CHROMOSOMAL ABERRATIONS IN BONE MARROW CELLS TRANSPLANTED INTO IRRADIATED MONKEYS

G. A. Udalov and I. L. Chertkov

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Chromosomal aberrations appeared in the bone marrow cells of female monkeys transplanted into irradiated thymectomized male monkeys. Similar aberrations were found in the hematopoietic cells after autografting of bone marrow.

This paper describes a study of the chromosomal apparatus of bone marrow cells transplanted into irradiated monkeys.

EXPERIMENTAL METHOD

Monkeys of the species Papio hamadryas and Macaca rhesus were used in the investigation. The monkeys were irradiated with γ rays in a dose of 800-1500 R, and 1.5-2 h later they were grafted with autologous or allogeneic bone marrow in a dose of $(2\text{-}5) \cdot 10^8$ viable nucleated cells/kg body weight. Full details of the methods of irradiation and of obtaining and transplanting the bone marrow will be described elsewhere.

The donors for allografting were females and the recipients were males. Since allogeneic bone marrow in monkeys induces an acute form of secondary disease, with differentiation of hematopoietic cells into lymphocytes, and with rapid allergic death of the developing immunocytes [2], this part of the investigation was carried out on monkeys thymectomized 1.5-3 months before the experiment, to prevent the development of acute secondary disease.

For the karyologic investigation 1-1.5 ml of bone marrow suspension was aspirated from the head of the humerus and incubated for 30-50 min at 37°C in 20 ml of Hanks' solution with 8 μg/ml colchicine and 10 units/ml heparin. Hypotonicity was created by the addition of 100 ml of 0.44% sodium citrate solution for 20 min. After centrifugation, the cells were fixed with a mixture of ethyl alcohol and acetic acid (3:1) and dried films were made by igniting the fixing solution [5].

EXPERIMENTAL RESULTS

The normal karyotype was studied in four monkeys of the species P. hamadryas and two of M. rhesus. In both species of monkeys the modal number of chromosomes was 42. Cells of the male were readily distinguishable from those of the female by the presence of the single acrocentric chromosome (the Y chromosome) in the set (Fig. 1). In both species a pair of marker chromosomes was present, the upper branches of which were connected with the centromere by almost invisible isthmuses. The number of aneuploid cells was about 5%, and these were entirely hypodiploid cells. In 3 of the 216 metaphases studied. deletions were found, and only in 1 was there a chromosomal fragment. On the whole these results agree with those given in the literature [1, 3, 4].

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TABLE 1. Chromosomal Analysis of Hematopoietic Cells of Irradiated Monkeys Receiving Transplanted Bone Marrow

Group	Species of monkey and dose of irradiation	Time after transplantation (in days)	Number of metaphases analyzed	Number of cells with specified number of chromosomes			Number of cells with structural changes in chromosomes*					Number of cells with deletions
				< 42	42	> 42	severely damaged	chromatid fragments	chromosomal fragments	chromatid translocations	chromosomal translocations	
Allogeneic bone marrow	P. hamadryas, 1000 R	2	20				20/0					
		6	0									
		8	30	1	28	–	1/0	2/2	7/6	–	–	2/2
		11	100	5	67	2	26/0	15/14	6/5	2/0	1/0	5/5
		15	50	1	43	–	6/0	4/4	5/4	–	–	2/2
		21	50	2	45	1	2/0	2/1	4/4	–	1/0	1/1
		24	10	–	10	–	–	–	–	–	–	–
	M. rhesus, 800 R	12	70	5	62	–	3/0	7/7	3/2	1/0	–	2/2
	M. rhesus, 800 R	12	55	10	45	–	–	2/2	1/1	–	–	–
	Total		385	24	300	3	58/0	32/30	26/22	3/0	2/0	12/12
Autologous bone marrow	P. hamadryas, 1,500 R	4	1				1					
		8	0									
		9	21	2	18	–	1	–	1	–	–	–
	M. rhesus, 800 R	21	100	4	94	–	2	6	9	–	–	4
	M. rhesus, 800 R	21	34	1	32	1	–	–	1	2	2	1
		74	35	1	34	–	–	1	–	–	–	1
	P. hamadryas, 900 R	371	33	1	32	–	–	–	–	1	–	1
		654	100	8	92	–	–	–	–	1	–	–
	Total		324	17	302	1	4	7	11	4	2	7

*Total number of cells given in numerator, number of identified donor's cells in denominator.

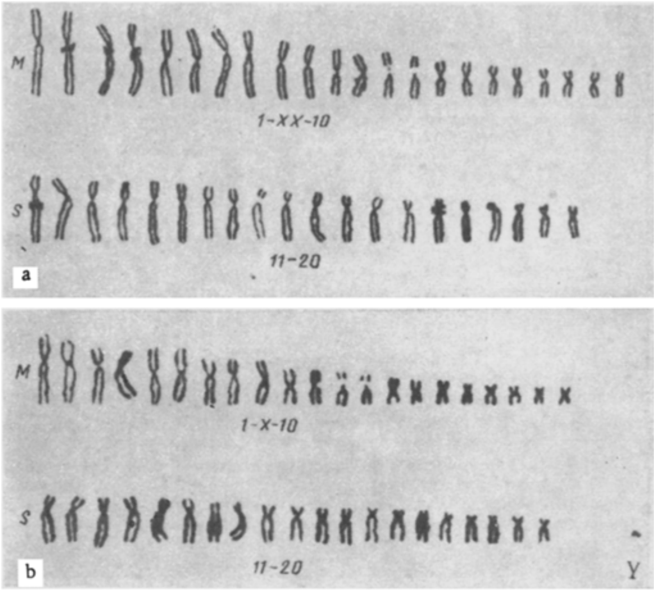

Fig. 1. Karyotype of intact monkeys (P. hamadryas): a) female; b) male.

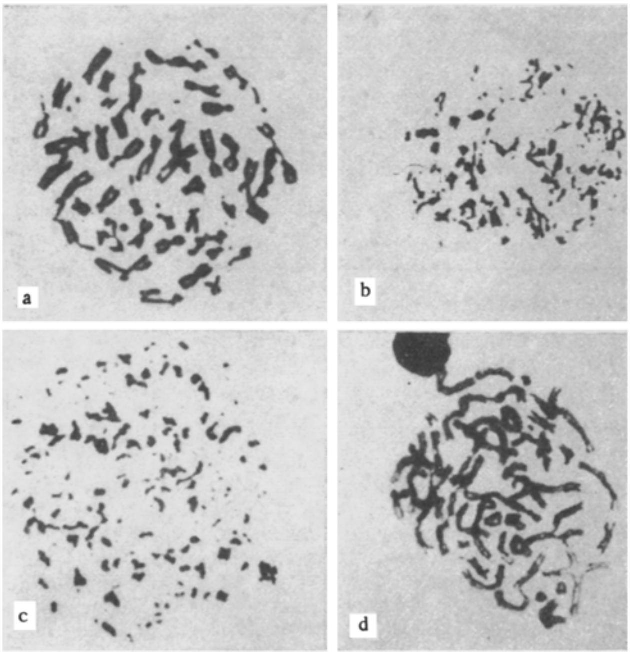

Fig. 2. Severely damaged cells in bone marrow of intact monkeys: a) multiple chromatid and isochromatid fragments; b, c) pulverization; d) fragments, chromatid translocations.

In the first 2–4 days after irradiation of the monkeys, only severely damaged cells with multiple chromosomal and chromatide fragments and translocations were observed; pulverization of the chromosomes was frequent (Fig. 2). These cells, with such severe injuries, were evidently those exposed to the direct action of the radiation, for they were found at these times in the control irradiated monkeys (without transplantation of bone marrow) also.

From the second week after irradiation, active repopulation of the graft took place (Table 1). Besides a very small number of severely damaged cells, many intact metaphases belonging to the bone marrow

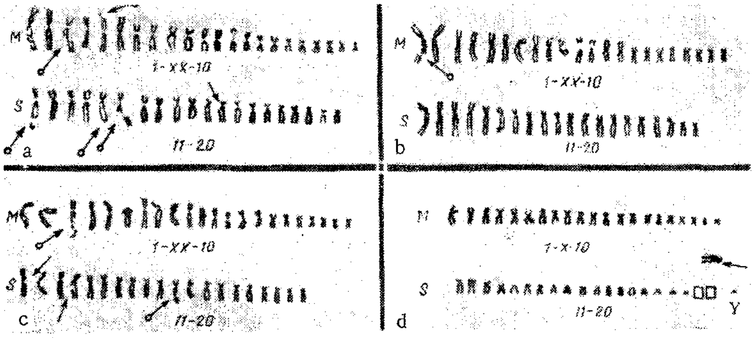

Fig. 3. Structure damage to chromosomes in hematopoietic cells of irradiated monkeys: a) P. hamadryas (XY), 11 days after transplantation. Donor's cell. ♂ isochromatid fragment; † chromatid fragment; b) the same monkey 15 days after transplantation. Donor's cell. ♂ isochromatid fragment; † deletion; c) M. rhesus (XY) 12 days after transplantation. Donor's cell. ♂ isochromatid fragment; d) P. hamadryas (XY) 371 days after autografting. † translocation (triradiate figure); two chromosomes of S group absent.

donor (XX) were found. Some of these cells showed structural disturbances of the chromosomes. In the analysis, a cell was regarded as identified only if all 42 chromosomes were present. If even one chromosome was lost, or if chromatid or chromosomal translocations were present, cells in which the Y chromosome was absent were regarded as unidentified. Even with this strict approach, chromosomal aberrations were found in all three monkeys in 7-30% of the donor's cells (Fig. 3). Chromatid and chromosomal ruptures were observed in about equal frequency; as Table 1 shows, translocations were much less common. If severely damaged cells, which are evidently recipient's cells lethally damaged by the direct action of the radiation, were disregarded, 19% of the cells (63 of 327) were found to carry structurally disturbed chromosomes, and all identified aberrant cells (52 of 63) belonged to the donor of the bone marrow. An increase in the number of cells with deletions (12 of 327 compared with a normal 3 of 215) also was found among the repopulating cells; in some cases these deletions were multiple and much larger in size than those observed in intact monkeys (Fig. 3a). By the end of the third week after irradiation, the number of damaged cells was reduced.

To exclude the effect of factors such as the foreign antigenic environment for the injected cells, thymectomy of the recipient, possible allogeneic inhibition, and so on, investigations were carried out with transplanted autologous bone marrow (Table 1). In these cases also cells carrying analogous chromosomal aberrations were found. Because of the absence of markers, it was impossible to establish directly that these cells belonged to the graft. However, with the dose of irradiation used (1.5-2 LD_{99}) all cells exposed to the direct action of the radiation and starting on mitosis at various times after irradiation carried very severe chromosomal injuries. Most probably cells with relatively slight damage belonged to the graft. This, of course, does not apply to the monkey investigated 1 and 2 years after irradiation. The two cells with chromatid translocations discovered in it (Fig. 3d) may be the progeny of unirradiated and of irradiated precursors. In these experiments also, if the severely damaged cells are disregarded, during the first 3 weeks after irradiation 21 of the 148 metaphases analyzed (14%) had chromatid and chromosomal fragments and translocations; the number of cells with deletions also was increased (5 of 148). The number of aberrant cells showed a decrease 2.5 months after transplantation (1 of 35). This close agreement between the relative number of aberrant cells after autologous and allogeneic grafting shows that the transplanted cells are evidently damaged after autografting also.

Chromosomal injuries observed after transplantation of both autologous and allogeneic bone marrow were not self-reproducing, but were lost at the first mitosis; no clones of damaged cells were found. It can accordingly be concluded that the donor's cells were damaged not only at the time of transplantation or soon after; in fact, in some cases 12-15 days after transplantation, up to 15-20% of the dividing donor's cells were aberrant. Since the total number of bone marrow cells at this time was largely restored by the graft (from 15,000 to 30,000/mm^3 compared with 200-1000/mm^3 at the depth of aplasia, 5-6 days after irradiation), it is clear that the total number of injured donor's cells discovered was greater than the total number of cells injected at the time of grafting and capable of prolonged proliferation.

Hence, at least during the first weeks after irradiation, factors are present in the body which can induce chromosomal aberrations in proliferating hematopoietic cells. No information is available regarding activity of this type in any hypothetical radiotoxins. The more probable cause of the injuries arising in the donor's cells is a viremia, which frequently develops in the severest types of radiation sickness. Under all conditions, and whatever the mechanism of these disturbances, the results must be taken into account in all radiobiological investigations. In particular, it must be concluded from them that not every cell with chromosomal aberrations found in an irradiated organism has been damaged by the direct action of the radiation.

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