Abstracts for Poster Exhibits

Rapid Production of eptiope-directed monoclonal antibodies

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We have developed a very efficient *in vitro* immunisation protocol which leads to a high number of specific mouse hybridoma clones. Spleen cells are stimulated by antigen in serum-free medium containing Ewing's Sarcoma Growth Factor (ESGF). Peptide antigens (A): We elicited monoclonal antibodies against the active sites of genetically engineered pancreatic secretory trypsin inhibitor (PSTI) variants by immunisation with heptapeptides comprising the protease binding sites. To identify repressor of primer protein (ROP) variants by monoclonal antibodies raised against the heptapeptide sequence of PSTI4, this sequence was inserted at the C-terminal end of the ROP protein (= ROP antigen). Protein antigens (B): The two proteins applied were recombinant human parathyroid hormone (hPTH) and ROP antigen. Monoclonal antibodies generated against the hPTH bound specifically to the N-terminal end of the protein. In the case of ROP antigen the antibodies were directed against the ROP part of the molecule and none could recognise the heptapeptide sequence of PSTI4.

Highly-active matrix-conjugated monoclonal antibodies for isolation and assaying of enzymes

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Many enzymes are present in physiological fluids in minute amounts. Their detection and quantitation are complicated by interference of foreign materials present in these fluids. Isolation of such enzymes by properly selected monoclonal antibodies (mAbs) prior to their assay should be of benefit. Thus, mAbs which do not interfere with the biological activity of the antigen and possess high affinity and specificity are desirable. Site-directed modification of such mAbs, followed by oriented immobilisation onto insoluble matrices was found to yield highly active mAbs–matrix conjugates. The carbohydrate moieties of anti-carboxypeptidase A and anti-horseradish peroxidase were chemically or enzymatically oxidised to yield reactive aldehyde groups located remote from the antigen binding sites of the antibodies. The oxidation procedure was optimised to achieve fully active modified mAbs. These were then immobilised onto hydrazide derivatives of agarose and Eupergit C. The antibody–matrix conjugates obtained possessed high antigen binding activities, close to the theoretical value of 2 mole of antigen per mole of matrix-conjugated antibody. The above approach should be applied as a general procedure for detection and isolation of enzymes in biological fluids.

Use of monoclonal antibodies to carcinoma-associated mucin in breast cancer

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Monoclonal antibodies to mucin glycoproteins have previously been shown to detect elevated levels of two new antigens, CAM26 and CAM29, in sera from breast cancer patients. This pilot study reports on CAM26 and CAM29 serum levels in 43 patients with breast cancer of all stages. The specimens were tested in the CAM26 and CAM29 Genetic Systems EIA. The cut-off level for CAM26 and CAM29 was respectively $\leq$100 U/ml and $\leq$20 U/ml, as defined in 30 healthy controls. According to the stage of disease, 30 patients were diagnosed as having operable breast cancer, preoperative stages T1NOMO–T4N2MO, and underwent CAM26 and CAM29 analysis before primary surgery. In 13 patients distant metastases (M+) were detected during the follow-up period. The median age of patients was 53. Only 2 out of 14 patients (14%) staging N− had elevated levels. Among patients staging N+ only 2 out of 16 (13%) patients showed CAM26. CAM29 was observed in 7 out of 13 patients (54%) with distant metastases (M+). None of 30 healthy controls had CAM26 and CAM29 elevated serum levels. Our data suggest that a combined marker elevation in the combination assay is highly indicative for advanced stage of malignant disease and marker elevation is found to correlate with extent of disease.

Evaluation of the new tumour-associated antigen CA-549 in breast cancer patients

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CA-549 is a new high molecular weight mucinous glycoprotein. In our investigation, we evaluated the clinical value of a sandwich-type monoclonal immunoradiometric assay (Hybri-BREScan, Hybritech) for 90 breast cancer patients with and without evidence of active tumour growth. The assay was tested for both analytical and clinical performance at multiple clinical trial sites. The test showed excellent reproducibility in intra- and interassay determinations as well as good linearity within the standard curve. The lowest detection limit was in the range of 1 U/ml, the cut-off for 95% specificity was 9.0 U/ml. At this specificity, there were 67% elevated values for patients with active cancer (n = 52), whereas there were only 14% elevated values for patients without evidence of disease (n = 29). In clinical follow-up studies, CEA and TPA were complementary to CA-549. These studies indicate that CA-549 is a useful test for monitoring breast cancer.

Value of MCA for serum diagnosis and radioimmunodetection

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We present results with the antibody MCA-b-12 which reacts with a mucin-like carcinoma antigen (MCA) expressed by breast cancer cells. The aim of the study was to investigate the clinical utility of serum determinations for detection of recurrent cancer and the value of the radiolabelled antibody for *in vivo* detection of tumours. The assay system and the antibody were received from Hoffmann La Roche AG, Basel, Switzerland. MCA was determined in 337 serum samples from patients with confirmed breast cancer. For immunodetection, 2 mg of antibody were labelled with 3–5 mCi by the iodo bead method and were infused in a 50 ml PBS solution. There was a 57% sensitivity of MCA at 95% specificity. In clinical follow-up, CEA (52% sensitivity) and TPA (51% sensitivity) were supplementary to MCA. Radioimmunodetection in 5 patients showed true-positive findings in 4 patients for detection of metastases in the skeleton. There was one false-positive result. Our results give evidence that MCA-b-12 is useful for monitoring patients with breast cancer and that localisation of tumour lesions will give additional information for the clinician.