Wednesday 20 November

WORKSHOP

Cancer vaccines, virotherapy, T-cell directed immunotherapy

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Dendritic cell therapy, clinical and histological aspects

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In the past decade, dendritic cells (DC) have been established as a major component of the cellular antitumor immune response, and culture techniques have allowed the generation of adequate numbers of DC for clinical studies. Current major problems are the low incidence and the relative unpredictability of clinical remissions, and with that the difficulty in patient selection. The availability of validated surrogate biologic markers to monitor their efficacy would greatly improve the development of DC-based vaccines. This would also be of great value in the testing of many other aspect of DC therapy, such as culture methods, schedule, dose, route of administration etc. Yet to date none are available. This is further hampered by the lack of T-cell specific antigens on most tumor types. The results of studies on tumor-specific T cell responses in the peripheral blood of vaccinated patients have been disappointing. This is not surprising given the fact that a relevant response, if any, should take place at the site of the tumor. The sampling of tumor tissue from cancer patients for these purposes imposes ethical and logistic problems.
We have investigated several variables of peptide-pulsed DC in melanoma patients. As also shown by others, we found that mature DC, when compared to immature DC, have improved migratory capacities as well as improved DTH reactions in the skin proliferative T cell- and humoral responses in peripheral blood to KLH which we use as a control epitope. More importantly, our preliminary results indicate that DTH testing is a promising surrogate marker to evaluate the efficacy of our DC vaccine in that tumor-specific T cell responses were detected in DTH biopsies taken from patients after vaccination. This was evident from the culture of T cells as well as tetramer staining on frozen sections of DTH biopsies. In these biopsies we only detected T cells directed against the immunogen which was presented on the DC used to evoke the DTH. Intriguingly, patients in whom these specific immune responses were detected had improved clinical outcome. Although the number of patients are yet too small for definite conclusions, these results show that DTH may be a promising tool for the immunomonitoring of DC vaccines.

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Cancer vaccines based on defined tumor antigens: specificity with an eye on prevention

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Molecular characterization of tumor antigens that are immunogenic in humans is an important goal of tumor immunologists. The incentive is to create effective tumor-specific immunogens and thus increase the immune response to a level necessary to reject the tumor. One such antigen is the MUC1 mucin. Expressed on normal ductal epithelial cells as a highly O-glycosylated molecule, composed almost exclusively of 20aa tandem repeats, MUC1 undergoes profound changes in glycosylation on tumor cells. This change determines the outcome of its processing and presentation by APC. MUC1 produced by tumor cells is not processed and presented by patient's APC, resulting in the lack of significant MUC1-specific immunity. Synthetic forms of MUC1 that can be introduced through vaccination, can be processed by DC and elicit MUC1-specific helper T cells, CTL and multiple antibody isotypes. MUC1 peptide vaccines have been tested in animal models and in cancer patients in phase I clinical trials. Animal studies have shown what all tumor immunologists have known for decades but have recently chosen to forget: vaccines work in prevention and not in therapy. MUC1 is expressed aberrantly not only on tumor cells but also on premalignant cells. Normal epithelial cells of the colon do not express MUC1 but polyps do and adenomatous polyps show all the changes in glycosylation and overexpression documented for colon tumor cells. Vaccinating individuals with MUC1 based vaccines to prevent polyp recurrence thereby preventing colon cancer, would be expected to have a larger impact then vaccinating people who have already developed cancer.
The second tumor antigen that is a candidate for preventive cancer vaccines is Cyclin B1. Cyclin B1 is a cell cycle regulatory protein that is aberrantly expressed in the cytoplasm of tumor cells where it is found in large amounts that are never encountered in normal cells. Tumors that aberrantly express Cyclin B1 belong to many different types, solid tumors as well as lymphomas, and they all have functionally inactive p53. Inactivation of p53 function and resulting overexpression of Cyclin B1 are very early changes in the transformation process and are characteristic of premalignant lesions as well as fully developed tumors. This suggest the possibility of vaccinating patients who have been diagnosed with prenoeplastic changes in hope of eliminating these lesions and preventing further transformation to cancer.

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Immunotherapy of herpesvirus reactivation

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Reactivation of chronic herpes virus infections often occurs on the background of transient or long-lasting immunosuppression supporting the role of immunity in anti-viral surveillance. Reactivation of Epstein-Barr virus (EBV), one of the best-characterized tumor associated human herpes viruses, serves as a prognostic marker for a number of EBV-associated malignancies. The frequency of EBV-associated post-transplant lymphoproliferative disease (PTLD) depends on the type of transplant and efficiency of immunosuppression. In bone marrow transplant recipients, PTLD can be treated or prevented by administration of in vitro expanded donor-derived EBV-specific cytotoxic lymphocytes (CTLs). Our data suggest that prophylactic administration of EBV specific CTLs of donor origin is the most efficient way to prevent PTLD development and control excessive EBV reactivation. Alternative approaches include the development of vector systems for transferring EBV specific T-cell receptors (TCR) to peripheral blood T-cells. In this case, the strategic choice of TCRs should be based on knowledge of TCR's peptide specificity, allo cross-reactivity and clonal composition of the specific TCR repertoire.
The mechanisms of EBV reactivation in vivo include proliferation of latently infected B-cells and lytic virus replication. The latter appears to be associated with a particularly high tumor risk. EBV infection of monocytes inhibits their development into dendritic cells and causes their apoptotic death. These findings suggest a novel mechanism for EBV interference with the development and maintenance of EBV-specific immune response and indicate that release of EBV virions could be an important factor of tumor progression.

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DNA vaccines for cancer

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More than 99% of cervical cancers have been associated with human papillomaviruses (HPVs), particularly HPV type 16. Two HPV oncogenic proteins, E6 and E7, are consistently co-expressed in HPV-expressing cervical cancers and are important in the induction and maintenance of cellular transformation. Therefore, immunotherapy that targets E7 and/or E6 proteins may provide an opportunity to prevent and treat HPV-associated cervical malignancies. It has been established that T cell-mediated immunity is one of the most crucial components to defend against HPV infections and HPV-associated lesions. Therefore, effective therapeutic HPV vaccines should generate enhanced E6/E7-specific T cell-mediated immune responses. It is now clear that dendritic cells (DCs) are the most potent professional antigen presenting cells (APCs) that prime helper and killer T cells *in vivo*. Thus, effective vaccines would most likely require a strategy that targets tumor antigens to DCs. Intradermal administration of DNA vaccines via gene gun represents an efficient way to deliver DNA vaccines into professional APCs *in vivo*. The gene gun approach enables delivery of DNA vaccines into epidermal Langerhans cells, which move into the draining lymph nodes to further activate T cells. Thus, this delivery method allows for direct targeting of genes of interest into professional APCs *in vivo*. We have successfully used this system to test several intracellular targeting strategies that enhance MHC class I and class II presentation and have generated impressive results. One major limitation of DNA vaccines is their intrinsic inability to amplify and spread *in vivo* as some replicating viral vac-