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SPONTANEOUS CHROMOSOMAL ABERRATIONS IN DIFFERENT TYPES OF CELLS FROM RHESUS MONKEYS

L. P. Kosichenko

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Because monkeys are widely used for experimental purposes the study of the spontaneous level of chromosomal aberrations is important for the establishment of cytogenetic norms, for the assessment of induced chromosomal mutations, and also to shed light on the nature of the mutation process [1]. However, information in the literature on this subject is scanty [4, 5, 9].

The spontaneous level of chromosomal aberrations in monkeys was studied in different types of somatic cells with different rates of proliferation: bone marrow, peripheral blood lymphocytes, and kidney epithelial cells.

EXPERIMENTAL METHOD

Cytogenetic norms were studied in 25 monkeys (12 males and 13 females) in which 4290 metaphases were analyzed in 33 experiments. In 16 animals chromosomes in a single tissue were studied: bone marrow, peripheral blood lymphocytes, or kidney epithelial cells. In another eight monkeys either the first two or the last two types of cells were studied simultaneously, and in the remaining monkey cells of all three types were investigated. Because of the very low mitotic activity, bone marrow was not studied in the remaining monkeys of this group.

Data on the spontaneous level of chromosomal aberrations in monkeys were obtained for the following age groups: immature monkeys aged 2-3 years, mature monkeys aged 6-10 years, middle-aged monkeys aged 14-19 years, and old monkeys aged 21-22 years. The material was always obtained at the same time of day – in the morning.

In each animal 100-200 metaphases were analyzed to discover structural aberrations in chromosomes identifiable without karyotypic analysis. Cells with 40-43 chromosomes and polyploids were examined; the normal karyotype of Macaca rhesus is 2n-42 [6, 10]. All types of aberrations were taken into account; stained regions of chromosomes displaced relative to their axis or length were interpreted as fragments.

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TABLE 1. Spontaneous Chromosomal Aberrations in Different Types of Somatic Cells from Monkeys

Type of cells	Number of experiments	Age of monkeys, years	Number of cells studied	Type of aberrations per 100 cells					No. of aberrations/100 cells	
				fragments		centric rings	interstitial deletions	abnormal chromosomes	limits of variation	total
				single	paired					
Bone marrow cells	3	2—3	400	0,66	0,17	—	—	—	0,5—1,0	0,83±0,45
	4	6—10	400	0,52	0,30	—	—	—	0,0—2,0	0,88±0,46
	6	14—19	1130	0,68	0,36	—	0,1	—	1,0—1,21	1,12±0,3
Total	13	—	1930	0,62±0,17	0,29±0,12	—	0,03±0,04	—	—	0,94±0,22
Blood lymphocyte	6	7—11	1005	0,39	0,34	—	—	0,66	1,0—2,0	1,39±0,37
	3	14—19	390	0,84	0,60	—	—	0,3	1,42—2,2	1,74±0,67
Total	9	—	1395	0,61±0,21	0,47±0,18	—	—	0,48±0,18	—	1,56±0,33
Kidney epithelial cells	8	7—11	880	0,96	0,47	—	—	0,09	0,8—2,13	1,52±0,41
	3	21—22	485	1,19	0,63	0,19	—	—	1,52—2,27	2,01±0,64
Total	11	—	1365	1,07±0,08	0,57±0,2	0,09± 0,08	—	0,03±0,04	—	1,76±0,36

The method of processing of the bone marrow was described previously [8]; blood lymphocytes were processed by Moorhead's method [11] with the addition of donors' blood of group AB (IV) in a volume equal to that of plasma. No antibiotics were added to the cultures. Cultures of kidney epithelial cells were obtained by the usual method and fixed on the 3rd-4th day after appearance of islets of growth. The times for fixation of the blood lymphocyte and kidney epithelial cell cultures were chosen to allow for maximal mitotic activity and minimal admixture of cells of the second and subsequent mitoses [3, 7].

The results are presented as generalized data obtained from clinically healthy monkeys receiving a general diet.

EXPERIMENTAL RESULTS

Chromosomal Aberrations. Examination of 1930 bone marrow metaphases revealed for the whole group of monkeys 0.94 ± 0.22 aberrations per 100 cells, in 1935 peripheral blood lymphocytes there were 1.56 ± 0.33 aberrations, and in 1365 kidney cells in culture there were 1.76 ± 0.36 aberrations (Table 1). The difference between the frequency of chromosomal aberrations in the bone marrow cells and kidney epithelial cells for the group as a whole was close to statistically significant. The mean spontaneous frequency of chromosomal aberrations in the peripheral blood lymphocytes did not differ significantly from that for bone marrow and kidney epithelial cells ($P > 0.05$). To rule out any possible individual differences in the level of spontaneous chromosomal aberrations these indices were compared in different types of cells in the same monkeys. The results indicate that in all the monkeys studied, despite the fact that the differences were not significant, the spontaneous frequency of chromosomal aberrations was as a rule higher in the kidney epithelial cells and lowest in bone marrow cells.

Another distinguishing feature of the spontaneous level of chromosomal aberrations was a tendency for the frequency of aberrations to increase as the monkeys grew older. The pattern observed was the same for all types of somatic cells. Examination of the spontaneous level of chromosomal aberrations in animals of different sexes likewise revealed no significant differences. For instance, in the bone marrow of females there were 0.78 ± 0.46 aberrations, compared with 0.98 ± 0.57 in the group of males; in peripheral blood lymphocytes of females there were 1.64 ± 0.34 aberrations compared with 1.39 ± 0.62 in males.

Types of Aberrations. As Table 1 shows, under normal conditions cells of each type were characterized mainly by acentric aberrations of the single and paired fragments type. However, in peripheral blood lymphocytes and kidney epithelial cells, by contrast with bone marrow, abnormal chromosomes also were found. These abnormal chromosomes appeared as a result of symmetrical chromosomal exchanges, as shown by the dimensions and morphology of the structurally changed chromosomes. Aberrations arising as a result of asymmetrical chromosomal exchanges (centric rings) also were found in the kidney epithelial cells of old monkeys.

Aneuploidy. Hypodiploid sets of chromosomes were present in between 10.27 ± 0.8 and 25.91 ± 1.18% of cells in different tissues. Only 0.13-0.23% of cells were hyperdiploids with 43 chromosomes. In cells of all types polyploid sets of chromosomes also were found, numbering 0.83 ± 0.24% in the bone marrow, 0.39 ± 0.22% in the blood lymphocytes, and 1.67 ± 0.34% in the kidney epithelial cells. Hyperdiploid sets of chromosomes, the most objective indicator of aneupoloidy, were thus absent in the monkeys examined. Accordingly, all deviations from the modal class of chromosomes were regarded as artefacts. Loss of chromosomes could arise during preparation of the specimens: during treatment with hypertonic solution, trypsinization of the material, and after other procedures. Similar deviations from the modal number of chromosomes have been observed in the case of human peripheral blood lymphocytes [2].

It can thus be concluded that the level of spontaneous chromosomal aberrations does not differ significantly in different types of somatic cells in Macaca rhesus. However, the results of the investigation indicate that in all experiments there was a tendency for the frequency of chromosomal aberrations to be higher in tissues with a low rate of proliferation than in the continually renewed bone marrow tissue. Differences in spontaneous frequencies of aberrations in monkeys, expressed as a lower frequency in the bone marrow and a higher frequency in kidney epithelial cells, have been observed by other workers also [5]. One factor determining the frequency of chromosomal aberrations in a tissue is its proliferative activity. The lower level of spontaneous chromosomal aberrations in the bone marrow cells of monkeys can be explained by the continuous elimination of injuries, whereas in peripheral blood lymphocytes and in kidney epithelial cells, which spend a longer time in interphase, conditions favor their accumulation. This is confirmed by the presence of symmetrical and asymmetrical chromosomal exchanges in these tissues. The larger number of single fragments also suggests that these aberrations appear in the course of replication of the chromosomes.

Another fact to be noted is that as the monkeys grow older there is a tendency in each tissue for the number of chromosomal aberrations to rise, in good agreement with data in the literature [4]. Analysis of the results of these experiments and of data in the literature also showed that the spontaneous level of structural mutations in chromosomes is mainly made up of aberrations of the single and paired fragment type. This feature sharply distinguishes the process of spontaneous mutation from changes observed in monkeys in the later period after exposure to radiation [8].

Accumulation of metabolic products in the body and a decrease in the intensity of mitotic activity with an increase in age of the monkeys are thus among the principal factors responsible for age variations in the spontaneous mutation process.

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