96

Expression Patterns and functional activity of the human 14C5 cell substrate adhesion molecule

Coene E., Willems K., Verbiest L., Wagner U.*, Schlebusch H.*, DePotter Ch.
N. Goormaghtigh Institute of Pathology, University Hospital, Ghent
*Dept.of Gyn.&Obst., University Bonn, S.Freud Str.25, 53105 Bonn

Cell substrate adhesion is a prerequisite for invasion and the subsequent formation of metastases. Therefore, we designed monoclonal antibodies (Mabs) against epitopes on the extracellular cell membrane domain of SK-BR-3 cells. One of the antibodies, called Mab 14C5, binds to an extracellular epitope of a plasma membrane antigen of SK-BR-3 and MCF-7 human breast cancer cells. This Mab 14C5 is able to inhibit cell substrate adhesion, not only on culture treated plastic but also on host tissue, and therefore prevents invasion and metastases. We evaluated the tissue distribution of the 14C5 antigen by immunohistochemistry. The antigen is specifically overexpressed in 64 % of invasive ductal adenocarcinomas of the breast (n=33), in all investigated cases of invasive squamous cell carcinoma (n=7) and in 40 % of basocellular carcinomas of the skin (n=5). The 14C5 molecule is located on the cell membrane of the carcinoma cells. However, when the tumour is characterized by a highly invasive phenotype, 65 % of the cases also show an extensive stromal expression on the fibroblasts between the tumour cells (n=71). This stromal expression is caused by the presence of the 14C5 antigen on the membrane of the adjacent fibroblasts. In normal tissues as well as in the stroma surrounding *in situ* carcinomas of the breast (n=15), no expression of the 14C5 antigen occurred. A 90 kDa protein was purified from lysates of human breast cancer cells using a 14C5 Mab sepharose column and is considered as the antigen, recognized by the Mab 14C5. The antigen was considered to be an effective target for passive and active immunotherapy and is therefore been introduced in in-vivo models to prove his efficacy as an immunological approach to tumor therapy in breast cancer.

97

COSTIMULATION WITH AGONISTIC ANTI-CD28 ANTIBODIES PREVENTS T CELL APOPTOSIS AND IS REQUIRED FOR EFFICIENT IMMUNOTHERAPY WITH CD3x19 BISPECIFIC ANTIBODIES IN B CELL LYMPHOMA

P.T. Daniel, A. Kroidl, A. Pezzutto, G. Moldenhauer*, B. Dörken. Department of Hematology, Oncology and Tumor-immunology, Robert-Rössle-Klinik, Virchow Klinikum, Humboldt University and Max Delbrück Center for Molecular Medicine, Berlin, and *German Cancer Research Center, Heidelberg

Bispecific antibodies (CD3x19) against the CD3 ε-chain of the T cell receptor/CD3 complex and the CD19 antigen on B cells can target non-tumor-specific T cells to B lymphoma cells. This induces T cell activation, generation of cytotoxic T cells (CTLs) and leads to lysis of the B lymphoma cells. This has been demonstrated *in vitro* and *in vivo* against B lymphoma cell lines but also in syngeneic B lymphoma cells. Further experiments showed that costimulation with agonistic anti-CD28 antibodies leads to better T cell activation and generation of CTLs. In a xenotransplant model in SCID mice, we observed that CD28 triggering in addition to CD3x19 administration decreases the number of T cells required for efficient B lymphoma cell elimination. In addition, we also found that co-stimulatory signals triggered by B7.1 or agonistic anti-CD28 antibodies abrogate the susceptibility for activation induced apoptosis of the CTLs during target cell recognition. This also explains the higher efficiency of costimulated CTLs in tumor cell elimination. We coined the term "veto" kill to describe the phenomenon that instead of further activation and clonal expansion of the CTLs after killing of the target the CTL itself is killed by apoptosis upon target cell contact. Thus, CD3x19 antibodies can target CTLs against B lymphoma tumor cells but a large proportion of the T cells dies by veto apoptosis. Blocking experiments showed that this effect is mediated by the Fas ligand which is produced by the T cells in an autocrine fashion. Costimulation with anti-CD28 antibodies efficiently prevented the veto kill of the activated CTLs during CD3x19 mediated T cell targeting. These data suggest that CTL-targeting by bispecific antibodies requires costimulation through CD28 to prevent veto apoptosis (one-hit-one-kill situation) and to allow clonal expansion.

98

The combination IFN-α/IL-2 and monoclonal antibody 17-1A induces a marked antibody dependent cellular cytotoxicity (ADCC) against the colorectal tumor cell line HT29

D.Flieger, S.Bungard, T.Sauerbruch, U.Spengler.
Department of General Internal Medicine, University Bonn, FRG.

Monoclonal antibodies specific for tumor associated antigens together with immunostimulating cytokines are promising therapeutic substances for the treatment of colorectal cancer because they can also induce attack of dormant tumor cells by immune cells. Thus, we investigated whether ADCC by MoAb 17-1A, which recognizes a tumor associated antigen, can be augmented by three day incubation with different concentrations of TNF-α, GM-CSF, M-CSF, IL-2, IFN-α and IFN-γ. ADCC was assessed by a new flow cytometric cytotoxicity assay (Flieger et al (1995) J.Immunol Methods, 180:1-13). All experiments were performed in triplicates with peripheral blood mononuclear cells from 5 healthy volunteers as effector cells. The antibody concentration of 50 µg/ml was used in all experiments, which was found optimal in titration experiments. All cytokines tested except IFN-γ were not cytostatic for HT29 alone. When immune cells were added, the cytokines IL-2, IFN-α and IFN-γ exerted some unspecific cytotoxicity towards the tumor cell line. The cytotoxicity of these cytokines in a concentration of 30 ng/ml was markedly increased by addition of antibody. However, the three other cytokines tested (TNF-α, M-CSF and GM-CSF) had no effect on ADCC at all. When the three effective cytokines were combined at lower concentrations the combination of IFN-α and IL-2 with antibody was found optimal in the induction of ADCC. Thus, in vitro testing allows to identify potentially useful combinations of cytokines and MoAb for the locoregional treatment of colorectal cancer.

#supported by Deutsche Krebshilfe#

99

MAb 14C5 against a human cell substrate adhesion molecule for inhibition of tumor growth in-vivo

Giffels P., Köhler S., DePotter Ch.*, Coene E.*, Nagel D., Krebs D., Schlebusch H., Wagner U.
*N. Goormaghtigh Institute of Pathology, University Hospital, Ghent
Dept.of Gyn.&Obst., University Bonn, S.Freud Str.25, 53105 Bonn

The cell substrate adhesion molecule (CA14C5) is typically present in high amounts on the tumour surface of in-situ and invasive breast cancer tissue and on other cancer tissues like adenocarcinoma of stomach, thyroidea and the colo-rectal tract. The 90 kd molecule is not found on normal tissues (exception: low-concentration on inflammatoric lesions). Furthermore the cell substrate adhesion receptor is involved in the metastatic process and blocking of the receptor by the antibody 14C5 results in a reversible loss of adhesion and invasion capacity in-vitro. We introduced the mAb 14C5 in an in-vivo model to prove his capacity for inhibition of invasion and metastasis. The human cancer antigen CA14C5 was introduced as a tumor-associated target for an immunotherapeutic approach in an in-vivo situation. Therefore, an immunocompetent animal model was chosen. The model uses a tumor cell line (HH16cl.1/2, rat adenocarcinoma/fibrosarcoma), which is overexpressing the human tumor-associated antigen CA14C5. In this model the passive immunotherapeutical approach is comprised by the application of the anti-tumoral antibody mAb14C5 in different concentrations after implantation of the tumor. 6 day old Sprague-Dawley rats (n=2x12) received tumor cells (2x10^6) subcutaneously. After one week series Ab1 received mAb 14C5 intraperitoneally at a dosage of 100 - 250 - 500 µg weekly (n=4 per group, total group n=12). A control group received polyvalent mouse IgG at the same dosage of 100 - 250 - 500 µg intraperitoneally weekly (n=4 per group, total group n=12). The tumor incidence in the used model was > 90%. The tumor growth was evaluated over a period of 60 days. 8 applications were administered in total.
The results showed a highly significant difference in the tumor growth as the 14C5 treated group developed a mean tumor size of 15.3±18.1 mm and the control showed a mean diameter 37.2±14.9 mm ($p<0.005$ t-test). Furthermore, the 14C5 treated animals showed a dose dependent inhibition of tumor growth with a significant reduction in the 500µg group.
In summary, the monoclonal antibody 14C5 against a human cell substrate adhesion molecule is able to inhibit tumor growth and invasion of CA14C5 overexpressing tumors in-vivo and therefore serves as a target for passive cytotoxic immunotherapy.