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Clonality, trafficking, and molecular alterations among Hprt mutant T lymphocytes isolated from control mice versus mice treated with N-ethyl-N-nitrosourea

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Abstract

Mutations in T lymphocytes (T-cells) are informative quantitative markers for environmental mutagen exposures, but risk extrapolations from rodent models to humans also requires understanding how T-cell development and proliferation kinetics impact mutagenic outcomes. Rodent studies have shown that patterns in chemical-induced mutations in the hypoxanthine-guanine phosphoribosyltransferase (Hprt) gene of T-cells differ between lymphoid organs. The current work was performed to obtain knowledge of the relationships between maturation events during T-cell development and changes in chemical-induced mutant frequencies over time in differing immune compartments of a mouse model. A novel RT?PCR based method was developed to determine the specific T-cell receptor beta (Tcrb) gene mRNA expressed in mouse T-cell isolates, enabling sequence analysis of the PCR product that then identifies the specific hypervariable CDR3 junctional region of the expressed Tcrb gene for individual isolates. Characterization of spontaneous Hprt mutant isolates from the thymus, spleen, and lymph nodes of control mice for their Tcrb gene expression found evidence of in vivo clonal amplifications of Hprt mutants and their trafficking between tissues in the same animal. Concurrent analyses of Hprt mutations and Tcrb gene rearrangements in different lymphoid tissues of control versus N-ethyl-N-nitrosourea-exposed mice permitted elucidation of the localization and timing of mutational events in T-cells, establishing that mutagenesis occurs primarily in the pre-rearrangement replicative period in pre-thymic/thymic populations. These findings demonstrate that chemical-induced mutagenic burden is determined by the combination of mutagenesis and T-cell clonal expansion, processes with roles in immune function and the pathogenesis of autoimmune disease and cancer.

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