A NOVEL CLASS OF UNSTABLE 6-THIOGUANINE-RESISTANT CELLS FROM DOG AND HUMAN KIDNEYS

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Thioguanine-resistant primary clones were grown from single cell suspensions obtained from dog and human kidneys by enzymatic digestion. In medium containing a relatively high concentration (10μg/ml) of thioguanine, thioguanine-resistant primary clones arose from each source at frequencies ranging from $10^{-4}$ to $10^{-5}$. A reduction in total hypoxanthine uptake was found in the thioguanine-resistant primary clones which had developed in thioguanine medium, consistent with a reduction in hypoxanthine phosphoribosyltransferase activity. When these thioguanine-resistant primary clones were subsequently grown in the absence of thioguanine and assayed for the thioguanine-resistant phenotype and hypoxanthine phosphoribosyltransferase activity, it was found that most were now thioguanine-sensitive and yielded cell-free extracts with substantial amounts of hypoxanthine phosphoribosyltransferase activity. In contrast, thioguanine-resistant human clones grown continuously in the presence of thioguanine yielded cell-free extracts with little or no detectable hypoxanthine phosphoribosyltransferase activity. Southern blot analysis demonstrated no structural alterations in the hypoxanthine phosphoribosyltransferase gene in thioguanine-resistant primary human kidney clones. These results

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2. Key words: hypoxanthine phosphoribosyltransferase; kidney primary clones; purine analogue resistance.
3. Abbreviations: AG, 8-azaguanine; APRT, adenine phosphoribosyltransferase; DAPI, 4'-6 diamino-2-phenylindole; DV, Dulbecco-Vogt; HAT, hypoxanthine, aminopterin, thymidine; HPRT, hypoxanthine phosphoribosyltransferase; PRPP, 5-phosphoribosyl 1-pyrophosphate; TG, 6-thioguanine; TG\textsuperscript{R}, thioguanine-resistant; TG\textsuperscript{S}, thioguanine-sensitive; TTP, thymidine triphosphate.
suggest that a novel mechanism(s) for thioguanine resistance and the control of hypoxanthine phosphoribosyltransferase expression may occur in dog and human kidney cells.

**INTRODUCTION**

Recent advances in somatic cell genetic techniques have allowed the isolation and clonal expansion of somatic cell variants that arise in vivo. As a result, it is now possible to analyze the molecular events underlying variations in cell phenotype in vivo. The most widely studied of these selective systems involves the isolation of peripheral blood T lymphocyte clones with deficiencies for the X-linked purine salvage enzyme hypoxanthine phosphoribosyltransferase (HPRT) (EC 2.4.2.8) (Albertini et al., 1982, 1985; Morley et al., 1982, Turner et al., 1985; Bradley et al., 1987). The purine analogue 6-thioguanine (TG) is often used as a selective agent for HPRT-deficient cells (Nelson et al., 1975). When sufficiently high concentrations of TG are used, the resultant TG-resistant (TG^r^) cells usually display severe and stable deficiencies in HPRT expression (Stout and Caskey, 1985). Molecular analyses of HPRT gene structure in TG^r^ human lymphocyte clones have demonstrated that HPRT deficiency can result from major structural alterations (including deletions) at the HPRT locus (Albertini et al., 1985; Turner et al., 1985; Bradley et al., 1987). Analyses of T-cell receptor gene rearrangement in these clones with a T-cell receptor beta chain probe has demonstrated that most TG^r^ lymphocytes arise in different T-cell clones in vivo, and thus are likely to be the result of independent mutational events (Nicklas et al., 1986).

We have previously reported a selective procedure for the isolation and clonal expansion of TG^r^ mouse primary kidney and skeletal muscle cells (Horn et al., 1984). These TG^r^ mouse cells demonstrated apparently stable HPRT deficiencies, consistent with a somatic mutational origin in vivo. In that study, the issue of stability was addressed in only a small sample of clones. Here we have extended this selective system to dog and human kidney cells. Surprisingly, most of the TG^r^ dog and human kidney primary clones selected in comparably high concentrations of TG were not stably TG^r^. These results suggest that the TG^r^ phenotype may be produced by mechanisms other than classical mutation in the HPRT gene of dog and human kidney cells.

**METHODS**

*Isolation and Selection of TG^r^-Dog and Human Kidney Cells.* Dog kidneys were from male and female beagle dogs (ages 82-182 months) maintained at Battelle Pacific Northwest Laboratories (Richland, WA). Several of the dogs had received a single