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Development of ribozymes and antisense oligonucleotides targeting genes involved in drug resistance and pathogenesis of B-cell lymphoma

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Pathogenesis and therapy resistance in malignant B-cell lymphoma are related to specific genetic alterations. We tested new experimental approaches with therapeutic potential to break some of the known alterations.
Ribozymes and antisense oligonucleotides against different target sites on the bcl-2, bcl-1 and mdr-1 mRNAs were tested for their potential to reduce the expression of the corresponding protein. In mdr-1 overexpressing cell lines P-glycoprotein expression was reduced up to 95% after incubation in the presence of 2 μM of the most efficient ribozyme.
Corresponding to this inhibition doxorubicin resistance was significantly reduced (MTT assay), uptake of doxorubicin and a fluorescent P-gp substrate (JC1) could be monitored. Investigations of ex vivo cultured cells from acute myeloid leukemia patients showed a correlation of P-gp expression and reduction of doxorubicin resistance after incubation in the presence of an anti mdr1 ribozyme.
Ribozyme-mediated reduction of the bcl-2 protein down to 20-30% was achieved within 144h. Colony growth was reduced and the number of apoptotic cells increased. Subsequent incubation in the presence of different concentrations of dexamethasone (0-500μM) showed a 80-90% reduction in viability as compared to untreated cells and control sequences.
Preliminary experiments to evaluate the impact of anti bcl-2 oligonucleotides and ribozymes on the clonogenic potential of hematopoetic stem cells revealed no differences between antisense compounds and control sequences.

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Virus-free B cell lines expressing mucin (MUC1) gene by transfection with a mini-EBV plasmid

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Epstein-Barr virus (EBV) readily infects human B lymphocytes, thus allowing the stable propagation of infected cells *in vitro*. These EBV-immortalized cells can be used as antigen presenting cells to study T cell responses. However, for the use in gene therapy approaches, these cells pose the problem of infectious virus which could be released into the supernatant. To avoid this problem we used a mini-EBV vector which contains all functional elements of EBV necessary for B cell immortalization to generate virus-free B cell clones. Upon transfection or infection of primary B cells these F-factor-based mini-EBV plasmids can induce proliferation as efficiently as wild-type virus. We constructed a mini-EBV plasmid carrying an expression cassette for the human mucin gene MUC1. Mucin is a tumor antigen expressed on breast and pancreatic carcinoma in an underglycosylated form. B cell lines were generated by infection of primary human tonsillar B cells with the MUC1 carrying mini-EBV plasmid packaged into an EBV coat. Immortalized B cell clones were expanded *in vitro* and confirmed to be free of helper virus by PCR analyses. The immortalized B cell clones expressed the relevant tumor specific epitopes of MUC1 as shown by flow cytometry. They further express the costimulatory ligands B7.1 and B7.2 necessary for an efficient T cell activation. We conclude therfore that such MUC1 transfected B cells can be used to stimulate T cells.

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MOUSE IL-2-TRANSFECTED PLASMOCYTOMA CELLS IN A THERAPY OF ALLOGENEIC TUMOR-BEARING MICE

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Non-tumorigenic BALB/c mouse plasmocytoma cells, engineered to secrete mIL-2 (X63-mIL-2 subline), were used for the peritumoral (p.t.) immunotherapy or combined chemoimmunotherapy of B6D2F1 mice inoculated previously s.c. with tumor tissue brei of colon adenocarcinoma 38 (C38), C57Bl/6 strain of origin. Transfected cells produced 2–3x10^3 LU/ml of mIL-2 in the standard conditions of CTLL-2 bioassay. The immunotherapy consisted of a series of 5 to 7 injections of X63-mIL-2 cells alone, administered once per week. The first injection usually contains 10^7 cells/mouse; in successive 5–7x10^6 cells were injected. In combination with chemotherapy, transfected cells were applied to mice with advanced tumor growth. Their administration started 3 days after one or two i.p. injections of 200mg/kg of cyclophosphamide and was scheduled similarly as in immunotherapy. Therapeutic effects of these procedures were described by retardation of tumor growth rate and life-span prolongation (ILS), as related to control mice, injected either with cell vehicle PBS(-), or with the wild type (non-transfected) plasmocytoma cells, as well as by tumor-free long-term survival (TF-LTS). The observed effects were dependent on the stage of tumor progression at the moment of treatment beginning. The majority of mice (10/13) whose treatment with X63mIL-2 cells started 3 days after C38 inoculation, that is before formation of visible tumor nodules, remained TF-LTS for more than three months. When the treatment was delayed to the days 9, 11 or 16 (the mass of palpable tumors was 40–60 mg) the rate of tumor growth was slowed down in a fraction of mice (44–80%, in different experiments) which resulted in ILS of 20–80%. Ten to 14% of mice rejected tumors and appeared to be "cured" (TF-LTS for more than 6 months). The combination chemoimmunotherapy applied in mice bearing tumors of 100–200 mg was more effective than the X63-mIL-2 cells or chemotherapy alone and resulted in a significant life-span prolongation (60 to >170%), and in 10–37% of TF-LTS. The majority of these mice appeared also to be resistant to a second challenge with colon 38 tumor tissue.

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Expression of B7.1 in Squamous Cell Carcinoma of the Head and Neck Enhances Proliferation and Cytotoxicity of Allogeneic and Autologous Lymphocytes

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It has been demonstrated recently that the transfection of B7.1 cDNA into tumor cells enhances their rejection both *in vitro* and *in vivo*. In order to see whether the expression of B7.1 (CD80) renders squamous cell carcinoma of the head and neck (SCCHN) more immunogeneic we transfected human SCCHN tumor cell lines with an expression plasmid encoding B7.1. Here, we show that CD80-positive tumor cells stimulate the proliferation and augment the cytotoxicity of allogeneic and autologous lymphocytes as tested in ^{51}Cr-release and MTT cytotoxicity assays. B7.1-induced proliferation can be blocked by the CTLA4Ig fusion protein. Parental non-transfected and LacZ-transfected tumor cells do not activate lymphocytes, thus strongly implicating the importance of this costimulatory molecule for tumor cell recognition and rejection by immune cells. In addition, B7.1-stimulated allogeneic and autologous lymphocytes lyse parental tumor cells very efficiently. As novel therapeutic strategies are needed to improve outcome in patients suffering with SCCHN, transfection of the B7.1 gene into tumor cells to increase activation of lymphocytes in vivo may be a promising approach. Experiments in animal models are in progress to evaluate this strategy.