X-Chromosome Inactivation and the Xg Locus

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Although the type of inactivation predicted by the inactive-X hypothesis has been demonstrated in man for a number of loci (see Lyon 1968 for review), it is not known whether the entire X chromosome undergoes inactivation. The evidence is particularly conflicting for the X-linked locus determining the red-cell antigen, Xg^a. The inactive-X hypothesis predicts that in a female heterozygous for the Xg^a gene and its thus far silent allele, Xg, both Xg(a+) and Xg(a−) cells should be found. If inactivation does not occur at this locus, all cells should type as Xg(a+). In fact, repeated failure of attempts to demonstrate a mixture of Xg(a+) and Xg(a−) red cells in normal Xg^a/Xg heterozygotes using techniques that can detect artificial 1:1 mixtures of cells from hemizygous Xg(a+) and Xg(a−) males (Gorman et al. 1963; Race and Sanger 1968) is consistent with the nonoccurrence of inactivation. More definitive results would be available from Xg^a testing of single red cells, but since this is not currently possible, other methods must be used. For example, one may study red cells with a clonal origin since, if fixed inactivation occurs, such cells should reflect the functional state of X-chromosome inactivation in the single precursor cell.

Cytogenetic studies (Sandberg et al. 1962; Trujillo and Ohno 1962; Tough et al. 1963; Whang et al. 1963) and genetic observations (Fialkow et al. 1967; Fialkow et al. 1969) strongly suggest that the red cells in patients with chronic myelocytic leukemia (CML) have a clonal origin. If the Xg locus undergoes fixed random inactivation, the phenotype in one-half of genetically proven Xg^a/Xg heterozygotes with CML should be Xg(a−); if the locus is not inactivated all such heterozygotes with CML should be Xg(a+). As reported elsewhere, all of 11 females with CML assumed to be heterozygous at the Xg locus on the basis of family studies were Xg(a+) (Fialkow et al. 1970). The probability that all 11 heterozygotes would be Xg(a+) in the presence of inactivation is one in 2,048. These and comparable data recorded by Lawler and Sanger (1970) provide no evidence for Xg inactivation.

On the other hand, apparently contradictory data were reported by Lee and his co-workers (1968). They made a diagnosis of hereditary X-linked microcytic anemia in a mother and two of her four daughters whose red cells consisted of two different populations. When the mother's cells were separated, both the normal and abnormal cells were Xg(a+). However, in the daughters, the normal cells were Xg(a−) and the abnormal cells were Xg(a+). The normal father was Xg(a−). These observations suggest that only one allele for the Xg locus was active in each cell.

Because of this conflicting evidence, it is important to seek other tests of inactiva-

Received February 27, 1970; revised March 20, 1970.

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tion at the Xg locus. The remaining portion of this communication describes data which provide further evidence that the Xg locus may not undergo random fixed inactivation.

The syndrome of hyperuricemia, mental retardation, choreoathetosis, and compulsive self-mutilation described by Lesch and Nyhan (1964) is inherited in an X-linked recessive manner. Affected males have virtual absence of hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) enzyme activity in red cells and fibroblasts (Seegmiller et al. 1967). Although skin fibroblasts in heterozygous females have the expected intermediate enzyme activity, the red cells have normal activity levels (Kelley et al. 1969; Nyhan et al. 1970). It has been suggested that the failure

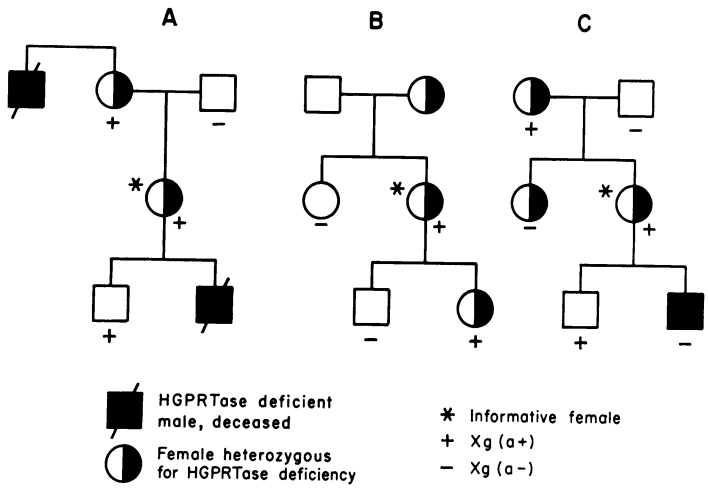

FIG. 1.—These are partial pedigrees abstracted from the literature: *A*, the informative female is III-15 reported by Nyhan et al. (1967); *B*, the informative female is I-8 in Family S reported by Henderson et al. (1969) and II-2 in the earlier report of this family by Shapiro et al. (1966); *C*, the informative female, reported by Greene et al. (1970), is the fourth daughter in Family VI and also the mother in Family VII (M. L. Greene, personal communication). The Xg^a phenotypes were not determined in the first generation in *B*, but since the sister of the informative female is Xg(a−), it can be inferred that their father was Xg(a−) and their mother, Xg(a+). Females are scored as heterozygous for HGPRTase deficiency on the basis of enzymatic study of their skin fibroblasts and/or genetic evidence. The genetic evidence that the mother of the informative female in *B* is heterozygous for HGPRTase deficiency is given in the pedigree reported by Shapiro et al. (1966) in which she is subject I-2.

to find intermediate activity in red cells in heterozygotes is due to selection against red-cell precursors in which the mutant allele is on the active X chromosome (Kelley et al. 1969). This notion receives strong support from a recent report by Nyhan and his co-workers who studied two females, each of whom was genetically proven to be heterozygous at both the HGPRTase and the glucose-6-phosphate dehydrogenase (G6PD) loci (Nyhan et al. 1970). From their father, these subjects inherited type B G6PD and the normal HGPRTase allele; from their mother, they inherited type A G6PD and the mutant HGPRTase allele. The red cells of both subjects had normal HGPRTase activity and displayed only type B G6PD, strongly suggesting that

these cells descended only from precursors in which the paternal X chromosome was active.

Since presumably the circulating red cells in HGPRTase heterozygotes are all derived from precursor cells in which the same X chromosome is active (the one with the normal HGPRTase allele), the system can be used to test for inactivation at the Xg locus. If this active X chromosome bears an *Xg* allele, the red cells of such females should type as Xg(a−) even if an Xg^a allele is present on the other X chromosome. Three females recorded in the literature are particularly informative. Each is heterozygous at both the HGPRTase and the Xg loci (see fig. 1). Each inherited the normal HGPRTase allele and the *Xg* allele from her father; therefore, according to the inactive-X hypothesis, their red cells should type as Xg(a−). However, the phenotype in all three subjects is Xg(a+). Furthermore, in a recently reported linkage study, six other females heterozygous at the HGPRTase locus who were shown to be Xg^a/Xg heterozygotes on the basis of phenotypes in the progeny were all Xg(a+) (Greene et al. 1970). No female known to be heterozygous at both loci was Xg(a−) (M. L. Greene, personal communication). These data, together with the previous observations, suggest that either the Xg locus does not undergo fixed random inactivation or that the Xg^a antigen is not synthesized in the red cells themselves. Although the latter possibility could explain many of the observations discussed here, there is currently no evidence to support a separate site of Xg^a antigen synthesis (Race and Sanger 1968).

SUMMARY

A new test for inactivation at the Xg locus based on data recorded in the literature is described. Females heterozygous for hypoxanthine-guanine phosphoribosyltransferase (HGPRTase) deficiency have a hemizygous normal phenotype in their blood cells. These cells are probably derived from precursors which all have the same X chromosome active (the one with the normal HGPRTase allele). If this X chromosome bears an *Xg* allele and the inactivated X chromosome bears an Xg^a allele, the inactive X hypothesis predicts that the red cells should have an Xg(a−) phenotype. However, each of three informative females was Xg(a+). These findings provide further evidence that this locus does not undergo random fixed inactivation or that the Xg^a antigen is not synthesized in the red cells themselves.

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