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Mechanism of Tumor Eradication by Transfer of Sensitized T Cells.
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Lymph nodes (LN) draining tumor vaccines are a rich source of sensitized T cells. Upon ex vivo stimulation with anti-CD3 and IL-2, they mature into potent effector cells. When systemically transferred, they are capable of eradicating advanced murine tumors established in the lung, skin and brain. In the absence of defined tumor antigens, we found that sensitized T cells belonged exclusively to a sub-population of cells with down-regulation of the homing molecule, L-selectin. With purified L-selectin^{low} cells, CD4 and CD8 T cells could independently mediate tumor regression but the combination of both subsets of T cells demonstrated the greatest therapeutic efficacy. Since most solid tumors do not express MHC class II molecules, we found that the transferred cells recognized tumor antigens indirectly through cross-priming by the host APCs. Extensive mechanistic studies have revealed that the transferred T cells must gain access to infiltrate into the tumor mass and this process is dependent on G protein-coupled chemokine receptors required for diapedesis, but is not immunologically specific. However, antigenically specific proliferation of infiltrating T cells at the tumor site is required for therapeutic efficacy. The proliferation of T cells may be enhanced by conjunctional treatment with IL-2 or mAb to ligate T cell costimulatory molecules such as OX-40R and 41BB. However, exogenous IL-2 treatment had deleterious effects on T cells by inhibiting their trafficking to tumors located in the brain but not in the lung. These studies have helped establish necessary principles and methodologies for the design of adoptive T-cell immunotherapy in the treatment of patients with renal cell carcinoma, malignant melanoma and high-grade gliomas. Clinical trial results suggest vaccine design to augment LN immune responses may prove to be critical for improved therapeutic outcome.

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A TGFβRII frameshift mutation derived CTL epitope recognised by HLA-A2 restricted CD8+ T cells

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Microsatellite instability (MSI) is recognised as genome-wide alterations in repetitive DNA sequences caused by defects in the DNA mismatch repair machinery. Such mutation patterns have been found in most analysed malignancies from patients with hereditary non-polyposis colorectal cancer (HNPCC), and in approximately 15% of sporadic colorectal cancers. In cancers with the MSI phenotype, microsatellite-like sequences in coding regions of various cancer-related genes including transforming growth factor β receptor type II (TGFβRII) are targets for mutations. The TGFβRII gene harbors a 10 bp polyadenine tract and mutations within this region are found in 90% of MSI+ colorectal cancers. The frameshift mutations result in new amino acid sequences prematurely terminating where a novel stop codon appears. In this study we have defined a new cytotoxic T lymphocyte (CTL) epitope (RLSSCVPVA), carrying a good HLA-A*0201 binding motif, and resulting from the most common frameshift mutation in TGFβRII. A CTL line and several CTL clones were generated from an HLA-A2+ normal donor by repeated stimulation of T cells with dendritic cells pulsed with the peptide. One of the CTL clones was able to kill a HLA-A2+ colon cancer cell line harbouring mutant TGFβRII. This epitope may be a valuable component in cancer vaccines directed at MSI+ carcinomas.

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T cell polarization in tumor vaccine draining lymph nodes (TVDLN) correlates with the tumor immunogenicity
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The TH1/TH2 paradigm is crucial for the understanding of the dichotomy of the acquired immune response in cell mediated (TH1) and humoral immunity (TH2). Here we investigated whether tumor immunogenicity determines the immune response in TVDLN. Three methylcholanthrene induced sarcomas (MCA304, 309, 310) and three prostate tumors (MPR3, 4, 5) with defined immunogenicity were examined for their potential to polarize T cells to a TH1 (IFN-γ) or TH2 (IL-4) phenotype. C57BL/6 mice were vaccinated s.c. with 10^6 tumor cells. TVDLN were harvested 8 days later and L-selectin^{lo} T cells selected by magnetic bead separation to enrich for tumor-sensitized T cells. Tumor-specific cytokine release of the activated T cells was determined by ELISA. Immunogenic tumors (MCA304, 309, MPR4) induced predominant TH1 T cells with high tumor-specific IFN- γ (284 pg/ml) and low IL-4 (32 pg/ml) release (INF- γ:IL-4 = 17.9, mean of 9 exps.). In contrast, weakly (MCA310) and non-immunogenic tumors (MPR3, 5) sensitized predominant TH2 T cells with tumor-specific IL-4 (90 pg/ml) and low IFN-γ (16.8 pg/ml) release (INF-γ:IL-4 = 0.57, mean of 9 exps.). There is a significant correlation ($p < 0.025$) between immunogenicity and the IFN-γ:IL-4 ratio (mean of 18 exps.). Poorly immunogenic tumors induced a predominant TH2 immune response, whereas immunogenic tumors exhibited a predominant TH1 response. Our results suggest that the failure of tumor vaccination is due to the nature of the immune response, not to its absence.

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Induction of T cell immune response after DNA immunization with human Cytochrome P450 CYP1B1 – a potential cancer therapy.
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Cytochrome P450 constitutes a large family of enzymes participating in the oxidative activation and/or deactivation of a range of endogenous compounds and xenobiotics. The human CYP1 gene family, one of the major P450 families, consists of three individual forms classified into two sub-families. CYP1B1, a member of one sub-family, is 543 aa long, located in the ER and mitochondria, and structurally distinct from the two members of the CYP1A sub-family. Various types of cancers show tumor-specific expression of CYP1B1. Immunohistochemistry demonstrate reactivity to multiple tumors including bladder, breast, colon, kidney, lung, esophagus, ovary, skin, stomach, uterus, brain and others. CYP1B1 DNA constructs, including selective mutations that inactivate its biologic activity, have been evaluated in HLA-A2 transgenic mice. Animals received 100ug DNA intramuscularly every 2 weeks and ELISpot assay was done 12 days after each boost (1-3 boosts). T cell response was tested against recombinant Vaccinia-CYP1B1 or peptide pulsed EL-4A2/Kb lymphoma target cells. High reactivity to the CYP1B1 epitope 190 and recVaccinia-1B1 was consistently found in immunized animals. None of these animals had pathologies distinct from the negative controls. The human CYP1B1 DNA vector formulated with our biodegradable polymer microspheres delivery system is currently evaluated as a candidate cancer vaccine. Results obtained using this DNA delivery system for induction of CTL targeting CYP1B1 will be discussed.