

A. Bendavid	D. Morvinski
I. Cohen	J. Lindner
T. Danon	C. Roodveldt
S. Gat	D. Schindler
A. Lindner	A. Schonenberger
V. Malina	T. Waks

Programming the nature of antibody and T cell receptor recognition

Department of Immunology

Tel. 972 8 934 3965

Fax. 972 8 947 4030

E-mail: zelig.eshhar@weizmann.ac.il

Our overall research goal in the recent years has been to study the nature of specific immune interactions and to genetically manipulate the specificity of immune-recognition molecules. We have focused our efforts on three major topics: Redirecting the specificity of T cells towards predefined antigens; Catalytic antibodies; and the Interactions between IgE and its high affinity receptor. In all these projects both basic and applicable aspects have been emphasized.

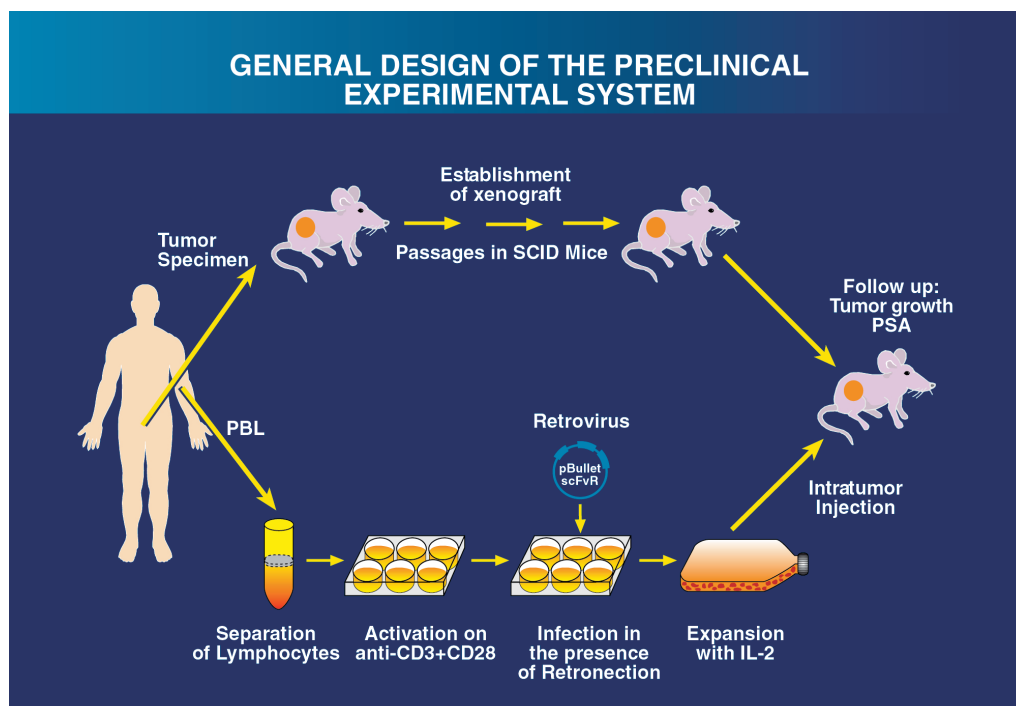
Redirecting effector lymphocytes to tumor cells using chimeric receptors with antibody specificity

To expand the recognition spectrum of effector lymphocytes and redirect them to predefined targets, we endowed T and natural killer (NK) cells with antibody-type specificity, using chimeric receptor genes. The modular structure of the chimeric receptor containing defined ecto-, spacer, transmembrane and cytoplasmic domains enabled its engineering to fit a desired task. Several configurations employing the antibody V region in the form of a single chain Fv (scFv) linked to triggering subunits of the Fc receptor (FcR) or TCR/CD3 complex have been constructed. We nicknamed T cells expressing such chimeric receptors T-bodies, and have demonstrated that they can undergo stimulation in an MHC-independent manner, yet restricted to the antibody specificity. In the recent years we have focused our efforts to optimize the chimeric receptor function and to further develop the T-body approach to cancer therapy. To target human tumors we have constructed several scFv's from antibodies recognizing tumor associated antigens that are over-expressed on human cancer cells, including anti-erbB2 antibodies. As an alternative to scFv as recognition units, we took advantage of the fact that human adenocarcinomas overexpress on their surface different combinations of the erb-B onco-receptor gene family and replaced the extracellular antibody recognition unit with the neuregulin ligand (NDF) which bind to these receptors. We found that optimal activity could be obtained with chimeric receptors made of the EGF-like domain of the NDF. T cells expressing the NDF based chimeric receptors were specific to heterodimers made of erbB2/3 or erbB2/4 on the surface of tumor cells. In order to bypass the T cell receptor (TCR) complex and its proximal triggering events (which are often impaired in T cells derived from tumor-bearing

patients), we joined the scFv through transmembrane stretch directly to intracellular kinases. Syk kinase was found to be the preferred protein-tyrosine kinase in this configuration. In another, yet novel design, we combined both antigen receptor signal with the co-stimulatory signal required for full T cell activation by inserting the cytoplasmic, transmembrane and part of the extracellular regions of the CD28 molecule in-between the scFv or NDF recognition units and the gamma or zeta signaling domains. Following introduction of all the various chimeric receptor configurations described above into murine and human effector lymphocytes these chimeric genes have been expressed as functional receptors and conferred non-MHC restricted, antibody (or ligand) specificity on the recipient cells. Specific killing and elimination of tumor cells have been demonstrated both *in vitro* and *in vivo* models. Notably, naive T cells derived from transgenic mice expressing the CD28 tripartite chimeric receptor undergo full stimulation by plastic-immobilized antigens and were more resistant than wild type cells to activation-induced apoptosis. To determine the clinical applicability of the chimeric receptor approach we have recently established a series of xenografts of human prostate tumors in SCID mice (together with Dr J. Ramon, Shiba Medical Center). Of special interest is a novel prostatic small cell tumor having neuroendocrine features which expresses members of the ErbB family and serve, together with other adeno carcinomas as targets for the neuregulin and anti-erbB2 redirected T cells. In the SCID system we have demonstrated the ability of the genetically engineered human T bodies to retard the growth of, and even reject, the prostate cancer xenografts in significant number of mice. Our future attempts will be focused on studying the *in-vivo* performance of lymphocytes harboring tumor-specific chimeric receptors and its optimization towards a clinical application as a new strategy for gene-immunotherapy of cancer.

Catalytic antibodies

Stretching our interest in the design of antibody binding specificity to enzyme catalytic activity led us to pioneer 'enzyme-like' catalytic monoclonal antibodies. Together with Dr. D. Tawfik, now in the Department of Biological Chemistry, innovative technologies were devised to prepare unique group



of efficient esterolytic antibodies (named D-antibodies (D-Abs)). Focusing on the catalytic mechanism of the D-Abs, we demonstrated recently that their mechanism includes both substrate-induced conformational isomerization as well as pre-equilibrium of the free antibody between active and nether-active forms. This finding suggested that antibodies may in general exhibit conformational diversity along with the well-described genetic diversity. Further, we improved up to 2000 fold the catalytic activity (kcat) of the D-Abs by introducing strong exogenous nucleophiles as peroxide. This work was complemented with structural studies, solving the X-ray structure of the D-Abs in presence or absence of their transition state analog haptens (in collaboration with M. Knossow, France). A special effort is given now to use the power of large combinatorial libraries and evolutionary pressure to obtain new antibody variable regions capable of catalyzing energetically and mechanistically demanding reactions.

Selected Publications

- Charbonnier JB., Golinelli-Pimpaneau B., Gigant B., Tawfik DS., Chap R., Schindler DG., Kim SH., Green BS., Eshhar Z. and Knossow M. (1997) Structural convergence in the active site of a family of catalytic antibodies. *Science* 275, 1140-1142.
- Eshhar Z. (1997) Tumor-specific T-bodies: towards clinical application. *Cancer Immunol. Immunother.* 45, 131-136.
- Fitzer-Attas CJ. et al. (1997) Direct T Cell Activation by Chimeric scFvSyk Promotes Syk Cbl Association and Cbl

- Phosphorylation. *J. Biol. Chem.* 272, 8551-8557.
- Fitzer-Attas C., Schindler DG., Waks T., and Eshhar Z. (1998) Harnessing Syk-family tyrosine kinases as signaling domains for chimeric scFv receptors; optimal design for T cell activation. *J. Immunol.* 160, 145-154.
- Mezzanzanica D., Canevari S., Mazzoni A., Figini M., Colnaghi M., Waks T., Schindler DG. and Eshhar Z. (1998) Gene transfer of variable regions from anti-human tumor antibody confers anti-carbohydrate antigen specificity to T cells. *Cancer Gene Therapy* 5, 401-407.
- Lindner AB., Eshhar Z. and Tawfik DS. (1999) Conformational changes affect binding and catalysis by ester-hydrolysing antibodies. *J. Mol. Biol.* 285, 421-430.
- Bitton N., Gorochoff G., Debre P. and Eshhar Z. (1999) Gene therapy approaches to HIV immunological strategies: Use of T bodies and universal receptors to redirect cytolytic T-cells. *Frontiers in Biosciences* 4, March 30.
- Pinthuis JH., Waks, T., Harmelin A., Beldegrun, A., Said, J., Ramon, J., and Eshhar, Z. (2000) WISH-PC2: A xenograft model of human prostatic small cell carcinoma. *Cancer Res.* 60, 6563-6567.
- Eshhar Z., Waks, T., Bendavid A. and Schindler, DG. (2001) Functional expression of chimeric receptor genes in human T cells. *J. Immunol. Methods*, 248, 67-76.

Acknowledgements

These studies were supported by grants from the US-Army, The EC 5th Program and CaPCURE.