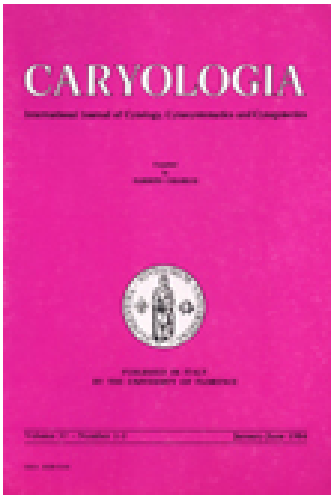

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Spontaneous Endoreduplication, Tetraploidy and Chromosome Breakage in Lymphocyte Cultures from Healthy Subjects

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SPONTANEOUS ENDOREDUPLICATION, TETRAPLOIDY AND CHROMOSOME BREAKAGE IN LYMPHOCYTE CULTURES FROM HEALTHY SUBJECTS

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SUMMARY — 42,703 metaphases of peripheral lymphocytes from 20 healthy subjects (10 women and 10 men) were examined in order to establish the frequency of endoreduplicated cells and of tetraploid cells without diplochromosomes. Frequencies were found to be 0.016% and 0.112%, respectively. The two sexes did not differ as to the frequency of tetraploid cells, with and without diplochromosomes (about 0.13% in either sex). In a total of 2,135 well spread metaphases examined, 26 cells (i.e. 1.2%) with chromosomal breaks were found. Again, no significant differences between the two sexes were found as to such chromosome abnormalities.

INTRODUCTION

Endoreduplication is a phenomenon occurring both in plant and animal tissues (GEITLER 1953; D'AMATO 1965). It has also been documented that endoreduplications are induced by chemical (TAKANARI and IZUTSU 1983) or physical agents (LÜCKE-HUHLE 1983). It is also well known that endoreduplication occurs spontaneously in mammalian cell cultures (GATTI *et al.* 1976 and other reports quoted by these Authors). Data on spontaneous endoreduplication levels in short term cultures of peripheral lymphocytes from healthy subjects have already been reported (OBE 1965; TURNER and WALD 1965; GRAY and DARTNALL 1965; TAKANARI and IZUTSU 1976; DOSIK *et al.* 1979).

Knowledge of the incidence of endoreduplications in peripheral lymphocytes from healthy subjects is useful for many purposes, including the study of human neoplasms. For this reason we are reporting here the results obtained from the analysis of a large number of metaphases (42,703) from 20 healthy subjects.

MATERIAL AND METHODS

Metaphases of PHA-stimulated peripheral lymphocytes from subjects who had not been recently exposed to radiations and who had not undergone any recent drug

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treatment were the object of the present study. Of the 20 subjects, 10 were females aged between 21 and 50 years, and 10 were males aged between 17 and 52 years. Leukocyte-rich plasma (1 ml) obtained from heparinized blood after gravity sedimentation, was diluted with TC 199 medium, added with 15% autologous plasma, antibiotics (penicillin 100 units/ml, streptomycin 100 μg/ml), and cultured for 72 hr. Colcemid (0.1 μg/ml) was added to all cultures 2 hr prior to cell harvesting. Other details of the method followed were described previously (CARBONE *et al.* 1982). A minimum of 100 metaphases per subject was examined for analysis of chromosome breaks which were identified following indications reported in ISCN (1978). Incidence of breaks was evaluated from all metaphases with well spread chromosomes. Tetraploid cells with or without diplochromosomes were considered separately. These cells were detected using low magnification and immediately checked (by at least two observers) under oil immersion (1,250 ×). Frequencies were calculated considering all metaphases observed at low magnification, according to the procedure suggested by DOSIK *et al.* (1979). Totals of 2,011 to 2,537 metaphases per subject were analysed. Statistical evaluation was carried out using the Student's t test.

RESULTS AND DISCUSSION

Results are summarized in Tables 1 and 2.

A total of 55 tetraploid cells with and without diplochromosomes (or 0.13%) was found over 42,703 metaphases from all subjects. The two sexes did not differ as to the frequency of these cells. Only one metaphase with diplochromosomes was found over 20,654 metaphases in the group of female subjects, while 6 endoreduplicated cells were observed over 22,049 cells of male subjects. No significant difference was found between the two sex groups as to frequencies of tetraploid cells with diplochromosomes ($P > 0.05$) or without diplochromosomes ($P > 0.5$).

26 cells with breaks were found over 2,135 well spread metaphases from all subjects, equal to a rate of about 1.2%. No significant differences between the two sex groups, as to such chromosome abnormalities, were observed ($P > 0.6$). Our results are in good agreement with data previously reported (GUNDY and VARGA 1983 and other reports quoted by these Authors).

29 cells with gaps (not included in Table 2) were found over 2,135 metaphases, equal to a rate of mean 1.4%.

Analytical data on the frequency of tetraploid cells with or without diplochromosomes in each subject are reported in Table 3. The frequency of cells with diplochromosomes was 7 over 42,703 metaphases of all subjects examined (or about 0.016%). This result is in good agreement with data reported by DOSIK *et al.* (1979).

In the present study endoreduplication was observed only in 6 out of 20 subjects (1 woman and 5 men). It is well known that this phenomenon occurs spontaneously only in a few subjects in short term cultures; in cultures pro-

TABLE 1 - *Frequency of tetraploid cells with or without diplochromosomes in lymphocyte cultures from 10 healthy women and 10 healthy men.*

Group	Cells examined	Tetraploid cells		
		with diplo-chromosomes (%)	without diplo-chromosomes (%)	Total (%)
Women	20,654	1 (0.005)	26 (0.13)	27 (0.13)
Men	22,049	6 (0.027)	22 (0.10)	28 (0.13)
Total	42,703	7 (0.016)	48 (0.11)	55 (0.13)

TABLE 2 - *Chromosome breaks in lymphocyte cultures from 10 healthy women and 10 healthy men.*

Group	Cells examined	Cells with breaks (%)	Range
Women	1,135	13 (1.15)	0-3%
Men	1,000	13 (1.30)	0-3%
Total	2,135	26 (1.22)	0-3%

TABLE 3 - *Tetraploid cells with or without diplochromosomes in individual subjects.*

Subjects identification	Sex/Age	Cells scored	Tetraploid cells		
			with diplo-chromosomes	without diplo-chromosomes	Total
1 (R.R.)	F/24	2,203	0	1	1
2 (P.E.)	F/22	2,045	0	1	1
3 (N.R.V.)	F/21	2,021	0	0	0
4 (M.G.)	F/22	2,020	0	2	2
5 (G.G.)	F/41	2,026	0	1	1
6 (C.G.)	F/40	2,063	1	4	5
7 (P.G.)	F/30	2,033	0	2	2
8 (G.M.)	F/33	2,030	0	3	3
9 (F.C.)	F/50	2,187	0	8	8
10 (B.A.)	F/25	2,026	0	4	4
11 (A.V.)	M/52	2,038	0	1	1
12 (B.G.)	M/33	2,011	0	1	1
13 (B.G.)	M/34	2,334	2	6	8
14 (P.A.)	M/33	2,128	0	1	1
15 (S.P.)	M/33	2,020	1	4	5
16 (V.G.)	M/26	2,537	1	2	3
17 (M.R.)	M/33	2,169	1	2	3
18 (R.P.)	M/17	2,520	0	1	1
19 (M.S.)	M/38	2,058	1	0	1
20 (M.L.)	M/22	2,234	0	4	4
Total		42,703	7 (0.016)*	48 (0.112)*	55 (0.129)*

F = Females; M = Males; * (%)

longed for some time, however, endoreduplications were observed in all subjects (TAKANARI and IZUTSU 1976).

Other data on spontaneous occurrence and cellular kinetics of endoreduplication in PHA-stimulated tonsillar lymphocytes were reported by TAKANARI and IZUTSU (1981). These Authors showed that endoreduplication frequency was very low: not more than 4 cells over 1,000 mitoses.

The two most comprehensive studies on the spontaneous occurrence of cells with diplochromosomes in peripheral lymphocytes (DOSIK *et al.* 1979 and the present work) show that endoreduplication frequency in healthy subjects is very low, averaging 0.013% and 0.016%, respectively. These data may be utilized for investigations on the occurrence of endoreduplications in human neoplasies and for the evaluation of the levels of endoreduplications induced by chemical and physical agents.

The phenomenon of endoreduplication may be influenced by various factors (GATTI *et al.* 1976; TAKANARI and IZUTSU 1976; LÜCKE-HUHLE 1983) and interesting hypoteses have been suggested (TAKANARI and IZUTSU 1976; LÜCKE-HUHLE 1983), but still it is not fully understood.

Number of Authors (SCHWARZACHER and SCHNEDL 1965; RIZZONI and PALITTI 1973; SUTOU and TOKUYAMA 1974; GATTI and OLIVERI 1976; TAKANARI and IZUTSU 1983; LÜCKE-HUHLE 1983) reported data on endoreduplication cycle. Studies using chemical or physical agents shown that endoreduplication was induced mainly during G_2 phase (RIZZONI and PALITTI 1973; SUTOU and TOKUYAMA 1974; TAKANARI and IZUTSU 1983; LÜCKE-HUHLE 1983).

Since high frequencies of cells with diplochromosomes were found among human and murine tumor cells (LEVAN and HAUSCHKA 1953; LEVAN 1956), in cases with ascertained leukaemia (BOTTURA and FERRARI 1963; REISSMAN *et al.* 1963) and in cells from patients with congenital anomalies with or without chromosome aberrations (Authors quoted by TAKANARI and IZUTSU 1976), the significance of such phenomenon was questioned.

It is well documented that some agents utilized in antiblastic therapy (6-mercapto purine, for example) induce polyploidy including endoreduplication (NASJLETI and SPENCER 1966). It is known, moreover, that increased frequencies of endoreduplicated cells were found in patients with leukemia previously treated with antimitotics (BOTTURA and FERRARI 1963; REISSMAN *et al.* 1963). In numerous cases of neoplasia, however, the phenomenon cannot be imputed to the therapeutic treatment; therefore the occurrence of endoreduplication could be ascribed to the conditions of growth of the neoplastic cell population (GATTI *et al.* 1976).

REFERENCES

BOTTURA C. and FERRARI I., 1963. — *Endoreduplication in acute leukemia.* Blood, 21: 207-212.

CARBONE P., GRANATA G., L. MARGIOTTA G., BARBATA G. and MAJOLINO I., 1982. — *Ph¹ duplication, t (13q−; 14q+) and trisomy 19 in a case with chronic myeloid leukemia in lymphoid blast crisis at presentation.* Haematologica, 67: 595-604.

D'AMATO F., 1965. — *Endopolyploidy as a factor in plant tissues development.* Proc. intern. Conference on Plant Tissue Culture, Berkeley, 449-462.

DOSIK H., STEIER W. and LUBINIECKI A., 1979. — *Inherited aplastic anaemia with increased endoreduplications: a new syndrome or Fanconi's anaemia variant?* Br. J. Haematol., 41: 77-82.

GATTI M., PECCI L. and OLIVERI G., 1976. — *«Spontaneous» endoreduplication in Chinese hamster cell cultures. I. Effect of growth conditions.* Caryologia, 29: 155-175.

GATTI M. and OLIVERI G., 1976. — *«Spontaneous» endoreduplication in Chinese hamster cell cultures. II. Analysis of the mitotic cell cycle.* Caryologia, 29: 177-186.

GEITLER L., 1953. — *Endomitose und endomitotische Polyploidisierung.* Protoplasmatologia, VI/C, Springer Wien.

GRAY J.E. and DARTNALL J.A., 1965. — *Preventing chromosomal endoreduplication.* Lancet, i: 915.

GUNDY S. and VARGA L.P., 1983. — *Chromosomal aberrations in healthy persons.* Mut. Res., 120: 187-191.

ISCN, 1978. — An International System for Human Cytogenetic Nomenclature. Birth Defects: Original Article Series, Vol. 14, No. 8, The National Foundation, New York; also in Cytogenet. Cell Genet., 21: 309-404.

LEVAN A., 1956. — *Chromosome studies on some human tumors and tissues of normal origin, grown in vivo ad in vitro at the Sloan-Kettering Institute.* Cancer, 9: 648-663.

LEVAN A. and HAUSCHKA T.S., 1953. — *Endomitotic reduplication mechanisms in ascites tumors of the mouse.* J. Natl. Cancer Inst., 14: 1-43.

LÜCKE-HUHLE C., 1983. — *Endoreduplication in Chinese hamster cells during α-radiation induced G_2 arrest.* Mut. Res., 119: 407-413.

NASJLETI C.E. and SPENCER H.H., 1966. — *Chromosome damage and polyploidization induced in human peripheral leukocytes in vivo ad in vitro with nitrogen mustard, 6-mercaptopurine, and A-649.* Cancer Res., 26: 2437-2443.

OBE G., 1965. — *Chromosome endoreduplication in normal controls.* Lancet, i: 53.

REISSMAN L.E., ZUELZER W.W. and MITANI M., 1963. — *Endoreduplication in a patient with acute monocytic leukemia.* Lancet, ii: 1038-1039.

RIZZONI M. and PALITTI F., 1973. — *Regulatory mechanism of cell division. I. Colchicine-induced endoreduplication.* Exptl. Cell Res., 77: 450-458.

SCHWARZACHER H.G. and SCHNEDL W., 1965. — *Endoreduplication in human fibroblast cultures.* Cytogenetics, 4: 1.

SUTOU S. and TOKUYAMA F., 1974. — *Induction of endoreduplication in cultured mammalian cells by some chemical mutagens.* Cancer Res., 34: 2615-2623.

TAKANARI H. and IZUTSU K., 1976. — *Studies on endoreduplication. I. Diplochromosomes in the peripheral lymphocyte culture.* Mie Med. J., 26: 59-68.

—, 1981. — *Studies on endoreduplication. II. Spontaneous occurrence and cellular kinetics of endoreduplication in PHA-stimulated tonsillar lymphocytes.* Cytogenet. Cell Genet., 29: 77-83.

—, 1983. — *Studies on endoreduplication. III. Endoreduplication induced by Mitomycin C in PHA-stimulated tonsillar lymphocyte cultures.* Mut. Res., 107: 297-306.

TURNER J.H. and WALD N., 1965. — *Endoreduplication and disease.* Lancet, i: 915.

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