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## **Tumor Growth in the Liver Depends on Antigen Load and Immunologic Ignorance**

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**Introduction:** The transforming T antigen (Tag) from the SV40 virus has been shown to immortalize cells in culture. Although such tumors grow in immunocompromised mice, when they are injected intravenously, subcutaneously, or intraperitoneally into immunocompetent C57BL/6 mice they are rejected by a vigorous cytotoxic T-lymphocyte (CTL) response to Tag. Hypothesizing that tumors that seed the liver directly might avoid immunologic destruction, we injected varying doses of a Tag-expressing, murine hepatocellular cancer (HCC) by intrasplenic routes into syngeneic immunocompetent C57BL/6 mice and compared the growth kinetics.

**Methods:** Three-month-old C57BL/6 mice were inoculated with syngeneic HCC cells expressing Tag of the SV40 virus (HCCT) through intravenous, subcutaneous, and intrasplenic injections. C57BL/6 mice were divided into different groups according to the number and site of tumor cells injected:  $5 \times 10^6$  cells (15 mice) and  $0.5 \times 10^6$  cells (15 mice) were injected intrasplenicly;  $5 \times 10^6$  cells (5 mice) and  $0.5 \times 10^6$  cells (5 mice) were injected subcutaneously; and  $5 \times 10^6$  cells (5 mice) and  $0.5 \times 10^6$  cells (5 mice) were injected intravenously. Tumor growth was evaluated with contrast-enhanced magnetic resonance imaging (MRI) at various time points after intrasplenic injection. Three animals from each cohort were vaccinated with WT-19 (renal tumor cell line expressing Tag) 14 days after intrasplenic injection of HCCT cells to assess the immunologic mechanism allowing hepatic tumor growth. Tetramer analysis was used to quantify Tag-specific CD8<sup>+</sup> T-cell proliferation, and T-cell function was assessed through

Tag-specific  $\gamma$ -interferon (IFN- $\gamma$ ) production. Autopsies were performed at 30 and 120 days after intrasplenic injection to examine for macroscopic and histologic evidence of tumor.

**Results:** The cohorts of mice injected with HCCT cells subcutaneously, intravenously, and  $5 \times 10^6$  cells intrasplenically did not demonstrate microscopic or radiographic evidence of tumor growth in any of the animals. In addition, the cohort injected intrasplenically with  $5 \times 10^6$  cells demonstrated a strong tumor antigen-specific immune response 9 and 21 days after intrasplenic injection. Interestingly, the group injected with  $0.5 \times 10^6$  cells did show microscopic and radiographic evidence of hepatic tumor growth in 12/15 animals. Immunohistochemistry staining of liver was used to confirm Tag expression in the tumor foci. This cohort did not show a tumor-specific immune response at 9 and 21 days after injection. After vaccination with WT-19 cells, this cohort of animals demonstrated strong tumor antigen-specific immune response as seen by CD8<sup>+</sup> T-cell proliferation and IFN- $\gamma$  production.

**Conclusion:** An otherwise immunogenic tumor when delivered to the liver in low doses, will grow. When this occurs, T cells are present and remain capable of producing a robust immune response when presented with the same tumor antigen. Our results suggest that immunologic ignorance, not tolerance, could be responsible for tumor growth and that this phenomenon may be related to the antigen load.