Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 6617–6621, November 1982
Genetics

T-cell cloning to detect the mutant 6-thioguanine-resistant lymphocytes present in human peripheral blood

(cellular specific-locus test/human mutagenicity monitoring/mutant lymphocytes/hypoxanthine-guanine phosphoribosyltransferase)

RICHARD J. ALBERTINI*, KAROL L. CASTLE†, AND WAYNE R. BORCHERDING†

*Department of Medicine, The University of Vermont College of Medicine, Given Medical Building, Burlington, Vermont 05405; and †The University of Wisconsin Medical School, University Hospital and Clinics, Division of Immunology, K4/443, 600 Highland Avenue, Madison, Wisconsin 53792

Communicated by James V. Neel, July 20, 1982

ABSTRACT **Rare thioguanine-resistant T lymphocytes, present *in vivo* in human peripheral blood, were isolated and grown *in vitro* as thioguanine-resistant cultured T cells. The conditions for their selection *in vitro* were such that thioguanine resistance had to have arisen *in vivo*. The mutant cells bore T-cell surface markers, maintained their thioguanine resistance *in vitro* in the presence or absence of selection, and were deficient in hypoxanthine-guanine phosphoribosyltransferase activity.**

Thioguanine-resistant T lymphocytes, present at low frequencies in human peripheral blood, are assumed to arise from somatic cell mutation *in vivo*. We have developed an autoradiographic method to quantify these variant cells and have proposed it for use as a direct mutagenicity test for man (1, 2). However, as judged by accepted criteria (3), the evidence that the variant lymphocytes are mutants has been indirect. These rare cells behave in assay as do all T lymphocytes from individuals with the Lesch–Nyhan syndrome that results from mutation in the X-chromosomal gene for hypoxanthine-guanine phosphoribosyltransferase [hypoxanthine phosphoribosyltransferase (HPRT), EC 2.4.2.8] (4–6).

It is now possible to clone human T lymphocytes directly and to propagate them *in vitro* (7). We describe here a method that uses crude T-cell growth factor and thioguanine selection to clone *in vitro* the thioguanine-resistant T lymphocytes that arise *in vivo* in normal individuals. Characterization of these cells provides direct evidence as to their mutant character. Lymphocyte cloning under selective conditions is a generalizable method for human direct mutagenicity testing.

MATERIALS AND METHODS

Tissue Culture Media. The basic medium was RPMI 1640 medium containing 25 mM Hepes buffer, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. Heat-inactivated fetal calf or human AB-negative (Biobee) serum was added to basic medium to produce nutrient medium. Growth medium was nutrient medium containing 25–40% crude T-cell growth factor, depending on factor potency. Lymphoblastoid cell line (LCL) cells that were x-irradiated (x-LCL) were added to growth medium at various densities to produce "growth medium and feeder cells." Test media contained growth medium and 6-thioguanine (Sigma) at various concentrations.

Preparation of Peripheral Blood Mononuclear Cells. Leukocyte residues from single-donor platelet pheresis packs (8) obtained from the American Red Cross Blood Services (Badger Region) were overlayered into sterile 50-ml centrifuge tubes containing equal volumes of Ficoll-Hypaque (Sigma or Winthrop; $\rho = 1.007$) and were centrifuged at 600 × *g* for 30 min. The recovered mononuclear cells were transferred to fresh tubes, washed with phosphate-buffered saline, incubated for 10 min with cold (4°C) 0.83% NH_4Cl, and again washed with phosphate-buffered saline. Mononuclear cells from single donors were suspended in basic medium for use as a source of test cells or for T-cell growth factor production.

Production and Assay of T-Cell Growth Factor. Mononuclear cells (single donor or pooled) received 1,000 rad (1 rad = 0.01 gray) x-irradiation (^{137}Cs) prior to suspension at 10^6 cells per ml in nutrient medium containing 1% fetal calf serum and 1% phytohemagglutinin M (PHA-M; Difco). X-LCL cells (4,000 rad) were added at 10^4 cells per ml as allogeneic stimulators (9). One-hundred-milliliter cell suspensions were inoculated into culture flasks and incubated for 48 hr under standard conditions at 37°C in humidified 5% CO_2/95% air. Culture supernatants (crude T-cell growth factor) were filtered (Millipore) and frozen in 100-ml volumes at −20°C. Factor was assayed as described (9).

Feeder Cells. A LCL, originally designated as WI-L2, deficient in hypoxanthine-guanine phosphoribosyltransferase activity and heterozygous at the thymidine kinase locus, was a gift from W. G. Thilly. LCL cells were maintained at densities between 5×10^5 and 10^6 cells per ml with nutrient medium containing 10% fetal calf serum. They received 9,000 rad x-irradiation (^{137}Cs) to produce x-LCL cells for use as feeder cells.

Cultured T Cells. Mass T-cell cultures, initiated by inoculating single-donor mononuclear cells at 5×10^5 to 10^6 cells per ml into flasks containing growth medium, 15% serum, and 10^4 x-LCL cells per ml, were diluted with this medium at appropriate intervals to maintain densities of growing T cells between 10^5 and 2×10^6 cells per ml. For cloning in microtiter wells (limiting dilution, with or without selection), fresh, single-donor mononuclear cells were initially "primed" by culturing 10^6 mononuclear cells per ml for ≈45 hr under standard conditions in flasks containing 15% serum and 1% PHA-M; this was followed by cell dilution in growth medium to produce suspensions containing 1, 10, or 10^5 primed cells (test cells) per 100 μl.

Detection of Thioguanine-Resistant T Lymphocytes. The 96 wells (7-mm round or 6-mm flat bottom) of microtiter plates (Linbro) were initially inoculated with 100 μl of growth medium containing serum and 10^4 x-LCL feeder cells with or without thioguanine. Primed test cells were then added in 100-μl volumes so that wells received 1 or 10 test cells per well (without thioguanine) or 10^5 test cells per well (with 5×10^{-6} M thiogua-

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Abbreviations: HPRT, hypoxanthine-guanine phosphoribosyltransferase; LCL, lymphoblastoid cell line; PHA, phytohemagglutinin; M_f, mutant frequency; x-LCL, x-irradiated LCL; TPA, 12-tetradecanoylphorbol 13-acetate; Hxan, hypoxanthine; CE, cloning efficiency.

nine). Thus, the primed cells of a single donor were distributed into nonselection plates (without thioguanine) and selection plates (with thioguanine) for cloning efficiency (CE) and mutant frequency (M_f) determinations, respectively. Wells of some plates contained, in addition, 10^{-9} M 12-tetradecanoylphorbol 13-acetate (TPA).

Wells were monitored microscopically for cell growth. After 10 days, some wells' contents were transferred with Pasteur pipettes to larger 17 × 17 mm wells (Linbro) containing 1 or 2 ml of growth medium, 15% serum, and x-LCL feeders, with or without thioguanine, as appropriate. Cell growth was monitored and suspensions were diluted appropriately and transferred to larger vessels to maintain optimal cell densities. Some colonies, isolated in thioguanine, were split and portions were maintained with and without thioguanine.

Media were partially replaced (100 μl) at day 14 or 15 in some untransferred microtiter wells. At 15–16 days, all untransferred wells were labeled with 0.4 μCi of [^{3}H]dThd (1 Ci = 3.7×10^{10} becquerels). After 12–16 hr, well contents were collected with a harvester and the radioactivity was counted by scintillation spectrometry (10).

Detection of Cell Surface Markers. Surface antigens were detected by using mouse monoclonal antibodies (Ortho or Coulter); this was followed by using goat anti-mouse fluorescein isothiocyanate-labeled antibodies (Monoclonal Antibodies). Cells were scored by fluorescence microscopy; 200 or more cells were counted.

HPRT Activity Assay. The method was a modification of that described by DeMars and Held (11). Cultured T lymphocytes (≈2×10^6), washed twice with 0.15 M KCl and suspended in 100–200 μl of cold 0.01 M Tris buffer (pH 7.4), were disrupted by sonication. After centrifugation for 30 sec in a Microfuge (8,700 × *g*), diluted supernatants were added to reaction mixtures containing 0.1 M Tris buffer (pH 8.4), 5×10^{-2} M $MgSO_4$, 1.9×10^{-3} M hypoxanthine (Hxan), and 10^{-4} M [^{14}C]Hxan (5 μCi/ml), 10^{-2} M 5-phosphorylribose 1-pyrophosphate, and distilled water. The reaction was stopped with 4 M formic acid after 2 hr of incubation in a 37°C water bath. Enzymatic conversion of Hxan to inosine and IMP was determined by liquid scintillation determination of radioactivity in Whatman 3MM paper spots after ascending chromatography of the reaction mixture in 5% Na_2HPO_4. Conversion was calculated as: % conversion = (cpm inosine + cpm IMP)/(cpm Hxan + cpm inosine + cpm IMP) × 100. The protein content of cell extracts was determined by a modification of the Lowry procedure (12, 13).

RESULTS

Mass Cultures. Mass T-cell cultures showed early doubling times of 12–15 hr, allowing single-donor populations in excess of 10^9 cells to be developed. Thioguanine sensitivity was determined for cells from two single-donor cultures. Growing T cells, inoculated into 17 × 17 mm wells at an initial density of ≈10^5 cells per ml, were tested with media consisting of growth medium, 15% serum, and serial 1:10 dilutions of thioguanine. After 5 days, the cells of one single-donor culture were inhibited by 2×10^{-6} M thioguanine—the lowest concentration tested. Cells of the other culture were resistant at this concentration but were inhibited at the next concentration (2×10^{-5} M thioguanine), indicating a minimal inhibitory thioguanine concentration between 2×10^{-6} M and 2×10^{-5} M for these cells (data not shown). To maximize recovery of resistant cells while inhibiting normals, a concentration of 5×10^{-6} M thioguanine was chosen for selection experiments.

Cloning Assays for Thioguanine-Resistant Lymphocytes. The wells of microtiter plates were inoculated to receive an average of 1 or 10 primed single-donor cells (nonselection plates) or 10^5 primed cells (thioguanine plates) from the same individual. Wells were definitively scored as positive (containing dividing cells) by scintillation spectrometry as described. CEs and M_fs were calculated by assuming a Poisson distribution of cells with the ability to form clones in wells.

Sample results with primed cells from a single normal donor are given in Figs. 1, 2, and 3. A microtiter plate that had received one primed cell per well is depicted in Fig. 1 (plate 0), as is its companion, a thioguanine-containing plate that had received 10^5 primed cells per well (plate R). The cpm of [^{3}H]dThd incorporation into DNA in the wells of plate 0 were distributed for the 96 wells as shown in Fig. 2. The distribution is bimodal, with the majority of wells (69) having cpm < 1,200 and the minority (27) having cpm > 1,200 (range, 1,245–50,950 cpm). Wells with cpm > 1,200 were considered to be positive. Seventeen of these wells had previously been screened as positive by inspection. A CE (x) of 0.33 was calculated for the T lymphocytes of this individual: $P_0 = e^{-x} = (96 - 27)/96$; x = 0.33.

Twenty-four wells of plate R (Fig. 1, columns 1 through 3, all rows) were transferred blindly to 24 17 × 17 mm wells of plate R1 (Fig. 1). The remaining 72 wells (Fig. 1, columns 4 through 12, all rows) were scored by scintillation spectrometry exactly as for plate 0. Fig. 3 shows the bimodal distribution of cpm for these 72 wells. With 1,200 cpm indicative of positivity (from plate 0), 24 of the 72 wells were positive (range, 1,405–43,145) and 48 were negative. Assuming that the separation of the rare preexisting thioguanine-resistant T lymphocytes present in the original inocula approximates a Poisson

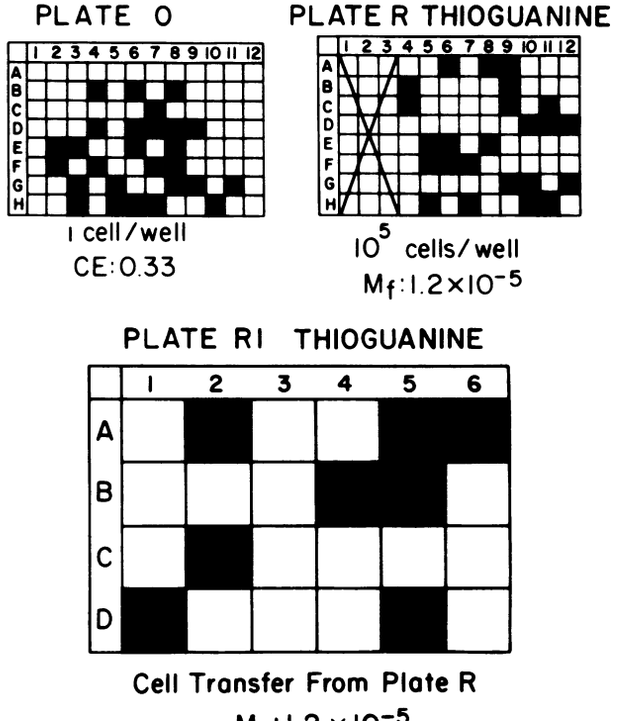

FIG. 1. Representation of microtiter plates inoculated in parallel with one primed mononuclear cell per well (plate 0) or 10^5 primed mononuclear cells per well (plate R) in growth medium/15% serum/10^{-9} M TPA and x-LCL feeder cells with (plate R) or without (plate 0) 5×10^{-6} M thioguanine. TPA was added to determine if it would enhance single T-cell cloning (14) or decrease possible metabolic cooperation (or both) (15). Media were partially replaced (100 μl) on day 14. The contents of 24 wells of plate R (columns 1 through 3, all rows) were transferred to the 24 thioguanine-containing wells of plate R1 (see text). Solid squares represent wells with growing colonies.

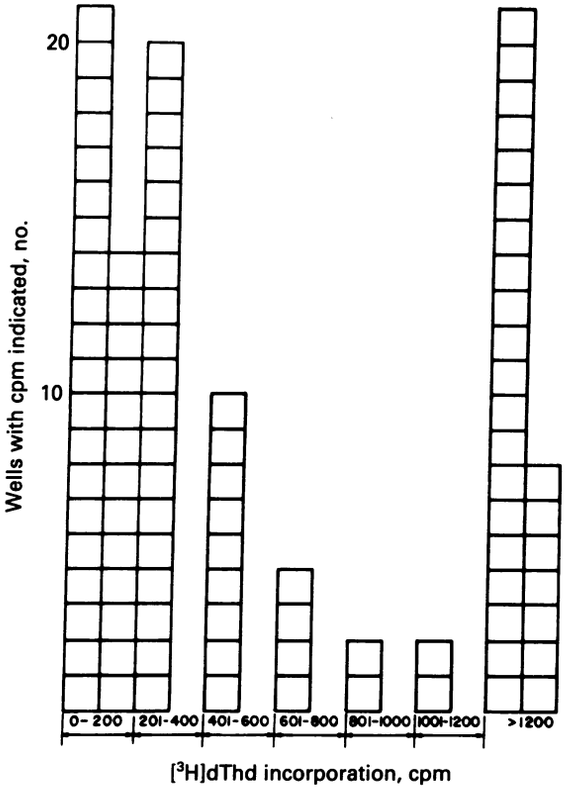

FIG. 2. Distribution of the cpm of [^{3}H]dThd incorporation into DNA among the 96 wells of plate 0 (Fig. 1).

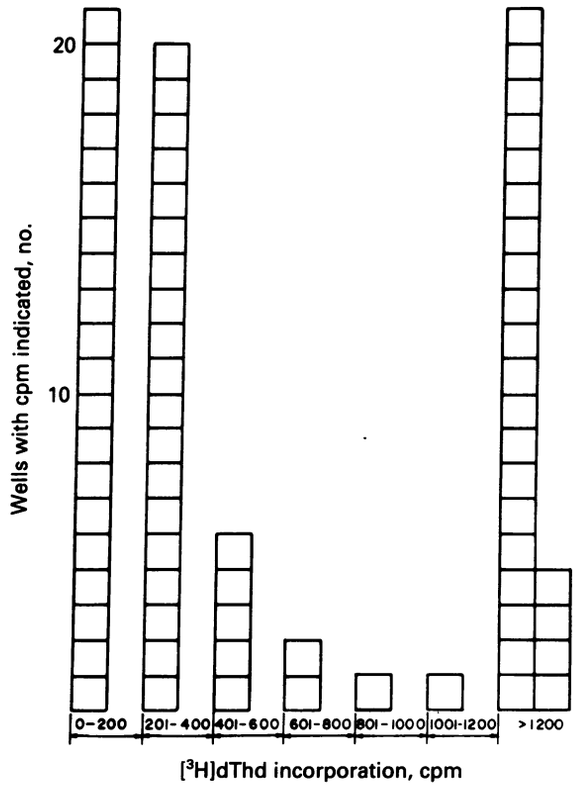

FIG. 3. Distribution of the cpm of [^{3}H]dThd incorporation into DNA among 72 wells of plate R (Fig. 1).

distribution among these 72 wells of plate R, a Poisson mean (x) of 0.405 thioguanine-resistant cells per well was calculated: $P_0 = e^{-x} = (72 - 24)/72$; $x = 0.405$. By using a CE of 0.33 (plate 0), a thioguanine-resistant T-lymphocyte M_f was determined from plates 0 and R: $M_f = 0.405/(0.33 \times 10^5) = 1.2 \times 10^{-5}$.

Eight of the 24 wells transferred to plate R1 were subsequently shown by clonal expansion to contain thioguanine-resistant growing T lymphocytes (Fig. 1). Making the assumptions indicated above allowed independent calculation of the thioguanine-resistant T-lymphocyte incidence (x) per well: $P_0 = e^{-x} = (24 - 8)/24$; $x = 0.405$. Again, the M_f was: $M_f = 0.405/(0.33 \times 10^5) = 1.2 \times 10^{-5}$.

These results were obtained in the context of a larger experiment that included eight sets of plates inoculated in parallel. For each set, M_fs were determined from scintillation spectrometry data and validated by cell transfer data. For six of the eight sets, the M_f, determined by these two methods, differed by <15% of their mean (data not shown). There appeared to be no effects of TPA that were independent of refeeding.

Characterization of Thioguanine-Resistant T-Cell Colonies. Growing T lymphocytes, isolated in selection experiments (as described) from nonselected colonies (initiated from 1 or 10 cells) or from thioguanine-selected (thioguanine resistant) colonies (initiated from 10^5 cells per well in 5×10^{-6} M thioguanine), were characterized as to (*i*) stability of the thioguanine resistance phenotype, (*ii*) HPRT activities, and (*iii*) lymphocyte surface markers. Some thioguanine-resistant colonies were split and portions were maintained in the presence (suffix TG) and absence of thioguanine.

Stability of the Thioguanine Resistance Phenotype. Four thioguanine-resistant colonies from two donors (A: male, age 39; B: female, age 46) were tested for stability *in vitro* of the resistance phenotype. Each colony was split and portions were maintained with and without thioguanine for 7 days prior to test (several doublings). Cells from these eight colonies and cells from two nonselected colonies were inoculated (5×10^4 cells per well) into 17 × 17 mm wells in growth medium and feeder cells, 15% serum, and serial 1:4 dilutions of thioguanine. After 8–9 days, all colonies initiated in thioguanine, regardless of donor or thioguanine maintenance, were completely resistant to 5×10^{-5} M and were partially resistant to 2×10^{-4} M thioguanine (Fig. 4, donor A colonies; data for donor B colonies not shown). By contrast, the two T-lymphocyte colonies initiated under nonselective conditions (representing parental cells) were completely inhibited by 3.1×10^{-6} M (Fig. 4) or 1.3×10^{-5} M (data not shown) thioguanine, with significant partial inhibition at lower concentrations.

HPRT Activities. HPRT activities were determined for cells of several "normal" (isolated under nonselective conditions) and thioguanine-resistant colonies initiated from either donor A or donor B. Several of the latter colonies were split and portions were maintained with (suffix TG) and without thioguanine as described above.

Table 1 shows that the two donor A colonies initiated under nonselective conditions had HPRT activities of 115 and 151 pmol of substrate converted per μg of protein per hr, respectively, and that the two donor B colonies, similarly isolated, had values of 153 and 144 pmol of substrate converted per μg of protein per hr, respectively. These represent parental, nonmutant values. By contrast, only one of the several thioguanine-resistant T-lymphocyte colonies isolated with thioguanine selection showed any HPRT activity and this was <15% of the parental value. The remaining nine independently maintained thioguanine-resistant colonies had no detectable HPRT activity. Thus, thioguanine-resistant T lymphocytes, maintained *in vitro* with or without thioguanine, are deficient in HPRT. As expected, the LCL cells were HPRT deficient.

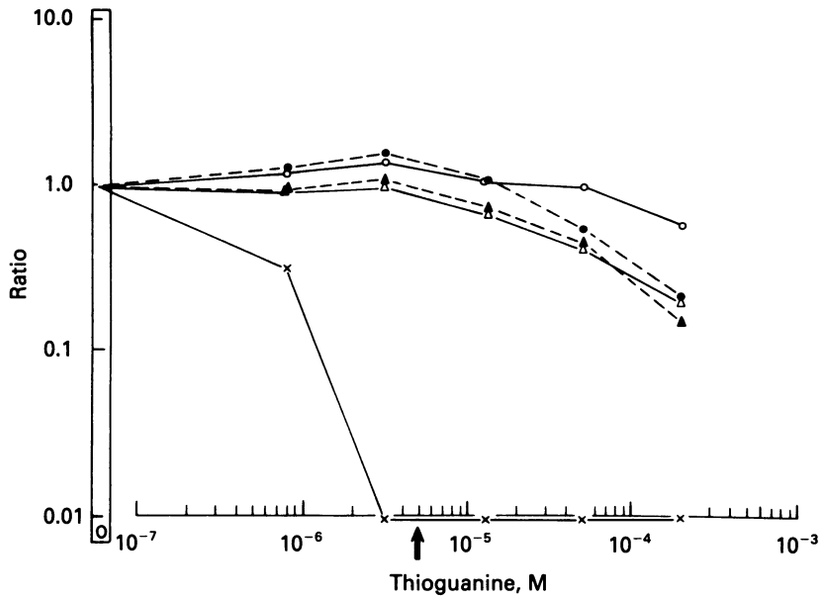

FIG. 4. Thioguanine sensitivities of donor A colonies. E1A4TG (●) and E1A4 (○), maintained *in vitro* with and without thioguanine, respectively, were derived from a single colony isolated under selective conditions (see text). Similarly, Q1A2TG (▲) and Q1A2 (△), maintained *in vitro* with and without thioguanine, respectively, were derived from a single colony isolated under selective conditions. Colony AF3 (×) was derived from a single colony (single-cell original inoculum) isolated under nonselective conditions. The original E1A4TG or E1A4 and AF3 isolates were from plates that had not contained 10^{-9} M TPA, whereas the original Q1A2TG or Q1A2 isolate was from a plate that had contained 10^{-9} M TPA. Arrow indicates the concentration of thioguanine used for selection experiments. Ratio = increase in cell number in thioguanine ÷ increase in cell number in growth medium.

Lymphocyte Surface Markers. Table 2 shows the results of lymphocyte surface-marker determinations. The nonselected T-lymphocyte colonies (initiated from single cells) were all T3-positive (a pan T-cell marker) as expected, with four being T4-positive, one being T8-positive, and one showing both the T4 and T8 markers. The latter colony most likely started from more than a single primed cell. The seven resistant colonies isolated in thioguanine were all T4-positive, T8-negative. The T3 marker was positive in the two instances tested. The Ia marker (indicating cell proliferation) was positive wherever tested. It was the only marker that was positive in LCL cells, demonstrating conclusively that these were not the thioguanine-resistant cells growing in thioguanine-containing plates, where x-LCL cells were present as feeder cells. The lymphocyte subpopulation composition of freshly isolated mononuclear cells from a single-donor platelet pack was within the expected range for adult peripheral blood.

Table 1. HPRT activity in cultured human T cells

	Donor	Maintenance*	HPRT specific activity†
Thioguanine-sensitive colonies			
AB10	A	NS	115
AF3	A	NS	151
HE3	B	NS	153
HE4	B	NS	144
Thioguanine-resistant colonies			
E1A4	A	NS	0
X1A4	B	NS	20
R1D1TG	A	S	0
R1D1	A	NS	0
Q1A2TG	A	S	0
Q1A2	A	NS	0
L1C5TG	B	S	0
L1C5	B	NS	0
Q1D5TG	A	S	0
R1D5TG	A	S	0
LCLs			0

Enzyme activity determinations were performed as indicated. Colonies with the same designations (e.g., R1D1) were isolated as single colonies and were subsequently split with portions maintained in 5 × 10^{-6} M thioguanine (suffix TG) and in the absence of thioguanine. AB10 and AF3 were initiated from single cells; HE3 and HE4 were from 10 cells. Thioguanine-resistant colonies were initiated from 10^5 cell inocula in thioguanine. Less than 5% substrate conversion (see text) is considered insignificant and is listed as 0.

* NS, nonselective; S, selective.

† Specific activity is given as pmol of [^{14}C]Hxan converted per μg of protein per hr.

DISCUSSION

The results presented here demonstrate that the thioguanine-resistant T lymphocytes of human peripheral blood have a stable phenotype that is associated with a deficiency of gene product—characteristics that traditionally have been used to define mutant somatic cells (3). However, "mutant" must be used in a broad sense because a stable epigenetic change leading to gene inactivation also may show such phenotypic characteristics (16).

Table 2. Lymphocyte surface-marker determinations

Sample	Marker* T3	T4	T8	Ia
Peripheral blood (adult normal range)	50–65	25–45	5–25	ND
Platelet pack: Mononuclear cell fraction (single donor)	50	32	24	12
Nonselected T-cell colonies				
A38 CA2	+	+	0	+
A38 CF9	+	0	+	+
A38 CG2	+	+	0	+
A38 FB4	+	+	+	+
A38 FC3	+	+	0	+
A38 FE6	+	+	0	+
Thioguanine-resistant T-cell colonies				
A55 GA2TG	+	+	0	ND
A55 GH9TG	+	+	0	ND
A79 L1C5TG	ND	+	0	ND
A79 L1C5	ND	+	0	ND
A79 Q1A2TG	ND	+	0	ND
A79 Q1A2	ND	+	0	ND
A79 Q1D5TG	ND	+	0	ND
Cultured LCLs: feeder cells	0	0	0	92

Lymphocyte surface markers were determined as indicated. A minimum of 200 cells was scored per determination.

* A + sign indicates that ≥99% of the cells showed the marker; numbers other than 0 indicate the percentage that showed <99% of the marker; 0 indicates absence of marker. ND, not done.

Nonetheless, with the availability of cultured T lymphocytes, the genetic mechanisms underlying thioguanine resistance and other phenotypic variations that occur in lymphocytes *in vivo* are open for investigation, as is possible heterogeneity of susceptibility to mutation among the T-cell subpopulations.

Obviously, it is critical to our interpretation of the results presented here that the mutant T lymphocytes that we isolated and studied *in vitro* actually arose *in vivo*. This conclusion seems justified for the following reasons: (*i*) There was little opportunity in selection experiments for mutations to occur and become fixed *in vitro* prior to thioguanine exposure. Priming in the absence of selection took ≤45 hr, allowing little or no cell division. Although ≈25–65% of T cells in PHA cultures after 72–96 hr are progeny of cells that have divided, there is little cell division prior to 40 hr (2, 17). (*ii*) For this same reason, there was virtually no opportunity for sufficient cell division *in vitro* prior to selection to allow mutants arising *in vitro* to overcome phenotypic lag. Cultured mammalian cells usually require several cell divisions to overcome phenotypic lag for thioguanine resistance; human B lymphoblasts can require as many as 16 divisions to optimally achieve this end (18). Rather than adding new mutants arising *in vitro*, it seems more likely that the method as described may miss very recent mutants arising *in vivo*. Direct mutagenicity testing will have to take account of this. (*iii*) Maximal cell growth, as judged by scintillation spectrometry data, was similar with or without thioguanine selection (maximal cpm, 43,145 vs. 50,950, respectively). This would be expected only if single cells began proliferating at similar times under the two conditions. It would not be expected if proliferation in the thioguanine-containing wells had to await mutation, fixation, and dilution of preexisting HPRT. (*iv*) We recently have recovered thioguanine-resistant T lymphocytes from "unprimed" fresh mononuclear cells inoculated immediately into thioguanine-containing wells (10^{-5} M thioguanine; 10^5 cells per well; data not shown). Unprimed cells spent virtually no time *in vitro* in the absence of thioguanine, are practically all in an arrested G_0 stage of the cell cycle, and must undergo an initial PHA- (present in our crude T-cell growth factor) induced G_0 to G_1 transformation with the acquisition of growth factor receptors in order to grow (19). This transformation step occurs *before* DNA synthesis (19) and appears to be the step inhibited by 2×10^{-4} M thioguanine in sensitive cells in the autoradiographic assay (20). It is difficult to see how clonal growth can occur in thioguanine unless resistance antedates transformation.

The cloning method, as presented here, is an approach being developed for human direct mutagenicity testing. It needs refinement before use in quantitative studies. For example, CE determinations made with a single donor's cells may differ if determined by a single-cell or by a 10-cell initial inoculum—i.e., the CE estimates are often lower with the higher inoculum. Because this difference may be small or absent for CEs < 10%, it may reflect simply the different culture conditions in microtiter wells containing different numbers of growing cells. With regard to culture conditions, we have not determined the extent to which replacement of medium is important in selection experiments. Also, the optimal thioguanine concentration to use for selection remains to be defined. In recent experiments, we have used a higher concentration than was used here (10^{-5} M). Finally, any advantages of using low concentrations of TPA remain to be determined.

The ability to perform mutagenicity studies with human T lymphocytes *in vitro* generalizes the potential use of the cells for human mutagenicity testing. The spontaneous mutation rate at the HPRT locus in these cells, as well as the expression curve characteristics for thioguanine resistance, may be assesed by established *in vitro* procedures to determine definitively the extent to which mutation *in vitro* influences *in vivo* M_fs determined as described. Furthermore, combined *in vivo–in vitro* test systems can be developed with cultured T lymphocytes, allowing human population heterogenicity with regard to mutability or mutagen sensitivity (or both) to be investigated. The problem of "phenocopies"—as seen with the autoradiographic assay for variant lymphocytes (20)—is obviated by the ability to propagate T lymphocytes *in vitro*. Actually, the autoradiographic method can be used more confidently for human mutagenicity monitoring if an outside reference for "true" variant frequencies is thus made available. Finally, mutagenicity studies with cultured T lymphocytes need not be limited to mutation at the HPRT locus. Rather, a multiplicity of markers may be developed with the goal of devising a multilocus system capable of human direct mutagenicity testing.

This work was supported in part by National Cancer Institute Grant CA 30688-01 (to R.J.A.) and was performed during sabbatical leave in the laboratory of Dr. Richard Hong, University of Wisconsin Clinical Science Center, Madison.

1. Albertini, R. J. (1980) *Teratog. Carcinog. Mutagen.* **1**, 25–48.
2. Strauss, G. H. & Albertini, R. J. (1979) *Mutat. Res.* **61**, 353–379.
3. Chu, E. H. Y. & Powell, S. S. (1976) *Adv. Hum. Genet.* **7**, 189–258.
4. Lesch, M. & Nyhan, W. L. (1964) *Am. J. Med.* **36**, 561–570.
5. Seegmiller, J. E., Rosenbloom, F. M. & Kelley, W. N. (1967) *Science* **155**, 1682–1684.
6. DeMars, R. (1971) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **30**, 944–955.
7. Paul, W. E., Sredni, B. & Schwartz, R. H. (1982) *Nature (London)* **294**, 697–699.
8. Nusbacher, J., Scher, M. L. & MacPherson, J. L. (1977) *Vox. Sang.* **33**, 9–15.
9. Inouye, H., Hank, J. A., Alter, B. J. & Bach, F. H. (1980) *Scand. J. Immunol.* **12**, 149–154.
10. Hartzman, R. J., Bach, M. L., Bach, F. H., Thurman, G. & Sell, K. G. (1972) *Cell. Immunol.* **4**, 182–187.
11. DeMars, R. & Held, K. R. (1972) *Humangenetik* **96**, 87–110.
12. Oyama, V. I. & Eagle, H. (1956) *Proc. Soc. Exp. Biol. Med.* **91**, 305–307.
13. Lowry, D. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
14. Singer, J. W., Ernst, C., Whalen, C. K., Steinmann, L. & Fialkow, P. J. (1981) *J. Immunol.* **126**, 1390–1392.
15. Warren, S. T., Yotti, L. P., Moskal, J. R., Chang, C. C. & Trosko, J. E. (1981) *Exp. Cell Res.* **131**, 427–430.
16. DeMars, R. (1974) *Mutat. Res.* **24**, 335–364.
17. Soren, L. (1973) *Exp. Cell Res.* **78**, 201–208.
18. Thilly, W. G., DeLuca, J. G., Hoppe, H., IV, & Penman, B. W. (1978) *Mutat. Res.* **50**, 137–144.
19. Maizel, A. L., Mehta, S. R., Hauft, S., Franzine, D., Lachman, L. B. & Ford, R. J. (1981) *J. Immunol.* **127**, 1058–1064.
20. Albertini, R. J., Allen, E. F., Quinn, A. S. & Albertini, M. R. (1981) in *Population and Biological Aspects of Human Mutation*, Birth Defects Institute Symposium 11, eds. Hook, E. B. & Porter, I. H. (Academic, New York), pp. 235–263.