62

Phenotypic and Functional Changes of Mouse Dendritic Cells after Infection with Vaccinia Virus: Comparison of Three Different Strains

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At present time genetically engineered viral vectors represent the most efficient means for ex vivo transduction of dendritic cells (DC). In this study we determined the transduction efficiency and the effect of transduction on the phenotype and function of mouse dendritic cells (DC) when the recombinant vaccinia virus (rVV) was used as a gene transfer vehicle. We compared three different strains of rVV: clone P13 of strain Praha generated from smallpox Sevac VARIE (P13) vaccine, Western Reserve strain (WR) and a modified VV Ankara (MVA), which is a highly attenuated VV strain. Mature DCs, generated according to the protocol described by Lutz et al. in the presence of GM-CSF and IL4, were infected at different multiplicities of infection (MOI) with active and replication-incompetent VV recombinants (2μg of psoralen, ultraviolet light, 366 nm, 5 minutes) expressing green fluorescence protein (GFP). The infection rate, the percentage of viability, phenotypic and functional changes were evaluated by flow cytometry analysis one to five days postinfection. The stimulatory capacity of infected DC was analyzed in allogeneic mixed leukocyte reaction (MLR). After quantification of the percentage of living cells infected with rVV (GFP positive) simultaneously expressing surface markers typical for mature DC, the best results were obtained with MVA. Active viruses were always more effective than UV-inactivated. In all cases the rVV infection did not alter the ability of DCs to express relevant cell surface markers. In contrast, infection with UV-inactivated rVV led to increased DC maturation. Virally transduced DCs retained their ability to stimulate T-cell proliferation in the MLR.

63

Plasmacytoid dendritic cells in tumor tissue as target for immuno-therapy with CpG oligonucleotides of head and neck squamous cell carcinoma

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Plasmacytoid dendritic cells (PDC, CD123+) PDC are the major producers of type I IFN (IFN-α and IFN-β) upon viral infection in humans. In previous studies we identified immunostimulatory CpG oligonucleotides (CpG ODN) as a unique microbial stimulus to stimulate PDC to produce the Th1 cytokines IFN-α and IL-12. Although head and neck squamous cell carcinoma (HNSCC) is infiltrated by immune effector cells, patients with HNSCC show a profound impairment of cell-mediated immune functions including T cells, NK cells and monocytes. In this study we examine the presence of PDC in tumor tissue and the immunological effects of CpG ODN on T cell subsets and antigen presenting cells in tumor-draining lymph nodes in patients with HNSCC. We found that single cell suspensions of healthy human nasal mucosa and of nasopharyngeal lymphoid tissue (adenoids) contained 0,2-1 % PDC as detected by flow cytometry. In patients with HNSCC we could identify 0,3-0,5 % PDC in single cell suspensions of freshly prepared tumor tissue. Next, we prepared single cell suspensions from tumor-draining lymph nodes and incubated them in the presence or absence of CpG-ODN. After 48 h CD4+, CD8+ and MHC II-positive antigen presenting cells were analyzed by flow cytometry. CpG ODN markedly increased activation of antigen presenting cells as demonstrated by increased expression of CD80 and CD69 and of CD4+ and CD8+ T cells as shown by increased expression of CD69. In conclusion we for the first time demonstrate the presence of PDC in solid tumor tissue. PDC which in the absence of an appropriate stimulus were reported to be involved in tolerization of T cells may play an important role in impairing a T cell mediated immune response against HNSCC. Our results suggest that immunotherapy with peritumoral injections of CpG ODN might help to overcome T cell anergy in patients with HNSCC.

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64

Effective immunotherapy of large established tumors with CpG oligonucleotides and dendritic cells in murine tumor models

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To test the therapeutic potential of dendritic cells (DC) and oligonucleotides containing immunostimulatory CpG motifs (CpG ODN) tumors were induced by s.c. injection of syngeneic C26 or Renca tumor cells in Balb/c mice. In a first protocol DC were coincubated with irradiated tumor cells and activated by CpG ODN 1826 in vitro. Injection of these DC on day 5 after tumor challenge induced a tumor-specific immune response which cured mice. In this protocol no CpG ODN was present in vivo. In a second protocol weekly injections of CpG ODN without DC into the margin of the tumor were performed. This monotherapy lead to rejection of local and distant established tumors as a result of the combined activation of innate effectors and tumor specific CD4 and CD8 T cells. In a third approach we combined both protocols. CpG ODN were coinjected with antigen-loaded DC in mice with large established tumors. In this setting the effect of DC alone was poor. Coinjection of CpG ODN potently improved efficacy of DC. The optimal regimen was found to be coinjection of DC and CpG at sites distant of the tumor and simultaneous injection of CpG ODN into the tumor margin. This therapy controlled tumor growth of tumors with 1.4 cm in diameter.In conclusion we established an optimized protocol based on a combination of DC and CpG ODN which allows the control of large established tumors. These results form the basis for clinical studies testing DC and CpG ODN for the treatment of human cancer.

65

DENDRITIC CELLS GENERATED IN THE PRESENCE OF IL-13 AND GM-CSF IN A GMP LARGE SCALE PRODUCTION PROCESS ARE POTENT TUMOR ANTIGEN STIMULATORS AND ARE WELL TOLERATED BY CANCER PATIENTS.

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IDM has developed a technology that allows the production of large amounts of clinical grade DC from bulk leukapheresis products. IDM's DC generation system is adapted to produce a pharmaceutical grade product (DΦ®) thanks to the stringent control of the procedure by a single-use cellular processor (CE mark holder). DΦ, generated from blood monocytes in serum free medium containing GM-CSF and IL-13, are irreversibly committed DC and their immature phenotype remains stable after withdrawal of cytokines and exposure to IL-10 in vitro. Functional and phenotypic maturation of DΦ is observed after treatment with several agents, some suitable for clinical use. Induction of tumor specific T cell responses in vitro can be induced by mature DΦ in the absence of exogenous cytokines. These tumor specific cells lyse EBV or T2 targets loaded with tumor-derived peptides as well as tumor cell lines. Two Phase I/II clinical trials using DΦ have been started. Prostate cancer patients received DΦ pulsed with rPSA. PSA-specific and clinical responses have been observed in some patients after vaccination. Melanoma patients have been treated with DΦ loaded with a melanoma line-derived lysate. No adverse reactions have been observed to these vaccines. Patient-derived DΦ have shown identical function and phenotype in vitro than healthy donor-derived DΦ.