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The Molecular Control of Haemopoiesis and Leukaemia

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The establishment of a cell culture system for the clonal development of haemopoietic cells made it possible to discover the proteins that regulate cell viability, growth and differentiation of different haemopoietic cell lineages and the molecular basis of normal and abnormal development in blood-forming tissues. These regulators include cytokines now called colony stimulating factors (CSFs) and interleukins (ILs). Different cytokines can induce cell viability, multiplication and differentiation, and haemopoiesis is controlled by a network of cytokine interactions. The multigene network includes positive regulators such as CSFs and ILs and negative regulators such as transforming growth factor β and tumour necrosis factor. The cytokine network which has arisen during evolution allows considerable flexibility depending on which part of the network is activated and the ready amplification of response to a particular stimulus. The CSFs and ILs induce cell viability by inhibiting programmed cell death (apoptosis). Programmed cell death is also regulated by the genes wild-type and mutant p 53, *c-myc* and *bcl-2*, and suppression or induction of this programme can result in tumour promotion or tumour suppression. Cytokines that regulate normal haemopoiesis can control the abnormal growth of certain types of leukaemic cells and suppress malignancy by inducing differentiation. Genetic abnormalities that give rise to malignancy in these leukaemic cells can be by-passed and their effects nullified by inducing differentiation and programmed cell death. The haemopoietic cytokines discovered in culture are active *in vivo* and are being used clinically to correct defects in haemopoiesis.

Key words: haemopoiesis, colony stimulating factors, cytokines, network, growth, differentiation, programmed cell death, evolution, leukaemia
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INTRODUCTION

THE DESCRIBED cultures thus seem to offer a useful system for a quantitative kinetic approach to haematopoietic cell formation and for experimental studies on the mechanism and regulation of haematopoietic cell differentiation [1].

In order to analyse the controls that regulate viability, multiplication and differentiation of normal haemopoietic cells to different cell lineages and the changes in these controls in disease, it is desirable and convenient to study the entire process in cell culture starting from single cells. Analysis of the molecular control of different types of haemopoietic cells, therefore, began with the development of a cell culture system for the cloning and clonal differentiation of different types of normal haemopoietic cells. This cell culture system was then used to discover a family of cytokines that regulate cell viability, multiplication and differentiation of different haemopoietic cell lineages, to analyse the origin of some haematological diseases, and to identify ways of treating these diseases with normal cytokines. I will mainly discuss cells of the myeloid cell lineages which have been used as a model system.

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CLONAL DEVELOPMENT OF NORMAL HAEMOPOIETIC CELLS IN CULTURE

In the cell culture system that was developed, normal cells from blood-forming tissues from mice were first cultured with feeder layers of other cell types such as normal embryo fibroblasts. These other cell types were chosen as possible candidates for cells that produce the regulatory molecules required for the cloning and differentiation of different haemopoietic cell lineages. The first such system [1, 2], using cells cultured in liquid medium (Figure 1, Table 1), showed that it was possible to obtain by this procedure clones containing mast cells or granulocytes in various stages of differentiation. To make it simpler to distinguish and isolate separate clones, this system was then applied to the cloning of different cell lineages in semi-solid medium containing agar [3–5]. Analysis of the first types of clones obtained in agar with these feeder layers showed clones containing macrophages, granulocytes, or both macrophages and granuloyctes, in various stages of differentiation. The macrophage clones in agar contained many metachromatic granules giving them an apparent morphological resemblance to mast cells [3–5]. However, these granules were not present when the cells were cloned in methylcellulose [4], and electron microscopy also demonstrated that these cells in agar were really macrophages that had phagocytosed agar [6]. The experiments also showed that these clones could originate from single cells [3–5, 7].

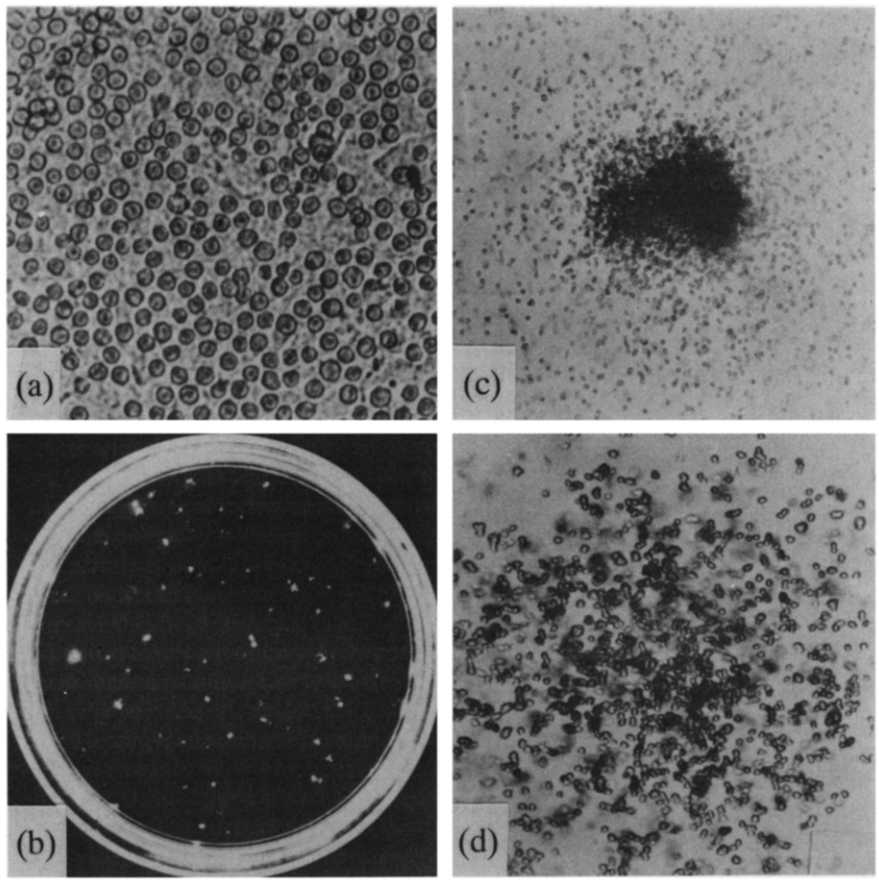

Figure 1. Cell culture system for cloning and clonal differentiation of normal haematopoietic cells. (a) Culture of mouse mast cells that have multiplied and differentiated on a feeder layer of mouse embryo cells [1]. (b–d) Clones of macrophages and granulocytes in cultures of normal haemopoietic cell precursors incubated with the appropriate inducer in semi-solid medium containing agar. (b) Petri dish with clones [3]. (c) Granulocyte clone and (d) macrophage clone [4].

Table 1. Establishment of the cell culture system for cloning and clonal differentiation of normal haemopoietic cells and the use of these cultures to discover colony stimulating factors

Cloning and differentiation in liquid medium (mast cells and granulocytes) [1]
Cloning and differentiation in agar (macrophages and granulocytes) [3–5]
Cloning and differentiation in methylcellulose (macrophages and granulocytes) [4]
Inducers for cloning and differentiation secreted by cells [3]
Inducers for cloning and differentiation in cell culture supernatants (macrophages and granulocytes) [4, 7]

This assay in agar [3–5] or methylcellulose [4] (Figure 1, Table 1) was then applied to cloning and clonal differentiation of normal human macrophages and granulocytes [8, 9] and to the cloning of all the other blood cell lineages, including erythroid cells [10], B lymphocytes [11] and T lymphocytes [12].

THE DISCOVERY OF COLONY STIMULATING FACTORS: CYTOKINES THAT CONTROL DEVELOPMENT OF DIFFERENT CELL LINEAGES

When blood-forming cells were cloned in a semi-solid substrate such as agar, another more solid agar layer was placed between the feeder layer cells and the cells seeded for cloning. This showed that the inducer(s) required for the formation of macrophage and granuloycte clones was secreted by the feeder layer cells and could diffuse through agar [3]. This finding led to the discovery (Table 1) that the inducers required for the formation of macrophage and granulocyte clones are present in conditioned medium produced by the feeder cells [4, 7]. These inducers were found in the conditioned medium from different types of normal and malignant cells (reviewed in [13, 14]). These media were then used to purify the inducers [15–19]. A similar approach was later used to identify the protein inducers for cloning of T lymphocytes [20] and B lymphocytes (reviewed in [21]). When cells were washed at various times after initiating the induction of clones, there was no further

development of either macrophage or granulocyte clones unless the inducer was added again [22]. The development of clones with differentiated cells thus requires both an initial and continued supply of inducer.

In cells belonging to the myeloid cell lineages, four different proteins that induce cell multiplication and can thus induce the formation of clones (colony inducing proteins) have been identified (reviewed in [23–27]). The same proteins have been given different names. After they were first discovered in cell culture supernatant fluids [4, 7], the first inducer identified was called *mashran gm* from the Hebrew word meaning to send forth with the initials for granulocytes and macrophages [28]. This and other growth-inducing proteins were then re-named including macrophage and granulocyte inducers (MGI) [15] - type 1, (MG1-1), are now called colony stimulating factors (CSF) [29], and one protein is called interleukin-3 (IL-3) [19] (Table 2). Of these four CSFs, one (M-CSF) induces the development of clones with macrophages, another (G-CSF) clones with granulocytes, the third (GM-CSF) clones with granulocytes, macrophages, or both macrophages and granulocytes, and the fourth (IL-3) clones with macrophages, granulocytes, eosinophils, mast cells, erythroid cells or megakaryocytes (Figure 2, Table 2). The CSFs induce cell viability and cell multiplication (reviewed in [25–27, 30, 31]) and enhance the functional activity of mature cells (reviewed in [29]). Cloning of genes from mice and humans for IL-3, GM-CSF, M-CSF and G-CSF has shown that these proteins are coded for by different genes (reviewed in [32]). Since the discovery of CSFs, other cytokines have been found, including various ILs and stem cell factor.

It appeared unlikely that a CSF that induces cell multiplication is also a differentiation inducer whose action includes stopping cell multiplication in mature cells. Indeed, a protein that acts as a myeloid cell differentiation inducer and does not have colony stimulating activity was identified and called macrophage and granulocyte inducers - type 2 (MG1–2) (reviewed in [24–26]). Studies on amino acid sequence of the purified protein, neutralisation by monoclonal antibody and myeloid cell differentiation-inducing activity of recombinant protein have shown that MG1–2 is interleukin 6 (IL–6) [26] and there are presumably other normal haemopoietic cell differentiation inducers. Studies on myeloid leukaemic cells have identified other differentiation-inducing proteins called D factor and differentiation-inducing factor (DIF) (reviewed in [26]). D-factor was identified as a protein that has also been called LIF and HILDA, and DIF was found to be a form of tumour necrosis factor (TNF). IL-6 can induce viability and differentiation of normal myeloid precursors, but LIF and TNF, which induce differentiation in certain clones of myeloid leukaemic cells, do not induce viability or differentiation of normal myeloid cells (reviewed in [26]) (Table 2).

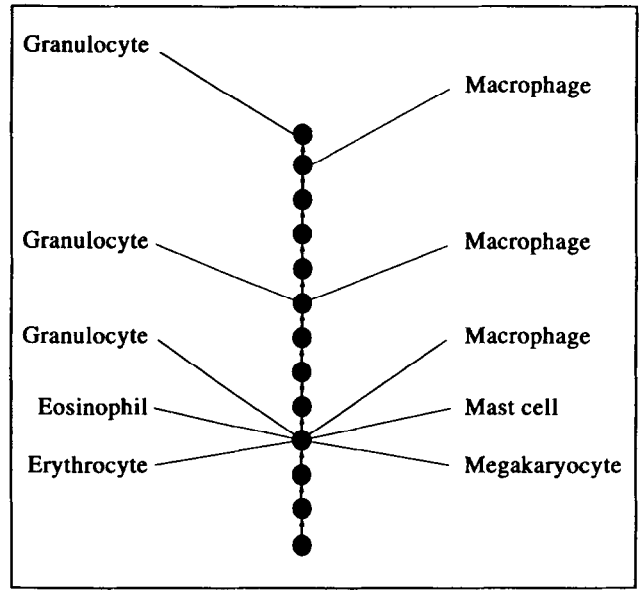

Figure 2. Myeloid haemopoietic precursor cells can be induced to form colonies by four different colony stimulating factors (CSFs). One (IL-3) induces the development of colonies in precursors that can develop into six cell types; the second (GM-CSF) the development of colonies in precursors that develop into two cell types; and the third (G-CSF) and fourth (M-CSF) in precursors that develop into one cell type.

CYTOKINE EVOLUTION

What one finds in nature today are the consequences of biological evolution. What is present is not only what the body uses today, but also what may be useful for further evolution. The production of different types of haemopoietic cells with a limited lifespan, both

Table 2. Induction of growth and differentiation of normal myeloid precursor cells by different haemopoietic cytokines

Nomenclature	Location on chromosome		Induction of colonies*	Induction of differentiation	
	Mouse	Human		Direct	Indirect†
MG1–1 M =CSF–1=M–CSF	3	5	+(M)	–	+
MG1–G =G-CSF	11	17	+(G)	–	+
MG1–1GM =GM–CSF	11	5	+(G,M)	–	+
IL-3	11	5	+(G,M,others)	–	+
MG1–2 =IL–6	5	7	–	+(G,M,Meg)	–
IL–1	2	2	–	–	+(G,M,Meg)
D–factor =HILDA=LIF	11	22	–	CD	CD
DIF =TNF	17	6	–	CD	CD

*Colonies with macrophages (M), granulocytes (G), granulocytes and macrophages (G,M) and granulocytes, macrophages, eosinophils, mast cells, megakaryocytes or erythroid cells (G,M, others), megakaryocytes (Meg). †The four CSFs, including IL-3 and IL-1, induce production of IL-6. CD, cell death. References in [26].

under normal conditions and in different emergency situations, such as infections, wound healing and various diseases, requires a system with considerable plasticity. The development of a multigene family of different interacting cytokines during evolution is, therefore, more useful for the functions required today and for adaptation to functions that may be required in the future, than the existence of only single cytokines with high specificity where a lack of function and lack of flexibility would be lethal. The evolution of a family of cytokines, some of which have overlapping functions, is thus a useful safeguard so that if one cytokine does not function effectively under certain conditions, another can take over.

The multigene family of proteins including the CSFs, ILs and erythropoietin, originating from ancestral gene(s), which now exists to regulate the development of haemopoietic cells in health and disease, is an effective system that contains such safeguards. For example, with the four CSFs, each of which is now coded for by a different gene, the production of macrophages can be initiated by M-CSF, GM-CSF or IL-3, and the production of granulocytes can be initiated by G-CSF, GM-CSF or IL-3 (reviewed in [23–26, 29]). All four CSFs can induce the production of IL-6 which does not induce the formation of colonies but can induce among myeloid cells their differentiation to macrophages, granulocytes or megakaryocytes [23–26, 33]. In a colony with differentiated cells, induction of growth by the CSFs is thus followed by production of another cytokine, IL-6, which can induce differentiation of different cell lineages. This induction of a differentiation factor by a growth factor serves as an effective mechanism to couple growth and differentiation (Figure 3). There are thus safeguards and economy in this system. IL-6 may switch on other, so far unidentified, factors that are required to determine the specificity of the final cell type. Another example of economy and safeguards is that the four CSFs and other cytokines, such as IL-1 and IL-6, which do not induce colonies, all function as viability factors by inhibiting programmed cell death (reviewed in [24–26, 31]), and that the CSFs, in addition to inducing cell viability and growth to form colonies, also enhance the function of mature cells (reviewed in [29]). There are different pathways for inducing viability by preventing programmed cell death [30, 31]. The use of alternative pathways to prevent programmed cell death provides a useful safeguard to regulate the number of cells in haemopoiesis.

A good way to obtain flexibility would be for different factors to function within a network of interactions and this is what the haemopoietic cytokines actually do (reviewed in [26, 34–36]).

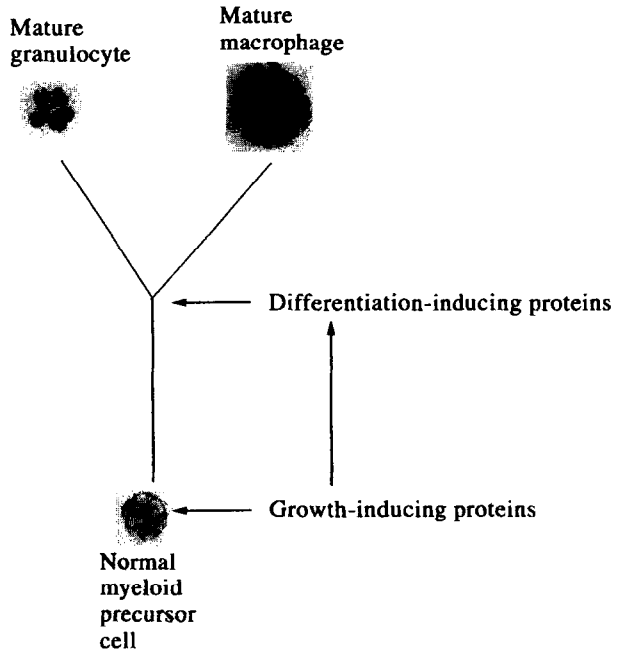

Figure 3. The four CSFs can induce growth of normal myeloid precursor cells to form colonies. They also induce in these cells production of another cytokine, IL-6, that induces differentiation. The induction of a differentiation inducer by the CSFs provides a mechanism to couple the multiplication of normal precursor cells and their differentiation.

NETWORK OF HAEMOPOIETIC CYTOKINES

Production of specific cell types has to be induced when new cells are required and has to stop when sufficient cells have been produced. This requires an appropriate balance between inducers and inhibitors of development. The network of interactions between haemopoietic cytokines (Figure 4) therefore, also includes cytokines that can function as inhibitors, such as tumour necrosis factor (TNF). Another inhibitory cytokine, transforming growth factor β1 (TGFβ1), which is also part of this network, can selectively inhibit the activity of some CSFs and ILs. It can also inhibit the production of some of these cytokines [37]. An important function of the network is to selectively control programmed cell death.

Parts of this network function not only within the haemopoietic cell system but also for some non-haemopoietic cell types. For example, in endothelial cells that make blood vessels there is an induction of IL-6 when new blood vessels are being formed and the production of IL-6 is switched off when angiogenesis has been completed [38]. The transient expression of IL-6 in the endothelial cells indicates a role for IL-6 in angiogenesis, in addition to its role in regulating the development of myeloid and lymphoid haemopoietic cells. IL-6 can also induce the production of acute phase proteins in liver cells [21]. The pleiotropic effects of a cytokine such as IL-6 raise the question whether these effects on different cell types are direct, or are indirect by IL-6 switching on production of other regulators that vary in the different cell types. Interpretation of experimental data on the effect of each cytokine, therefore, has to take into account that the regulator functions in a network of interactions, so as to avoid an incorrect assignment of a specific effect to a direct action of a particular cytokine. This network has also to be taken into account in the clinical use of these cytokines. What can be therapeutically useful may be due to the direct action of an injected cytokine, or to an indirect effect due to other cytokines that are switched on *in vivo*.

A network of interactions allows considerable flexibility, depending on which part of the network is activated. It also allows a ready amplification of response to a particular stimulus such as bacterial lipopolysaccharide (LPS) [26, 36]. This amplification can occur by autoregulation and by transregulation of genes for the haemopoietic cytokines [26]. There is also a transregulation by

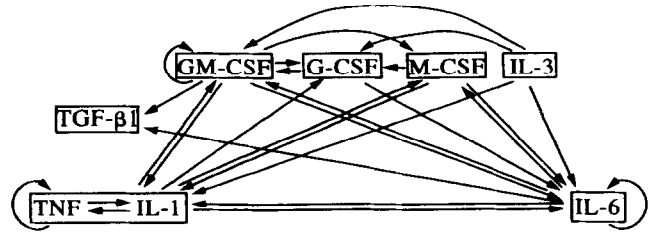

Figure 4. The network of interactions between heamopoietic cytokines [27].

these cytokines of receptors for other cytokines [34, 39]. In addition to the flexibility of this network both for the response to present-day infections and to infections that may develop in the future, a network may also be necessary to stabilise the whole system. Haemopoietic cytokines induce during differentiation sustained levels of transcription factors that can regulate and maintain gene expression in the differentiation program [40]. Interactions between the network of haemopoietic cytokines and transcription factors can thus ensure the production of specific cell types and stability of the differentiated state.

CONTROL OF LEUKAEMIA BY CYTOKINES THAT CONTROL NORMAL HAEMOPOIESIS

Can myeloid leukaemic cells still be induced to differentiate to mature non-dividing cells by cytokines that induce differentiation in normal myeloid cells? This question has been answered by showing that there are clones of myeloid leukemic cells that can be induced to differentiate to mature macrophages or granulocytes through the normal sequence of gene expression by incubation with the normal myeloid differentiation-inducing protein IL-6 (Figure 5). These are called D^+ clones (D for differentiation). The mature cells, which can be formed from all the cells of a leukaemic clone, then stop multiplying like normal mature cells and are no longer malignant *in vivo*. In addition to D^+ clones that can be induced to differentiate by IL-6, there are other D^+ clones from other myeloid leukaemias that can be induced to differentiate by incubation with GM-CSF, IL-3 or G-CSF (Table 3) (reviewed in [24–26]). In these clones the growth inducers presumably induce production of an appropriate differentiation inducer. D^+ leukaemic cells that respond to IL-6 can also be induced to differentiate by IL-1α and IL-1β, and this is mediated by the endogenous production of IL-6 [26].

Studies in animals and humans have shown that normal differentiation of D^+ myeloid leukaemic cells to mature non-dividing cells can be induced not only in culture but also *in vivo*. These leukaemias, therefore, grow progressively when there are too many leukaemic cells for the normal amount of differentiation inducer in the body. The development of leukaemia can be inhibited in mice with these D^+ leukaemic cells by increasing the amount of differentiation-inducing protein, either by injecting it or by injecting a compound that increases its production by cells in the body [41–43] and reviewed in [24–26]. Induction of differentiation *in vivo* like *in vitro* can occur directly, or by an indirect mechanism that involves induction of the appropriate differentiation-inducing protein either by the same cells or by other cells in the body. After injection of myeloid leukaemic cells into fetuses, D^+ leukaemic cells can participate in haemopoietic cell differentiation in apparently healthy adult animals [44, 45].

The D^+ myeloid leukaemic cells have an abnormal chromosome composition, and suppression of malignancy in these cells was not associated with chromosome changes. It was obtained by induction of the normal sequence of cell differentiation by a normal myeloid regulatory protein. In this suppression, the stopping of cell multiplication by inducing differentiation to mature cells bypasses genetic changes that produced the malignant phenotype [46], such as changes in the requirement for a normal cytokine for viability and growth, and a block in the ability of growth inducer to induce differentiation inducer. The study of different clones of myeloid leukaemic cells has also shown that in addition to D^+ clones there are differentiation-defective clones called D^- clones. Some D^- clones are induced by a normal myeloid cytokine to an intermediate stage of differentiation which then slows down the growth of the cells, and others could not be induced to differentiate even to this

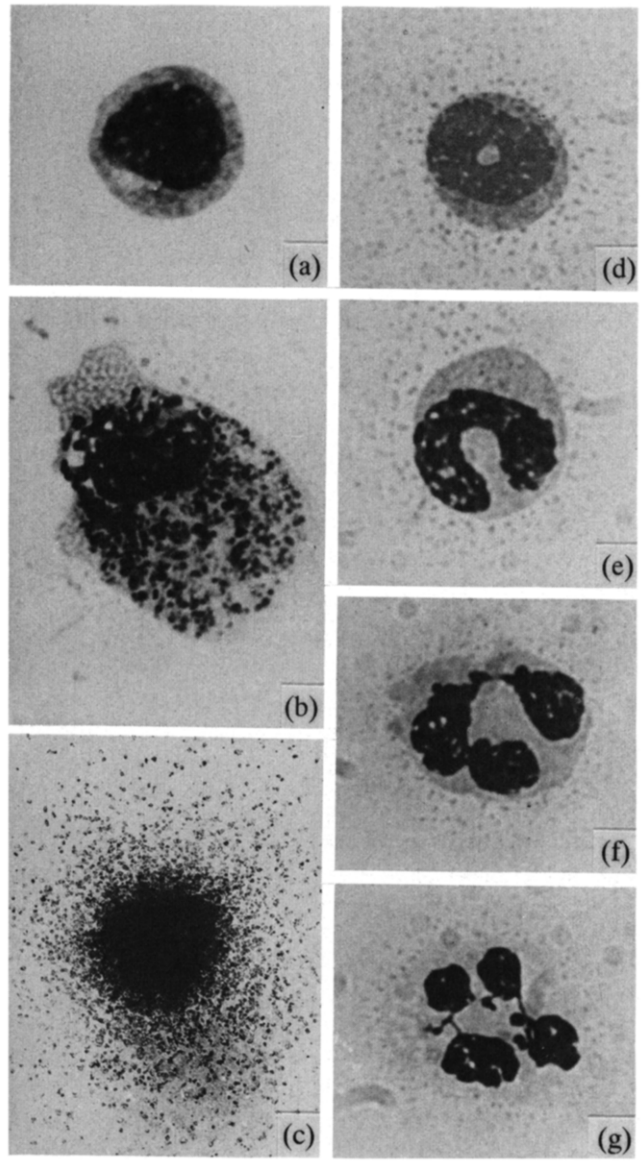

Figure 5. Differentiation of myeloid leukaemic cells to non-malignant mature macrophages or granulocytes by normal myeloid differentiation-inducing protein IL-6. (a) Leukaemic cell; (b) macrophage; (c) colony of cells with macrophages; (d–g) stages in differentiation to granulocytes [74].

intermediate stage (Figure 6). Since a normal differentiation inducer can induce differentiation to mature non-dividing cells in the D^+ clones, it has been suggested that D^+ clones are the early stages of leukaemia and that the formation of D^- clones may be later stages in the further progression of malignancy (reviewed in [24–26]). Genetic changes which make cells defective in their ability to be induced to differentiate by the normal differentiation inducer thus occur in the evolution of myeloid leukaemia. But even these D^- cells can be induced to differentiate by other compounds, either singly or in combination, that can induce the differentiation program by alternative pathways. The stopping of cell multiplication by inducing differentiation by these alternative pathways bypasses the genetic changes that inhibit response to the normal differentiation inducer (reviewed in [24–26]). Studies on the genetic changes in D^- clones of myeloid leukaemias have shown that differentiation defectiveness may be due to changes in homeobox genes. These include re-arrangement of the *Hox-2.4* homeobox

Table 3. Differentiation of myeloid leukaemic cells by different haemopoietic cytokines

Myeloid leukaemia	Differentiation after culture with							
	IL-6	IL-1	D-factor/LIF	TNF	IL-3	GM-CSF	G-CSF	M-CSF
M1-clone 11	+	+*	–	–	–	–	±*	–
M1-clone T22	+	+*	+*	–	–	–	±*	–
7-M12	–	–	–	–	+*	+*	–	–
WEHI-3B	–	–	–	–	–	–	+*	–
HL-60	–	–	–	+*	–	–	±*	–

*IL-1 induces differentiation indirectly in clone 11. It is suggested that the induction of differentiation in WEHI-3B by G-CSF, in clone 7-M12 by GM-CSF or IL-3, in clone T22 by D-factor/LIF, in HL-60 by TNF and partial differentiation by G-CSF in some clones may also indirect. +, induced to differentiate; –, not induced to differentiate; ±, partial differentiation. References in [26].

gene which results in abnormal expression of this gene in the leukaemic cells [47]. This abnormal expression inhibits specific pathways of myeloid cell differentiation [48]. In other leukaemias with a deletion in one chromosome 2 [49] there is a deletion of one copy of *Hox-4.1* [50].

Studies with a variety of chemicals other than normal haemopoietic cytokines have shown that many compounds can induce differentiation in D^+ clones of myeloid leukaemic cells. These include certain steroid hormones, chemicals such as cytosine arabinoside, adriamycin, methotrexate and other chemicals that are used today in cancer chemotherapy, and irradiation. At high doses these compounds used in cancer chemotherapy and irradiation kill cells by inducing programmed cell death, whereas at low doses they can induce differentiation. Not all these compounds are equally active on the same leukaemic clone [51, 52]. A variety of chemicals can also induce differentiation in clones that are not induced to differentiate by a normal haemopoietic cytokine, and in some D^- clones induction of differentiation requires combined treatment with different compounds [52]. In addition to certain steroids and chemicals used today in chemotherapy and radiation therapy, other compounds that can induce differentiation in myeloid leukaemic cells include insulin, bacterial lipopolysaccharide, certain plant lectins, tumour-promoting phorbol esters and retinoic acid [24, 51–53]. In addition to the normal myeloid cytokines, the steroid hormones, insulin and retinoic acid are physiological compounds that can induce differentiation. It is possible that all myeloid leukaemic cells no longer susceptible to the normal haemopoietic cytokines by themselves can be induced to differentiate by the appropriate combination of compounds.

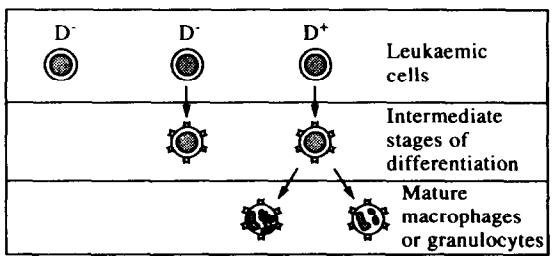

Figure 6. Classification of different clones of myeloid leukaemic cells according to their ability to be induced to differentiate by normal haemopoietic cytokines. Some differentiation-defective (D^-) clones can be induced by these cytokines to intermediate stages by differentiation, whereas other D^- clones are not induced to differentiate by these cytokines even to an intermediate stage [24, 52].

Table 4. Control of programmed cell death by a tumour suppressor gene and oncogenes

Deregulated expression of	Programmed cell death		
	Induction	Enhancement	Suppression
Wild-type p53	+	–	–
c-*myc*	–	+	–
Mutant p53	–	–	+*
bcl-2	–	–	+

*Mutant p53 suppresses the enhancement of programmed cell death by deregulated c-*myc* [62]. Other references in [31].

REGULATION OF PROGRAMMED CELL DEATH

Normal myeloid precursor cells depend on haemopoietic cytokines for viability, multiplication and differentiation (reviewed in [24–26, 31]). Withdrawal of these cytokines leads to death by programmed cell death [54] (apoptosis) [55]. Although viability factors such as the CSFs are also growth factors, viability and growth are separately regulated (reviewed in [31]). Certain myeloid leukaemic cells are growth factor-independent and do not require an exogenously added cytokine for cell viability and growth. Induction of differentiation in these leukaemic cells with IL-6 induces in the differentiating cells a growth factor-dependent state so that the cells lose viability by apoptosis following withdrawal of IL-6 [30, 39, 56–58]. This induction of the program for cell death occurs before terminal differentiation, and the differentiating cells can be rescued from apoptosis and continue to multiply by re-adding IL-6, or by adding IL-3, M-CSF, G-CSF or IL-1 [39]. The differentiating leukaemic cells can also be rescued from apoptosis by the tumour-promoting phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) but not by the non-promoting isomer 4-α-TPA [30]. TPA rescued the differentiating cells from apoptosis by a different pathway than rescue with these cytokines. TPA can thus act as a tumour promoter by inhibiting programmed cell death [30]. The programme for cell death is present in normal myeloid precursor cells and in more differentiated cells including mature granulocytes and macrophages. Induction of programmed cell death in myeloid leukaemic cells is a physiological process that can be used to suppress leukaemia.

Programmed cell death can also be induced in myeloid leukaemic cells without inducing differentiation. Wild-type p53 protein is a product of a tumour suppressor gene which is no longer expressed

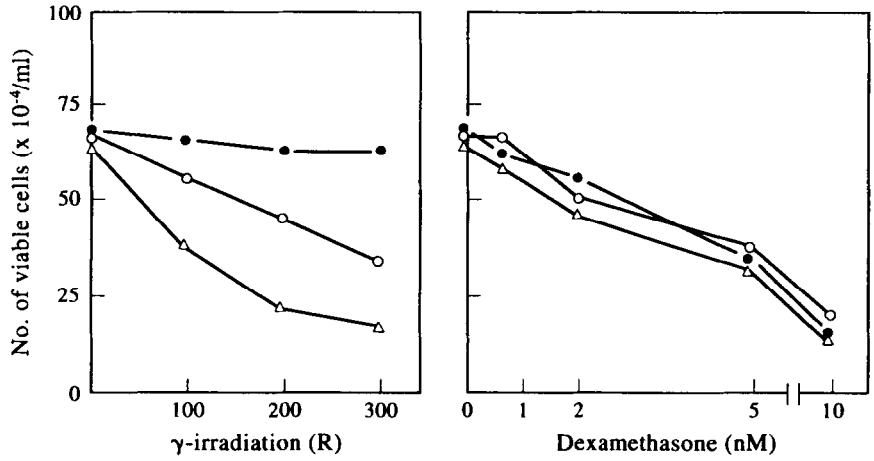

Figure 7. Thymocytes from mice deficient in wild-type p53 are more resistant to induction of programmed cell death by γ-irradiation but not by dexamethasone. p53-deficient homozygous mice (●), p53-deficient heterozygous mice (○) and normal mice (△) [61].

in many types of tumours, including myeloid leukaemias (reviewed in [59]). There is a clone of myeloid leukaemic cells that completely lacks expression of p53 protein and mRNA [60]. This p53-negative clone of myeloid leukaemic cells was transfected with DNA encoding a temperature-sensitive p53 mutant (Ala to Val change at position 135). The Val 135 mutant behaves like other p53 mutants at 37.5°C but like wild-type p53 at 32.5°C. There was no change in the behaviour of the transfected cells at 37.5°C but activation of the wild-type p53 protein at 32.5°C resulted in apoptotic cell death. This induction of apoptosis was not associated with differentiation [60]. Apoptosis can, therefore, be induced in myeloid leukaemic cells not only by a differentiation-associated process, but also by expression of wild-type p53 (Table 4) in undifferentiated leukaemic cells. This induction of apoptosis by wild-type p53 was inhibited by IL-6 [60]. These results show that wild-type p53-mediated apoptosis in these myeloid leukaemic cells is a physiological process. Experiments with p53 knock-out mice have shown that wild-type p53 is also involved in mediating apoptosis in normal myeloid precursors deprived of the appropriate cytokine concentration required for cell viability [61]. The induction of apoptosis in myeloid leukaemic cells by various cytotoxic agents can be enhanced by deregulated expression of c-*myc* [62]. The oncogene mutant p53 [62] and *bcl*-2 ([63–64] and reviewed in [65]) (Table 4) suppress the enhancing effect on cell death of deregulated c-*myc*, and thus allow induction of cell proliferation and inhibition of differentiation which are other functions of deregulated c-*myc*. The suppression of cell death by mutant p53 and *bcl*-2 increases the probability of developing tumours. Treatments that downregulate the expression or activity of mutant p53 and *bcl*-2 in tumour cells should be useful for cancer therapy [31]. Experiments with p53 knock-out mice have also shown that there are wild-type p53-dependent and p53-independent pathways of inducing apoptosis [61, 66, 67] (Figure 7). These and other experiments have shown that there are alternative pathways to apoptotic cell death [31]. Alternative pathways to regulate apoptosis can be useful to control selective cell viability.

CLINICAL USE OF HAEMOPOIETIC CYTOKINES

Identification of the myeloid cell regulatory cytokines has suggested novel possibilities for therapy [23–26, 51, 68–70]. The concentration of these proteins can be increased *in vivo* either by injecting one of these cytokines or by injecting a compound that induces their production (reviewed in [26]). Injection of a CSF such as G-CSF or GM-CSF stimulates myelopoiesis under normal circumstances and after suppression of myelopoiesis induced by compounds used in chemotherapy, irradiation therapy and treatments used for immune depression such as cyclosporine A. In non-malignant haematological abnormalities, myeloid regulatory cytokines were shown to be clinically useful in restoring quicker the normal number and function of the myeloid blood cell population in patients with suppressed myelopoiesis which occurs after cytotoxic cancer therapy and immune suppression used for transplantation. These treatments destroy cells that produce these cytokines and suppress myelopoiesis. Addition of these cytokines *in vitro* to bone marrow cells before grafting and/or their injection *in vivo* can also increase the success of bone marrow transplant grafts, and increase survival in patients deficient in these proteins and the cells that produce them. Because of the important functions of mature cells such as granulocytes and other myeloid cells, the increased function of mature cells induced by CSFs can also be clinically helful to patients with deficiencies in myeloid cell functions (reviewed in [26]). The finding that granulocyte development can be induced in culture in cells from patients with infantile genetic granulocytosis [8, 71] has led to promising clinical results with G-CSF in children with this genetic disease. It has also been shown that injection of erythropoietin, which stimulates the development of erythroid cells, can correct the anaemia in patients with end-stage renal disease of progressive renal failure (reviewed in [26]).

CSFs and interleukins such as IL-1 and IL-6 can control the abnormal growth of certain types of leukaemic cells and suppress malignancy by inducing differentiation (reviewed in [24–26, 51]). Induction of programmed cell death in leukaemic cells, either with [30, 31] or without [31, 60, 72] inducing differentiation, is also a process that can be used to suppress malignancy. Induction of a requirement for one of these cytokines for viability of the leukaemic cells followed by withdrawal of the cytokine [30, 39, 56–58] causes death of the leukaemic cells, and this would also be useful for therapy. The existence of the cytokine network has to be taken into account in the clinical use of cytokines [26, 36, 72].

It can be concluded that starting with the original cell culture assays [1–5] for the clonal development of haemopoietic cells and discovey of the myeloid haemopoietic cytokines in cell culture supernatants [4, 7], the study of these cytokines has now progressed to their clinical use for therapy.

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News

A Workshop Report

Cancer Screening in the European Union

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INTRODUCTION

THE PURPOSE of this report is to present an account of cancer screening in Europe.

Primary prevention should be given the highest priority in the fight against cancer; however, the reduction in the incidence of cancer that can be achieved by interventions, based on present scientific knowledge of risk factors, is limited. This is, unfortunately, particularly true for some common cancers, such as those of the colon, rectum, breast and prostate. Early detection and screening programmes must, therefore, be considered the best second choice for reducing mortality from certain cancers.

Early detection and screening programmes involve contacting, examining and possibly treating healthy, symptom-free people. Society should, therefore, not use resources on cancer screening programmes unless there is sufficient scientific evidence to ensure that the population will benefit. Any scientific evaluation should cover reduction in the number of cases of late-stage disease and of death from the specific cancer (the aim of such programmes), any possible side-effects, such as unnecessary treatment, and an overall evaluation of the potential impact of the programme on the general health of the target population, for instance in years of life saved.

It is hoped that this report will provide useful background information for politicians, administrators and the public. There is an emphasis on different health care systems and recommendations are made not only for individuals but also for society.