BISPECIFIC MONOCLONAL ANTIBODIES FOR TWO PHASE RADIOIMMUNOTHERAPY

K. Bosslet, A. Steinstraesser, P. Hermentin, L. Kuhlmann, A. Bruynck, M. Magerstaedt, G. Seemann, A. Schwarz and H.H. Sedlacek

Abstract

Starting from the moderate therapeutic effects of MAb guided radioimmunotherapy in the treatment of carcinomas, we presented a two phase radioimmunotherapy concept based on bispecific MAbs which might be more effective. To work out this concept we first showed that penetration into human carcinoma xenografts as well as long term storage of intact MAb outside the carcinoma cells are possible. Epitope saturation was however not yet obtained despite the large MAb doses injected i.v for 10 days. Second we generated hybridomas producing high avidity anti metal chelate MAbs (anti DTPA-Y). These hybridomas were fused with hybridomas producing MAbs selective for CEA or GIT-mucin, and stable bispecific MAb producing quadromas were obtained. THE purification of these bispecific anti Cea x anti DTPA-Y or anti GIT-mucin x anti DTPA-Y MAbs, their detailed molecular characterization and potential value we are going to work out.

Introduction

Immunoscintigraphy using Tc-99m-labelled MAbs (34) is going to become a routine diagnostic method for the specific in vivo detection of tumors (3,4,25,26) or inflammatory processes (21,21,22,5)

In contrast, radioimmunotherapy using MAbs tagged with I-131 or Y-90 has not yet been accepted in oncology as a treatment modality despite a few encouraging early reports (14,13). This is mainly due to the small amounts of radiolabelled Mabs bound to the tumor site and the unfavourable whole body distribution and metabolisation of intact immunoglobulins or their
fragments (37). If a radioactive α- or β-emitter or a toxic drug is directly coupled to the MAb or its fragment the above mentioned unfavourable whole body distribution would bring much harm to many non tumor tissues especially bone marrow.

It may be possible to circumvent these problems using one of the recently suggested two phase approaches (1,2,16,17,32,12). These approaches assume a long term non toxic targeting phase of a modified MAb and a second short term toxic binding or activation phase of a small hydrophilic molecule.

In this report we show that it is possible to target significant amounts of MAb to human colon carcinoma xenografts and to store the MAb for many days at the tumor site. If such a MAb would have two arms with different specificities, one against the tumor, the second against a small hydrophilic metal chelate it should be possible to target a quickly - distributing and penetrating chelate very efficiently to the tumor. Because of its short plasma half life and its complete extracellular distribution, a molecule like DTPA-Y90 should bring little harm to the normal tissue but would hopefully be retained at the anti DTPA arm of the bispecific MAb.

Furthermore this paper presents our efforts to generate bispecific anti tumor x anti DTPA-Y90 MAb s to be able to optimize the condition for the concept of two phase radioimmunotherapy.

**Material and Methods**

**Immunohistochemical investigations**

Thin sections (6 μm) from cryopreserved human tumor xenografts were investigated using the highly sensitive APAAP-technique (Cordell et al. 1984) without any fixation.

**Percolation and saturation studies**

Human colon carcinoma (CoCa4) bearing nude mice (three animals/group) were 10x injected i.v. with 250 μg each of Mabs BW 431, BW 494 or BW 227 (6,7) for ten consecutive days. Tumors were resected at various days after stop of i.v. injection and processed for immunohistochemical investigations.

**Production of DTPA specific MAb**

As immunogen, 19 moles of SCN-benzyl-DTPA were coupled to 1 mole of human serum