The role of antibodies in the diagnosis of cancer

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Whereas the techniques of diagnostic radiology image the physical characteristics of cancer, the role of antibodies in diagnosis is to target aspects of the essential cancerousness of a cancer cell that makes it different from normal. The requirements for radioimmunoscintigraphy include the following: an antigen specific to the cancer rather than tumour-associated antigens may be developed through the identification of oncoproteins related to cancer oncogenes. A monoclonal antibody of high avidity against the cancer antigen, at present hybridoma derived, may be humanised by recombinant DNA techniques or made with the polymerase chain reaction and the bacterial bioengineer. It may be made bifunctional by the quadroma or by genetic engineering. The radiolabel has progressed from ^{131}I through ^{123}I and ^{111}In to ^{99m}Tc routinely available at low cost and giving low radiation, with radiolabelling techniques giving *in vivo* stability. The gamma camera has improved through SPECT and computer assisted image analysis. The result is a routine nuclear medicine technique for imaging cancer which is benefiting the management of patients with gynaecological, gastrointestinal, bladder cancers, melanoma, cerebral or hepatic involvement.

Session 3. Chairman: K. Bosslet, Marburg, FRG

Growth and differentiation of normal and malignant epithelia

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Analysis of cell differentiation is a very topical problem, and several aspects have been made much easier with the advent of monoclonal antibodies. We have used a panel of such reagents, together with riboprobe *in situ* hybridisation, to examine the phenotype of a novel cell lineage we have recently described which is induced in the human gastrointestinal tract by ulceration (*Nature* 1990, **343**, 82–85). These cells display several interesting differentiation antigens, and, in addition to secreting epidermal growth factor/urogastrone (EGF/URO), also co-express pS2 and hSP (human spasmolytic polypeptide) molecules with many disulphide bridges which are themselves putative growth factors. Our studies indicate that pS2 and hSP secretion is regulated by EGF/URO production. The results will be discussed in relation to the role of these factors in ulcer-healing and neoplasia.

Molecular analysis of the carcinoembryonic antigen gene

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Carcinoembryonic antigen (CEA) is a large glycoprotein (MW180kDa) that has excited interest as a marker for various cancers, particularly those of the gastrointestinal tract. In the 25 years since first characterised by Gold and Freedman, assays have been developed and refined to test for the presence of CEA in serum samples for potential use in screening, diagnosis, prognosis and monitoring. A major difficulty in the development of a specific and sensitive assay for CEA has been the numerous further antigens, closely related to the CEA molecule, which are also detected in sera and affected tissues. We have adopted an alternative approach in the characterisation of CEA by isolating the genomic sequence for the protein moiety of the molecule. The CEA gene contains nine exons encoding aminoacids and one for a 3′ untranslated fragment. Sequence comparison of CEA with other published family members reveals high nucleotide homology, e.g. 90%, 70% and 50% for normal cross reacting antigen (NCA), biliary glycoprotein (BGP) and the pregnancy specific glycoproteins (PSGs), respectively. Hybridisation of a non-repeating 5′ genomic CEA probe to human DNA digests identified about a dozen homologous bands, even at high stringency, supporting the idea of a large family of related proteins. The CEA gene family has been localised to the long arm of chromosome 19 following hybridisation of the 5′ probe to digests of human/rodent hybrid DNA and also by hybridisation to human metaphase chromosomes by the direct *in situ* method.

Antigenic sites in carcinoembryonic antigen

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Fifty-two well characterised monoclonal antibodies against carcinoembryonic antigen (CEA) from eleven different research groups were analysed for epitope reactivities in a collaborative effort to map antigen sites in CEA. Using solid-phase immunoassays about 60% of all possible combinations of Mabs were investigated as inhibitors or as the primary binding antibody. Concordant and discordant inhibition patterns between Mabs were determined using a computer program. Forty-three of 52 Mabs could be classified into one of 5 essentially non-interacting epitope groups containing between 4 and 15 Mabs each. These epitopes were peptide in nature. Non-classifiable Mabs were, with one exception, directed against carbohydrate epitopes (4 Mabs) or were inactive. The reactivities of the Mabs against different normal adult tissues have recently been investigated by immunohistochemistry. Apparently CEA specific Mabs belonged to epitope group 1 and 2.

The application of "pin technology" to the rapid biochemical and immunological screening of peptides—a review

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Unlike most methods of simultaneous peptide synthesis, the multipin strategy originally described by Geysen *et al.* (*Proc Natl Acad Sci USA* 1984, **81**, 3998) may readily accommodate the concurrent synthesis of a thousand or more peptides. The peptides are synthesised on polyethylene pins in an 8 × 12 microtitre plate array facilitating direct immunological screening using appropriate ELISA techniques. The approach was developed to allow elucidation of antibody epitopes to single aminoacid resolution. Recent developments have utilised the pin-peptides as ligands for affinity fractionation of polyclonal sera.

Further adaptation now permits fully deprotected peptides to be cleaved from the pins in such a fashion that the peptides may be used directly in sensitive cell mediated assays. Although intended for the study of T-cell epitopes, the method should prove generally applicable where large numbers of free peptides are required in the 10–100 nmol range.

Antibody geometry and form: biologically active subunits of antibodies

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Our work concerns the molecular analysis of anti-receptor antibodies which induce metabolic effects on cells that they bind to. We have found it possible to isolate the CDR 2 of the light chain in peptide form and couple it to the CDR 2 of the heavy chain. These peptides have all of the biologic and immunologic activities of the intact anti-receptor immunoglobulin. CDRs were able to inhibit growth of a variety of transformed cells. Strategies using CDR 2 loops might help to design new pharmaceutics.