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Monocyte-derived Dendritic Cells to induce immunity to cancer

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Dendritic Cells (DC) are a promising approach to induce immunity. We have explored fully mature monocyte-derived DC to induce immunity to melanoma. In two initial clinical trials we used Mage-3 peptide-loaded DC and found unequivocal evidence for CTL induction in the majority of the far-advanced stage IV melanoma HLA-A1 or -A2.1 patients that had all been progressive despite standard chemo(immuno-)therapy (= expected median survival 4 months). These results thus provided the "proof of principle" that DC vaccination can expand tumor antigen-specific CD8+ T cells even in advanced cancer patients. Regression of some metastases was observed in ~ 50% of the HLA-A1+ patients after 5 bi-weekly vaccinations, and upon further vaccination and follow up prolonged survival now appears likely. As a next step we have systematically improved the DC vaccine by a) identifying and characterizing optimal maturation stimuli, b) generation of DC from leukapheresis in a closed system, and c) development of an effective freezing procedure to generate antigen-loaded cryopreserved DC aliquots. After these optimizations we initiated a clinical trial where melanoma patients are vaccinated with DC loaded with both MHC class I and II restricted peptides. The immunomonitoring results show that the DC appear particularly effective in inducing potent tumor-specific Th1 cells (both effector and memory cells). We will next explore RNA-transfected DC as a modified electroporation technique allows a 80% transfection rate. We are confident that the strategy to i) first improve the DC vaccine in vitro, and then ii) test it in clinical pilot trials employing DCs pulsed with *defined* antigens in conjunction with established immunomonitoring methods will allow to rationally optimize DC vaccination strategies.

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Heat shock proteins and dendritic cells: effects on maturation and antigen presentation

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Heat shock proteins (HSP) can elicit a specific immune response due to their ability to chaperone antigenic peptides as HSP-peptide complexes (PC). HSP can also act independently from associated peptides in a cytokine-like manner. Due to this dual role chaperone function and cytokine-like activity, HSP are regarded as chaperokines. We could show that DC pulsed with HSP70-PC Tyr+ could activate tyrosinase specific CTL. In contrast DC treated with HSP70-PC (Tyr-) were unable to stimulate tyrosinase specific CTL. The activation observed was the result of receptor mediated endocytosis of HSP70-PC. In comparison to DCs pulsed with tyrosinase peptide alone DCs pulsed with HSP70-PC(Tyr+) exhibited a more than hundredfold greater capacity to stimulate tyrosinase specific T cells.

Heat shock proteins can act as danger signals to DCs .We investigated the effect of recombinant rHSP70 and rHSC70 on the differentiation and maturation of monocyte-derived DCs. We found that only inducible HSP70 but not the constitutively expressed HSC70 stimulated the maturation of immature monocyte-derived DC. Cytokine-driven differentiation of monocytes into DC was suppressed by inducible HSP70. In addition immature DC but not mature DC were able to bind rHSP70. Recombinant HSP70 matured antigen-pulsed DC were more efficient antigen-presenting cells. These findings demonstrate the specific ability of rHSP70 to induce the maturation of immature DC.

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IMMUNOTHERAPY OF MALIGNANT DISEASES WITH PEPTIDE PULSED DENDRITIC CELLS.

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Dendritic cells (DC) are the most potent APC with the unique capacity to initiate primary immune responses. For clinical use DC can be generated in vitro from CD34+ peripheral blood progenitor cells or monocytes. Vaccination of patients with cancer using DC was shown to be effective for B-cell lymphoma, renal cell carcinoma, prostate cancer and malignant melanoma. We provide evidence that patients with advanced breast and ovarian cancer can be efficiently vaccinated with autologous DC pulsed with HER-2/neu- or MUC1-derived peptides. In 5 of 10 patients, peptide-specific cytotoxic T lymphocytes (CTL) could be detected in the peripheral blood using both intracellular IFN-gamma staining and Cr-release assays. In addition, in one patient vaccinated with the MUC1-derived peptides, CEA- and MAGE-3 peptide-specific T-cell responses were detected after several vaccinations. In a second patient immunized with the HER-2/neu peptides, MUC1-specific T lymphocytes were induced after 7 immunizations, suggesting that antigen spreading in vivo might occur after successful immunization with a single tumor antigen. Currently we are analyzing the effect of T-helper epitopes and IL-2 on the CTL induction using peptide pulsed DC. In this ongoing trial one patient with metastatic renal cell carcinoma (RCC) developed a partial remission of the metastatic sites was induced after the first 4 vaccinations with MUC1 peptides pulsed DC, that was ongoing after the next cycles containing IL-2. Vaccine-induced peptide specific T-cell responses in vivo were detected in the PBMNC of this patient and in peptide specific DTH reactions. Another RCC patient developed a regression of a pulmonary metastasis after four vaccinations while being stable with abdominal leasions. This studies demonstrate that peptide pulsed DC can be effective in cancer patients and induce significant clinical and immunological responses, especially in patients with RCC.

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