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Phil. Trans. R. Soc. Lond. B 1990 **327**, 85-98
doi: 10.1098/rstb.1990.0045

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Phil. Trans. R. Soc. Lond. B **327**, 85–98 (1990)
Printed in Great Britain

The role of growth factors in self-renewal and differentiation of haemopoietic stem cells

By T. M. Dexter, C. M. Heyworth, E. Spooncer and I. L. O. Ponting

Cancer Research Campaign Laboratories, Paterson Institute for Cancer Research, Christie Hospital, Withington, Manchester M20 9BX, UK.

Haemopoietic stem cells *in vivo* proliferate and develop in association with stromal cells of the bone marrow. Proliferation and differentiation of haemopoietic stem cells also occurs *in vitro*, either in association with stromal cells or in response to soluble growth factors. Many of the growth factors that promote growth and development of haemopoietic cells *in vitro* have now been molecularly cloned and purified to homogeneity and various techniques have been described that allow enrichment (to near homogeneity) of multipotential stem cells. This in turn, has facilitated studies at the mechanistic level regarding the role of such growth factors in self-renewal and differentiation of stem cells and their relevance in stromal-cell mediated haemopoiesis.

Our studies have shown that at least some multipotential cells express receptors for most, if not all, of the haemopoietic cell growth factors already characterized and that to elicit a response, several growth factors often need to be present at the same time. Furthermore, lineage development reflects the stimuli to which the cells are exposed, that is, some stimuli promote differentiation and development of multipotential cells into multiple cell lineages, whereas others promote development of multipotential cells into only one cell lineage. We suggest that, in the bone marrow environment, the stromal cells produce or sequester different types of growth factors, leading to the formation of microenvironments that direct cells along certain lineages. Furthermore, a model system has been used to show the possibility that the self-renewal probability of multipotential cells can also be modulated by the range and concentrations of growth factors present in the environment. This suggests that discrete micro-environments, preferentially promoting self-renewal rather than differentiation of multipotential cells, may also be provided by marrow stromal cells and sequestered growth factors.

Introduction

The mature cells of the rapidly regenerating tissues (such as the haemopoietic system, the intestine and the skin) are post-mitotic cells with a relatively short life-span (Lord & Dexter 1988). In the bone marrow, for example, about 4×10^{11} mature myeloid cells need to be produced each hour, to replace the circulating blood cells that are lost as a result of natural ageing. In these regenerating tissues, the integrity of the system is maintained by a relatively small population of stem cells that can either undergo self-renewal (to produce more stem cells) or undergo differentiation, proliferation and development (leading to extinction of proliferative potential). The stem cells of the haemopoietic system are further characterized by their potential to develop into eight distinct cell lineages (figure 1). The self-renewal ability of stem cells ensures that their number persists throughout adult life; indeed, in the haemopoietic system, studies have shown that neither the number nor the quality of stem cells decline with age (Schofield *et al.* 1986). Clearly, these birth and death processes need to be balanced: if too

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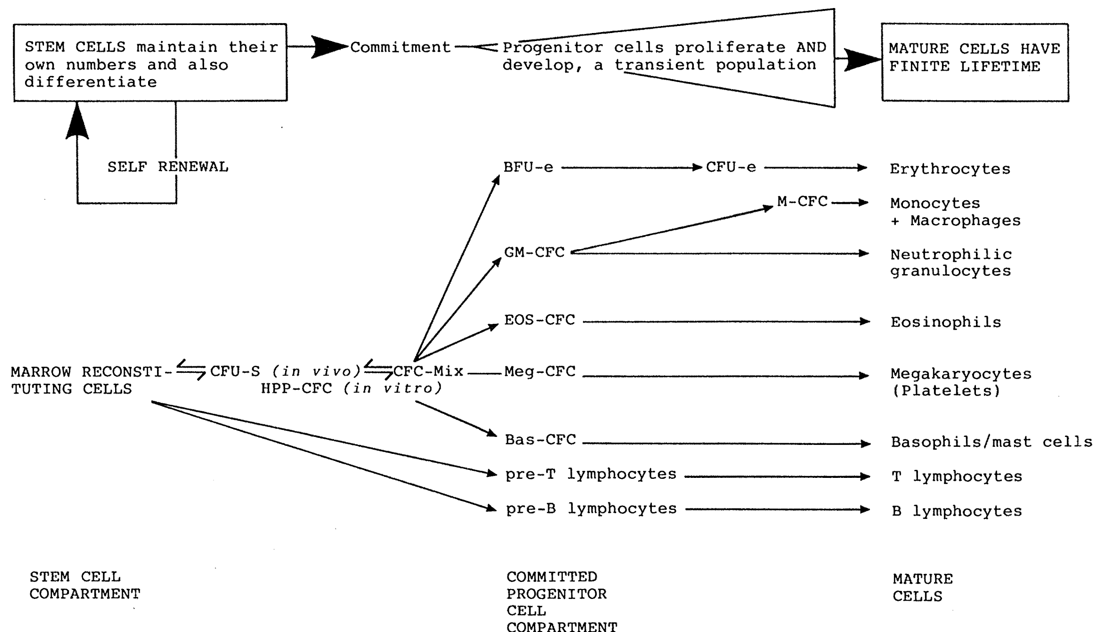

FIGURE 1. The structure of the haemopoietic system. The stem cell compartment contains CFU-S (colony-forming unit-spleen), HPP-CFC (high proliferative potential colony-forming cells) and CFC-mix (colony-forming cell mixed). These cells are identified by different assay techniques but it is likely that there is a considerable overlap between these populations. Committed progenitor cells include BFU-e (burst forming unit-erythroid), CFU-e (colony-forming unit-erythroid, a more mature erythroid colony-forming cell), GM-CFC (granulocyte–macrophage colony forming cells), M-CFC (Macrophage-CFC), Eos-CFC (Eosinophil-CFC), Meg-CFC (Megakaryocyte-CFC) and Bas-CFC (Basophil-CFC).

many stem cells undergo self-renewal, the population of primitive cells must expand and mature cell output would be compromised. If, on the other hand, too many stem cells undergo differentiation, the stem cell pool could rapidly become exhausted. Either circumstance, therefore, would rapidly result in life-threatening consequences and one of the more fundamental problems to be resolved in biology is how self-renewal and differentiation of stem cells are regulated (Till & McCulloch 1980). Furthermore, an understanding of the processes involved has clear implications in tumourigenesis, which in its most simple form, represents an 'uncoupling' of growth and differentiation control mechanisms (Sachs 1978). To approach the problem requires a detailed knowledge of the structure of the haemopoietic system and the various cell-mediated processes, extracellular matrix molecules and soluble growth regulatory factors that regulate proliferation and differentiation of stem cells.

The structure of the haemopoietic system

The existence of pluripotential haemopoietic cells capable of extensive self-renewal had been inferred for many years, based upon the ability of (relatively few) bone marrow cells to fully reconstitute the haemopoietic system after transfer into irradiated hosts. Such cells have recently been formally demonstrated using replication-defective retroviruses as 'insertional markers' in transplanted bone marrow cells to follow: (*a*) longevity of the grafted cells; (*b*) the clonal nature of haemopoiesis, and (*c*) the multi-lineage developmental potential of transplanted cells (Snodgrass & Keller 1987). These transplantable stem cells which contribute to long-term haemopoiesis, have been referred to as 'reconstituting' stem cells.

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A more quantitative assay for primitive cells was developed by Till & McCulloch (1961), who demonstrated the ability of transplanted bone marrow cells to form 'nodules' in the spleen of potentially lethally irradiated mice. For many years, these spleen colony-forming cells (or CFU-S) were referred to as stem cells. Whereas this is probably true for at least some of the CFU-S, it is now known that the CFU-S population is heterogeneous both in respect to self-renewal ability and also in lineage potential. For example, cells that give rise to spleen nodules 12–13 days after transplantation are more primitive (that is, are multipotential and have greater self-renewal capacity) than cells that give rise to spleen colonies 7–8 days after transplantation (Till & McCulloch 1961; Magli *et al.* 1982; Molineux *et al.* 1986). Furthermore, a cell that gives rise to CFU-S and is high in marrow reconstituting ability, has recently been characterized (Ploemacher & Brons 1988). It seems, then, that the stem cell system is best viewed as a continuum and that self-renewal capacity is progressively lost as the cells become more developmentally restricted. Whether or not self-renewal capacity is lost concomitantly with lineage restriction will be discussed later.

Another useful assay for multipotential cells with a (limited) capacity for self-renewal is provided by an *in vitro* culture system. When marrow cells are cultured in a soft-gel matrix, in the presence of an appropriate growth stimulus, a small proportion of the cells undergo clonal proliferation and development to produce colonies containing mature cells of multiple cell lineages (Metcalf 1988). Such colony-forming cells (or CFC-mix) are closely related to CFU-S: the colonies produced can contain (in addition to mature myeloid cells) undifferentiated cells that can produce more mixed-myeloid colonies *in vitro* or spleen colonies *in vivo* (Humphries *et al.* 1981). It is likely, however, that most of the CFC-mix have lost their lymphoid potential and are perhaps best considered as myeloid-restricted multipotential cells.

Multipotential cells differentiate to produce progenitor cells restricted to only one or two of the haemopoietic lineages (Metcalf 1988). Although some data have been presented, which suggest that this occurs in a defined sequence (Brown *et al.* 1988), other data support a stochastic model (Till *et al.* 1964; Nakahala *et al.* 1982; Suda *et al.* 1984). The progenitor cells are recognized by their ability to produce clonally derived colonies *in vitro* (colony-forming cells) and are classified according to their lineage potential (figure 1). Like the CFC-mix, the growth and development of progenitor cells *in vitro* requires the continuous presence of growth promoting agents; in the absence of these growth factors, the cells die.

Growth factors

Many of the growth factors required for survival, proliferation and development of multipotential and lineage restricted progenitor cells *in vitro* have been molecularly cloned and purified to homogeneity (Metcalf 1985; Clark & Kamen 1987). They are all polypeptides which, in their natural form, are glycosylated. It should be noted, however, that the sugar groups do not appear to be essential for biological activity either *in vitro* or *in vivo*: recombinant growth factors, produced in *Escherichia coli*, are as effective stimuli as native material (Metcalf 1985).

One of these growth factors, interleukin-3 (IL-3), promotes proliferation and differentiation of multipotential cells as well as progenitor cells of several different lineages (table 1). Because of this, it is commonly referred to as multi-colony stimulating factor or (multi-CSF). Granulocyte–Macrophage (GM) colony stimulating factor (GM-CSF), preferentially stimulates the proliferation and development of GM-CFC and Eos-CFC when used at a

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Table 1. Target cells for haemopoietic growth factors

(The haemopoietic cell growth factor IL-3 (interleukin-3), GM-CSF (granulocyte–macrophage colony stimulating factor), G-CSF (granulocyte colony stimulating factor), M-CSF (macrophage colony stimulating factor) and epo (erythropoietin) when cultured in the absence of other growth factors can act to varying degrees (✓or ±) on a variety of different cell types as defined in the legend to figure 1.)

	IL-3	GM-CSF	G-CSF	M-CSF	Epo
CFU-S	✓	?	—	—	—
CFC-mix	✓	±	—	—	—
GM-CFC	✓	✓	✓(G)	✓(M)	—
Eos-CFC	✓	✓	—	—	—
BFU-E	✓	±	—	—	±
CFU-E	±	—	—	—	✓
Meg-CFC	✓	±	—	—	±
mast cells	✓	—	—	—	—
neutrophils	±	✓	✓	—	—
macrophages	±	✓	±	✓	—

(relatively) low concentration. At higher concentrations, GM-CSF also promotes proliferation in (a few) CFC-mix, BFU-E and Meg-CFC (Metcalf *et al.* 1980). In other words, the target cells for this growth factor overlaps with the cells stimulated by IL-3.

G-CSF, when initially isolated, was described as a pluripoietin (Welte *et al.* 1985) because of the wide range of colony types studied *in vitro*. Subsequent work, however, has shown that the multi-lineage stimulating activity of the G-CSF is only seen in the presence of other growth and 'synergistic' factors (to be discussed later) and that the primary direct effect of G-CSF is the stimulation of neutrophil development from precursor cells (Metcalf & Nicola 1983). M-CSF, on the other hand, acts upon GM-CFC and M-CFC and facilitates their growth and development down the macrophage lineage (Stanley & Guilbert 1981). The earliest described haemopoietic 'growth factor,' erythropoietin, promotes proliferation and haemoglobinization of erythroid precursor cells: leading to the production of mature, enucleate erythrocytes (Johnson 1989).

In addition to these classically defined colony stimulating factors, a variety of other cytokines (for example interleukin-1 (IL-1), interleukin-4, interleukin-5 and interleukin-6) have been described that can influence the growth and differentiation of myeloid cells. Many of these are lymphokines that directly influence the proliferation or differentiation of B or T lymphocytes. Although (with the exception of IL-5), these lymphokines do not directly stimulate myeloid cell development on their own, they can all synergise (to a greater or lesser extent) with the colony-stimulating factors. Furthermore, it has recently been shown that transforming growth factor (TGF-β) (a cytokine with both growth stimulatory and growth inhibitory effects on a wide variety of cell types) can inhibit the proliferation of multipotential and lineage-restricted haemopoietic progenitor cells in response to the colony-stimulating factors: the effect is cytostatic rather than cytotoxic (Keller *et al.* 1988; Hampson 1989). Factors active on lymphocytes and also the effects of TGF-β are covered in more detail in this symposium (see Honjo *et al.* and Roberts *et al.*). However, what has emerged from this work is that multipotential cells and lineage-restricted cells can respond to a variety of growth stimulatory and inhibitory factors showing that the receptors for these growth factors are widely distributed among the various populations of haemopoietic cells. This has clear implications for differentiation and development of stem cells.

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Role of growth factors in stem cell differentiation

Based upon an analysis of haemopoietic cell development *in vivo*, Curry & Trentin (1967) coined the term 'haemopoietic inductive microenvironment' (HIM). Essentially their thesis was that the local environment (of the bone marrow or spleen) was responsible for directing stem cells along particular pathways of lineage development, that is, specific microenvironments promoting erythroid cell development: other environments promoting granulocyte development and so on. This idea is in contrast to the model proposed by Till & McCulloch (Till *et al.* 1964; Korn *et al.* 1973) who suggested that haemopoiesis is engendered randomly (HER): a stochastic model for stem cell differentiation. As biological events such as stem cell self-renewal or differentiation are almost certainly governed by the laws of probability, Till & McCulloch are correct in proposing a stochastic model. However, if the extent to which an event is likely to occur (for example, development of stem cells along a particular lineage) is influenced by external stimuli in the local environment, then programatically, the HER model has some justification. Based upon our recent data, we propose a deterministic model for stem cell differentiation, which reflects the ability of external stimuli (such as growth factors) to influence self-renewal and lineage development of stem cells.

The first question we approached related to the ability of multipotent haemopoietic cells to directly respond to growth factors. Because marrow cells are heterogeneous and include cell populations (for example, T lymphocytes, macrophages and marrow stromal cells) that are known to produce a variety of cytokines (Quesenberry *et al.* 1987; Rennick *et al.* 1987), the experiments required a relatively pure population of multipotent cells, free of accessory (factor-producing) cells. This was achieved using fluorescence activated cell sorting to enrich for 12-day CFU-S (Visser *et al.* 1984; Lord & Spooncer 1986). These cells are multipotential, can undergo extensive self-renewal, and can reconstitute haemopoiesis in irradiated mice. Thus, although they may not be the most *primitive* haemopoietic stem cells, they do possess the characteristics (self-renewal ability and a multipotential nature) of immediate interest. Spleen colony assays demonstrated that these cells were essentially 'pure' (Visser *et al.* 1984; Lord & Spooncer 1986).

When cultured *in vitro*, in the presence of IL-3, between 6% and up to 40% of the cells were able to undergo clonal proliferation and develop into colonies containing mature cells of multiple myeloid lineages. Thus at least a proportion of these cells possess receptors for (and can respond to) IL-3. In this context, it should be noted that perhaps all the sorted cells have the potential to respond to IL-3, but the culture conditions are suboptimal for their development.

When plated in GM-CSF, we observed about 25% of the number of clones seen in IL-3 (figure 2). Significantly, the colonies developing in GM-CSF contained only macrophages, neutrophils and occasional eosinophils. In M-CSF, colony formation was somewhat better (about 30% of that seen in IL-3) and, in this case, only mature macrophages (and occasional neutrophils) were present in the colonies. G-CSF was a poor stimulus for colony development: the few colonies produced, however, contained only neutrophils. IL-1 (a lymphokine that directly influences T-cells and various stromal cell types) (Dinarello 1984; Rennick *et al.* 1987) was not effective in stimulating the 12-day CFU-S. These data suggest that (some of) the multipotential cells can respond to GM-CSF, M-CSF and (to a much lesser degree) G-CSF as well as IL-3 and that lineage development is a reflection of the stimulus to which the cells are

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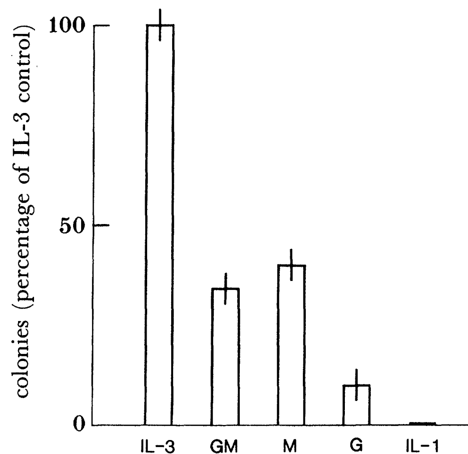

FIGURE 2. The effect of individual haemopoietic growth factors on colony formation from a highly enriched population of pluripotent haemopoietic cells. Cells were cultured in soft agar for ten days after which time the number of clonal colonies were counted. All growth factors were used at maximal plateau concentrations (IL-3 100 U ml^{-1}; GM-CSF 50 U ml^{-1}; M-CSF 20 U ml^{-1}; IL-1 10 U ml^{-1}). Results are expressed as a percentage of the number of colonies formed in the presence of IL-3 (mean ± standard deviation of at least six observations).

exposed. Alternatively, perhaps some lineage-restricted cells had 'co-purified' with 12-day CFU-S.

To test this further, we combined IL-1 (previously shown to be a powerful synergistic stimulus for primitive cells (Stanley *et al.* 1986; Mochizuki *et al.* 1987) with each of the other growth factors. The results (figure 3) demonstrate little or no effect when combined with either IL-3 or with G-CSF, but a dramatic synergism when combined with either GM-CSF or M-CSF. Lineage development, however, was not influenced by the co-treatment with IL-1 (that is GM-CSF and IL-1 stimulated primarily neutrophil and macrophage development: M-CSF and IL-1 promoted mainly macrophage development) and the data suggest that IL-1 can 'recruit' more primitive cells to a GM-CSF or M-CSF responsive state (Heyworth *et al.* 1988).

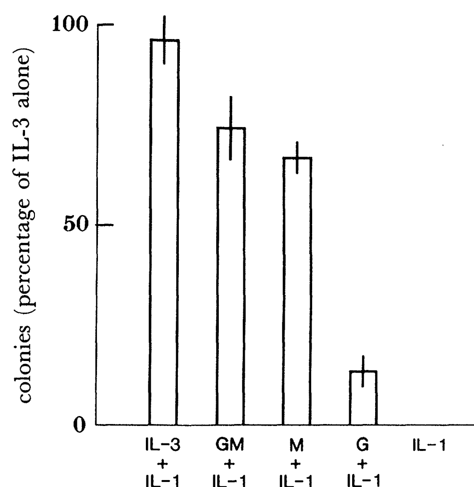

FIGURE 3. The effect of IL-1 and haemopoietic growth factors on colony formation from a highly enriched population of pluripotent stem cells. Results shown are the mean ± standard deviation from at least six observations.

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We also examined the effects of combining G-CSF (itself a poor stimulus for colony formation by the sorted cells) with the other growth factors (figure 4). No effect was seen when added together with IL-3, but (as for IL-1) a profound synergism was seen when G-CSF was combined with either GM-CSF or with M-CSF. Again, however, lineage development was not significantly altered. Thus both IL-1 and G-CSF can synergize with GM-CSF and M-CSF and recruit proliferation and development of cells unable to respond to either factor when used alone. In recent experiments, as yet preliminary, we have found that these growth factors must be added at the same time to have this effect, that is, the ability to respond to combinations of IL-1 and M-CSF does not involve sequential changes in the target cells that allow the cells to respond first to one growth factor and then to the other.

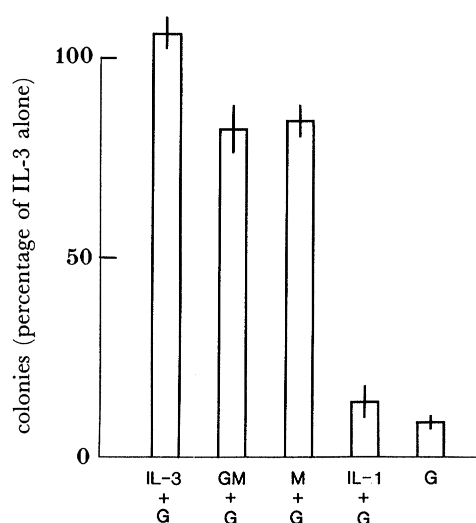

FIGURE 4. The effect of combinations of haemopoietic growth factors plus G-CSF on colony formation from a highly enriched population of pluripotent stem cells. Results are the mean ± standard deviation from at least six observations.

To investigate whether the cells that can respond to combinations of growth factors (such as M-CSF and IL-1 or M-CSF and G-CSF) are also the cells that can respond to IL-3, we cultured the cells in combinations of three growth factors (IL-3, G-CSF and M-CSF or IL-3, M-CSF and IL-1). The results (figure 5) demonstrate that these were non-additive: colony formation did not exceed that found in IL-3 alone. From these data, we conclude that the cells responding to GM-CSF and G-CSF or IL-1 and the cells responding to M-CSF and G-CSF or IL-1 represent a cell population that can also respond to IL-3.

What these data infer, of course, is that some multipotent cells have receptors for multiple growth factors, but that in some instances, more than one growth factor must be present to elicit a response. But, also of great significance, is that the outcome of the response (in terms of lineage development) is a reflection of the growth factors present, that is, IL-3 induces the development of multiple cell lineages whereas GM-CSF and IL-1–G-CSF or M-CSF and IL-1–G-CSF promote development only into neutrophils and macrophages, respectively. In other words, differentiation and lineage-development of multipotent cells is determined by the range and concentration of growth factors in the environmental milieu. Therefore, the observations made by Curry & Trentin (1967) could reflect differences in the capacity of

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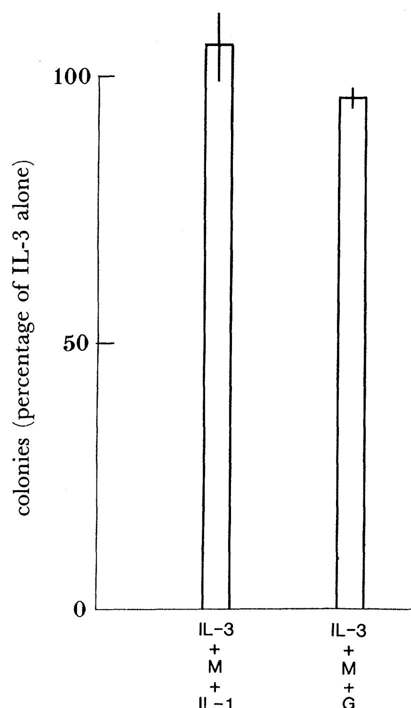

FIGURE 5. The effect of IL-1 and G-CSF, respectively, on the IL-3 and M-CSF stimulated formation of colonies from pluripotent haemopoietic stem cells. Results shown are the mean ± standard deviation from at least six observations.

marrow stromal cells and spleen stromal cells to produce or sequester different species of growth promoting agents. Furthermore, as the stromal cells of the bone marrow are themselves heterogeneous (Lichtman 1981; Dexter 1982; Allen & Dexter 1983), the demonstration of specific cellular associations (for example, between alkaline phosphatase positive reticular cells and developing granulocytes) may represent the more obvious results of qualitative or quantitative differences in the ability of the environment (in this case, the stromal cells) to 'present' growth factors to the haemopoietic cells (Gordon *et al.* 1987; Roberts *et al.* 1988). It is worth noting, in this context, that components of the membrane-associated extracellular matrix (particularly the heparan sulphate proteoglycans) of the stromal cells, appear to be important molecules for binding IL-3 and GM-CSF and presenting these, in a biologically active form, to the appropriate target cells (Gordon *et al.* 1987; Roberts *et al.* 1988) (figure 6).

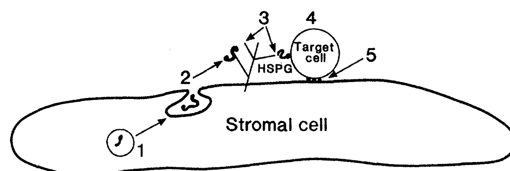

FIGURE 6. A model for stromal cell mediated control of haemopoietic cell proliferation. Stromal cells produce (1) and secrete (2) haemopoietic growth factors (for example, IL-3 and GM-CSF) that are bound by extracellular matrix proteoglycans containing heparan sulphate (3) residues. These heparan sulphate moieties bind and present haemopoietic growth factors to target haemopoietic cells (4) that proliferate and develop. Attachment of the target haemopoietic cells to stromal cells (5), possibly mediated by specific cell adhesion molecules, may precede interaction of the target cell with the growth factor (3).

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It is not unreasonable to suggest therefore, that in the bone marrow environment the stromal cells and extracellular matrix molecules secrete or sequester microenvironments that preferentially promote the development of different cell lineages.

Role of growth factors in stem cell self-renewal

The work presented so far has emphasized the influence of growth factors upon differentiation and lineage-development. What about self-renewal? As discussed previously, in normal 'steady-state' haemopoiesis, self-renewal and differentiation are balanced. When stem cells divide, on average, half of the daughter cells will retain stem cell characteristics and half will undergo differentiation and development. How is this controlled? And do growth factors have a role to play in this process?

Many of the growth factors discussed previously, for example, GM-CSF, M-CSF, G-CSF, promote proliferation with concomitant differentiation and development of progenitor cells: little or no self-renewal occurs. However, a limited degree of 'self-renewal' has been demonstrated after culture of CFU-S in IL-3 alone or in IL-3 in combination with other growth factors (Spivak *et al.* 1985; Clark-Lewis & Schrader 1988). Whether or not this represents self-renewal of stem cells or generation of cells such as CFU-S from an earlier precursor cell has not been adequately resolved. To our knowledge, the only *in vitro* system where maintenance and self-renewal of reconstituting haemopoietic stem cells can reasonably be inferred to occur is in 'long-term' bone marrow cultures. In this system, prolonged haemopoiesis occurs (in the absence of added growth factors) in association with marrow stromal cells. From these, and other data, the idea of a 'stem cell niche' evolved (Schofield 1978) where a microenvironment that facilitates self-renewal and 'protects' the stem cells from differentiation. Although the nature of the proposed 'stem-cell niche' is unknown, some recent data does indeed suggest that growth factors may have a prominent role to play.

Several years ago, it was demonstrated that certain haemopoietic cells, produced within the stromal environment of long-term marrow cultures, would proliferate *in vitro* in the absence of stromal cells, provided that IL-3 was supplied to the cells. In this way, continuously growing, IL-3 dependent, clonally derived cell lines could be obtained routinely. Invariably, these cell lines have a diploid karyotype and are non-leukaemic (Dexter *et al.* 1986). Many of the cell lines have a primitive morphology but others are obviously from the mast cell lineage (Schrader *et al.* 1981). When deprived of IL-3 the cells die.

In subsequent work, we have shown that it is also possible to establish IL-3-dependent cell lines that are multipotent and possess many characteristics in common with normal stem cells. These cells (FDCP-mix) differ from other IL-3 dependent cell lines in several important aspects. First, when initially isolated, they will produce spleen colonies containing cells of multiple haemopoietic lineages when injected into suitably conditioned mice (Spooncer *et al.* 1986). Second, if cultured on marrow stromal cells (in the absence of IL-3) the FDCP-mix cells attach to the stroma and undergo proliferation and multi-lineage development. Third, they can be induced to express this differentiation potential in clonogenic assay systems *in vitro* that encourage the development of multipotent cells (Spooncer *et al.* 1986). In other words, the FDCP-mix cell lines behave like normal 'stem' cells in many aspects. Furthermore, the clear morphological differentiation of these cells that is induced either on stromal cells or in clonogenic assay systems suggested that they could be useful as model systems for approaching the mechanisms underlying self-renewal and differentiation.

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Self-renewal clearly occurs in the presence of IL-3 (figure 7): in liquid culture the cells grow exponentially with a doubling time of about 24 h and more than 99% of cells have positive morphology. Maturing cells are only found infrequently. However, when the concentration of IL-3 is reduced from 100 U ml^{-1} to 1 U ml^{-1}, only limited proliferation is seen, and after seven days culture in these conditions, about 15% of the cells consist of maturing granulocytes and macrophages. In these circumstances, self-renewal is still occurring at the expense of differentiation (figure 8), that is, 'p' the probability of self renewal is greater than 0.5. Clearly, however, the concentration of IL-3 does have an influence upon self-renewal of the FDCP-mix cells.

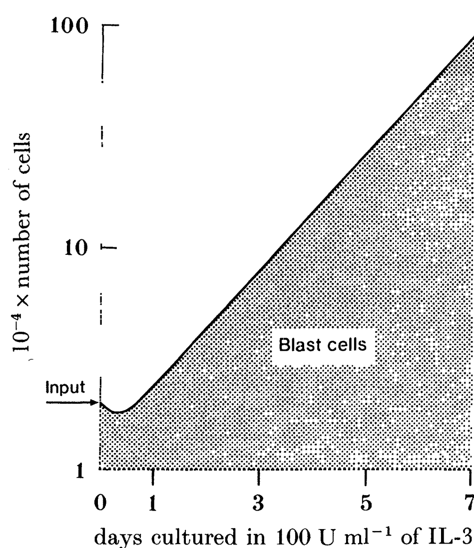

FIGURE 7. Maintenance of primitive cell morphology during the proliferation of FDCP-mix A4 multipotent stem cells in high concentrations of IL-3 (100 U ml^{-1}).

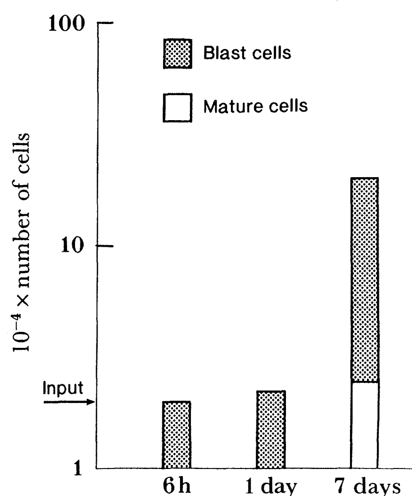

FIGURE 8. The effect of low concentrations of IL-3 on the growth and differentiation of FDCP-mix A4 cells.

To determine if 'p' could be further modulated by the presence of other growth factors, we combined a low concentration of IL-3 with two 'downstream' regulatory molecules, GM-CSF and G-CSF. The result was dramatic: in these circumstances, the cells proliferate at the same rate (and to the same extent) as in the presence of a high concentration of IL-3 (figure 9).

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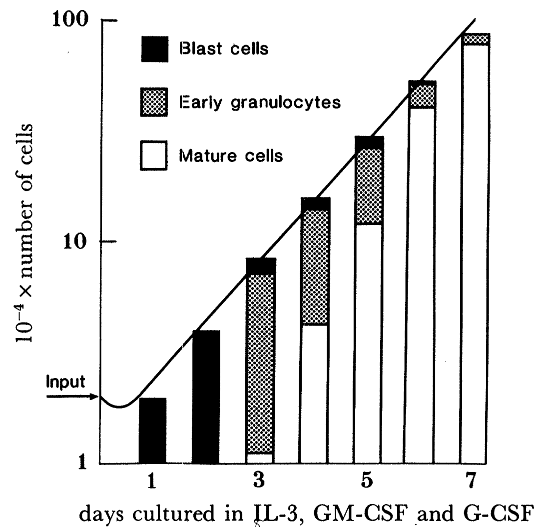

FIGURE 9. The effect of low concentration of IL-3 (1 U ml^{-1}) and GM-CSF (50 U ml^{-1}) and G-CSF (1000 U ml^{-1}) on the proliferation and development of FDCP-mix A4 cells.

For the first two days, only primitive cells are produced. Subsequently, however, myeloblasts and promyelocytes are observed, followed subsequently by the emergence of mature neutrophils and macrophages. After seven days of culture, primitive 'blast' cells are conspicuously absent. These conditions, therefore, appear to preferentially favour cell differentiation and development rather than self-renewal. Indeed, the clonogenic cells (that is, the self-renewing 'stem' cells) present in these cultures have disappeared after five to six days of culture (figure 10).

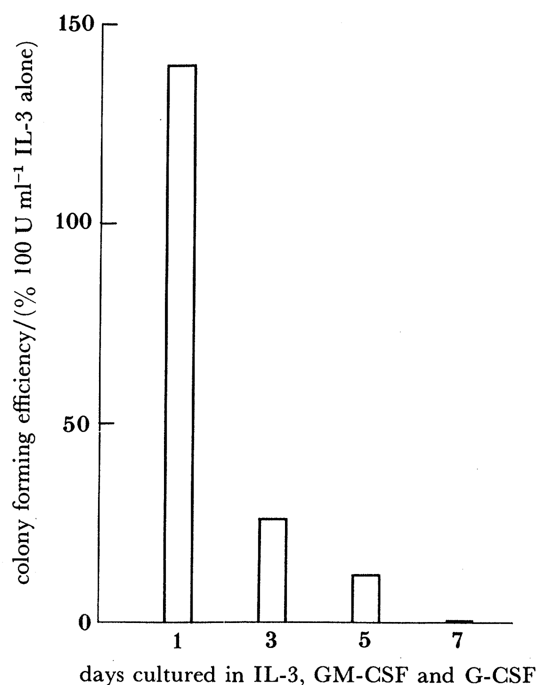

FIGURE 10. The effect of low concentration of IL-3 (1 U ml^{-1}) plus GM-CSF (50 U ml^{-1}) and G-CSF (1000 U ml^{-1}) on the proliferative potential of FDCP-mix A4 cells, as assessed by the ability to form colonies in soft agar gels.

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The data show, therefore, that in the presence of high concentration of IL-3, the FDCP-mix cells undergo preferential self-renewal and that, if the IL-3 concentration is reduced, less self-renewal occurs. This is further exaggerated when a low concentration of IL-3 is combined with GM-CSF and G-CSF.

The pivotal role of IL-3 in this process was further demonstrated by culturing the cells in a constant concentration of GM-CSF and G-CSF and varying the concentration of IL-3 (figure 11). As the IL-3 concentration is increased (irrespective of the presence of GM-CSF and G-CSF) less differentiation and increased self-renewal occurs.

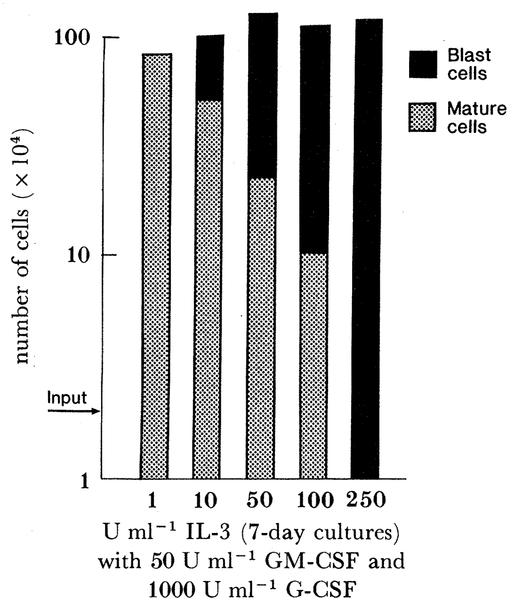

FIGURE 11. The effect of varying IL-3 concentration on the proliferation (self-renewal) and differentiation of FDCP-mix A4 cells in the presence of GM-CSF and G-CSF over a seven-day incubation period.

What does this mean in terms of regulation of stem cell growth and development in the bone marrow? At this time, it is perhaps unwise to extrapolate the results found with the FDCP-mix cells to the control of normal stem cells. In particular, it is unlikely that self-renewal of normal stem cells could be maintained for prolonged periods *in vitro* simply by adjusting the concentration of IL-3. However, the results with the FDCP-mix cells may be indicating a possible mechanism whereby normal stem cell growth and development are regulated. As discussed previously, it is likely that the bone marrow is 'compartmentalized' into discrete cellular 'niches' that can operationally be defined in terms of their ability to promote growth and development of one or the other myeloid cell lineages. In this context, growth factors have an obvious role to play; not only for differentiation and development, but also for self-renewal of stem cells. Based upon the data obtained from the FDCP-mix cells, and the inferences drawn, it is likely that these processes are determined by the range and concentration of growth promoting agents that are present in the local milieu. The problem now, of course, is to define these environments in molecular terms.

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