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GROWTH FACTOR REGULATION OF NORMAL HEMATOPOIESIS

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The development of hematopoietic cells in vivo is believed to be regulated by a complex network of cytokines. The longterm culture (LTC) system, in which hematopoiesis can be sustained for several months by factors produced by stromal fibroblasts, offers a useful model to define mechanisms that may operate in vivo. Addition to these cultures of a variety of known growth factors either alone or in combination can trigger quiescent primitive clonogenic progenitors (CFC) into S-phase and may also enhance the overall output of granulopoietic cells. Although more primitive cells that are precursors of CFC (identified as LTC-initiating cells or LTC-IC) can also be triggered by some of these factors, we have found that their continuous addition to standard LTC does not increase (or decrease) LTC-IC numbers over a 5 week period. However, our recent studies also suggest that this may be related to the way in which the LTC-IC interact with the stromal regulatory cells since strategies that prevent direct interactions between these cells can lead to net LTC-IC expansions of 5 to 10-fold over a 4 week period in the presence of IL-3 and Steel factor.

The clinical use of factors such as IL-3 and G-CSF or GM-CSF has allowed the period of cytopenia that follows myeloablative therapy to be significantly reduced and allowed mobilization of both CFC and LTC-IC into the peripheral blood where they can be harvested in sufficient numbers for use in stem cell transplantation. Further advances in the in vitro manipulation of such cells, including progenitors of the megakaryocyte lineage exploiting the recently cloned thrombopoietin, should soon make feasible the large scale expansion in vitro of both primitive marrow repopulating cells as well as more mature progenitor populations for a variety of clinical indications.

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DIFFERENTIATION AND PROLIFERATION IN HAEMOPOIETIC CELLS.

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The haemopoietic malignancies are made up of a variety of different diseases in which the regulatory factors which balance the survival, proliferative, differentiation and developmental responses of lymphoid and myeloid cells are disrupted. Many of the leukaemias are not associated with disregulated or uncontrolled proliferation as such, rather the *development* of the leukaemic cell is blocked or occurs in a discordant fashion.

In order to develop new strategies for treatment of leukaemia we have studied the signals elicited by growth factors leading to macrophage or neutrophil development in committed macrophage/neutrophil progenitor cells. We have identified a cellular signalling pathway, involving activation and nuclear translocation of an enzyme known as protein kinase Cα, which is specifically associated with macrophage development.

A second approach has been to study the effects of leukaemogenic oncogenes such as BCR/ABL on responses to growth inhibitors and promoters. Progenitor cells from patients with Chronic Myeloid Leukaemia (CML) are non-responsive to the growth inhibitory effects of MIP-1α even thou
gh they express receptors for this chemokine. We have shown that this desensitization to MIP-1α is caused by ABL protein tyrosine kinase activity. MIP-1α non-responsiveness in CML cells may allow the development of novel forms of treatment for CML in which the normal stem cells are protected from the effects of cell cycle specific drugs by MIP-1α whilst the CML cells are still sensitive to these drugs.

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GM-CSF/IL3 FUSION PROTEIN (PIXY 321) AND OTHER GROWTH FACTORS IN ACUTE MYELOID LEUKEMIA BLASTS.

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In vitro priming with growth factors (GFs) of acute myeloid leukemia (AML) cells has been reported to increase percentage of cycling cells and enhance cytotoxic effects of cycle specific drugs. Proliferative response may be heterogeneous for different GFs. Aim of our study was to investigate in vitro proliferative effects of the novel cytokine GM-CSF/IL-3 fusion protein (PIXY 321) on 20 de novo AML. The same evaluation was performed using other GFs as well as Stem Cell factor (SCF), GM-CSF, IL-3, G-CSF and the GF combinations SCF+PIXY and GM-CSF+IL-3. GFs were used in liquid colture for 48-72 hours to determine both proliferative effects on clonogenic cell growth (CFU-L), and cell cycle changes by flow cytometric DNA/RNA (acridine-orange). AML blasts, after GFs priming, were exposed to different concentrations of Ara-C and cytotoxicity was measured as percentage of CFU-L inhibition.

Among the single cytokines PIXY was able to induce the best proliferative effect on clonogenic cell growth (3.59 mean fold increase) comparable to that obtained by GM+IL3. The combination of SCF+PIXY induced an higher proliferative response than other GF conditions (mean fold increase of S phase= 6.72) and the most pronounced effects in more than 50% of the cases. Cytotoxic effects of Ara-C were also enhanced by SCF+PIXY, although benefit in leukemic cell killing was different from case to case and for the different cytokines tested. In conclusion this in vitro study showed activity of PIXY on AML blast cells priming and Ara-C sensitization.

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Identification and characterization of murine stem cells with preferential short-term or long-term bone marrow repopulating ability.

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In order to separate cells with short term repopulating ability (STRA) from those with long term repopulating ability (LTRA) transplant experiments in mice were performed with subsets of relatively mature and relatively immature hematopoietic progenitor cells. Mouse (Balb-C) bone marrow cells were enriched for progenitor cells using Wheat Germ Agglutinin (WGA) and the monoclonal antibody 15.1.1. that recognizes relatively mature myeloid cells. The WGA^+ $15.1.1.^-$ cells were further purified using Rhodamine (Rh) 123 fluorescence. Cells were separated into Rh^-, dull or bright cells. The Rh^- cells were subjected to a second incubation with Rh in the presence of Verapamil (VP) to block the energy dependent efflux pump encoded by the multi drug resistance (MDR-1) gene. Part of the Rh^- cells remained Rh^- ($Rh^-/Rh(VP)^-$) whereas approximately 70% of the cells became Rh dull ($Rh^-/Rh(VP)^+$). Decreasing numbers of cells were then transplanted into lethally irradiated (8.5 Gy) recipients. Radioprotection was mainly present in the Rh^- fraction, to a latter extent in the Rh dull fraction, whereas the Rh bright cells had very poor radioprotective capacity. The 50% survival rate was reached after transplantation of 30-50 Rh^- cells, approximately 300-500 Rh dull cells, 10-30 $Rh^-/Rh(VP)^+$ cells and 100-300 $Rh^-/Rh(VP)^-$ cells. In additional experiments, $Rh^-/Rh(VP)^-$ cells obtained from male donor animals and $Rh^-/Rh(VP)^+$ obtained from female donor animals were mixed and transplanted into lethally irradiated female recipient animals. These experiments have indicated that STRA after transplantation is mainly derived from the $Rh^-/Rh(VP)^+$ cells, whereas LTRA in recipients at 6 months after transplantation was mainly present in the $Rh^-/Rh(VP)^-$ cells. These experiments indicate the existence of a population of cells that have preferential term (STRA) or long term repopulating capacity (LTRA).

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