Accumulation of the MAb BW431/26 in human colon tumours after *in vivo* and *ex vivo* application

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For evaluation of monoclonal antibody (MAb) enrichment in human colon carcinoma a postoperative *ex vivo* perfusion system was established. The aim was to investigate the MAb BW431/26 and to compare its *in vivo* and *ex vivo* accumulation. The major vessel of the resected colon segment was canulated and the arterial supply of the tumour confirmed by angiography. In addition 7 patients received MAb BW431/26 two days prior to the operation. After resection the radioactivity in the tumour was determined and all colon segments were subsequently perfused using the Tc^{99} labelled MAb BW431/26. Immunoscintigraphy of patients could detect the tumour in 3/7 cases *in vivo*. However, in all resected colon segments it was demonstrated that the MAb had localised specifically, reaching a mean tumour/non-tumour ratio of 4.1:1. Additionally, metastatic lymph nodes were localised.

Proliferative responses of EBV transformed B cells to lymphokines and mitogens

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An attractive approach to the analysis of the human B cell repertoire at the clonal level involves transformation with EBV. As our interests focus on the generation of human monoclonal antibodies reactive with tumour-associated antigens, an obvious advantage of EBV transformation in this case is the larger population of B cells surveyed for antibody production after EBV infection as compared to the more random sampling inherent in hybrid formation. Despite these advantages there are several problems that limit the usefulness of EBV transformation of B cells. For instance EBV infected B cells demonstrate very low cloning efficiency particularly at low limiting dilution numbers. However, the most serious difficulty is the loss of antibody secretion after the initial phase of polyclonal expansion of these cells. With the recognition that B-cell growth and differentiation are controlled by a number of different cytokines and soluble factors we studied the effects of the tumour promoter 12-0-tetradecanoylphorbol-13-acetate (TPA) and three different lymphokines IL-2, IL4, and IL-6. TPA demonstrated a dose dependent inhibition of EBV-B cell proliferation, with a plateau at 2.5% FCS. These can also grow efficiently in serum free medium (0% FCS). The effects of the three cytokines on EBV transformed lymphocytes showed only a marginal effect on proliferation, particularly with IL-4 alone. Preactivation of lymphoblastoid cells with insolubilised anti-IgM resulted in similar proliferative responses to IL-4 and IL-6 as without preactivation. Although these results demonstrate only marginal effects of cytokines on EBV-B cell proliferation and inhibition with TPA, we are currently studying their effects on the differentiation and antibody secretion of these cells. It appears that EBV infection of B cells leads to an autonomous proliferation with loss of the ability to respond to different lymphokines when compared to normal B cells.

***In vitro* proliferation of T-cells from patients treated with murine monoclonal antibodies (mAbs)**

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We investigated the cellular immune responses in patients receiving multiple (at least two) mAb administrations for the treatment of malignancies. The *in vitro* T-cell proliferation responses were found to be elevated after the second or subsequent therapies, when compared with the pre-therapy values. Patients that received only one mAb therapy and normal controls did not show any significant or dose dependent proliferation to mAbs *in vitro*. When T-cell proliferation was studied in the presence of the therapeutic mAb (HMFG1) and in the presence of an isotypically related but idiotypically unrelated mAb their post-therapy proliferation appeared to be higher for the administered mAb (HMFG1). Stimulation indexes were calculated and found to be significantly increased post-therapy with HMFG1 when compared to the irrelevant 11.4.1 mAb at a range of concentrations (10–1000 $\mu g/ml^{-1}$). These differences may be due to idiotypic and/or allotypic determinants on the mAb administered for therapy. The T-cell responses of the same patients using non-specific T-cell mitogens, such as PHA, were found to differ significantly pre- and post-therapy suggesting that T-cells become committed to proliferation after mAb therapy.

Idiotypic replica of an anti-human tumour-associated antigen monoclonal antibody

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CaMBr1 is a tissue-specific and tumour-associated saccharidic epitope, defined by mAb MBr1 (Ab1), expressed on glycoconjugates of the human mammary carcinoma cell line MCF-7 and of normal and neoplastic mammary epithelial cells. An anti-anti-idiotypic monoclonal Ab3, 2G-3, identifying a human breast tumour associated antigen, was raised by using as immunogen a mouse anti-idiotypic monoclonal Ab2, A3B10, which behaves as the internal image of CaMBr1. Reactivities of monoclonal Ab1 and Ab3 on normal and neoplastic tissues have been compared. Mab 2G-3, as well as MBr1, define a saccharidic epitope on glycoconjugates extracted from MCF-7 cells and show MBr1-like reactivity on normal and neoplastic tissues. Experimental evidence, however, suggests that the fine immunoreactivity of MBr1 is with glycolipids and 2G-3 with glycoproteins. In western blot analysis of MCF-7 lysates, both antibodies recognised a major protein band of 41 kDa and a minor band of 53 kDa, but while MBr1 also recognised the glycolipid band on both lysates and GLP extracts from MCF-7, 2G-3 in both cases failed to react with the glycolipid band. Similar results were confirmed with TLC.

We suggest that a possible biological explanation for our findings could reside in the nature of the immunogens used to raise the two mAb (glycolipid vs. protein "internal image"). The present results further support the use of "internal image" monoclonal antibodies as Ag surrogates of tumour associated molecules to obtain antitumour mAb which, as in this instance, are endowed with unique specificity. These reagents might provide a better understanding of the structure and the functional relevance of a given tumour-associated epitope.