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Enhanced Effect of Cyclophosphamide on Burkitt Lymphoma Cell Lines *in Vivo*[1] (39586)

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We have recently described a modified host-mediated assay system using human lymphoid cells cultured in diffusion chambers implanted into mice (1). Using this system and cytogenetic analysis as an indicator of the possible mutagenic effects of various agents, preliminary studies demonstrated that cyclophosphamide (CY) caused a significantly higher number of chromosome aberrations in the Burkitt lymphoma (BL) cell line B35M than in the normal-derived cell line SP-163. This result suggested that BL-derived cells were more sensitive to the chemotherapeutic drug CY than other lymphoid cell lines, and the present studies were initiated to investigate this possibility.

Reports on the cytostatic action of CY, an alkylating agent, or its ability to induce chromosome damage *in vitro* vary. Connors *et al.* (2) suggest that CY must undergo a metabolic conversion *in vivo* to yield the active antitumor and mutagenic agent, and the studies of Perry and Evans (3) using Chinese hamster cells (line CHO) support this notion. However, Nasjletti and Spencer (4) reported that CY could induce chromosome aberrations in cultures of human leukocytes, while Bishun (5) described similar effects in a BL cell line *in vitro*. Therefore, we have investigated the CY sensitivity of our cell lines *in vitro* as well as in diffusion chambers.

Materials and methods. Seven human lymphoid cell lines were used, three BL and four non-BL. The three BL cell lines were B35M, HR1K, and Raji (6), while the four non-BL cell lines were Wentzl (7), derived from a patient with Down's syndrome, SP-163 (8), B411 (9), and RPMI-1788 (9). The latter three lines were all derived from the blood of normal individuals. The cells were cultured in medium RPMI-1640 supplemented with 10% fetal calf serum but without antibiotics.

The technique of culture in diffusion chambers (DC) has been described (1). The dry-sterilized DC were filled with 0.33 ml of a suspension containing approximately 5×10^5 cells. The filled DC were implanted into the peritoneal cavity of 6- to 8-week-old C3H/St mice under ether anesthesia. One day after DC implantation, the mice were injected ip with CY at a concentration of either 100 or 150 mg per kg body weight. Twenty-two hours after CY treatment, the animals received 0.5 ml of 0.04% colchicine ip, and the DC were removed 2 hr later, 24 hr after CY treatment. The DC were then treated with 0.5% Pronase in Hank's balanced salt solution containing 5% ficol for 60–90 min to disperse the cell aggregates. After removing the cells from the DC, the cell viability and number from each DC were determined. Cells were then treated with hypotonic solution and fixed in acetic acid:methanol (1:3). Flame-dried slides were made and stained with Giemsa.

The cytogenetic and cytostatic effects of CY *in vitro* were assessed in suspension cultures containing 10 ml of cells initially at 5×10^5 cells/ml. Cyclophosphamide at final concentrations of 1, 10, 50, and 100 μg/ml was compared to control cultures. The cell viability counts and cytogenetic analyses were made 24, 48, and 72 hr after the addition of CY. Colcemid (0.04 μg/ml) was added 2 hr before collecting cells for chromosomal analyses. Cells with gaps, breaks, exchanges, dicentrics, and rings were recorded.

Results. With the exception of RPMI-1788, all the human lymphoid cell lines

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TABLE I. CHROMOSOME ABERRATIONS INDUCED BY CYCLOPHOSPHAMIDE IN BURKITT CELL LINES (B35M, HR1K, AND RAJI) AND IN NON-BURKITT LINES (SP-163, WENTZL, AND B411) CULTURED IN DIFFUSION CHAMBERS AND IMPLANTED INTO MICE.[a]

Cell line	Dose (mg/kg)	No. of chambers	Total cells analyzed	Gaps (G)% (#G/cell)	Breaks (B)% (#B/cell)	Exchanges (E)% (#E/cell)	Dicentrics (D)% (#D/cell)	Rings (R)% (#R/cell)	Multiple abberrations %	% Cells with aberr-ations
B35M	150	3	100	23.3 (0.37)	49.6 (1.20)	20.4 (0.25)	4.1 (0.04)	- -	7.9	70.0
	0	4	180	3.8 (0.04)	3.2 (0.03)	- -	- -	- -	-	3.9
HR1K	150	3	85	24.7 (0.54)	42.4 (1.04)	10.6 (0.18)	3.5 (0.06)	1.2 (0.01)	9.4	57.6
	0	3	80	3.8 (0.05)	2.5 (0.05)	- -	- -	- -	-	5.0
Raji	150	4	55[b]	29.1 (0.47)	43.6 (1.31)	14.5 (0.27)	5.5 (0.05)	- -	14.5	70.9
	100	1	11[b]	27.3 (0.45)	45.5 (1.09)	9.1 (0.18)	9.1 (0.09)	- -	18.2	81.8
	0	2	60	5.0 (0.07)	3.3 (0.03)	1.7 (0.02)	- -	- -	-	5.0
SP-163	150	5	136	6.0 (0.06)	15.5 (0.25)	2.5 (0.03)	1.5 (0.02)	0.5 (0.01)	-	22.6
	0	6	150	4.0 (0.04)	5.0 (0.05)	- -	2.0 (0.02)	- -	-	6.0
Wentzl	150	4	100	5.0 (0.06)	6.0 (0.09)	5.0 (0.05)	3.0 (0.04)	- -	-	15.0
	0	3	100	3.0 (0.04)	3.0 (0.03)	1.0 (0.02)	- -	- -	-	6.0
B411	150	2	30[b]	6.7 (0.07)	6.7 (0.10)	3.3 (0.03)	3.3 (0.03)	- -	3.3	20.0
	100	3	100	5.0 (0.07)	6.0 (0.08)	2.0 (0.02)	- -	- -	-	14.0
	0	4	100	3.0 (0.05)	5.0 (0.05)	2.0 (0.02)	- -	- -	-	5.0

[a] Breaks include chromatid or chromosome breaks and fragments. Cells with exchange, dicentric, or ring may have breaks which were also included in the cells with breaks. Cells having more than eight aberrations (multiply aberrations) were not recorded in the other categories.

[b] Mitoses were suppressed; only a few metaphases could be scored.

grew well in DC implanted in mice. Line RPMI-1788 was, therefore, not included in the *in vivo* cytogenetic studies. Cell lines B35M, HR1K, and SP-163 proliferated more rapidly in DC than lines Raji, Wentzl, and B411. The former three lines increased three to four times the initial concentration in 2 days while the latter three lines approached one doubling. Cyclophosphamide reduced the growth rate of all the cell lines.

The effects of CY on the chromosomes of cells from the six cell lines cultured in DC are shown in Table I. It is evident that treatment with CY caused a high incidence of chromosomal aberrations in all six lines, but that the BL cell lines were uniformly more sensitive to CY than the non-BL cell lines. The incidence of aberrations in the BL cell lines ranged from 58–82% while this was only 14–23% in the non-BL lines. This difference in sensitivity between the two groups of cell lines was highly significant ($P < 0.001$).

Studies carried out in tissue culture were uniformly negative. All the cell lines grew well, including RPMI-1788, and CY in various concentrations from 1–100 μg/ml did not affect their growth rates. Cytogenetic analysis of 50 metaphases from control flasks and flasks treated with various durations and concentrations of CY in all the cell lines revealed no effects of CY on chromosomes.

Discussion. Our results have shown that BL cells carried in mice in DC were significantly more sensitive to CY than non-BL cells. The reasons for such a high sensitivity are presently under investigation. The *in vitro* studies would suggest that the BL cells do not activate CY directly, although the presence of an inhibitor of this activation in the tissue culture medium has not been ruled out. It is possible that the BL cells stimulate the host's activation of CY and result in a higher concentration of the active metabolite in the peritoneal fluid, a possibility which can be tested using the peritoneal fluid directly. It has been reported (10) that certain cancer cells are more sensitive to CY *in vivo* than normal cells, and, thus, the enhanced sensitivity observed in the present work may reflect an intrinsic property of the BL cell unrelated to activation phenomena. For example, the BL cells used in the present studies are all hyperdiploid. Zech *et al.* (11) recently reported that cells from many BL and BL biopsies had a marker chromosome, 14q+. The non-BL cell lines SP-163 and B411 are diploid while the Wentzl line retains the characteristic constitution of Down's syndrome. Our studies, therefore, open several avenues for future investigation while demonstrating further specific effects of cyclophosphamide *in vivo* but not *in vitro*.

Summary. Cells from three Burkitt lymphoma lines and three normal-derived human lymphoid lines were cultured in diffusion chambers implanted into mice. Cytogenetic analysis was made on all these cell lines following treatment of the animals with cyclophosphamide. The Burkitt lymphoma cell lines were uniformly more sensitive to cyclophosphamide than the normal-derived cell lines, having a chromosome aberration incidence of 58–82% compared to 14–23% for the latter cell lines. Studies in tissue culture failed to reveal either the cytostatic effect of cyclophosphamide or an effect on chromosomes.

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