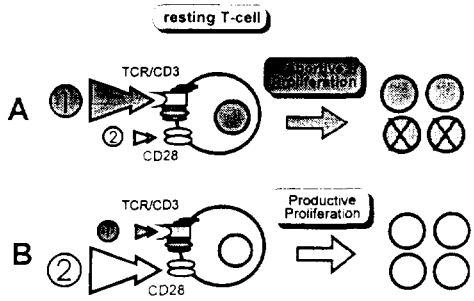
70

INDUCTION OF APOPTOSIS AND ANERGY IN RESTING HUMAN T-LYMPHOCYTES AFTER CD3-TRIGGERING AND ITS MODULATION BY CD28 AND CYTOKINES.

Y.Müller, H.Wolf, W.Wilmanns and G.Jung.

A puzzling feature of the T-cell-receptor (TCR)/CD3-complex, a central regulator of T-cell activity, is its "signaling dichotomy", i.e. the potential to transduce both positive and negative signals. We previously described the induction of anergy in resting human T-cells after high density triggering of the CD3-complex. Here we report the concommitantly occuring increase in cell death in these cultures to be due to apoptosis, which becomes apparent *after* initial cell proliferation. Thus it appears that a high dose TCR/CD3-stimulus is a negative signal for resting T cells leading to "abortive proliferation" followed by anergy and/or cell death. If initial proliferation is induced by a low dose CD3-stimulus in the presence of an accessory signal cell death is markedly reduced and "productive proliferation" may occur. Even during high dose anti-CD3 induced stimulation viability is (variably) modulated by the addition of costimuli. While PMA and anti-CD28 strongly reduce cell death, cytokines such as IL-2 and IL-4 have little effect. However, PMA, anti-CD28 and IL-4 effectively prevent the induction of hyporesponsiveness in anti-CD3 stimulated T-cells whereas IL-2 does not. The figure below summarizes our model of the T-cell signaling dichotomy and its implications for subsequent proliferation and responsiveness



Medizinische Klinik III der Universität München and Institut für Klinische Hämatologie, GSF-Forschungszentrum für Umwelt und Gesundheit, München, FRG

71

RECOMBINANT HUMAN STEM CELL FACTOR PROTECTS CD34 POSITIVE HUMAN MYELOID LEUKEMIA CELLS FROM THE CHEMOTHERAPY-INDUCED APOPTOSIS.

H.T.Hassan, S.Grell, U.Borrmann-Danso and M.Freund
Department of Hematology and Oncology, University of Hannover, Germany.

Adult acute myeloid leukaemia(AML) patients with CD34 positive cells were shown to have significantly low remission and survival rates. Also, in patients with myelodysplasia CD34 expression was associated with transformation to AML and poor survival rate. Since, human recombinant stem cell factor (SCF) has been shown to stimulate the proliferation of mainly CD34 positive AML cells, we have investigated whether the poor response of CD34 positive myeloid leukemia cells to chemotherapy could be due to SCF. The effect of SCF on the apoptosis induced by each of three commonly used chemotherapeutic drugs in the treatment of AML: Cytarabine, Daunorubicin and Carboplatin was examined in the human CD34 positive myeloid leukemia cells MHH225. The MHH225 cells were seeded at a concentration of 100 thousand cells per ml. at day 0 in 24-well tissue culture plates at 37°C in humidified incubator for 96 hours under CO_2. Stem cell factor was added at a concentration of 200 ng/ml, which has been found to be the optimum concentration for the proliferation of these CD34 positive leukemia cells. Apoptosis was determined after 96 hours by measuring the expression of APO-1 (CD95) antigen in treated MHH225 cells in the presence or absence of human recombinant Stem cell factor in three separate experiments.

Chemotherapy Drug (Concentration)	**Apoptosis (%)** in Human CD34 + Leukaemia Cells		
	Without SCF	With SCF	p
-Cytarabine(0.5 µg/ml)	**79.1 ± 6.0**	**37.4 ±5.1**	**< 0.01**
-Daunorubicin(0.05 µg/ml)	**86.2 ± 8.1**	**42.8 ± 4.6**	**< 0.01**
-Carboplatin (0.05 µg/ml)	**93.1 ± 5.7**	**38.0 ± 3.2**	**< 0.01**

The present table shows the significant inhibition of chemotherapy-induced apoptosis in the presence of SCF. The SCF significantly reduced the induced-apoptosis by more than 50% in all CD34 positive human leukemia cells treated by any of the three chemotherapeutic drugs. The present results suggest that the poor response of patients with CD34 positive leukemia cells could be at least partially due to less apoptosis in the presence of Stem cell factor.

72

FURTHER CHARACTERISATION OF TWO DIFFERENT RESISTANT HUMAN LEUKEMIA CELL LINES WITH RESPECT TO APOPTOSIS INDUCTION

O.Stötzer, V.Nüssler, U.Wallner, R.Pelka-Fleischer, M. Darsow, E. Gullis, W. Wilmanns
GSF- Forschungszentrum für Umwelt und Gesundheit, Institut für Klinische Hämatologie and Medizinische Klinik III, Klinikum Grosshadern, Munich

AIM: Determination of the potential influence of bcl-2 expression on (multi)-drug-resistance conferred by other mechanisms like P-glycoprotein overexpression or topoisomerase-II-mutations.

INTRODUCTION: Deregulation of bcl-2 leads to extended viability of cells by blocking apoptotic cell death. Studies with a variety of chemotherapeutic agents have shown that bcl-2 is able to confer multidrug resistance to cells that would otherwise exhibit sensitivity to these compounds.

METHODS: Analysis of endonuclease activation was done quantitatively by diphenylamine reagent assay and Hoechst 3342 dye/propidium iodide staining, detection of characteristic oligonucleosomal fragmentation patterns was done by DNA agarose gel electrophoresis; BCL-2 protein detection was performed by ECL-western blot analysis and indirect alkaline phosphatase immunostaining.

CELL LINES:

HL-60:	human acute myeloblastic leukemia (FAB M3);
HL-60/AMSA:	50-100-fold amsacrine resistant subline; resistant due to topoisomerase-II-mutation;
CCRF-CEM:	human acute T-lymphoblastic leukemia;
CCRF-CEM-ACT:	840-fold actinomycin-D resistant subline; resistant due to P-glycoprotein overexpression.

RESULTS: Characteristic patterns of oligonucleosomal DNA-fragmentation can be observed in both sensitive cell lines after drug exposition but not in the resistant sublines. HL-60 and HL-60/AMSA show a higher rate of spontaneous apoptosis than CCRF-CEM and CCRF-CEM-ACT. DNA-fragmentation can be detected already 4hrs after starting drug treatment in HL-60 but not in CCFR-CEM. 20hrs after treatment the extent of DNA-fragmentation is the same in both lines. Both resistant sublines, HL-60/AMSA as well as CCRF-CEM-ACT are expressing more BCL-2 protein than the corresponding sensitive cell lines. We observed a decrease in BCL-2 protein level 4hrs after drug treatment in HL-60 but not in HL-60/AMSA, CCRF-CEM and CCRF-CEM-ACT. Additionally HL-60 cells seem to express an extra protein at 19kD, which might be BAX. BAX is another protein of the BCL-2 family, which is discussed to antagonize the apoposis-blocking function of BCL-2.

SUMMARY: Expression of BCL-2 protein seems to be a common phenomenon in drug-resistant leukemia cells and might play an additional role to well known resistance factors in conferring multi-drug-resistance.

73

Recombinant single chain immunotoxins specific for the EGF receptor and ErbB-2.

Mathias Schmidt[1], Nancy E. Hynes[2], Bernd Groner[1], and Winfried Wels[1]

[1]Institute for Experimental Cancer Research, Tumor Biology Center, P.O. Box 1120, D-79011 Freiburg, Germany. [2]Friedrich Miescher Institute, P.O. Box 2543, CH-4002 Basel, Switzerland.

The EGF receptor and ErbB-2 (HER-2, Neu) are members of the growth factor receptor family of tyrosine kinases. Both receptors are overexpressed in a variety of human cancers of epithelial origin. Their extracellular accessibility and their elevated expression levels on tumor cells make these receptors excellent targets for directed tumor therapy.

We have cloned the variable domains of the monoclonal antibodies (mAb) FRP5 and 225 binding to, respectively, the extracellular domain of the ErbB-2 and EGF receptors by reverse transcription of hybridoma cell RNA and specific cDNA amplification using PCR techniques. Fusion genes coding for single-chain antibody molecules (scFv) were made by joining the light and heavy chain variable domains with a synthetic nucleotide linker. Recombinant antibody-toxin genes were constructed by the addition of sequences encoding truncated *Pseudomonas* exotoxin A (ETA) devoid of its own cell binding domain to the scFv encoding DNA (1,2). Likewise, a recombinant growth factor-toxin specific for the EGF receptor was constructed by PCR amplification of cDNA encoding amino acids 1 to 50 of human transforming growth factor (TGF) α and fusion with the modified ETA gene. The bacterially expressed immunotoxins scFv(FRP5)-ETA, scFv(225)-ETA, and TGFα-ETA bind specifically to the appropriate receptors and display potent cell killing activity on human tumor cells in vitro. At low doses the immunotoxins inhibited specifically the growth of established tumor xenografts expressing the appropriate receptor molecules in a variety of nude mouse tumor models.