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pBM1.1, A NEW pET-BASED VECTOR FOR HIGH EFFICIENCY EXPRESSION OF ETA' FUSION TOXINS

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During the last several years, recombinant immunotoxins (rITs), anti-tumor agents composed of monoclonal antibody fragments or cytokines coupled to plant or bacterial toxins have been designed.
Our aim was to develop a new bacterial expression system for high level production of rITs. We constructed a series of pET-based vectors for pelB-directed periplasmic secretion under the control of the strong T7*lac* promotor. Expression in BL21(DE3) allows a tightly regulated IPTG-induction of protein synthesis (Studier and Moffat, 1986).
A enterokinase-cleavable His-cluster has first been introduced into this setup for purification by metal chelate chromatography. A major modification results from the insertion of a specifically designed multiple cloning site. It contains only rare restriction enzyme recognition sites used for cloning of immunoglobulin variable region genes (Chaudhary et al., 1990) as well as SfiI and NotI restriction sites for directed insertion of scFv available from established bacteriophage systems.
Finally, we deleted two internal SfiI and one XhoI consensus sites in a deletion mutant of *Pseudomonas aeruginosa* Exotoxin A (Wels et al., 1992). Every single structural element of this vector can be directionally replaced by alternative gene cassettes in only one cloning step.
The fidelity of IPTG-induction, and high level expression were demonstrated using human interleukin-9 coding region fused to ETA' (Klimka et al., 1996). The biologic activities of new anti-CD25 and anti-CD30 rITs produced by this system are actually examined in our laboratory.

Chaudhary VK, Batra JK, Gallo MG et al.: PNAS 87, 1066-1070 (1990)
Klimka A, Barth S, Drillich S et al.: Cytokines Mol. Ther. 2, 139-146 (1996)
Studier FW, Moffat BA: J Mol Biol 189, 113 (1986)
Wels W, Harwerth IM, Mueller M et al.: Cancer Res 52, 6310-6317 (1992)

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Tumor specific targeting of a cell line with Natural Killer cell activity by asialoglycoprotein receptor gene transfer

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A targeting of immunological effector cells, like Natural Killer (NK)- or T-cells, to tumor cells could be an efficient strategy of adoptive immunotherapy. Accordingly, we investigated whether a gene transfer of a tumor specific receptor into a cell line with NK-cell acticity leads to a tumor specific targeting of these cells. As receptor we used the human asialoglycoprotein receptor (ASGP-R) which recognizes desialylated carbohydrate structures on tumor cells. Receptor-cDNA's were cloned into the plasmids pREP4 and pREP8. Electroporation was used to transfect the NK-cell line. After selection, all cells expressed the ASGP-R-chains as determined by flow cytometry. *In vitro* studies showed a significant increase of the binding activity of ASGP-R-transfected cells to the colon carcinoma cell line SW480, to the leukemic cell lines KG1 and K562 compared to *mock*-transfected cells. Furthermore, cytotoxicity assays were performed to compare the lytic activity of ASGP-R-transfected NK-cells *versus mock*-transfected cells. We could show that ASGP-R transfected NK-cells can be directed against tumor cells by gene transfer of ASGP-R.

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CYTOKINE GENE TRANSFER STUDIES IN A MURINE B CELL LYMPHOMA MODEL

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Vaccination with autologous tumor cells genetically modified to express cytokines has become an attractive approach for gene therapy of cancer. Recent studies using murine B16 melanoma cells showed that vaccination with transduced tumor cells elicits a systemic anti-tumor immunity. Transduction with GM-CSF has been determined to be most effective, and a dose effect has been established for some cytokines.
As the prognosis of B cell lymphomas is still unsatisfactory, we are studying the effect of vaccination with cytokine gene-modified lymphoma cells. The immunoglobulin idiotypes (Id) expressed on B cell lymphomas represent tumor-specific antigens, which, however, do not induce a potent anti-tumor response. One goal of tumor immunotherapy is to augment the immunogenicity of the Id, e.g. by improving its presentation by professional APC's using GM-CSF-secreting tumor cells.
Since a common obstacle to gene therapy are the insufficient expression rates achieved with the hitherto available vector systems, we first set out to establish a dose-response correlation in the murine MPC11 lymphoma model. We therefore created tumor cells expressing different levels of GM-CSF. On the basis of two different vectors, pSV2gpt and BCMGSNeo, we generated two constructs carrying the GM-CSF cDNA under the control of the Ig and the CMV promoter, respectively. After transfection into MPC11 cells, the pSV2gpt-GM-CSF clones produced significantly lower doses than the BCMGSNeo-GM-CSF clones. As determined by ELISA, the expression rates of GM-CSF ranged from 0.14 to 1.25 ng/ml/10^6 cells/24hrs for pSV2gpt and from 25 to 300 ng per ml/10^6 cells/24hrs for BCMGSNeo. The low productivity of the pSV2gpt construct is in contrast to previous experiments where Ig genes or Ig-cytokine fusion genes were efficiently expressed in this vector. This indicates a tightly regulated interaction between Ig genes and their autologous promoters. When, in contrast, an IL-4 cDNA was inserted into the pSV2gpt vector, a high expression level was obtained. This also shows that the expression in the pSV2gpt vector depends on intragenic regulation elements. For studying the dose effect of GM-CSF, BALB/c mice were vaccinated twice with irradiated MPC11-GM-CSF cells (of four different production levels) before tumor challenge. First *in vivo* results will be discussed.