

Induction of CD4-independent CD8⁺ Memory T Cells by HspE7 Fusion Protein

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Long lasting, protective memory T cells can be induced after microbial infection, and such immunological memory is considered to be a critical component of many vaccine strategies. In addition, CD8⁺ memory T cells can be induced in the absence of CD4⁺ T cells if antigen presenting cells are directly activated, for example by microbial infection or CD40 ligation. HspE7 is a fusion protein comprised of *M. bovis* BCG Hsp65 fused to the E7 antigen of human papillomavirus (HPV) type 16. HspE7 is capable of inducing antigen-specific CD8⁺ T cells and anti-tumor responses in mice and can activate murine DC *in vitro*. Moreover, HspE7 displays significant clinical activity in phase II trials for the immunotherapy of HPV-related diseases. Therefore, we investigated the ability of HspE7 to induce antigen-specific CD8⁺ memory T cells, and examined the role of CD4⁺ T cells in this process by comparing CD8⁺ responses in Class II^{-/-} and wild-type mice. Mice were given two subcutaneous immunizations with HspE7 without exogenous adjuvant. At various times following the last immunization, splenocytes were restimulated with an E7-derived CTL epitope peptide and the effector cells were assayed for lytic activity using peptide-pulsed and E7-expressing target cells. All immunization regimens tested induced memory CTL responses that lasted for at least 17 weeks after the last immunization. In these experiments, the optimal CTL response was primed with two immunizations given at an interval of 8 weeks. HspE7 elicited high levels of CTL activity in both wild-type and Class II^{-/-} mice, although the level of target cell lysis by CTL from Class II^{-/-} mice was somewhat lower. Similar results were obtained when the antigen-specific effector cells were assayed for IFN- γ release by ELISPOT. The CTL response peaked 1-3 weeks after the booster immunization and declined to approximately one-half of the peak activity by 13-17 weeks post-immunization in wild-type and Class II^{-/-} mice. These results demonstrate that HspE7 induces CD4-independent antigen-specific CD8⁺ memory T cells, although the induction of CD8⁺ T cells with cytolytic and IFN- γ secreting activities is greater in the presence of CD4⁺ T cells.