Induction of protective humoral immunity against murine melanoma B16F10.

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Polyclonal and monoclonal antibodies (mAbs) have been raised against B16F10 cells collected from growing tumors *in vivo* or growing in culture media supplemented with NMS to avoid xenogeneic reactivity. Ab binding to glutaraldehyde-fixed melanoma cells and Melan A melanocytes were assayed using CL-ELISA for increased sensitivity. Most of the reactivity of antitumor polyclonal IgG (92%) was inhibited by a carbohydrate pool consisting of melibiose, mannose, lactose and sialic acid. Two IgG2a mAbs, A4 and B11, had their reactivity to melanoma cells completely and specifically inhibited by melibiose. MAb A4 did not bind to alpha-galactosyl residues abundantly expressed in a protozoan mucin used as substrate, and its binding to tumor cells was not affected by alpha-galactosidase treatment or addition of alpha-methyl-galactopyranoside or raffinose. By subcloning the A4 hybridoma, a third mAb was isolated (A4M). In contrast to A4G, the reactivity of A4M with tumor cells was not inhibited by carbohydrates. MAb A4G, but not A4M, was cytotoxic *in vitro* in a complement-mediated reaction and effectively neutralized melanoma cells protecting syngeneic mice against tumor development *in vivo*. MAb A4G reacted not only with murine but also with human melanoma cells. In some cell lines, A4G induced apoptosis as shown by DNA degradation and morphological alterations including cell detachment from the substrate. Using phage display assays the described mAbs

reacted with different epitopes. As shown by FACS and confocal microscopy, A4M reacted mostly with intranuclear components and recognized a protein of approximately 30 kDa from the nuclear extract, probably histone. MAb A4G reacted with intracellular components, and specifically with a protein pair of 75-77kDa (similar to TRP proteins expressed in melanotic cells). These mAbs are thus important tools for further studies on antitumor adjuvant therapy associated with other immunological and chemotherapeutic procedures.

Key words: monoclonal antibodies, B16F10, antitumor, carbohydrates, phage-display, protection