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Increased bleomycin-induced chromosome damage in lymphocytes of patients with common variable immunodeficiency indicates an involvement of chromosomal instability in their cancer predisposition

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Summary. To study mutagen-induced chromosome instability in cancer disposition, late S and G_2 lymphocytes of 15 patients with common variable immunodeficiency and 14 healthy controls were exposed to bleomycin in vitro. The groups did not differ in the frequency of spontaneous chromosome aberrations. In bleomycin-treated samples we found higher numbers of break events per cell and increased frequency of cells with aberrations compared to the control group. A slightly reduced breakage of chromosome group D was noted in patients. These results support the hypothesis that a higher incidence of cancer in patients with genetically determined immunodeficiencies may be explained by an increased mutagen-induced chromosome instability in at least some of them.

Introduction

Several classical chromosome-breakage syndromes (ataxia telangiectasia, Fanconi's anaemia or Bloom syndrome) and some other disorders with mutagen hypersensitivity and involving risk of cancer [7] suggest that chromosomal instability may increase the probability of mutational events and thus play an important role in carcinogenesis. Since cellular responses to mutagen action, such as deoxyribonucleic acid (DNA) repair and replication, are under polygenic control in mammalian cells, a gradient of chromosomal or genetic instability is likely to exist in the human population [9]. Both tumour cells and fibroblasts or lymphocytes derived from patients with a number of cancer-prone genetic disorders, when X-irradiated during the G_2 phase of the cell cycle, have more chromatid breaks and gaps during the postirradiation period than cells from unaffected individuals [17–19]. Biochemical and cytogenetic studies indicate that this increased chromatid damage results from deficient DNA repair [6, 18]. The frequent association between higher spontaneous or mutagen-induced chromosome instability and immunodeficiency has been extensively reported (see, e.g. [12, 13, 15, 21, 25, 27]), but is not fully understood. Enhanced bleomycin-induced chromatid damage in G_2 peripheral lymphocytes of some cancer patients has been found by several authors [2, 10, 11]. To determine the importance of mutagen-induced chromatin instability in cancer susceptibility, we used this radiomimetic agent to expose the late S and G_2 lymphocytes of 15 patients with common variable immunodeficiency (CVID).

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Materials and methods

Patients and controls. Fifteen unrelated patients with CVID and 14 healthy control subjects were involved in the study. The patients with CVID had decreased serum levels of immunoglobulin classes G, A, and M, and in 13 cases a defect in the T cell system was also present, as indicated by decreased skin reactivity or anergy in the tests of delayed hypersensitivity and by a decreased $CD4^+/CD8^+$ ratio. Controls had no symptoms suggesting disorders associated with chromosomal instability (absence of neurological and haematological disease, frequent infections or immunodeficiency, malformations, and signs of premature ageing). No controls acknowledged a history of chemotherapy or radiation, and detailed family history revealed no excess of cancer or spontaneous abortions among their first- and second-degree relatives. The mean age ($\pm$SD) of the patients and controls was 35 ± 16 and 29 ± 3 years, respectively.

Cultures. Whole blood cultures were initiated with RPMI 1640 medium (ÚSOL, Czechoslovakia) supplemented with 20% bovine serum, 1% phytohaemagglutinin (Wellcome) and antibiotics (penicillin and streptomycin). All samples were incubated for 72 h in the dark at 37° C. Bleomycin (Nippon Kayaku Inc.) was added to a final concentration of 30 μg/ml for the last 5 h of culture. Before cell harvesting, Colcemid (Serva) treatment (0.05 μg/ml) was given for 2 h. The cells were then treated with 0.075 M potassium chloride at 37° C for 20 min and fixed in methanol/glacial acetic acid (3:1, v/v) with two changes of fixative. The suspensions were finally dropped on to slides and air-dried. The preparations were Giemsa-stained and G-banded to determine the exact locations of break points of chromosomes 1–3.

Scoring aberrations. Chromosome analyses were made on coded preparations. Only well-spread metaphases with 44–47 chromosomes were evaluated. Samples of 100 or more mitotic cells were examined per person with the exception of three CVID patients and one control. Gaps were not enumerated. The pulverized cells (here defined as

cells with more than seven break events) were disregarded in the final computation, but their frequency was recorded. The following criteria were used to distinguish gaps and breaks. (a) When the length of the achromatic region was equal to or shorter than the width of the chromatid, the lesion was considered a gap; when the achromatic segment was longer than the width of the chromatid, the lesion was regarded as a break (Chatham Workshop Conference, 1971). (b) If a lesion was a gap, according to the previous definition, and if there was a clear misalignment of the chromatid distal to the centromere it was counted as a break [8]. For the calculation of aberration rates, chromatid breaks and acentric fragments were considered as single-break events and rare chromatid exchanges or chromosome-type aberrations as two-break events. The frequency of breakage was then expressed as the number of break events per cell. The break events were assigned to the chromosome arm and group. The number of breaks that could not be classified with certainty was recorded and was not included in the distribution analysis. For the presence of structural abnormalities, 10 G-banded mitotic cells from each person were karyotyped. To compare the instability of both groups, we used the unpaired Student's *t*-test, the frequency of aberration types was evaluated by the χ^2 test.

Results

The difference in the proportion of cells with spontaneous aberrations between both groups studied was not shown to be significant (1.48% in controls, 1.90% in patients, $P > 0.05$). No constitutional abnormalities in chromosome structure were found, the centromeric heterochromatin instability of chromosomes 1, 9 or 16 was not detected. There was no significant difference in the proportion of chromatid-type spontaneous aberrations between the groups studied.

The chromosomal instability in bleomycin-treated samples is shown in Table 1. The mean frequency of the cells with aberration was higher in the patients with CVID than in the control group. The proportion of pulverized cells was also increased in the patients but the difference was small. Aberrant lymphocytes of the patients with CVID (without pulverizations) had more aberrations than

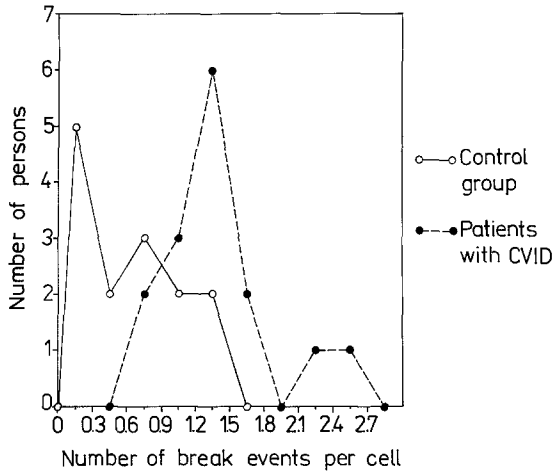

Fig. 1. Distribution of numbers of break events per cell in controls and patients with common variable immunodeficiency (CVID)

the cells of controls (1.97 vs 1.67). The mean number of break events per cell was twice as high in the patients with CVID than in controls (1.32 vs 0.66, $P < 0.005$), but there was a broad overlap in individual values between the two groups (Fig. 1). The distribution of aberration types was also different in both groups ($P < 0.01$), "two-break events" being found slightly more frequently in the patients with CVID (Table 1).

The relationship between the numbers of break events per cell and the proportion of pulverized cells (Fig. 2) showed positive correlations both in the patients and in controls ($r = 0.867$, $P < 0.001$, and 0.870, $P < 0.001$, respectively).

The distribution of a total of 1347 mapped break events on the chromosomes or chromosome groups in the patients did not differ from that of 671 found in controls, except for a decrease of breakage of chromosome regions Dq (8.2 vs 11.2%, $P < 0.05$). The slight increase in relative breakage of the arms 2q (8.6 vs 7.5%) and Bq (11.1 vs 8.8%) in the patients was not found to be significant. The proportion of breaks which could not be assigned to a chromosome or chromosome group with certainty was similar in

Table 1. Bleomycin-induced chromosomal instability in both groups

Parameter	Controls ($n = 14$)	Patients ($n = 15$)	Significance
Number of cells analysed	1363	1369	(*t*-test)
Number of cells with aberrations (mean ± SD)	37.5 ± 23.7%	62.6 ± 21.3%	$P < 0.01$
Number of pulverized cells (mean ± SD)	5.3 ± 5.1%	8.7 ± 8.7%	Not significant
Number of aberrations	822	1570	(χ^2 test)
Number of chromatid breaks (%)	797 (97.0)	1477 (94.1)	$P < 0.01$
Number of chromatid exchanges (%)	2 (0.2)	14 (0.9)	
Number of chromosome type aberrations (%)	23 (2.8)	79 (5.0)	

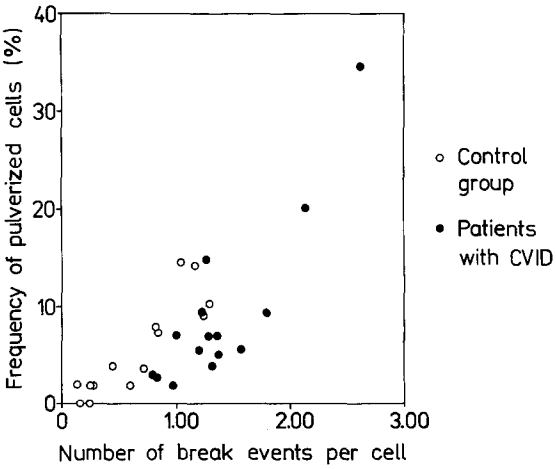

Fig. 2. Relationship between number of break events per cell and frequency of pulverizations (%) in both groups

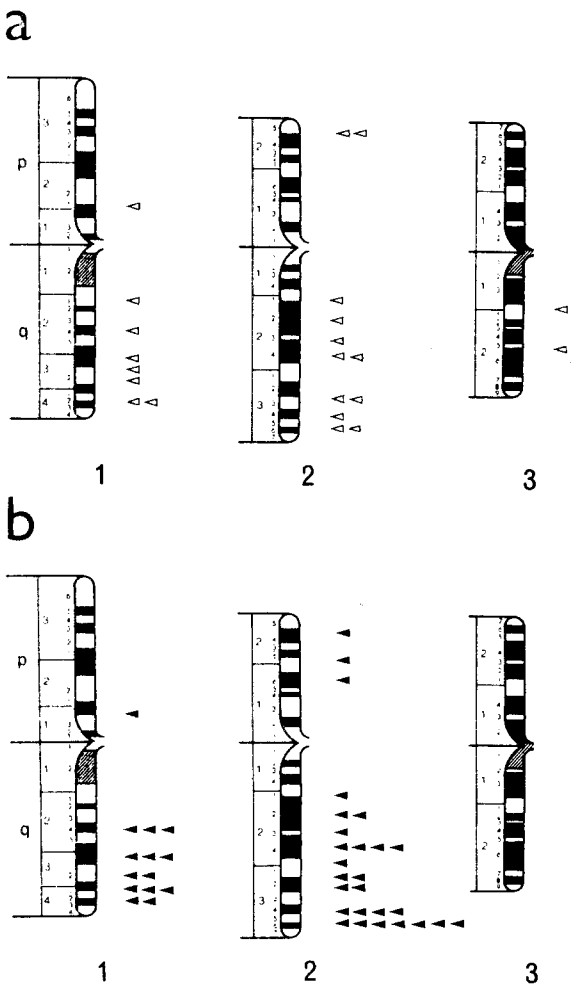

Fig. 3. Repeated break points on chromosomes 1–3 found in individuals of the control group **(a)** and CVID patients **(b)**

both groups (14±8% in the patients, 16±6% in controls), as was the percentage of break events on the short arms only (23.8% and 22.5% respectively).

The repeated break points on chromosomes 1–3 found in an individual are presented in Fig. 3. The regions most frequently involved in breakage were 1q2, 1q3, 2q2, and 2q3. We could not find any clear difference between the two groups.

Discussion

A high incidence of cancer in patients (both with and without organ transplant) being maintained for a long period with immunosuppressive drugs and in patients with genetically determined immunodeficiency states [20, 22, 24] was considered to support the immune-surveillance theory. In contrast, the observations that athymic nude mice lacking T cell function do not develop many spontaneous tumours and that immunodeficient patients exhibit a very restricted range of neoplasms [3, 22] seem to stand against this view. At present, concepts in tumour immunology are changing fundamentally, and there is no need to postulate rather obscure strong immune or natural resistance barriers [4]: if a tumour cell arises de novo, which is considered to be a very infrequent situation [3], it may or may not be attacked by immune or natural resistance mechanisms with their limited potential. However, data to support the idea that these mechanisms induce regression of spontaneously arising tumours do not seem sufficient. Therefore it is logical to reconsider the possibility that immunodeficiency and malignancy may occur in parallel, and a common cause makes the cells more susceptible to malignant transformation; our results support this view.

We were not able to demonstrate the difference in spontaneous instability in the lymphocytes of either group studied here. However, in bleomycin-treated samples the response of some patients was clearly higher than in the preselected healthy control group. The relationship of the number of individual break events per cell to a possible risk of cancer may be further elucidated in a prospective follow-up of the patients; no malignant disease has been diagnosed in our group. An increased response in these values in several patients (Fig. 1) may also reflect an aetiological heterogeneity of CVID.

Mutagen-induced chromosome instability may exhibit a considerable tissue-specificity [7]. We did not analyze chromosome instability in cell types other than predominantly T cells. It would be of interest to discover whether CVID patients are more susceptible to T cell lymphomas, but to our knowledge, reliable data are not available in the literature. A case of diffuse T cell lymphoma in a patient with CVID was reported by Durham et al. [5].

Although the immunodeficient patients were more frequently X-rayed than controls and the synergic effect of X-irradiation and bleomycin has been described [1], it is not likely that a low dose of X-irradiation *in vivo* could influence chromosomal breakage after the described dose of bleomycin *in vitro*.

Taalman et al. [23] investigated X-ray-induced chromosomal breakage in ten asymptomatic patients with IgA deficiency. They could not establish a relationship between chromosomal radiosensitivity and IgA deficiency in spite of the fact that two patients exhibited an approximately 1.5 times higher incidence of lymphocytes with aberrations than the normal controls. It is probable that only some patients with immunodeficiency (those at risk of malignancy?) exhibit more or less increased spontaneous or suitably induced chromosomal breakage, as expressed by the number of cells with aberrations or by the number of break events per cell. Furthermore, the incidence of cancer in patients with CVID has been reported to be higher than in patients with isolated deficiency of IgA (see, e.g. [14, 22]).

The importance of pulverized cells in bleomycin-treated samples is not clear. In our study their frequency and the number of break events per cell corresponded well in both groups, indicating that the higher number of these cells represents an increased chromosomal sensitivity of a given genotype. However, the difference between the frequency of pulverized cells in our patients and controls was only small in comparison with the number of aberrant cells or the number of aberrations per cell, which is probably due to the fact that heavily damaged cells do not enter the mitotic phase. The fact that bleomycin, in contrast to gamma irradiation, induces extreme variation of DNA strand breakage from cell to cell has not been fully explained [16].

Several described cases of immunodeficiency, facial abnormalities, and centromeric heterochromatin instability [12, 13, 15, 25] as well as some findings of constitu-

tional chromosome abnormalities in persons with immunodeficiency, congenital malformations, and mental retardation (see, e.g. [23]) suggest a possible association. Although our patients did not show congenital anomalies, we could not demonstrate any constitutional chromosome abnormalities.

Unlike the results of our previous work [26], only small differences in the distribution of breaks on chromosomes or chromosome groups between both groups were noted. They do not seem sufficient to indicate a relationship between preferential mutagen-induced damage in a chromosome region and the CVID phenotype. Furthermore, the difference observed could be invalidated by the proportion of unclassified breaks. Although the location of repeated break points on chromosomes 1–3 in an individual examined suggests their non-random distribution, a difference between the two groups could not be established; where a high number of induced breaks was achieved in an individual we noticed that such a person exhibited repeated break points in typical locations.

In conclusion, our results further support the hypothesis of a gradient of genetic instability in the population, and point to a high complexity in the cellular response to the radiomimetic agent. The different response of bleomycin-induced chromosome aberrations between our groups indicates that increased chromatid instability after DNA damage in some patients with CVID might be responsible for their cancer susceptibility.

References

1. Alalawi FA, Chapman IV (1977) Combined effects of bleomycin and X-rays on DNA synthesis in asynchronous Ehrlich ascites cells in suspension. Br J Cancer 36: 78–83
2. Cherry LM, Hsu TC (1983) Bleomycin-induced chromosome damage in lymphocytes of medullary thyroid carcinoma patients and their family members. Anticancer Res 3: 367–372
3. Den Otter W (1985) Tumor cells do not arise frequently. Cancer Immunol Immunother 19: 159–162
4. Den Otter W (1986) Immune surveillance and natural resistance: an evaluation. Cancer Immunol Immunother 21: 85–92
5. Durham JC, Stephens DS, Rimland D, Nassar VH, Spira TJ (1987) Common variable hypogammaglobulinemia complicated by an unusual T-suppressor/cytotoxic cell lymphoma. Cancer 59: 271–276
6. Gantt R, Parshad R, Price FM, Sanford KK (1987) Biochemical evidence for deficient DNA repair leading to enhanced G_2 chromatid radiosensitivity and susceptibility to cancer. Radiat Res 108: 117–126
7. German J (1983) Chromosome mutation and neoplasia. Alan R Liss, New York, pp 452
8. Harnden DG, Klinger HP (1985) An international system for human cytogenetic nomenclature (ISCN). Karger, New York, p 66
9. Hsu TC (1983) Genetic instability in the human population: a working hypothesis. Hereditas 98: 1–9
10. Hsu TC (1987) Genetic predisposition to cancer with special reference to mutagen sensitivity. In Vitro Cell Dev Biol 23: 591–603
11. Hsu TC, Cherry LM, Samaan NA (1985) Differential mutagen susceptibility in cultured lymphocytes of normal individuals and cancer patients. Cancer Genet Cytogenet 17: 307–313
12. Hultén M (1978) Selective somatic pairing and fragility at 1q12 in a boy with common variable immunodeficiency. Clin Genet 14: 294
13. Jaspers NGJ, Taalman RDFM, Baan C (1988) Patients with an inherited syndrome characterized by immunodeficiency, microcephaly, and chromosomal instability: genetic relationship to ataxia telangiectasia. Am J Hum Genet 42: 66–73
14. Kinlen LJ, Webster ADB, Bird AG, Haile R, Peto J, Soothill JF, Thompson RA (1985) Prospective study of cancer in patients with hypogammaglobulinaemia. Lancet i: 263–266
15. Maraschio P, Zuffardi O, Fior TD, Tiepolo L (1988) Immunodeficiency, centromeric heterochromatin instability of chromosomes 1, 9, and 16, and facial abnormalities: the ICF syndrome. J Med Genet 25: 173–180
16. Östling O, Johanson KJ (1987) Bleomycin, in contrast to gamma irradiation, induces extreme variation of DNA strand breakage from cell to cell. Int J Radiat Biol 52: 683–691
17. Parshad R, Gantt R, Sanford KK, Jones GM, Tarone RE (1982) Repair of chromosome damage induced by X-irradiation during G_2 phase in a line of normal human fibroblasts and its malignant derivative. J Natl Cancer Inst 69: 409–414
18. Parshad R, Sanford KK, Jones GM (1983) Chromatid damage after G_2 phase x-irradiation of cells from cancer-prone individuals implicates deficiency in DNA repair. Proc Natl Acad Sci USA 80: 5612–5616
19. Parshad R, Gantt R, Sanford KK, Jones GM (1984) Chromosomal radiosensitivity of human tumor cells during the G_2 cell cycle period. Cancer Res 44: 5577–5582
20. Penn I (1974) Occurrence of cancer in immune deficiencies. Cancer 34: 858–866
21. Seemanova E, Passarge E, Beneskova D, Houstek J, Kasal P, Sevcikova M (1985) Familial microcephaly with normal intelligence, immunodeficiency, and risk for lymphoreticular malignancies: a new autosomal recessive disorder. Am J Med Genet 20: 639–648
22. Spector BD, Perry GS, Kersey JH (1978) Genetically determined immunodeficiency diseases (GDID) and malignancy: report from the Immunodeficiency-Cancer Registry. Clin Immunol Immunopathol 11: 12–29
23. Taalman RDFM, Weemaes CMR, Hustinx TWJ, Scheres JMJC, Clement JME, Stoelinga GBA (1987) Chromosome studies in IgA-deficient patients. Clin Genet 32: 81–87
24. Thomas L (1982) On immunosurveillance in human cancer. Yale J Biol Med 55: 329–333
25. Valkova G, Ghenev E, Tzancheva M (1987) Centromeric instability of chromosomes 1, 9 and 16 with variable immune deficiency. Support for a new syndrome. Clin Genet 31: 119–124
26. Vorechovsky I, Zaloudik J (1989) Increased breakage of chromosome 1 in lymphocytes of patients with testicular cancer after bleomycin treatment in vitro. Brit J Cancer (in press)
27. Wegner RD, Metzger M, Hanefeld F, Jaspers NGJ, Baan C, Magdorf K, Kunze J, Sperling K (1988) A new chromosomal instability disorder confirmed by complementation studies. Clin Genet 33: 20–32

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Note added in proof:

Two patients in our group have died since this paper was submitted for publication: one patient, aged 53, suddenly died of bronchopneumonia with no signs of mediastinal enlargements, but he eluded autopsy. His number of break events per cell after bleomycin treatment was 2.61, frequency of pulverized cells 34%. The second patient, aged 56, died of myeolid leukaemia which developed soon after myelodysplastic syndrome had been diagnosed. The number of break events per cell was 1.37, frequency of pulverizations 5%.