Adhesive Interactions in the Regulation of Haemopoiesis

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Introduction

The role of stomal cells in the regulation of haemopoiesis has been amply demonstrated both *in vivo* and *in vitro*. In normal adult humans haemopoietic activity is restricted to parts of the skeleton, particularly the sternum and pelvis [1] and the success of clinical bone marrow transplantation shows that stem cells can seek out haemopoietic tissue. *In vitro*, the production of haemopoietic stem cells in the culture system described by Dexter et al. [2] and the formation of colonies of human blast cells on stromal feeder layers [3] provide convenient systems for investigating regulatory interactions between haemopoietic cells and the stromal cells of the haemopoietic microenvironment.

The distribution of haemopoiesis *in vivo*, the "homing" of transplanted cells and the close association between haemopoietic cells and stromal cells in *in vitro* culture systems suggest that specific binding interactions are involved in the microenvironmental regulation of haemopoiesis. Indeed, a capacity to bind to stroma by blast colony-forming cells (Bl-CFC) in the stromal feeder layer assay [3] is a prerequisite for detection in this culture system (see Methods). In contrast to normal haemopoietic stem cells, leukaemic stem cells are not restricted to the skeleton, circulate in the bloodstream and proliferate in extramedullary sites. For example, large numbers of circulating stem and progenitor cells are characteristic of the chronic phase of chronic myeloid leukaemia (CML) [4–7]. Thus, there may be a failure in leukaemia of the binding interactions that normally hold stem cells under the regulatory influence of the haemopoietic microenvironment.

Here, we will summarise information relevant to the stroma-mediated regulation of normal haemopoiesis and its dysregulation in leukaemia.

Materials and Methods

Normal bone marrow cells have been obtained, with informed consent, from donations of marrow for allogeneic transplantation and peripheral blood cells from chronic phase CML patients at presentation. The normal bone marrow cells and the CML blood cells were separated using Lymphoprep (Nyegaard, Oslo) to obtain the mononuclear cell fraction.

The Bl-CFC assay is set up in a series of stages. First, confluent stromal layers are grown in 35 mm petri dishes or in wells from normal marrow mononuclear cells (5×10^5/ml) in α-medium (GIBCO) supplemented with 10% fetal calf serum, 10% horse serum (GIBCO) and 2×10^{-6}/ml mononuclear cells are added to the stromal layers and incubated with them for 2 h. The stromal layers are then washed thoroughly to remove any cells that have not bound to the stroma, covered with a layer of 0.3% agar in α-medium supplemented with 15% fetal calf serum and incubated for

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5–7 days. Some cells that bind to the stroma produce colonies of more than 20 blast cells that can be scored by inverted phase contrast microscopy.

Implications of Stroma-Dependent Blast Colony Formation for the Regulation of Haemopoiesis

Clearly, Bl-CFC can bind to stromal layers *in vitro* and form colonies and, since we believe that these progenitor cells represent an early stage in haemopoiesis [8], the assay can be used as a model to investigate further the microenvironmental regulation of stem cell activity. It is presumed that binding of Bl-CFC to stroma is mediated via a cell adhesion molecule (CAM) and that proliferation is stimulated by endogenous growth factors produced by stromal cells of by exogenous growth factors. As discussed by Gordon and Greaves [9] a regulatory unit can be envisaged wherein stem and progenitor cells bind to specific microenvironments, according to their stage of development and lineage of differentiation, which in addition to binding cells also sequester the appropriate growth factor so that it can be presented to the immobilised target cell.

The Cell Adhesion Mechanism

A CAM responsible for binding Bl-CFC to stroma has not been identified and studies in this area are difficult because there are no representative stromal or haemopoietic cell lines that can be exploited. Neither is binding inhibited by antibodies to any known CAMs [10]. However, we have made a number of studies of the functional properties of the Bl-CFC/stroma cell adhesion mechanism, showing it to be: heparan sulphate dependent [11]; calcium, magnesium and serum independent [10]; partially sensitive to trypsin, partially sensitive to phosphatidylinositol-specific phospholipase C (PI-PLC) [10]; tissue specific [12]; species nonspecific [13]; modulated during haemopoietic cell maturation [14]; regulated during development [12].

Sequestration of Growth Factors by the Stromal Microenvironment *In Vitro*

Either endogenously produced or exogenous growth factors could be responsible for stimulating progenitor proliferation in the Bl-CFC assay. There is evidence that both mechanisms could operate by binding to the extracellular matrix (in particular the glycosaminoglycan fraction) produced by the stromal cells in vitro. Examples of this evidence are that: endogenous growth factor can be extracted using salt [15]; haemopoietic growth factors bind to glycosaminoglycans; GM-CSF binds to hyaluronic acid, IL-3 binds to heparan sulphate (unpublished data); binding is selective, saturable and retains the biological activity of the growth factor [15]. The model for the microenvironmental regulation implies that different growth factors, as well as different progenitor cells, have different binding specificities and this has been confirmed using stromal layers [16] and glycosaminoglycan-coated sepharose beads (unpublished observations).

Binding Properties of Bl-CFC in CML

Large numbers of Bl-CFC circulate in chronic phase CML [7], indicating that their capacity to interact with stromal layers is defective. This idea has been explored further, and several differences between the binding properties of normal and CML Bl-CFC have emerged. These are:

- Bl-CFC circulate in CML, normal Bl-CFC do not [7]
- CML-Bl-CFC bind to stroma grown without methylprednisolone, normal Bl-CFC do not [17]
- CML-Bl-CFC bind transiently to stromal layers grown with methylpred-

nisolone, normal cells bind irreversibly [18]
- CML-Bl-CFC are insensitive to PI-PLC treatment, normal cells are partially sensitive to PI-PLC treatment

The finding that Bl-CFC in CML are insensitive to treatment with PI-PLC indicates that they lack a functional phosphatidylinositol-anchored component of the cell adhesion mechanism. In support of this idea, preliminary results have shown that normal PI-PLC-treated Bl-CFC (i.e., rendered deficient in the PI-anchored cell adhesion component to resemble CML cells) bind transiently to cultured stromal layers. Moreover, these treated cells, like CML cells, can interact with stromal layers grown without methylprednisolone.

Summary and Conclusions

The Bl-CFC assay system provides a method for investigating regulatory interactions between normal and abnormal haemopoietic cells and their stromal microenvironment. Normally, regulation may be achieved by specific binding interactions between haemopoietic cells and stroma together with the presentation of appropriate growth factors. In CML, early progenitor cells do not interact properly with stromal cells and this may be attributable to an absence of a functional PI-linked component of the cell adhesion mechanism. We propose that abnormal expression of CAMs in CML may contribute to the dysregulation and extramedullary distribution of stem cells in this disease.

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