Abstracts of Oral Papers

SESSION 1 : CANCER

Anti-tumor immunity derived from a genetically engineered fusion protein possessing the mouse/human chimeric antibody molecule and the cytokine moiety

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To target IFN-*(tau)* to tumor cells, recombinant antibody techniques were used to construct a RM_4/IFN-*(tau)* fusion protein possessing the chimeric $F(ab')_2$ - (RM_4) recognizing the human tumor associated TAG72 antigen and the IFN-*(tau)* moiety. The recombinant cDNA of IFN-*(tau)* was linked to the 3' end of the chimeric heavy-chain gene fragment (M_4) containing the V_H, the C_{H1} and the hinge region to form a fused heavy-chain gene fragment M_4-IFN-*(tau)*. Transfection of M_4 - IFN-*(tau)* into a mouse myeloma derived cell line $V_K C_K$ which produced the chimeric light chain of the same antibody, allowed the transfectant cell line $V_K C_K/RM_4$ secreting the fusion protein RM_4/IFN-*(tau)*. Our data showed that the RM_4/IFN-*(tau)* trained the TAG72-binding reactivity and the IFN-*(tau)* activity as meaured in ELISA, Western blotting, FACS analysis, up-regulation of the CEA-expression and in antiviral assay. Therefore, the recombinant fusion protein RM_4/IFN-*(tau)* was proved to be bifunctional. Since the secretion of fusion protein by the transfectant cell line $V_K C_K/RM_4$ creates a local high concentration of IFN-*(tau)* in tumor, this cell line thus became a good model to study the possible anti-tumor immune mechanisms derived from the fusion protein RM_4/IFN-*(tau)*. Our data showed that the RM_4/IFN-*(tau)* secreted by $V_K C_K/RM_4$ myeloma cells curtailed its tumorigenicity in BALB/c mice and further induced persistant protective immune responses against a subsequent graft of the parental $V_K C_K$ tumor. This protective immunity became complete 10 days and lasted up to at least 6 months subsequent to the $V_K C_K/RM_4$ tumor inoculation. Our results also demonstrated that the CD_8 CTLs play a major role in the reduction of tumorigenicity. The adoptive transfer of T-lymphocyte-enriched spleen cells or CTLs also conferred the significant protection against tumor growth of parental $V_K C_K$ cells ($p < 0.01$). Therefore, this study suggests that the fusion protein RM_4/IFN-*(tau)* may be useful in cancer immunotherapy due to its capacity of targeting IFN-*(tau)* to human tumors expressing the human tumor-associated TAG72 antigen to induce a subsequent immune destruction of tumor cells.

Cloning and expression of a tumor reactive human IgG4

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The human monoclonal antibody GM4 was generated by fusing the SHFP-1 human lymphoblastoid cell line with pooled lymphocytes from cancer patients isolated using immunomagnetic bead technology. Immunohistochemical staining of tumor and normal tissue indicated that this human IgG4 antibody preferentially reacted with melanomas. Further studies indicated that GM4 recognized an epitope in the carboxyl terminus of a 'vimentin-like antigen' (VLA), termed AgGM4. To generate a recombinant derivative of this human Mab, we isolated and expressed the complete heavy chain and light chain genes. The entire coding sequence for both the heavy and light chain genes was isolated by RT-PCR using a set of degenerate 5' signal sequence specific primers and a 3' constant region primer. High level antibody expression was achieved in Chinese hamster ovary (CHO) cell lines using the dihydrofolate reductase gene (dhfr) as a selectable amplifiable marker, and the drug methotrexate (MTX) to select for gene amplification. These lines have been adapted to grow in protein-free media. Recombinant GM4 reacted with human tumor cells and AgGM4 in a manner similar to the antibody produced by the parent hybridoma cell line, indicating that the specificity of the antibody was not altered during molecular cloning.

A human anti-idiotypic MAb against anti-GD_2 chimeric antibody ch14.18 from a patient treated for neuroblastoma

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Neuroblastoma is a tumor of neuroectodermal origin with a poor prognosis in advanced stages of disease. In clinical phase-I and -II trials with a mouse and a chimeric MAb against GD_2, 14G2a and ch14.18 respectively, objective responses including complete remissions were observed. Most patients developed significant levels of antibodies against the injected therapeutic antibody human antimouse antibody in the case of the mouse, and anti-idiotypic reactivity in the case of the chimeric construct. To investigate this immune response further, we cloned human antibodies from treated patients. PBLs of a patient treated with ch14.18 were EBV transfected and thereafter fused with the mouse-human heteromyeloma K6H6/B5 by somatic cell hybridization to produce stable human hybridomas. Hybridoma supernatants were screened in an ELISA for reactivity and the mouse antibody 14G2a which is composed of the same variable region domains as ch14.18 but a murine instead of the human Fc part. Cells of positive wells were further subcloned by limiting dilution using human IL-6 as a supporting cytokine. One stable subclone (anti-Id) was further propagated and the hybridoma supernatant partially purified using protein-G affinity chromatography. The subclass of this clone was determined in ELISA to be IgG_2 with a kappa light chain. In further ELISAs, the specificity of the anti-Id was analysed. The anti-Id showed a strong reaction with ch14.18 but a weaker reaction with 14G2a. No cross-reactivity with human Fc-fragments was observed. Whether the anti-Id is able to compete with GD_2 for binding to either 14G2a or ch14.18 is currently being investigated. However, this anti-idiotypic antibody reacting with ch14.18 will be helpful in establishing a specific, easy to use assay to determine pharmacokinetic data [1] in further clinical trials with ch14.18 or similar fusion proteins, respectively.[Supported in part by DFG grants #GA 167/5-1 and 167/6-1.]

1. Uttenreuther-Fischer MM *et al. Cancer Immunol Immunother* 1995; **41:** 29-36

A novel monoclonal antibody with immune-stimulatory and anti-tumor properties

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We have raised a novel monoclonal antibody (BAT MAb) to Daudi B lymphoblastoid cell line. This antibody stimulates both human and mouse lymphocytes. BAT MAb has a striking antitumor effect on different mouse tumor models. This is manifested by tumor regression and by prolonged survival. Adoptive transfer of splenocytes from mice which had been inoculated with B-16 melanoma and treated with BAT MAb into recipients bearing either melanoma or 3LL carcinoma tumors also resulted in tumor regression.

BAT MAb is also effective in the regression of human tumors. BAT failed to induce regression of SK-28 human melanoma inoculated (i.v.) into SCID mice. Engraftment of human PBL into SCID mice rendered the tumor bearing mice (pulmonary lesions) responsive to BAT.

Experimental *in vivo* data support the notion that the tumor and BAT synergizes in the activation of the host immune system.

In vitro studies in which mouse or human lymphocytes were co-cultured with tumor cells and BAT MAb, showed a synergistic effect in the induction of lymphocyte proliferation. FACS analysis revealed that BAT induced the generation of CD3/CD56 positive lymphocytes. These cells may also be involved in the anti-tumor effect.

These findings form a base for the potential clinical use of this antibody.

Mono- and bi-specific 'single chain' antibody fragments for use in cancer therapy

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Although MAbs have proven their use in many applications, they have serious limitations in clinical applications due to their nonhuman nature. In cancer therapy the hope lies in the design of smaller therapeutic antibodies that have less side effects, better tumor access and faster clearance rates. Therefore, 'single chain' anti- body fragments(scFv) could be a promising tool for biotherapy. Because of their size reduction of about 50%, scFv's are less immunogenetic, show better tumor penetration capacities and can be produced in bacteria (high yields). scFv's can be easily coupled to therapeutic molecules, such as toxins, to fulfill the role of delivery agent. Otherwise, two scFv's with different specificity could be covalently linked, resulting in bispecific single chain antibody gragments (biscFv). These antibodies can form a bridge between the effector and target cell. Our aim is to construct (bi)scFv antibodies with improved potentials for their use in breast cancer therapy. On the one hand, scFv's against MCF7-associated antigens (5D10 and 11F9) were made to be used in tumor cell targeting. On the other hand, scFv's specific for immune cells (CD3- and IL-2R positive T cells) were assembled. These scFv's are useful in triggering the immune system. Both advantages are joint in genetically engineered bispecific scFv fragments. We were able to construct scFv's from the variable light and heavy chain gene segments in one single PCR reaction. For this purpose, we designed universal (linker) primers based on consensus regions within the variable light and heavy chains. The primers possess restriction sites for direct cloning and, in the case of the linker-primers, for easy linker exchange. In addition we created a eukaryotic expression cassette for the (bi)scFv. This cassette contains the variable immunoglubulin leader sequence, an intron and a cloning site for the (bi)scFv's, 5' to the variable chain JH_2-sequence. Using this cassette the eukaryotic expression vector pST was created. This EBV-based vector is maintained extrachromosomally in primate and canine cells and expresses high levels of protein from the RSV-promoter. These such obtained (bi)scFv's were fully characterized by SDS-PAGE, Immunoblot, FACS analysis (bindings characteristics, Ca^{2+} uptake), ADCC test, proliferation test (thymidine uptake) and adhesion test (bi-specificity). *In vitro* tests will be performed to see if native T cells can be activated to eliminate MCF-7 tumor cells.

Human anti-PEM (polymorphic epithelial mucin) antibodies produced by specific B-lymphocytes isolated from tumor-draining lymph nodes

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PEM is a high molecular weight transmembrane glycoprotein expressed at the apical surface of several siplex epithelia. The extracellular domain of the protein core of PEM is made up of contiguous repetitions of a 20 amino-acids sequence named tandem repeat (TR). Tumor cells of epithelial histotype express new PEM glycoforms with shorter O-linked sugar side chains resulting in the exposition of new antigenic determinants and in an increase of immunogenicity of this self-protein. Experimental and clinical evidence shows that PEM is able to evocate cellular and humoral immune response in patients with PEM-expressing tumors. We first describe a humoral immune response in ovarian cancer patients and established B-cell clones producing human anti-PEM antibodies. These recognize the TR aminoacid sequence APPAH, which is distinct from the one immunodominant in mice, APDTRPAP. Antibody producing B-cell clones were obtained by infection of tumor draining lymph node B-cells. However, EBV methodology presents some limits, such as low efficiency/frequency of immortalization, prevalence of the IgM isotype and shut-off of immunoglobulin production. B-cell expressing anti-TR antibodies were isolated to increase the specificity of the B-cell population and to detect the potential IgG immune response towards this antigen in cancer patients. B-cells expressing membrane bound immunoglobulins were selected form freshly dissected lymph nodes using as a 'catcher' the 60mer peptide corresponding to 3TR, i.e. the minimum number of TRs able to assume the protein conformation and containing every possible linear epitope. The rescue of labelled B-cells was performed using an immunomagnetic method based on biotin-conjugate 60mer peptide and streptavidin-coated magnetic microbeads (Miltenyi). Highly sensitive immunoassay was used to test culture supernatants from such selected EBV immortalized B-lymphocytes for specificity and isotype. The immunoglobulins were detected by their ability to bind the biotin-conjugate 60mer linked to streptavidin-coated plates. Our results show that the described procedure allows the isolation of PEM specific B-lymphocytes. The immunoglobulins produced by these B-cell clones are both of IgM and IgG isotype. These results indicate the presence of a memory humoral immunity against the TR of PEM and confirm the role of PEM in the anti-tumor response of cancer patients. [Supported by AIRC and ACRO, CNR; A.R. is supported by AIRC.]

Receptor analysis of the idiotype antibodies of the MALT lymphomas by hybridomas

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Introduction: The immunoglobulin receptor produced by the MALT type lymphoma ß cells is thought to provide crucial information for the elucidation of its pathogenesis. Therefore, tumor cells are fused with appropriate partners to obtain sufficient amounts of the immunoglobulin for further analysis. In many earlier studies, hybridomas of the lymphoma cells have been produced and were typed by an ELISA assay in respect to isotype identity with the secreted lymphoma antibody. Recently, a high intra tumor mutational dynamic process in some MALT lymphomas has been demonstrated. This may lead to several subclones differing only by point mutations. Also the fusion of bystander cells with the same isotype may lead to misleading results. Thus, this study aims at a comparison between tumor antibodies and the antibodies produced by the different lymphoma-derived hybridomas at the DNA level.
Materials and Methods: Total RNA was extracted and cDNA was produced from 5 MALT type lymphomas and the 90 hybridomas using standard methods. PCR was performed using VH family specific FR1/JHb primers. When a single band was detected in the expected size range (about 300 bp), it was cut out and sequenced directly.
Results: After somatic hybridization and the check of the idiotype often multiple bands were detectable, indicating the presence of multiple clones within the hybridoma. After subcloning and a new check of the idiotype, a single band within the expected size range was detectable in most cases. The sequencing of the PCR products showed their identity with the lymphoma clone. Single mutations reflected the ongoing mutational dynamic within the MALT type lymphoma.
Conclusion: The hybridomas may serve as a tool for the elucidation of the pathogenesis of the MALT lymphoma as well as the intra tumor heterogeneity and mutational dynamics. They may serve as a powerful analytical tool to get results which otherwise may only be obtained by single cell analysis. To reach monoclonality within the hybridoma at least one step of subcloning with limiting dilution and subsequent check of the idiotype is needed.

Antihuman-TAA-antibodies can isolate p53 antigen from the serum of cancer patients and can be used as a new tool for colon cancer diagnosis

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The early cancer detection remains highly problematic, and modern methods do not always show positive results. We developed a new serological method for cancer detection using antibodies generated against the human tumor-associated antigens (TAA). The method is based on the determination of the serum-level of tumor-associated p53 kD cytoplasmic antigen in the cancer and non-cancer patients. The correlation coefficient between this protein and the total serum amount of TAA or total serum protein ranged from 0.55 to 0.93. The serum level of p53 protein was shown to be related to cancer. Therefore, the determination of its serum concentration can serve as a screening tool for cancer detection. The serum level of p53 protein ranges between 0.24 and 0.94 mg/ml in patients with non-cancer diseases, and between 1.0 to 2.0 mg/ml in patients with polyposis and in a high risk group, respectively, increased over 2.0 mg/ml in primary colon cancer patients and up to 5.0 mg/ml in cancer patients with metastases. The sensitivity and specificity of our method are 92% and 96% respectively, and accuracy is 88%. A strong correlation has been found between Duke's stage in colon cancer and the serum level of p53 protein. The presence of p53 protein in the cytoplasm of cells from patients with non-cancer disease may explain why p53 protein is presented in their sera. Our method can be useful to detect cancer development either as a primary illness or as a recurent disorder. It is possible to follow up patients with chronic diseases and to detect transformation of these diseases into cancer, or to follow-up former cancer patients in order to detect as early as possible incidence of recurrent cancer. It should also be emphasized that our method allows the detection of patients with polyposis or those of high risk groups who exhibit a tendency to develop colon cancer.