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FLT3 LIGAND: A NEW REGULATOR OF HEMATOPOIETIC STEM CELLS

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Flt3 ligand is a hematopoietic growth factor that as a single agent has been shown to stimulate the proliferation and mobilization of stem and progenitor cells. Flt3 ligand also synergizes well with either GM-CSF or G-CSF to mobilize early cells to peripheral blood. Cells mobilized with flt3 ligand provide long term reconstitution of multiple hematopoietic lineages when transplanted into lethally irradiated recipient mice. Studies of serum levels of flt3 ligand in humans suggest that it may be a key physiological regulator of stem cells *in vivo*.

More recent data indicate that flt3 ligand plays an important role in the generation of both dendritic cells and NK cells. Injection of soluble, recombinant CHO-derived human flt3 ligand into mice stimulates the production of dendritic cells *in vivo*, and flt3 ligand synergizes with other cytokines *in vitro* to generate dendritic cells. Analysis of mice carrying a targeted disruption in the flt3 ligand gene (KO mice) revealed a significant reduction in the number of dendritic cells in the spleen. In addition, spleens of the KO mice had a complete deficiency of natural killer (NK) cell activity, suggesting that flt3 ligand is a physiological regulator of NK cell development.

Since flt3 ligand plays a role in generating both NK and dendritic cells, its potential anti-tumor effects were examined in several model systems. Expression of flt3 ligand on the surface of a murine breast cancer cell line resulted in the specific rejection of tumors expressing this protein [K. Chen et al Blood 86, 244a (1995)]. Injection of flt3 ligand in a murine fibrosarcoma model led to elimination or decreased growth rate of the tumors. The mechanism responsible for this anti-tumor effect likely involves both NK cells and dendritic cells. These data suggest that flt3 ligand may have clinical potential as an immunotherapeutic agent for cancer as well as a stem cell mobilizing agent.

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IRREGULAR PRODUCTION OF CYTOKINES IN HAIRY LEUKEMIA: EFFECTS OF IFN-α

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The treatment of hairy cell leukemia (HCL) with rhIFN-α still represents the best example of successful cancer biotherapy. However, the mechanism of action of IFN-α in this disease is not completely revealed. We have recently shown that the hematopoietic insufficiency in HCL is accompanied by an inadequate in vitro production of cytokines such as G-CSF, GM-CSF, IL-6 and TNF-α and, as demonstrated with IL-6, that rhIFN-α is capable to restore this defect. It is intriguing to speculate that there is a deficiency of endogenous interferon in HCL. Therefore we studied the expression of IFN-α at the mRNA and protein level. Using a sensitive RT-PCR technique we found that IFN-α mRNA could barely be detected in PBMC from HCL patients as compared to healthy donors. However, treatment of the cells with rhIFN-α or poly I:C, a potent inducer of endogenous IFN-α, resulted in a significant expression of IFN-α mRNA and secretion of IFN protein. In a second serious of experiments we tested the expresion of TGF-β1, which is known as a supressor of hematopoiesis. We found that TGF-β1 mRNA is highly expressed in a HCL as compared to controls. Interestingly, if PBMC from HCL patients were treated with rhIFN-α or with poly I:C, a significat downregulation of TGF-β1 mRNA was observed. On the other hand, HC-cultures treated with hTGF-β1 showed a decrease in IFN-α mRNA level. Since a feature, in variably associated with HCL, is bone marrow fibrosis and since TGF-β in association with other factors such as bFGF is capable to induce fibrotic processes, we tested whether HCL is acompanied be an abnormal expression of bFGF. While the cytokine was not or barely present in serum of healthy donors, higher levels were found in HCL patients. In contrast to PBMC from HD cells from HCL patients expressed bFGF at the mRNA and protein level. Cell separation studies revealed that preparations enriched for hairy cells (CD19/CD11c) are the main producers of bFGF. Furthermore, a high molecular weight bFGF (23 KD), an isoform that is commonly associated with a high secretion pattern and malignancy, was detected by western blotting. In sumary, the results of our studies point to an unbalanced expression of hematopoietic growth factors and other cytokines in HCL. They may help to understand some of the characteristic pathological conditions seen in this disease and may shed a light on the mechanism of action of IFN-α in HCL therapy.

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STEM CELL FACTOR (r-metHuSCF) IN COMBINATION WITH FILGRASTIM (r-metHuG-CSF) ENHANCES PERIPHERAL BLOOD PROGENITOR CELL (PBPC) MOBILISATION FOLLOWING CHEMOTHERAPY: A RANDOMISED STUDY

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We report here that SCF plus filgrastim following chemotherapy enhances PBPC mobilisation compared with filgrastim alone. 48 chemotherapy-naive patients with FIGO stages Ic-IV epithelial ovarian cancer were randomised to receive either SCF and filgrastim or filgrastim alone following cyclophosphamide 3g/m^2. The dose of SCF was cohort dependent, with 12 patients in each cohort, 9 of whom received filgrastim (5μg/kg) plus SCF and the remaining 3 receiving filgrastim (5μg/kg) alone. The dose of SCF was 5,10,15, and 20μg/kg in cohorts 1 to 4 respectively. Growth factors were administered daily from day 3 post-chemotherapy until WBC was ≥4x10^9/L, when all patients underwent an apheresis, the product of which was divided into 4 aliquots, one aliquot being reinfused following each subsequent dose-intensive cycle of chemotherapy. We have demonstrated a statistically significant increase in colony-forming cells and CD34+ cells as the dose of SCF was increased. Likewise there was a significant enhancement in yields of CD34+ cells, CFU-GM, BFU-E and LTC-IC in those patients receiving the higher doses of SCF plus filgrastim compared with those receiving filgrastim alone. The median number of days (range) peripheral blood CD34+ cells, CFU-GM and BFU-E remained above specified threshold values are presented below.

	Cyclophosphamide 3g/m^2 followed by				
Number of days for:	**filgrastim 5μg/kg**	**filgrastim 5μg/kg + SCF 5μg/kg**	**filgrastim 5μg/kg + SCF 10μg/kg**	**filgrastim 5μg/kg + SCF 15μg/kg**	**filgrastim 5μg/kg + SCF 20μg/kg**
CFU-GM	5	4	5	8	9*
>5x10^3/ml	(0-7)	(2-5)	(1-8)	(5-10)	(6-10)
BFU-E	5	5	6	7	9*
>5x10^2/ml	(0-7)	(2-5)	(2-9)	(6-10)	(6-10)
CD34+	3 5	3	4	6	7*
>50x10^3/ml	(0-5)	(0-5)	(0-8)	(3-9)	(6-8)

* p<0.001 for linear regression on SCF dose across all 5 groups

This has an important clinical implication in that the higher doses of SCF offer a greater 'window of opportunity' in which to perform the apheresis to achieve high yields.