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VACCINATION OF RHESUS MONKEYS WITH AN ANTI-IDIOTYPE ANTIBODY MIMICKING LEWIS Y: ENHANCEMENT OF THE ANTI-TUMOR IMMUNE RESPONSE BY REPEATED ADMINISTRATIONS OF RECOMBINANT HUMAN GM-CSF.

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A murine monoclonal internal image anti-idiotypic antibody (MMA 383) has been raised against the anti-Lewis Y antibody ABL 364. The Lewis Y (LeY) carbohydrate antigen is frequently expressed on human epithelial cancer. Following repeated vaccinations of rhesus monkeys with MMA 383, a pronounced IgG immune response specific for LeY positive tumor cells is raised. The present study was designed to determine the role of recombinant human (rh) GM-CSF (Leucomax®) as adjuvant for vaccination of rhesus monkeys with a single suboptimal dose of MMA 383. GM-CSF was chosen since this cytokine is known to upregulate MHC class II antigens on antigen-presenting cells and to induce proliferation and maturation of dendritic cells. One group of monkeys was vaccinated with a single low dose of MMA 383 only (10µg/kg) adsorbed on alum. A second group in addition to this vaccination received rhGM-CSF (5µg/kg) on the vaccination day only. A third group in addition received rhGM-CSF on 4 consecutive days (5µg/kg/day), starting with the vaccination day. Sera were taken before, 2 weeks and 4 weeks after vaccination and the influence of rhGM-CSF on induction of specific antitumor antibodies was determined: Due to the suboptimal single vaccination chosen, in the sera of rhesus monkeys treated with MMA 383, at week 2 no tumor specific antibodies were detected and at week 4 only a low titer was observed. Concomitant treatment with rhGM-CSF for 1 day had no significant influence on induction of specific antitumor antibodies. In striking contrast, if rhGM-CSF was given for 4 consecutive days, a pronounced humoral antitumor immune response was found already at week 2 which was further increased at week 4. Apparently, for enhancement of antitumor antibodies a sustained concnetration of rhGM-CSF is required.

In conclusion, rhGM-CSF may act as powerful and selective adjuvant for therapeutic vaccinations of cancer patients with anti-idiotype vaccines if a certain concentration is maintained for a few days.

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ADJUVANT HIGH DOSE EPIRUBICIN AND CYCLOPHOSPHAMIDE WITH G-CSF SUPPORT IN PATIENTS WITH LOCALLY ADVANCED BREAST CANCER

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In breast cancer patients with $\geq$ 10 positive axillary nodes or peri-nodular involvement the prognosis following adjuvant chemotherapy is poor. In this prospective randomized multicenter study until now 51 patients at our hosipital in pre- and postmenopause with $\geq$ 10 positive axillary nodes or perinodular infiltration were randomized to receive 4 cycles EC 90/600 mg/m2 every 3 weeks and 3 cycles CMF (arm A) or a more intensive chemotherapy including **4x EC 120/600 mg/m2 every 2 weeks with G-CSF support 5 µg/kg from day 2 to 12** (arm B) to reduce myelosuppression. 25 patients received arm B. In 5 patients leucopenia $< 1000/\mu l$ occured. In 13 patients leucocyte nadir was $< 2000/\mu l$. 11 out of 97 cycles had to be delayed because of thrombocytopenia. No cases of cardiac toxicity have been recorded **yet**. Alopecia was observed in 100% of subjects. Nausea and vomiting occured in 75% of the patients despite prophylactic Ondansetron and Dexamethason medication. Chemotherapy was administered via port-a-cath systems. In both arm A and B one extravasate occured.

We were able to increase the delivered dose-intensity for Epirubicin from 28 to 52 mg/m2/week. At a median follow up of 9 months 0/25 patients relapsed in arm B, 3/26 patients relapsed in arm A.

Conclusions:

1. This dose intensified Epirubicin containing regimen with G-CSF support is tolerable and safe.
2. The addition of G-CSF (5 µg/kg day 2 to 12) results in an increase in delivered dose-intensity of 86%.
3. A significant improvement of disease free survival or overall survival by dose-intensity concepts has not yet been proven in randomized trials.
   Patient accruel of this multicenter randomized trial should be complete by 7/95.

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A HEAT INDUCIBLE HSP72 ASSOCIATED IMMUNOGENIC DETERMINANT ACTS AS A TUMOR SPECIFIC RECOGNITION STRUCTURE FOR NK CELLS

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Intracellularly, heat shock proteins (HSP) perform a variety of chaperoning functions e.g. they play a role in the translocation, assembly and disassembly of other proteins. Recently, data are accumulating that HSP, especially members of the HSP60, 70, 90 and 100 families are also found on the cell surface. Despite the fact that HSP are among the most highly conserved proteins, it has been demonstrated that they act as specific target structures on tumor cells eliciting an anti-cancer immune response (Srivastava et al., 1994). In an attempt to study the immunological consequences of a local hyperthermia treatment on sarcomas, we established an in vitro model using different human sarcoma cells. We could show that nonlethal heat shock leads to a HSP72 cell surface expression, selectively on human tumor cells (see abstract Wiesnet et al.), but not on normal cells. Despite a reduced MHC class I expression after heat shock the immunogenicity of heat stressed tumor cells was substantially increased, as demonstrated in a standard Cr-51 release assay. By antibody blocking studies this increased immunogenicity could be correlated with the cell surface expression of HSP72; an HSP72 specific MAb was able to inhibit the increased lysis of heat shocked tumor cells, whereas an MHC class I specific MAb has no inhibitory effect. In addition, separation of the effector cell population into a CD3- NK and a CD3+ CTL population clearly demonstrates that the increased lysis of heat shocked sarcoma cells is mediated by CD3-, non-MHC restricted NK cells. Preliminary data obtained from a combined thermochemotherapy of patients suffering from sarcomas (RHT91, Issels et al.) are encouraging in both, local tumor control and long term survival within a 3 year time interval. These observations led us to the hypothesis that in vivo thermochemotherapy might be able to induce HSP as well; this might imply a role of HSP in eliciting an immune response against cancer.

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A STRESS INDUCIBLE HSP72 CELL SURFACE EXPRESSION IS OBSERVED SELECTIVELY ON HUMAN TUMOR CELLS

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Heat is one possible stress factor that induces the synthesis of heat shock proteins (HSP), especially of members of the HSP70 family. HSP are highly conserved proteins with a wide phylogenetic distribution that function as molecular chaperones. Beside their intracellular localization, more recently members of the HSP60, 70 and 90 family were found to be expressed at the cell surface. The aim of the present study was to analyse the effects of nonlethal heat shock on intracellular induction and cellular distribution of members of the HSP70 family (HSP72 and HSP73, respectively) in tumor versus normal cells. As tumor cells we used Ewing's sarcoma cells, HOS58 and MG63 osteosarcoma cells and HT29 colon carcinoma cells; as normal cells we used PBL, EBV transformed B-LCL and fibroblasts derived from healthy human individuals. We could show by 2D SDS-PAGE of metabolically labeled cellular extracts of tumor and normal cells that the relative rate of HSP72 and HSP73 induction was comparable in both cell types (about 20x for HSP72; about 3x for HSP73). Furthermore, the total amount of HSP72 in tumor and normal cells after nonlethal heat shock was also comparable. In contrast, tumor cells differ substantially from normal cells in their HSP72 expression pattern. By flowcytometric analysis of heat shocked tumor and normal cells using a HSP72 we found that only tumor cells express HSP72 at the cell surface. A tumor specific cell surface expression of HSP72 could be confirmed by selective cell surface radioiodination of heat treated tumor cells and by specific immunoprecipitation using the HSP72 monoclonal antibody. Our observations might imply a role of HSP72 as a tumor specific, heat inducible target structure to elicit an immune response against tumors (see abstract Botzler et al.).

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