Characterisation of the mouse/human chimaeric antibody MBr1

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The murine MAb MBr1 (IgM/k), directed against human breast and ovarian carcinomas, was "humanised" in the perspective of a therapeutic approach. The immunoglobulin variable domains were amplified by polymerase chain reaction, cloned for sequencing and expressed in myeloma cells NSO as a simple mouse/human chimaeric antibody (human IgG1/k). The chimaeric and the murine MBr1 show the same reactivity on tumour and normal cells and they recognise the same molecules on the reference cell line MCF7. Both antibodies are able to cross-inhibit their binding on reference target cell. These data suggest that the "humanisation" of MBr1 antibody did not influence the specific binding of the variable domains. In addition unlike the murine antibody, the chimaeric MBr1 mediated tumour cell lysis by ADCC due to the human constant region of IgG1 isotype. Therefore this antibody could be a good candidate for cancer therapy.

Production of reshaped human antibodies for cancer diagnosis and therapy

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It is now widely accepted that monoclonal antibodies (MABs) for *in vivo* diagnostic and therapeutic use in humans should be preferably of human origin. Unfortunately these are difficult to obtain by conventional hybridoma technology. It is, however, possible to "reshape" existing human MABs at the genetic level into MABs with a desired specificity by means of CDR grafting (Jones PT *et al.*, *Nature* **321**, 1986, 522–525; Verhoeyen M *et al.*, Science 1988, **239**, 1534–1536; Reichmann L *et al.*, *Nature* 1988, **332**, 323–327). We used CDR grafting to transplant the antigen binding sites of the murine MABs H17E2 and HMFG1 onto the framework regions of a human antibody. Both MABs have already shown their potential clinical value. H17E2 reacts with placental alkaline phosphatase (PLAP) which is found particularly in ovarian cancer and seminoma of testis. HMFG1 reacts with a mucin occurring in a number of tumour types including ovarian and breast.

Both reshaped human MABs are functional and reacting with their specific antigens. The affinity of the reshaped human anti-PLAP antibody seems to be similar to that of the original mouse antibody (H17E2). Affinity studies on the reshaped human anti-HMFG1 are in progress.

Session 5. Chairman: K. Sikora, London, UK

Progress with chemoimmunoconjugates

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Chemoimmunoconjugates have been constructed by conjugation of anti-tumour monoclonal antibodies to several classes of anti-cancer drugs including anthracyclines, anti-folates and vinca alkaloid analogues. Drugs have been directly linked to antibody but in addition drug-carrier conjugates have been investigated in order to increase the level of drug deposition in tumours. The pharmacokinetic properties of these drug–carrier conjugates are critically important for tumour delivery. This will be considered with reference to studies using branched polypeptides on a polylysine backbone for the delivery of cytotoxic drugs such as daunomycin. Polypeptides with glutamic acid side chains showed significantly prolonged blood survival indicating their potential as drug carriers. Drug–carrier conjugates are also being considered for targeting with bispecific antibodies. The approach will be reviewed with reference to studies on a bispecific antibody against methotrexate and a cell membrane antigen (gp72) expressed on colorectal and ovarian carcinoma cells. The bispecific antibody enhanced the cytotoxicity of methotrexate polymer conjugates for tumour cells indicating the potential of this approach for designing "intelligent" drug conjugates.

Molecular analysis of the antibody variable region

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The ability to clone rearranged immunoglobulin genes from hybridoma cell lines underlies the engineering of the binding properties of a given mAb. In the case of antibodies specific for certain haptens, we and others have found that even single aminoacid exchanges introduced by site-directed mutagenesis may lead to profound changes in binding. These observations may be used to explain many of the selective forces which operate during immune diversification *in vivo*; antigenic selection following somatic hypermutation accounts for the predominance of increased affinity antibodies upon secondary challenge with antigen, whereas the conservation of certain aminoacid residues at regions normally associated with hypervariability (e.g. at the V_H-D junction) ensures antigenic specificity irrespective of antibody idiotype. These results also suggest that only a small number of residues within the variable region actually make contact with antigen. If this is the case, it would be feasible to design small variants of antibodies which would retain binding specificity. We are currently using these studies to investigate the binding of therapeutic antibodies to their tumour-associated antigens.

Recombinant immunotoxins

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Ricin's cytotoxicity is due to (a) the ability of its A chain to catalytically inactivate ribosomes and (b) the delivery of the A chain to a cell by the B chain. In contrast to Pseudomonas exotoxin A, there is no clearly defined membrane translocation domain in ricin. Nevertheless, since ricin A chain is cytotoxic if delivered to a cell by a suitable antibody, then at least limited translocation activity resides in the A chain. Is this ability retained in fusion proteins?

A variety of ricin A chain-based fusion molecules have been expressed in attempts to link ribosome inactivating activities with cell-binding functions. In at least one case, further engineering resulted in proteins with acceptable cytotoxicity. The success of these experiments augurs well for the future design of recombinant ricin-based therapeutic molecules.