A COMPLEX MOSAIC WITH t(13;18)(p11;p11),
+13p-, +18p-, r(13) *etc.* IN A MALE INFANT

II. INCREASED DISSOCIATION OF DICENTRIC
CHROMOSOME BY MITOMYCIN C

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Summary When cultured lymphocytes of a patient with 45,XY,t(13;18)(p11.1-2;p11.2) were treated with mitomycin C (MMC), dissociations of t(13;18) chromosomes were increased as compared with controls. Furthermore, various other anomalies involving t(13;18), 13 and 18 chromosomes were observed, which seemed to have resulted from breakage-fusion-bridge (BFB) cycles due to MMC.

INTRODUCTION

Dicentric (dic) chromosomes have a tendency to cause mosaics of multiple anomalies by centromere inactivation, nondisjunction, or dissociation of dic chromosome. The aim of the present study was to investigate the effects of mitomycin C (MMC), ethidium-bromide (EB) and 5-bromodeoxy-uridine (BrdU) to dic chromosomes. The alkylating antibiotic MMC induces chromosome aberrations such as attenuation, constriction, gap, breakage and exchange more frequently in the centromeric regions especially in human chromosomes 1, 9 and 16, and satellite and telomeric regions containing repetitive DNA (Brøgger and Johansen, 1972; Morad *et al.*, 1973; Bourgeois, 1974; Ishii and Bender, 1978). EB is intercalated in the DNA strand unspecifically. BrdU is incorporated instead of thymidine into newly replicated DNA. In the present study using the lymphocytes from a patient with 45,XY,t(13;18)(p11;p11), the dissociation of t(13;18) was found to increase with MMC and EB treatments.

MATERIALS AND METHODS

The peripheral lymphocytes from the patient were cultured as follows; 0.3-0.5 ml of whole heparinized blood was added to 5 ml of RPMI 1640 medium containing 20% fetal calf serum, 2% phytohemagglutinin (PHA), and 100 IU penicillin; all

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cultures were incubated at 37°C in a 5% CO₂ atmosphere for 72 or 96 hr.

MMC was added to the culture at a final concentration of 0.01 µg/ml 24 hr before harvesting. BrdU was added to the culture at a final concentration of 2 µg/ml at the beginning of the incubation, and the cultures were kept in the dark. EB was added to the culture at a final concentration of 10 µg/ml 2 hr before harvesting. Furthermore, the combined treatment of both MMC and BrdU or that of both MMC and EB was also done. For observation of metaphases colcemid was added to the cultures two hours before harvesting. Anaphase chromosomes were observed without colcemid. Hypotonic treatment with 0.075 M KCl was followed by fixation with methanol-acetic acid. G-banded preparations were obtained with trypsin (Seabright, 1971), and nonbanded ones with Giemsa staining.

RESULTS

The karyotype of the lymphocytes of the patient was 45,XY,t(13;18) in modal cells. The details of the clinical features and karyotypes of the patient are described in the accompanying article (Uehara and Kida, 1986). In MMC-treated cultures, the metaphases with t(13;18) chromosomes bearing two constrictions decreased, while the metaphases with t(13;18) chromosomes having only one constriction at centromere of chromosome 18 increased, when compared with untreated control cultures (Table 1).

The karyotypes observed in minor cell lines and the percentages of each type of the cells are shown in Table 2. In MMC- or EB-treated cultures, the frequencies of dissociation of t(13;18) chromosomes [46,XY,13p-,18p- or breaks in t(13;18)] were increased when compared with controls (Fig. 1), but no synergistic effect of MMC and EB was observed in the combined treatment. In BrdU-treated cultures,

Table 1. Frequencies of constrictions at centromeres in t(13;18) chromosome after treatments with chemicals.

Sites of constrictions ^a	Percentage of cells after treatment with					
	None	MMC	BrdU	BrdU+ MMC	EB	MMC+ EB
	(1) ^{b,*}		(2) ^{b,*}		(3) ^{b,*}	
(A) Centromeres 13 & 18	59	43	56	35	53	43
(B) Centromere 18	30	43	32	52	31	40
(C) Centromere 13	7	7	7	8	10	7
Total number of cells	44	30	108	102	93	30

^a (A), two centromeres of chromosome 13 and chromosome 18 in t(13;18); (B), a centromere of chromosome 18 in t(13;18); (C), a centromere of chromosome 13 in t(13;18). ^b (1), 96 h culture; (2) and (3), 72 hr culture. * Significant at level $p < 0.001$ in (2)-(A) and (B); not significant in other columns.

Table 2. Abnormal karyotypes observed in lymphocytes after treatments with chemicals.

Karyotypes or number of chromosomes	Percentage of cells after treatment with					
	None	MMC	BrdU	BrdU+MMC	EB	MMC+EB
	(1)		(2)		(3)	
[Diploid]						
45,XY,t(13;18)	90	71	85	80	86	83
Trisomy						
46,XY,t(13;18),+13p—	0	4	1	3	4	1
46,XY,t(13;18),+18p—	2	4	2	1	2	2
46,XY,t(13;18),t(13;18)	0	0	3	1	0	0
Dissociation						
46,XY,13p—,18p—	0	12	0	7	6	5
Breakage of t(13;18)	2	2	0	4	3	5
Total dissociation	2	14	0	11	9	10
Other anomalies						
46,XY,r(13),18p—	0	1	0	1	1	0
46,XY,t(13;18),+i(13)	0	0	0	0	0	1
45,XY,t(13;18) ^a	2	1	3	0	1	0
Other anomalies with treatment						
45,46 or 47 chromosomes	0	0	0	2	0	4
[Tetraploid]						
90	2	0	1	2	1	1
92	0	0	0	0	0	0
Breakage of other sites ^b	0	18	0	0	0	10
Total no. of cells	45	48	98	101	101	97

(1), 96 h culture; (2) and (3), 72 hr culture. ^a Large intercentromeric region of t(13;18). ^b Other sites of breakages were chromosomes 1, 9, 16, D *etc.*; percentages of breakages in each chromosomes were below 2% (including exchange).

dissociation of t(13;18) chromosome was not observed, but 46,XY,t(13;18),+t(13;18) was observed in 3% (Fig. 1). In BrdU-MMC-treated cultures, 47,XY,t(13;18),+t(13;18),+13(?) (Fig. 1) and ring formation of t(13;18) were observed (Table 2). In MMC-EB-treated cultures the following chromosomal anomalies were observed: 46,XY,-13,-18,+13(?),+13(?) and 47,XY,t(13;18),+13(?),+18, with and without attenuated elongation of 13(?) and terminal association of t(13;18) and 18, and a chain-like chromosome instead of t(13;18) (Table 2, Fig. 1).

Table 3 shows break point distribution in the dissociation of t(13;18) chromosomes in control and MMC- and EB-treated cultures. Breaks were most frequently

Fig. 1. Various types of abnormalities of t(13;18) chromosomes observed after the chemical treatment with MMC, EB or BrdU. *Upper*: Left, dissociation of t(13;18); Center, duplication of t(13;18); Right, duplication of t(13;18) and acrocentric chromosomes. *Lower*: Left, attenuated elongation of 13(?) and terminal association of t(13;18) and 18; Right, chain-like chromosome instead of t(13;18).

Table 3. Break point distribution in dissociation of t(13;18) after chemical treatments.

Break point	Number of breaks after treatment with		
	None (control)	MMC	EB
Centromere 18 ^a	3	6	0
Intercentromeric region ^b	1	8	2
Centromere 13 ^c	6	14	3
Number of cells examined	300	246	100

^a Sites near centromeric region of chromosome 18 in t(13;18). ^b Intercentromeric region of two centromeres in t(13;18). ^c Sites near centromeric region of chromosome 13 in t(13;18).

observed in the sites near centromeric region of chromosome 13. Frequencies of anaphase-bridge, breakage, lagging and elongation of the intercentromeric region were slightly higher in MMC-treated cultures than in the controls, but the difference was not statistically significant.

DISCUSSION

As to mechanisms for loss of function of a centromere in dicentric chromosome, both genetic inactivation and centromere deletion have been proposed (Distèch *et al.*, 1972; Therman *et al.*, 1974; Cohen *et al.*, 1975). Dewald *et al.* (1979) observed that breakage of dic chromosomes occurred spontaneously, and suggested the existence of a repair mechanism that can bind loose ends of DNA occurring as a result

Fig. 2. Diagrammatic representation of various types of spontaneous and chemical induced abnormalities of the tdc(13;18) chromosomes. (1), tdc(13;18); (2), tdc(13;18), +18p-; (3), tdc(13;18), +13p-; (4), tdc(13;18), +tdic(13;18); (5), tdc(13;18), +13p-, +18p-; (6), 13p-, 18p-; (7), r(13), 18p-; (8), tdc(13;18) with large intercentromeric region; (9), tdc(13;18), +tdic(13;18), +13p-; (10), tdc(13;18), +i(13) with large intercentromeric region; (11), -13, -18, +13p-, +13p-.

of anaphase bridge and breakage, without substitution of a new centromere; the absence of a primary constriction is associated with deletion of a centromere.

In our observations of MMC-treated cultures, cells with t(13;18) chromosomes having two constrictions at two centromeres were decreased and cells with t(13;18) chromosomes having only one constriction at the centromere of chromosome 18 were increased, compared with controls. This phenomenon seemed to be a result of increased breaks of t(13;18) chromosomes observed in anaphase and metaphase: dicentric chromosome of the t(13;18) showed a tendency for breakage especially in the centromere of chromosome 13 with the MMC treatment. When the breakage at the centromere of chromosome 13 is repaired, the centromere of chromosome 13 may change to a nonfunctional centromere by inactivation or deletion.

Dissociation of dic chromosomes was increased not only with MMC treatment, but also with EB, compared with untreated controls. Nevertheless after treatment with EB, cells with t(13;18) chromosomes having two constrictions were not decreased and cells with t(13;18) chromosomes having only one constriction at the centromere of chromosome 18 were not increased, compared with controls. This may be due to different effects of EB and MMC at different stages of the cell cycle. The effect of these two chemicals was not synergistic for dissociation of t(13;18) chromosomes. It is therefore suggested that such chemical treatment appears to enhance a latent tendency to dissociate of t(13;18) already existing in these chromosomes.

In BrdU-treated cultures, dissociation of t(13;18) chromosomes was not observed, but an increase in the t(13;18) chromosome being in double was found. These two translocation chromosomes might be affected for the dissociation and non-disjunction after BrdU and MMC treatment.

The process of occurrence of various types of spontaneous and induced abnormalities in the 13/18 translocated chromosomes can be explained by breakage-fusion-bridge (BFB) cycles, as illustrated in Fig. 2. These abnormalities may be arisen through two or three cycles of cell divisions.

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