Int. J. Dev. Biol. 61: 127-136 (2017)
doi: 10.1387/ijdb.160262yg

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

The Leo Sachs' legacy: a pioneer's journey through hematopoiesis

JOSEPH LOTEM and YORAM GRONER*

Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

ABSTRACT Leo Sachs spent almost his entire scientific career in Israel, at the Weizmann Institute of Science, and became a worldwide renowned scientist for his pioneering studies in normal hematopoiesis, its breakdown in leukemia and the suppression of malignancy by inducing differentiation, thereby bypassing genetic defects that give rise to malignancy. The cell culture system he established in the early 1960s for the clonal development of normal hematopoietic cells, made it possible to discover the proteins that regulate the viability, proliferation and differentiation of different blood cell lineages, the molecular basis of normal hematopoiesis and the changes that drive leukemia. His studies established significant general concepts including: a) the value of a multi-gene cytokine network in regulating the viability, number and development of different cell types; b) the existence of alternative pathways that give flexibility to development in both normal and cancer cells; c) the response of some cancer cells to normal regulators of development; d) suppression of myeloid leukemia by inducing differentiation, bypassing malignancy-driving genetic defects; e) identification of chromosomes that control tumor suppression; f) discovering apoptosis as a major mechanism by which WT-p53 suppresses malignancy and g) the ability of hematopoietic cytokines to suppress apoptosis in both normal and leukemic cells. It is gratifying that Leo had the good fortune to witness his pioneering discoveries and ideas move from the basic science stage to effective clinical applications, augmenting normal hematopoiesis in patients with various hematopoietic deficiencies, in patients requiring hematopoietic stem cell transplantation and in the suppression of malignancy by inducing differentiation and apoptosis.

KEY WORDS: *apoptosis, cytokine, differentiation, hematopoiesis, leukemia*

Introduction

Leo Sachs (Fig. 1) was born in Leipzig, Germany in 1924. Fortunately, he emigrated in 1933 with his family from Nazi Germany to England where he grew up. Some years after his schooling, he decided to move to Israel. His original intention was to help found a kibbutz, and to prepare himself for this purpose he worked in England as a farm laborer, mainly milking cows. In 1948 he obtained a B.Sc. degree in agriculture and agricultural botany at the University of Wales in Bangor and in 1951 went on to obtain a Ph.D. degree studying plant genetics and cytogenetics at the Trinity College, Cambridge University. He then decided to change direction to work on cytogenetics and development in mammals, including humans, and he was appointed a Research Scientist in Genetics at the John Innes Institute, England, working on mammalian chromosomes. Upon arrival in Israel in 1952, he joined the Weizmann Institute of Science as a geneticist, where he was given the task of initiating research on genetics and development in the Department of Experimental Biology, established just two years earlier by Dr. Isaac Berenblum. With only a bench, a microscope and his experience with mammalian and human sex chromosomes (Sachs 1953, 1954), he decided to determine whether the spot of condensed chromatin in the nucleus, called a chromocenter, can be used to diagnose sex in non-dividing adult human somatic cells. The chromocenter was expressed during different stages of human fetal development, perhaps making it a useful marker for prenatal diagnosis of sex. Specifically, he asked whether cells in human amniotic fluid were sufficiently well preserved to be used

Abbreviations used in this paper: APL, acute promyelocytic leukemia; ATRA, *aII trans* retinoic acid; CSF, colony-stimulating factor; DIF, differentiation-inducing factor; HSC, hematopoietic stem cell; IL, interleukin; LIF, leukemia-inhibiting factor; MGI, macrophage and granulocyte inducer; TGFβ, transforming growth factor beta; TNF, tumor necrosis factor.

***Address correspondence to:** Yoram Groner. Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel. Tel: 972-8-934-3972. Fax: 972-8-934-4108. E-mail: yoram.groner@weizmann.ac.il

Accepted: 10 August, 2016.

ISSN: Online 1696-3547, Print 0214-6282

Fig. 1. Leo Sachs (1924-2013).

for prenatal diagnosis of sex and for a general prenatal diagnosis of human diseases. Amniotic fluid cells from fetuses of different ages were collected by amniocentesis and were shown to contain sufficient numbers of well-preserved fetal cells, which could be used for prenatal diagnosis of sex (Serr *et al*., 1955; Danon *et al.,* 1956; Sachs and Danon, 1956; Sachs *et al*., 1956b) and also of fetal blood group antigens (Sachs *et al*., 1956a). As Leo suggested at the time (Sachs and Danon, 1956), this first ever method can be applied to prenatal diagnosis of other genetic properties of the fetus and is now widely used for prenatal diagnosis in pregnant women.

Encouraged by Dr. Berenblum to find his own way in science and with an ensured supply of mice provided to him to expand his activities, Leo conducted some experiments on the chromosomes (Sachs and Gallily, 1955) of tumors with different abilities to be transplanted. His interest in regulation of normal and abnormal development led him to realize that it would be advantageous to choose a broad approach and, therefore, he decided to try and develop some new experimental systems that could be used to study these processes. This pioneering kind of research would become a characteristic of Leo's studies throughout his scientific career, in his studies on Polyoma and SV40 tumor viruses, chemical carcinogenesis *in vitro*, chromosome changes and reversibility of malignancy (reviewed in Sachs, 1995) and control of normal hematopoiesis and its breakdown in leukemia, which will be described in the following sections. In 1960, he was promoted to Associate Professor and established the section of Genetics and Virology, and in 1962, he became a Full Professor and head of the Department of Genetics, a position he held until his retirement. In 1968 he was appointed the Otto Meyerhof Professor of Molecular Biology and between 1974 -1979 he served as the Dean of the Faculty of Biology. Throughout his scientific career Leo supervised and mentored many Ph.D. students, some of who became prominent scientists on their own. Leo continued his work until his passing in the Department of Molecular Genetics that was created by the merger of the Genetics and Virology Departments in 1979.

Leo will always be remembered by the scientific communities in Israel and the world for his pioneering studies in several areas, most notably in normal hematopoiesis and leukemia, for which he received many awards including the Israel Prize for Natural Sciences in 1972; Member of the Israel Academy of Sciences and Humanities in 1975; Rothchild Prize in Biological Sciences 1977; Wolf Prize in Medicine (with J.L. Gowans and C. Milstein) in 1980; Bristol-Myers Award for Distinguished Achievement in Cancer Research, New York in 1982; The Royal Society Wellcome Foundation Prize, London (with D. Metcalf) in 1986; The Alfred P. Sloan Prize, General Motors Cancer Research Foundation, New York (with D. Metcalf) in 1989; Foreign Associate of USA National Academy of Sciences in 1995; The Warren Alpert Foundation Prize, Harvard Medical School, Boston (with D. Metcalf) in 1997; Fellow of the Royal Society, London in 1997; and the Emet Prize for Life Sciences, Medicine and Genetics (with A. Hershko and A. Chiechanover) in 2002. He also received honorary degrees from the Bordeuax University, France, in 1985 (Doctor Honoris Causa), Lund University, Sweden, 1997 (Doctor of Medicine Honoris Causa) and University of Wales, Bangor in 1999 (Honorary fellow) and gave numerous invited lectures including the Harvey Lecture in Rockefeller University, New York, 1972; Bob Smith Lecture in University of Texas System Cancer Center, Houston, 1976; General Motors Cancer Research Foundation Lecture, Walter and Eliza Hall Institute, Melbourne 1986; Anniversary Lecture, Swedish Society of Hematology, Stockholm 1996; Guest Lecture at the founding Congress of the Spanish Society for Developmental Biology, Leioa, Spain 1996 (Fig. 2); and the Ham-Wasserman lecture, American Society of Hematology, San Francisco 2000. The latter lecture can be accessed at https://www.youtube.com/watch?v=IzfqD5IvHc0

Hematopoiesis and leukemia

It became clear to Leo that in order to study the development of normal and tumor cells, it was necessary to develop cell culture systems in which cells could be cloned and made to differentiate into unique cell lineages. He decided to use hematopoietic cells that give rise to blood cells of different lineages throughout the life of an individual. Mouse leukemia originating from different cell lineages induced by viruses and other agents could also be obtained, so it was possible to study *in vitro* the development in clones of normal hematopoietic as well as leukemic cells. He could also study the effects of infection of normal cells with leukemia viruses. The first viruses he used were the Moloney and Rauscher viruses. Since neither the normal hematopoietic nor the leukemic cells survived in the standard cell culture medium *in vitro*, he used feeder layers of different types of mouse cells as possible sources of factors that the hematopoietic cells may require. This procedure worked out well both for leukemic (Ginsburg and Sachs, 1961 a,b) and normal hematopoietic cells (Ginsburg and Sachs, 1963; Pluznik and Sachs, 1965). Leo's analysis of the molecular control of normal hematopoiesis therefore began with the development of a cell culture system for the cloning and clonal differentiation of different types of normal hematopoietic cells (Ginsburg and Sachs, 1963). This cell culture system made

it possible to discover a family of cytokines that regulate cell viability, multiplication, and differentiation of different hematopoietic cell lineages, to analyze the origin of hematological diseases, and to identify ways of treating these diseases with normal cytokines.

Establishing the first in vitro *system for clonal development of normal hematopoietic cells*

In the cell culture system that was developed, normal cells from mouse blood-forming tissues were first cultured with feeder layers of other cell types, such as normal embryo fibroblasts. These feeder layers were chosen as a possible source of cells that may produce regulatory molecules required for the cloning and differentiation of different hematopoietic cell lineages. The first such system, which used cells cultured in liquid medium (Ginsburg and Sachs, 1963), showed that it was possible to obtain clones containing mast cells or granulocytes in various stages of differentiation. The cultures also showed differentiation to macrophages. To formulate his basic idea Leo wrote as the concluding sentence of this 1963 paper *"The described cultures thus seem to offer a useful system for a quantitative kinetic approach to hematopoietic cell formation and for experimental studies on the mechanism and regulation of hematopoietic cell differentiation"* (Ginsburg and Sachs, 1963).

The mast cell clones described above were obtained from cultures of thymus and the granulocyte clones from cultures of spleen. Infection of the thymus with Moloney virus and of the spleen with Rauscher virus increased the number of mast cells and granulocytes, respectively, indicating that this virus infection enhanced cell survival and/or multiplication (Ginsburg and Sachs, 1963). *In vivo* experiments with the Rauscher virus showed that the virus that infected nucleated erythroid cells could be assayed and cloned by a spleen colony-forming assay (Pluznik and Sachs, 1964). The culture system using feeder layers was then applied to the cloning of different cell lineages in semisolid medium containing agar (Pluznik and Sachs, 1965), which made it simpler to distinguish and isolate separate clones and was then also used by others (Bradley and Metcalf, 1966). Analysis of the first types of clones obtained in agar showed clones containing macrophages, granulocytes, or both macrophages and granulocytes in various stages of differentiation. The macrophage clones in agar contained many metachromatic granules, giving them an apparent morphological resemblance to mast cells (Pluznik and Sachs, 1965; Ichikawa *et al*., 1966). However, these metachromatic granules were not observed when the cells were cloned in methylcellulose (Ichikawa *et al*., 1966), and electron microscopy revealed that these "mast" cells in agar colonies were in fact macrophages that had phagocytized agar (Lagunoff *et al*., 1966). The experiments also showed that hematopoietic cell colonies grown *in vitro* can originate from single cells (Ginsburg and Sachs, 1963; Pluznik and Sachs, 1966; Paran and Sachs, 1969) and are therefore *bona fide* cell-clones. This agar or methylcellulose colony-forming assay was then applied to cloning and clonal differentiation of normal human macrophages and granulocytes (Paran *et al*., 1970; Pike and Robinson, 1970) and other blood cell lineages including erythroid cells (Stephenson *et al*., 1971), B-lymphocytes (Metcalf *et al*., 1975), T-lymphocytes (Fibach *et al*., 1976; Gerassi and Sachs, 1976) and megakaryocytes (Freedman *et al*., 1981).

Discovery of colony-stimulating factors and differentiation-inducing factors

When hematopoietic cells were cloned in soft agar, a harder agar layer was placed between the feeder layer cells and the hematopoietic cells seeded for cloning. This procedure revealed that the inducer(s) required for the formation of macrophage and granulocyte clones were secreted by the feeder layer cells and diffused through the agar (Pluznik and Sachs, 1965). This finding led to the discovery that the inducers required for the formation of macrophage and granulocyte clones are present in the conditioned medium produced by the feeder cells (Ichikawa *et al*., 1966; Pluznik

Fig. 2. Leo Sachs (front row, first on the right) as a guest lecturer *at the founding Congress of the Spanish Society of Developmetnal Biology (SEBD) in Leioa, Spain, (1996).*

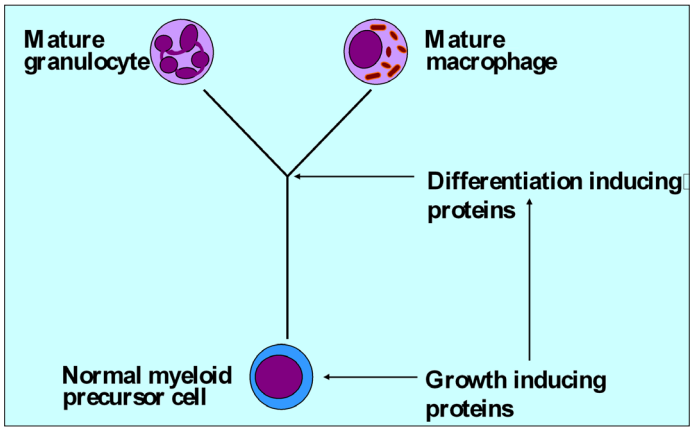
and Sachs, 1966). These inducers were found in the conditioned medium from various types of normal and malignant cells (reviewed in Paran *et al*., 1968; Sachs, 1974), which were then used to purify the different inducers (Landau and Sachs, 1971; Burgess *et al*., 1977; Stanley and Heard, 1977; Lipton and Sachs, 1981; Ihle *et al*., 1982). A similar approach was later used to identify the protein inducers for cloning of T-lymphocytes (Mier and Gallo, 1980) and B-lymphocytes (reviewed in Hirano *et al*., 1990). 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 re-added (Paran and Sachs, 1968). The development of clones with differentiated cells thus requires both an initial and a continued supply of inducer.

In cells belonging to the myeloid cell lineages, several different proteins that induce cell proliferation giving rise to colonies (colony-inducing proteins) have been identified (reviewed in Sachs, 1986, 1987 a,b, 1990, 1992, 1995). The same proteins were given different names. After their discovery in conditioned medium (Pluznik and Sachs, 1966; Ichikawa *et al*., 1966), the first inducer identified was given the name "Mashran gm" based on the Hebrew word meaning "to send forth" with the initials for granulocytes and macrophages (Ichikawa *et al*., 1967). This and other colony-inducing proteins were then renamed as macrophage and granulocyte inducers (MGI) (Landau and Sachs, 1971) and MGI-type 1 (MGI-1) (Lotem *et al.,* 1980) and are now collectively called colony-stimulating factors (CSFs) (reviewed in Metcalf, 1985; Sachs, 1987b; Witte, 1990). Of the different CSFs identified, M-CSF induces the development of clones with macrophages, G-CSF clones with granulocytes, GM-CSF clones with granulocytes, macrophages, or both macrophages and granulocytes and stem cell factor (SCF) clones with blast cells and granulocytes at different stages of maturation. Another factor called interleukin-3 (IL-3) (Ihle *et al*., 1982) had CSF activity that induced clones with macrophages, granulocytes, eosinophils, mast cells, erythroid cells, or megakaryocytes. All CSFs induce cell viability and proliferation (reviewed in Sachs, 1987b, 1990, 1992; Sachs and Lotem, 1993; Sachs 1995) of myeloid precursor cells and enhance the functional activity of mature cells (reviewed in Metcalf, 1985). Cloning of human and mouse *CSF* genes revealed that distinct genes encode each of the CSFs (reviewed in Clark and Kamen, 1987; Lennartsson and Ronnstrand, 2012).

It seemed to Leo and his colleagues quite unlikely that a CSF that induces cell proliferation can also simultaneously induce terminal cell differentiation that involves arresting cell proliferation in mature cells. Therefore, they looked for a protein that acts as a myeloid cell-differentiation inducer but does not have significant CSF activity. When such a protein was discovered, it was given the name macrophage and granulocyte inducer-type 2 (MGI-2) (Lotem *et al*., 1980; and reviewed in Sachs, 1987 a,b, 1990, 1995). The amino acid sequence of a 22 amino acid CNBr-cleaved peptide derived from purified mouse MGI-2 protein (Shabo *et al*., 1988) proved identical to that of recombinant mouse IL-6 (Wong and Clark, 1988). Moreover, recombinant mouse IL-6 possessed myeloid cell differentiation-inducing activity that could be inhibited by monoclonal anti-MGI-2 antibody, showing that the myeloid differentiation-inducing protein MGI-2 is identical to IL-6 (Shabo *et al*., 1988). Studies on myeloid leukemic cells have identified other differentiation-inducing proteins called D-factor and differentiation-inducing factor (DIF) (reviewed in Sachs, 1990). D-factor has also been called human interleukin for DA cells (HILDA) and leukemia inhibiting factor (LIF) (Moreau *et al.,* 1988), and DIF was found to be a form of tumor necrosis factor (TNF) (reviewed in Sachs, 1990). Whereas IL-6 can induce viability and differentiation of normal myeloid precursors, LIF and TNF, which induce differentiation in certain clones of myeloid leukemic cells, do not induce viability or differentiation of normal myeloid cells (reviewed in Sachs, 1990).

Hematopoietic cytokine network

The existence of a family of hematopoietic cytokines raised the question whether these cytokines interact with each other. Leo found that all CSFs induce the production of IL-6 in normal myeloid precursor cells (Lotem and Sachs, 1982, 1983), which can then induce these cells to differentiate to macrophages, granulocytes, or megakaryocytes (Sachs, 1986, 1987 a,b, 1990; Lotem *et al*., 1989). This induction of a differentiation factor such as IL-6 by a CSF growth factor can thus serve as an effective mechanism to couple growth and differentiation (Fig. 3). IL-6 may switch on other, yet unidentified, factors that may be required to determine the specificity of the terminally differentiated cell type. Thus, one



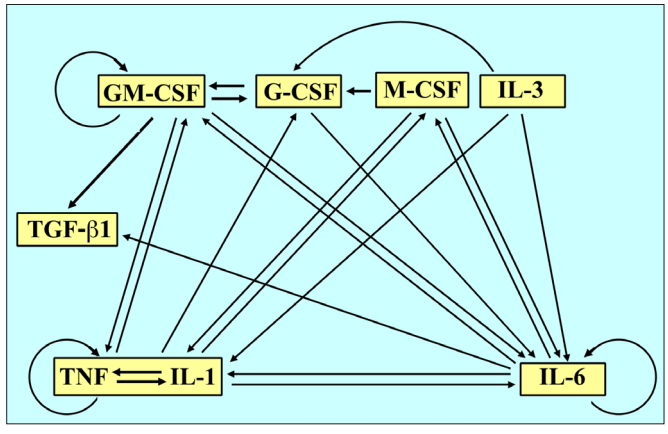


Fig. 3 (left). Induction of differentiation-inducing proteins by growth-inducing proteins *serves as a coupling mechanism in normal myeloid cell development.*

Fig. 4 (right). Cytokine network of interactions in myeloid cell development.

of the concepts that emerged from Leo's studies on hematopoiesis is that the different hematopoietic cytokines function in a network of interactions (Lotem *et al*., 1991b and reviewed in Sachs, 1990, 1995; Sachs and Lotem, 1994) (Fig. 4).

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, which is achieved by cytokines that can function as inhibitors, such as TNF and transforming growth-factor β1 (TGFβ1), which can selectively inhibit the activity and production of some CSFs and ILs (Lotem and Sachs, 1990) (Fig. 4). This network and the pleiotropic effects of its cytokines such as IL-6, IL-1, TNF and TGFβ1 outside the hematopoietic system (reviewed in Sachs, 1995) must to be taken into account in the clinical use of these cytokines. What can be therapeutically effective 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 released during certain infections (reviewed in Sachs, 1990). This amplification can occur both by auto-regulation and by trans-regulation of hematopoietic cytokine genes (Sachs, 1990) and also involves cytokine-induced up-regulation of different cytokine receptors (Lotem and Sachs, 1986, 1989). These cytokine-induced changes during differentiation are mediated by induction of sustained levels of transcription factors that can regulate and maintain gene expression in the differentiation program (Shabo *et al*., 1990). In addition to the flexibility of this network in response to different infections, it may also contribute to the stability of the hematopoietic system.

Normal cytokines and other compounds that control differentiation of myeloid leukemia cells

The next two major questions Leo asked were: (1) Can the cytokines that control normal hematopoiesis also control the behavior of leukemic cells? (2) Are there are other compounds that may have effects similar to these cytokines? Leo showed that there are human and murine myeloid leukemic cells that can be induced to differentiate to mature granulocytes and macrophages by media containing normal hematopoietic cytokines (Paran *el al*., 1970; Fibach *et al*., 1972) (Fig. 5). The clones of myeloid leukemic cells that could be induced to differentiate to mature macrophages or granulocytes by a normal myeloid differentiation-inducing cytokine were designated D+ clones (D for differentiation) (Fibach *et al*., 1973). The temporal sequence of acquired differentiation-associated functions in these D+ leukemic cells was the same as in normal myeloid precursor cells (Lotem and Sachs, 1977). Importantly, the mature cells that have developed from these leukemic clones stopped dividing, behaving like normal mature cells (Fibach, *et al*., 1973; Fibach and Sachs, 1974, 1975; Lotem and Sachs, 1977). These cells were no longer malignant *in vivo* (Fibach and Sachs, 1975). In addition to these D+ clones that can be induced to terminally differentiate by IL-6 and to partially differentiate with G-CSF, Leo found D+ clones from another myeloid leukemia that can be induced to differentiate with GM-CSF or IL-3 but not with IL-6 or G-CSF (reviewed in Sachs, 1987 a,b, 1990).

The D+ clones that respond to these CSFs are presumably induced by the CSFs to produce appropriate endogenous differentiation inducer(s) other than IL-6. The D+ leukemic cells that respond to IL-6 can also be induced to differentiate by IL-lα and IL-lβ, mediated by the endogenous production of IL-6 (reviewed in Sachs, 1990).

Subsequent studies have shown that differentiation of D+ myeloid leukemic cells to mature non-dividing cells can be induced not only by adding cytokines in culture, but also occurs *in vivo* (Lotem and Sachs, 1978, 1984, 1988). However, when several thousand of these leukemic cells were injected into adult syngeneic mice they grew progressively and eventually killed the host, presumably because the amount of differentiation inducers in the body was too low to overcome the overload of leukemic cells failing to induce terminal differentiation of all leukemic cells. When sufficient amounts of differentiation-inducing protein or compounds that induce their production by cells in the body were injected, the development of

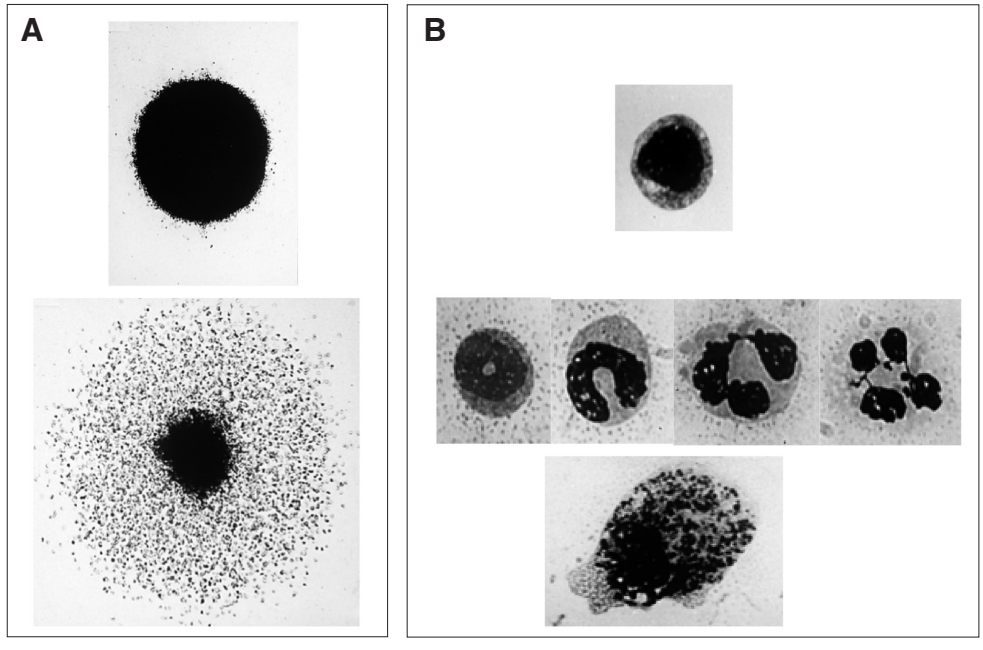


Fig. 5. Induction of differentiation of myeloid leukemic cells to mature macrophages and granulocytes by normal hematopoietic cytokines. (A) *myeloid leukemic D+ clone grown in agar without (top) or with (bottom) normal hematopoietic cytokines. The dispersed colony contains cells that have migrated out of the center of the colony and contains mature cells, whereas the compact colony contains only undifferentiated blast cells.* **(B)** *Morphology of cells within myeloid leukemic colonies. (Top), Undifferentiated blast cell from a colony of D+ myeloid leukemic cells grown without hematopoietic cytokins; (Middle and bottom), cells at different stages of differentiation to mature granulocytes (middle, left to right: myelocte, metamyelocte and mature granulocytes with segmented nuclei) or mature macrophage (bottom) from a colony of D+ myeloid leukemic cells grown with hematopoietic cytokines.*

leukemia by these D+ leukemic cells could be inhibited and mice survival was prolonged (Lotem and Sachs, 1981 and reviewed in Sachs, 1987 a,b, 1990). Induction of differentiation of D+ clones *in vivo* can occur either directly by the cytokine to which the cells respond *in vitro*, 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 (Lotem and Sachs, 1988). Remarkably, when these D+ myeloid leukemic cells were injected into mouse fetuses, they were shown to participate in normal hematopoietic cell differentiation to mature granulocytes and macrophages in apparently healthy adult animals that did not develop leukemia (Gootwine *el al*., 1982; Webb *et al*., 1984). The D+ myeloid leukemic cells have an abnormal chromosome composition (Fibach *et al*., 1973; Hayashi *et al*., 1974; Azumi and Sachs, 1977), yet suppression of malignancy in these cells during differentiation was not associated with regaining a normal diploid chromosome complement. Thus, the stopping of cell multiplication by inducing differentiation to mature cells bypasses genetic changes that produced the malignant phenotype (Sachs, 1987c), such as loss of a requirement for a normal cytokine for viability and growth and a block in the ability of growth inducer to promote production of a differentiation inducer.

The study of different clones of myeloid leukemic cells has shown that in addition to D+ clones there are differentiation-defective (D-) clones (Paran *et al*., 1970; Fibach *et al*., 1973). A variety of D- clones have been used to genetically dissect the differentiation program and analyze the differentiation blocks in leukemic cells (reviewed in Sachs, 1995). Some D- clones are induced by a normal myeloid differentiation-inducing cytokine to an intermediate stage of differentiation, which then slows down cell proliferation, while other D- clones could not be induced to differentiate even to this intermediate stage. These observations indicated that there are different blocks in the differentiation program of myeloid leukemic clones (Lotem and Sachs, 1974). The fact that a normal differentiation inducer can induce differentiation to mature non-dividing cells in the D+ clones, has led Leo to suggest that D+ clones represent early stages of leukemia development and that the appearance of D- clones may represent later stages in the progression of malignancy (reviewed in Sachs, 1987 a,b, 1990). He then went on to show that even such D- cells can be induced to differentiate by other compounds, either singly or in combination, that can induce the differentiation program by alternative pathways (reviewed in Sachs, 1982, l987a). The stopping of cell proliferation by inducing differentiation via these alternative pathways bypasses the genetic changes that inhibit response to the normal differentiation inducer (reviewed in Sachs, 1987 a,b, 1990).

Leo's studies on the genetic changes in various myeloid leukemic D- clones have shown that differentiation defectiveness may be due to aberrant expression of a homeobox gene such as *Hox2.4/ Hoxb8* (Blatt *et al*., 1988; Ben-David *et al*., 1991). Forced expression of *Hoxb8* in D+ myeloid leukemic cells inhibits specific pathways of myeloid cell differentiation (Blatt *et al*., 1992). Moreover, it was subsequently shown that introduction of *Hoxb8* into normal bone marrow cells enhances the self-renewal of myeloid progenitors and is sufficient for their progression to myeloid leukemia *in vivo*, which is associated with acquisition of mutations conferring autocrine production of IL-3 (Perkins and Cory, 1993). More recent studies have shown that deregulation of other *Hox* genes by chromosomal translocations also occurs in human leukemia (reviewed in Rice and Licht, 2007).

In his studies with a variety of chemicals, other than the normal hematopoietic cytokines, Leo found that many compounds could induce differentiation in D+ clones of myeloid leukemic cells. These include glucocorticoid hormones and chemicals used in cancer chemotherapy such as cytosine arabinoside, doxorubicin, methotrexate and even irradiation. At high doses, irradiation and these chemotherapeutic compounds kill cells by inducing apoptosis, whereas at low doses they induce differentiation. Not all these compounds are equally active on the same leukemic clone (reviewed in Sachs, 1978, 1982). In addition, other compounds that can induce differentiation in myeloid leukemic cells include insulin, bacterial lipopolysaccharide, certain plant lectins, tumor-promoting phorbol esters (reviewed in Sachs, 1978, 1982, 1987a) and retinoic acid (Breitmann *et al*., 1980, 1981 and reviewed in Degos, 1992). Of special interest is the finding that a variety of chemicals or their combination can also induce differentiation in some D- clones (reviewed in Sachs, 1982). These results indicate that besides normal myeloid cytokines, other physiological compounds including steroid hormones, insulin, and retinoic acid can also induce differentiation of myeloid leukemic cells. These findings raise the possibility that in fact all D- myeloid leukemic cells, which are no longer susceptible to the normal hematopoietic cytokines alone, can be induced to differentiate by the appropriate combination of other compounds. Thus, Leo's experiments with myeloid leukemic cells have shown that there are different pathways of gene expression for inducing differentiation and that genetic changes which inhibit induction of differentiation by one compound need not affect differentiation induced by another compound through alternative pathways.

Cytokines and control of programmed cell death

Normal myeloid precursor cells depend on hematopoietic cytokines for viability, proliferation and differentiation (reviewed in Sachs, 1987 a,b, 1990; Sachs and Lotem, 1993). Withdrawal of these cytokines leads to cell death by apoptosis. The program for cell death in factor-deprived cells is present not only in normal myeloid precursor cells but also in more differentiated progeny including mature granulocytes and macrophages. Although viability factors such as the CSFs also induce growth, the viability and growth processes are separately regulated (reviewed in Sachs and Lotem, 1993). Cancer cells, like their normal counterparts, are not immortal. Even tumor cell lines that have been sub-cultured for years contain cells that will eventually die by apoptosis. The ability to sub-culture these cell lines for long periods of time is maintained because more cells survive and divide than those that die. While most myeloid leukemic cells initially maintain a normal requirement for hematopoietic cytokines for viability and growth, certain myeloid leukemic clones have become independent on these factors and therefore do not require an exogenously added cytokine for cell viability and growth. Yet, even in such factor-independent leukemic cells, the endogenous program for cell death is not lost and can be activated in different ways. Leo's studies have shown that induction of differentiation in D+ leukemic cells by IL-6 induces a viability-factor dependent state in the differentiating cells, so that the cells undergo apoptosis following withdrawal of IL-6 (Fibach and Sachs, 1976; Lotem and Sachs, 1982, 1983, 1989). This regaining of the normal program for cell death in the IL-6-induced leukemic cells occurs before their terminal differentiation and the

differentiating cells can be rescued from apoptosis and continue to divide by re-adding IL-6 or replacing it with IL-3, M-CSF, G-CSF or IL-1 (Lotem and Sachs, 1989). Induction of apoptosis in differentiating myeloid leukemic cells deprived of viability factors thus constitutes activation of a normal physiological process that can potentially be used to suppress leukemia.

Cancer cells can be induced to undergo apoptotic cell death by different cytotoxic agents including chemotherapeutic compounds and irradiation, which is the basis for cancer therapy. Leo's studies with murine myeloid leukemic cell lines (Lotem and Sachs, 1992) and primary leukemic cells from human acute myeloid leukemia patients (Kaplinsky *et al.,* 1996) have shown that this cytotoxic agents induced apoptosis can also be inhibited by different CSFs and ILs acting as viability factors. Studies by others have also shown that various cytokines can inhibit cytotoxic agents induced apoptosis in other cells types such as T-lymphocytes and neurons (reviewed in Lotem and Sachs, 1999). Using growth-factor independent myeloid leukemic cells that lack expression of the tumor suppressor p53, Leo and colleagues have shown that transfection with a plasmid encoding a temperature-sensitive mutant p53 (Ala to Val change at position 135), induces apoptosis when cells are shifted from 37.5°C to 32.5°C, at which the Val 135 mutant protein assumes wild-type (WT) p53 conformation and activity (Yonish-Rouach *et al*., 1991). This WT-p53-induced apoptosis was not associated with differentiation induction but was effectively inhibited by IL-6 (Yonish-Rouach *et al*., 1991), suggesting that p53-mediated apoptosis in these myeloid leukemic cells is in fact a normal physiological process. Subsequent experiments using p53-deficient mice have confirmed that WT-p53 is physiologically involved in mediating apoptosis in normal myeloid precursors deprived of the appropriate cytokine concentration required for cell viability and also following DNA damage by γ-irradiation (Lotem and Sachs, 1993a). In addition, these experiments have also shown that γ-irradiation induces apoptosis in normal thymocytes by a p53-dependent process and that there are also WT-p53-independent pathways of inducing apoptosis in these cells (Clarke *et al*., 1993; Lotem and Sachs, 1993a; Lowe *et al*., 1993). Leo's studies have also revealed that WT-p53-induced apoptosis in myeloid leukemic cells can be inhibited by antioxidants, protease inhibitors and calcium mobilizing compounds, the latter acting via activation of calcineurin, a calcium-calmodulin-dependent phosphatase (Lotem and Sachs, 1997, 1998, 1999) (Fig. 6). Of particular interest is Leo's finding that inhibition of p53-induced apoptosis by IL-6 and calcium mobilizing compounds is not due to a general block of the wild-type p53-induced changes in gene expression but rather is mediated by induction of anti-apoptotic genes such as *Bcl3, Stat3 and Spp1*, that override the p53-induced apoptotic program (Lotem *et al*., 2003).

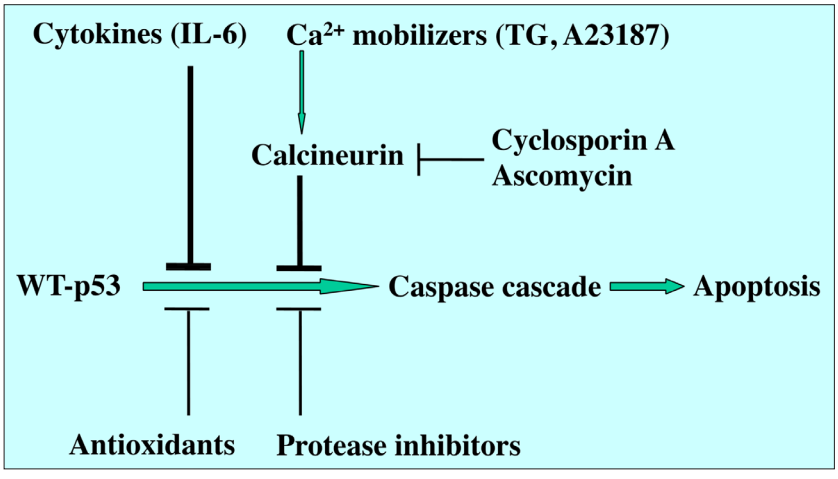


Fig. 6. inhibition of WT-p53-induced apoptosis in myeloid leukemic cells *by hematopoieic cytokines, Ca^{2+} mobilizers, protease inhibitors and antioxidants. TG, thapsigargin.*

The induction of apoptosis in myeloid leukemic cells by various cytotoxic agents, including those used in cancer therapy (Lotem and Sachs, 1992), is enhanced by overexpression of c-myc (Lotem and Sachs, 1993b). Conversely, the mutant p53 (Lotem and Sachs, 1993b) and bcl-2 oncogenes (reviewed in Korsmeyer, 1992) suppress the enhancing effect of deregulated c-myc on apoptosis and thus allow c-myc-mediated induction of cell proliferation and inhibition of differentiation (Lotem and Sachs, 1993b). The suppression of cell death by mutant p53 and bcl-2 increases the probability of developing tumors. Treatments that down-regulate the expression or activity of mutant p53 and Bcl-2 in tumor cells can thus be useful to increase susceptibility of tumors to cytotoxic cancer therapy (Sachs and Lotem, 1993; Lotem and Sachs, 1994). Leo showed that Bcl-2 protein expression was strongly and rapidly reduced in myeloid leukemic cells by treatment with cytokines such as IL-6 and G-CSF and by the steroid hormone dexamethasone (Lotem and Sachs, 1993b, 1994) and in human acute promyelocytic leukemic cells by *all-trans*-retinoic acid (Chomienne *et al*., 1992). The cytokine-mediated decrease in Bcl-2 expression enhanced leukemic cell susceptibility to apoptosis induction by cancer chemotherapeutic compounds (Lotem and Sachs, 1994). Collectively, Leo's studies on the cytokine and gene regulation of apoptosis in normal and leukemic myeloid cells have shown that there are alternative pathways to apoptotic cell death, which can be useful to selectively control cell viability of cancer cells and thus may have important implications in cancer therapy (Sachs and Lotem, 1993).

Therapeutic use of hematopoietic cytokines

The identification of myeloid cell regulatory cytokines has led Leo to suggest novel possibilities for therapy (reviewed in Sachs, 1978, 1986, 1987 a,b, 1990, 1995, 1996). Directly injecting these cytokines or inducing their production *in vivo* by other compounds (Fibach and Sachs, 1974; Lotem and Sachs, 1978, 1981, 1984) could elevate their *in vivo* levels and thereby enhance myelopoiesis. The finding that apparently normal granulocyte development can be induced in culture of cells from patients with infantile genetic agranulocytosis (Paran *et al*., 1970; Barak *et al*., 1971) has led to promising clinical results with G-CSF in children with this genetic disease as well as in acquired idiopathic neutropenia and aplastic anemia patients (reviewed in Mehta *et al.,* 2015). Moreover, G-CSF and GM-CSF were also shown to be clinically effective treatments for irradiation, chemotherapy or immune suppression-associated neutropenia (reviewed in Mehta *et al.,* 2015). Because of the important functions of mature cells, such as granulocytes and other myeloid cells, the increased CSF-mediated survival and function of mature cells can also be clinically helpful to patients with deficiencies in myeloid cell production, functions and viability (reviewed in Sachs, 1990).

Induction of apoptosis of cancer cells is the basis for

most if not all cancer therapy regimens. As indicated in the previous section, hematopoietic cytokines including various CSFs, IL-6 and IL-1 can inhibit apoptosis of cancer cells induced by various cancer chemotherapy compounds and irradiation (Lotem and Sachs, 1992; Sachs and Lotem, 1993). The clinical use of cytokines to correct therapy-associated suppression of normal hematopoiesis should therefore be carefully timed to avoid protection of the malignant cells from the cytotoxic action of therapeutic compounds (Lotem and Sachs, 1992).

The bone marrow contains hematopoietic stem cells (HSC) that can repopulate all hematopoietic lineages in lethally irradiated hosts (reviewed in Dzierzak *et al*., 1998; Weissman, 2000), creating the basis for bone marrow transplantation. The finding that G-CSF and GM-CSF can enhance the migration of HSCs from bone marrow to peripheral blood (reviewed in Horsfall *et al*., 2000; Gazitt, 2001), by decreasing the chemokine SDF-1 and increasing CXCR4 in bone marrow (Petit *et al*., 2002), has simplified the procedure for HSC harvesting for transplantation into patients with immunodeficiency and in cancer patients following chemotherapy and irradiation.

Differentiation therapy: from hypothesis to practice

Failure to terminally differentiate together with enhanced viability and proliferation characterizes most, if not all, cancer cells of every origin. Whether the induction of differentiation could be a valid treatment strategy for cancer was hotly debated before the advent of differentiation therapy. Based on his discovery in the early 1970s that myeloid leukemic cells could be reprogrammed by various cytokines and other compounds to resume normal differentiation and to become non-dividing mature granulocytes or macrophages (reviewed in Sachs, 1978, 1987 a,b, 1990, 1995), Leo hypothesized that treatment with agents that induce cancer cells to complete differentiation could be a potential therapeutic option for patients with cancer (Sachs, 1978). In the early 1980s, Breitman and colleagues showed that retinoic acid, a derivative of vitamin A, could induce human promyelocytic cells of the HL-60 leukemic cell line (Breitman *et al*., 1980) and primary cells from human acute promyeloicytic leukemia (APL) patients (Breitman *et al*., 1981) to terminally differentiate *in vitro* to mature neutrophil granulocytes. Subsequently, a clinical trial showed that *all-trans* RA (ATRA) produced remarkable complete remission in more than 95% of APL patients with evidence for leukemic cell morphological maturation in all patients (Huang *et al*., 1988). Currently, ATRA combined with anthracycline-based chemotherapy can achieve complete remission in 90%–95% of APL patients and overall 5-year disease-free survival in up to 75% of patients (Zhou *et al*., 2005). Both ATRA and arsenic trioxide, another differentiation inducing and clinically effective compound in APL, act by inducing degradation of the PML-RARα oncogenic fusion protein that drives APL development, allowing the cells to regain normal differentiation and undergo apoptosis and thus achieve complete remissions *in vivo* (Zhu *et al*., 2001; Zhou *et al*., 2005).

References

AZUMI, J.I. 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.

BARAK, Y., PARAN, M., LEVIN, S. AND SACHS, L. (1971). *In vitro* induction of myeloid proliferation and maturation in infantile genetic agranulocytosis. *Blood* 38: 74-80.

BEN-DAVID, L., ABERDAM, D. SACHS, L. and BLATT, C. (1991). A deletion and a rearrangement distinguish between the intracisternal A- particle of Hox2.4 and that of interleukin-3 in the same leukemic cells. *Virology* 182: 382-387.

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.

BRADLEY, T.R. and METCALF, D. (1966). The growth of mouse bone marrow cells in vitro. *AustJ Exp Biol Med Sci* 44: 287-299.

BREITMAN, T.R., COLLINS, S.J. and KEENE, B.R. (1981). Terminal differentiation of human promyelocytic leukemic cells in primary culture in response to retinoic acid. *Blood* 57: 1000-1004.

BREITMAN, T.R., SELONICK, S.E. AND COLLINS, S.J. (1980). Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc Natl Acad Sci USA* 77: 2936-2940.

BURGESS, A.W., CAMAKARIS, J. and METCALF, D. (1977). Purification and properties of colony-stimulating factor from mouse lung-conditioned medium. *J Biol Chem* 252: 1998-2003.

CHOMIENNE, C., BARBEY, S., BALITRAND, N., DEGOS, L. and SACHS, L. (1992). Regulation of bcl-2 and cell death by all-trans-retinoic acid in acute promyelocytic leukemic cells. *Proc. Am. Assoc. Cancer Res.* 33: 41.

CLARK, S.C. and KAMEN, R. (1987). The human hematopoietic colony-stimulating factors. *Science* 236: 1229-1237.

CLARKE, A.R., PURDIE, C.A., HARRISON, D.J., MORRIS, R.G., BIRD, C.C., HOOPER, M.L. and WYLLIE, A.H. (1993). Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature* 362: 849-852.

DANON, M., SACHS, L. and SERR D.M. (1956). Analysis of amniotic fluid cells for diagnosis of foetal sex. *Br Med J* 2: 795-798.

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.

DZIERZAK, E., MEDVINSKY, A., and DE BRUIJN, M. (1998). Qualitative and quantitative aspects of haematopoietic cell development in the mammalian embryo. *Immunol Today* 19: 228-236.

FIBACH, E. and SACHS, L. (1974). Control of normal differentiation of myeloid leukemic cells. IV. Induction of differentiation by serum from endotoxin treated mice. *J Cell Physiol* 83: 177-185.

FIBACH, E. and SACHS, L. (1975). Control of normal differentiation of myeloid leukemic cells. VIII. Induction of differentiation to mature granulocytes in mass culture. *J Cell Physiol* 86: 221-230.

FIBACH, E. and SACHS, L. (1976). Control of normal differentiation of myeloid leukemic cells. XI. Induction of a specific requirement for cell viability and growth during the differentiation of myeloid leukemic cells. *J Cell Physiol* 89: 259-266.

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., GERASSI, E. and SACHS, L. (1976). Induction of colony formation *in vitro* by human lymphocytes. *Nature* 259: 127-129.

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

FREEDMAN, M.H., MCDONALD, T.P. and SAUNDERS, E.F. (1981). Differentiation of murine marrow megakaryocyte progenitors (CFUm): humoral control *in vitro*. *Cell Tissue Kinet* 14: 53-58.

GAZITT, Y. (2001). Recent developments in the regulation of peripheral blood stem cell mobilization and engraftment by cytokines, chemokines, and adhesion molecules. *J Hematother Stem Cell Res* 10: 229-236.

GERASSI, E. and SACHS, L. (1976). Regulation of the induction of colonies *in vitro* by normal human lymphocytes. *Proc Natl Acad Sci USA* 73: 4546-4550.

GINSBURG, H. and SACHS, L. (1961a). Long-term cultivation in tissue culture of leukemic cells from mouse leukemia induced by Moloney virus or by x rays. *J Natl Cancer Inst* 27: 1153-1171.

GINSBURG, H. and SACHS, L. (1961b). *In vitro* culture of a mammalian leukemia virus. *Virology* 13: 380-382.

GINSBURG, H. and SACHS, L. (1963). Formation of pure suspensions of mast cells

in tissue culture by differentiation of lymphoid cells from the mouse thymus. *J Natl Cancer Inst* 31: 1-39.

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.

HAYASHI, M., FIBACH, E. and SACHS, L. (1974). Control of normal differentiation of myeloid leukemic cells. V. Normal differentiation in aneuploid leukemic cells and the chromosome banding pattern of D+ and D minus clones. *Int J Cancer* 14: 40-48.

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

HORSFALL, M.J., HUI, C.H., TO, L.B., BEGLEY, C.G., BASSER, R.L. and SIMMONS, P.J. (2000). Combination of stem cell factor and granulocyte colony-stimulating factor mobilizes the highest number of primitive haemopoietic progenitors as shown by pre-colony-forming unit (pre-CFU) assay. *Br J Haematol* 109: 751-758.

HUANG, M.E., YE, Y.C., CHEN, S.R., CHAI, J.R., LU, J.X., ZHOA, L., GU, L.J. and WANG, Z.Y. (1988). Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* 72: 567-572.

ICHIKAWA, Y., PLUZNIK, D.H. and SACHS, L. (1966). *In vitro* control of the development of macrophage and granulocyte 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.

IHLE, J.N., KELLER, J., HENDERSON, L., KLEIN F. and PALASZINSKI, E. (1982). Procedures for the purification of interleukin 3 to homogeneity. *J Immunol* 129: 2431-2436.

KAPLINSKY, C., LOTEM, J. and SACHS, L. (1996). Protection of human myeloid leukemic cells against doxorubicin-induced apoptosis by granulocyte-macrophage colony stimulating factor and interleukin 3. *Leukemia* 10: 460-465

KORSMEYER S.J. (1992). Bcl-2 initiates a new category of oncogenes: regulators of cell death. *Blood* 80: 879-886.

LAGUNOFF, D., PLUZNIK, D.H. and SACHS, L. (1966). The cloning of macrophages in agar: Identification of cells by electron microscopy. *J Cell Physiol* 68: 385-388.

LANDAU, T. and SACHS, L. (1971). Characterization of the inducer required for the development of macrophage and granulocyte colonies. *Proc Natl Acad Sci USA* 68: 2540-2544.

LENNARTSSON, J. and RONNSTRAND, L. (2012). Stem cell factor receptor/c-Kit: from basic science to clinical implications. *Physiol Rev* 92: 1619-1649.

LIPTON, J.H. and SACHS, L. (1981). Characterization of macrophage- and granulocyte-inducing proteins for normal and leukemic myeloid cells produced by the Krebs ascites tumor. *Biochim Biophys Acta* 673: 552-569.

LOTEM, J. and SACHS, L. (1974). Different blocks in the differentiation of myeloid leukemic cells. *Proc Natl Acad Sci USA* 71: 3507-3711.

LOTEM, J. and SACHS, L. (1977). Control of normal differentiation of myeloid leukemic cells. XII. Isolation of normal myeloid colony-forming cells from bone marrow and the sequence of differentiation to mature granulocytes in normal and D+ myeloid leukemic cells. *J Cell Physiol* 92: 97-108.

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. (1982). Mechanisms that uncouple growth and differentiation in myeloid leukemia cells: restoration of requirement for normal growth-inducing protein without restoring induction of differentiation-inducing protein. *Proc Natl Acad Sci U S A* 79: 4347-4351.

LOTEM, J. and SACHS, L. (1983). Coupling of growth and differentiation in normal myeloid precursors and the breakdown of this coupling in leukemia. *Int J Cancer* 32: 127-134.

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. (1986). Regulation of cell surface receptors for different hematopoietic growth factors on myeloid leukemic cells. *EMBO J* 5: 21632170.

LOTEM, J. and SACHS, L. (1988). Control of *in vivo* differentiation of myeloid leukemic cells. *Leukemia* 2: 24S-37S.

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 beta 1 in normal and leukemic myeloid cells. *Blood* 76: 1315-1322.

LOTEM, J. and SACHS, L. (1992). Hematopoietic cytokines inhibit apoptosis induced by transforming growth factor beta 1 and cancer chemotherapy compounds in myeloid leukemic cells. *Blood* 80: 1750-1757.

LOTEM, J. and SACHS, L. (1993a). Hematopoietic cells from mice deficient in wild-type p53 are more resistant to induction of apoptosis by some agents. *Blood* 82: 1092-1096.

LOTEM, J. and SACHS, L. (1993b). 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. and SACHS, L. (1994). Control of sensitivity to induction of apoptosis in myeloid leukemic cells by differentiation and bcl-2 dependent and independent pathways. *Cell Growth Differ* 5: 321-327.

LOTEM, J. and SACHS, L. (1997). Cytokine suppression of protease activation in wild-type p53-dependent and p53-independent apoptosis. *Proc Natl Acad Sci USA* 94: 9349-9353.

LOTEM, J. and SACHS, L. (1998). Different mechanisms for suppression of apoptosis by cytokines and calcium mobilizing compounds. *Proc Natl Acad Sci USA* 95: 4601-4606.

LOTEM, J. and SACHS, L. (1999). Cytokines as suppressors of apoptosis. *Apoptosis* 4: 187-196.

LOTEM, J., GAL, H., KAMA, R., AMARIGLIO, N., RECHAVI, G., DOMANY, E., SACHS, L. and GIVOL, D. (2003). Inhibition of p53-induced apoptosis without affecting expression of p53-regulated genes. *Proc Natl Acad Sci USA* 100: 6718-6723.

LOTEM, J., LIPTON, J.H. and SACHS, L. (1980). Separation of different molecular forms of macrophage-and granulocyte-inducing proteins for normal and leukemic myeloid cells. *Int J Cancer* 25: 763-771.

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

LOTEM, J., SHABO, Y. and SACHS, L. (1991b). The network of hemopoietic regulatory proteins in myeloid cell differentiation. *Cell Growth Differ* 2: 421-427.

LOWE, S.W., SCHMITT, E.M., SMITH, S.W., OSBORNE, B.A. and JACKS, T. (1993). p53 is required for radiation-induced apoptosis in mouse thymocytes, *Nature* 362: 847-849.

MEHTA, H.M., MALANDRA, M. and COREY, S.J. (2015). G-CSF and GM-CSF in neutropenia. *J Immunol* 195: 1341-1349.

METCALF, D. (1985). The granulocyte-macrophage colony-stimulating factors. *Science* 229: 16-22.

METCALF, D., NOSSAL, G.J., WARNER, N.L., MILLER, J.F., MANDEL, T.E., LAYTON, J.E. and GUTMAN, G.A. (1975). Growth of B-lymphocyte colonies in vitro. *J Exp Med* 142: 1534-1549.

MIER, J.W. and GALLO, R.C. (1980). Purification and some characteristics of human T-cell growth-factor from phytohemagglutinin-stimulated lymphocyte-conditioned media. *Proc Natl Acad Sci USA* 77: 6134-6138.

MOREAU, J.F., DONALDSON, D.D., BENNETT, F., WITEK-GIANNOTTI, J., CLARK, S.C. and WONG, G.G. (1988). Leukaemia inhibitory factor is identical to the myeloid growth factor human interleukin for DA cells. *Nature* 336: 690-692.

PARAN, M. and SACHS, L. (1968). The continued requirement for inducer for the development of macrophage and granulocyte colonies. *J Cell Physiol* 72: 247-250.

PARAN, M. and SACHS, L. (1969). The single cell origin of normal granulocyte colonies in vitro. *J Cell Physiol* 73: 91-92.

PARAN, M., ICHIKAWA, Y. and SACHS, L. (1968). Production of the inducer for macrophage and granulocyte colonies by leukemic cells. *J Cell Physiol* 72: 251-254.

PARAN, M., SACHS, L., BARAK, Y. and RESNITZKY, P. (1970). *In vitro* induction of granulocyte differentiation in hematopoietic cells from leukemic and nonleukemic patients. *Proc Natl Acad Sci USA* 67: 1542-1549.

PERKINS, A.C. and CORY, S. (1993). Conditional immortalization of mouse myelomonocytic, megakaryocytic and mast cell progenitors by the Hox2.4 homeobox gene. *EMBO J* 12: 3835-3846.

PETIT, I., SZYPER-KRAVITZ, M., NAGLER, A., LAHAV, M., PELED, A., HABLER, L., PONOMARYOV, T., TAICHMAN, R.S., ARENZANA-SEISDEDOS, F., FUJII, N. *et al*. (2002). G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol* 3: 687-694.

PIKE, B.L. and ROBINSON, W.A. (1970). Human bone marrow colony growth in agar-gel. *J Cell Physiol* 76: 77-84.

PLUZNIK, D.H. and SACHS, L. (1964). Quantitation of a murine leukemia virus with a spleen colony assay. *J Natl Cancer Inst* 33: 535-546.

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.

RICE, K.L. and LICHT, J.D. (2007). HOX deregulation in acute myeloid leukemia. *J Clin Invest* 117: 865-868.

SACHS, L. (1953). The giant sex chromosomes in the mammal Microtus agrestis. *Heredity* 7: 227-238.

SACHS, L. (1954). Sex-linkage and sex chromosomes in man. *J Human Genet* 18: 255-261.

SACHS, L. (1974). Regulation of membrane changes, differentiation and malignancy in carcinogenesis. *Harvey Lectures* 68: 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. (1982). Normal developmental programmes im 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). The molecular control of blood cell development. *Science* 238: 1374-1379.

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

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

SACHS, L. (1990). The control of growth and differentiation in normal and leukemic blood cells. *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.

SACHS, L. (1995). The adventures of a biologist: Prenatal diagnosis, hematopoiesis, leukemia, carcinogenesis and tumor suppression. *Adv Cancer Res* 66: 1-40.

SACHS, L. (1996). The control of hematopoiesis and leukemia: from basic biology to the clinic. *Proc Natl Acad Sci USA* 93: 4742-4749.

SACHS, L. and DANON M. (1956). Antenatal identification of heredity disorders, *Lancet* 267: 1013.

SACHS L. and GALLILY, R. (1955). The chromosomes and transplantability of tumors. I. Fundamental chromosome numbers and strain specificity in ascites tumors. *J Natl Cancer Inst* 15: 1267-1289.

SACHS, L. and LOTEM, J. (1993). Control of programmed cell death in normal and leukemic cells: new implications for therapy. *Blood* 82: 15-21.

SACHS, L. and LOTEM, J. (1994). The network of hematopoietic cytokines. *Proc Soc Exp Biol Med* 206: 170-175.

SACHS, L., FELDMAN, M. and DANON, M. (1956a). Prenatal identification of blood group antigens. *Lancet* 271: 356.

SACHS, L., SERR, D.M. and DANON, M. (1956b). Prenatal diagnosis of sex using cells from the amniotic fluid. *Science* 123: 548.

SERR, D.M., SACHS, L. and DANON, M. (1955). The diagnosis of sex before birth using cells from the amniotic fluid (a preliminary report). *Bull Res Counc Isr* 5B: 137-138.

SHABO, Y., LOTEM, J., RUBINSTEIN, M., REVEL, M., CLARK, S.C., WOLF, S.F., KAMEN, R. and SACHS, L. (1988). The myeloid blood cell differentiation-inducing protein MGI-2A is interleukin-6. *Blood* 72: 2070-2073.

SHABO, Y., LOTEM, J. and SACHS, L. (1990). Induction of genes for transcription factors by normal hematopoietic regulatory proteins in the differentiation of myeloid leukemic cells. *Leukemia* 4: 797-801.

STANLEY, E.R. and HEARD, P.M. (1977). Factors regulating macrophage production and growth. Purification and some properties of the colony stimulating factor from medium conditioned by mouse L cells. *J Biol Chem* 252: 4305-4312.

STEPHENSON, J.R., AXELRAD, A.A., MCLEOD, D.L. AND SHREEVE, M.M. (1971). Induction of colonies of hemoglobin-synthesizing cells by erythropoietin in vitro. *Proc Natl Acad Sci USA* 68: 1542-1546.

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

WEISSMAN, I.L. (2000). Translating stem and progenitor cell biology to the clinic: barriers and opportunities. *Science* 287: 1442-1446.

WITTE, O.N. (1990). Steel locus defines new multipotent growth factor. *Cell* 63: 5-6.

WONG, G.G. and CLARK, S.C. (1988). Multiple actions of interleukin 6 within a cytokine network. *Immunol Today* 9: 137-139.

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.

ZHOU, G.B., ZHAO, W.L., WANG, Z.Y., CHEN, S.J. and CHEN, Z. (2005). Retinoic acid and arsenic for treating acute promyelocytic leukemia. *PLoS Med* 2: e12.

ZHU, J., LALLEMAND-BREITENBACH, V. and DE THE, H. (2001). Pathways of retinoic acid- or arsenic trioxide-induced PML/RARalpha catabolism, role of oncogene degradation in disease remission. *Oncogene* 20: 7257-7265.

Further Related Reading, published previously in the *Int. J. Dev. Biol.*

Hematopoietic stem cell emergence in the conceptus and the role of Runx1
Gena Sweiers, Marella de Bruijn and Nancy A. Speck
Int. J. Dev. Biol. (2010) 54: 1151-1163

Gene regulatory networks governing hematopoietic stem cell development and identity
John E. Pimanda and Berthold Gottgens
Int. J. Dev. Biol. (2010) 54: 1201-1211

Key apoptosis regulatory proteins are down-regulated during postnatal tissue development
Shane D. Madden, Maryanne Donovan and Thomas G. Cotter
Int. J. Dev. Biol. (2007) 51: 415-424

The chemokine network in cancer- much more than directing cell movement
Hagen Kulbe, Neil R. Levinson, Fran Balkwill and Julia L. Wilson
Int. J. Dev. Biol. (2004) 48: 489-496

The role of p53 in vivo during skeletal muscle post-natal development and regulation: studied in p53 knockout mice
Jason D. White, Rachel Collins, Royce Vermeulen, Marilyn Davies and Miranda D. Grounds
Int. J. Dev. Biol. (2002) 46: 577-582