

Mutation Rate of Normal and Malignant Human Lymphocytes¹

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ABSTRACT

The genetic stability of normal and neoplastic lymphocytes was compared by using base-line mutation frequency and mutation rate/cell generation. Mutations at the hypoxanthine-guanine phosphoribosyltransferase locus were studied by enumerating thioguanine-resistant cells in a clonogenic assay. The base-line ("spontaneous") mutation frequency was 1.52×10^{-6} , 6.38×10^{-6} , and 1.06×10^{-6} for normal cells from three individuals and was 1.16×10^{-3} , 6.08×10^{-5} , and 3.06×10^{-5} for the three malignant cell lines, Jurkat (JM), HRIK, FMC-Hu1B, respectively. The mutation cell/generation rate was 24.6×10^{-8} , 15×10^{-8} , and 5.5×10^{-8} for lymphocytes from the three normal individuals, and 666.4×10^{-8} , 52.8×10^{-8} , and 131×10^{-8} for the three malignant cell lines. The results suggest that neoplastic lymphocytes are more genetically unstable than normal lymphocytes.

INTRODUCTION

The development of cytotoxic drug resistance is a major cause of failure to cure malignancy. Although the nature of the development of drug resistance is poorly understood, recent studies suggest that the abnormality may be genetically determined (1, 2). It has been suggested, based on chromosomal analysis, that malignant cells are genetically unstable and that the genetic instability may be the underlying mechanism in the development of cytotoxic drug resistance and tumor progression (3).

Recently we (4) and other workers (5) have shown that the population of normal human lymphocytes contains a small subpopulation of TG^R cells which are mutated at the hypoxanthine-guanine phosphoribosyltransferase locus. The frequency of mutant cells rises after exposure to X-rays (6) and is increased in patients treated with cytotoxic drugs and radiotherapy (7).

In the present study we compared the genetic stability of malignant lymphocytes to that of normal lymphocytes by determining the frequency of spontaneously occurring mutant cells and by determining the rate of generation of new mutants during proliferation in culture.

MATERIALS AND METHODS

The following established leukemia and lymphoma cell lines were studied in the logarithmic phase of growth: two Burkitt's lymphoma B-cell lines, FMC-HU-1B and HRIK; and JM (Jurkat), an acute lymphoblastic leukemia T-cell line. Human lymphocytes were obtained from peripheral blood samples of five healthy volunteer donors.

Mutation Assay. Full details of T-lymphocyte cloning and detection of TG^R lymphocyte clones have been described previously (8, 9), but in brief lymphocytes were separated from peripheral blood using Ficoll-Hypaque (Pharmacia, Uppsala Sweden) and cloned at a limiting dilution in microwells with or without TG. Ninety-six microwells containing a mean of 2 cells per well were set up without TG, and 480-960 microwells, each containing a mean of 2×10^4 cells per well, were set

up with TG. All microwells contained McCoy's Medium 5A with PHA, irradiated peripheral blood mononuclear cells as feeder cells, and conditioned medium which provided a source of interleukin-2. After 14-20 days of culture, microwells were assessed as positive or negative for growth using an inverted microscope, and the cloning efficiency was calculated using Poisson statistics (10). The mutation frequency was calculated as the ratio of the cloning efficiency with and without TG.

For estimating the mutation frequency in malignant lymphocytes a similar technique to that of normal lymphocytes was followed. However, the malignant lymphocytes were cloned in the presence of bone marrow stromal cells as described before (11). Briefly, the culture conditions included Iscove's modification of Dulbecco's medium (GIBCO, Grand Island, NY), irradiated human bone marrow stromal cells, and human AB-serum. Due to the possibility that the malignant cells may differ from normal cells in their TG sensitivity, dose-response curves for each of the malignant lines were determined to select the appropriate dose of TG required for the selection of mutants for each cell line. Control cultures were set up with a mean of 2 cells per well without TG, and test cultures were set up with varying concentrations of TG and cell numbers as described before (7). After 14-20 days of culture, cloning efficiency was calculated as for normal lymphocytes, and the clone survival in the presence of TG was estimated as a fraction of the control.

Estimation of Mutation Generation Rate. For normal lymphocytes, clones were produced by plating freshly isolated cells at a concentration of 2 cells per well in microtiter plates with PHA, conditioned medium, and irradiated peripheral blood mononuclear cells as feeder cells. After growth for 14 days, cells from 6-8 individual positive wells were picked off and separately expanded in individual microtiter plates in the presence of irradiated feeder cells, PHA, and conditioned medium (8). After growth for a total period of 30-36 days, the cells were harvested, and the mutation frequency was separately determined for each replicate.

For each malignant cell line studied, clones were produced by plating 2 cells per well in 96-well microtiter plates in the presence of irradiated bone marrow feeder cells (11). After 14 days, cells from 8-10 positive wells were separately expanded in tissue culture flasks without the use of bone marrow stromal cells. After further culture for 14-21 days, the flasks were harvested, and the total cell number in each flask was determined. These cells from individual flasks were then separately plated in microtiter plates with or without TG as described above for the estimation of TG^R mutation frequency.

Preliminary experiments showed that the phenotypic expression time for TG^R cells was approximately 5-7 days (data not shown). The rate of mutation was calculated by the equation described by Luria and Delbruck in Formula 8 of Ref. 12: $r = aN_d \ln(N_d/Ca)$, where r is the mean number of TG^R cells, a is the mutation rate (to be solved for), N_d is the number of cells in the growing cultures (at the time of testing), and C is the number of cultures. N_d/Ca was derived from the tables provided by Capizzi and Jameson (13).

RESULTS

The cell lines FMC-Hu-1B, HRIK, and JM were chosen after screening a number of malignant cell lines in order to exclude any already resistant to TG and to determine the final concentration of TG to be used in the mutation assay. As shown in Fig. 1 the concentrations of 2.5 and 10 $\mu\text{g/ml}$ for normal and malignant lymphocytes, respectively, were well along the plateau of the TG dose-response curve. This figure also suggested that the JM cell line had a significantly higher number of TG^R cells compared to normal lymphocytes and to the cell lines HRIK and FMC-HU-1B.

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³ The abbreviations used are: TG^R, thioguanine resistant; TG, thioguanine; PHA, phytohemagglutinin.

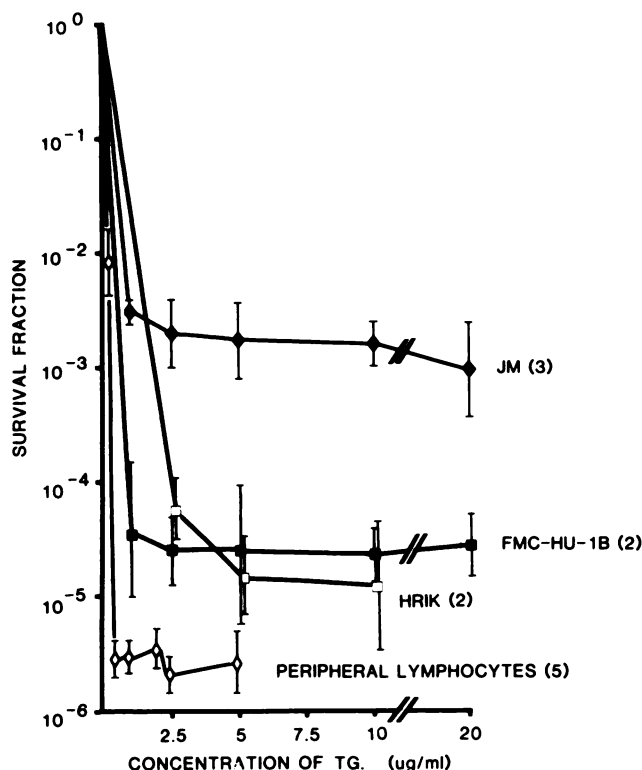


Fig. 1. Sensitivity of normal and neoplastic lymphocytes to TG. Points, mean sensitivity to TG of three neoplastic lymphocyte lines and of lymphocytes from five normal individuals as measured by the fraction of clones surviving in the presence of various concentrations of TG; bar, 2 SE. Numbers in parentheses, number of experiments.

Table 1 "Spontaneous" mutation frequency

Cells		Mutation frequency (mean)
Normal lymphocytes	Previous data (4, 13)	117 individuals
	Present data	3 individuals
		1.52×10^{-6}
		6.38×10^{-6}
	1.06×10^{-6}	
Neoplastic lymphocytes	JM	4 experiments
		1.167×10^{-4}
		1.169×10^{-4}
		3.60×10^{-3}
		8.04×10^{-4}
HRIK	4 experiments	
	5.538×10^{-5}	
	1.40×10^{-4}	
	3.28×10^{-6}	
	4.452×10^{-5}	
FMC-Hu-1B	4 experiments	
	3.312×10^{-5}	
	5.75×10^{-5}	
	1.17×10^{-5}	
	1.99×10^{-5}	

Table 1 shows the "spontaneous" mutation frequency in normal and malignant cells. *In vivo* mutation frequency in normal lymphocytes was estimated in 117 healthy normal individuals (previous data from Refs. 4 and 14) and in the 3 subjects in the present study. The 3 malignant cell lines had spontaneous *in vitro* mutation frequencies which were 10- to 1000-fold greater than the *in vivo* mutation frequency of normal lymphocytes.

Table 2 shows the results of the experiments to calculate the mutation generation rates. The rates were calculated from the mean mutations observed in 6-9 replicate cultures after expansion for 30-36 and 17-30 days for normal and malignant

lymphocytes, respectively. The rate of mutation/cell/generation was 2- to 120-fold greater in malignant cells compared with normal lymphocytes.

DISCUSSION

It has been known for several years that most types of drug-resistant tumor cells result from some type of permanent genetic change occurring in the initial drug-sensitive line. Using the fluctuation test, Luria and Delbruck (12) showed that drug-resistant bacterial cells appeared to arise spontaneously and randomly and that a similar phenomena may well occur within tumor cell populations (15). Only a few studies have directly compared the mutation rates of normal *versus* malignant cells. Goldberg and Defendi (16) compared the mutation rates of a nontumorigenic, aneuploid Chinese hamster cell line and various viral transformants of these cells. No difference in the mutation rates of the untransformed and polyoma virus-transformed cells was observed, whereas polyoma plus SV-40 virus transformants had a 4- to 25-fold increase in mutation rates. Cifone and Fidler (17) observed that a highly malignant, murine melanoma cell line had a higher mutation rate than a cell line with a lower metastatic potential. More recently, Elmore *et al.* (18) compared the TG and Na⁺-K⁺-adenosine triphosphatase mutation rates of normal, diploid human skin fibroblasts and a chemically induced transformed variant of the normal fibroblasts. They observed no difference in the mutation rates of the two cell lines.

To our knowledge this is the first study which compares the mutation rate of spontaneous human tumor cells to that of their normal counterpart. The possibility exists that the malignant cell lines arose from patients who had been treated with TG or mercaptopurine, and therefore the cell lines may already have had a higher population of TG^R cells. However, we used a concentration of TG which was well along the plateau of the TG dose-response curve. Also, the clones studied in the cell expansion studies were started from a single cell which must have been sensitive to TG as shown by the sensitivity of the overwhelming majority of the final cell population. Therefore preexisting mutants could not have affected the calculation of mutation rate.

Determination of the mutation rates of cells in culture can be influenced by various cultural and cellular factors such as cell karyotype, cloning efficiency, cell size, confluence arrest, and mutation expression time and is based on the assumption that there is no selection against mutant cells during culture. In our study, the cloning efficiencies of normal and malignant lymphocytes were comparable, and the lymphocytes did not have the problems of confluence arrest and loss of cells during trypsinization for transfer as is seen in fibroblast cultures. Mutation phenotypic expression time was 5-7 days, and since the culture period was 17-36 days in our experiments, the influence of mutation expression time would be negligible in the calculation of mutation generation rates.

Chromosomal ploidy level may influence the frequency of mutants (18) and, if the recessive gene mutations such as TG^R are being studied, cells with hyperdiploidy and multiple gene copies are likely to show a lower level of mutation compared with normal diploid cells. Since the malignant cells in our study showed a greater rate of mutation, despite perhaps having aneuploid populations, altered gene number is unlikely to explain our observations.

For comparison of the mutation rates of different cells, it is also important that the cells have similar growth rates. In the

TG^R MUTANTS IN HUMAN LYMPHOCYTES

Table 2 Rate of generation of TG^R mutants

Experiment	Normal lymphocytes (individuals)			Malignant lymphocytes		
	1	2	3	JM	FMC-Hu-1B	HRIK
No. of replicates	7	7	6	9	6	7
Initial no. of cells/replicate	2	2	2	2	2	2
Final no. of cells × 10 ⁶ /replicate (mean)	19.2	19.2	19.2	19.2	18.3 (17.6–19.2) ^a	18.8 (18.7–19.2)
No. of replicates with <i>n</i> TG ^R cells/ 10 ⁶ cells						
0	3	2	3	0	1	0
0.1–2	2	5	4	0	0	2
2.1–4	1	0	0	0	5	1
4.1–10	0	0	0	0	0	2
10.1–20	0	0	0	3	0	2
20.1–40	0	0	0	3	0	0
>40	0	0	0	3	0	0
Mean no. of mutants/10 ⁶ cells	0.82	0.46	0.11	46.9	2.1	6.7
CaN, (from tables in Ref. 13) ^b	28.3	20.5	7.4	1151.5	57.2	172.7
Mutation rate (mutants/cell genera- tion) × 10 ⁻⁸	24.6	15	5.5	666.4	52	131

^a Numbers in parentheses, range.

^b See text for details.

present study the numbers of cell generations occurring during the expansion phases of both normal and malignant lymphocytes were similar. Our recent studies have shown that the spontaneous TG^R mutants in normal cells show a number of genetic lesions, such as gene deletion and gene amplification (19). Further studies are under way to determine whether the spontaneously arising TG^R mutants in malignant lymphocytes show a heightened degree of DNA and/or cytogenetic abnormality.

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