

Clonal Stability of Blood Cell Lineages Indicated by X-Chromosomal Transcriptional Polymorphism

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Summary

The idea that stem cells oscillate between a state of activity and dormancy, thereby giving rise to differentiating progeny either randomly or in orderly clonal succession, has important implications for understanding normal hematopoiesis and blood cell dyscrasias. The degree of clonal stability in individuals also has practical implications for the evaluation of clonal lymphomyeloproliferative diseases. To evaluate the clonality pattern of the different types of blood cells as a function of time we have validated the applicability, sensitivity, and reproducibility of a thermostable ligase reaction to detect transcripts of the G6PD allele on the active X-chromosome in normal heterozygous females. While the ratio of the two X-chromosome-derived allelic transcripts varied widely among hemopoietic and nonhemopoietic tissues in a given individual, this allelic ratio was virtually identical in all types of mature myeloid and lymphoid cells. Longitudinal studies indicated constancy of the G6PD allelic ratio in blood cells over a 912-d period of observation in healthy females. The individual variability observed in this allelic ratio suggests that the progeny of a relatively small number of original embryonic hemopoietic stem cells, approximately eight, contribute to the sustained production of all types of blood cells in healthy individuals.

One view of hematopoietic stem cell differentiation holds that all of the stem cells in an individual contribute continuously to hematopoiesis throughout life (1). An alternate theory of clonal succession suggests that hematopoiesis is maintained by an active subset of stem cells with dormant stem cells in reserve. As the active clones are exhausted, possibly through terminal differentiation, a proportion of the dormant reserve cells becomes active to maintain hematopoiesis until they also become depleted, and the process repeats itself.

The serial evaluation of hematopoiesis presented here is based on a clonal assay identifying the active X chromosome. Early in development, one X chromosome in each primordial cells of female embryo is inactivated (2). The molecular events leading to X-chromosomal inactivation are not fully understood, but it is known that some X-chromosomal genes escape the inactivation process which is initiated by the Xi-specific transcripts (XIST) gene in the inactivation center (2). The polymorphism of consistently inactivated genes, such as the glucose-6-phosphate

dehydrogenase gene (G6PD),¹ can be used for clonality studies. The clonality of functional stem cells can thus be determined by transcriptional analysis of the active X chromosome in females heterozygous for an X chromosome exonic polymorphism. A common C/T polymorphism is present in G6PD in exon 11 at position 1311 (3, 4). This polymorphism does not change the peptide sequence and is therefore silent. The G6PD polymorphism is present in all races and ethnic groups, suggesting an ancient origin of this mutation.

We have developed a method for detection of this polymorphism by a ligase detection reaction (LDR) that is based on the ability of a thermostable ligase to covalently bind with DNA oligonucleotides only when they are perfectly complementary to the target DNA in the ligation region (5–8). The polymorphism is detected in this PCR-based assay by the ligation product of allele-specific oligo-

¹Abbreviations used in this paper: G6PD, glucose-6-phosphate dehydrogenase gene; LDR, ligase detection reaction.

nucleotide primers of different lengths that are ligated to the 5' common radiolabeled oligonucleotide. This sensitive assay was used to examine the active G6PD alleles in the different populations of blood cells in females heterozygous for this polymorphism. Our earlier studies indicated that the usage ratio for the two X-chromosome alleles varies widely in samples of different tissues in a heterozygous female, while the different types of hematopoietic cells may share the same allelic ratio (5, 6).

In the present study serial determinations of the C/T allele ratios of the different lineages of peripheral blood cells were made to examine the issue of relative clonal stability of hematopoietic cells in normal females. These data also allowed estimation of the number of founder hematopoietic stem cells in females after embryonic X-chromosomal inactivation.

Materials and Methods

Study Group, Isolation of Blood Cells and RNA Preparation. Peripheral blood samples were collected from healthy volunteers after obtaining informed consent. EDTA anti-coagulated blood (5 ml) was obtained for preparation of myeloid cells and heparinized blood (5 ml) was used for mononuclear cell preparation. Myeloid populations (granulocytes, reticulocytes, platelets) were separated by differential centrifugation, isopyknic density gradient separation, and dextran sedimentation using standard protocols (9). Mononuclear cells were purified further by immunofluorescence-directed sorting of the nonadherent mononuclear population into CD3⁺ T lymphocytes, CD19⁺ B lymphocytes, CD56⁺ NK cells and CD14⁺ monocytes using a FACS[®] Star plus (Becton Dickinson and Co., San Jose, CA). Reticulocytes were lysed in NH₄HCO₃/NH₄Cl buffer, followed by acid precipitation of RNA (10) that was further purified by guanidium thiocyanate extraction. Leukocyte and platelet RNAs were prepared by acid guanidium thiocyanate extraction using standard protocols (11).

Transcriptional Analysis of the Active X-Chromosome. The LDR analysis of G6PD transcripts was performed using genomic DNA or cDNA templates prepared by the reverse transcriptase PCR from total cellular RNA (12). The initial analysis of samples obtained over the first 360 d used previously described experimental conditions (5, 6). When additional analyses of clonality encompassing the entire 912-d interval were performed at 580 and 912 d, repeat analyses of stored RNA aliquots of the day 0 sample were included. These analyses used the GeneAmp PCR System 9600 (Perkin-Elmer Corp., Norwalk, CT) and modification of the PCR conditions for amplification of cDNA fragments to include denaturation at 94°C for 5 min before the first cycle, then primer annealing at 60°C for 30 s, extension at 72°C for 30 s, and denaturation for 30 s at 94°C. After completion of 30 cycles the sample was subjected to a final 6-min incubation at 72°C. After the first amplification with external primers, 1 µl of the first amplification reaction mixture was used as the template for the second round of amplification with an identical PCR program. To evaluate the amplification products, 10 µl of the reaction mixture was withdrawn and the sample electrophoresed in a 1% agarose gel.

Three oligonucleotides were used for the LDR: a 23-mer with a 3'-G-ending specific for the C allele, a 25-mer with 3'-A-ending for the T allele, and a 21-mer labeled at the 5' end by T4 kinase as an adjacent oligonucleotide ligating with both allele-

specific oligonucleotides. The reaction conditions allowed detection of both alleles using either PCR amplified genomic DNA or cDNA as the templates. The T allele was detected by a 46-nucleotide fusion product and the C allele by a 44-nucleotide product, as described (5, 6). The radiolabeled reaction products were separated on 15% polyacrylamide gel containing 7 M urea and identified by autoradiography. The ratio of C and T alleles was determined by using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Study Design and Statistical Analysis. Relative stability of the C/T ratios was determined by examining the differences between two time intervals (e.g., day 180 minus day 0) using the (one-sample) Wilcoxon signed rank test. Separate analyses were performed for isolated samples of each cell type. Since the C/T ratio proved to be virtually identical for the different types of blood cells in each subject, the primary analysis provided the mean of the differences between the cell types for each subject. The measurements for each of the different blood cell types in a given individual at a given time were used for calculation of the standard error of measurement.

For estimation of the number of embryonic stem cells generated after X-chromosomal inactivation, the probability of each C/T heterozygous cell choosing to express the C allelic transcript was assumed to be random (1 in 2), and the proportion of cells expressing the C allele was assumed to be stable after X-chromosome inactivation given that our experimental data indicated constancy of the proportion of C allelic cells within the reticulocyte, platelet, granulocyte, and mononuclear cell populations over the period of observation. If K is used to denote the number of stem cells present at the time of X-chromosome inactivation, the percentage of active C allelic cells at the time of X-chromosome inactivation would have a mean 1/2 and variance 1/4K. It is reasonable to assume that the percentage of active C allelic cells from a heterozygous subject is approximately normally distributed with mean 1/2 and variance $\tau^2 = 1/4K$. This variance formula $\tau^2 = 1/4K$ was used to estimate the number of stem cells at the time of X-chromosome inactivation. Allowing for possible minor bias of the allele detection technique and measurement errors, the measured percentage of C allelic cells in a components-of-variance model (13, 14) could be used to obtain an estimate and confidence intervals for τ^2 , and to determine the value for K. The test for changes in the proportion of C allele usage over time using Wilks' Lambda is as described by Rao (13), analysis of variance by the analysis of variance program (13), components of the variance model described by Graybill (15) and signed rank test by standard methods (16).

Results

Relative Frequency of G6PD C/T Heterozygosity. Genomic DNA samples from 117 female individuals of diverse ethnic backgrounds were analyzed to determine the heterozygote frequency for the G6PD C/T polymorphism. 49 were healthy volunteers. The remainder were patients and their relatives who were examined for possible acquired or congenital hematological disorders. In this ethnically diverse group of individuals, 91 had only the C allele, 5 had the T allele, and 21 had both alleles. Heterozygous individuals were found in all of the different ethnic groups examined: Caucasian, African-American, Ashkenazi-Jewish, Chinese, and Vietnamese. The observed gene frequen-

cies of these alleles were 78% C, 18% C/T, and 4% T. The C allele frequency in this analysis of 234 X-chromosomes from widely diverse ethnic subjects is comparable to that found by two other laboratories that analyzed 208 and 36 X-chromosomes, respectively (3, 17).

Reproducibility and Relative Sensitivity of the G6PD Allele Measurement. To estimate the reproducibility of the assay for G6PD allelic usage, five aliquots of reticulocyte RNA from a heterozygous female were processed separately for first-strand cDNA synthesis, PCR amplified, and the ratio of G6PD allelic transcripts revealed by measurement of the LDR products by autoradiographic densitometry. The five independent measurements yielded a mean of 72.16% with a 2.06% SD of measurement error, thus indicating excellent reproducibility of this LDR assay.

When genomic DNA from 10 females heterozygous for the G6PD exon 11 C/T polymorphism was analyzed by the LDR assay, a minor bias of genomic DNA was detected in favor of the C allele: 54.9% with an SD of 2.4%.

G-6-PD Allelic Usage by Different Types of Blood Cells. 49 female volunteers were screened for heterozygosity of G6PD alleles. The heterozygous state was found in 10 healthy individuals, who volunteered for further study. For each of these heterozygous females, remarkably consistent C/T ratios were observed in preparations of their reticulocytes, platelets, granulocytes, and PBMC (Fig. 1 and Table 1). Blood mononuclear cells from five of the healthy subjects were further purified into monocytes, B, T, and NK lymphocyte preparations, the transcript C/T ratios of which closely paralleled those observed for the myeloid cell lineages (Table 1, individuals 2, 6, 7, 8, 10). Notably, however, the fixed ratio for the different blood cell types in an individual varied widely between the different heterozygous individuals. The ratios of C and T allelic transcripts ranged from 2.2:7.8 to 8.5:1.5 or from 21 to 87%, when expressed as proportion of C allele (Table 1).

Evaluation of the Relative Stability in G6PD Allelic Distribution among Hematopoietic Cells. The C/T transcript ratios in different myeloid and lymphoid cells were determined for two to six of the heterozygous subjects (based on the subjects' availability) at 0, 180, 360, 580, and 912 d. The analyses performed over the first 360 d (Table 2) indicated the stability of the C/T transcript ratios throughout this period of observation. Changes in the C/T ratio at the different follow-up times were also tested separately for each cell type, and none was found to be significant at the 0.05 level in a two-sided signed rank test (15). Analyses encompassing the entire 912-d span (with additional timepoints of 580

Table 1. The ratio of G6PD Allelic Transcripts Is Relatively Invariant for Different Blood Cell Types in a Healthy Heterozygous Female, But Is Highly Variable between Individuals

Heterozygous females	Blood cell types*							
	R	P	G	M	Mo	B	T	NK
1	49	44	51	53				
	±2.5	±1.2	±2.2	±3.0				
2	59	51	52	59	58	64	59	62
	±3.5	±0.7	±1.5	±2.3				
3	84	84	84	84				
	±2.2	±1.85	±1.8	±2.1				
4	76	78	78	78				
	±0.9	±1.2	±1.2	±1.2				
5	66	73	75	75				
	±1.8	±1.2	±1.0	±0.6				
6	85	84	85	84	87	85	81	80
	±0	±0	±0.5	±1.0				
7	61	68	70	70	66	70	72	76
8	54	60	56	57	57	60	58	54
9	21	21	30	29				
10	72	68	71	68	68	71	67	71

*The cells isolated for transcript analysis as described in Materials and Methods, were: R, reticulocytes; P, platelets; G, granulocytes; M, mononuclear cells; Mo, monocytes; B, B lymphocytes; T, T lymphocytes; NK, NK cells. Data expressed as percentage of the C allele usage ± standard error of values obtained at three different time intervals (see Table 2). Monocytes and lymphocytes of subjects 2 and 6 and all of the cells from subjects 7–10 were analyzed only on one occasion.

and 912 d) used different PCR equipment and a slightly different program (see Materials and Methods), and these results are shown in Table 2, in parentheses.

The data indicate remarkable stability of the C/T ratios in the myeloid cell lineages, reticulocytes, platelets, granulocytes, and monocytes, for the entire 912-d of follow-up. To determine whether the constancy of the C/T ratios holds true for lymphoid cells also, we used immunofluorescence cell sorting to separate mononuclear cell preparations from individuals 2 and 6 into pure monocyte, B, T, and NK cell fractions (Table 1, and Figs. 1 and 2). The same C/T ratio was observed among all lymphoid types and monocytes and these ratios were constant at 550 d when compared to day 0 (Fig. 2). The C/T transcript ratios in lymphoid cells were found to parallel those observed for myeloid cells.

Estimation of the Hemopoietic Stem Cell Number at the Time of X-Chromosome Inactivation. Based on the differences observed in the ratio of the X-chromosome C/T allelic transcripts among normal heterozygous females, the number of the progenitor cells at the time of X-chromosome inactivation would intuitively appear to be relatively small, since a large number of stem cells would result in the pres-

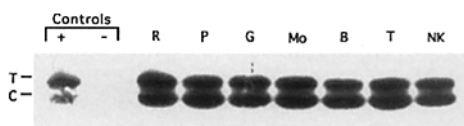


Figure 1. Ligase detection analysis of circulating blood cells. Ligase detection analysis of reverse transcribed total cellular RNA from reticulocytes (R), platelets (P), granulocytes (G), monocytes (Mo), B lymphocytes (B), T lymphocytes (T), NK cells (NK) in individual 8. The 46-bp product corresponds to T allele and the 44-bp product to C allele.

Table 2. Frequency Analysis of G6PD C Allele Transcripts in Blood Cells of Heterozygous Females (C/T)

Heterozygous females	Day	Blood cell types			
		R	P	G	M
1	0	51 (48)	45 (39)	48	47
	180	44 (45)	46 (49)	55	54
	360	52 (34)	42 (39)	49	57
	580	(38)	(41)		
2	0	63	52 (45)	55 (49)	58
	180	62	50 (43)	52 (48)	55
	360	52	50 (42)	50 (46)	63
	580		(43)	(44)	
	912		(47)	(41)	
3	0	87 (76)	85 (76)	86	87
	180	86 (76)	87 (76)	85	85
	360	80 (78)	81 (79)	82	80
	580	(76)	(80)		
4	0	77	80	80	76
	180	76	78	78	78
	360	74	76	76	80
5	0	69	71	73	75
	180	67	73	76	76
	360	63	75	76	74
6	0	85 (72)	84 (74)	84 (84)	83
	180	85 (76)	84 (75)	85	85
	360	(70)	(72)	85	85
	580	(70)	(72)		
	912		(71)	(83)	

The ratio of G6PD allelic transcripts in blood cells remains constant in heterozygous individuals over 360-, 580-, and 912-d intervals. The numbers represent G6PD allelic transcripts expressed as percentage of the C allele. Stability of the C/T transcript ratios throughout 360-d period of observation was observed, on the basis of insignificant differences between individual timepoints: the two-sided P value for the interval of 180 vs 0 d is 0.875, that for 360 vs 0 d is 0.25, that for 360 vs 180 d is 0.125. Numbers in parentheses represent analysis performed at 580 d and 912 d, using different PCR conditions. Fresh cells were used as a starting material at these days, while stored aliquots of RNA were used on days 0, 180, and 360. The individuals were chosen based on their availability.

ence of equal proportions of both allelic transcripts (assuming a random mode of X-chromosome inactivation). On the other hand, if only a very small number of stem cells existed, such as two or three, one would frequently observe a single allelic transcript due to the chance selection of the same allele by a limited number of stem cells. Our data argue against either of these extremes. When calculations were performed using the mathematical model described in Materials and Methods, the number of pluripotent hemopoietic stem cells present in the female embryo at the time of X-chromosome inactivation was estimated to be

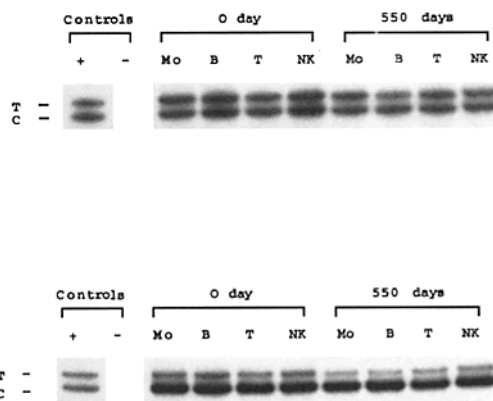


Figure 2. Stability of G6PD C/T allelic ratios in mononuclear cells. Ligate detection analysis of reverse-transcribed total cellular RNA from monocytes (Mo), B lymphocytes (B), T lymphocytes (T), NK cells (NK) in two individuals at days 0 and 550 (subjects 2 and 6). The day 0 C/T allelic ratios (C%) for individual 2 (top) were 49, 50, 51, and 51 for Mo, B, T, and NK cells, and 50, 55, 49, and 51, respectively, for day 550. The day 0 C/T ratios (C%) for individual 6 (bottom) were 69, 68, 72, and 70 for Mo, B, T, and NK cells, and 76, 77, 73, and 72, respectively, for day 550.

between seven and eight (80% confidence interval for 3 to 14 cells and a 90% confidence interval for 2 to 16 cells).

The probability of all seven or eight stem cells expressing a single allele (resulting in “pseudoclonality”) is 0.0078, as calculated by a standard formula of binomial probability (13). In studies of 200 samples of apparently nonclonal tissues taken from heterozygous females we did not encounter such an event.

Statistical Evaluation of Data Assuming Balanced Amplification of Stem Cells after X-Chromosomal Inactivation. The above data indicate constancy of the allelic ratio over an extended

Table 3. Mathematical Model of Functional Stem Cells Needed to Maintain Constancy of the G6PD Allelic Ratio in Blood Cells

Total number of stem cells	Percentage of functioning stem cells at any one point in time	Probability of achieving G6PD allelic constancy
8	100.0	1.000
14	95.0	1.000
28	95.0	1.000
56	90.0	0.977
112	77.5	0.957
224	62.5	0.962
448	47.5	0.953
896	30.0	0.951
1792	17.5	0.954
3584	12.5	0.977
7168	5.5	0.957
14336	3.0	0.965
28672	1.5	0.963
57344	1.0	0.985

period of time. This index of clonal stability could be explained by alternative interpretations. First, all of the stem cells could contribute continuously to the process of hematopoiesis. Second, an extensive, balanced amplification of the original stem cell pool could occur so that the large size of this pool would obscure the fact that only a minor portion of the stem cells are actively engaged in hematopoiesis at any given time. For the latter possibility to fit our data additional assumptions need to be made: (a) All of the original stem cells would need to divide and none of the derivative subclones could gain proliferative or survival advantage. (b) The final number of functional stem cells would need to be relatively large. Table 3 provides a mathematical evaluation of the stem cell pool sizes required to maintain constancy of the G6PD allelic usage ratio in the myeloid and lymphoid cell progeny. For each given number of stem cells (that is, the size of the stem cell pool in existence), one can use the hypergeometric distribution table (12) to estimate the percentage of stem cells that must give rise to the differentiated progeny to maintain a high probability of G6PD allelic constancy in all of the different blood cell lineages. For example, contribution of 30% of 896 stem cells to hematopoietic division and differentiation would result in a high probability (0.951) of relative stability in the C and T allelic ratio over many stem cell divisions. For a very low percentage of stem cells, 1% for example, to function at a given time and still maintain constant usage of the two X-chromosomal alleles, the total stem cell pool would need to contain ~60,000 pluripotent stem cells. Calculation of the values of the probabilities appearing in Table 3 is based on the condition of balanced usage of the X-chromosome alleles, but these values tend to be conserved even when the allelic ratio is skewed.

Discussion

The results of this transcriptional analysis of the G6PD allele usage confirm that the ratio of the X-chromosome-derived allelic transcripts varies between different tissues and for different heterozygous females (5, 6, 18–21). In contrast, we find that this allelic ratio is remarkably constant for all of the myeloid and lymphoid (T, B, and NK) cell lineages within a normal heterozygous individual. These data provide support for the principle that myeloid lineages are derived from the same hematopoietic progenitor that contributes to the lymphoid lineages in humans (9, 22–24), a concept that has been verified by direct labeling of individual stem cells by retroviral insertion (25).

Validity of the Ligase Detection Assay for Monitoring Allelic Transcriptional Ratios. Somatic selection has been implicated in studies of the distribution of G6PD-deficient cells in Sardinian females (26) and apparent selection against G6PD-deficient cells has also been observed in aging South African females (27). These observations were based on studies of G6PD polymorphism that resulted in abnormal peptide properties, while the polymorphism that we have studied here is translationally silent and cannot be discerned at the protein level.

The transcriptional analysis of G6PD alleles used in the present studies has several attractive features for studies in humans under normal and disease-related conditions. Our data confirm that normal females of all ethnic groups can be informative; the thermostable ligase technique is sensitive, reproducible, and quantitative; and the method allows the analysis of transcripts in nonnucleated cells such as reticulocytes and platelets. In contrast to strategies employing cell culture of individual hematopoietic colonies (28), this ligase detection assay can be used to assess the stem cell contribution to all aspects of hematopoiesis by individual analyses of reticulocyte, platelet, granulocyte, monocyte, B, T, and NK cell progeny. In addition, any isolatable subpopulation of the different lineages can be compared by this sensitive assay.

The current studies of normal hematopoiesis, unperturbed by extraneous stimuli, also have an advantage over measurements of G6PD isoenzymes (28) in that these values may be influenced by factors such as a variable half-life of G6PD isoenzymes (29) and by differences in the processing of RNA transcripts of G6PD protein in various tissues (30).

Estimation of the Stem Cell Pool Size. Our data and that of Puck et al. (21) indicate significant variation in the balance of X-chromosome inactivation (31, 32) among mesodermal ancestors of the hematopoietic stem cells in normal individuals. Skewing of the ratios of X-chromosome alleles thus cannot be used as evidence of oligoclonality as has been suggested (33). This conclusion is also supported by studies of HPRT and PGK genomic polymorphisms in normal females (34).

Mathematical analysis of the ratios observed for the transcripts of the G6PD alleles allowed us to estimate the number of hematopoietic stem cell founders derived from the original embryonic cells that randomly undergo X-chromosome inactivation. Based on the variability of the allelic ratio in 10 healthy females heterozygous for this polymorphism, it can be estimated that seven or eight pluripotent hematopoietic stem cells may exist in female embryos near the time of X-chromosome inactivation during the blastocyst developmental stage (18–20). An earlier estimate of 19 hematopoietic progenitors at the time of X-chromosome inactivation (20) was based on an assay that could not detect the full extent of normal skewing of the ratios of G6PD alleles (5, 6, 21, 29, 30). Our estimate is in close agreement with that of Puck et al. (21), who examined the expression of another X-chromosome-encoded enzyme, HPRT, in hybrid cell clones of human T lymphocytes and hamster fibroblasts to estimate that ~10 hematopoietic stem cells are present at the time of X-chromosome inactivation. While these estimates in humans contrast with those obtained in repopulation studies of irradiated mice suggesting that one or two stem cells may account for all of the mature hemopoietic cells (25), they are congruent with the results of human bone marrow transplantation studies (35). This could indicate a species difference, but it is more likely that the murine bone marrow transplantation model does not accurately reflect the conditions of normal hematopoiesis.

Stability of G6PD Allele Usage by Blood Cell Lineages. Remarkable stability of the G6PD allele usage was observed in normal heterozygous females over a two and a half year interval. These observations in healthy women differ from those made previously in irradiated mice transplanted with hematopoietic stem cells, marked by a retroviral transfection marker (25) or a recognizable chromosomal feature (36), and in bone marrow-transplanted cats (28). In these experimental models, significant subsequent fluctuations of G6PD isoenzymes were seen in blood cells over time. There could be several reasons why the direct extrapolation of these findings in animal models may not be valid in humans. Despite usefulness for tracing the potentiality of a given hematopoietic precursor (25), manipulations of bone marrow progenitors by retroviral transfection, bone marrow transplantation, or chemotherapy, could disturb the homeostatic mechanisms governing hematopoiesis and thus unreliably reflect normal steady state hematopoiesis.

The identical allelic ratio observed in the short-lived myeloid cells, long-lived B lymphocytes, and even longer-lived T lymphocytes, suggests long-term stability of the pattern of X-chromosomal allelic transcript usage in healthy females. Nevertheless, the data do not exclude the possibility of clonal succession. Indeed, data in mice indicate that a significant portion or even a majority of pluripotent stem cells may be in G₀ phase at any given point in time (37). It is theoretically possible that after the initial X-chromosomal inactivation in the female embryo, the initial pool of approximately eight self-renewing hemopoietic stem cells is markedly and evenly amplified. Given the generation of a sufficiently large pool, a minority of the stem cells (but still a significant number) could be actively engaged in hematopoiesis, while the others remain dormant without changing the allelic balance. This theoretical possibility would require that the founder stem cells undergo many nondifferentiating cell divisions after X-chromosomal inactivation and that none of the subclones would gain prolif-

erative or survival advantage; i.e., that clonal selection of stem cells does not take place normally. Under this scenario (mathematically modeled according to a hypergeometric distribution in Table 3), the percentage of cells expressing one allele could remain unchanged even with the occurrence of clonal succession given the activity of a significant number of stem cells at any one time.

The observation of the same X-chromosome transcriptional ratio in T cells and other types of circulating blood cells suggests that the thymus is populated by a number of precursor cells that is sufficiently large as not to distort the C/T ratio present in the original stem cell pool. Our observations further indicate that the clonal expansion of T and B lymphocytes selected by routine antigenic stimulation in the peripheral lymphoid tissues does not significantly affect this ratio. This picture is quite distinct from the disease state patterns in which these ratios may vary for myeloid or lymphoid lineages or both in myeloproliferative and other clonal hematopoietic diseases, as well as in certain infectious and rheumatoid disorders (5, 7, 22, and our unpublished results). Thus, temporal analysis of the ratio of X-chromosome-derived blood lineage cells in an informative female allow monitoring of clonal and oligoclonal expansion or contraction that may transiently or permanently distort the clonal balance.

In conclusion, we have verified the reproducibility and quantitative analytical potential of a transcriptional assay of the active X-chromosome in G6PD heterozygous females (5, 6) and used it to evaluate stem cell input into normal myelopoiesis and lymphopoiesis. The data showing consistency of a fixed pattern of X-chromosomal usage by all of the lymphoid and myeloid cell lineages in a normal individual indicate that this analysis can be used to detect aberrations in stem cell differentiation after hematopoietic stem cell transplantation and in a variety of inherited and acquired gene defects that can affect the clonal distribution of hematopoiesis.

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