Regulators of normal development and tumor suppression

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ABSTRACT Identification of normal growth and differentiation-inducing proteins and how they interact in normal development has made it possible to identify the molecular basis of normal development and the mechanisms that uncouple growth and differentiation so as to produce tumor cells. When normal cells have been changed into tumor cells, the malignant phenotype can again be suppressed. Results on the molecular control of growth and differentiation in normal myeloid hematopoietic cells, changes in the normal developmental program in myeloid leukemia, and the suppression of malignancy in myeloid leukemia and sarcomas, have shown that (A) malignancy can be suppressed either with or without genetic changes in the tumor cells, (B) suppression of malignancy by inducing differentiation does not have to restore all the normal controls, and (C) genetic abnormalities which give rise to malignancy can be bypassed and their effects nullified by inducing differentiation which stops cells from multiplying.

KEY WORDS: *normal development, tumor suppression, tumor cell differentiation, hematopoiesis, leukemia, sarcomas*

Introduction

The multiplication and differentiation of normal cells are controlled by different regulatory molecules. These regulators have to interact to achieve the correct balance between cell multiplication and differentiation during embryogenesis and the normal functioning of the adult individual. The origin and progression of malignancy result from genetic changes that uncouple the normal balance between multiplication and differentiation so that there are too many growing cells. This uncoupling can occur in various ways (Sachs, 1980, 1987a). When cells have become malignant, how can malignancy be suppressed so as to revert malignant back to non-malignant cells? Malignant cells can have different abnormalities in the controls for multiplication and differentiation. Do all the abnormalities have to be corrected, or can they be by-passed in order to suppress malignancy? I will mainly discuss results with normal and leukemic myeloid hematopoietic cells and sarcomas that have been used as model systems to answer these questions.

Normal growth- and differentiation-inducing cytokines

An understanding of the mechanisms that control multiplication (growth) and differentiation of normal cells would seem to be an essential requirement to elucidate the origin and reversibility of malignancy. The establishment of a cell culture system for the clonal development of normal hematopoietic cells (Ginsburg and Sachs, 1963; Pluznik and Sachs, 1965; Bradley and Metcalf, 1966; Ichikawa *et al.*, 1966) and discovery of the regulators of this clonal development in cell culture supernatants (Ichikawa *et al.*, 1966; Pluznik and Sachs, 1966) has led to the identification of a family of myeloid cell growth and differentiation-inducing cytokines (Fig. 1, Table 1). In cells belonging to the myeloid cell lineages, different proteins that induce cell multiplication and can thus induce the formation of clones were identified. The same proteins have been given different names. After they were first discovered in cell culture supernatant fluids, the first inducer identified was called *mashran gm*, from the Hebrew word meaning to send forth, with the initials for granulocytes and macrophages (Ichikawa *et al.*, 1967). This and other growth-inducing cytokines were then re-named including macrophage and granulocyte inducers (MGI) - type 1, (MGI-1), and are now called colony stimulating factors (CSFs). One of these cytokines is called interleukin-3 (IL-3) (Sachs, 1982, 1986, 1987c, 1990, 1992; Metcalf, 1985).

There are four CSFs. One (M-CSF), induces the development of colonies with macrophages, another (G-CSF), colonies with granulocytes, the third (GM-CSF), colonies with granulocytes, macrophages, or both macrophages and granulocytes, and the fourth, (IL-3), colonies with macrophages, granulocytes, eosinophils, mast cells, erythroid cells, or megakaryocytes (Fig. 2, Table 2). The CSFs induce cell viability and cell multiplication (Sachs, 1987c, 1990, 1992; Lotem *et al.*, 1991) and enhance the function of mature cells (Metcalf, 1985). Cloning of genes from mice and

Abbreviations used in this paper: CSF, colony stimulating factors; IL, interleukin; TGFß, transforming growth factor ß; TNF, tumor necrosis factor.

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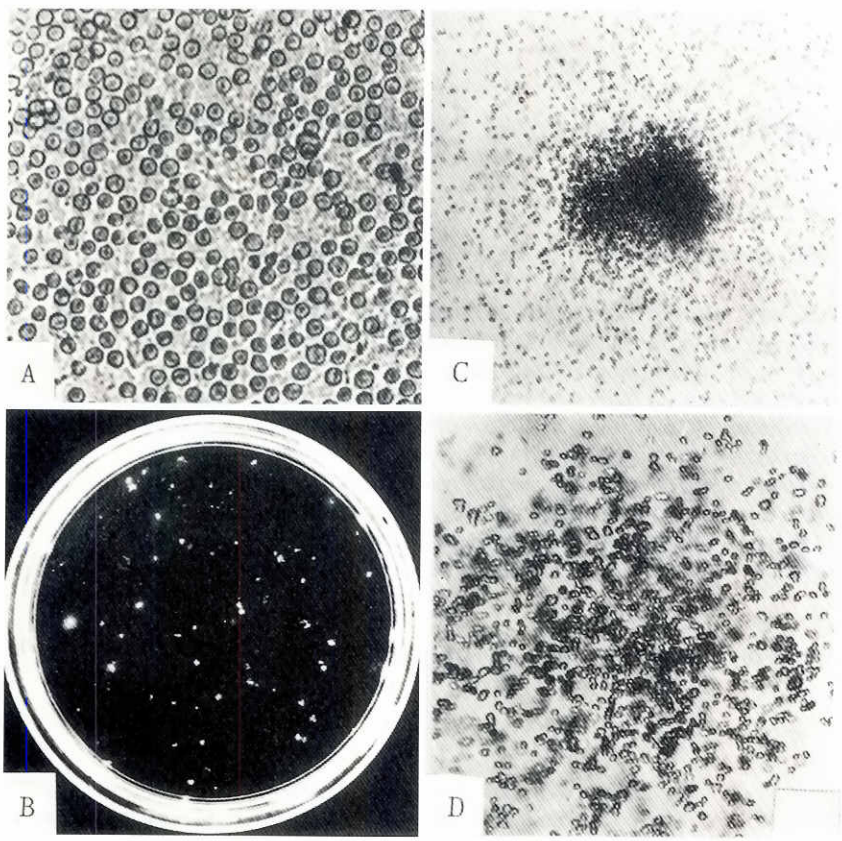

Fig. 1. Cell culture system for cloning and clonal differentiation of normal hematopoietic cells. (A) *Culture of mouse mast cells that have multiplied and differentiated on a feeder layer of mouse embryo cells (Ginsburg and Sachs, 1963).* **(B-D)** *Clones of macrophages and granulocytes in cultures of normal hematopoietic cell precursors incubated with the appropriate inducer.* ***(B)*** *Petri dish with clones (Pluznik and Sachs, 1965).* **(C)** *Granulocyte clone and* ***(D)*** *macrophage clone (Ichikawa et al., 1966).*

humans for GM-CSF, G-CSF, M-CSF and IL-3 has shown that these cytokines are coded for by different genes (Clark and Kamen, 1987). Since the discovery of CSFs, other cytokines have been found including various cytokines called interleukins with different numbers and a cytokine that has been called stem cell factor.

Cytokine network

How do normal myeloid precursor cells induced to multiply by the CSFs develop into clones that contain mature differentiated cells that stop multiplying when they terminally differentiate? 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 it was found that all four CSFs can induce in normal myeloid precursors the production of another cytokine, interleukin 6 (IL-6), which we had previously called macrophage and granulocyte inducer-type 2 (MGI-2) (Sachs, 1982, 1987b,c, 1990). IL-6 does not induce the formation of colonies but can induce among myeloid cells their differentiation to macrophages, granulocytes, or megakaryocytes (Sachs, 1987c, 1990; Lotem *et al.*, 1989). 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 (Fig. 3) and there are presumably other differentiation inducers in addition to IL-6. The four CSFs and other cytokines, such as IL-1 and IL-6, which do not induce colonies of normal myeloid cells, all function as viability factors by preventing programmed cell death (apoptosis) (Lotem and Sachs, 1989; Lotem *et al.*, 1991; Sachs, 1992).

Hematopoietic cytokines function within a network of interactions (Sachs, 1987c, 1990, 1992). This network includes CSFs, IL-1, IL-6 and also cytokines that can function as inhibitors, such as tumor necrosis factor (TNF) (Fig. 4). Another cytokine that can function as an inhibitor which is also part of this network, transforming growth factor ß1 (TGF-ß1) (Fig. 4), can selectively inhibit the activity of some CSFs and interleukins. TGF-ß1 can also inhibit the production of some of these cytokines (Lotem and Sachs, 1990). 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 or viral infection.

Parts of this network function not only within the hematopoietic cell system but also for some non-hematopoietic 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 (Motro *et al.*, 1990). 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 hematopoietic cells. IL-6 can also induce the production of acute phase proteins in liver cells (Hirano *et al.*, 1990). The pleiotropic effects of a cytokine such as IL-6 raises 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

TABLE 1

ESTABLISHMENT OF THE CELL CULTURE SYSTEM FOR CLONING AND CLONAL DIFFERENTIATION OF NORMAL HEMATOPOIETIC CELLS AND DISCOVERY OF THE MOLECULAR REGULATORS OF THIS CLONAL DEVELOPMENT IN CELL CULTURE SUPERNATANTS

Cloning and differentiation in liquid medium (mast cells and granulocytes)	(Ginsburg and Sachs, 1963).
Cloning and differentiation in agar (macrophages and granulocytes)	(Pluznik and Sachs, 1965; Bradley and Metcalf, 1966).
Cloning and differentiation in methyl-cellulose (macrophages and granulocytes)	(Ichikawa et al., 1966).
Inducers for cloning and differentiation secreted by cells	(Pluznik and Sachs, 1965).
Inducers for cloning and differentiation in cell culture supernatants (macrophages and granulocytes)	(Pluznik and Sachs, 1966; Ichikawa et al., 1966).

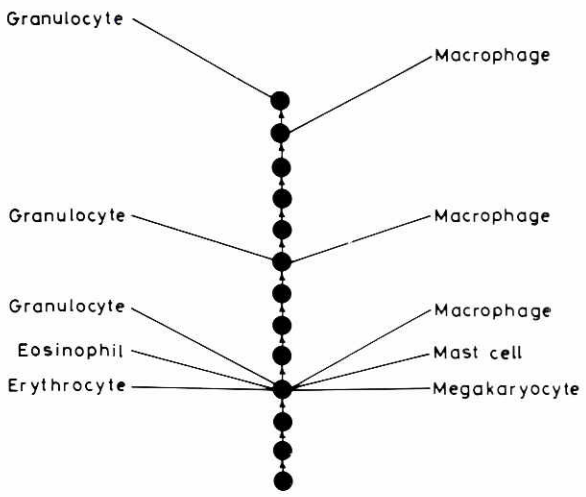

Fig. 2. Myeloid hematopoietic precursor cells can be induced to multiply by four different colony stimulating factors (CSFs). *One (IL-3) induces the development of colonies in precursors that can develop into six cell types; the second (GM-CSF) the development of colonies in precursors that develop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

effect of each cytokine, therefore, has to take into account that the cytokine 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*.

Growth-inducing cytokines and tumor development

The normal myeloid growth inducers can be produced by various cell types (Sachs, 1974). However, these growth inducers are not made by the normal myeloid precursors, so that the normal precursors require for cell viability and growth the production of cytokines by other cell types. When cells become malignant they have escaped some normal control, which can be associated with changes from an induced to a constitutive expression of certain genes (Sachs, 1980, 1982, 1987b). In myeloid leukemia, different clones of malignant cells have been identified which have shown the various types of changes that can occur in the normal response to growth-inducing cytokines. There are different leukemic clones that (A) need less or have become independent of normal growth inducer for growth, (B) constitutively produce their own growth inducer, which seems to be a rare event, or (C) are blocked in the ability of growth inducer to induce production of differentiation inducer (Sachs, 1978, 1980, 1982, 1987b, 1992). One way in which tumors can develop is thus by changes in the production of or response to a normal growth-inducing cytokine which gives a growth advantage to the leukemic over the normal cells.

Growth-inducing cytokines induce cell viability and cell multiplication (Sachs, 1987c, 1990, 1992, Lotem *et al.*, 1991). Independence from normal growth inducer or constitutive production of their own growth inducer can also explain the survival and growth of metastasizing malignant cells in places in the body where the growth inducer required for the survival of normal cells is not present. In cells that are malignant but may still need some growth inducer, the organ preference of metastasis can be due to production of the required growth inducer in the organ where the metastasis occurs.

Induction of differentiation in tumor cells

The different types of myeloid leukemic cells include clones that have changed their normal requirement for growth inducer and in which growth inducer no longer switches on production of differen-

TABLE 2

INDUCTION OF GROWTH AND DIFFERENTIATION OF NORMAL MYELOID PRECURSOR CELLS BY DIFFERENT HEMATOPOIETIC CYTOKINES

Nomenclature	Location on chromosome		Induction of colonies*	Induction of differentiation	
	Mouse	Human		Direct	Indirect**
MGI-1M= CSF-1= M-CSF	3	5	+(M)	-	+
MGI-1G= G-CSF	11	17	+(G)	-	+
MGI-1GM= GM-CSF	11	5	+(G, M)	-	+
IL-3	11	5	+ (G,M, others)	-	+
MGI-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, which include IL-3, induce production of IL-6. IL-1 also induces IL-6. CD= cell death. References in Sachs (1990).

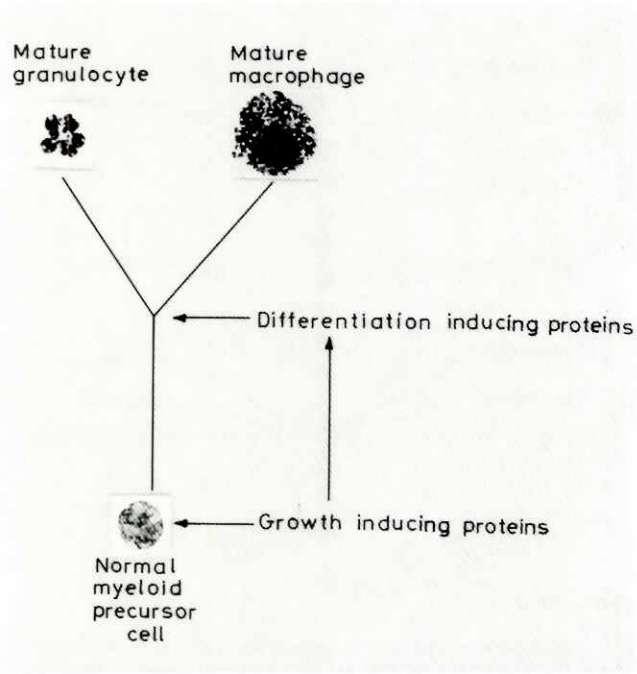

Fig. 3. The four CSFs can induce growth of normal myeloid precursor cells to form colonies. *They also induce in these cells the production of another cytokine, IL-6, that induces differentiation. The induction of a differentiation inducer by the CSFs provides a mechanism to couple the multiplication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

tiation inducer, but which can still be induced to differentiate to mature non-dividing cells by a normal differentiation-inducing cytokine (Fig. 5). These clones, which are called D^+ clones (D for differentiation) (Fibach *et al.*, 1973) can be induced to differentiate normally to mature macrophages or granulocytes via the normal sequence of gene expression that occurs during differentiation by incubating the cells with a normal myeloid differentiation-inducing cytokine (Sachs, 1974, 1978, 1982, 1987b,c, 1990). The mature cells, which can be formed from all the cells of a leukemic clone, then stop multiplying like normal mature cells and are no longer malignant *in vivo* (Lotem and Sachs, 1981, 1984; Sachs, 1982, 1987b,c). Studies in animals have shown that normal differentiation of D^+ myeloid leukemic cells to mature non-dividing cells can be induced not only in culture but also *in vivo* (Lotem and Sachs, 1978; Sachs, 1978, 1982, 1987c). These leukemias, therefore, grow progressively when there are too many leukemic cells for the normal amount of differentiation inducer in the body. The development of leukemia can be inhibited in mice with these D^+ leukemic cells by increasing the normal amount of differentiation-inducing cytokine, either by injecting it or by injecting a compound that increases its production by cells in the body (Lotem and Sachs, 1981, 1984; Sachs, 1982, 1987b). After injection of these myeloid leukemic cells into fetuses, the leukemic cells can participate in hematopoietic cell differentiation in apparently healthy adult animals (Gootwine *et al.*, 1982; Webb *et al.*, 1984).

In addition to the D^+ leukemic clones that can be induced to differentiate by a normal myeloid differentiation-inducing protein, there are other D^+ clones that can be induced to differentiate by incubating the leukemic cells with a normal myeloid growth-inducing cytokine (Table 3). In these leukemic clones the growth inducers can presumably still induce production of an appropriate differentiation inducer. Not all clones respond to the same cytokine even though they may have the appropriate receptors. The results show that most, and probably all, the physiological regulatory cytokines of normal myeloid hematopoietic cell development can regulate differentiation of D^+ myeloid leukemic cells (Table 3) (Lotem and Sachs, 1988; Sachs, 1990).

The study of different clones of myeloid leukemic cells has also shown that in addition to D^+ clones, there are differentiation-defective clones called D^- clones (Fibach *et al.*, 1973) (Fig.6). Some D^- clones are induced by a normal myeloid regulatory 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 intermediate stage (Sachs, 1974, 1978, 1982, 1987b, 1990). Since 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 leukemia and that the formation of different types of D^- clones may be later stages in the further progression of malignancy (Sachs, 1978). Studies on changes in the synthesis of cellular proteins in normal myeloid precursors and D^+ and D^- myeloid leukemic cells have shown that there have been changes from inducible to constitutive gene expression in the leukemic cells, and that the most differentiation-defective D^- clones showed the highest amount of constitutive gene expression (Liebermann *et al.*, 1980; Sachs, 1980, 1982, 1987b). The cellular protein changes during differentiation of normal myeloid precursors are induced as a series of parallel multiple pathways of gene expression. It can be assumed that normal differentiation requires synchronous initiation and progression of these multiple parallel pathways. The presence of constitutive instead of induced gene expression for some pathways can be expected to produce asynchrony in the co-ordination required for differentiation. Depending on the pathways involved, this asynchrony can then produce blocks in the induction and termination of the differentiation program (Liebermann *et al.*, 1980; Sachs, 1980, 1982, 1987a,b). Studies on the genetic changes in differentiation-defective clones of myeloid leukemias have shown that differentiation defectiveness may be due to changes in homeobox genes. These include rearrangement of the Hox-2.4 homeobox gene which results in abnormal expression of this gene in the leukemic cells (Blatt *et al.*,

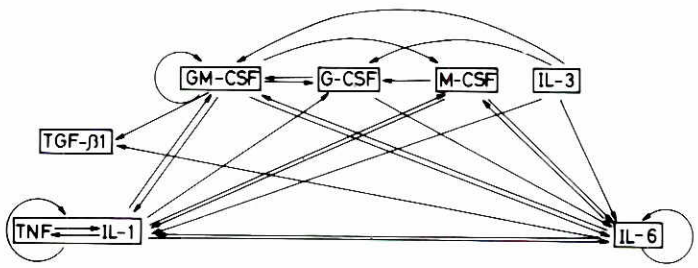

Fig. 4. The network of interactions between hematopoietic cytokines (Sachs, 1992).

TABLE 3

DIFFERENTIATION OF MYELOID LEUKEMIC CELLS BY DIFFERENT HEMATOPOIETIC CYTOKINES

Myeloid leukemia	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 clones 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 be indirect. +, induced to differentiate; -, not induced to differentiate; ±, partial differentiation. References in Sachs (1990).

1988). This abnormal expression inhibits specific pathways of myeloid cell differentiation (Blatt *et al.*, 1992). In other leukemias with a deletion in one chromosome 2 (Azumi and Sachs, 1977), there is a deletion of one copy of Hox-4.1 (Blatt and Sachs, 1988).

Different pathways for inducing differentiation

Studies with a variety of chemicals other than normal hematopoietic regulatory cytokines have shown that many compounds can induce differentiation in D^+ clones of myeloid leukemic cells. These include certain steroid hormones, chemicals such as cytosine arabinoside, adriamycin, methotrexate and other chemicals that are used today in cancer chemotherapy, and also x-irradiation. At high doses these compounds used in cancer chemotherapy and x-irradiation kill cells, whereas at low doses they can induce differentiation. Not all these compounds are equally active on the same leukemic clone (Sachs, 1978, 1982). A variety of chemicals can also induce differentiation in clones that are not induced to differentiate by a normal hematopoietic regulatory cytokine, and in some clones induction of differentiation requires combined treatment with different compounds (Sachs, 1982). The results show that although the response to induction of differentiation by a normal myeloid regulatory protein has been altered, the D^- clones have not lost all the genes for differentiation. In addition to certain steroids and chemicals used today in chemotherapy and radiation therapy, other compounds that can induce differentiation in myeloid leukemic cells include insulin, bacterial lipopolysaccharide, certain plant lectins, phorbol esters and retinoic acid (Sachs, 1978, 1982, 1987b; Degos, 1992). In addition to the normal myeloid regulatory cytokines, the steroid hormones, insulin and retinoic acid are physiological compounds that can induce differentiation. It is possible that all myeloid leukemic cells no longer susceptible to the normal regulatory cytokines by themselves can be induced to differentiate by the appropriate combination of compounds.

The ability of a variety of compounds to induce differentiation in malignant cells is not restricted to myeloid leukemic cells. Erythroleukemic cells can be induced to differentiate by various chemicals (Marks and Rifkind, 1978). Erythroprotein, a cytokine that induces the production of hemoglobin in normal erythroid cells, does not induce hemoglobin in these erythroleukemias. These

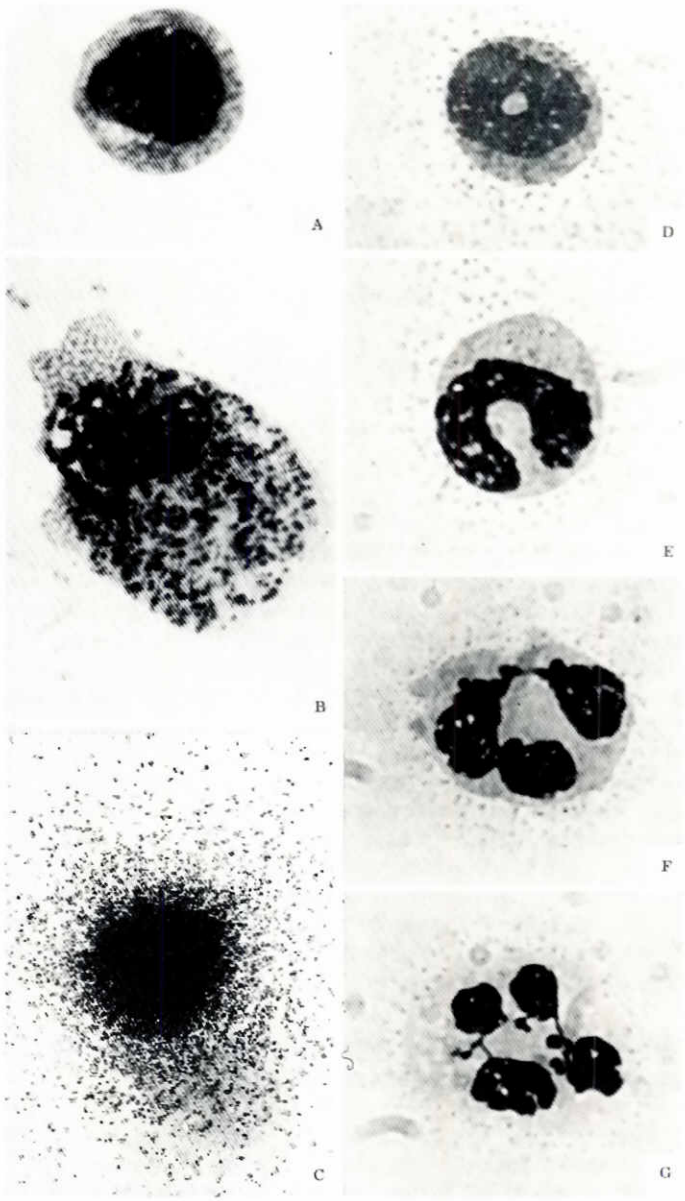

Fig. 5. Differentiation of myeloid leukemic cells to non-malignant mature macrophages or granulocytes by normal myeloid differentiation-inducing protein. (A) *Leukemic cell;* **(B)** *macrophage;* **(C)** *colony of cells with macrophages;* **(D-G)** *stages in differentiation to granulocytes (Fibach et al., 1972)*

erythroleukemias are thus like D^- myeloid leukemias that are not induced to differentiate by a normal myeloid regulatory cytokine. It has also been shown that some of the compounds that induce differentiation in leukemic cells can induce differentiation in tumors derived from other types of cells (Gardner, 1983).

Not only do different compounds induce differentiation but there are different ways of inducing it. In myeloid leukemic cells, some compounds induce differentiation by inducing the production of a normal differentiation-inducing cytokine in the D^+ leukemic cells, whereas others such as the steroid hormones induce differentiation

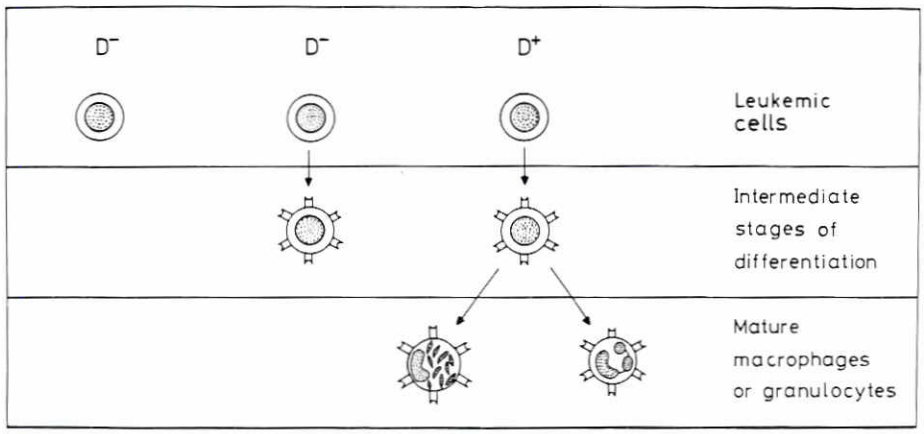

Fig. 6. Classification of different types of clones of myeloid leukemic cells according to their ability to be induced to differentiate by normal myeloid differentiation-inducing proteins. *Some differentiation-defective (D^-) clones can be induced by normal differentiation-inducing proteins to intermediate stages by differentiation, whereas other D^- clones are not induced to differentiate by these proteins even to an intermediate stage (Sachs, 1982, 1987b).*

without inducing this cytokine (Sachs, 1987b). Different compounds can induce different parts of the differentiation program. When combined treatment with different compounds is required, this combined treatment then produces by complementation the appropriate gene expression that is required for differentiation. This complementation of gene expression can occur at the level of mRNA production and mRNA translation (Sachs, 1987b). Studies with different mutants of myeloid leukemic cells have also shown that the constitutive cellular protein changes which inhibit differentiation by the steroid hormone dexamethasone are different from the constitutive cellular protein changes that inhibit differentiation by a normal differentiation-inducing cytokine (Cohen and Sachs, 1981; Sachs, 1987b). These experiments have identified different pathways of gene expression for inducing differentiation, and have also shown that genetic changes which inhibit differentiation by one compound need not affect differentiation by another compound that uses alternative pathways.

Chromosome changes, differentiation and bypassing of genetic defects in tumor suppression

Evidence has been obtained with various types of tumors, including teratocarcinomas (Lehman *et al.*, 1974; Gardner, 1983; Pierce *et al.*, 1987; Pierce and Speers, 1988) and sarcomas (Sachs, 1974) in addition to myeloid leukemias (Sachs, 1974, 1978), that tumor cells have not lost all the genes that control normal growth. This was first shown in sarcomas by the finding that it was possible to reverse the malignant to a non-malignant phenotype with a high frequency in cloned sarcoma cells whose malignancy had been induced by chemical carcinogens, x-irradiation, or by a tumor-inducing virus (Fig. 7) (Rabinowitz and Sachs, 1968, 1970a,b). In sarcomas induced after transformation of normal fibroblasts in culture with chemical carcinogens (Berwald and Sachs, 1963) or x-irradiation (Borek and Sachs, 1966), this reversibility of malignancy included reversion to the limited life-span found with normal fibroblasts (Rabinowitz and Sachs, 1970b). Chromosome studies on normal fibroblasts, sarcomas, revertants from sarcomas which had regained a non-malignant phenotype, and re-revertants, showed that the difference between these malignant and non-malignant cells is controlled by the balance between genes located on specific chromosomes that at that time were called genes for expression (E) and genes for suppression (S) of malignancy (Fig. 8) (Rabinowitz and Sachs, 1970a; Hitotsumachi *et al.*, 1971; Yamamoto *et al.*, 1973; Sachs 1974; Bloch-Shtacher and Sachs, 1976). When there is enough S to neutralize E malignancy

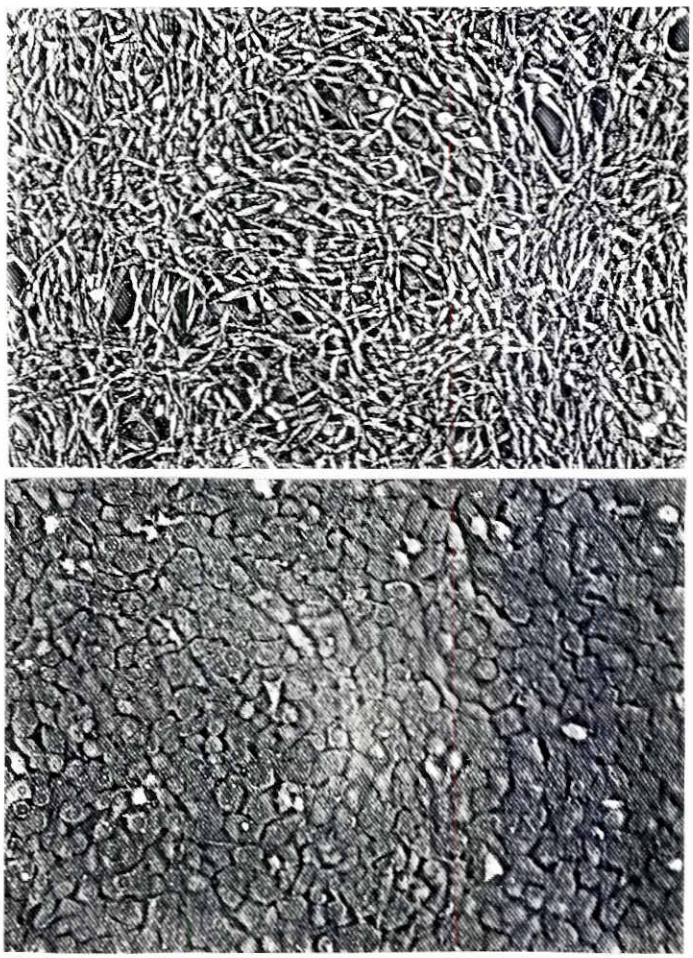

Fig. 7. Cultures of sarcoma cells (top) and a non-malignant revertant (bottom) (Rabinowitz and Sachs, 1968).

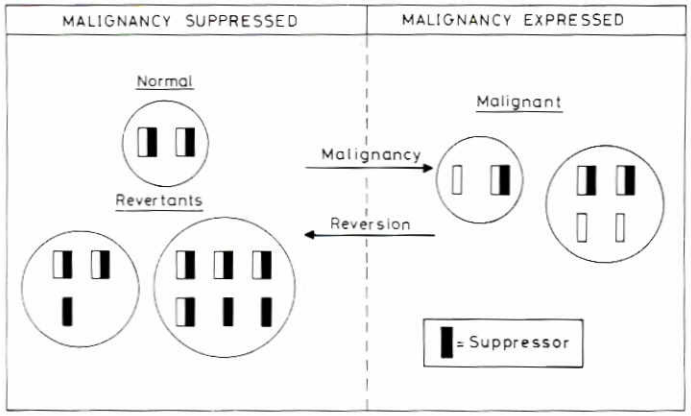

Fig. 8. The expression and suppression of malignancy by chromosome segregation resulting in a change in gene dosage owing to a change in the balance of specific chromosomes. *From experiments with normal fibroblasts, sarcoma cells and their non-malignant revertants. Genes for ❐ expression and ■ suppression of malignancy (Rabinowitz and Sachs, 1970a).*

is suppressed, and when the amount of S is not sufficient to neutralize E malignancy is expressed. These early experiments have shown (Rabinowitz and Sachs, 1970a; Hitotsumachi *et al.*, 1971; Yamamoto *et al.*, 1973; Sachs, 1974, 1987a) that in addition to genes on specific chromosomes for expression of malignancy (E) (oncogenes), there are other genes on specific chromosomes, S genes (suppressor genes), that can suppress the action of oncogenes. Among the activities of these genes is the ability of an oncogene to suppress programmed cell death and the ability of a tumor suppressor gene to activate programmed cell death (Yonish-Rouach *et al.*, 1991; Lotem and Sachs, 1993).

The suppression of malignancy in sarcomas (Sachs, 1974) was obtained by chromosome segregation, resulting in a change in gene dosage due to a change in the balance of specific chromosomes. This suppression of malignancy by chromosome segregation, with a return to the gene balance required for suppression of the malignant phenotype, occurred without hybridization between different types of cells. The non-malignant cells were thus derived from the malignant cells by genetic segregation. Suppression of malignancy associated with chromosome changes including changes in gene balance has also been found after hybridization between different types of cells (Klein, 1981; Evans *et al.*, 1982; Kitchin *et al.*, 1982; Stanbridge *et al.*, 1982; Benedict *et al.*, 1984). These studies on cell hybrids have led to similar conclusions to those obtained from the reversal of malignancy in sarcomas without hybridization between different cell types.

The D^+ myeloid leukemic cells have an abnormal chromosome composition (Azumi and Sachs, 1977). But suppression of malignancy in these cells, which also occurred in certain clones with a high frequency, was not associated with chromosome changes. Suppression of malignancy in these D^+ leukemic cells was obtained by induction of the normal sequence of cell differentiation by a normal myeloid regulatory cytokine. In this suppression of the malignant phenotype, the stopping of cell multiplication by inducing differentiation to mature cells by-passes genetic changes in the requirement for the normal growth inducer, and a block in the ability of growth inducer to induce differentiation inducer, that produced the malignant phenotype. Genetic changes which make cells defective in their ability to be induced to differentiate by the normal differentiation inducer occur in the evolution of myeloid leukemia. 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. Also in these cases the stopping of cell multiplication by inducing differentiation by these alternative pathways by-passes the genetic changes that inhibit response to the normal differentiation inducer. This by-passing of genetic defects is presumably also the mechanism for the suppression of malignancy by inducing differentiation in erythroleukemias and other types of tumors.

Studies on the chromosomes of myeloid leukemic cells have shown that the change from D^- to D^+ and *vice versa*, i.e. the ability to be induced to differentiate to mature non-dividing cells by a normal myeloid regulatory cytokine, is controlled by the balance between genes that allow induction of differentiation and genes that

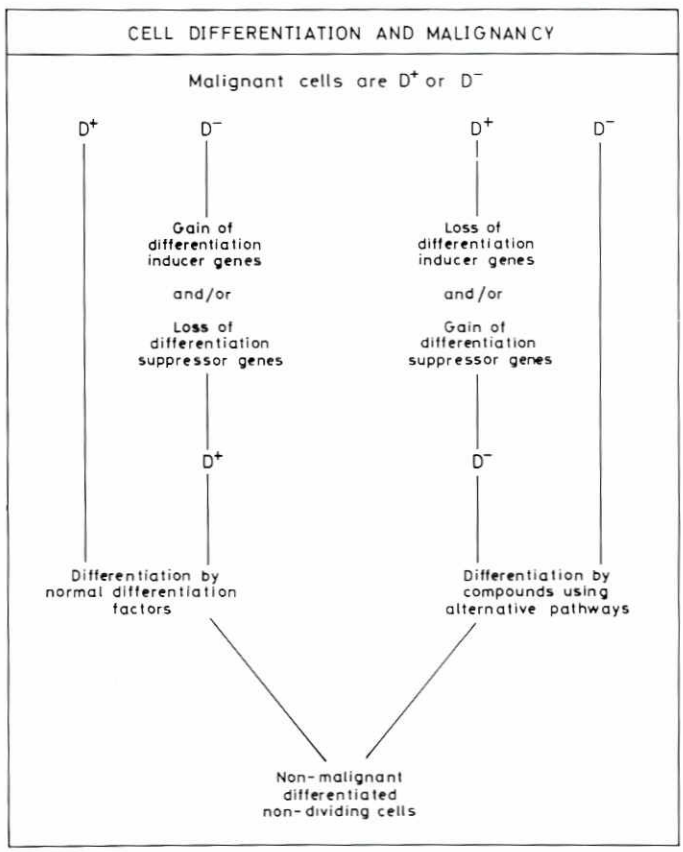

Fig. 9. Suppression of malignancy by inducing differentiation can be achieved in different ways. *Leukemic cells can (D^+) or cannot (D^-) be induced to differentiate to mature non-dividing cells by normal regulatory proteins. The D^- cells can, however, be induced to differentiate by compounds that induce differentiation by alternative pathways. Chromosome changes that change the balance between genes for induction and genes for suppression of differentiation can change cells from D^- to D^+ and vice versa (Sachs, 1987a).*

suppress differentiation (Azumi and Sachs, 1977). It was then also shown in hybrids between different cell types that chromosome changes can suppress malignancy by restoring the ability of the cells to be induced to differentiate to non-dividing cells *in vivo* in a location in the body where the cells are exposed to what is presumably the normal differentiation inducer (Stanbridge, 1984; Harris, 1985). The appropriate chromosome changes also change these hybrid cells from D^- to D^+. Chromosome changes can thus change tumor cells from D^- to D^+, so that the cells can then be induced to differentiate when exposed to normal differentiation inducer.

It can, therefore, be concluded from studies on the molecular regulators of growth and differentiation in normal development, changes in the normal development program in tumor cells, and suppression of malignancy that (A) malignancy can be suppressed by inducing differentiation either with or without genetic changes in the malignant cells (Fig. 9), (B) this suppression does not have to restore all the normal controls, and (C) genetic defects that give rise to malignancy can be by-passed and their effects nullified by inducing differentiation which stops cells from multiplying.

Acknowledgments

It is a particular pleasure to contribute to this special issue dedicated to G. Barry Pierce on the occasion of his «retirement». His contributions to research and his personality have long been an example to the scientific community, and even more so to those who have the privilege of knowing him personally. I am sure he will continue to be an example in whatever field of activity he chooses to pursue.

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References

AZUMI, J. and SACHS, L. (1977). Chromosome mapping of the genes that control differentiation and malignancy in myeloid leukemic cells. *Proc. Natl. Acad. Sci. USA 74:* 253-257.

BENEDICT, W.F., WEISSMANN, B.E., MARK, C. and STANBRIDGE, E.J. (1984). Tumorigenicity of human HT1080 fibrosarcoma x normal fibroblast hybrids: chromosome dosage dependency. *Cancer Res. 44:* 3471-3479.

BERWALD, Y. and SACHS, L. (1963). *In vitro* cell transformation with chemical carcinogens. *Nature 200:* 1182-1184.

BLATT, C. and SACHS, L. (1988). Deletion of a homeobox gene in myeloid leukemias with a deletion in chromosome 2. *Biochem. Biophys. Res. Commun. 156:* 1265-1270.

BLATT, C., ABERDAM, D., SCHWARTZ, R. and SACHS, L. (1988). DNA rearrangement of a homeobox gene in myeloid leukaemic cells. *EMBO J. 7:* 4283-4290.

BLATT, C., LOTEM, J. and SACHS, L. (1992). Inhibition of specific pathways of myeloid cell differentiation by an activated Hox-2.4 homeobox gene. *Cell Growth Differ. 3:* 671-676.

BLOCH-SHTACHER, N. and SACHS, L. (1976). Chromosome balance and the control of malignancy. *J. Cell. Physiol. 87:* 89-100.

BOREK, C. and SACHS, L. (1966). *In vitro* cell transformation by x-irradiation. *Nature 210:* 276-278.

BRADLEY, T.R. and METCALF, D. (1966). The growth of mouse bone marrow cells *in vitro*. *Aust. J. Exp. Biol. Med. Sci. 44:* 287-300.

CLARK, S.C. and KAMEN, R. (1987). The human hematopoietic colony-stimulating factors. *Science 236:* 1129-1237.

COHEN, L. and SACHS, L. (1981). Constitutive gene expression in myeloid leukemia and cell competence for induction of differentiation by the steroid dexamethasone. *Proc. Natl. Acad. Sci. USA 78:* 353-357.

DEGOS, L. (1992). All-trans-retinoic acid treatment and retinoic acid receptor alpha gene rearrangement in acute promyelocytic leukemia: a model for differentiation therapy. *Int. J. Cell Cloning 10:* 63-69.

EVANS, E.P., BURTENSHAW, M.D., BROWN, B.B., HENNION, R. and HARRIS, H. (1982). The analysis of malignancy by cell fusion. X. Reexamination and clarification of the cytogenic problem. *J. Cell Sci. 56:* 113-130.

FIBACH, E., HAYASHI, M. and SACHS, L. (1973). Control of normal differentiation of myeloid leukemic cells to macrophages and granulocytes. *Proc. Natl. Acad. Sci. USA 70:* 343-346.

FIBACH, E., LANDAU, T. and SACHS, L. (1972). Normal differentiation of myeloid leukemic cells induced by a differentiation-inducing protein. *Nature New Biol. 237:* 276- 278.

GARDNER, R.L. (1983). Teratomas in perspective. *Cancer Surv. 2:* 1-19.

GINSBURG, H. and SACHS, L. (1963). Formation of pure suspension of mast cells in tissue culture by differentiation of lymphoid cells from the mouse thymus. *J. Natl. Cancer Inst. 31:* 1-40.

GOOTWINE, E., WEBB, C.G. and SACHS, L. (1982). Participation of myeloid leukaemic cells injected into embryos in haematopoietic differentiation in adult mice. *Nature 299:* 63-65.

HARRIS, H. (1985). The suppression of malignancy in hybrid cells: the mechanism. *J. Cell Sci. 79:* 83-94.

HIRANO, T., AKIRA, S., TAGA, T. and KISHIMOTO, T. (1990). Biological and clinical aspects of interleukin 6. *Immunol. Today 11:* 443-449.

HITOTSUMACHI, S., RABINOWITZ, Z. and SACHS, L. (1971). Chromosomal control of reversion in transformed cells. *Nature 231:* 511-514.

ICHIKAWA, Y., PLUZNIK, D.H. and SACHS, L. (1966). *In vitro* control of the development of macrophage and granulocytes colonies. *Proc. Natl. Acad. Sci. USA 56:* 488-495.

ICHIKAWA, Y., PLUZNIK, D.H. and SACHS, L. (1967). Feedback inhibition of the development of macrophage and granulocyte colonies. I. Inhibition by macrophages. *Proc. Natl. Acad. Sci. USA 58:* 1480-1486.

KITCHIN, R.M., GADI, I.K., SMITH, B.L. and SAGER, R. (1982). Genetic analysis of tumorigenesis. X. Chromosome studies oof transformed mutants and tumor-derived CHEF/18 cells. *Somat. Cell Genet. 8:* 677-689.

KLEIN, G. (1981). The role of gene dosage and genetic transposition in carcinogenesis. *Nature 194:* 313-318.

LEHMAN, J.M., SPEERS, W.C., SWARTZENDRUBER, D.E. and PIERCE, G.B. (1974). Neoplastic differentiation: Characteristics of cell lines derived from a murine teratocarcinoma. *J. Cell. Physiol. 84:* 13-28.

LIEBERMANN, D., HOFFMAN-LIEBERMANN, B. and SACHS, L. (1980). Molecular dissection of differentiation in normal and leukemic myeloblasts. Separately programmed pathways of gene expression. *Dev. Biol. 79:* 46-63.

LOTEM, J. and SACHS, L. (1978). *In vivo* induction of normal differentiation in myeloid leukemia cells. *Proc. Natl. Acad. Sci. USA 75:* 3781-3785.

LOTEM, J. and SACHS, L. (1981). *In vivo* inhibition of the development of myeloid leukemia by injection of macrophage and granulocyte inducing protein. *Int. J. Cancer 28:* 375-386.

LOTEM, J. and SACHS, L. (1984). Control of *in vivo* differentiation of myeloid leukemic cells. IV. Inhibition of leukemia development by myeloid differentiation-inducing protein. *Int. J. Cancer 33:* 147-154.

LOTEM, J. and SACHS, L. (1988). *In vivo* control of differentiation of myeloid leukemic cells by recombinant granulocyte-macrophage colony stimulating factor and interleukin 3. *Blood 71:* 375-382.

LOTEM, J. and SACHS, L. (1989). Induction of dependence on hematopoietic proteins for viability and receptor upregulation in differentiating myeloid leukemic cells. *Blood 74:* 579-585.

LOTEM, J. and SACHS, L. (1990). Selective regulation of the activity of different hematopoietic regulatory proteins by transforming growth factor ß1 in normal and leukemic myeloid cells. *Blood 76:* 1315-1322.

LOTEM, J. and SACHS, L. (1993). Regulation by *bcl*-2, c-*myc* and p53 of susceptibility to induction of apoptosis by heat shock and cancer chemotherapy compounds in differentiation competent and defective myeloid leukemic cells. *Cell Growth Differ. 4:* 41-47.

LOTEM, J., CRAGOE, E.J. and SACHS, L. (1991). Rescue from programmed cell death in leukemic and normal myeloid cells. *Blood 78:* 953-960.

LOTEM, J., SHABO, Y. and SACHS, L. (1989). Regulation of megakaryocyte development by interleukin 6. *Blood 74:* 1545-1551.

MARKS, P. and RIFKIND, R.A. (1978). Erythroleukemia differentiation. *Annu. Rev. Biochem. 47:* 419-448.

METCALF, D. (1985). The granulocyte-macrophage colony-stimulating factors. *Science 299:* 16-22.

MOTRO, B., ITIN, A., SACHS, L. and KESHET, E. (1990). Pattern of interleukin 6 gene expression *in vivo* suggests a role for this cytokine in angiogenesis. *Proc. Natl. Acad. Sci. USA 87:* 3092-3096.

PIERCE, G.B. and SPEERS, W.C. (1988). Tumors as caricatures of the process of tissue renewal: prospects for therapy by directing differentiation. *Cancer Res. 48:* 1996-2004.

PIERCE, G.B., ARECHAGA, J., JONES, A., LEWELLYN, A. and WELLS, R.S. (1987). The fate of embryonal-carcinoma cells injected into mouse blastocysts. *Differentiation 33:* 247-253.

PLUZNIK, D.H. and SACHS, L. (1965). The cloning of normal «mast» cells in tissue culture. *J. Cell. Comp. Physiol. 66:* 319-324.

PLUZNIK, D.H. and SACHS, L. (1966). The induction of clones of normal «mast» cells by a substance from conditioned medium. *Exp. Cell Res. 43:* 553-563.

RABINOWITZ, Z. and SACHS, L. (1968). Reversion of properties in cells transformed by polyoma virus. *Nature 220:* 1203-1206.

RABINOWITZ, Z. and SACHS, L. (1970a). Control of the reversion of properties in transformed cells. *Nature 225:* 136-139.

RABINOWITZ, Z. and SACHS, L. (1970b). The formation of variants with a reversion of properties of transformed cells. V. Reversion to a limited life span. *Int. J. Cancer 6:* 388-398.

SACHS, L. (1974). Regulation of membrane changes, differentiation and malignancy in carcinogenesis. *The Harvey Lectures Vol. 68.* Academic Press, New York, pp. 1-35.

SACHS, L. (1978). Control of normal cell differentiation and the phenotypic reversion of malignancy in myeloid leukaemia. *Nature 274:* 535-539.

SACHS, L. (1980). Constitutive uncoupling of pathways of gene expression that control growth and differentiation in myeloid leukemia: a model for the origin and progression of malignancy. *Proc. Natl. Acad. Sci. USA 77:* 6152-6156.

SACHS, L. (1982). Normal developmental programmes in myeloid leukaemia: regulatory proteins in the control of growth and differentiation. *Cancer Surv. 1:* 321-342.

SACHS, L. (1986). Growth, differentiation and the reversal of malignancy. *Sci. Am. 254:* 40-47.

SACHS, L. (1987a). Cell differentiation and by-passing of genetic defects in the suppression of malignancy. *Cancer Res. 47:* 1981-1986.

SACHS, L. (1987b). The molecular regulators of normal and leukaemic blood cells. The Wellcome Foundation Lecture 1986. *Proc. R. Soc. Lond. B 231:* 289-312.

SACHS, L. (1987c). The molecular control of blood cell development. *Science 238:* 1374-1379.

SACHS, L. (1990). The control of growth and differentiation in normal and leukemic blood cells. The 1989 Alfred P. Sloan Prize of the General Motors Cancer Research Foundation. *Cancer 65:* 2196-2206.

SACHS, L. (1992). The molecular control of hematopoiesis: from clonal development in culture to therapy in the clinic. *Int. J. Cell Cloning 10:* 196-204.

STANBRIDGE, E.J. (1984). Genetic analysis of tumorigenicity in human cell hybrids. *Cancer Surv. 3:* 335-350.

STANBRIDGE, E.J., DER, C.J., DOERSEN, C-J., NISHIMI, R.Y., PEEHL, D.M. WEISSMAN, B.E. and WILKINSON, J.E. (1982). Human cell hybrids: analysis of transformation and tumorigenicity. *Science 215:* 252-259.

WEBB, C.G., GOOTWINE, E. and SACHS, L. (1984). Developmental potential of myeloid leukemia cells injected into mid-gestation embryos. *Dev. Biol. 101:* 221-224.

YAMAMOTO, T., RABINOWITZ, Z. and SACHS, L. (1973). Identification of the chromosomes that control malignancy. *Nature New Biol. 243:* 247-250.

YONISH-ROUACH, E., RESNITZKY, D., LOTEM, J., SACHS, L., KIMCHI, A. and OREN, M. (1991). Wild type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin 6. *Nature 352:* 345-347.