This hypothesis is also in agreement with data in the literature [4]. The results require further verification on a more extensive material, for evidence has been obtained on the genetic heterogeneity of FA [7, 9]. Accumulation of data on the relationship between CA and SCE in FA will allow the character of this relationship and the mechanism of CA formation in this disease to be presented in a clearer form.

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