Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 670–674, January 1989
Medical Sciences

Evidence for increased *in vivo* mutation and somatic recombination in Bloom's syndrome

(glycophorin A/cancer-prone syndromes/*in vivo* mutagenesis/cancer genetics)

RICHARD G. LANGLOIS*, WILLIAM L. BIGBEE*, RONALD H. JENSEN*, AND JAMES GERMAN†

*Lawrence Livermore National Laboratory, Biomedical Sciences Division L-452, University of California, P.O. Box 5507, Livermore, CA 94550; and †Laboratory of Human Genetics, The New York Blood Center, New York, NY 10021

Communicated by Alfred Knudson, October 11, 1988 (received for review August 15, 1988)

ABSTRACT **The glycophorin A assay was used to estimate the frequency of mutations that accumulate *in vivo* in somatic cells of persons with Bloom's syndrome (BS). This assay measures the frequency in persons of blood type MN of variant erythrocytes that lack the expression of one allelic form of glycophorin A, presumably due to mutational or recombinational events in erythroid precursor cells. Samples of blood from persons with BS showed dramatic 50- to 100-fold increases in the frequency of variants of three types, those with a hemizygous phenotype, those with a homozygous phenotype, and those with what appears to be partial loss of the expression of one locus. The high frequency of homozygous variants, genetic evidence for altered allelic segregation of a specific biochemical locus, provides evidence for increased somatic crossing-over *in vivo* in BS. An increased generation of functional hemizygosity and homozygosity in their somatic cells may play an important role in the extreme cancer risk of persons with BS.**

Bloom's syndrome (BS) (1) is a rare autosomal recessive genetic disorder of growth that greatly increases the affected individual's chance of developing cancer. In the 130 affected individuals in the Bloom's Syndrome Registry, 57 malignant neoplasms have been detected, at a mean age at diagnosis of 24.7 years (2). A wide spectrum of tumor types has been observed in affected individuals, including lymphoid and myeloid leukemias, lymphomas, squamous cell carcinomas, and adenocarcinomas (3).

In addition, a distinctive array of cytological abnormalities in a variety of cell types, signifying a remarkable degree of genomic instability, is a constant feature of BS (4, 5). These include high spontaneous frequencies of chromosome breaks and rearrangements (5–8), sister-chromatid exchanges (SCEs) (5, 9–11), micronucleated cells (12, 13), and cells with specific-locus mutations (14–17). A unique cellular abnormality in untreated BS cells *in vitro* is a dramatic increase over normal in the frequency of a certain type of quadriradial configuration (Qr) in metaphase cells (5, 18). The Qrs of BS are the microscopically visible consequence of interchanges that, earlier in the cell cycle, occurred between homologous chromosomes at apparently homologous sites; they are interpreted as cytological evidence for an increased frequency of somatic crossing-over in BS (18–21). Heterozygous carriers of the BS mutation (*bl*/+) are clinically and cytologically normal (22). Although the primary genetic defect responsible for BS may not have been defined, DNA ligase I activity has been reported to be decreased (23–25), and this very possibly is responsible for the genomic instability of BS. The complex cytogenetic abnormalities readily demonstrable in BS cells *in vitro* raise the possibility that somatic cell mutation (point mutation, chromosome segmental rearrangement, and somatic recombination) is also substantially increased *in vivo* in BS, and this could explain the extreme cancer risk.

In the present study we have used the glycophorin A (GPA) assay to measure the incidence *in vivo* in BS of cells with mutant phenotypes. GPA, a cell-surface protein of human erythrocytes which occurs in two forms, M and N, is the product of codominantly expressed alleles at a locus that has been mapped to chromosome 4q28–q31 (26–28). In the GPA assay, pairs of fluorescently tagged monoclonal antibodies specific for the M and N allelic forms of GPA are used to label erythrocytes from heterozygous MN donors. Flow cytometry and sorting are used to enumerate and purify rare single-color cells that lack the expression of one allelic form of this protein, presumably because they are progeny of mutated erythroid precursor cells. We have shown previously that the frequency of such variant cells is increased in individuals who have been exposed excessively to mutagenic chemicals or ionizing radiation (29, 30). We also have presented evidence that variant cells of two types can be detected and distinguished from one another: hemizygous phenotype variants that lack expression of one allele and express the remaining allele at a one-copy level, and homozygous phenotype variants that lack expression of one allele and express the remaining allele at a two-copy level (28, 29). Thus, the GPA assay provides measurements of the frequency of both gene-expression loss (null) mutations and presumed chromosomal mutations that lead to homozygosity at the GPA locus.

MATERIALS AND METHODS

Blood Samples. Blood samples were obtained from individuals of blood type MN—i.e., heterozygous at the GPA locus—who had the following genotypes with respect to the BS mutation: seven affected BS homozygotes (*bl*/*bl*); two obligate BS heterozygotes (*bl*/+); and one normal control (+/+). The *bl*/*bl* individuals were 4 to 41 years old. They included individuals from several ancestries—three Japanese, two Western European, one Ashkenazi Jewish, and one Mexican. Three of the *bl*/*bl* individuals have developed cancer; one of them was treated with radio- and chemotherapy 10 years ago, whereas the other six have had no known excessive exposure to mutagens.

GPA Analysis. The frequencies of GPA variant cells were measured as previously described (29). Briefly, blood samples from heterozygous MN donors were fixed and labeled with two GPA-specific monoclonal antibodies, each conjugated with a different fluorescent dye. Approximately 10^6 erythrocytes were analyzed by flow cytometry in each assay run to enumerate variant cells that show normal fluorescence

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GPA, glycophorin A; BS, Bloom's syndrome; VF, variant-cell frequency; SCE, sister-chromatid exchange; Qr, quadriradial configuration.

from one antibody but reduced fluorescence from the second antibody. Flow sorting and visual identification of single-color variant cells was used to determine variant-cell frequencies (VFs).

Four independent versions of the assay, each employing different antibodies or cell fixation methods, were used in the present study to measure both M-loss and N-loss variants. Detailed results are presented from the "1W1" assay for M-loss variants (NØ and NN phenotypes) and the "2W2" assay for N-loss variants (MØ and MM phenotypes), which have been described earlier (29). These assays used the following antibody combinations: 1W1, the M-specific antibody 6A7 conjugated with biotin followed by Texas red conjugated with avidin (6A7-B-AvTR) and the (M+N)-specific antibody 10F7 conjugated with fluorescein (10F7-F); 2W2, the N-specific NN3-B-AvTR and the M-specific 9A3-F. Two new antibody combinations, both detecting M-loss variants, were also used for the analysis of these samples to confirm that different labeling methods yield comparable VFs. The "1W2" assay uses the M-specific 6A7-B-AvTR and the (M+N)-specific R1.3-F (31); the "2W3" assay also uses 6A7-B-AvTR and the N-specific antibody NN3-F.

RESULTS

Representative flow distributions of erythrocytes obtained from two persons with BS are shown in Fig. 1. Artificial mixtures of erythrocytes from normal donors differing in M,N type were used to define the rectangular windows in the figure, which correspond to the expected labeling of variant cells with either a hemizygous or a homozygous phenotype. It is clear that many cells are present in both the hemizygous (NØ, MØ) and the homozygous (NN, MM) windows. The homozygous variants form a discrete population in the variant window; in contrast, the hemizygous variants exhibit a wide range of labeling intensities along the *y* axis, suggesting that this population contains both hemizygous and partial-loss phenotype cells. VFs from the 1W1 and 2W2 assays on all donors in the study, as well as a summary of control values from previous studies, are reported in Table 1. Dramatic elevations in VF were observed for both hemizygous and homozygous variants in all BS blood samples, with comparable elevations of both M-loss and N-loss variants. Near-normal VFs were observed for both of the BS heterozygotes. Similar results were obtained with two other versions of the

Table 1. VFs in BS homozygotes (*bl/bl*), heterozygotes (*bl/+*), and normal individuals (+/+)

Genotype	Donor identification no.*	VF × $10^{6\dagger}$ NØ	MØ	NN	MM
Homozygous (*bl/bl*)	86	680	820	994	988
	97	1128	1088	2304	1690
	78	334	1067	323	624
	14	609	1208	632	1116
	7	402	277	702	738
	127	466	548	579	595
	20	483	647	304	598
	Mean	586	808	834	907
Heterozygous (*bl/+*)	M(97)	6	6	1	14
	F(97)	14	38	17	60
Control‡ (+/+)	—	12	12	22	19
Normal‡ mean ±SD	—	11 ±7	10 ±5	17 ±14	10 ±11

*Identification numbers are the same as those used to identify persons with BS (*bl/bl*) in the Bloom's Syndrome Registry (2). The heterozygotes (*bl/+*) are the mother (M) and father (F) of homozygote no. 97.

†The reported VFs are the numbers of microscopically identified variant cells per million cells analyzed after correction for the sorting efficiency as described previously for the 1W1 and 2W2 assay systems (29). All numbers correspond to the average of duplicate analyses.

‡Two estimates of variant frequencies in normal persons are reported in the table. The "Control" is the mean value for a single normal donor who was assayed on each day that BS samples were assayed. The "Normal" is the mean and standard deviation of control populations from several previously reported studies (29, 30, 32).

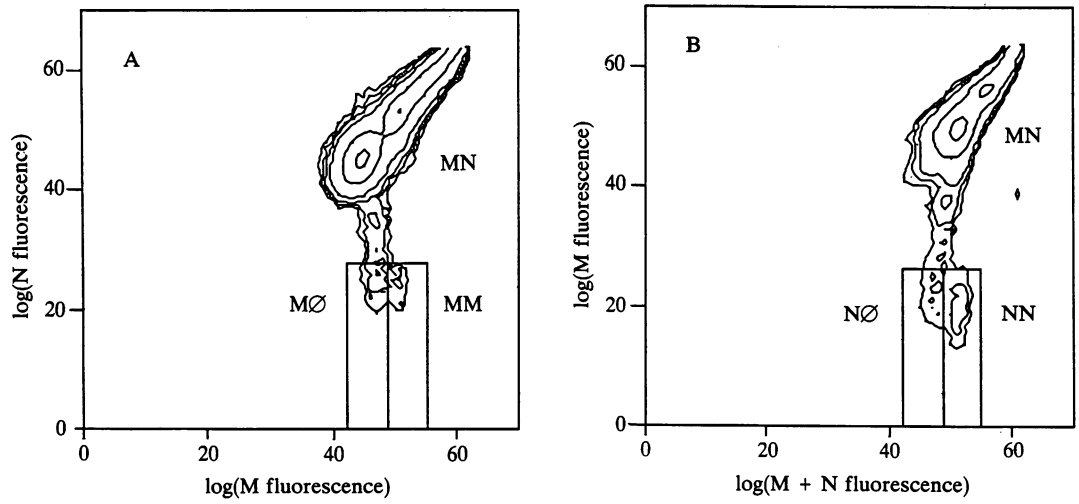

FIG. 1. Bivariate flow distributions of erythrocytes from persons with BS labeled with pairs of fluorescent, GPA-specific monoclonal antibodies. Intensities of green fluorescence (*x* axis) and red fluorescence (*y* axis) are indicated in channel numbers on a logarithmic scale; an increase in six channels corresponds to a doubling of intensity. Subpopulations of cells produce peaks in the distribution, and the height of each peak is indicated by contours. The large peaks correspond to normal MN cells and doublets of MN cells having both green and red fluorescence, while the small peaks correspond to single-color variant cells, which lack one allelic form of GPA. The two rectangular windows indicate the regions where variant cells should appear if one allele is expressed at less than 10% of the normal level and the second allele is expressed at either a one-copy level (hemizygous NØ or MØ variants, left window) or a two-copy level (homozygous NN or MM variants, right window). (*A*) Results from a 2W2 assay on BS individual 14. (*B*) Results from a 1W1 assay on BS individual 97. The experimental details for both assays have been described elsewhere (29).

assay. Mean VFs for (*bl/bl*), (*bl/+*), and (+/+) donors with the 1W2 assay were the following: N∅ = 1157, 13, and 10 per million; NN = 1081, 15, and 51 per million. Mean VFs for the 2W3 assay were the following: N∅ = 803, 20, and 8 per million; NN = 763, 11, and 8 per million. Thus, comparable elevations in VF were observed in BS individuals with all four versions of the assay. While a small age-dependent increase in VF has been observed in normal donors (32), no significant age effects were detected in this BS population.

The homozygous VFs for the BS donors are the highest values ever detected for this variant cell type with the GPA-assay system, with mean VFs 49- to 91-fold higher than normals. Hemizygous VFs are similarly elevated 53- to 81-fold over normals and include the highest values yet detected for this variant-cell type; comparably elevated frequencies were observed in a few of the heavily exposed survivors of the atom bomb at Hiroshima (30). Our observation that each of the BS blood samples studied displayed the same distinctive flow distribution of variant cells as well as a dramatically elevated VF suggests a common cellular abnormality in BS individuals with different ethnic backgrounds. This finding is consistent with recent complementation studies, which indicate that the same locus is mutated in BS individuals of Jewish, Western European, and Japanese origins (33).

Given the complex pattern of variant cells shown in Fig. 1, the distributions of labeling intensities for variant cells shown in Fig. 2 were used to determine what variant-cell phenotypes are present in BS and to provide some insight into the genetic alterations that might be responsible for them. For cells that lack expression of one allele, the distribution of labeling intensities for the remaining allele shows two discrete but overlapping populations at labeling levels expected for both one and two copies of the remaining allele (Fig. 2 *A* and *B*). This suggests that these populations represent two distinct variant-cell phenotypes rather than a broad distribution of labeling intensities for one variant-cell type. While doublets of one-copy variants could produce the peak at the intensity expected for two-copy variants, visual observation of the cells sorted from these regions confirmed that two-copy variants are single cells with brighter fluorescence than one-copy variants. Since it has been shown that individuals who have inherited a defect in one GPA allele express the remaining allele at a one-copy level (28, 31, 34), it is probable that these two variant-cell types are the progeny of two different populations of erythroid precursor cells having one or two copies of the remaining allele.

Variants that express one allele at a two-copy level appear to lack expression of the second allele totally (Fig. 2 *C* and *D*), which is consistent with a homozygous phenotype. In contrast, variants that express one allele at a one-copy level exhibit a wide range of labeling intensities for the second allele (Fig. 2 *E* and *F*), suggesting that some cells have a complete-loss hemizygous phenotype while others show only a partial loss of expression of the affected allele. One feature that was consistently observed in all BS analyses is a small subpopulation immediately below the MN peak (Fig. 1, approximate coordinates $x = 45$, $y = 37$). This location corresponds to partial-loss variants with normal labeling of one allele and about 25% of the normal labeling of the second

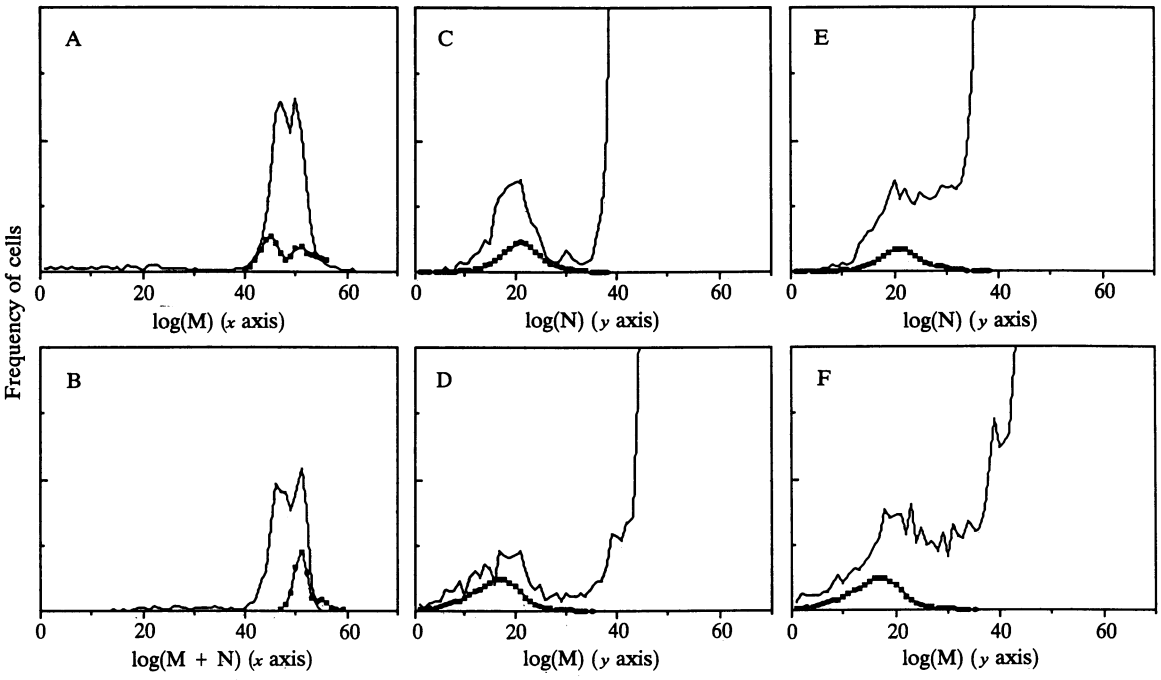

FIG. 2. Labeling characteristics of variant cells from BS individual 7 analyzed with the 2W2 assay (*A*, *C*, *E*) and from BS individual 78 analyzed with the 1W1 assay (*B*, *D*, *F*). Bivariate distributions like those in Fig. 1 were used to determine the distribution of green fluorescence (*x* axis) or red fluorescence (*y* axis) for specific subpopulations of variant cells. Distributions of normal cells (■–■) with a reduced vertical scale (about 1000-fold) are included to show the intensity of specific cell types. (*A*) Distribution of green fluorescence for normal MN cells and doublets of MN cells with one and two copies of GPA(M), respectively (■–■), and for variant cells lacking GPA(N) ($y <$ channel 25) (—). (*B*) Distribution of green fluorescence for normal MN cells with two copies of GPA(M+N) (■–■) and for variant cells lacking GPA(M) ($y <$ channel 25) (—). In both cases the variant cells are concentrated in two partially resolved peaks that exhibit intensities expected for the remaining allele being expressed at a one-copy or two-copy level. (*C*) Distribution of red fluorescence for normal MM cells (■–■) and homozygous variant cells with two copies of GPA(M) ($x =$ channels 49–54) (—). (*D*) Distribution of red fluorescence for normal NN cells (■–■) and homozygous variant cells with two copies of GPA(N) ($x =$ channels 49–54) (—). In both cases, homozygous variant cells are concentrated into one peak with the intensity expected for complete loss of expression of one allele. (*E* and *F*) Distribution of red fluorescence for hemizygous variant cells ($x =$ channels 42–48) compared with normal MM or NN cells as in *C* and *D*. In this case, while some hemizygous variants are concentrated at an intensity expected for complete allele loss, others have a greater red intensity, suggesting only partial loss of expression of an allele. The large truncated peaks in *C–F* correspond to normal MN cells.

allele. Partial-loss variants could be either cells that express one GPA allele at a reduced level or cells that express an altered form of one GPA allele, leading to reduced antibody binding to the protein product of that allele.

Thus, BS individuals appear to have marked elevations over normal individuals in a variety of variant-cell phenotypes. While it seems reasonable that the genomic instability seen in BS cells could produce increased VFs for all these variant-cell types, it is surprising that the homozygous and hemizygous VFs are so similar. This similarity in homozygous and hemizygous VFs contrasts with previous studies, in which we observed larger elevations in hemizygous variants compared with homozygous variants in individuals with ataxia–telangiectasia (35) and in individuals exposed to radiation (30) or chemotherapy (29).

DISCUSSION

The high frequency of homozygous variants in BS blood samples provides evidence for elevated somatic recombination in BS, and it provides estimates for the *in vivo* frequency of recombination in hemopoietic cells of these individuals. While genetic mechanisms other than crossing-over could yield homozygous variants (36), it is the most likely explanation, given the high frequency in BS of Qrs (5, 18–20) and the evidence for elevated viral recombination in virus-infected BS cells (37), altered segregation of cytological satellites on acrocentric chromosomes (38), and what may be "twin-spots" on the skin of some BS patients (39). Our results provide genetic evidence in BS for the altered segregation of a specific biochemical locus producing homozygous variants, as expected from somatic crossing-over (18, 21, 36). Somatic crossing-over also may occur in normal individuals but at a much lower frequency, given earlier reports of rare Qrs in normal individuals (18) and our observation of a low frequency of homozygous GPA variants in normal individuals. If all homozygous GPA variants are produced by somatic recombination, then about 0.1% of the erythroid cells in BS individuals are progeny of cells that have been altered by a recombinational event at some point between the GPA locus and the centromere of chromosome 4. While this frequency of somatic recombination is at least 50-fold higher than the frequency in normal individuals, it still is much lower than the frequency of about 50% expected for meiotic recombination in this 60-centimorgan region (26). If the frequency of somatic recombination for different chromosome regions is related to meiotic map distances, then, as a result of recombination, more than 5% of BS cells may contain segments of homozygosity somewhere in the genome (26).

Hemizygous and partial-loss variants also are markedly elevated in BS. An increased frequency of hemizygous GPA variants is consistent with previous reports of increased spontaneous genomic instability and chromosome breakage in BS. BS cells have been reported to have a 10- to 20-fold elevation of chromosome aberrations (6–8) and a 10- to 25-fold excess of micronucleated cells (12, 13). However, the variants we have detected in BS by the GPA assay appear to differ from variants induced by genotoxic exposure to ionizing radiation, which also leads to increased chromosome aberrations. In a previous study of atom-bomb survivors (30) we observed that both GPA variants and chromosome aberrations were increased about 15-fold in high-dose survivors and that most of these induced variants were complete-loss variants. In contrast, persons with BS have a much greater increase in GPA variants (50- to 80-fold) compared to chromosome aberrations (10- to 20-fold), and furthermore, many of these variants display a partial-loss phenotype. Thus, additional genetic mechanisms besides chromosomal breakage may be responsible for this unusual pattern of variant-cell types in BS.

One mechanism that could yield hemizygous and partial-loss variants in BS is unequal recombination between either sister chromatids or chromatids of homologous chromosomes (21, 40). If the mechanisms by which SCEs or homologous exchanges occur are error prone and if the exchanges can occur between partially homologous DNA sequences, then an unequal cross-over spanning the GPA locus could produce hemizygous daughter cells, while errors involving smaller mismatches could lead to the expression of an altered protein or to altered regulation of the expression of one allele. Supporting this hypothesis of unequal chromatid exchanges is recent molecular evidence for unequal SCE within a region of human DNA composed of tandemly repeated nucleotide sequences in BS cells proliferating *in vitro* (41).

The region around the GPA gene may be unusually susceptible to unequal recombination in view of many reports that have described individuals who heritably express an altered glycophorin protein apparently resulting from an unequal meiotic cross-over event between the GPA gene and the closely linked and partially homologous glycophorin B (GPB) gene (28, 31, 42, 43). Measurements of the hybrid protein on cells from four of these individuals with "Lepore-type" hybrids showed that the hybrid protein was expressed at about 25% of normal for one GPA allele (28, 31). This corresponds to the labeling characteristics of the small discrete population of variant cells commented on earlier, that lying below the MN peak that was observed consistently in all seven BS samples in the present study (Fig. 1). Elevated unequal somatic recombination in the GPA–GPB region provides a possible explanation for this variant population. Thus, elevated crossing-over and SCE in BS, both equal and unequal, could contribute to the unusually high frequencies for all the variant GPA phenotypes that we have observed.

An elevated frequency of mutations of the types that produced both the homozygous and the hemizygous GPA variant cells detected in the present study could be a major contributor to the extreme cancer risk of BS. Early reports suggested that chromosomal mechanisms including recombination might be important in unmasking recessive defects leading to neoplasia (18, 44–46). These suggestions have been confirmed by recent studies demonstrating the importance of deletion, missegregation, and recombination in the development of rare childhood tumors such as retinoblastoma and Wilms tumor (36, 47–49). Allele-loss mechanisms also appear to be important in the development of common tumor types, including large intestinal, breast, bladder, and lung tumors, for there, also, DNA analyses have demonstrated losses of heterozygosity (or induced homozygosity) for specific chromosomal regions in the tumor tissue (50–53). In light of these observations, it is interesting that the small, and young, population of BS individuals includes three cases of Wilms tumor, six of large intestinal cancer, and four of breast cancer (2, 3, 54). The existence of three apparently unrelated individuals with Wilms tumor is particularly striking because of the rarity of this tumor type in the general population (55). The exceptionally high incidence of Wilms tumor in BS is consistent with there being elevated frequencies of both the first and second mutational steps hypothesized for this tumor type (47, 55), suggesting that similar dramatic elevations in incidence might be expected for other cancer types where multiple mutational events are required for tumor development. Thus, the rare entity BS, with its strikingly elevated genomic instability and enormous cancer risk, provides a useful model for studying the relationship between somatic cell mutation and carcinogenesis in the general population.

We dedicate this paper to the memory of Sam Latt, a pioneer in SCE and other aspects of cytogenetics, a respected colleague and friend, who died on August 28, 1988. We thank B. Nisbet, S. Tyler,

and R. Barlett for technical assistance. We also thank Dr. P. A. W. Edwards for providing the R1.3 monoclonal antibody. Work at Lawrence Livermore National Laboratory was performed under the auspices of U.S. Department of Energy Contract W-7405-ENG-48 and was partially supported by U.S. Environmental Protection Agency Grant R811819-02. Work at The New York Blood Center was supported by National Institutes of Health Grants HD04134 and CA38036. Several Japanese blood samples were obtained with the assistance of Prof. H. Takebe and Dr. K. Tatsumi (Kyoto) during a Fellowship awarded J.G. by the Japan Society for the Promotion of Science.

1. German, J. (1974) in *Chromosomes and Cancer*, ed. German, J. (Wiley, New York), pp. 601–617.
2. German, J., Bloom, D. & Passarge, E. (1989) *Clin. Genet.* **35,** 57–69.
3. German, J. (1983) in *Chromosome Mutation and Neoplasia*, ed. German, J. (Liss, New York), pp. 97–134.
4. German, J. & Schonberg, S. (1980) in *Genetic and Environmental Factors in Experimental and Human Cancer*, eds. Gelboin, H., Macmahon, B., Matsushima, T., Sugimura, T., Takayama, S. & Takebe, H. (Japan Sci. Soc. Press, Tokyo), pp. 175–186.
5. Ray, J. H. & German, J. (1983) in *Chromosome Mutation and Neoplasia*, ed. German, J. (Liss, New York), pp. 135–167.
6. Evans, H. J., Adams, A. C., Clarkson, J. M. & German, J. (1978) *Cytogenet. Cell Genet.* **20,** 124–140.
7. Kuhn, E. M. (1980) *Hum. Genet.* **54,** 335–341.
8. Shiraishi, Y. & Sandberg, A. A. (1977) *Cytogenet. Cell Genet.* **18,** 13–23.
9. Chaganti, R. S. K., Schonberg, S. & German, J. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 4508–4512.
10. German, J., Schonberg, S., Louie, E. & Chaganti, R. S. K. (1977) *Am. J. Genet.* **29,** 248–255.
11. Tsuji, H., Heartlein, M. W. & Latt, S. A. (1988) *Mutat. Res.* **198,** 241–253.
12. Rosin, M. P. & German, J. (1985) *Hum. Genet.* **71,** 187–191.
13. Frorath, B., Schmidt-Preuss, U., Siemers, U., Zollner, M. & Rudiger, H. W. (1984) *Hum. Genet.* **67,** 52–55.
14. Gupta, R. S. & Goldstein, S. (1980) *Mutat. Res.* **73,** 331–338.
15. Warren, S. T., Schultz, R. A., Chang, C.-C., Wade, M. H. & Trosko, J. E. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3133–3137.
16. Vijayalaxmi, Evans, H. J., Ray, J. H. & German, J. (1983) *Science* **221,** 851–853.
17. Ben-Sasson, S. A., Cohen, T. & Voss, R. (1985) *Cancer Genet. Cytogenet.* **15,** 237–242.
18. German, J. (1964) *Science* **144,** 298–301.
19. German, J., Crippa, L. P. & Bloom, D. (1974) *Chromosoma* **48,** 361–366.
20. Therman, E. & Kuhn, E. M. (1976) *Cytogenet. Cell Genet.* **17,** 254–267.
21. German, J. (1982) in *Gene Amplification*, ed. Schimke, R. T. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 307–312.
22. Kuhn, E. M. & Therman, E. (1979) *J. Med. Genet.* **16,** 219–222.
23. Willis, A. E. & Lindahl, T. (1987) *Nature (London)* **325,** 355–357.
24. Chan, J. Y. H., Becker, F. F., German, J. & Ray, J. H. (1987) *Nature (London)* **325,** 357–359.
25. Willis, A. E., Weksberg, R., Tomlinson, S. & Lindahl, T. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8016–8020.
26. Cook, P. J., Noades, J. E., Lomas, C. G., Buckton, K. E. & Robson, E. B. (1980) *Ann. Hum. Genet.* **44,** 61–73.
27. German, J., Metaxas, M. N., Metaxas-Buhler, M., Louie, E. & Chaganti, R. S. K. (1979) *Cytogenet. Cell Genet.* **25,** 160 (abstr.).
28. Langlois, R. G., Bigbee, W. L. & Jensen, R. H. (1985) *J. Immunol.* **134,** 4009–4017.
29. Langlois, R. G., Bigbee, W. L. & Jensen, R. H. (1986) *Hum. Genet.* **74,** 353–362.
30. Langlois, R. G., Bigbee, W. L., Kyoizumi, S., Nakamura, N., Bean, M. A., Akiyama, M. & Jensen, R. H. (1987) *Science* **236,** 445–448.
31. Merry, A. H., Hodson, C., Thompson, E., Mallinson, G. & Anstee, D. J. (1986) *Biochem. J.* **233,** 93–98.
32. Jensen, R. H., Bigbee, W. L. & Langlois, R. G. (1987) in *Banbury Report 28: Mammalian Cell Mutagenesis*, eds. Moore, M. M., DeMarini, D. M. & Tindall, K. R. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 149–159.
33. Weksberg, R., Smith, C., Anson-Cartwright, L. & Maloney, K. (1988) *Am. J. Hum. Genet.* **42,** 816–824.
34. Furthmayer, H. (1978) *Nature (London)* **271,** 519–524.
35. Bigbee, W. L., Langlois, R. G., Swift, M. & Jensen, R. H. (1989) *Am. J. Hum. Genet.*, in press.
36. Cavenee, W. K., Dryja, T. P., Phillips, R. A., Benedict, W. F., Godbout, R., Gallie, B. L., Murphree, A. L., Strong, L. C. & White, R. L. (1983) *Nature (London)* **305,** 779–783.
37. Bubley, G. J. & Schnipper, L. E. (1987) *Somatic Cell Mol. Genet.* **13,** 111–117.
38. Therman, E., Otto, P. G. & Shahidi, N. T. (1981) *Chromosoma* **82,** 627–636.
39. Festa, R. S., Meadows, A. T. & Boshes, R. A. (1979) *Cancer* **44,** 1507–1510.
40. Wahlstrom, J., Kyllerman, M., Hansson, A. & Taranger, J. (1985) *Hum. Genet.* **70,** 186–188.
41. German, J. & Groden, J. (1988) *Am. J. Hum. Genet.* **43,** A183 (abstr.).
42. Siebert, P. D. & Fukuda, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6735–6739.
43. Dahr, W., Beyreuther, K., Moulds, J. & Unger, P. (1987) *Eur. J. Biochem.* **166,** 31–36.
44. German, J. (1965) *Science* **148,** 506–507.
45. Knudson, A. G. (1973) *Adv. Cancer Res.* **17,** 317–352.
46. Passarge, E. & Bartram, C. R. (1976) in *Cancer and Genetics*, ed. Bergsma, D. (Liss, New York), pp. 177–180.
47. Raizis, A. M., Becroft, D. M., Shaw, R. L. & Reeve, A. E. (1985) *Hum. Genet.* **70,** 344–346.
48. Koufos, A., Hansen, M. F., Copeland, N. G., Jenkens, N. A., Lampkin, B. C. & Cavenee, W. K. (1985) *Nature (London)* **316,** 330–334.
49. Dryja, T. P., Rapaport, J. M., Epstein, J., Goorin, A. M., Weichselbaum, R., Koufos, A. & Cavenee, W. K. (1986) *Am. J. Hum. Genet.* **38,** 59–66.
50. Fearon, E. R., Hamilton, S. R. & Vogelstein, B. (1987) *Science* **238,** 193–197.
51. Ali, I. U., Lidereau, R., Theillet, C. & Callahan, R. (1987) *Science* **238,** 185–188.
52. Fearon, E. R., Feinberg, A. P., Hamilton, S. H. & Vogelstein, B. (1985) *Nature (London)* **318,** 377–380.
53. Naylor, S. L., Johnson, B. E., Minna, J. D. & Sakaguchi, A. Y. (1987) *Nature (London)* **329,** 451–454.
54. Cairney, A. E. L., Andrews, M., Greenberg, M., Smith, D. & Weksberg, R. (1987) *J. Pediatr.* **111,** 414–416.
55. Knudson, A. G. & Strong, L. C. (1972) *J. Natl. Cancer Inst.* **48,** 313–324.