Persistence of Phosphoglucomutase (PGM) Polymorphism in Long-Term Lymphoid Lines[1] (34557)

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(Introduced by H. L. Hodes)

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It is now possible to establish long-term lymphoid cell lines from peripheral blood of patients with a wide variety of benign and malignant lymphoproliferative states (1–4). Such lines have been established in our laboratory and maintained as continuous suspension cultures for nearly 2 years. In each instance, the cells display a lymphocyte morphology (5), synthesize immunoglobulins (6), and readily phagocytize particulates from the culture environment (7). In addition, all of these lines retain an essentially normal diploid chromosome complement of 46. In contrast, the 20 established human cell lines examined by Gartler (8) were heteroploid and were maintained as monolayer cultures. All of these heteroploid lines were found to display the homozygous (or hemizygous) PGM_1 1 phenotype and the "A" variety of glucose-6-phosphate dehydrogenase (G6PD) upon electrophoretic screening. The "A" isozyme of G6PD has not been found in Caucasians. Gartler (8, 9) reasoned that since HeLa (PGM_1 1, and G6PD-A), derived from a Negro, was the first heteroploid line isolated (10) and is widely distributed (11), it was most likely that the heteroploid lines examined were contaminated with HeLa, giving rise to identical enzyme types in all these lines.

We decided to examine our established diploid lymphoid cell lines for their PGM type as a measure of their genetic variation. Also, follow-up studies, using red cell enzyme content of peripheral blood samples from cell line donors were performed, to evaluate persistence of PGM phenotypes in the cell lines. Since PGM_3 (third locus) has never been reported in red blood cells, our comparison of PGM phenotypes of cell lines and donors would only be valid for the first two loci of PGM activity. It is recognized, however, that a comparison of all three PGM loci would be possible if the donor material were skin fibroblasts.

In the electrophoretic system of Spencer *et al.* (12), up to seven bands of enzyme activity can commonly be found representing the first two PGM loci. They have been designated by the letters a–g, starting with the slowest (most cathodal) band. The second locus is represented by the three bands e–g, and variants here are very rare (exception to this would be the pygmy population) (13). Enzymes from homozygotes at the first locus will have two additional bands, a and c (PGM_1 1-1), or b and d, PGM_1 2-2. Heterozygotes at this locus will have the four bands a–d (PGM_1 2-1), as well as the three bands of the second locus (see Fig. 1).

Materials and Methods. Several human, long-term diploid cell lines were used (Table I). These included AL-1 (derived from a patient with Burkitt's tumor) (14); LK-1D (derived from a patient with lymphoblastic leukemia) (15); IM-1 (derived from a patient with lymphosarcoma) (16); 14 cell lines isolated from nine patients with heterophile-positive infectious mononucleosis (7, 17); 3 cell lines isolated from a family (three patients) displaying homozygous and heterozygous forms of Chediak-Higashi syndrome (3); 6 cell lines derived from six patients with mumps, measles, herpes simplex, and

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TABLE I. PGM phenotypes in long-term lymphoid lines.

Cell line[a]	Disease	PGM_1 type	Age in continuous culture (months)
PGLC-33H	Infectious mononucleosis	2-1	28
PGLC-42D		1-1	26
PGLC-42F		1-1	26
PGLC-44B		2-1	25
PGLC-50B		1-1	24
PGLC-51D		1-1	24
PGLC-51E		1-1	22
PGLC-53B		1-1	24
PGLC-53D		1-1	23
PGLC-54B		1-1	23
PGLC-55B		1-1	23
PGLC-55E		1-1	22
PGLC-55F		1-1	21
PGLC-56B		2-1	23
IM-1	Lymphosarcoma	1-1	20
LK-1D	Acute lymphoblastic leukemia	1-1	36
AL-1	American Burkitt lymphoma	2-1	36
GCB-1	Homozygous Chediak-Higashi	1-1	22
GCB-2	Heterozygous Chediak-Higashi	1-1	22
GCS-1	Chronic leukocytosis	2-2	22
GCS-2	Chronic leukocytosis	1-1	33
GCS-4	Heterozygous Chediak-Higashi	1-1	18
PGIP-1	Herpes zoster	1-1	12
PGIP-3	Mumps	1-1	12
PGIP-7	Herpes simplex	1-1	12
PGIP-8A	Herpes zoster	1-1	12
PGIP-6	Measles	1-1	12
PGIP-4	Herpes zoster	1-1	12

[a] Cell lines, isolated from the same individual (or the same family) at different times during the course of an illness, have the same number but different letter coding.

herpes zoster (2); and 2 cell lines isolated from two patients with chronic leukocytosis (which display cytogenetic instability) (18).

Cells were grown as suspension cultures in RPMI-1640 medium, supplemented with 20 % fetal calf serum, penicillin and streptomycin. The cultures were maintained by regularly transferring into fresh medium at 3 to 4-day intervals. Cultures used for enzyme studies were fed the preceding day. The majority of these cell lines have been in continuous culture for at least 1 year. Cultures handled in this fashion displayed greater than 90 % viability as evidenced by trypan blue staining.

The cells were isolated by centrifugation at 250*g* for 15 min. Cell pellets, containing 3 to

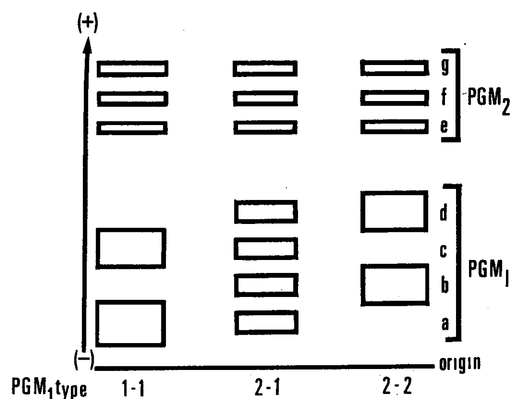

FIG. 1. Diagram of a starch-gel human PGM electrophoretic pattern demonstrating the three most common phenotypes of the first locus PGM_1. Isozymes e–g are determined by the PGM_2 allele of the PGM_2 locus (12).

5×10^7 cells, were then washed three times in physiological saline. Cells were finally resuspended in an equal volume of saline and freeze-thawed six to seven times in an ethanol-Dry Ice mixture. The cell suspensions were then centrifuged, and the enzyme active supernatant removed for electrophoresis. Red blood cells were isolated from cell line donor peripheral blood samples by centrifugation. Red cells were then washed three times in isotonic saline and lysed in a manner identical to that described for the suspension cultures.

Horizontal starch-gel electrophoresis was carried out according to the system previously described for a 17-hr duration at 5.5 V/cm (12). Gel cooling during electrophoresis was accomplished by using metal cooling plates appropriately insulated with plastic bags and applied to the top and bottom surfaces of the gel. Ethylene glycol-water mixture at a temperature of 4° was circulated through the cooling plates. Whatman No. 17 filter paper inserts were used to apply samples into the gel.

After electrophoresis, the gels were cut horizontally and stained according to Hopkinson and Harris (19). Characterization of PGM_1 and PGM_2 components was possible after 20 to 30 min (in the dark) at 37°. Phenotypic identification was done with reference to Spencer *et al.* (12).

Results. The result of PGM_1 typing of the various lines examined is listed in Table I.

Eleven of the 14 infectious mononucleosis cell lines (from six individuals), displayed the homozygous PGM_1 1-1 phenotype for the first locus. The lymphosarcoma, acute lymphoblastic leukemia, homozygous Chediak-Higashi, both heterozygous Chediak-Higashi, all three herpes zoster, the mumps, herpes simplex, measles, and one chronic leukocytosis lines also proved to be homozygous PGM_1 1-1.

Three of the infectious mononucleosis cell lines (from three patients) exhibited a heterozygous PGM_1 2-1 phenotype.

The only incidence of the homozygous PGM_1 2-2 variety was in one patient with chronic leukocytosis (GCS-1).

TABLE II. Correlation of PGM types in cell line donors.

Cell line[a]	PGM_1 type	Donor RBC PGM_1 type
CGS-1	2-2	2-2
PGIP-1	1-1	1-1
PGLC-42D	1-1	
PGLC-42F	1-1	1-1

[a] PGLC-42D and PGLC-42F lines were derived from the same patient during the same illness.

All lines demonstrated PGM_2 1-1 phenotype for the second locus.

Follow-up studies on three cell line donors, who were available, were performed utilizing their red cell enzyme as a measure of their current PGM status. These results, given in Table II, show no change in PGM type for the donors of PGLC-42D and PGLC-42F (both from one donor), PGIP-1, and GCS-1.

Discussion. Only a small number of cell line donors were available for comparison, but in each instance phenotypic persistence of PGM_1 phenotype was demonstrated. Since PGM_1 2-1 expresses itself in some of the established lines which are diploid, it seems most likely the PGM_1 1 phenotypes represent a homozygous, not hemizygous, genotype. The apparent increase in the PGM_1^1 gene frequency is most likely explained by sampling error. However, since all three common PGM_1 phenotypes were seen among the 28 lymphoid lines, the possibility of any cross-contamination seems remote.

Four individuals who were selected for repeated culture were all of the homozygous PGM_1 1-1 type. These lines upon repeated checking were genetically stable with respect to ploidy and PGM_1 phenotype. The persistence and identity to the donor is indicative of the genetic stability of these lines. This genetic stability would be most useful for future studies, such as somatic cell hybridization. In addition, it seems likely that the viral etiology of the PGIP-1, PGLC-42D, and PGLC-42F cell lines does not involve any genotypic alteration of the PGM loci in question.

Since PGM_1 1-1, 2-1, and 2-2 phenotypes were demonstrated in these diploid lines, es-

tablishment here cannot be due to any cross-contamination by a common cell type, as is alleged to have occurred from HeLa cells in the permanent heteroploid lines examined by Gartler (9). Nor can establishment of our diploid suspension cell lines be viewed as selecting a PGM_1 1-1 phenotype or a PGM_1^1 allele, as many have happened in the established monolayer heteroploid lines. In addition, establishment does not necessarily imply any alteration in ploidy, as all of our lines are diploid and have been carried from one to two years in continuous culture. It is considered by Hayflick (20), that normal diploid cells can only undergo a restricted number of cell divisions, and, therefore that any attempt at continuous culture would fail unless spontaneous or induced transformation occurred.

Summary. Long-term lymphoid lines are useful in studying the relationship of an individual's genotype *in vivo* and *in vitro*. The persistence of the PGM_1 phenotype in these established lines serves as a consistent, reliable genetic marker which can be used in a variety of studies and be related directly back to the cell line donor. This experimental advantage was heretofore not available in established heteroploid monolayer lines, or in diploid fibroblasts, which are not capable of continuous vigorous growth beyond a finite number of generations.

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