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CHROMOSOME ABERRATIONS IN LYMPHOID TISSUES OF DIFFERENT ORIGIN AND IMMUNE RESPONSIVENESS OF *MACACA RHESUS* MONKEYS INFECTED WITH POLIOVIRUS TYPE 1

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Summary. — A virulent strain of poliovirus type 1 induced aberrations in the chromosomal apparatus of both blood lymphocytes and bone marrow cells in infected *Macaca rhesus* monkeys. In addition, there were significant differences in the type and degree of aberrations, occuring in bone marrow cells and blood lymphocytes. Changes were also observed in the mitotic activity of bone marrow cells. Poliovirus inhibited the lymphocyte blast transformation (LBT) to phytohaemagglutinin (PHA), but enhanced the LBT to virus-specific antigen and the reactivity of neutrophils.

Key words: *monkeys*; *chromosome aberrations*; *immunity*; *lymphoid tissue*; *poliovirus*

Introduction

The degree of both spontaneous and induced mutations greatly depends on genotype of the host (Dubinin, 1966; Keskinova and Rapoport, 1968). Differences were also reported in the degree of cytogenetic changes in different tissues of the same host both under normal and mutagenic conditions (Kerkis *et al.*, 1962). At present, great majority of the studies on cytogenetic alterations in mammals *in vivo* have been done in lymphoid cells (Prokofieva-Belgovskaya, 1969; Blyumkin and Zhdanov, 1973). Their populations are extremely heterogeneous which apparently causes differences in the degree and persistence of cytogenetic disorders induced by the mutagens not excluding viruses (Kerkis and Skorova, 1973). Great variations in the sensitivity of different cells to viruses were found in the same host and even among the cells of lymphoid tissue (Grivs, 1980). As shown in our previous paper, Sabin's attenuated trivalent vaccine was able to induce cytogenetic alterations in lymphocytes simultaneously with the rise of antibody levels in immunized *M. rhesus* monkeys (Ilyinskikh *et al.*, 1978).

The present work was aimed at the elucidation of the degree of cytogenetic alterations in lymphoid cells of different origin and at the study of immune responsiveness of *M. rhesus* monkeys infected with virulent strains of poliovirus type 1.

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Materials and Methods

Virus and animals. Macaca rhesus monkeys were inoculated into musculus quadriceps femoris with 10^6 $TCID_{50}$/ml (in a volume of 2.5 ml) of a virulent strain 855/69 (4140) of poliovirus isolated from faeces of a sick child. Studies were conducted at the Sverdlovsk Research Institute of Viral Infections and in the U.S.S.R. Acad. Med. Sciences Institute of Poliomyelitis and Viral Encephalitides on intratypic serodifferentiation according to Wecker's and McBride's methods; according to genetic markers the strain was classified as a "wild" variant of poliovirus type 1 (Gracheva *et al.*, 1975; Zlobin, 1978). Altogether 14 monkeys were examined out of which 8 controls had received placebo.

Cytogenetic investigations. The animals were sacrificed under hexenal anesthesia at the first appearance of clinical symptoms of poliomyelitis (3—7 days post inoculation). Cytogenetic alterations were studied in PHA-stimulated lymphocytes and lymphoid bone marrow cells by the methods of Moorhead *et al.* (1963) and of Ford and Woolam (1960). In each animal, 100 metaphases of either cells were analysed, which is sufficient for complete cytogenetic characterization of these tissues (Prokofieva-Belgovskaya, 1969). In addition, the mitotic regimen was studied in lymphoid bone marrow cells with identification of mitosis pathology according to the method of Blyumkin and Zhdanov (1973). All results were analysed by Student's method.

Immune response. The following aspects of the immune response were studied along with virus distribution in monkeys: LBT to PHA according to the method of Cheredeev (1976), spontaneous blast transformation and blast transformation to virus-specific antigen (thermally inactivated strain 4140 of poliomyelitis virus) according to Hungerford *et al.* (1959), index of neutrophil damage according to Fradkin (1962), plasmocytic reaction in the spleen and regional lymph nodes according to Zemskov (1964) and levels of virus neutralizing antibodies in blood (antibody titres were determined in HEp-2 cells by dr. Ya. B. Beikin, staff member of the Sverdlovsk Research Institute of Viral Infections; for details see Beikin *et al.*, 1980).

Results

Inoculation of *M. rhesus* monkeys with the poliovirus strain 4140 resulted in development of pareses and paralyses as early as within 3—7 days. The virus induced strong chromosomic impairments both in bone marrow cells and in the blood lymphocytes of sick animals. As shown in Table 1 the number of bone marrow cells with chromosomal aberrations varied from 38 to 57 (mean 47.1 $\pm$ 1.8%) against 6—19 in the controls (mean 12.9 $\pm$ $\pm$ 3.4%; $P < 0.01$); in blood lymphocytes the aberrations varied from 42 to 78 (mean 63.3 $\pm$ 4.9%) against 3—15 in controls (mean 8.4 $\pm$ 1.3%; $P < 0.01$). In the bone marrow cells, most prevalent chromosomal aberrations consisted of premature chromatid disjunctions of the centromere (26.7 $\pm$ 5.7% against 3.8 $\pm$ 1.4% in the controls; $P < 0.01$). In leukocytes this type of aberrations — absent in controls — amounted to 4.7 $\pm$ 2.0%. Besides, in the bone marrow cells of infected but not control monkeys, cells appeared with chromatid type of chromosome breakage (1.0 $\pm$ 0.0%). Among the cells with altered chromosome numbers, the infected monkeys had an increased number of hypoploid cells (15.0 $\pm$ 1.0% against 4.5 $\pm$ $\pm$ 1.3% in the controls; $P < 0.01$). There was no increase in the frequency of other types of cytogenetic impairments in the bone marrow cells. A considerably more variable spectrum of changes in number and structure of chromosomes was observed in blood lymphocytes among which 27.7 $\pm$ $\pm$ 2.0% (in controls 5.8 $\pm$ 2.0%; $P < 0.01$) had an altered chromosome number and 36.3 $\pm$ 2.0% (in controls 2.6 $\pm$ 0.7%; $P < 0.01$ had structural aberrations of chromosomes). The most frequent were cells with hypoploid karyotype (24.0 $\pm$ 1.7% against 5.0 $\pm$ 1.7% in controls; $P < 0.01$), then

Table 1. Cytogenetic aberrations in bone marrow cells and blood lymphocytes of M. rhesus monkeys infected with poliovirus

Cells from	Number of cells (M ± m) with structural disorders of chromosomes				
	Total	Chromosome breakage	Chromatin breakage	Exchanges	Premature chromatid disjunction
Bone marrow					
Control monkeys	6.1 ± 1.9	0.0 ± 0.0	2.3 ± 1.0	0.0 ± 0.0	3.8 ± 1.4
Infected monkeys	29.7 ± 5.3*	1.0 ± 0.0	2.0 ± 0.6	0.0 ± 0.0	26.7 ± 5.7*
Lymphocytes					
Control monkeys	2.8 ± 0.7	1.2 ± 0.8	)1.6 ± 0.7	0.0 ± 0.0	0.0 ± 0.0
Infected monkeys	36.3 ± 2.0*	11.3 ± 0.9*	18.3 ± 1.5*	2.0 ± 0.9	4.7 ± 2.0

	Number of cells (M ± m) with quantitative chromosome aberrations			
	Total	Hypoploid	Hyperploid	Polyploid
Bone marrow				
Control monkeys	6.8 ± 1.9	4.5 ± 1.3	1.0 ± 0.3	1.3 ± 0.4
Infected monkeys	17.0 ± 2.5*	15.0 ± 1.0*	1.3 ± 1.2	0.7 ± 0.0
Lymphocytes				
Control monkeys	5.8 ± 2.0	5.0 ± 1.7	0.2 ± 0.0	0.6 ± 0.4
Infected monkeys	27.7 ± 2.0*	24.0 ± 1.7*	2.0 ± 0.6*	1.7 ± 0.9

Significant differences from controls are designated by asterisks: $p < 0.01$ (*) and at $p < 0.05$ (**) respectively.

Table 2. Immune response and changes in the mitotic regimen of bone marrow cells of M. rhesus monkeys infected with poliovirus

Bone marrow cells	Immune response						
	LBT		Plasmocytic reaction			Index of neutrophil	Antibody levels (1og)
	PHA	Antigen	Spontaneous	Spleen	Lymph nodes		
Control monkeys	70.8 ± 6.4	0.2 ± 0.1	0.2 ± 0.1	2.0 ± 0.7	2.5 ± 0.7	0.07 ± 0.01	0
Infected monkeys	53.7 ± 6.7**	4.3 ± 0.7*	1.0 ± 0.6	6.6 ± 2.7	4.0 ± 1.6	0.16 ± 0.07**	1.4048 ± 0.29

	Mitotic regimen	Mitotic regimen P + M / A + T	Pathology of mitosis (%)
Control monkeys	1.5 ± 0.3	2.7 ± 0.2	6.0 ± 1.4
Infected monkeys	9.0 ± 0.6*	4.4 ± 0.8**	18.0 ± 4.2*

$\frac{P + M}{A + T}$ = ratio of mitotic phases (P = prometaphase, M = metaphase, A = anaphase, T = telophase).
For further explanations see Table 1.

with chromatid breaks (18.3 ± 1.5% against 1.6 ± 0.7 in controls; $P < 0.01$) and chromosome breaks (11.3 ± 0.9% against 1.2 ± 0.8% in controls; $P < 0.01$).

Investigation on the immune response of infected monkeys (Table 2) showed inhibition of blastic transformation to PHA (53.7 ± 6.7% against 70.8 ± 6.4% in control; $P < 0.05$), enhancement of blastic transformation in response to virus specific antigen (from 0.2 ± 0.1% to 4.3 ± 0.7%; $P < 0.01$), and increase of neutrophil damage (from 0.07 ± 0.01 to 0.16 ± ± 0.07; $P < 0.05$). The level of antibody to poliovirus in the infected monkeys was 1.4048 ± 0.29 log. The infected monkeys also showed a trend for increased plasmocytic reaction (in the spleen to 6.6 ± 2.7% and in lymph nodes to 4.0 ± 1.6% against 2.0 ± 0.7% and 2.5 ± 0.7% in the controls, respectively). However, because of considerable individual deviations of this value from the mean level, the results did not differ significantly from controls ($P > 0.05$).

In the bone marrow of the infected monkeys a marked increase in the number of dividing cells was observed (9.0 ± 0.6‰ against 1.5 ± 0.3‰ in controls; $P < 0.01$). Changes in the ratio of mitosis phases due to an increased number of prometaphases and a decrease in the number of anatelophases ($P < 0.05$) were accompanied by an increased number of cells with pathologic divisions mainly at the expense of colchicine-like metaphases (11.7 ± 3.8%); such changes were absent in controls.

Discussion

The results obtained indicate that virulent poliovirus strain induced cytogenetic disorders in both bone marrow cells and blood lymphocytes of infected *M. rhesus* monkeys. A previous report showed that the Sabin's live trivalent poliomyelitis vaccine had also exerted a mutagenic effect on monkey lymphocytes (Ilyinskikh *et al.*, 1978). Thus, virulent and attenuated poliovirus strains are capable of inducing chromosome aberrations. Our own results and the data from literature indicate that the sensitivity of host to infection plays a decisive role in their induction (Nichols and Levan, 1965; Ilyinskikh, 1975). The ability of poliovirus to induce premature chromosome disjunction in the centromere of bone marrow cells was first experimentally demonstrated in mice by Mikhailova and Gorshunova (1969).

Since the replication of poliovirus in mouse cells is considerably lower than in monkeys, it may be suggested that this type of aberration is not so much due to virus replication as due to some effect of non-replicating virus or its components on the centromere area of chromosomes or on the division apparatus of the animal bone marrow cells. This is also supported by our observation of colchicine-like effect of poliovirus on dividing cells of the bone marrow. Colchicine is known to destroy the cell division apparatus and to induce chromosome condensation (Poroshenko, 1977).

Some research workers believe that premature disjunction of chromatids in poliovirus infection is due to allocycly of sister chromatids (Bartsch *et al.*, 1969). Since disjunction in the centromere was not observed in all chromo-

somes of bone marrow cells, it cannot be excluded that certain chromosomes undergo premature condensation which may be caused either by asynchronous replication in the S-period of interphase (Prokofieva-Belgovskaya, 1969) or by non-simultaneous onset of chromosome disjunction in the mitosis (Schaop and Forere, 1979).

Premature disjunction of chromatids was observed largely only in the bone marrow whose cells comprise the bulk of B lymphocytes and their precursors. Therefore, it is quite possible to assume that this type of chromosomal aberration is caused by the process of rearrangement of humoral immunity in response to poliovirus vaccination. T lymphocytes are known to respond much less than B lymphocytes to poliovirus invasion (Lash *et al.*, 1978). It should be noted that an increase in the number of bone marrow cells with colchicine mitosis seems to be reversible (Alov *et al.*, 1974), because, as follows from our data, it does not lead to the appearance of polyploid cells, although these changes might possibly occur at later stages of the infectious process. The infected monkeys were shown to have a reduced number of PHA-transformed cells known to represent T lymphocytes (Cheredeev, 1976). There is some evidence indicating that chromosomal apparatus aberrations lead to a decrease of the proliferative responses of affected lymphocytes to PHA (Prokofieva-Belgovskaya, 1969). Despite of rising antibody levels, the infected monkeys showed no significant enhancement of plasmocytic reaction in the spleen and lymph nodes which appears to agree with the data on the inhibiting effect of poliovirus on splenocytes (Khozinsky *et al.*, 1978). According to the current concepts, the degree of neutrophil damage and LBT response to viral antigen characterize the extent of sensitization of the host (Fradkin, 1978; Vershigora, 1980). Apparently a considerable number of lymphocytes is capable of specific response to virus antigen despite of the increase of cytogenetic aberrations in immunocompetent tissues.

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