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# A Chromosome Study of 6-Thioguanine-Resistant Mutants in T Lymphocytes of Hiroshima Atomic Bomb Survivors

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1989年から，放射線影響研究所の業績報告書は，従来の日英両文を併記した方式では発行しない．主要な報告書については，今後も日英両文で発表するが，それぞれ別に発行する．内容が高度に専門的であり，一般の関心が少ないと思われる報告書については英文のみとし，日本文の要約を添付する．

これにより，広島・長崎の原爆電離放射線被曝の人体に及ぼす晩発性生物学的影響に関する最近の知見を今までよりも速やかにお知らせできることと思う．

*The Radiation Effects Research Foundation (formerly ABCC) was established in April 1975 as a private nonprofit Japanese Foundation, supported equally by the Government of Japan through the Ministry of Health and Welfare, and the Government of the United States through the National Academy of Sciences under contract with the Department of Energy.*

財団法人　放射線影響研究所
業　績　報　告　書

RERF TR 5-89
研究課題　4-83

# 被爆者末梢血6-TG耐性突然変異T-リンパ球における染色体分析§

# A Chromosome Study of 6-Thioguanine-Resistant Mutants in T Lymphocytes of Hiroshima Atomic Bomb Survivors

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## 要　約

原爆被爆者末梢血中における6-TG耐性突然変異Tリンパ球の頻度を測定するシステムから6-TG耐性及び野性型（非TG抽出）のTリンパ球コロニーを得て（有意な原爆放射線量を受けていない遠距離被爆者では，耐性コロニー18，野性型コロニー9，近距離被爆者では，耐性コロニー45，野性型コロニー19），その染色体分析をGバンド法を用いて行った．種々の交換型異常及び数的異常が観察されたが，HPRT遺伝子が存在するX染色体に異常が見られたのは6群で，対照の遠距離被爆者耐性コロニーで2群〔45，X/46，XX；46，X，ins（X）〕，近距離被爆者耐性コロニーで3群〔45，X/46，XX/46，X，＋mar；46，X，t（Xq＋；14q－）；46，Y，t（Xq－；5q＋）〕，近距離被爆者野性型コロニーで1群〔45，X/47，XXX〕であった．構造異常が識別されたX染色体では，いずれもq26付近で切断再結合が生じており，これはHPRT遺伝子座位と一致していた．RBG法によるDNA複製パターンの分析を行ったところ，構造異常をもつX染色体が早期複製パターンを，正常X染色体が後期複製パターンを示すという結果が得られた．

§要約以外の訳文はない．

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# A Chromosome Study of 6-Thioguanine-Resistant Mutants in T Lymphocytes of Hiroshima Atomic Bomb Survivors§

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## Summary

Cytogenetic characterizations were made of lymphocyte colonies established from somatic mutation assays for 6-thioguanine (TG) resistance in Hiroshima atomic bomb survivors. G-banded chromosomes were analyzed in both TG-resistant ($TG^r$) and wild-type (not TG-selected) colonies. Included were 45 $TG^r$ and 19 wild-type colonies derived from proximally exposed A-bomb survivors, as well as colonies from distally exposed control individuals who were not exposed to a significant level of A-bomb radiation (18 $TG^r$ and 9 wild-type colonies). Various structural and numerical abnormalities of chromosomes were observed in both $TG^r$ and wild-type colonies. Aberrations of the X chromosome, on which the hypoxanthine guanine phosphoribosyltransferase (HPRT) locus is present, were found in six colonies: two resistant colonies from controls [45,X/46,XX; 46,X,ins(X)], three resistant colonies [45,X/46,XX/46,X,+mar; 46,X,t(Xq+;14q−); 46,Y,t(Xq−;5q+)], and one wild-type colony [45,X/47,XXX] from proximally exposed persons. In cases with exchange aberrations, each of the break points on the X chromosome was situated proximally to band q26 where the HPRT locus is known to be assigned. DNA replicating patterns were also studied, and it was found that abnormal X chromosomes showed early replicating patterns, while normal X chromosomes showed late replicating patterns.

## Introduction

Somatic mutations induced by radiation and chemical substances are thought to play an important role in the etiology of cancer. With the development of culture techniques for clonal proliferation of human peripheral T lymphocytes, it has become possible to detect in vivo mutations at the X chromosome-linked

§**The complete text of this report will not be available in Japanese.**

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HPRT locus.[1,2] Recently, Hakoda et al[3] have modified these techniques and have established an efficient and reproducible system to determine the mutant frequency of peripheral T lymphocytes, using resistance to TG as a marker for HPRT-deficient mutants. This method has been used to study the relationship between HPRT mutant frequency and A-bomb radiation dose, and a shallow but significant positive correlation has been observed.[4]

It is well known that cells with radiation-induced chromosome aberrations can persist in circulating lymphocytes of A-bomb survivors long after radiation exposure, and that the frequency of these aberrant cells is proportional to the estimated dose received by each individual.[5,6] Furthermore, stable-type aberrations, such as translocations, inversions, and deletions, are exclusively found.

The present report describes the results of chromosome studies on $TG^r$ and wild-type T lymphocyte colonies obtained from Hiroshima A-bomb survivors. Here, the term "wild-type colonies" refers to colonies that were not TG-selected. A possible association between HPRT deficiency and specific chromosomal change is discussed.

## Materials and Methods

The study sample consisted of eight proximally exposed Hiroshima A-bomb survivors (two females and six males) whose estimated doses were 1 Gy or more (T65DR). These were compared to six distally exposed individuals (four females and two males) with estimated doses of less than 0.01 Gy.[7] In the present study, these distally exposed persons were studied as demographically matched negative controls. These individuals are participants of the Adult Health Study (AHS) program, who received biennial physical examinations at RERF.[8] None had received either radiation therapy at any time in the past or extensive diagnostic radiation in the preceding year.

Peripheral T lymphocytes were cultured according to modifications of the methods of Albertini et al[1] and Morley et al.[2] Culture methods and the results of the study on somatic mutation assay have been described in detail elsewhere.[3,4] After the measurement of mutant frequencies, T cell colonies were cultured for two more weeks to obtain a sufficient number of cells for cytogenetic analysis. A total of 91 colonies were examined: 45 $TG^r$ and 19 wild-type colonies derived from the proximally exposed individuals, as well as 18 $TG^r$ and 9 wild-type colonies from the control individuals. Both $TG^r$ and wild-type colonies were characterized in cells from four of eight proximally exposed individuals; only $TG^r$ colonies were examined for the remaining four. Similarly, both $TG^r$ and wild-type colonies were investigated in two control persons. Only $TG^r$ colonies were studied in the remaining four individuals.

Chromosome slides were routinely prepared using an air-dried method, following exposure to Colcemid (0.05 $\mu$g/ml) for four hours. The slides were

treated one week later with a 0.1% trypsin solution (GIBCO) for 15-20 sec at 30°C and then stained with a 2% Giemsa solution for 15 minutes. In each case, 10 to 50 metaphases were analyzed. Both C-banding[9] and silver-staining[10] methods were also used for precisely identifying break points in several cases. In addition, DNA replication patterns were studied by using the RBG banding method.[11] In this method, cells were labeled with BrdU (30 $\mu$g/ml) for 8-9 hours before harvest, and air-dried slides were prepared as described above and stained by the fluorochrome plus Giemsa technique.[12] Here, the term "abnormal clone" has been used when identical abnormal karyotypes were observed in at least two or more cells in the same colony.

## Results

Various structural and numerical abnormalities of chromosomes were observed in both TG$^r$ and wild-type colonies. The majority of the abnormal clones were found in colonies derived from the proximally exposed group (Table 1). Out of a total of 64 colonies studied in this group, 30 colonies were found to have abnormal clones: 9 of 19 in wild-type colonies and 21 of 45 in TG$^r$ colonies. On the other hand, of the 27 colonies studied in the control group (9 in wild-type colonies and 18 in TG$^r$ colonies), only 2 TG$^r$ colonies contained abnormal clones, and none were observed in wild-type colonies.

Table 1. Results of chromosome analyses in T cell colonies

| | Control | | Exposed | |
|---|---|---|---|---|
| | Wild-type | 6-TG$^r$ | Wild-type | 6-TG$^r$ |
| Number of persons | 2 | 6 | 4 | 8 |
| Number of colonies | 9 | 18 | 19 | 45 |
| Total number of colonies with abnormal clone | 0 | 2 | 9 | 21 |
| Number of clones with structural abnormal X | 0 | 1 | 0 | 2 |
| Number of clones with numerical abnormal X | 0 | 1 | 1 | 1 |

Individual structural aberrations were distributed among all chromosomes except for numbers 10 and 22. A total of 75 break points observed in abnormal clones are shown on the diagram of individual human chromosomes in Figure 1. The statistical analysis was only conducted on 52 break points found in TG$^r$ colonies obtained from the proximally exposed group. The observed number of break points on each chromosome was compared with the expected number of

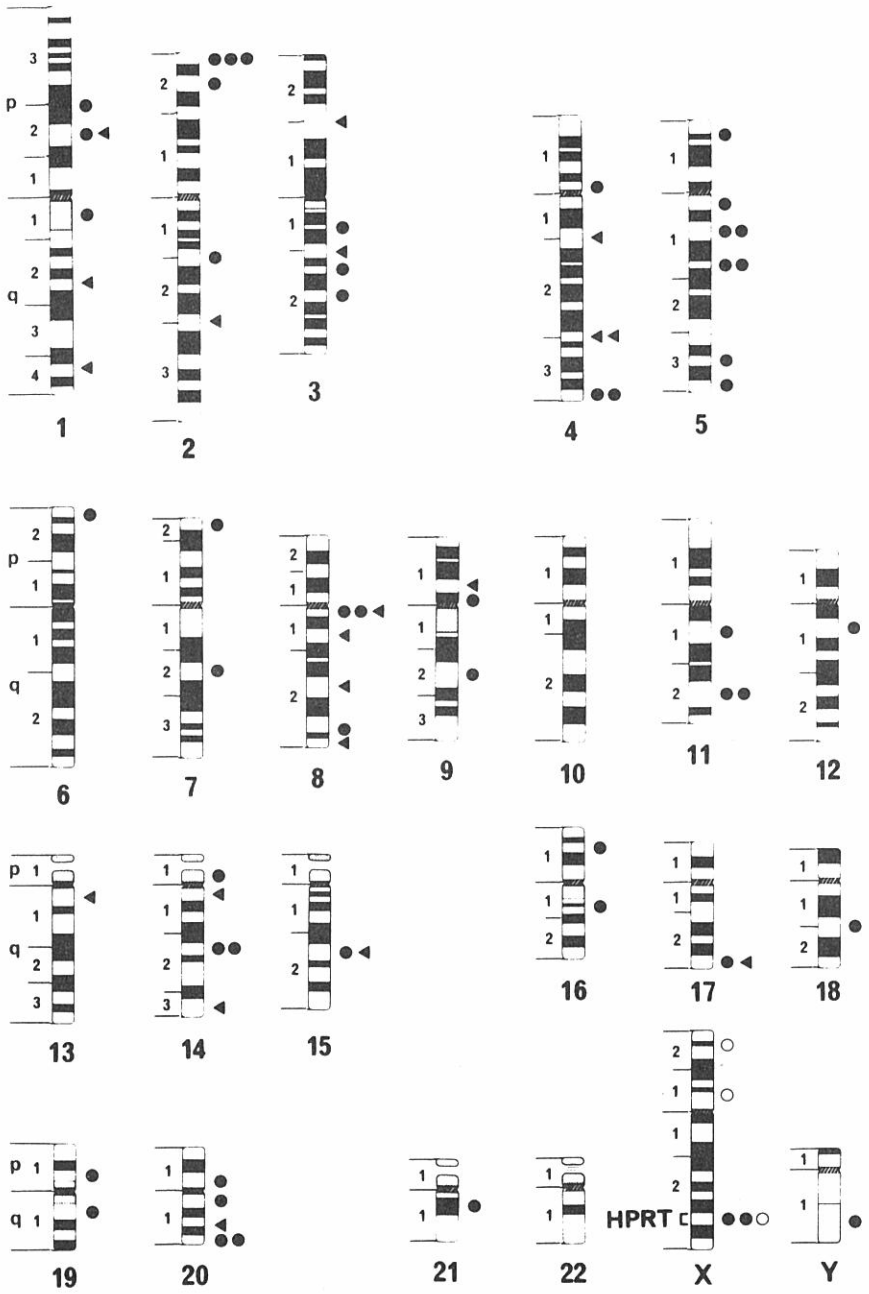


*Figure 1. Distribution of break points observed in abnormal clones. ● : $TG^r$ clones in exposed group; ◀ : wild-type clones in exposed group; and ○ : $TG^r$ clones in control group.*

break points which were calculated by the value of the relative chromosome length measured by Caspersson et al in ISCN.[13] The results of a $\chi^2$ test demonstrated that interchromosomally these break points were randomly distributed ($p>0.20$). A $\chi^2$ test was also performed on chromosomes individually. The results of this test showed that a significant number of breaks was observed in chromosome 5 ($p<0.01$) and in chromosome 20 ($p<0.01$). No excessive number of break points was observed in the X chromosome ($p>0.70$).

Aberrations of the X chromosome were found in six colonies: two cases in $TG^r$ colonies in controls, one case in wild-type colonies, and three cases in $TG^r$ colonies in the proximally exposed group (Table 1). Two cases (38-6, 38-$4^r$) were obtained from one individual, and the other four cases were derived from different persons (Table 2). As also shown in Table 2, structural aberrations of the X chromosome were observed in three $TG^r$ colonies (35-$3^r$ in controls, R-$2^r$ and 38-$4^r$ in the proximally exposed). The first case (35-$3^r$) appears to be an insertion within an X chromosome. It could not be determined, however, whether the insertion was direct or inverted at the 400-band level. The other two cases (R-$2^r$, 38-$4^r$) were characterized by reciprocal translocations between the X chromosome and chromosomes 5 and 14, respectively. These three colonies were reexamined several months later, and consistent results were obtained (Table 2). Partial karyotypes of these aberrations are shown in Figure 2. Each of the break points on the X chromosome was located proximally to q26 where the HPRT locus is assigned in man.[14]

Numerical abnormalities were noted in three colonies; all involved the X chromosome and were mosaic (Table 2). The first case (D-$3^r$), a $TG^r$ colony from the control, revealed the mosaic of X chromosome monosomy (45,X) and the X chromosome trisomy (47,XXX). The second case (38-6), a wild-type colony from the proximally exposed, showed the mosaic of X chromosome monosomy (45,X) and the normal cell (46,XX). The third case (30-$5^r$), a $TG^r$ colony from the proximally exposed, was characterized by three different karyotypes: the X chromosome monosomy (45,X), the normal cell (46,XX), and the X chromosome monosomy accompanied by the minute chromosome, the origin of which was unknown (46,X,+mar). In each case, the X chromosome monosomy was observed in the majority of cells examined, and this observation was found to be consistent throughout the serial examinations (Table 2).

The DNA replicating pattern was also determined in cases of female origin by the RBG banding method.[11] In two cases (35-$3^r$, 38-$4^r$) in which structural aberrations of the X chromosome were observed, it was found that the abnormal X chromosome showed an early DNA replicating pattern. In each case, the corresponding normal X chromosome showed a late replicating pattern (Figure 3). In the case of numerical abnormalities of the X chromosome (D-$3^r$, 38-6, 30-$5^r$), the one X chromosome always showed an early replicating pattern.

Table 2. Aberrations of the X chromosome in T cell colonies

| Origin | Colony | Kerma (Gy) | | Age and sex | Examination No. | No. of cells analyzed | Karyotype | No. of cells of each karyotypic group |
|---|---|---|---|---|---|---|---|---|
| | | T65D | DS86[1)] | | | | | |
| Control TG$^r$ | D-3$^r$ | 0 | 0 | 70 F | I | 22 | 45,X/46,XX | 20/2 |
| | | | | | II | 50 | 45,X/46,XX | 46/4 |
| | | | | | III | 23 | 45,X/46,XX | 21/2 |
| | 35-3$^r$ | 0 | 0 | 58 F | I | 20 | 46,X,ins(X)(q26p11.2p22) or 46,X,inv ins(X)(q26,p22p11.2) | 20 |
| | | | | | II | 35 | 46,X,ins(X)(q26p11.2p22) or 46,X,inv ins(X)(q26,p22p11.2) | 35 |
| Exposed wild-type | 38-6 | 2.37 | 1.21 | 54 F | I | 22 | 45,X/47,XXX | 13/9 |
| | | | | | II | 20 | 45,X/47,XXX | 14/6 |
| | | | | | III | 14 | 45,X/47,XXX | 10/4 |
| Exposed TG$^r$ | R-2$^r$ | 1.73 | ND[2)] | 75 M | I | 33 | 46,Y,t(X;5)(q26;q35) | 33 |
| | | | | | II | 20 | 46,Y,t(X;5)(q26;q35) | 20 |
| | 38-4$^r$ | 2.37 | 1.21 | 54 F | I | 16 | 46,X,t(X;14)(q26;q22) | 16 |
| | | | | | II | 14 | 46,X,t(X;14)(q26;q22) | 14 |
| | 30-5$^r$ | 4.04 | 2.01 | 71 F | I | 25 | 45,X/46,XX/46,X,+mar[3)] | 15/7/3 |
| | | | | | II | 20 | 45,X/46,XX/46,X,+mar[3)] | 9/7/4 |

1) Revised dose estimates[23]

2) Not determined

3) mar: minute chromosome

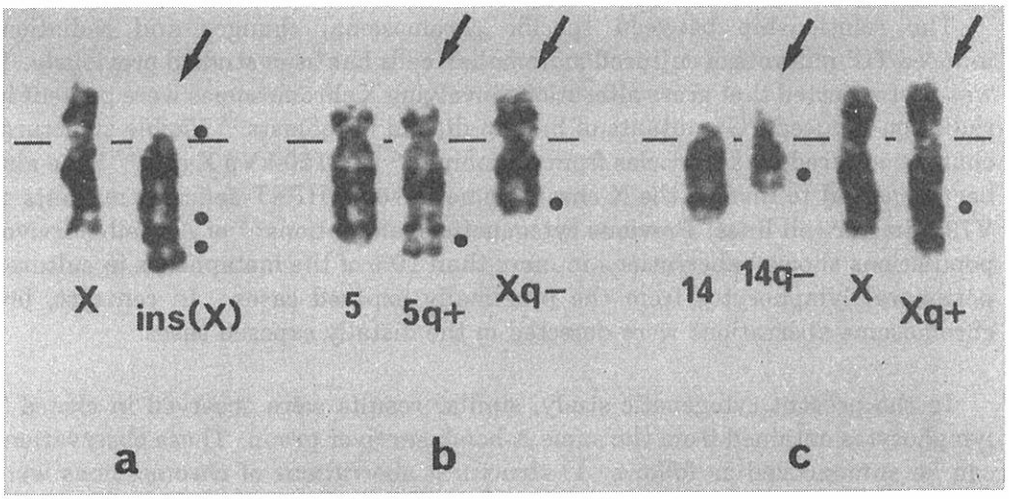


*Figure 2. Partial karyotypes of structural aberrations of the X chromosome observed in TG$^r$ colonies. ● : break points. a: ins(X)(q26p11.2p22) or inv ins(X)(q26p22p11.2) found in a colony (35-3$^r$) from the controls; b: t(X;5)(q26;q35) found in a colony (R-2$^r$) from the exposed; and c: t(X;14)(q26;q22) found in a colony (38-4$^r$) from the exposed.*

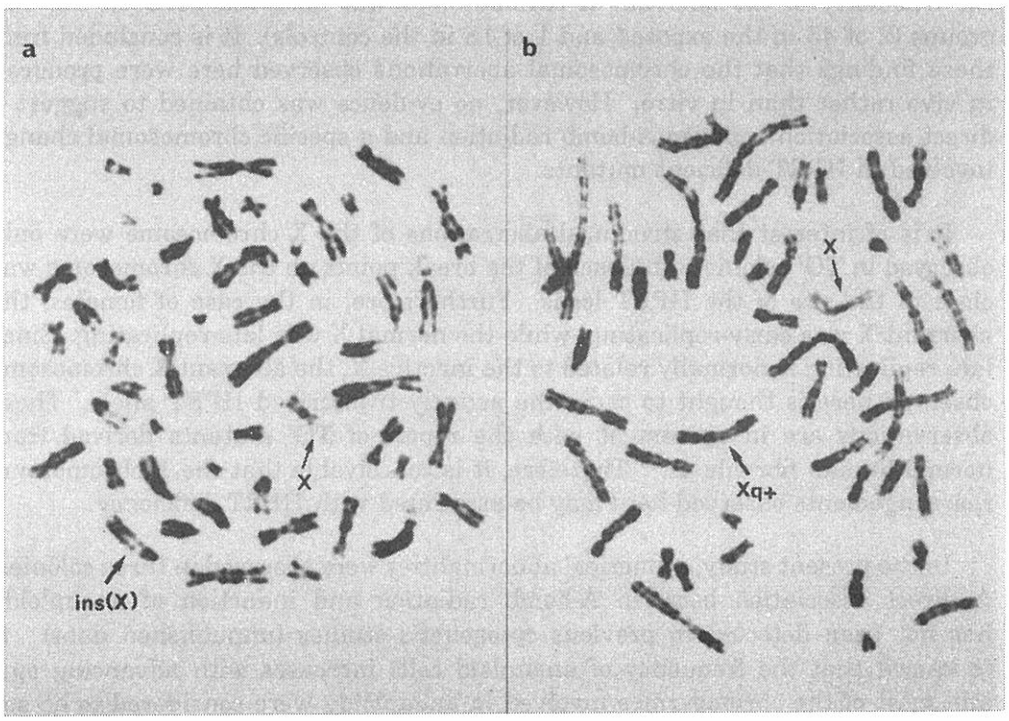


*Figure 3. R-banded metaphases with structural aberrations of the X chromosome observed in TG$^r$ colonies. Abnormal X shows the early DNA replicating pattern, while normal X shows the late replicating pattern in both cases. a: ins(X) or inv ins(X) found in a colony (35-3$^r$) from the controls; and b: t(X;14) found in a colony (38-4$^r$) from the exposed.*

## Discussion

The relationship between specific chromosomal changes and radiation-induced $TG^r$ mutants of cultured mammalian cells has been studied previously. It was first reported that gross alterations involving X chromosomes were present in radiation-induced $TG^r$ mutants of human diploid fibroblasts.[15] Visible structural changes induced by $\alpha$ particles from plutonium[16] and 150-kVp X rays[17] have also been reported to involve the X chromosome in some HPRT-deficient mutants of V79 hamster cell lines. Previous cytogenetic examinations[5,6] of A-bomb survivor populations showed aberrations in more than 10% of the metaphases in cultured peripheral lymphocytes from the proximally exposed cases. In contrast, few chromosome aberrations were detected in the distally exposed cases.

In the present cytogenetic study, similar results were observed in cloned T lymphocytes obtained from the same A-bomb survivor group. These observations can be summarized as follows: 1) structural aberrations of chromosomes were found in not only $TG^r$ colonies (21 of 45) but also wild-type colonies (9 of 19) in the proximally exposed group; 2) a few aberrations were observed in $TG^r$ colonies (2 of 18) and no aberrations were detected in wild-type colonies (0 of 9) in the control; 3) a small number of structural aberrations of the X chromosome were observed in $TG^r$ colonies from both proximally exposed and controls, but no difference in the frequency of the aberrant X chromosomes was observed between the two groups (2 of 45 in the exposed and 1 of 18 in the controls). It is concluded from these findings that the chromosomal aberrations observed here were produced in vivo rather than in vitro. However, no evidence was obtained to suggest a direct association between A-bomb radiation and a specific chromosomal change involved in HPRT-deficient mutants.

It is of interest that structural aberrations of the X chromosome were only observed in $TG^r$ colonies, and each of the break points on the X chromosome was close to the site of the HPRT locus. Furthermore, in the case of females, the aberrant X was early-replicating, while the normal X was late-replicating. Since late replication is normally related to the inactive X, the aberrant X chromosome observed here is thought to carry the actively transcribed HPRT allele. These observations are in agreement with the report of $TG^r$ mutants derived from normal human fibroblasts.[15] Therefore, it is conceivable that the X chromosome rearrangements observed here may be associated with HPRT deficiency.

In the present study, numerical abnormalities were observed in three colonies. A direct association between A-bomb radiation and induction of aneuploidy has not been detected in previous cytogenetic studies (unpublished data). It is known that the frequency of aneuploid cells increases with advancing age, and most of the chromosomes involved in aneuploidy were considered to be sex chromosomes.[18] In this study, most of the donors were older than 50 years of age, and numerical abnormalities were found to include only X chromosomes, not only in $TG^r$ colonies but also in wild-type colonies. It seems likely, therefore, that the loss or gain of an X chromosome observed in this study may be the consequence of

aging rather than radiation exposure, though a possibility that such chromosome aberrations are relevant to radiation exposure cannot be ruled out. Alternatively, observed numerical abnormalities may simply represent a culture effect since all of these colonies were mosaic.

It may be worth noting that breaks on chromosome 5 were only detected in $TG^r$ colonies. Thacker and Cox[19] reported that, of the 18 independent X/autosome translocations observed in radiation-induced $TG^r$ mutants of human diploid fibroblasts, 5 (28%) were of the t(Xq;5q) type. Their observations were somewhat different from our cases in which only one colony had a t(Xq;5q) aberration. The other colonies showed different types of aberrations involving chromosome 5: such as, inv(5), del(5), and translocations between chromosome 5 and other autosomes. Although our observation on aberrations involving chromosome 5 may merely reflect sampling errors because of the paucity in the number of aberrations, it is conceivable that the mutant cells with those aberrations may acquire some growth advantage in prolonged cultures containing 6-thioguanine.[19]

Recently, Muir et al[20] investigated chromosome changes in the HPRT-deficient human lymphocyte clones. Of 17 X ray-induced mutants analyzed, only 1 mutant showed an aberration involving the X chromosome. No other X chromosome abnormalities was observed in 47 spontaneous mutants. Turner et al[21] also described in vivo somatic mutations in human lymphocytes obtained from healthy adults. They found major gene alterations of the HPRT gene by Southern analysis; however, chromosome studies showed no structural abnormalities of the X chromosome. An analogous observation was also reported in Lesch-Nyhan patients.[22] In the present study, only a few X chromosome aberrations were observed in a number of HPRT mutant colonies. Molecular analyses of the remaining mutant colonies are required to determine the nature of these HPRT mutant genes.

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