PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Induction and Inhibition of Differentiation in Normal and Leukaemic Cells

D. Metcalf

Phil. Trans. R. Soc. Lond. B 1990 **327**, 99-109
doi: 10.1098/rstb.1990.0046

Phil. Trans. R. Soc. Lond. B **327**, 99–109 (1990)
Printed in Great Britain

The induction and inhibition of differentiation in normal and leukaemic cells

By D. Metcalf, F.R.S.

The Walter and Eliza Hall Institute of Medical Research, P.O. Royal Melbourne Hospital, Victoria 3050, *Australia*

For granulocytic-macrophage progenitor populations and their progeny, five glycoproteins have been identified: GM-CSF, G-CSF, multi-CSF, M-CSF and IL-6 that can regulate their proliferative activity, maturation and functional activities. The same glycoproteins also have a capacity to induce irreversible differentiation commitment in normal bipotential granulocyte–macrophage progenitors and in some myeloid leukaemic cell lines, which suggests that common cellular processes exist in both situations.

The leukaemia inhibitory factor (LIF) is a glycoprotein, with intriguing properties, which can either induce differentiation in some myeloid leukaemic cell lines or prevent differentiation in normal totipotential embryonic stem cells. The data from the LIF studies suggest a genetic mechanism controlling self-generation that is relatively simple and may be common to all cells. However, the actual cellular response observed appears to depend on the nature of the responding cell.

Introduction

Haemopoietic populations comprise eight distinct cell lineages and are maintained by the extensive capacity for self-generation of a small population of multipotential haemopoietic stem cells (see Metcalf & Moore 1971). These stem cells also generate larger numbers of progenitor cells that are irreversibly committed to a single or restricted number of differentiation lineages. The formation of progenitor cells by stem cells is the major differentiation commitment event in haemopoiesis. However, further differentiation commitment occurs in progenitor cells during their initial cell divisions, at which time the progenitor cells usually become restricted to a single lineage of differentiation. Thereafter, although each progenitor cell can generate up to 10^5 progeny, this proliferation involves no further commitment events even though the cells pass through spectacular morphological changes (maturation) as they progress to fully mature, often post-mitotic, cells.

Analysis of progenitor cell behaviour is feasible because such cells are clonogenic in primary semisolid cultures and the growth factors necessary to stimulate this cell division and the maturation of their progeny are well characterized. Unfortunately, no methods exist for the exclusive culture of multipotential stem cells. Colonies formed by these cells are small (Nakahata & Ogawa 1982) and difficult to identify in cultures containing much larger numbers of colonies formed by progenitor cells.

Despite these difficulties, an analysis has been made of the pattern of differentiation commitment of the initial progeny of multipotential stem cells. This revealed (Suda *et al.* 1984) the occurrence of all conceivable combinations of differentiation commitment in the progeny, leading to the conclusion that progressive differentiation commitment and lineage restriction do not proceed by a fixed sequential pattern, but instead occur in a random (stochastic)

fashion. The observed random pattern of differentiation commitment need not imply that the process is incapable of being manipulated by external signalling. However, a problem in pursuing this possibility with stem cells is that present knowledge of the regulators operating on these cells is rather incomplete.

For these reasons, most of the information available at present on the molecular control of differentiation commitment comes from two types of model systems. In the first, normal bipotential granulocyte–macrophage progenitors become committed to the exclusive formation either of granulocytes or macrophages. In the second differentiation, commitment is monitored in immortalized haemopoietic cell lines or myeloid leukaemic cell lines and, for both types of cell, their abnormal nature requires the data to be interpreted with some caution.

Before proceeding, it is necessary to refer briefly to the haemopoietic growth factors regulating the behaviour of granulocytic and macrophage populations.

The molecules and receptors regulating granulocyte and macrophage populations

The identification of these molecules was made possible by the development of semisolid culture techniques in which specific progenitor cells are stimulated to proliferate and generate colonies of maturing granulocytes and macrophages (see the review by Metcalf (1984)). Analysis of colony formation in such cultures revealed that these cells were incapable of spontaneous proliferation and could only proliferate if stimulated by the addition of various sera, cells or media conditioned by such cells.

Biochemical fractionation of conditioned media able to stimulate granulocyte–macrophage colony formation led to the recognition, and finally the purification of four distinct glycoproteins with colony-stimulating activity (Metcalf 1987). These are termed the colony-stimulating factors (CSFs) and are listed in table 1 with a fifth molecule, interleukin-6, which was originally detected as a proliferative factor for plasmacytoma cells, but has recently been shown to have colony-stimulating activity, not unlike that of G-CSF (Ikebachi *et al.* 1987; Metcalf 1989).

Table 1. The haemopoietic growth factors acting on murine granulocyte–macrophage populations

factor	acronym	relative molecular mass[a]
granulocyte–macrophage colony-stimulating factor	GM-CSF	21000–23000
granulocyte colony-stimulating factor	G-CSF	25000
macrophage colony-stimulating factor	M-CSF (CSF-1)	40000–70000 (dimer)
multipotential colony-stimulating factor	multi-CSF (IL-3)	20000–28000
interleukin-6	IL-6	22000–29000

[a] The relative molecular masses of native glycosylated molecules vary according to tissue source.

The five glycoproteins all have somewhat similar polypeptide chain or subunit lengths of relative molecular mass 13000–20000 although one, M-CSF, is a dimer of two identical subunits. The polypeptide chains are held in a biologically active configuration by mandatory disulphide bridges and substitution of serine for cysteine in these bridges leads to loss of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

biological activity. The subunits of M-CSF are also linked by mandatory disulphide bridges; the individual subunits are without biological activity. The molecules contain a high proportion of carbohydrate (up to 60%) and different cells, when synthesizing CSF appear to add varying amounts of carbohydrate. The carbohydrate may influence the half-life of the molecules in the serum, but does not influence the type or range of actions of the molecules (see reviews by Metcalf (1988) and Nicola (1989)).

There is no significant sequence homology between the CSFs and only minor homology between IL-6 and G-CSF. Each is encoded by a single gene and there is an intriguing clustering of these genes. In man, the genes for GM-CSF, multi-CSF, M-CSF and the M-CSF receptor (*c-fms*) are grouped close together on the long arm of chromosome 5, together with the genes for two allied haemopoietic growth factors, IL-4 and IL-5. In the mouse a similar clustering of GM-CSF, multi-CSF, M-CSF, IL-4 and IL-5 genes occurs on chromosome 11. A similarity in general pattern of exon–intron structure of the CSF genes and their grouping raises the possibility that the CSF genes might have had a common ancestral origin.

Distinct receptors exist for each CSF and IL-6 and these do not allow cross-binding of inappropriate molecules (Nicola 1989). Only one has been identified, the M-CSF receptor, which is the *c-fms* proto-oncogene product (Sherr *et al.* 1985). This receptor has the general characteristics of the epidermal growth factor receptor with an intracellular tyrosine kinase domain that becomes activated after binding of M-CSF. For at least two of the CSFs, the receptors are too small to encode a conventional tyrosine kinase domain and signalling must be achieved by a different mechanism, with some initial evidence that suggests involvement of GTP-binding protein (see review by Nicola (1989)).

Responding granulocytic and macrophage cells do not exhibit large numbers of receptors (a few hundred per cell) and, under equilibrium binding conditions, only a small fraction of these receptors needs to be occupied to achieve a biological response (Nicola & Metcalf 1988). Different subsets of granulocyte–macrophage cells can handle the same CSF-receptor complex in a different manner and the same cell type can handle different CSF–receptor complexes in a quite different manner (Nicola *et al.* 1988). The signalling system is complex because most granulocytic and macrophage cells simultaneously exhibit receptors for at least three CSFs and the CSF receptors are linked in *trans*-modulation interactions where occupation by one type of CSF of its receptor leads to down-modulation of other CSF receptors in a hierarchical sequence (Nicola 1989).

The multiple functional actions of the CSFs

Proliferative stimulation of the precursors of granulocytes and macrophages requires the constant presence of CSF (Metcalf 1984). Some experiments appear to show that interrupted stimulation by CSF can achieve some cell division but the relatively long intracytoplasmic half-life of bound CSF–receptor complexes may enable sufficiently sustained signalling in such experiments to achieve the observed proliferation (Nicola 1989). The CSFs are active at low concentrations (in the picograms per millilitre range) and a direct relationship has been documented between CSF concentration and the length of the cell cycle (Metcalf 1980; 1985). Withdrawal of cells from CSF, at least for CSF-dependent cell lines, leads to a failure of most cells to complete the cell cycle in progress at the time (Metcalf 1985).

There is considerable overlap in the actions of these molecules. Thus GM-CSF and multi-

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

CSF can stimulate the proliferation of most granulocyte–macrophage progenitors with the formation of both granulocyte and macrophage progeny; M-CSF, acting on essentially the same progenitor population, stimulates the formation mainly of macrophages, whereas G-CSF has a more restricted range of responsive progenitor cells and is mainly a proliferative stimulus for granulocyte formation (Metcalf 1987). IL-6, at least when stimulating murine cells, has an action rather similar to that of G-CSF (Metcalf 1989).

A feature of the biology of the colony stimulating factors is that each CSF is multifunctional *in vitro* (see review by Metcalf (1988)). Thus the CSFs, in addition to controlling cell division, also influence the viability of progenitor cells and their progeny probably by maintaining membrane integrity, induce differentiation commitment and maturation and can increase the functional activity of mature granulocytes and macrophages.

Whereas for some CSFs only a single type of receptor exists, these differing cellular responses require considerable complexity in the signalling cascades that can be initiated by the binding of CSF to its receptor.

The actual effects observed after CSF stimulation depend on the gene programming of the cell responding. For example, a progenitor cell responds to CSF stimulation by proliferation and perhaps differentiation commitment, whereas a post-mitotic polymorph responds merely by functional activation. The receptors and the CSF concentrations required can be identical, but the particular response elicited appears to be determined by the cell itself.

The induction of differentiation commitment in normal granulocyte–macrophage progenitor cells

In early studies using highly enriched populations of granulocyte–macrophage progenitor cells it was observed that, in cultures stimulated by M-CSF, most progenitors proliferated and formed colonies comprised of macrophages. When GM-CSF was used as the stimulus, equivalent numbers of colonies developed, but many were composed wholly or partly of granulocytes (Horiuchi *et al.* 1979).

These observations suggested that the type of CSF used was able to determine the differentiation lineage entered by the progeny of the progenitor cells, a differentiation commitment action. However, the interpretation is complicated by the existence of three types of granulocyte–macrophage progenitors, unipotential progenitors, precommitted either to granulocyte or macrophage formation, and a population of bipotential progenitors able to form both types of progeny (Metcalf 1984).

The accumulation of less equivocal evidence regarding the ability of CSFs to determine progenitor cells commitment required attention to be concentrated on those progenitors demonstrable as being bipotential. By studying the behaviour of matching daughter or granddaughter cells of progenitors after micromanipulation and separate culture, two phenomena were demonstrable: (i) high GM-CSF concentrations were able to stimulate the formation by one daughter cell of progeny that included granulocytes whereas low GM-CSF concentrations stimulated the formation by the other daughter only of macrophages (Metcalf 1980), and (ii) initial stimulation of progenitor cell divisions by M-CSF tended to result in progeny that generated only macrophages, even if the cells were subsequently stimulated by GM-CSF, whereas initial stimulation by GM-CSF led to the formation of progeny, some of which continued to form granulocytes even when stimulated by M-CSF (Metcalf & Burgess 1982).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

This latter phenomenon became more evident after stimulation of three to four divisions by the initial CSF.

These observations suggested three features of the commitment event: its irreversibility, its asymmetry and its likely dependence on cell cycling and passage of the cells through chromosomal duplication.

CSF-induced differentiation commitment can lead to interesting consequences when combinations of CSFs are used. Thus combination of G-CSF with M-CSF can lead to the formation of curious mixed granulocyte–macrophage colonies in which discrete subclones of granulocytic cells are embedded in an otherwise macrophage colony (Metcalf *et al.* 1983). A reasonable inference is that the phenomenon is the consequence of competition for commitment of the initial progeny of the original bipotential cell, a competition process that occurs despite the overall potentiation of growth resulting from combination of the two stimuli. Thus, in these cultures, there is an overall size increase in both granulocytic and macrophage colonies as well as granulocyte–macrophage colonies (Metcalf 1984).

The ability of combinations of CSFs to induce additive or superadditive proliferative responses suggests that the portion of the signalling cascade initiated by binding of CSF to its receptor which stimulates cell division may be shared in common with other CSF–receptor complexes. However, the differentiation commitment action would probably require a specific product of the signalling cascade to bind to a particular regulatory region for the gene complex involved in commitment. For this function, the evidence would suggest that qualitatively different signalling products would be necessary to achieve commitment into two distinct lineages and that such signal products would not be shared between all CSF–receptor complexes.

Whereas these *in vitro* experiments show an ability of CSFs to induce differentiation commitment in progenitor cells, it should be considered whether this process is likely to occur *in vivo* and a significant mechanism controlling the usual commitment process in granulocyte–macrophage progenitors, most of which reside in the bone marrow. An obvious requirement is that the CSFs in the circulation can obtain access to marrow cells or that adequate local production of CSFs occurs in the bone marrow. M-CSF is present in the serum in significant concentrations (Bartocci *et al.* 1986) but there are some observations suggesting that circulating M-CSF may not have free access to marrow cells (Shadduck *et al.* 1989). The situation with multi-CSF is quite obscure because it has not been detected in the serum or tissues (Metcalf *et al.* 1987). However, autoradiographic studies using intravenously injected ^{125}I-labelled multi-CSF did demonstrate its ability to reach and bind to marrow cells *in vivo* (Metcalf & Nicola 1988).

Both G-CSF and GM-CSF can be present in the serum and elevated levels can occur in states of perturbation, for example, during infections (Metcalf 1987). Of possibly more importance, the stromal cells in the marrow have a clear capacity to produce M-CSF (Lanotte *et al.* 1982) and, particularly after induction, to produce G-CSF and GM-CSF and IL-6 (Rennick *et al.* 1987; Lee *et al.* 1988).

Whereas no technique exists for assaying CSF concentrations in the tissue fluid of the marrow, the above observations suggest that necessary concentrations of CSFs and IL-6 are likely to be available in the locality of marrow progenitor cells and that the above *in vitro* phenomena could quite reasonably be expected to occur *in vivo*.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Differentiation commitment in myeloid leukaemic cell lines

Studies on several myeloid leukaemic cell lines have provided intriguing evidence of the ability of haemopoietic regulators to induce differentiation. Established murine or human myeloid leukaemic cell lines can be maintained in continuous culture because of the ability of at least a proportion of the cells to exhibit a high level of self-renewal cell divisions. The frequency of such cells may range from a low percentage to almost 100% of the population and as single cells of this type are able to initiate and sustain subclones of the cell lines, they are referred to as stem cells.

It is unclear how closely such leukaemic stem cells resemble normal multipotential haemopoietic stem cells. The likelihood is that the term 'leukaemic stem cell' is somewhat misleading and should be interpreted to mean little more than a cell that can initiate and sustain continuously proliferating lines of leukaemic cells. Not all clonogenic leukaemic cells have an ability for indefinitely sustained proliferation and often there is also a low percentage of cells in the line that exhibits quasi-normal maturation to identifiable mature cells. Most of the commonly used myelo-monocytic cell lines appear to possess no capacity to differentiate into cells of other lineages, so the clonogenic cells in such lines might be better regarded as neoplastic progenitor cells, differing from normal progenitors in exhibiting a sustained capacity for self-generation.

In at least some experimental models, clonogenic leukaemic cells appear to have the option of forming two types of progeny: cells such as the parental cells (self-renewal division) or cells characterizable as having undergone differentiation commitment (differentiative division). In at least one model, analysis showed that asymmetrical divisions occurred, one daughter remaining a stem cell, the other becoming differentiated (Metcalf 1982).

In their simplest form, the leukaemic cell lines provide models in which the occurrence of differentiation induction can be monitored by determining the frequency of clonogenic cells in colonies forming the clonogenic cells of the line. For self-sustaining normal haemopoietic stem cells, self-renewal divisions do not exceed 50%, but for leukaemic stem cells, always exceeds 50% and can approach 100%. This abnormally high self-renewal capacity is not exclusive to leukaemic cells and does not identify the leukaemic state. Many continuous haemopoietic cell lines have stem cells with an equally high capacity for self-renewal (Metcalf 1985), but are not leukaemogenic and even normal stem cells can exhibit levels of self-renewal *in vivo* in excess of 50% when called upon to replenish the haemopoietic tissues after depletion by irradiation or cytotoxic drugs (Metcalf & Moore 1971).

The progeny arising from differentiative divisions are altered cells that can be identified by one of a variety of altered properties: (i) the cells may remain clonogenic but exhibit a restricted capacity for self-generation or a restricted capacity for continuing cell division; (ii) the cells may exhibit proliferative capacity, but generate cells some or all of which undergo morphological or functional maturation; (iii) the cells may lose all proliferative capacity and undergo maturation; or (iv) the cells may die without evidence of maturation (Metcalf 1982).

It is unclear whether this variety of altered states represents qualitatively different differentiation commitment events or is merely a continuum of a single event becoming more evident with further divisions or simply being more evident in some cells than others because of underlying heterogeneity in cells of the leukaemic line. The variety of changes identifying the occurrence of a differentiative division does emphasize that maturation is not a necessary

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

outcome of such divisions and, with certain cell lines, marked or complete suppression of self-renewal with extinction of the leukaemic population can be accomplished without evidence of morphological maturation (Begley *et al.* 1987).

Although some reservations should be maintained whether differentiation induction in leukaemic cell lines is necessarily a valid model of differentiation induction in normal stem or progenitor cells, the data so far available support the conclusion that the two processes can be very similar.

In the initial studies using the murine myelomonocytic leukaemic cell line M1, culture of clonogenic cells in the presence of various types of conditioned media led to the formation of colonies of differentiating granulocytes macrophages (Ichikawa 1969; 1970), and in subsequent studies, such cells were shown to acquire membrane markers and functional activity consistent with such morphological differentiation (see review by Sachs (1987)).

During the period when the regulators of normal granulocyte and macrophage populations were being identified and purified, some confusion arose regarding the identity of the regulators able to induce differentiation in M1 leukaemic cells. This was compounded by the use of a second leukaemic cell line, the WEHI-3B, which exhibited similar phenomena, but in response to apparently different regulators. The situation has now been clarified and differentiation in M1 cells can be induced by the leukaemia inhibitory factor, interleukin-6 (IL-6), but less efficiently by G-CSF (Metcalf *et al.* 1988; Shabo *et al.* 1988; Metcalf 1989). WEHI-3B cells can also be induced to differentiate by G-CSF, IL-6, but less efficiently by GM-CSF (Metcalf 1979, 1989; Metcalf & Nicola 1982). Table 2 lists information for two human myeloid leukaemic cell lines arranging the regulators in order of their apparent activity.

TABLE 2. THE HAEMOPOIETIC GROWTH FACTORS WITH DIFFERENTIATING-INDUCING ACTIVITY FOR MYELOID LEUKAEMIC CELL LINES

		differentiating-inducing activity[a]	
species	cell line	high	moderate to low
mouse	M1	LIF, IL-6	G-CSF
	WEHI-3B	G-CSF, IL-6	GM-CSF
man	HL60	—	GM-CSF, IL-6, LIF
	U937	GM-CSF, IL-6	G-CSF, LIF

[a] Differentiation-inducing activity assessed from ability to suppress self-renewal of clonogenic cells. Factors are listed in order of relative activity.

In the WEHI-3B system, the leukaemic cells are now autonomous and do not depend on extrinsic CSF for proliferative stimulation. G-CSF exhibits the opposing effects of initially stimulating WEHI-3B cell proliferation (similar to its action on normal cells) but then inducing relatively late in the incubation period (3–4 days) a proportion of the colony cells to exhibit maturation to granulocytes and macrophages, a process visible in semisolid cultures as a migration of these cells away from the compact centre of undifferentiated leukaemic cells (Metcalf 1982). Use of G-CSF does not reduce the number of colonies developing in such cultures.

Analysis of daughter and grand-daughter cells of WEHI-3B cells dividing in the presence of G-CSF indicated that induction of differentiation was an asymmetrical process, requiring passage of the cells through a full cell cycle (Metcalf 1982). Having occurred, differentiation induction was irreversible.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

When IL-6 induces differentiation commitment in WEHI-3B cells, the range of subsequent cellular behaviour appears similar to that induced by G-CSF except that events appear to move more rapidly with clear evidence of cellular maturation in affected clones within 48 h (Metcalf 1989).

The possibility that quite different molecular mechanisms might achieve differentiation induction in leukaemic cells was raised by studies using a second murine leukaemic cell line, the M1. Both LIF and IL-6 can induce an identical response in this cell line (Metcalf 1989). As concentrations are increased there is a progression of observable changes. With low concentrations there is a reduction in colony size; with higher concentrations there is the induction of colonies containing a halo of maturing macrophages and at even higher concentrations, progressive reduction in colony numbers with a final complete suppression of all clonogenic cells, the cultures contained no surviving viable cells. Reduction in clonogenicity is detectable within 3–6 h of exposure of M1 cells to LIF and as in the case of the action of G-CSF on WEHI-3B cells, LIF-induced changes on M1 cells are irreversible. With the M1 subline used, only macrophage differentiation appeared possible. A clue regarding a possible mechanism for the rapid cellular suppression observed was that cells exposed to otherwise suppressive concentrations of LIF or IL-6 could survive and proliferate to form colonies of maturing macrophages if M-CSF (but not GM-CSF, multi-CSF or G-CSF) was added to the cultures (Metcalf *et al.* 1988; Metcalf 1989). This suggests that, among other changes, LIF or IL-6 may have induced the expression of M-CSF receptors on differentiating M1 cells and thereby induced an M-CSF-dependent state. Normal M-CSF-dependent macrophages are especially sensitive to death after withdrawal of M-CSF (Metcalf & Merchav 1982) and the leukaemic cells may also exhibit a similar phenomenon.

The dramatic loss of clonogenicity in M1 cells after exposure to LIF or IL-6 suggests that a quite different mechanism may be involved from that occurring in the WEHI-3B/G-CSF system. Does this difference occur because of the regulator used or because of the responding leukaemic cells. The fact that IL-6 induces differentiation responses in WEHI-3B cells without suppression of clone numbers and that G-CSF can enhance the actions of LIF or IL-6 on M1 cells, without altering the basic pattern of the response in M1 cells, suggests that the nature of the responding cells rather than the inducing factor dictates the type of response observed.

The notion that leukaemic cells (and possibly normal cells) face an all-or-none decision regarding differentiation commitment when passing through a certain phase of the cell cycle is supported by evidence from the induction of differentiation in leukaemic cells by using chemical inducers such as DMSO or butyrate (Boyd & Metcalf 1984; Von Melchner & Hoffken 1985). In these systems the location of the decision point in the cell cycle was the G_1-S interface, suggesting the involvement of an early event in chromosomal duplication, leading to permanent modification of a specific regulatory sequence.

The two models discussed may suggest a special importance of G-CSF, IL-6 and LIF in differentiation commitment of myeloid leukaemic cells, but this results largely from the use of two particular cell populations that happen to respond poorly or not at all to other regulators. Studies on HL60, and particularly U937 cells, place the various regulators in a quite different hierarchy. For example, with human U937 leukaemic cells, GM-CSF is by far the most active suppressing agent (Maekawa & Metcalf 1989) and data have shown, by using human acute myeloid leukaemic populations, that M-CSF in many instances is the most effective suppressing agent (Miyauchi *et al.* 1988).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

It seems reasonable to conclude that all of the regulators (with the possible exception of multi-CSF) may have the potential to suppress self-generation in myeloid leukaemic populations, with the nature of the particular leukaemic population determining which is the most effective regulator.

Suppression of differentiation commitment by LIF

The basic bioassay used to monitor the purification of LIF was its ability to induce differentiation in M1 leukaemic cells (Tomida *et al.* 1984; Hilton *et al.* 1988). However, a remarkable situation has recently been documented with the demonstration that LIF has a powerful action in inhibiting differentiation commitment in normal totipotential embryonic stem cells (Williams *et al.* 1988; Smith *et al.* 1988).

The concentrations of LIF required to inhibit differentiation in embryonic stem cells are identical to those required to induce differentiation in M1 cells and LIF receptors on the two cells types, are present in comparable numbers and have an identical binding affinity.

This conflicting action of LIF, according to the target cell responding is remarkable, but not entirely different from the opposing actions of the CSFs in being both proliferative stimuli and inducers of differentiation to maturing post-mitotic cells.

This raises the possibility that a rather simple switch mechanism may control the decision whether to self-generate or generate progeny that are committed: commitment being loosely defined as an altered state in which the cell behaves in one of the variety of ways listed above. If the switch mechanism is envisaged as a regulatory element of a gene or gene sequence normally ensuring self-generation then the activated LIF–receptor complex might initiate production of a binding protein able to interact with this regulatory sequence. The consequence of this interaction is either to inactivate the regulatory sequence or to allow it to remain operative. This hypothesis requires that the gene programme active in the target cell itself dictates the actual outcome of the interaction with the regulatory sequence, a situation similar to that determining which type of cellular response occurs following CSF stimulation of normal cells.

The ability of a single regulator to influence self-generation in two such different cells as normal embryonic stem cells and myeloid leukaemic cells suggests that the mechanism controlling self-generation might be common to all cells and not unique for each cell type. If so, two mechanisms would prevent all cells in the body responding to a single agent such as LIF: (i) the requirement for expression of membrane receptors for the initiating regulator molecule, and (ii) the pattern of transcriptional activity pre-existing in the responding cell that might not only determine what type of response occurs, but whether any response occurs at all.

There are many myeloid leukaemic cell lines expressing receptors for the various CSFs or LIF that are refractory to suppression by these agents and these may represent instances where the pattern of transcriptional activity of relevant genes prevents differentiation induction.

References

Bartocci, A., Pollard, J. W. & Stanley, E. R. 1986 Regulation of colony-stimulating factor 1 during pregnancy. *J. exp. Med.* **164**, 956–961.

Begley, C. G., Metcalf, D. & Nicola, N. A. 1987 Purified colony-stimulating factors (G-CSF and GM-CSF) induce differentiation in HL60 cells with suppression of clonogenicity. *Int. J. Cancer* **39**, 99–105.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Boyd, A. W. & Metcalf, D. 1984 Induction of differentiation in HL60 cells: a cell cycle dependent all-or-none effect. *Leukemia Res.* **8**, 27–43.
Hilton, D. J., Nicola, N. A., Gough, N. M. & Metcalf, D. 1988 Resolution and purification of three distinct factors produced by Krebs ascites cells which have differentiation-inducing activity on murine myeloid leukemic cell lines. *J. biol. Chem.* **263**, 9238–9243.
Horiuchi, I., Nagata, K. & Ichikawa, Y. 1979 'Determination' of differentiation of bipotent hemopoietic progenitor cells *in vitro*. *Expl Cell Res.* **123**, 247–252.
Ichikawa, Y. 1969 Differentiation of a cell line of myeloid leukemia. *J. cell. Physiol.* **74**, 223–234.
Ichikawa, Y. 1970 Further studies on the differentiation of a cell line of myeloid leukemia. *J. cell. Physiol.* **76**, 175–184.
Ikebachi, K., Wong, G. G., Clark, S. C., Ihle, J. N., Hirai, Y. & Ogawa, M. 1987 Interleukin-6 enhancement of interleukin-3-dependent proliferation of multipotential hemopoietic progenitors. *Proc. natn. Acad. sci. U.S.A.* **84**, 9035–9039.
Lanotte, M., Metcalf, D. & Dexter, T. M. 1982 Production of monocyte/macrophage colony-stimulating factor by preadipocyte cell lines derived from murine marrow stroma. *J. cell. Physiol.* **112**, 123–127.
Lee, F., Yokota, T., Chiu, C. P., De Vries, J., Banchereau, J., Arai, N., Coffman, R., Rennick, D. & Arai, K. 1988 The molecular cloning of interleukins-4 and 6: Multifunctional hemopoietic growth factors. *Behring Inst. Mitt.* **83**, 8–14.
Maekawa, T. & Metcalf, D. 1989 Clonal suppression of HL60 and U937 cells by recombinant human leukemia inhibitory factor in combination with GM-CSF or G-CSF. *Leukemia* **3**, 270–276.
Metcalf, D. 1979 Clonal analysis of the action of GM-CSF on the proliferation and differentiation of myelomonocytic leukemic cells. *Int. J. Cancer* **24**, 616–623.
Metcalf, D. 1982 Clonal analysis of proliferation and differentiation of paired daughter cells: action of granulocyte–macrophage colony-stimulating factor on granulocyte–macrophage precursors. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5327–5330.
Metcalf, D. 1982 Regulator-induced suppression of myelomonocytic leukemic cells: clonal analysis of early cellular events. *Int. J. Cancer* **30**, 203–210.
Metcalf, D. 1984 *The hemopoietic colony stimulating factors*. Amsterdam: Elsevier.
Metcalf, D. 1985 Multi-CSF-dependent colony formation by cells of a murine hemopoietic cell line: specificity and action of multi-CSF. *Blood* **65**, 357–362.
Metcalf, D. 1987 The molecular control of normal and leukaemic granulocytes and macrophages. *Proc. R. Soc.* B**230**, 389–423.
Metcalf, D. 1988 *The molecular control of blood cells*. Cambridge, Massachusetts: Harvard University Press.
Metcalf, D. 1989 Actions and interactions of G-CSF, LIF and IL-6 on normal and leukemic murine cells. *Leukemia* **3**, 349–355.
Metcalf, D. & Burgess, A. W. 1982 Clonal analysis of progenitor cell commitment to granulocyte or macrophage production. *J. cell. Physiol.* **111**, 275–283.
Metcalf, D. & Merchav, S. 1982 Effects of GM-CSF deprivation on precursors of granulocytes and macrophages. *J. cell. Physiol.* **112**, 411–418.
Metcalf, D. & Moore, M. A. S. 1971 Haemopoietic Cells. Amsterdam: North-Holland.
Metcalf, D. & Nicola, N. A. 1982 Autoinduction of differentiation in WEHI-3B leukemia cells. *Int. J. Cancer* **30**, 773–780.
Metcalf, D. & Nicola, N. A. 1988 Tissue localization and fate in mice of injected multipotential colony-stimulating factor. *Proc. natn. Acad. Sci. U.S.A.* **85**, 3160–3164.
Metcalf, D., Hilton, D. J. & Nicola, N. A. 1988 Clonal analysis of the action of the murine leukemia inhibitory factor on leukemic and normal murine hemopoietic cells. *Leukemia* **2**, 216–221.
Metcalf, D., Merchav, S. & Wagemaker, G. 1983 Commitment by GM-CSF or M-CSF of bipotential GM progenitor cells to granulocyte or macrophage formation. In *Experimental hematology today 1982* (ed. S. J. Baum, G. D. Ledney & S. Theirfelder), pp. 3–9. Basel: Karger.
Metcalf, D., Begley, C. G., Nicola, N. A. & Johnson, G. R. 1987 Quantitative responsiveness of murine hemopoietic populations in vitro and in vivo to recombinant multi-CSF (IL-3). *Expl Hematol.* **15**, 288–295.
Miyauchi, J., Wang, C., Kelleher, C. H., Wong, G. G., Clark, S. C., Minden, M. D. & McCulloch, E. A. 1988 The effects of recombinant CSF-1 on the blast cells of acute myeloblastic leukemia in suspension culture. *J. cell. Physiol.* **135**, 55–62.
Nakahata, T. & Ogawa, M. 1982 Identification in culture of a class of hemopoietic colony-forming unit with extensive capability to self-renew and generate multipotential colonies. *Proc. natn. Acad. Sci. U.S.A.* **79**, 3843–3847.
Nicola, N. A. 1990 Hemopoietic growth factors and their receptors. *A. Rev. Biochem.* (In the press.)
Nicola, N. A. & Metcalf, D. 1988 Binding internalization and degradation of ^{125}I-multipotential colony stimulating factor (interleukin-3) by FDC-P1 cells. *Growth Factors* **1**, 29–39.
Nicola, N. A., Peterson, L., Hilton, D. J. & Metcalf, D. 1988 Cellular processing of murine colony-stimulating factor (multi-CSF, GM-CSF, G-CSF) receptors by normal hemopoietic cells and cell lines. *Growth Factors* **1**, 41–49.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Rennick, D. M., Yang, L., Gemmel, L. & Lee, F. 1987 Control of hemopoiesis by a bone marrow stromal cell clone: lipopolysaccharide- and interleukin 1-inducible production of colony stimulating factors. *Blood* **69**, 682–691.

Sachs, L. 1987 The molecular regulators of normal and leukaemic blood cells. *Proc. R. Soc. Lond.* B**231**, 289–312.

Shabo, Y., Lotem, J., Rubenstein, M., Revel, M., Clark, S. C., Wolf, S. G., Kamen, R. & Sachs, L. 1988 The myeloid blood cell differentiation-inducing protein MGI-2A is interleukin-6. *Blood* **72**, 2070–2073.

Shadduck, R. K., Waheed, A. & Wing, E. J. 1989 Demonstration of a blood-bone marrow barrier to macrophage colony-stimulating factor. *Blood* **73**, 68–73.

Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T. & Stanley, E. R. 1985 The c-fms proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor CSF-1. *Cell* **41**, 665–676.

Smith, A. G., Heath, J. K., Donaldson, D. D., Wong, G. G., Moreau, J., Stahl, M. & Rogers, D. 1988 Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. *Nature, Lond.* **336**, 688–690.

Suda, J., Suda, T. & Ogawa, M. 1984 Analysis of differentiation of mouse hemopoietic stem cells in culture by sequential replating of paired progenitors. *Blood* **64**, 393–399.

Tomida, M., Yamamoto-Yamiguchi, Y. & Hozumi, M. 1984 Purification of a factor inducing differentiation of mouse myeloid leukemic M1 cells from conditioned medium of mouse fibroblast L929 cells. *J. biol. Chem.* **259**, 10978–10982.

Von Melchner, H. & Hoffken, K. 1985 Commitment to differentiation of human promyelocytic leukemia cells (HL60): an all-or-none event preceded by reversible losses of self-renewal potential. *J. cell. Physiol.* **125**, 573–581.

Williams R. L., Hilton, D. J., Pease, S., Willson, T. A., Stewart, C. L., Gearing, D. P., Wagner, E. F., Metcalf, D., Nicola, N. A. & Gough, N. M. 1988 Myeloid leukemia inhibitory factor (LIF) maintains the developmental potential of embryonic stem cells. *Nature, Lond.* **336**, 684–687.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES