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Adoptive Gene-Immunotherapy of Advanced Cancer Using Engineered Lymphocytes

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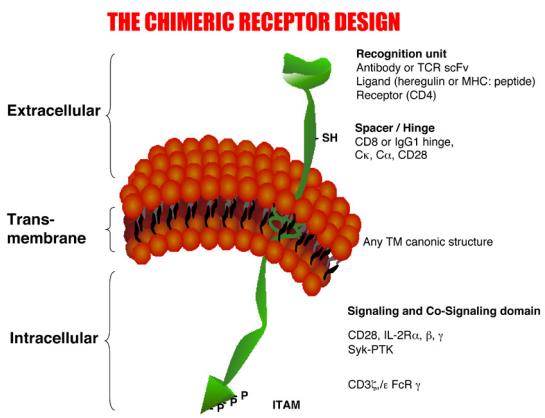
The immune system has been evolved not only to selectively recognize foreign from self-antigens but also to differentiate self from modified-self, such as occurs following viral infection and neoplastic transformation. Accordingly, the immune system is equipped with versatile arsenal to identify and eliminate malignant cells that express tumor associated antigens. T cells are the major mean the immune system uses to recognize and efficiently and completely eradicate both large tumors as well as disseminated metastasis. Yet, functional, specific T cells are rare in most malignancies due to the fact many spontaneous tumors escape and evade their attack. Anti-tumor antibodies that can be more readily prepared are less effective, especially in the elimination of solid tumors. Based on our ability to genetically program and redirect the recognition of T cells using chimeric receptors, we have endowed T cells with antibody-type anti-tumor specificity and thereby combined the advantages of both cellular and humoral arms of the immune response to combat cancer. These studies have been performed in animal models using human breast and prostate cancer xenografts attempting to treat advanced disseminated cancer, a disease stage that is by-and-large incurable. The various prostate cancer xenografts that we have established are also being used to study the patho-physiology of this disease.

Redirecting effector lymphocytes using chimeric receptors with antibodies specificity

To expand the recognition spectrum of effector lymphocytes and redirect them to predefined targets we expressed in T and NK cells chimeric receptor (CR) genes with antibody specificity. The modular structure of the CR allowed it's engineering to fit a desired specificity and task. Several of the configurations we prepared are depicted in the Figure. A most useful configuration is the tripartite CR (TPCR) that uses a scFv of a given antibody, the extracellular hinge region, transmembrane and cytoplasmic domains of CD28 linked to either the FcRγ or the CD3ζ intra-cellular moieties that include the ITAM activation motif. This tripartite CR combines the stimulatory and co-stimulatory signals needed for full activation of T cells. As confirmed in transgenic mice, naïve, unprimed T cells underwent non-MHC restricted stimulation for proliferation, high IL-2 production and is rescue from apoptosis upon encountering plastic-bound antigen as well as specific killing of target cells *in-vitro*. *In-vivo* application of the antigen elicited DTH responses without priming. Interestingly, transgenic mice expressing TPCR that cross reacts with thymic antigens demonstrated unexpected cellularity, reflecting the power of usage of CR to perturb the normal homeostasis.

To determine the clinical applicability of the CR approach we have used erbB2 as target TAA of choice as it is associated with the uncontrolled growth and commonly expressed in human adenocarcinomas, while its expression level is increased in advanced metastatic stages of the



disease. We have expressed erbB2-specific CR in human lymphocytes using retrovectors in a procedure that yielded high transduction efficiency in T and NK cells. These engineered lymphocytes (nicknamed T-Bodies) were studied for their ability to reject pre-established human breast and prostate cancer xenografts in SCID mice. Intratumoral administration of the T-bodies resulted in a significant cure of s.c. or orthotopic xenografts. To mimic bone metastasis (the favorite dissemination site of these malignancies) we have generated bone lesions by the injection of tumor cells into the tibia of SCID mice. Systemic administration of the T-bodies was effective in retarding the growth of prostate cancer bone lesions and prolonging the life span of the mice, yet, only after mild cyto-reductive pretreatments that enhanced the migration of the T-bodies to the bones. We currently study the mechanism beyond these pre-treatments and focus on the optimization of the *in-vivo* performance of the T-bodies towards their clinical application as a new strategy for adoptive gene-immunotherapy of cancer. New strains of transgenic mice harboring different erbB2- specific TPCR that we have recently generated will be extremely helpful reseach asset.

Pathophysiology of prostate cancer

Prostate cancer (PC) is a slow growing tumor that appears in adult men and is the second cause of death of men in the Western World. At its early stages, surgery, irradiation and androgen ablative therapies are quite effective and curative. However, at its advanced stages it is incurable. We took advantage of several PC xenografts that we have established (together with Prof. J. Ramon Sheba Medical Center and Dr I. Leibovich, Meir Hospital) to probe several features that are involved in the transition from androgen dependent to androgen refractory, from primary to metastatic tumor and from irradiation sensitive to resistant growth. The PC xenografts were derived from samples taken from patients at various stages of their disease and represent different types of PC- from the common adenocarcinoma, the less common and more aggressive prostatic Small Cell Carcinoma (with neuroendocrine (NE) cell features) to the very rare Clear-Cell carcinoma. We characterized these xenografts and could derive variants that differ in their sensitivity to ionizing irradiation (single dose or fractionated) and variants that could grow in castrated SCID mice with no external supply of androgens. Interestingly, we found that the NE tumor can facilitate, in a paracrine manner, the transition of PC adenocaricinomas from androgen-dependent to androgen independent, both *in vitro* and *in-vivo*. We characterize now the NE molecules involved in the biochemical pathways relevant to this process. To determine whether there is genetic disposition in PC for resistance to irradiation and for metastasis, we compare the transcription pattern and gene amplification of the different xenografts and their variants (with Prof. E. Domany, Physics of Complex Systems and Prof. Avi Orr Tel Aviv Medical Center). These studies should help to understand the mechanism(s) involved in the processes and lead to the development of new therapies.

Selected Publications

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Pinthus, J.H., et al. 2003. Immuno-gene therapy of established prostate cancer using chimeric receptor redirected human lymphocytes. Cancer Res. 15: 2470-6.

Morvinski D, et al. 2004. Full activation of naïve T cells of transgenic mice expressing a tripartite chimeric receptor combining both stimulatory and co-stimulatory domains. Submitted.

Pinthus, J. H., et al. 2004. Adoptive Immunotherapy of Prostate Cancer Bone Lesions Using Redirected Effector Lymphocytes. Submitted.

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