

Variation in sister chromatid exchange frequencies and cell-cycle kinetics between human whole blood and plasma leukocyte cultures

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ABSTRACT

The effects of bromodeoxyuridine (BUdR) concentration and the length of the synthesis (S) period on the baseline frequency of sister chromatid exchanges (SCEs) and the proliferation rate of human whole blood (WBC) and plasma leukocyte cultures (PLC) were studied. An immunofluorescent method was used to induce sister chromatid differentiation. This method employs a low concentration of BUdR for chromosome labelling during the first cell cycle *in vitro* (3.3 μM), and fluorescein isothiocyanate (FITC)-conjugated anti-BUdR monoclonal antibody for detection. WBC and PLC were allowed to grow during the first cell cycle (48 h) in the presence of BUdR, and the culture medium was then replaced with BUdR-free culture medium, and cells were cultured for an additional cell cycle (24 h) until harvesting (72 h). Chromosomes were counterstained with propidium iodide and DAPI. PLC showed at least a two-fold increase in SCE frequency over WBC values and cell proliferation was slower in PLC than in parallel WBC, independent of the concentration of BUdR of the culture medium, at least in the range of the base analogue employed (3.3 μM -33.0 μM). Moreover, an estimation of the length of the S period, made by analyzing the proportion of labelled cells that were synthesizing DNA during different harvesting times, revealed that PLC have a markedly lengthened S period compared to WBC. Accordingly, the rate of DNA-fork displacement of lymphocytes in PLC could be slower than in WBC, resulting in an increase in SCE frequency of these cultures.

INTRODUCTION

Spontaneous sister chromatid exchange (SCE) induction and the large variability in the baseline frequency of SCE have been discussed by several authors (Carrano *et al.*, 1980, Latt *et al.*, 1980; Gebhart, 1981; Larramendy and Reigosa, 1986; Larramendy *et al.*, 1990, 1993). All of them agree in demonstrating that

several factors can affect and modulate baseline SCE frequency in lymphocyte cultures. Among such factors, the concentration of bromodeoxyuridine (BUdR) (Latt, 1974; Lambert *et al.*, 1976; Kato, 1977; Carrano *et al.*, 1980; Davidson *et al.*, 1980; Kligerman *et al.*, 1982; Morgan and Wolff, 1984), temperature (Kato, 1980; Speit, 1980; Gutierrez *et al.*, 1981; Abdel-Fadil *et al.*, 1982), serum (Kato, 1977; Kato and Sandberg, 1977; Bianchi *et al.*, 1979; Ghosh and Nand, 1979; Mutchinick *et al.*, 1980; McFee and Sherill, 1981; Larramendy *et al.*, 1993), culture media (Bianchi *et al.*, 1979, 1981), and the number of white (Kligerman *et al.*, 1982) as well as red blood cells (RBCs) (Mehnert *et al.*, 1984; Larramendy and Reigosa, 1986; Larramendy *et al.*, 1990), can be included.

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Whole blood (WBC), plasma leukocyte (PLC), and mononuclear leukocyte cultures (MLC) are routinely used for SCE assays. However, discrepancies in the baseline SCE frequency and cell-cycle kinetics of the same lymphocytes occur even when the aforementioned sources of variation are avoided or minimized (Ray and Altenburg, 1978; Wilmer *et al.*, 1983; Mehnert *et al.*, 1984; Wilmer *et al.*, 1984; Larramendy and Reigosa, 1986; Larramendy *et al.*, 1990, 1993).

We found that human but not porcine PLC and MLC, exhibited nearly a two-fold increase in the baseline frequency of SCEs compared to those observed in WBC (Larramendy and Reigosa, 1986). The titration of either human or pig RBCs in human PLC and MLC exerted a RBC dose-dependent decrease in the frequency of SCEs. PLC and MLC possessed similar baseline levels of SCEs when lymphocytes were co-cultured with concentrations of RBCs equivalent to those present in WBC. Since the presence of either human or pig RBCs in human PLC and MLC decreased the SCE frequency to that of WBC, the existence of a common component and/or a modulating effect of the red cells was suggested.

We have also observed that the period of co-culturing of RBCs and leukocytes modulates the cell-cycle kinetics of both human and pig lymphocytes, whereas the baseline frequency of SCEs is affected only in human leukocytes (Larramendy *et al.*, 1990). We found that the low SCE baseline frequency of porcine RBC-free cultures is independent of the lapse between lymphocyte stimulation and the addition of RBCs to the cultures.

Recently, we investigated the proliferative response and the SCE baseline frequency of porcine and human lymphocytes with human and pig plasma in the culture medium. We demonstrated that the baseline frequency of SCEs was not affected by the plasmas (Larramendy *et al.*, 1993).

In the present study we analyzed whether the delay of the cell-cycle kinetics and the increase of the SCE baseline frequency observed in human lymphocytes cultured under PLC are correlated with a lengthening of the synthesis (S) period and/or with the concentration of BUdR during culturing.

MATERIAL AND METHODS

Blood samples

Human blood samples were obtained from healthy donors (age 20-40 years) selected as recommended elsewhere (Bianchi *et al.*, 1979). From

each donor, 50-100 ml of blood was drawn by venipuncture (Blood Bank of Buenos Aires Province, La Plata, Argentina).

Whole blood cultures (WBC)

Human WBC were set up as described previously (Larramendy and Reigosa, 1986). Briefly, 1.0 ml of whole blood was seeded in 9.0 ml of complete culture medium (80% Ham's F10 (Gibco, Grand Island, NY, USA), 20% fetal calf serum (Gibco), 0.3 ml phytohemagglutinin M (Gibco), 100 units/ml penicillin, 100 µg/ml streptomycin (Gibco)). The final concentration of cells in culture was approximately 6.0×10^5 leukocytes/ml and 4.5×10^8 RBCs/ml. During the last 3 h of culture, the cells were treated with 0.1 µg/ml colchicine (Sigma Chemical Co., St Louis, MO, USA). After 72 h of culture, the cells were harvested, exposed to a hypotonic solution (0.075 M KCl, 37°C, 15 min), and fixed in methanol:acetic acid (3:1). Cultures were set up in duplicate for each sample of each donor, and at least two different donors were used in each experiment.

Plasma leukocyte cultures (PLC)

Human PLC were set up according to a procedure reported previously (Larramendy and Reigosa, 1986). Briefly, after gravity sedimentation of whole blood (approximately 20-30 ml sample) for 1-2 h at room temperature, 1.0 ml of plasma-leukocyte suspension was added to 9.0 ml of complete culture medium. The final concentration of leukocytes was approximately 1.2×10^6 cells/ml. Harvesting was performed at the times specified for WBC.

Experimental variations

Assessment for measuring the sensitivity to bromodeoxyuridine (BUdR)

WBC and PLC were set up according to the standard protocol reported above. BUdR was added to the cultures at a final concentration of 33 µM (about 10 µg/ml, normal dose) and 3.3 µM (about 1 µg/ml, low dose), following the culture procedure reported elsewhere (Larramendy *et al.*, 1995). Briefly, cultures were grown for 48 h in darkness at 37°C in a 5% CO₂ atmosphere, then washed three times with Hanks balanced salt solution and incubated in BUdR-free culture medium for an additional 24 h until conventional harvesting. As in previous reports (Bianchi and Larramendy, 1983; Bianchi *et al.*, 1986), human lymphocytes were grown for one cell cycle in

BUdR-containing medium and one generation in BUdR-free medium. Endogenous thymidine synthesis during the first generation in the presence of BUdR was blocked by incorporation of 10 μM fluorodeoxyuridine (Sigma) and 200 μM deoxycytidine (Sigma). Harvesting was performed as described above, after 3 h of colchicine treatment.

Assessment for measuring the length of the S period

WBC and PLC were performed according to the standard protocol reported above, and cultured for 48 and 72 h. Afterwards, cells were treated with tritiated thymidine ($^3\text{H-TdR}$) at a final concentration of 2 mCi/ml (New England, USA, sp. act. = 20 Ci/mmol) for 30 min. At the end of the $^3\text{H-TdR}$ treatment, the cells were washed three times with Hanks solution, harvested without colchicine treatment and fixed in methanol-acetic acid (3:1). Slides were prepared by the air-drying method, and then mounted with AR10 Kodak stripping film. After a 12-18 day exposure at 4°C , the autoradiograms were developed in D-19 Kodak developer, fixed and stained with 5% aqueous Giemsa solution. The proportion of interphase cells that have incorporated $^3\text{H-TdR}$ was determined by analyzing at least 2000 cells per sample and expressed as the percentage of labelled cells among all interphases.

Fluorescence-plus-Giemsa (FPG) method for sister chromatid differentiation

All chromosome spreads from WBC and PLC grown in the presence of 33 μM BUdR were processed using a combination of the procedures of Perry and Wolf (1974) and Korenberg and Freedlander (1974) to obtain sister chromatid differentiation.

Immunofluorescence method for sister chromatid differentiation

All chromosome spreads from WBC and PLC grown in the presence of 3.3 μM BUdR were processed following the one-step immunofluorescent procedure recently described in detail elsewhere (Larramendy *et al.*, 1995). Briefly, slides were first denatured in 70% formamide (Sigma) in 2 x SSC (pH 7.0) at 70°C for 2 min. Immediately, the slides were dehydrated in a series of ice-cold 70, 94 and 100% ethanol (5 min each), and then air-dried. Afterwards, the slides were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-BUdR monoclonal antibody (Boehringer Mannheim, GmbH-Biochemica, Mannheim, Germany)

diluted 1:100 in PBS containing 0.5% Tween 20 (Sigma) during 45 min at 37°C . Unbound anti-BUdR-FITC antibody was washed out by incubating the slides in three changes of PBS-Tween 20 (5 min each) at room temperature. Finally, the slides were washed in distilled water, air-dried, and the cells were counterstained simultaneously with 1 $\mu\text{g/ml}$ of propidium iodide (Sigma) and 0.2 $\mu\text{g/ml}$ of 4,6-diamino-2-phenylindole dihydrochloride (DAPI, Sigma) in PBS containing 0.5% Tween 20 for 7 min, and then mounted in fluorescence antifading buffer (Johnson and de Nogueira-Araujo, 1981).

The slides were examined under a Zeiss Axiophot fluorescence photomicroscope equipped with Zeiss 02 and 09 filters.

Sister chromatid exchange analysis and cell-cycle progression

All slides were coded and a single observer scored the SCE frequencies from at least 50 second diploid metaphases per sample. A minimum of 200 metaphase cells per sample were scored to determine the frequency distribution of cells according to the number of cell-cycles performed. The SCE data were subjected to an analysis of variance. Tukey's test for comparisons was used to analyze the significance of the differences between the mean SCE frequencies. A χ^2 -test was applied to the cell-cycle progression data. Differences were considered statistically significant if the P-value was 0.05 or less.

RESULTS AND DISCUSSION

The lymphocyte culture system employed and the immunofluorescent method for differentiation of the sister chromatids from metaphase chromosomes allow the scoring of SCEs and the analysis of cell-cycle progression and cell proliferation rates of cells grown at low BUdR concentration (Larramendy *et al.*, 1995). In order to achieve sister chromatid differentiation, only first single cycle labelling with BUdR was employed, DNA synthesized after base analogue pulse-treatment has been washed out will not be labelled. Thus, the SCE and the analysis of the cell-cycle progression can be studied by the differential quenching of the fluorescence of the fluorescein and the propidium iodide. Photographs illustrating the staining pattern achieved with this technique are presented in Figure 1A-D. According to previous results (Larramendy *et al.*, 1995), the DNA of one chromatid of each second human lymphocyte metaphase becomes unifilarly substituted

with BUdR, and its sister chromatid remains unsubstituted. Then, after the immunofluorescence differentiation, the fluorescein-stained BUdR-substituted chromatids appeared yellow-greenish and the unsubstituted chromatids, not stained with fluorescein, carried the orange-reddish color of the propidium iodide, showing a characteristic immunofluorescent sister chromatid differentiation pattern (Figure 1C). On

the other hand, when cells completed only one cell cycle during the culture period, the antibody labelled the two BUdR-substituted chromatids, and the fluorescein uniformly stained all the chromosomes in the metaphase (Figure 1A). Figure 1D shows the immunofluorescence pattern reflected by a cell which had undergone three cell cycles in our culture system. Approximately, half of the chromosomes possess

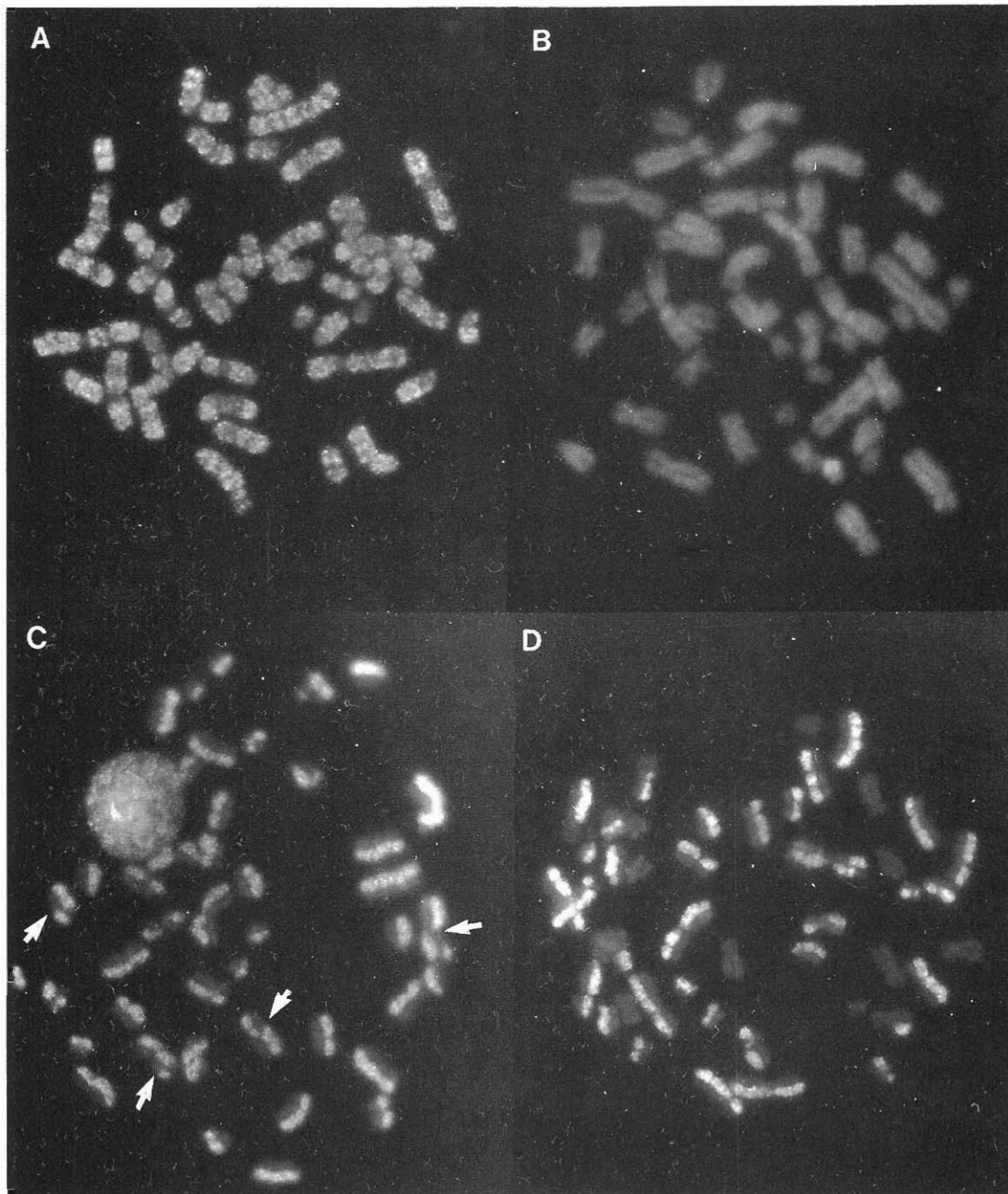


Figure 1 - Immunofluorescent sister chromatid differentiation patterns of human lymphocytes allowed to grow for the first cell cycle *in vitro* at a low BUdR concentration (3.3 μ M). Chromosomes were stained with FITC-conjugated anti-BUdR monoclonal antibody, and counterstained with DAPI and propidium iodide. The differential quenching of the fluorochromes reveals an early-replicating first (1A), late-replicating first (1B), second (1C) and third (1D) mitosis. Arrows indicate sister chromatid exchanges detected by the antibody.

harlequin staining while the rest appear unbounded by the antibody, and carry the orange-reddish color of the propidium iodide, reflecting that their chromatids have not been substituted with BUdR (Figure 1D).

In addition to the three differentiation patterns observed in both pig (Larramendy *et al.*, 1995) and human cells (Figure 1A,C,D), a fourth staining pattern shown in Figure 1B was also found. All the chromosomes belonging to the metaphase are characterized by being uniformly stained orange-reddish by the propidium iodide revealing an unsubstituted-BUdR condition in the chromatids. Accordingly, these cells represent a lymphocyte subpopulation which started the onset of DNA synthesis after removal the BUdR from the culture medium, and then are observed in their first mitosis by the time of harvesting. Taking into account the two different fluorescent patterns at first mitosis, it is possible to differentiate an early-replicating lymphocyte subpopulation (E-1st) (Figure 1A) from a late-replicating subset (L-1st) (Figure 1B).

There were no differences in the frequency of SCEs between the two concentrations of BUdR (33 μ M or 3.3 μ M) employed in the culture medium ($P > 0.05$) (Table I). Similarly, no significant differences in the baseline SCE frequency were observed between cells cultured with or without fluorodeoxyuridine and

deoxycytidine incorporated simultaneously with the BUdR. On the other hand, PLC exhibited a nearly two-fold increase in SCE frequencies compared to those of WBC. These observations confirm and verify the differential response of human lymphocytes depending on whether they are cultured together with red blood cells or not, previously reported by us (Larramendy and Reigosa, 1986; Larramendy *et al.*, 1990, 1993), and Mehnert *et al.* (1984). However, these studies did not address the effect of BUdR concentration on the frequency of SCEs. Accordingly, it seems valid to conclude that the basal frequency of SCEs in human lymphocytes from WBC and PLC is independent of the concentration of BUdR in the culture medium, at least within the range of 3.3-33 μ M. Similar findings have been previously reported for human lymphocytes by Tucker *et al.* (1986) as well as for pig cells by Larramendy *et al.* (1995). Measuring the baseline frequency of SCEs in leukocyte cultures as a function of BUdR concentration, these authors were unable to correlate the amount of the thymidine analogue incorporated into the DNA with the frequency of spontaneous SCEs. Deviating from the method employed in the aforementioned studies, our results showing no differences in baseline SCE frequency were obtained with BUdR concentrations that did not induce good sister chromatid

Table I - Frequencies of sister chromatid exchanges (SCEs) and analysis of cell-cycle progression in human lymphocytes grown in the presence of two different concentrations of 5-bromodeoxyuridine (BUdR) during the first cell cycle in culture.

Donor	Culture type	BUdR μ M	FU ^a 10 μ M	DC ^b 200 μ M	SCE/cell ^c	% Cells in metaphase ^d				
						1st	E-1st	L-1st	2nd	\geq 3rd
A	WBC	33	+	+	4.96 \pm 0.50	83	NE	NE	17	0
	WBC	33	-	-	5.26 \pm 0.38	74	NE	NE	25	1
	WBC	3.3	+	+	5.05 \pm 0.39	80	45	35	20	0
	WBC	3.3	-	-	4.75 \pm 0.37	71	35	36	26	3
	PLC	33	+	+	11.30 \pm 0.58	90	NE	NE	10	0
	PLC	33	-	-	10.84 \pm 0.52	88	NE	NE	12	0
	PLC	3.3	+	+	11.05 \pm 0.63	94	32	62	6	0
	PLC	3.3	-	-	11.25 \pm 0.42	93	33	60	7	0
B	WBC	33	+	+	5.92 \pm 0.52	80	NE	NE	20	0
	WBC	33	-	-	5.50 \pm 0.35	76	NE	NE	21	3
	WBC	3.3	+	+	5.05 \pm 0.45	82	42	40	18	0
	WBC	3.3	-	-	5.20 \pm 0.65	77	40	37	22	1
	PLC	33	+	+	11.85 \pm 0.65	94	NE	NE	6	0
	PLC	33	-	-	12.05 \pm 0.55	96	NE	NE	4	0
	PLC	3.3	+	+	12.20 \pm 0.42	93	32	61	7	0
	PLC	3.3	-	-	11.98 \pm 0.52	95	36	59	5	0

^aFU = Fluorodeoxyuridine.

^bDC = Deoxycytidine.

^cResults are presented as means \pm standard errors of the means.

^dWhen the immunofluorescent method for sister chromatid differentiation was applied, the proportion of cells in their first mitosis (1st) was estimated as the frequency of E-1st + L-1st. For details, see explanation in text.

NE = Not possible to evaluate.

differentiation in chromosomes with conventional fluorescence-plus-Giemsa procedures. In PHA-stimulated cells, Santenson *et al.* (1979) found no sister chromatid differentiation when 5 μM BUdR was employed. Similarly, Bianchi and Lezana (1976) and Miller (1988) have observed difficulty in identifying the differences of stainability between the sister chromatids of human chromosomes when doses of BUdR lower than 17 μM were employed. It has been addressed that sister chromatid differentiation of sufficient quality for SCE analysis started at BUdR concentration ranging from 7 to 15 μM for human B lymphocytes (Miller, 1988).

To determine whether different concentrations of BUdR would modify the proliferation rate of human lymphocytes *in vitro*, the proportions of cells in their first, second or subsequent divisions were calculated in the samples (Table I). The results showed no alteration of the proliferation rate of lymphocytes according to the concentration of BUdR ($P > 0.05$). On the other hand, Table I also demonstrates that in PLC an increase in the proportion of cells in their first mitosis and a decrease in the frequency of cells in second mitosis were observed when compared to the lymphocyte proliferation kinetics in the corresponding WBC ($P < 0.05$). These results clearly demonstrate that, regardless of the donor, lymphocytes in erythrocyte-free cultures proliferate more slowly than in parallel WBC. Similar observations have been reported previously for human (Mehnert *et al.*, 1984; Larramendy *et al.*, 1990, 1993) and for pig cells (Larramendy *et al.*, 1990, 1993, 1995). They found that mononuclear leukocytes isolated by centrifugation in lymphodex (Mehnert *et al.*, 1984) or cultured in PLC (Larramendy *et al.*, 1990, 1993, 1995) exhibited a slower cell-cycle progression than WBC. The mean generation time was estimated to be approximately 17.5 h and 25.0 h for WBC and PLC, respectively (Larramendy *et al.*, 1990). Thus, there was a difference of about 43% in the length of the cell cycle between the two different types of culture. Furthermore, the present results differed from our previous observations (Larramendy *et al.*, 1990, 1993) in that there were now an increase in the proportion of cells in their first and second mitosis and almost no lymphocytes in their third or further division. Accordingly, the lymphocyte proliferation was slower under the prevailing culture condition than in those previously reported by us. Our results regarding the alteration of cell-cycle progression in relationship with the employed culture condition are in good agreement with similar observations recently reported by us for pig leukocytes (Larramendy *et al.*, 1995).

However, since neither changes in the culture medium composition nor alterations in the sampling

time were introduced in the lymphocyte culture system, other hypothesis must be proposed to explain the differences in the proliferation kinetics of human WBC and PLC. The culture procedure employed in the present report was very simple, but it did require a change of the culture medium and several cell washes within the incubation period. It may reasonably be assumed that this disruption in the continuity of the culture is responsible for the aforementioned observations. Moreover, a differential loss of cells with faster than average generation time or a selective death of early replicating lymphocytes cannot be ruled out. The differential analysis performed between early-(E-1st) and late-replicating (L-1st) first mitosis clearly shows that more than 62% of the first mitosis observed in PLC at the sampling time have started the onset of DNA replication after the change of the culture medium. This observation could therefore be consistent with the latter aforementioned hypothesis. Finally, Wilmer *et al.* (1983) suggested that mononuclear leukocytes in culture might produce cell regulatory factor(s) upon mitogenic stimulation. It is possible, but also speculative, that this(these) factor(s) were eliminated by the change of culture medium, keeping its(their) concentration low and thus inducing the alteration in the proliferation. Whether the existence and relative concentration of any of such factor(s) at lower concentration than normal causes the delay in the cell-cycle progression, however, remains unknown.

The molecular mechanisms that give rise to SCEs are not yet completely understood. Years ago, Painter (1980) proposed a replication model for the mechanisms leading to SCEs. He suggested that during the S phase double-strand breaks are originated at the junctions between completely and partially duplicated replicon clusters. Accordingly, agents that induce blocks to DNA-fork displacement will allow more time for double-strand breaks to occur at junctions favoring an abnormal recombination of DNA strands with the subsequent appearance of SCEs. On the other hand, agents that inhibit initiation of whole clusters will rarely cause it at all. Besides, in studies using human PHA-stimulated lymphocytes several authors have observed that the faster-dividing cell populations have significantly lower SCE frequencies than the slowest-growing cells which exhibit the greatest SCE rates (Ishii and Bender, 1978; Snope and Rary, 1979; Deknudt and Kamra, 1983). We report here a delay in cell-cycle kinetics observed in lymphocytes from erythrocyte-free cultures compared to the WBC progression (Table I). In our studies, too, the greatest SCE response is shown by the slowest growing cells (PLC) (Table I). Thus, Painter's hypothesis could furnish an explanation for

the high frequency of SCE observed in human PLC if we assume that the lengthening of the generation time observed in these cells reflects a lengthening of their S period.

It has been established that the length of each phase of the cell cycle is approximately equal to the total fraction of cells in that phase at any instant multiplied by the total cell-cycle time (Sisken, 1964). Table II shows the fraction of $^3\text{H-TdR}$ labelled cells that were synthesizing DNA during the brief exposure to base analogue. Their frequency in the population reflects, then, the fraction of the cell cycle that is occupied by the S phase. The mean length of the S period was estimated to be approximately 3.6 h and 5.07 h for human WBC and PLC, respectively. These results clearly demonstrate that human lymphocytes in PLC have a markedly lengthened S period compared to WBC. Accordingly, the rate of DNA-fork displacement of lymphocytes in PLC could be slower than in WBC, resulting in an increase of SCEs.

Unfortunately, the information available on the lymphocyte proliferative characteristics is quite conflicting. For instance, although after stimulation of blood lymphocytes with lectins, the cultures contain cells that have divided different numbers of times as demonstrated by the presence of a mixture of first, second, third and subsequent division metaphases at advanced harvesting time intervals, confirming that even though peripheral lymphocytes are considered a GO synchronized cell population *in vivo*, once they are stimulated, sooner or later they show an asynchronous pattern of growth (Sasaki and Norman, 1966; Bianchi and Lezana, 1976; Crossen and Morgan, 1977, 1979; Bianchi *et al.*, 1979; Morimoto and Wolff, 1980; Bianchi and Larramendy, 1983). According to a different type of evidence, it has been postulated the presence of diverse subpopulations of peripheral blood lymphocytes with cell-cycle times ranging from less than 12 h up to 48 h or more (Sasaki and Norman, 1966; Crossen and Morgan, 1979; Morimoto *et al.*, 1983; Larramendy *et al.*, 1990). In this respect, it is almost accepted that the heterogeneity with respect to different generation metaphases at a given sampling time in culture is a reflection of a difference in the times the cells enter their first DNA-synthesis period, and that the cells have about the same generation time thereafter (Crossen and Morgan, 1979; Morimoto and Wolff, 1980; Mutchinick *et al.*, 1980; Morimoto *et al.*, 1983). So far, the different first metaphase patterns (Figures 1A and 1B) observed

Table II - Estimation of the length of the S period of human lymphocytes grown in plasma leukocyte (PLC) and whole blood cultures (WBC). Lymphocytes were grown in PLC and WBC, and exposed to a 20-min pulse $^3\text{H-TdR}$ treatment 48 h and 72 h after stimulation.

Donor	Culture type	Harvesting time (h)	Percentage of labelled interphases ^a	Estimated length of cell cycle (h) ^b	Estimated length of S period ^c
C	WBC	48	19.8	17.5	3.46
	WBC	72	20.2	17.5	3.53
	PLC	48	18.5	25	4.62
	PLC	72	19.9	25	4.97
D	WBC	48	17.8	17.5	3.11
	WBC	72	20.6	17.5	3.50
	PLC	48	19.2	25	4.80
	PLC	72	19.3	25	4.82
E	WBC	48	23.4	17.5	4.09
	WBC	72	24.3	17.5	4.25
	PLC	48	22.2	25	5.55
	PLC	72	22.7	25	5.67

^a After developing the autoradiograms, the frequency of labelled cells at each harvesting time was evaluated, and expressed as a percentage of labelled interphases among all interphases.

^b After Larramendy *et al.* (1993).

^c Following Sisken (1964).

in our cultures represent the first fluorescent evidence of this phenomenon.

Regardless of the explanation for our findings, the primary significance of the observations is to emphasize the incomplete knowledge we have of the mechanism(s) leading to SCEs. Furthermore, the experiments demonstrate that variations in the behavior of lymphocytes in culture exist. The results of these experiments also point out that the sources of variation involved in any short-term lymphocyte culture must be totally controlled when such methods are employed as monitoring assays for clastogenesis.

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RESUMO

Os efeitos da concentração de bromodeoxiuridina (BUdR) e o comprimento do período de síntese (S) na frequência basal de intercâmbios de cromátides irmãs (SCEs) e na taxa de proliferação de culturas de sangue total (WBC) e de leucócitos plasmáticos (PLC) humanos foram estudados.

Um método imunofluorescente foi usado para induzir a diferenciação de cromátides irmãs. Este método emprega uma baixa concentração de BUdR para a marcação de cromossomos durante o primeiro ciclo celular *in vitro* (3,3 μM), e o anticorpo monoclonal anti-BUdR conjugado com fluoresceína isotiocianada (FITC) para a detecção. Permitiu-se que WBC e PLC crescessem durante o primeiro ciclo celular (48 h) na presença de BUdR; o meio de cultura foi então substituído pelo meio de cultura sem BUdR e as células foram cultivadas por um ciclo celular adicional (24 h) até a colheita (72 h). Os cromossomos foram contrastados com iodeto de propidium DAPI. PLC mostrou no mínimo um aumento em dobro na frequência de SCE sobre os valores de WBC e a proliferação celular foi mais lenta em PLC que em WBC paralela, independente da concentração de BUdR no meio de cultura, pelo menos na faixa do análogo de base empregada (3,3 μM -33,0 μM). Além disso, uma estimativa do comprimento do período S, feita pela análise da proporção de células marcadas que estavam sintetizando DNA durante diferentes tempos de colheita, revelou que PLC tem um período S marcadamente extenso, comparado a WBC. Conseqüentemente, a taxa de deslocamento de fork do DNA de linfócitos em PLC poderia ser mais lenta que em WBC, resultando em um aumento na frequência de SCE destas culturas.

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