Immune response and immunoprotection against murine melanoma B16F10-Nex2

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The murine melanoma cell line B16F10-Nex2 is syngeneic in the C57Bl/6 mouse host, is highly aggressive *in vivo*, and has low immunogenicity. These characteristics are shared by most human melanomas. As with human melanoma cells, *in vivo* growing B16F10-Nex2 cells induce a nonprotective host immune response, unable to eliminate the tumor cells. Innate immunity (macrophages, NK and NKT effector cells) is, however, stimulated as are humoral and cellular (antibodies and CD4+ and CD8+ T lymphocytes, respectively) tumor-specific adaptive immunity in human patients, factors which open the possibility of further stimulating these responses towards a protective immunotherapy. Our goal is to better understand the protective B16F10-Nex2 immune response and to manipulate its inducing and regulatory components to finally achieve reduction of tumor development *in vivo* and consequently, increase the survival time of tumor-challenged mice.

Activation of the innate immune response may directly eliminate tumor cells and improve adaptive immune responses, as has recently been demonstrated by several research groups. Our results suggest that glycolipid components of B16F10-Nex2 can stimulate NKT cells. These components were purified using classical lipid separation protocols, and a fraction containing neutral and acidic glycolipids and phospholipids had a component that was presented via CD1d to murine NKT-cells, leading to the production of IL-2 and IL-4 by these cells. NKT cells had previously been associated with anti-tumor activity in IL-12-treated mice. Certain immunization protocols can induce anti-melanoma adaptive immune response. Anti-B16F10-Nex2 monoclonal antibodies (IgG) were produced,

exhibiting cytotoxic and immunoprotective activities *in vitro* and *in vivo*, respectively. These antibodies (MAbA4) recognize antigens expressed on the tumor cell membrane and cytosol (m.w. 75-77 kDa), and CL-ELISA reactivity of tumor cells with MAbA4 was completely abrogated by melibiose. The same immunization protocol induced nonprotective monoclonal antibodies (IgM), reactive with histones at the nuclei of tumor cells. Cytokines have an important role in the evolution/rejection of tumor cells *in vivo*, and pro-inflammatory interleukins, mainly IFN-□, are protective against murine melanoma. We developed a model to study the direct activity of IFN-□ on tumor cells in vivo, and our results showed, for the first time, that this cytokine can eliminate subcutaneous development of melanoma cells independently of its activity on the host immune system. Overall, our results suggest that anti-B16F10-Nex2 innate and adaptive immune response can be efficiently stimulated to eliminate tumor cell development *in vivo*. Effector cells and mediators were elicited by tumor cell components and whole cells and were correlated with decreased tumor development and increased survival of the animal hosts.

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