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G2 phase cell cycle disturbance as a manifestation of genetic cell damage

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Abstract. The predominant cell cycle change induced by X-rays and clastogens in peripheral blood mononuclear cells is the accumulation of cells in the G2 phase of the cell cycle. We show that this accumulation consists of cells that are either delayed or arrested within the G2 phase. Since both X-rays and DNA crosslinking chemicals are known to damage DNA, the G2 phase inhibition caused by these agents is thought to be one of the primary manifestations of (unrepaired) DNA damage. This interpretation is supported by two additional findings. *(1)* Older individuals have elevated baseline levels of mononuclear blood cells that are delayed and/or arrested in the G2 phase of the cell cycle. This coincides with the increased chromosomal breakage rates reported for older individuals. *(2)* Irrespective of their age, individuals with inherited genetic instability syndromes (such as Fanconi anemia and Bloom syndrome) exhibit elevated G2 phase cell fractions. We show that the method used to detect such induced or spontaneous cell cycle changes, viz. BrdU-Hoechst flow cytometry, is a rapid and highly sensitive technique for the assessment of genetic cell damage.

Introduction

The biological relevance of DNA damage induced by X-rays or chemicals in the living organism is not limited to physical damage, but rather involves a number of complex changes that affect all levels of the cellular hierarchy. For example, most eukaryotic cells respond to genotoxic stress with the induction of a large number of genes. Examples of primary response genes (i.e., those that do not require de novo protein synthesis) are c-fos, c-jun, jun-B, and Egr-1. Secondary response genes include those coding for collagenase, gadd153, gadd45, and plasminogen activator. Many of these response genes are known to be involved in the control of cell proliferation and/or DNA repair (for a review, see Holbrook and Fornace 1991). In view of such complex pattern of cellular changes in response to genetic injury, the assessment of genetic cell damage should not be limited to the mere analysis of physical lesions in DNA or chromosomes. We therefore present a cell kinetic approach for the evaluation of genetic cell damage. This approach detects changes in cell proliferation that are a probable primary manifestation of DNA damage in living cell systems. As such, the cell kinetic approach may yield more biologically relevant information than most methods concerned with the direct assessment of physical DNA damage.

Dedicated to Professor Ulrich Wolf on the occasion of his 60th birthday

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Materials and methods

Cell culture

Voluntary blood donors of various ages were recruited from the authors' families and friends. Patient blood samples were obtained from primary care physicians wishing to confirm or rule out diagnosis of a chromosomal breakage syndrome. Peripheral blood mononuclear cells were isolated from 5–10 ml heparinized venous blood using Ficoll gradient centrifugation (Ficoll-Paque, Pharmacia, Uppsala, Sweden). Mononuclear blood cell cultures were started using 100000 cells per ml medium (RPMI 1640, Gibco, Grand Island, NY) supplemented with 1% autologous serum, 15% heat-inactivated fetal bovine serum (Gibco), equimolar amounts of 10^{-4} *M* 2′-deoxycytidine and 5-bromo-2′-deoxyuridine (Sigma, Deisenhofen, Germany) and 1.2 μg/ml phytohemagglutinin (PHA) (Wellcome Diagnostics, Burgwedel, Germany). The 50-ml culture flasks were wrapped in aluminum foil for light protection and incubated for the desired length of time at 37.5°C in 5% CO_2/high humidity incubators (Hereaus, Hanau, Germany). For harvesting, the cell suspension was pelleted by centrifugation and resuspended in RPMI media supplemented with 10% fetal bovine serum and 10% dimethylsulfoxide as a cryoprotectant. Samples were stored at –20°C for 2–24 weeks prior to flow cytometric analysis.

Exposure to radiation and chemicals

X-irradiation was carried out immediately after Ficoll gradient separation with mononuclear cells suspended at 100000 cells/ml in RPMI 1640 medium containing 1% autologous serum. Irradiation

was performed at ambient temperature using a Müller MG 150 generator (CHF Müller, Hamburg, Germany) at 110 kV and 10 mA (5 mm aluminum filter). This set-up yields a dose-rate of 1.0 Gy/min. Mitomycin C (MMC) and diepoxybutane (DEB) were purchased from Sigma and dissolved in phosphate-buffered saline at neutral pH. The desired concentration of these agents was added 22 h after the cultures were set up. All cultures were handled using latex gloves and vertical laminar flow hoods.

Bivariate BrdU-Hoechst/ethidium bromide flow cytometry

For flow cytometric analysis, cells were thawed, centrifuged and resuspended at a concentration of 4×10^5 cells/ml in staining buffer. The staining buffer contained 0.1 *M* TRIS-HCl pH 7.4, 0.154 *M* NaCl, 0.5 m*M* $MgCl_2$, 1 m*M* $CaCl_2$, 0.1% NP40, and 0.2% bovine serum albumin (Sigma). Staining was performed in two steps. First, cells were stained with 1.2 µg Hoechst 33258 (Sigma) per ml buffer for 15 min, and then they were stained for the same length of time with 1.5 µg ethidium bromide (Serva, Heidelberg, Germany) per ml buffer. Both staining steps were executed at 4°C. Flow cytometry was performed with an ICP-22 arc-lamp instrument (Phywe, Göttingen, Germany) interfaced to a PDP 11/23 microcomputer (Digital Equipment, Maynard, MA). At a flow rate of around 300 cells/s, the instrument simultaneously records (1) the extent of BrdU substitution into DNA by the degree of quenching of Hoechst 33258 fluorescence, and (2) the cell-cycle-dependent oscillation of DNA content via ethidium bromide fluorescence. This bivariate analysis allows the quantitation of cell numbers in each G1, S and G2/M phase of three successive cell cycles, using specialized cell cycle software developed by P. S. Rabinovitch (University of Washington, Seattle; distributed by Phoenix Flow Systems, San Diego, CA). Details of the BrdU-Hoechst flow cytometric technique have been published in recent reviews (Rabinovitch et al. 1988; Kubbies et al. 1989; Poot et al. 1990a).

Cell kinetic analysis

For the cell kinetic evaluation of temporally spaced cell cycle data, a modified model was used that assumes an exponential rate of exit (Smith and Martin 1973) from the quiescent phases (G0–G1) and from each of the subsequent cell cycle phases. In order to account for a variable fraction of non-cycling cells that is typically present in primary cell cultures (such as used in this study), Rabinovitch (1983) modified the original Smith-Martin model such that the percentage of non-cycling cells at any given time *(t)* after the start of the culture can be calculated as $(1-f) \times 10^{p(t-T)} + f$, where f is the fraction of non-dividing cells, p is the rate of exit from one compartment into the next (transition rate), and T is the minimum cell cycle compartment duration. The mean cell cycle compartment duration then equals $T + 0.301/p$. The cell cycle analysis software developed by Rabinovitch ("Mulit2D") plots the data obtained from temporally spaced cell harvests as a log of the percentage of cells in a given compartment vs the time after the start of the culture, and fits this data with a nonlinear least-squares technique. The program performs the calculation of the standard error of each of the parameters in the least-squares fit by the use of an error matrix as described by Bevington (1969:

Results

Cell cycle inhibition resulting from radiation

In order to analyze cell cycle defects caused by radiation, freshly isolated peripheral blood mononuclear cells were exposed to increasing doses of X-rays and incubated in the presence of bromodeoxyuridine for 72 h. Flow cytometry was used to generate typical bivariate cytograms that reveal the distribution of cells throughout three consecu-

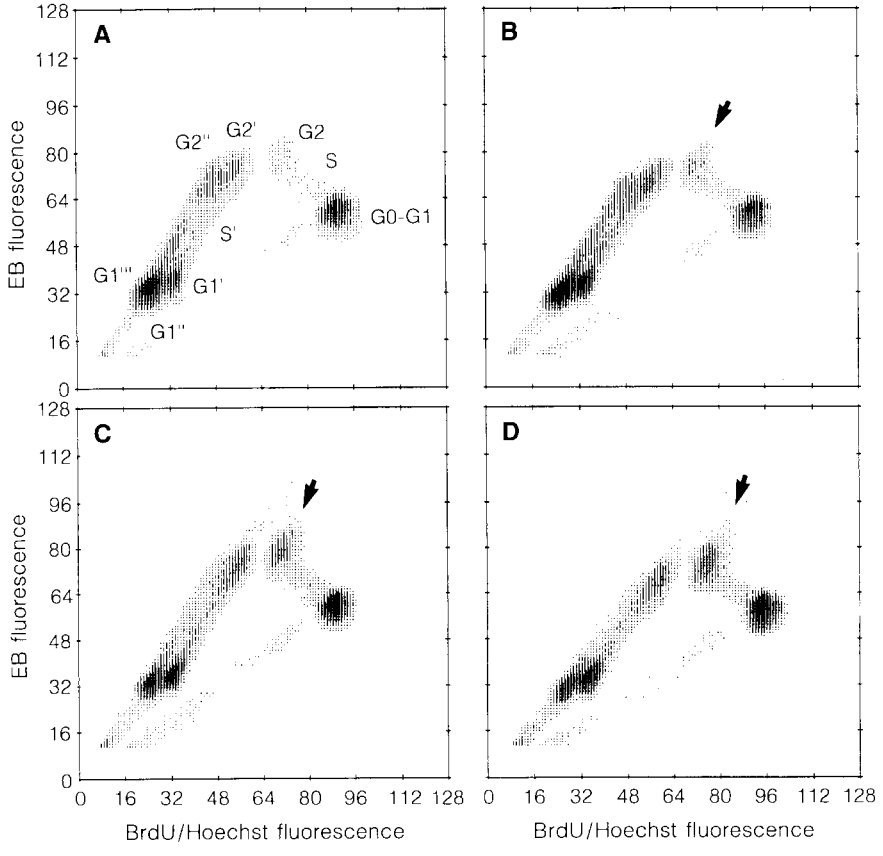

Fig. 1A–D. Bivariate cytograms showing the cell cycle distribution of control and irradiated human peripheral blood mononuclear cells 72 h after polyclonal activation by PHA. Individual cell cycle compartments are denoted by corresponding *capital letters* in **A** (control). Note increasing accumulations of cells in the G2 phase compartment of the first cell cycle with increasing doses of radiation *(arrows)*. **B** 1.5 Gy; **C** 2.5 Gy; **D** 4.0 Gy

Table 1. The 72-h cell cycle distributions of human peripheral blood mononuclear cells exposed to increasing doses of X-ray prior to activation by PHA. YA, Young adult blood donors (age 1–55 years); OLD, elderly blood donors (age 55–85 years). Data are mean percentages and standard deviations. GF denotes the growth fraction (all cycling cells except G0/G1 phase compartment)

Donor group	*n*	X-ray dose (Gy)	Cell cycle compartment		
			G0–G1	G2	G2/GF
YA	41	0	32.0 ± 18.3	2.8 ± 1.0	0.04 ± 0.02
YA	40	1.5	33.0 ± 16.5	5.6 ± 2.0[b]	0.09 ± 0.03[b]
YA	32	2.5	37.6 ± 15.8	8.8 ± 3.9[b]	0.14 ± 0.05[b]
YA	35	4.0	43.6 ± 18.4[a]	15.1 ± 7.3[b]	0.26 ± 0.09[b]
YA	23	6.0	38.3 ± 8.6	27.4 ± 6.3[b]	0.45 ± 0.10[b]
YA	8	10.0	48.9 ± 11.0	33.0 ± 10.6[b]	0.64 ± 0.12[b]
OLD	16	0	63.9 ± 15.5	3.5 ± 1.7	0.09 ± 0.02
OLD	16	1.5	68.1 ± 12.9	4.0 ± 1.8	0.13 ± 0.02[b]
OLD	13	2.5	71.6 ± 12.8	4.7 ± 1.9	0.17 ± 0.03[b]
OLD	13	4.0	77.8 ± 11.0[a]	5.5 ± 3.0	0.24 ± 0.04[b]

[a] $P < 0.01$
[b] $P < 0.001$
Student's *t*-test

tive cell cycles. As shown in Fig. 1, exposure to X-rays causes significant changes in these 72-h cytogram patterns. At a dose of 1.5 Gy (panel B), these changes mostly affect the S and G2 phases of the first cell cycle, with increasing numbers of cells accumulating in these respective compartments. At higher doses (2.5 and 4.0 Gy, panels C and D), there is additional impairment of cell cycle progression. This is seen in the decreasing number of cells that reach the third cell cycle. Less evident by visual inspection of these cytograms is the dose-dependent increase of cells that fail to respond to the mitogen. Such non-responding cells are represented by the G0–G1 cell fraction, as indicated in panel A for the (mock-irradiated) control culture.

Quantitative evaluation of cytograms derived from a series of young and older blood donors (Table 1) shows that, in the case of young donors, a significant G2 phase increase occurs at a radiation dose as low as 1.5 Gy. At a dose of 10 Gy, cell growth is severely impaired: nearly half of the cells fail to respond to the mitogenic stimulus and thus remain in the non-cycling (G0–G1) compartment. Of these cells, 33% are halted in the G2 phase of the first cell cycle. In contrast, the G2 phase of the older donors seems less sensitive to the effects of radiation (Table 1). This, however, is a spurious result, since older individuals show larger non-cycling cell fractions (and, consequently, smaller growth fractions) than young blood donors. The proliferation-independent assessment of the radiation effect (via the G2/GF ratio) yields almost identical data for young and old donors (Table 1, right-hand column).

Measurements of the cell cycle distribution at a single time point (i.e. 72 h) provide only a static picture of the growth disturbance induced by increasing doses of radiation. In order to obtain data on the manner in which radiation affects the duration of individual cell cycle phases and in which it affects the fraction of cells that enter but do not exit from a given cell cycle phase, the analysis of temporally spaced cell harvests is required. Such sequential analysis permits the construction of a series of cell cycle compartment transition curves (Kubbies et al. 1985a). In the examples of such curves shown in Fig. 2, the log percentages of cells within a given cell cycle compartment are plotted as a function of time after the mitogenic stimulus. Exposure to a radiation dose of 4 Gy changes

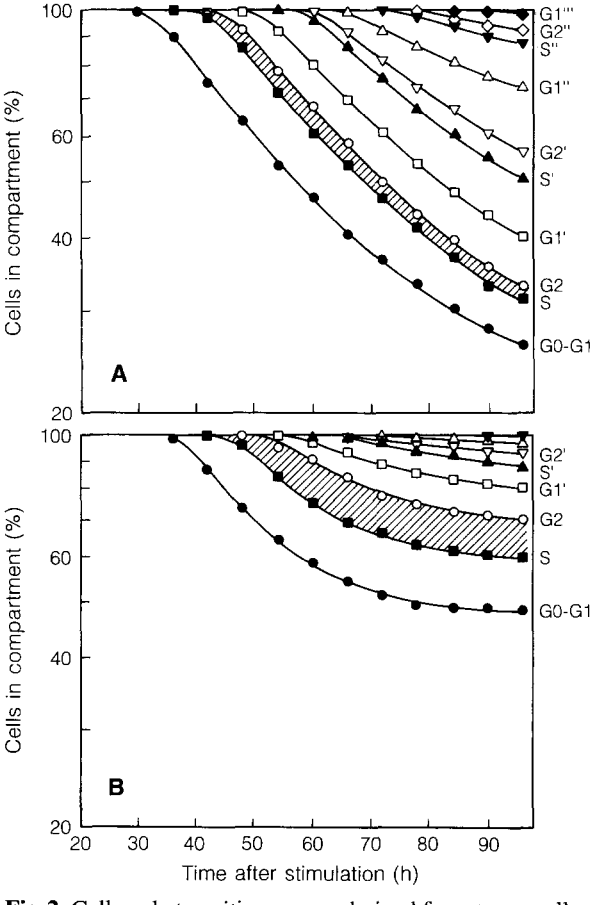

Fig. 2. Cell cycle transition curves derived from temporally spaced samples of control (**A**) and X-irradiated (**B**; 4.0 Gy) human peripheral blood mononuclear cells. The curves were constructed using the Rabinovitch modification of the Smith-Martin transition probability model. The *shaded segment* delineates the time course of entry into and exit from the G2 phase of the first cell cycle

Table 2. Cell kinetic changes caused by X-irradiation in human peripheral blood mononuclear cell cultures. Data are derived from temporally spaced samples fitted to the modified model of exponential cell transit

Cell cycle compartment	Dose (Gy)	Mean duration (h)	Cell cycle arrest (%)	No. samples tested
G0–G1	0	45.6 ± 7.2	27.9 ± 14.7	6
	1.5	46.3 ± 6.4	31.8 ± 12.5	6
	4.0	51.4 ± 5.7	42.6 ± 10.4	3
S	0	8.6 ± 1.3	2.1 ± 1.8	6
	1.5	8.7 ± 0.6	3.8 ± 2.6	6
	4.0	9.5 ± 0.5	7.5 ± 3.4[a]	3
G2	0	4.0 ± 0.5	1.2 ± 1.7	6
	1.5	4.9 ± 0.9	2.1 ± 1.8	6
	4.0	6.8 ± 1.9[a]	8.8 ± 2.7[a]	3

[a] $P < 0.01$
Student's *t*-test

the X and Y axis intercepts, and the shape of these curves. As highlighted by the shaded areas, cells traversing the G2 phase of the first cell cycle undergo the most prominent changes after exposure to X-rays.

Using the Rabinovitch modification of the Smith-Martin cell kinetic model, the following cell cycle parameters were computed from such temporally spaced flow cytometric data: minimal and mean compartment duration, transition probability, and compartment-specific cell cycle arrest. Table 2 summarizes changes of mean cell cycle compartment duration and compartment arrest that are caused by increasing doses of radiation. The mean duration of the G2 phase transit increases significantly at a dose of 4 Gy, as does the percentage of cells that become arrested within the S and the G2 phases of the first cell cycle. These results indicate that the dose-dependent inhibition of cells in the G2 phase caused by X-rays (Fig. 1) consists of two distinct cell kinetic components: *(a)* an increased duration of G2 phase transit for some cells, and *(b)* a permanent arrest of an increasing fraction of cells within the G2 phase compartment.

Cell cycle inhibition caused by DNA crosslinking agents

As in the case of radiation, exposure to DNA crosslinking agents causes a dose-dependent accumulation of cells in the G2 phase of the first cell cycle. At the highest dosage used, severe impairment of cell cycle progression is evident by the increasing lack of cells within the third cell cycle (Table 3). MMC and DEB also elicit a dose-dependent increase of the non-cycling (G0–G1) cell fraction. As shown in Table 3, a statistically significant accumulation of cells in the G2 phase of the first cell cycle is first seen at a concentration of 50 ng/ml for DEB; in the case of MMC, exposure to only 5 ng/ml causes a significant G2 phase alteration. The tenfold greater sensitivity of 72-h cell cultures to MMC, as opposed to DEB, is also apparent at the higher concentration ranges (Table 3). Again, cell kinetic studies with temporally spaced samples indicate that the dose-dependent accumulation of cells within the G2 phase compartment consists of two components, i.e., prolongation of G2 phase transit and permanent arrest within that compartment of the cell cycle (data not included).

Cell cycle defects related to endogenous DNA lesions

Cell cycle defects related to aging. During the assessment of radiation-induced cell cycle alterations, it was noticed that older individuals had higher baseline levels of first cycle G2 phase cells than younger individuals. This is shown in Table 1. The excess of first cycle cells of older donors is in part the result of a delayed mitogen response that is characteristic for older donors (Kubbies et al. 1985a; Schindler et al. 1988). However, Fig. 3 shows that

Table 3. Effects of crosslinking agents diepoxybutane (DEB, **A**) and mitomycin C (MMC, **B**) on the 72-h cell cycle distributions of human peripheral blood cells of young adult (YA; ages 1–55 years) blood donors. Data are mean percentages and standard deviations

Donor group	*n*	Crosslinking agent (ng/ml)	Cell cycle compartment		
			G0–G1	G2	G2/GF
A DEB					
YA	30	0	23.1 ± 8.1	3.2 ± 1.7	0.04 ± 0.02
YA	24	10	20.6 ± 7.8	3.5 ± 1.7	0.04 ± 0.03
YA	29	50	23.1 ± 7.6	6.2 ± 2.9[b]	0.08 ± 0.04[b]
YA	28	100	23.9 ± 9.3	9.7 ± 4.7[b]	0.14 ± 0.09[b]
YA	17	500	34.8 ± 12.7[b]	28.0 ± 11.0[b]	0.42 ± 0.11[b]
B MMC					
YA	18	0	20.0 ± 5.6	3.5 ± 1.8	0.04 ± 0.02
YA	7	0.5	19.4 ± 4.4	5.2 ± 0.8	0.07 ± 0.01
YA	14	1	20.7 ± 3.8	3.8 ± 1.8	0.05 ± 0.02
YA	6	5	21.0 ± 5.4	6.8 ± 2.0[b]	0.09 ± 0.03[b]
YA	17	10	22.6 ± 4.7	7.5 ± 4.5[a]	0.10 ± 0.06[b]
YA	6	50	26.4 ± 5.6	26.8 ± 4.4[b]	0.37 ± 0.07[b]
YA	13	100	33.7 ± 10.2[b]	29.2 ± 9.4[b]	0.43 ± 0.08[b]

[a] $P < 0.01$
[b] $P < 0.001$
Student's *t*-test

the proliferation-independent assessment of the G2 phase cell fraction via the G2/GF ratio reveals a definite (and growth-independent) increase of G2 phase cells as a function of donor age ($r = 0.93$; $P < 0.001$).

Cell cycle defects resulting from inherited genetic instability. A striking example of a spontaneous cell cycle lesion is the G2 phase defect in Fanconi anemia (FA) (Dutrillaux et al. 1982; Kubbies et al. 1985b). Figure 4 illustrates the characteristic cell cycle lesion in FA cells. The cytogram shows that FA cells accumulate not only in the G2 phase of the first cell cycle, but also within the G2 phase compartments of the second and third cell cycles (arrows). The ratio between the sum of all G2 phases and the growth fraction serves as a proliferation-independent parameter that permits the quantitative assessment of the cell cycle defect in FA cells. As shown by the bar graph of Fig. 4, this ratio provides a clear distinction between a series of 25 patient and 93 control cultures (Seyschab et al., in preparation).

A further example of a spontaneous G2 phase arrest was observed in 72-h mononuclear blood cell cultures from patients with Bloom syndrome (BS). However, in contrast to FA, the cell cycle lesion detected in BS mononuclear blood cells appears to be limited to the second cell cycle G2 phase. This peculiar behavior is illustrated in Fig. 5, which also shows that BS blood cell cultures tend to respond less well to polyclonal activation by PHA than the respective controls. As a manifestation of this poor mitogen response, a prominent G0–G1 cell-fraction is the hallmark of BS cell cultures. The bar graph of Fig. 5 summarizes the cell cycle findings in our BS patients. It also shows that the proliferation-independent assessment of the (in this case) second cell cycle G2 phase defect (via the G2′/GF ratio) permits a clear distinction between BS and control cell cultures.

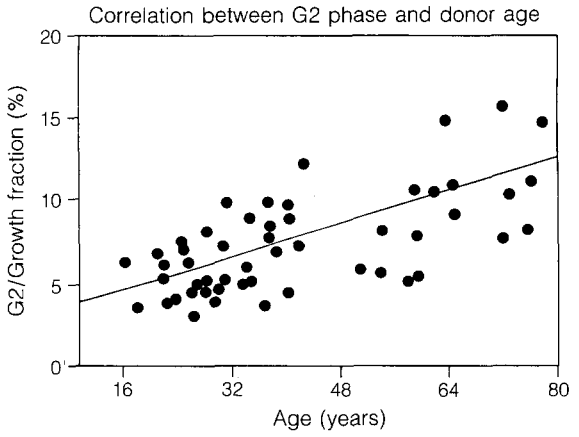

Fig. 3. Proliferation-independent measurement of the first cell cycle G2 phase fraction (G2/GF ratio) as a function of donor age in 72-h peripheral blood mononuclear cell cultures from 52 healthy cell donors aged 16–78 years. $Y = 0.12X + 4.24$

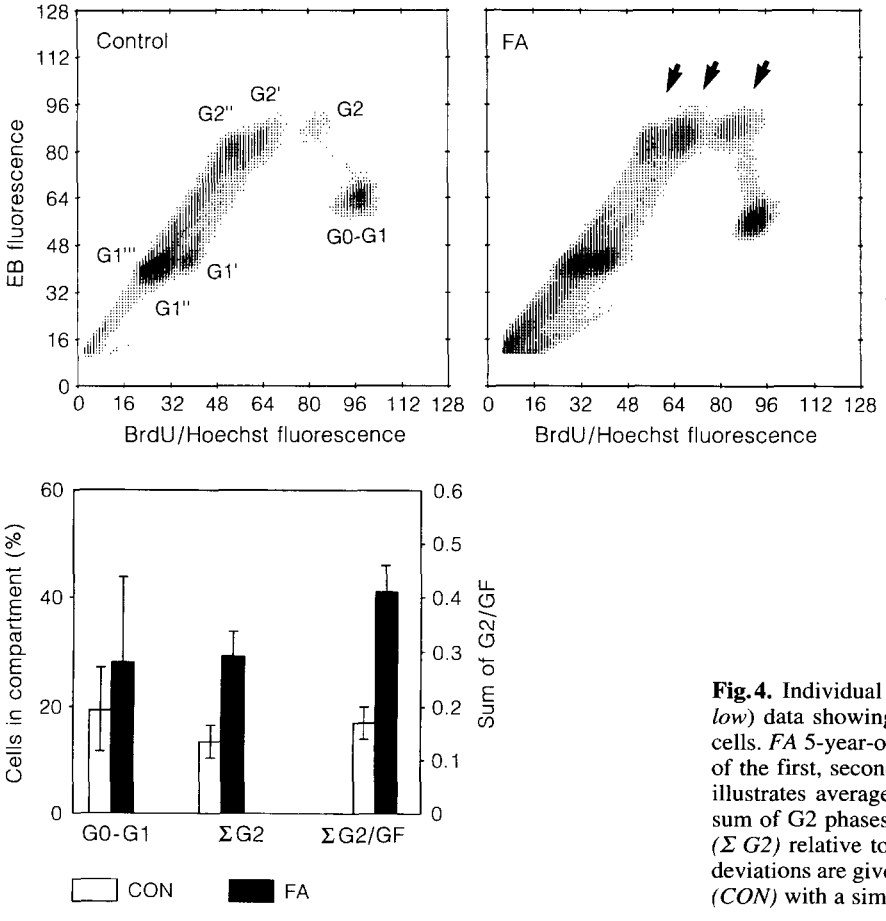

Fig. 4. Individual (cytograms, *above*) and cohort (bar graph, *below*) data showing G2 phase blockage in FA mononuclear blood cells. *FA* 5-year-old patient with FA. *Arrows* denote the G2 phases of the first, second and third cell cycles *(G2–G2″)* The bar graph illustrates averages of the non-cycling cell fraction *(G0/G1)*, the sum of G2 phases *(Σ G2)*, and the ratio of the sum of G2 phases *(Σ G2)* relative to the growth fraction *(GF)*. Means and standard deviations are given for 25 FA patients *(solid bars)* and 94 controls *(CON)* with a similar age distribution *(open bars)*

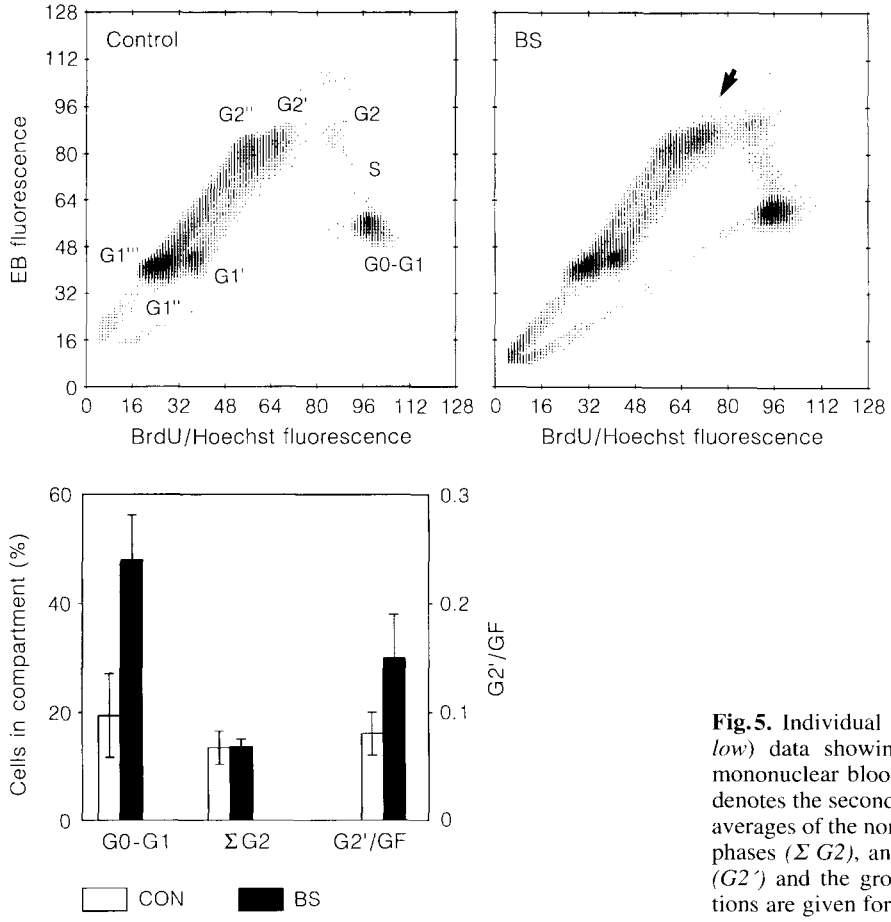

Fig. 5. Individual (cytograms, *above*) and cohort (bar graph, *below*) data showing the second cycle G2 phase lesion in BS mononuclear blood cells. *BS* 11-year-old patient with BS. *Arrow* denotes the second cycle G2 phase *(G2')*. The bar graph illustrates averages of the noncycling cell fraction *(G0/G1)*, the sum of all G2 phases *(Σ G2)*, and the ratio between the second cycle G2 phase *(G2')* and the growth fraction *(GF)*. Means and standard deviations are given for 3 BS patients *(BS)* and 94 controls *(CON)*

Discussion

One of the primary changes induced by ionizing radiation in somatic cells is the formation of single and double strand DNA breaks (Hagen 1986). Cells affected by such damage are thought to become delayed in the G2 phase of the cell cycle in order to allow for repair of their DNA lesions (Rowley 1985). This interpretation has received cogent support by the isolation of the RAD9 family of genes in *Saccharomyces cerevisiae* (Weinert and Hartwell 1988). Rad9 mutants do not respond to X-irradiation by the usual G2 phase delay. The intact RAD9 gene product thus obviously recognizes DNA damage and causes the affected cell to be delayed in the G2 phase of the cell cycle. Such a repair feedback pathway appears to operate in most eukaryotic cells, including mammalian cells. Its purpose is to prevent cells from entering mitosis unless and until their DNA damage is repaired (Hartwell and Weinert 1989). Direct evidence for the augmentation of DNA repair enzymes in response to genotoxic injury has been obtained in a number of studies (e.g., Elledge and Davis 1990; Nehls et al. 1991). Whether the affected cell will suffer permanent G2 phase arrest or whether it will be merely delayed may depend on the extent of the sustained damage and on the efficiency of the repair systems involved. Examples that support this notion are the G2 arrests found in human cancer cells (Dutrillaux et al. 1991) and in the human radiosensitivity syndromes (Seyschab et al. 1992).

Regarding the likely molecular mechanism of the G2 phase arrest, it has recently been shown that DNA damage causes an alteration of cyclin B expression, either at the transcriptional or at the translational level (Muschel et al. 1991). A related finding is that elevation of tyrosine kinase activity in response to DNA damage leads to persistent phosphorylation of $p34^{cdc2}$, the product of the cdc2 gene (Lock and Ross 1990). This in turn prevents activation of the maturation-promoting factor complex (which includes cyclin B), and therefore inhibits entry into mitosis (Kornbluth et al. 1992).

As with ionizing radiation, DNA damage appears to be the primary reason for the G2 phase inhibition caused by exposure to DNA cross linking agents (Rao 1980; Konopa 1988). The results of the present study illustrate the unparalleled sensitivity of the BrdU-Hoechst flow cytometric assay in detecting genetic damage caused by these crosslinking agents. For example, a concentration of 100 ng/ml DEB causes few if any chromosome breaks in healthy blood donors (Auerbach et al. 1989). In contrast, the flow cytometric assay revealed a highly significant G2 phase inhibition at half that concentration (Table 3). In the case of MMC, the superior sensitivity of the flow cytometric assay (compared with standard cytogenetics) is even more striking. A dose as low as 1 ng/ml elicits a cell

cycle response that is distinctive. How can the high sensitivity of the flow cytometric assay be explained? This assay not only analyses a large number of individual cells, but also covers, in addition to a larger cell cycle window (entire interphase), a much more extended timeframe (i.e., a maximum of three cell cycles).

It should be stressed that G2 phase inhibition is only one of the cell cycle changes that are caused by exposure to X-rays and crosslinking agents. To a lesser degree, but consistently affected by each of these agents, is the S-phase compartment of the cell cycle. Like the G2 phase defect, S-phase inhibition consists of two components (i.e., cell cycle delay and complete cell cycle blockage; data not shown). In addition, the exit of cells from their resting stage (G0–G1 phase) was found to be impaired, in a dose-dependent fashion, by both X-rays and crosslinking agents. The cell kinetic effects of these agents therefore are complex, but at least some of this complexity can be resolved by the flow cytometric assay.

Having established a close correlation between induced DNA damage and G2 phase inhibition, we present several examples that suggest that endogenous genetic damage may have a similar pattern of cell cycle expression. The first example concerns the finding of increased G2 phase cell fractions as a function of cell donor age (Fig. 3). The observed increase of this ratio with donor age has received independent confirmation by studies performed previously by Grossmann et al. (1989). Although these authors did not specifically comment on this fact, their corrected first cycle G2 plus S phase fractions, with one exception, were higher in their elderly than in their young donors. Moreover, Grossmann et al. (1989) found that the age-dependent decrease in T-lymphocyte proliferation was mostly attributable to a diminished proliferation of the CD8+ subset of T-cells. Again, this subset showed higher first cycle S and G2 phase fractions in older compared with young donors. The combined evidence from these studies suggests that, with increasing age, an increasing fraction of peripheral blood mononuclear cells accumulates in the S and G2 phases of the cell cycle. This inhibition of cell cycle progression must be viewed in the context that subsets of human peripheral blood mononuclear cells can be long-lived, and that these resting cells have only low levels of nucleotide precursors required for DNA repair (Cohen and Thompson 1986). It is conceivable that some of these long-lived cells accumulate DNA damage that cannot be fully repaired during their in vivo lifetime. Increased frequencies of random chromosomal breakage as a function of donor age have indeed been observed in human peripheral blood lymphocytes (Prieur et al. 1988). Of course, we have not ruled out the possibility of an age-dependent change in a cytokine response that specifically affects the G2 phase of the cell cycle of a specific subset of mononuclear blood cells. Although there are examples of such G2 phase inhibitors in other cell types (e.g., Gelfant 1977; Kinzel et al. 1990), a G2-phase-specific cytokine has not as yet been uncovered in our cell system.

An even more striking example of how endogenous DNA damage manifests itself as an accumulation of cells in the G2 phase of the cell cycle is provided by cells that are homozygous for the FA gene defect. Findings of Joenje's group (Joenje et al. 1981; Joenje and Gille 1989) and our own cell culture studies (Schindler and Hoehn 1988; Hoehn et al. 1989) suggest that the DNA damage in FA cells might result from some defect in oxygen metabolism. An alternative is that the spontaneous cell cycle arrest of FA cells reflects a primary cell cycle disturbance that is aggravated by DNA damage. Such genuine cell cycle mutants abound in yeast, but at least one mammalian G2 phase mutant (resulting from a point mutation in the cdc2 gene) has been described (Th'ng et al. 1990).

The cell cycle disturbance observed in BS cells is of a different nature and quality than that found in FA cells. In BS peripheral blood mononuclear cells, the disturbance affects the G2 phase of the second rather than first cell cycle (Fig. 5), although accumulations in the first cycle G2 phase have been noted in BS fibroblasts (Poot et al. 1990b). Moreover, in contrast to FA cells, BS cells are hypersensitive toward bromodeoxyuridine (Heartlein et al. 1987), a property that suffices to explain both their high sister chromatid exchange rates and their cell cycle behavior when grown in the presence of BrdU.

In summary, this study shows that exogenous and endogenous DNA damage manifests itself as a disturbance of cell proliferation. This disturbance affects mostly, but not exclusively, the G2 phase of the cell cycle. Whereas some of the affected cells are only delayed in their transit through G2, others are completely arrested. This different cell cycle behavior may reflect the degree to which DNA repair is possible and successful in such cells. Unlike any other technique, the BrdU-Hoechst flow cytometric assay permits the rapid and quantitative cell-by-cell assessment of the cell cycle consequences of genetic cell damage.

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