Immunization of mice with a 14-mer peptide TKDNNLLGRFELSG, termed "TKD," comprising amino acids 450-461 (aa(450-461)) in the C terminus of inducible Hsp70, resulted in the generation of an IgG1 mouse mAb cmHsp70.1. The epitope recognized by cmHsp70.1 mAb, which has been confirmed to be located in the TKD sequence by SPOT analysis, is frequently detectable on the cell surface of human and mouse tumors, but not on isogenic cells and normal tissues, and membrane Hsp70 might thus serve as a tumor-specific target structure. As shown for human tumors, Hsp70 is associated with cholesterol-rich microdomains in the plasma membrane of mouse tumors. Herein, we show that the cmHsp70.1 mAb can selectively induce antibody-dependent cellular cytotoxicity (ADCC) of membrane Hsp70(+) mouse tumor cells by unstimulated mouse spleen cells. Tumor killing could be further enhanced by activating the effector cells with TKD and IL-2. Three consecutive injections of the cmHsp70.1 mAb into mice bearing CT26 tumors significantly inhibited tumor growth and enhanced the overall survival. These effects were associated with infiltrations of NK cells, macrophages, and granulocytes. The Hsp70 specificity of the ADCC response was confirmed by preventing the antitumor response in
tumor-bearing mice by coinjecting the cognate TKD peptide with the cmHsp70.1 mAb, and by blocking the binding of cmHsp70.1 mAb to CT26 tumor cells using either TKD peptide or the C-terminal substrate-binding domain of Hsp70.