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Selective cytotoxicity of A Diphtheria Toxin-Interleukin-3 Fusion Protein on Acute Myeloid Leukemia Stem Cells

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Substantial efforts have recently been made to develop new therapies for AML that would specifically target leukemic cells but avoid primitive normal hematopoietic progenitors. We now show the ability of a diphtheria toxin (DT) fusion protein engineered by fusing the human IL3 cDNA to a truncated DT with a (G4S)2 linker (DTLIL3) to selectively kill leukemic stem cells. AML cells from 7 newly-diagnosed patients were exposed for 24h to 50ng/ml DTLIL3 prior to culture in the AML-CFC, and the primitive AML LTC-IC and SC-IC assays and injection into NOD/SCID mice. The mean (range) % kill of AML-CFC, LTC-IC and SC-IC was 82 (47-100); 55 (28-91) and 76 (59-92), respectively, with most surviving progenitors being cytogenetically normal. AML cell engraftment in the NOD/SL-IC assay at wks 4 and 8 was reduced by a mean (range) % of 97 (93-100) and 63 (0-100), respectively. DTLIL3-treated cells from 2 samples showed an eradication of leukemic engraftment in mice up to wk 16. In contrast, 3 normal bone marrow showed a mean % CFC kill of 49 and 64 with 50 or 250ng/ml DTLIL3, respectively, while no significant kill was observed in the progenitor LTC-IC, SC-IC and CRU assays. Thus, DTLIL3 selectively kills primitive leukemic progenitors from most AML patients sparing their normal counterparts and is a novel immunotherapeutic strategy for patients with AML as well as an agent for purging leukemia cells.

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Human angiogenin fused to human CD30 ligand (Ang-CD30L) exhibits specific cytotoxicity against CD30-positive lymphoma

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A number of different immunotoxins composed of cell-specific targeting structures coupled to plant or bacterial toxins have increasingly been evaluated for immunotherapy. Since these foreign proteins are highly immunogenic in man, we have developed a new CD30 ligand-based fusion toxin (Ang-CD30L) using the human ribonuclease angiogenin. The completely human fusion gene was inserted into a pET-based expression plasmid. Transformed *E.coli* BL21(DE3) were grown under osmotic stress conditions in the presence of compatible solutes. After IPTG induction, the 37 kDa His_{10}-tagged Ang-CD30L was directed into the periplasmic space and functionally purified by a combination of metal ion affinity followed by enterokinase cleavage of the His_{10}-Tag and molecular size-chromatography. The characteristics of the recombinant protein were assessed by ELISA, flow cytometry and toxicity assays showing specific activity against $CD30^+$ Hodgkin-derived cells. Ang-CD30L showed RNase activity *in vitro* and exhibited highest specific cytotoxicity against L540 cells (IC_{50} = 8 ng/ml) as determined by cell proliferation assays. This is the first report on the specific cytotoxicity of a recombinant completely human fusion toxin with possibly largely reduced immunogenicity for the treatment of CD30-positive malignancies.

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Assessing the cancerostatic potency of rViscumin towards human tumor xenografts and cell lines *in vitro*

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Cancerostatic activity of rViscumin -recombinant mistletoe lectin- was assessed in several independent *in vitro* experiments with human xenografts and tumor cell lines. rViscumin was found to be strongly cytotoxic at picomolar concentrations. For 42 human xenografts the mean IC_{50} was determined in a clonogenic assay to be 0.9 ng/ml (IC_{70} 3 ng/ml) corresponding to 15 pM (50 pM). The mean IC_{50} for a set of 20 cell lines derived from different tumour entities (Freiburg panel) was 0.16 ng/ml (IC_{70} 0.4 ng/ml). In the NCI 60 cell line panel the mean GI_{50} for rViscumin (NSC D-716789-A) was 1 ng/ml, the mean TGI 7.6 ng/ml and the mean LC_{50} 40.5 ng/ml (average of 4 experiments). On a molar basis rViscumin is more potent than taxol in the NCI 60 panel by a factor of 1500 and compared to doxorubicin by a factor of 5000.
rViscumin was strongly cytotoxic towards cell lines with resistance to standard therapeutic agents, such as adriamycin and vindesin. Those cell lines were even more sensitive to rViscumin than the parental cell lines. Thus, multi-drug resistance mechanisms do not hamper the rViscumin drug effect potentially offering new treatment options. COMPARE analyses with the cell panels show no relevant correlation to known molecular targets, nor to the mode-of-action of standard cytostatic agents.
In both cell panels prostate carcinoma cell lines, in the Freiburg panel also colon, breast, small cell and non small cell lung cancer lines were relatively sensitive compared to the mean value. Ovarian cancer cell lines were identified as relatively sensitive in the NCI panel. In conclusion, the data suggest that rViscumin is a highly potent new drug with strong cancerostatic activity and a unique mode of action.

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Systemic Tumor Regression mediated by Effector T cells generated from Perforin/IFN-γ double knock out Mice is TNF dependent

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Understanding the mechanism(s) of T cell-mediated tumor regression is critical for the development of effective immunotherapy. Recently, we reported that Effector T cells (T_E) from perforin or IFN-γ knock out (k/o) mice mediated significant tumor regression and cured mice of B16BL6-D5 (D5) pulmonary metastases. Since perforin or IFN-γ might compensate for each other, we developed mice deficient in both genes (PIG k/o). PIG k/o and wild type C57BL/6 (wt) mice were vaccinated with D5-G6 (stable mGM-CSF secreting D5 subclone) and tumor vaccine-draining lymph nodes were harvested on day 8, activated with anti-CD3, expanded in IL-2 and adoptively transfered into wt or PIG k/o D5 tumor-bearing mice (TBM). Animals were monitored for survival, or sacrificed to count pulmonary metastases (PM). In 3 of 3 experiments adoptive transfer of T_E from wt or PIG k/o regressed PM (Controls >250) in wt and PIG k/o TBM. In survival experiments PIG k/o T_E cured 50% of wt TBM with established D5 PM, and 100% of survivors were immune to a D5 s.c. rechallenge after 120 days. PIG k/o T_E also cured 50% of PIG k/o TBM. No tumor-specific IL-4, IL-10, IL-5 or TNFα was detectable by ELISA from PIG k/o T_E, but RT-PCR detected tumor-specific TNFα, suggesting a possible role for this molecule. 2h anti-CD3 stimulation resulted in upregulation of membrane-bound Lymphotoxin β and TNF on PIG k/o T_E. In 3 of 3 experiments the therapeutic efficacy of PIG k/o T_E was significantly reduced by blocking TNF in vivo, suggesting a critical role for this molecule in T cell-mediated tumor regression in the absence of perforin and IFN-γ. This work was supported by 1RO1CA80964-03 and 1RO1CA92254-01(BAF). C.H.P. is a Chiles Foundation visiting Fellow.