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Local and metastatic growth and in vivo differentiation of human myeloid leukemia cells transplanted in nude mice

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Summary. Cells from the established human myeloid cell lines KG-1, KG-1a, and HL-60, transplanted subcutaneously (sc) into nude mice, developed discrete tumors (myelosarcomas). These myelosarcomas had a host's age-associated pattern of growth identical to that of experimental tumors produced by sc transplantation of cells derived from malignant solid neoplasias. Thus, leukemia cells yielded either localized myelosarcomas at the site of inoculation or a disseminated neoplastic growth after inoculation in *adult* (more than 4 weeks old) or *newborn* (1–3 days old) nude mice, respectively. Human myeloid leukemia cells proliferating in the nude mice preserved the human karyotype and a surface antigenic determinant and did not influence the hematopoietic tissues of the host.

The KG-1 and HL-60 cell lines consistently attained varying degrees of differentiation along the myeloid series in vitro, and these features were maintained during proliferation in the mice. Furthermore, cells of the variant subline KG-1a, which had a blastic morphology, developed signs of differentiation that were not seen in culture. The presence of readily identifiable markers, such as cytoplasmic granules containing myeloperoxidase, in the cell lines tested makes these models particularly useful for studying the influence of a biological environment on cell differentiation and its influence on tumor growth. These experimental systems are also suitable for investigating the mechanism(s) of metastases and for in vivo experimental therapeutic trials.

Key words: Human myeloid leukemia cell lines – Nude mice – Transplantation – Metastasis – In vivo differentiation

Cultured human leukemia cells transplanted into immunodeficient mice have tumorigenic properties and show a host's age-dependent pattern of neoplas-

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tic growth. Thus, leukemia cells proliferate either as single or metastatic neoplasms after transplantation into *adult* or *newborn* nude mice, respectively (for review, see Lozzio and Machado 1982). In contrast with in vitro proliferation, neoplastic cells transplanted into mice replicate and, supported by a biological framework in which they induce vascularization and stromal reaction, form actual tumors. Therefore, established human leukemia cell lines are, within the restrictions of any experimental system, a suitable resource for developing models for the study of neoplastic growth and for screening therapeutic agents (Lozzio et al. 1982 and 1983; Machado et al. 1982a, b, 1983). However, while human non-myeloid leukemia cells proliferate well in mice, experimental neoplasias composed of myelogenous leukemia cells are difficult to generate and maintain by mouse-to-mouse passages (Lozzio and Machado 1982). To date, the only experimental tumor formed by atypical human myeloid cells and maintained by long-term serial transplantation (more than nine years) into immunodeficient mice is the K-562 myelosarcoma (Lozzio et al. 1976; Machado et al. 1977 and 1982a).

With the use of procedures similar to those followed in developing the K-562 myelosarcoma, we have now succeeded in obtaining three reproducible myelosarcomas composed of the KG-1 (Koeffler and Golde 1978), KG-1a (Koeffler et al. 1980), and HL-60 (Gallagher et al. 1979) human myeloid leukemia cell lines transplanted into immunodeficient mice. Since these cell lines attain varying degrees of differentiation in vitro, our tumor models are particularly suitable for investigating the influence of a biological environment on the maturation of malignant myeloid cells and whether such changes affect neoplastic growth.

Material and methods

Culture methods

The KG-1 line and subline KG-1a, established from the bone marrow tissue of a patient with erythroleukemia that evolved into acute myelogenous leukemia (AML) (Koeffer and Golde 1978; Koeffler et al. 1980), were cultured in α-medium with 20% inactivated fetal calf serum (IFCS). HL-60 cells, a line derived from the peripheral leukocytes of a patient with acute promyelocytic leukemia (APML) (Gallagher et al. 1979), were cultured in RPMI 1640 containing 20% IFCS.

Heterotransplantation

Human myeloid leukemia cells were transplanted into inbred BALB/c nude mice of both sexes. Mice were conventionally termed *newborn* (1–3 days old) or *adult* (over 4 weeks old), according to age at the time of transplantation. All procedures were carried out in specific pathogen-free (SPF) enclosures in which the mice were housed. A 50 µl aliquot containing 10^7 viable cells from suspension cultures of KG-1, KG-1a, and HL-60 cell lines was inoculated subcutaneously (sc) in the dorsal region of *newborn* nude mice. Pieces of the sc myelosarcomas developed by the KG-1 and HL-60 cells were serially transplanted into *adult* nude mice. The tumors developed from the KG-1a cells could only be passaged in *newborn* mice.

Cells and tumors characterization rate of growth

Two diameters of the sc tumors were measured twice a week with a caliper and the volume was calculated at fixed intervals. The presence of neoplastic nodules in the lungs and kidneys

of the mice was determined via autopsies performed at 30–120 day intervals after transplantation.

Light microscopy. Slices of sc tumors and of internal organs with metastases were fixed in 10% formaldehyde and processed for light microscopy. Sections were stained with hematoxylin-eosin and Gomori's silver reticulin stain.

Ultrastructure. Small (1 mm^3) blocks of sc and visceral tumors were fixed in 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) for 2 h, rinsed 3 times in buffer, and post-fixed in 2% aqueous osmium tetroxide. After dehydration, specimens were embedded in Epon 812. Ultra-thin sections were cut and stained with uranyl acetate and lead citrate for electron microscopy examination.

Aliquots of 10^7/ml malignant hematopoietic cells proliferating in culture were fixed with equal volumes of glutaraldehyde in 0.05 M sodium cacodylate buffer for 1 h. Then the cells were post-fixed in osmium tetroxide and embedded.

Histochemical assays. Aliquots of cells and blocks of tumors, fixed for only 1 h in a glutaraldehyde-sodium cacodylate mixture, were incubated in myeloperoxidase (MPO) (Novikoff and Goldfischer 1968) and acid phosphatase (Barka and Anderson 1962) media. After incubation, the specimens were processed as described above.

Assessment of human cell features. Chromosome analyses were performed using G-banding techniques (Sumner et al. 1971) in cultured cells prior to transplantation and in cells recovered from sc tumors and metastases subcultured in tissue culture media (EM 15A).

A heterologous antibody, raised to K-562 cells and which cross-reacts with other myeloid cell lines (Lozzio et al. 1977), was used to determine the presence of leukemia antigen receptors on cells recovered from tumors and metastases. This determination was made by detecting antibody-dependent, complement-mediated cytotoxicity (ADCMC) on recultured cells. Also, sections of tumors fixed in 10% formaldehyde in methanol containing 0.3% hydrogen peroxide were used to determine immunoperoxidase activity.

Hematological studies. To ascertain the lack of involvement of mouse hematopoietic tissues by the human leukemia cells, total and differential mouse peripheral leukocytic counts were determined with a Particle Data counter. Differential bone marrow counts were made on smears stained by the May-Gründwald-Giemsa technique. In addition, sections of bone marrow, spleen, lymph nodes and liver of tumor-bearing nude mice were examined by light microscopy.

K-562 human myeloid leukemia cells. Results from our experiments with the transplantation of the K-562 human myeloid leukemia cell line were compared with the characteristics of tumors formed by the new cell lines tested. Culture conditions of K-562 cells have been extensively reported (Lozzio and Lozzio 1975; Lozzio and Machado 1982). Our methods for the transplantation of K-562 cells and tumor passages were identical to those described above.

Results

Incidence and pattern of neoplastic growth. KG-1, KG-1a and HL-60 cells transplanted sc into *newborn* nude mice developed well-defined, localized tumors at the site of inoculation. Some mice also had disseminated tumors in the lungs and kidneys (Table 1). The length of the latent phase following transplantation varied for each cell line (Fig. 1). After the lag period, the neoplastic cells began to proliferate steadily, increasing the size of the sc nodule. The speed of this proliferation, as well as the slope of the curve corresponding to logarithmic growth, was peculiar for each cell line and did not vary significantly through five serial mouse-to-mouse passages. The analysis of the rate of growth of the sc tumors shows that the HL-60 cells

Table 1. Incidence and distribution of neoplastic processes arising from human myeloid leukemia cells injected neonatally into nude mice

Cell line	Nature	Tumors/mice injected[a]	Mice with SC tumors	Mice with visceral disseminations		
				Lungs	Kidneys	Brain
K-562	Pluripotential (CML)	89/136	35	74	15	38
KG-1	Myelogenous (AML)	31/ 39	31	5	1	0
KG-1a	Myelogenous (AML)	34/ 35	34	17	1	0
HL-60	Promyelocytic (APL)	2/ 21	2	2	2	0

CML and AML: Chronic and acute myelogenous leukemia, respectively
APL: Acute promyelocytic leukemia

[a] Subcutaneous (sc) tumor and/or visceral disseminations. All figures have been rounded to the nearest unit

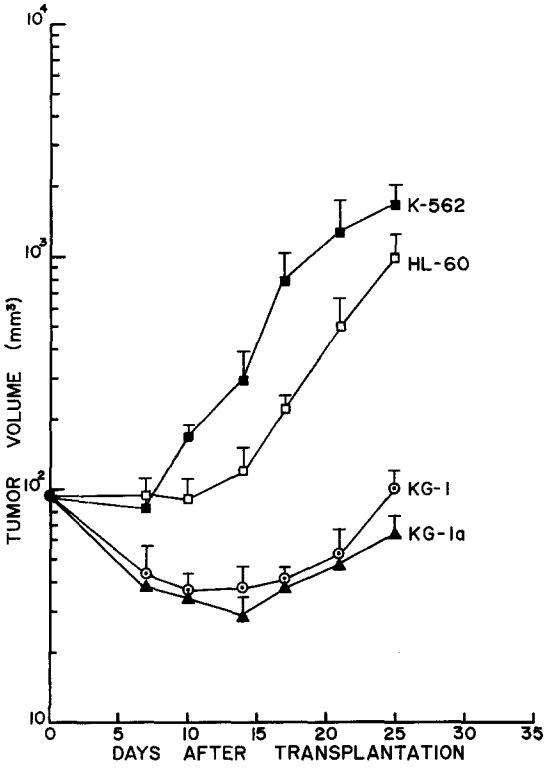

Fig. 1. Growth rates of sc myelosarcomas developed after transplantation of HL-60, KG-1 and KG-1a human leukemia cells into nude mice. Growth of sc K-562 myelosarcomas at similar intervals is included for comparative purposes

gave rise in a shorter period of time to tumors significantly larger than those formed by KG-1 and KG-1a cells. As shown in Fig. 1, both the rate of growth and the volume of HL-60 myelosarcomas at similar intervals paralleled those of the tumors yielded by the highly undifferentiated K-562 cells in previous experiments.

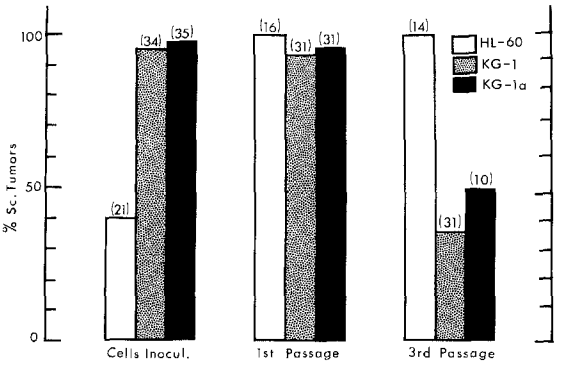

Fig. 2. Incidence of sc myelosarcomas after the initial cell inoculation, and first and third mouse-to-mouse passages. Figures in parentheses correpond to the number of mice inoculated. KG-1a myelosarcomas were serially transplanted only in newborn nude mice

Although the incidence of tumors yielded by HL-60 cells after the first inoculation in *newborn* mice was low, it increased dramatically during ensuing serial passages (Fig. 2). The KG-1 and KG-1a lines, on the other hand, showed a decreasing incidence of tumorigenesis from the first inoculation through the following serial passages. It must be emphasized that KG-1a myelosarcomas grew well only in *newborn* nude mice while KG-1 and HL-60 myelosarcomas, initially developed in *newborn* hosts, were serially passaged in *adult* nude mice.

Light microscopy. HL-60 myelosarcomas were composed of nests of large cells with clear nuclei that had prominent nucleoli and smaller cells displaying indented or banded darker nuclei (Fig. 3a). Mitotic figures were numerous. Groups of tumor cells appeared supported by a fine fibrillar stroma which harbored a network of neocapillaries. The border of the tumor infiltrated the surrounding host tissues, including the muscle fibrils of the thoracic wall of the mouse. Pulmonary metastases formed by HL-60 myelosarcomas had morphologic features similar to those of sc tumors. The metastatic nodules, which at early stages were small discrete formations, coalesced into larger masses later in the evolution (Fig. 3b).

KG-1 and KG-1a sc myelosarcomas had similar morphologic characteristics (Figs. 4a and 5a). They were composed of nests and cords of undifferentiated cells that had a more heterogeneous aspect than did the HL-60 myelosarcomas. This was mainly due to the intermingling of the cells which had large or elongated nuclei and smaller cells with pachychromatic and necrotic nuclei. A number of mitotic figures was observed, and infiltration of the surrounding host subcutaneous tissues by neoplastic cells was a common feature.

Pulmonary metastases of KG-1 and KG-1a myelosarcomas (Figs. 4b and 5b) had a pleomorphic cellular aspect similar to the sc myelosarcomas. Like the pulmonary metastases of HL-60 myelosarcomas, the KG-1 and KG-1a lung tumors were enlarged by the confluence of discrete nodules. These metastatic nodules frequently surrounded bronchioles and small branches of the pulmonary vein.

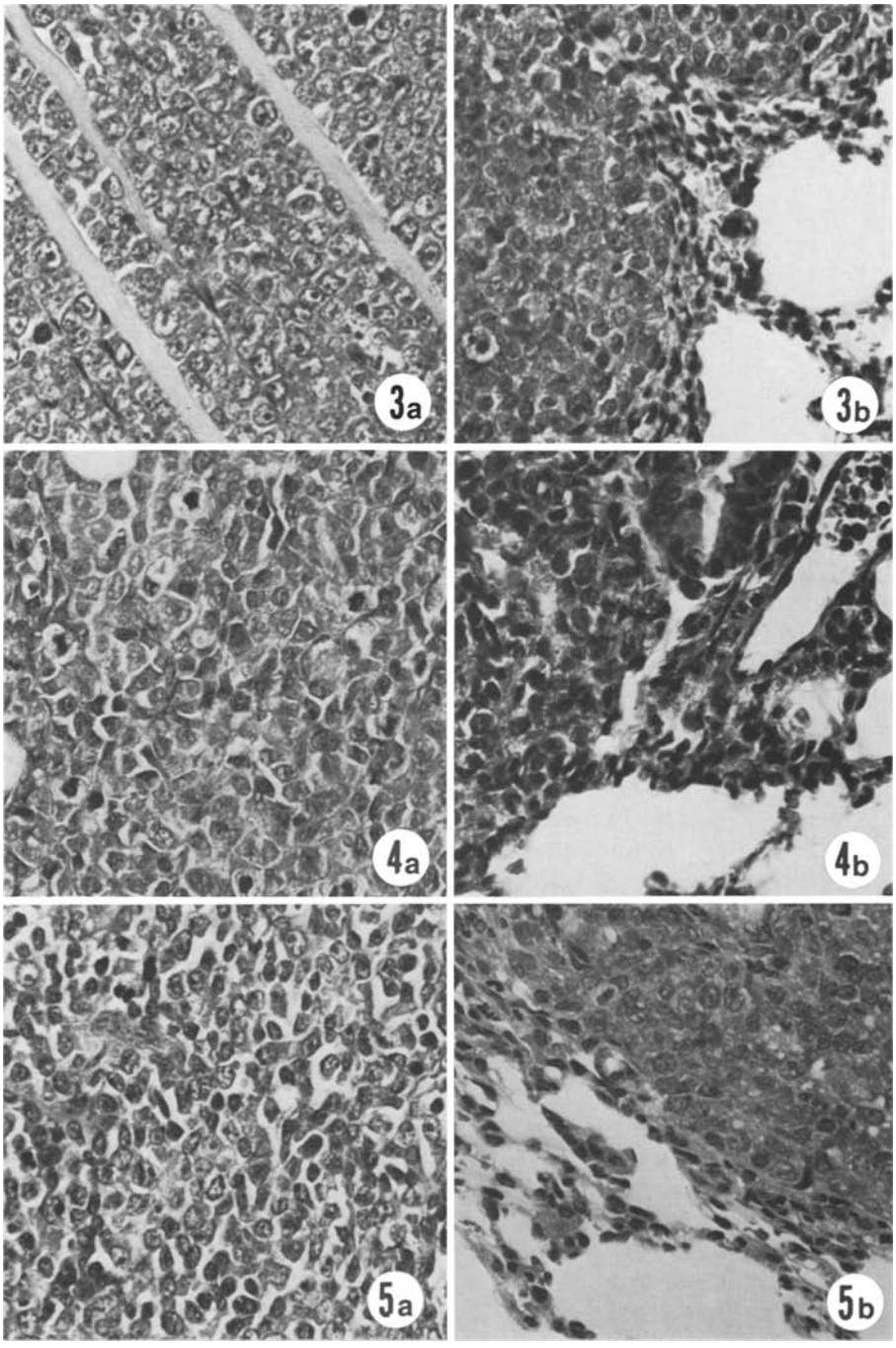

Fig. 3. **a** Section of an sc HL-60 myelosarcoma. The clear longitudinal bands correspond to residual fragments of muscular tissue of the thoracic wall of the nude mouse infiltrated by the neoplastic cells (H & E, ×250). **b** Pulmonary metastases of an HL-60 myelosarcoma formed by confluent neoplastic nodules (H & E, ×250). (This and following illustrations correspond to tumors proliferating 25 days after transplantation)

Fig. 4. **a** SC KG-1 myelosarcoma infiltrating the adjoining fatty tissue of the host (H & E, ×250). **b** Metastases of KG-1 myelosarcoma in lung (H & E, ×250)

Fig. 5. **a** Section of an sc KG-1a myelosarcoma showing intermingling of preserved and picnotic nuclei (H & E, ×250). **b** Pulmonary metastases of a KG-1a myelosarcoma (H & E, ×250)

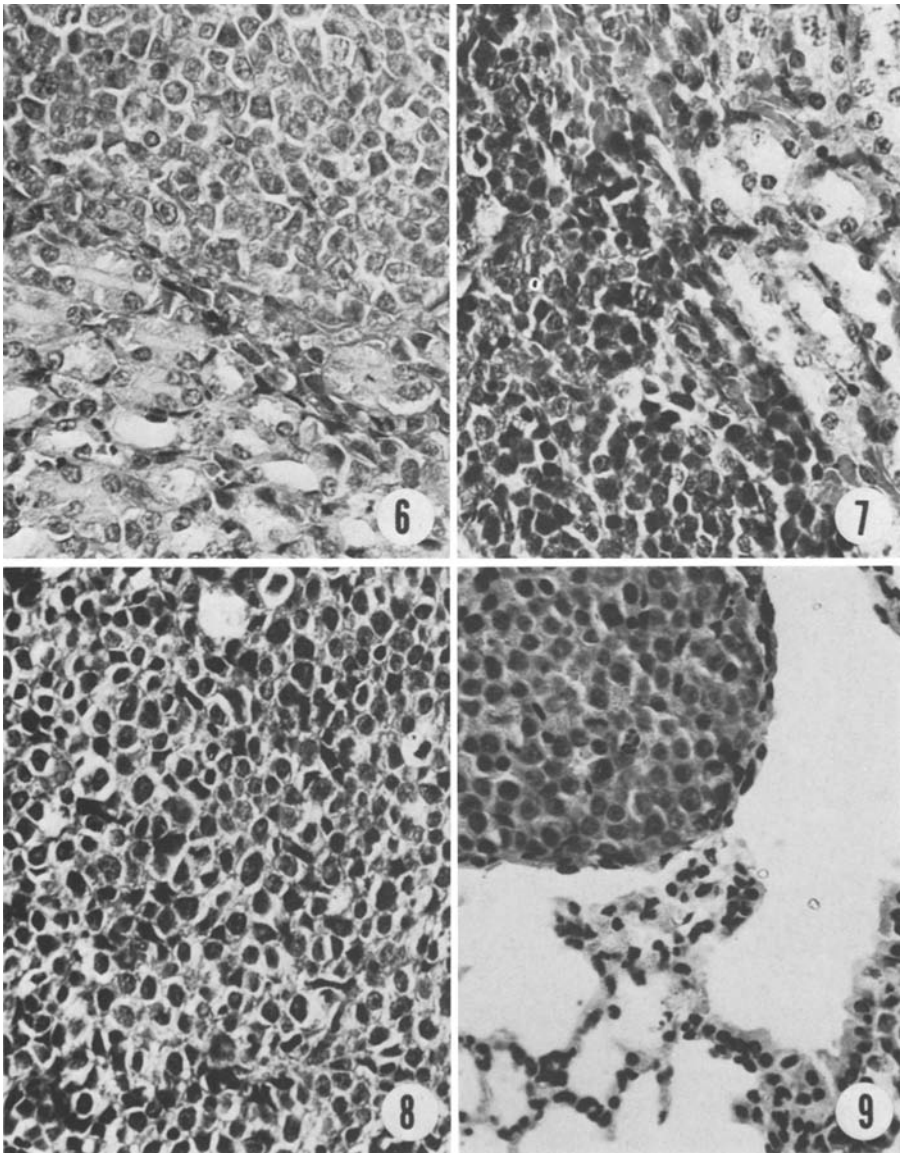

Fig. 6. Well-limited metastatic nodule of an HL-60 myelosarcoma in kidney (H & E, ×250)

Fig. 7. Renal metastases of a KG-1 myelosarcoma. The neoplastic growth shows ill-defined borders (H & E, ×250)

Fig. 8. Section of a small "dormant" HL-60 myelosarcoma. The tumor is composed of densely packed cells; necrosis is not observed (H & E, ×250)

Fig. 9. Pulmonary metastases from a large HL-60 myelosarcoma that had remained in "dormant" stage for several mouse-to-mouse passages (H & E, ×250)

Renal metastases of the HL-60, KG-1 and KG-1a myelosarcomas displayed cellular characteristics similar to the sc tumors (Figs. 6 and 7). These metastatic nodules were located in the cortico-medullar borderline of the kidney.

Dormant stage in HL-60 myelosarcomas. Occasionally, pieces of HL-60 tumors did not show significant growth and either appeared as palpable subcutaneous nodules or produced tumors a few millimeters in diameter, even as late as four weeks after transplantation. These sc nodules were retransplanted into other nude mice but continued to reproduce a similar restricted growth. Finally, after several mouse-to-mouse passages, the growth rate of the transplants increased and large HL-60 myelosarcomas were again formed. Histological studies of the "dormant" HL-60 sarcomas ruled out necrosis. Mitotic figures were scarce but the tumor cells preserved the characteristic features and the vascularization was well developed (Fig. 8). SC HL-60 myelosarcomas that recovered a fast rate of growth developed lung metastases (Fig. 9). The "dormant" growth was a random phenomenon and occurred only in some pieces of a tumor while the remaining fragments of the same HL-60 sarcoma transplanted simultaneously into other nude mice proliferated well. This arrest of tumor growth could not be associated with clinical signs of disease in the mice.

Ultrastructure and histochemistry of cultured cells. The three cell lines used in these studies contained morphologic and enzymatic markers of the myeloid series that facilitated their identification. Thus, cultured HL-60 cells had specific cytoplasmic granules of variable density (Fig. 10a). The granules, as well as the perinuclear space and the cysternae of the smooth encoplasmic reticulum (SER) of HL-60 cells, showed a positive MPO reaction (Fig. 10, inset). Each culture aliquot was composed of cells whose morphology corresponded to promyelocytes and myelocytes. More advanced forms with banded nuclei and small granulocytic-like cells were occasionally observed.

Although found in smaller numbers than in the HL-60 cells, KG-1 cells also contained cytoplasmic granules. The granules of the KG-1 cells were either homogeneously electron-dense or presented a low density and honeycomb appearance (Fig. 11). Most of them gave a positive MPO reaction (Fig. 11, inset). Multivesicular bodies (MVB), which did not react for acid phosphatase or MPO, were frequently found in KG-1 cells. The morphologic and cytochemical features of this cell line indicated that it was mainly composed of myeloblasts with some promyelocytes.

KG-1a cells, a variant of the KG-1 line, were highly undifferentiated in vitro and did not contain granules (Fig. 12). MPO reaction was not observed in the perinuclear space or SER cysternae. KG-1a cells contained numerous MVB, similar to those observed in KG-1 cells. As in the parent line, MVB of KG-1a cells were negative for MPO and acid phosphatase.

Ultrastructure and histochemistry of tumors. HL-60 and KG-1 human leukemia cell lines proliferating in mice kept the morphological and cytochemical

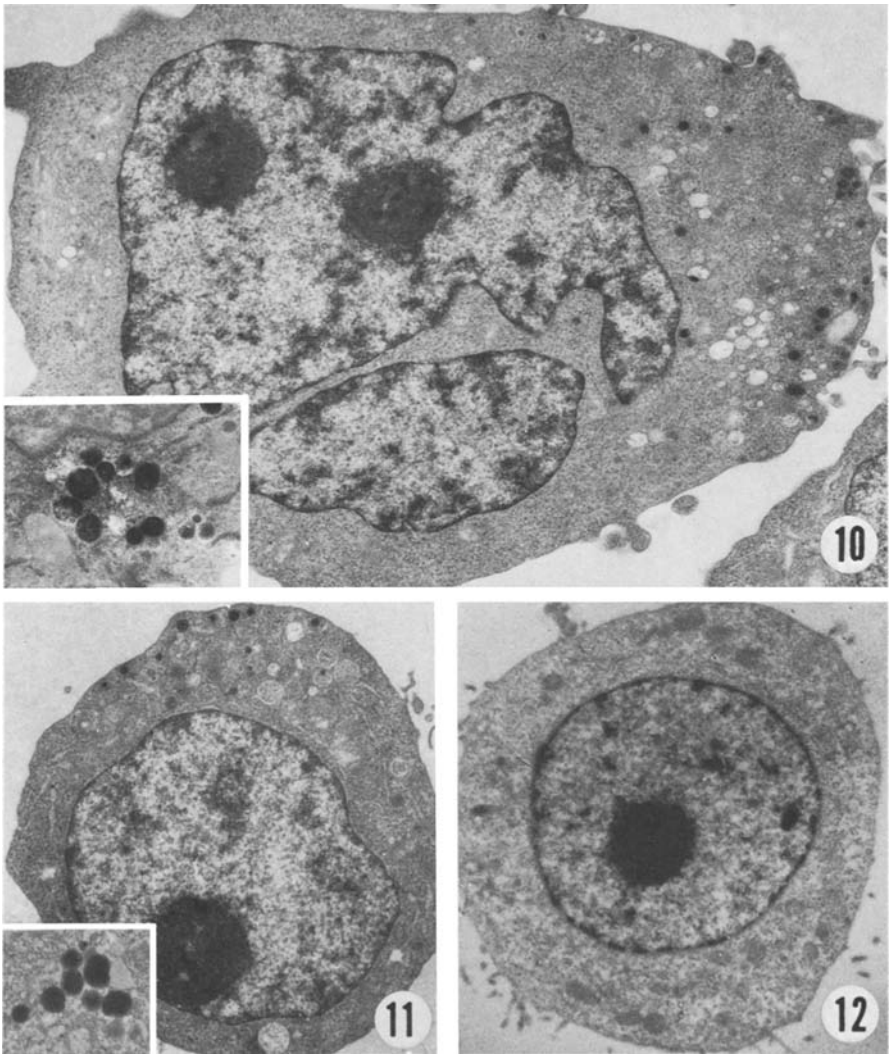

Fig. 10. HL-60 cell in vitro showing numerous cytoplasmic granules and a segmented nucleus (×8,000). *Inset*: MPO reaction is seen in granules as well as ER and perinuclear space (×15,000)

Fig. 11. Representative KG-1 cell in vitro has some cytoplasmic granules, a rounded nucleus, and marginated nucleolus (×7,000). *Inset*: Positive MPO reaction of cytoplasmic granules (×15,000)

Fig. 12. KG-1a cell in vitro is highly undifferentiated. Cytoplasmic granules are not observed (×7,000)

features attained in vitro. In contrast, the KG-1a cell line developed in vivo signs of differentiation not seen in culture.

HL-60 cells forming myelosarcomas had large round or indented nuclei containing euchromatin and one or more large nucleoli with prominent homogeneous nucleolar bodies (Fig. 13). The Golgi apparatus in many cells

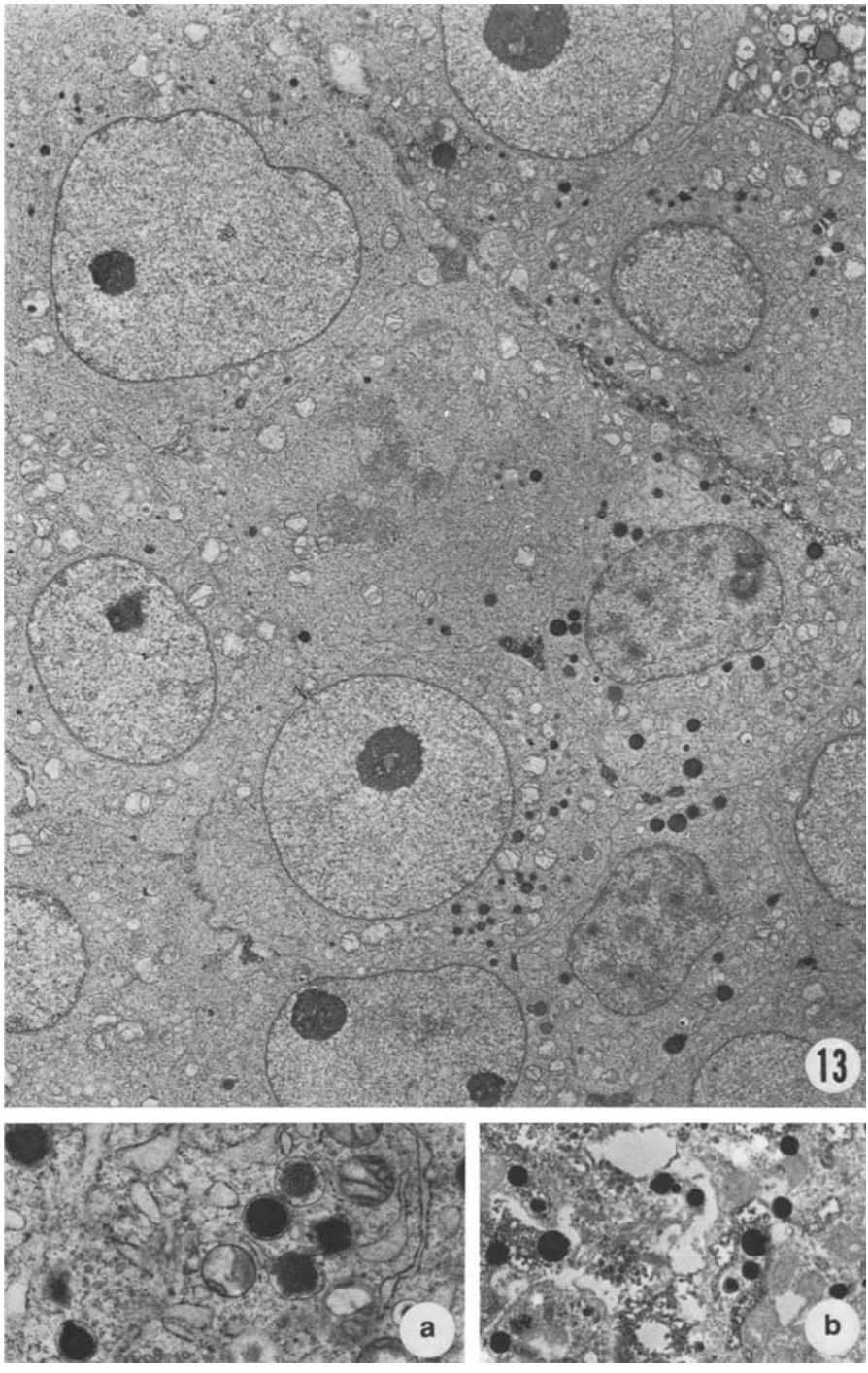

Fig. 13a, b. *Low power* micrograph of an sc HL-60 tumor (×5,000). **a** Higher magnification shows cytoplasmic granules of varying densities and dilated RER (×16,000). **b** Cytoplasmic granules were strongly reactive for MPO (×14,000)

was markedly developed and the rough endoplasmic reticulum (RER) showed signs of secretory activity not observed in HL-60 cells in vitro. Thus, RER cysternae appeared dilated and filled with either homogeneous or granulated light material (Fig. 13, inset a). SER cysternae had a vesicular appearance. Cytoplasmic specific granules were numerous. Most of them had an electron-dense central area surrounded by a clear halo and a single membrane while others had partially extracted or light cores. In some cells, large and irregular dense granules, similar to the early stages of Auer rod formation, were observed. The cytochemical reactions demonstrated that the cytoplasmic granules contained MPO in varying concentrations, as revealed by the density of the precipitates in the granular cores (Fig. 13, inset b). Positive MPO reaction in the perinuclear envelope and SER as observed in vitro was not present in the HL-60 cells forming myelosarcomas in mice.

KG-1 myelosarcoma cells also had generally round large nuclei with euchromatin (Fig. 14). The large nucleoli of these cells had a reticular structural pattern. A characteristic of the cytoplasm of KG-1 cells forming myelosarcomas was the parallel arrangement of membrane-bound narrow cysternae and fibrillar profiles with numerous attached and free ribosome-like bodies (Fig. 14, inset a). In different planes of sections, these arrangements appeared as either longitudinal or concentric arrays of cysternae. The cytoplasmic granules in KG-1 myelosarcoma cells were more homogeneous than in cultured cells. Round and elongated granules had an electron-dense matrix and a few of them had low density cores. Early stages of Auer rod formation were represented by several dense granules enclosed in single membrane-bound vacuoles (Fig. 14, inset a). KG-1 cells in vivo also contained MVB although they were less numerous than in cultured cells. The MPO reaction was strongly positive in the electron-dense granules although some granular cores did not contain the enzyme. Some light granules not easily detected by routine stains became evident with a positive MPO reaction (Fig. 14, inset b). Acid phosphatase and MPO reactions were negative in MVB.

The ultrastructural features of KG-1a myelosarcomas were similar to those of tumors formed by the parent cell line KG-1. However, the cytoplasm of KG-1a cells had a more developed RER than was found in vitro. The cysternae of RER appeared peculiarly rigid and irregularly dilated. Cross sections of the cells revealed that the cysternae were interconnected with an angular branching arrangement. The outstanding feature of KG-1a cells composing myelosarcomas was the presence of electron-dense, homogeneous cytoplasmic granules of small size that were not seen in vitro (Fig. 15). These granules gave a positive MPO reaction, thereby ascertaining the myeloid origin of the cells (Fig. 15, inset). Acid phosphatase- and MPO-negative MVB were also present in KG-1a cells.

The ultrastructural studies demonstrated the close relationships that developed between the leukemia cells forming myelosarcomas. Thus, interdigitations of cytoplasmic extensions, close parallel arrangements of cell membranes, and electron-dense intercellular areas were common features of the tumors. The leukemia cells were in close contact with the fibroblasts derived

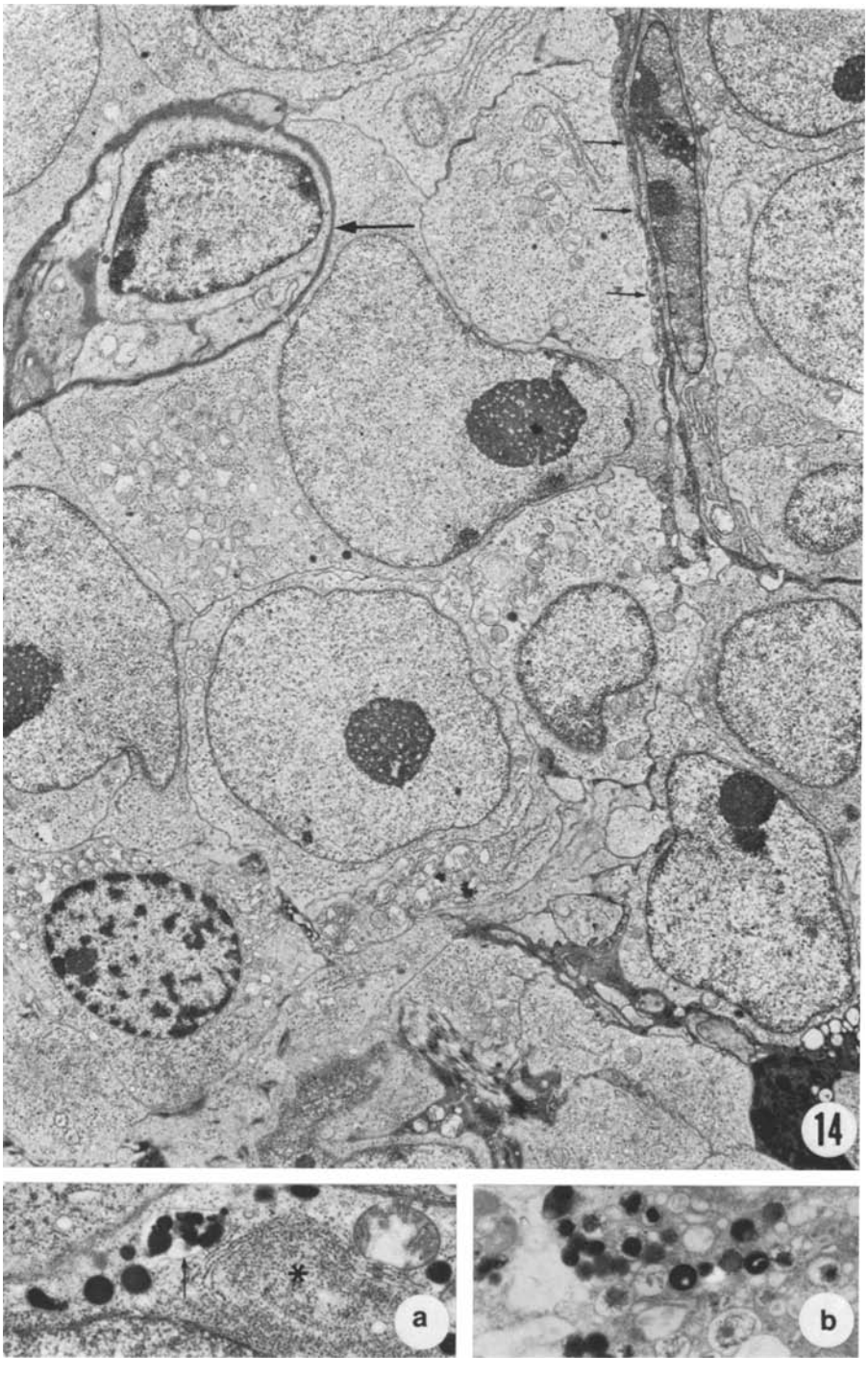

Fig. 14a, b. Low power of an sc KG-1 tumor showing a neocapillary (*large arrow*) and a mouse fibroblast (*small arrows*), which form the support network for the tumor (×7,000). **a** Individual cytoplasmic granules are seen in this micrograph of an sc KG-1 tumor cell as well as an Auer rod-like structure (*arrow*) and whorl-like ER structure (*asterisk*) (×15,000). **b** MPO reaction in cytoplasmic granules (×14,000)

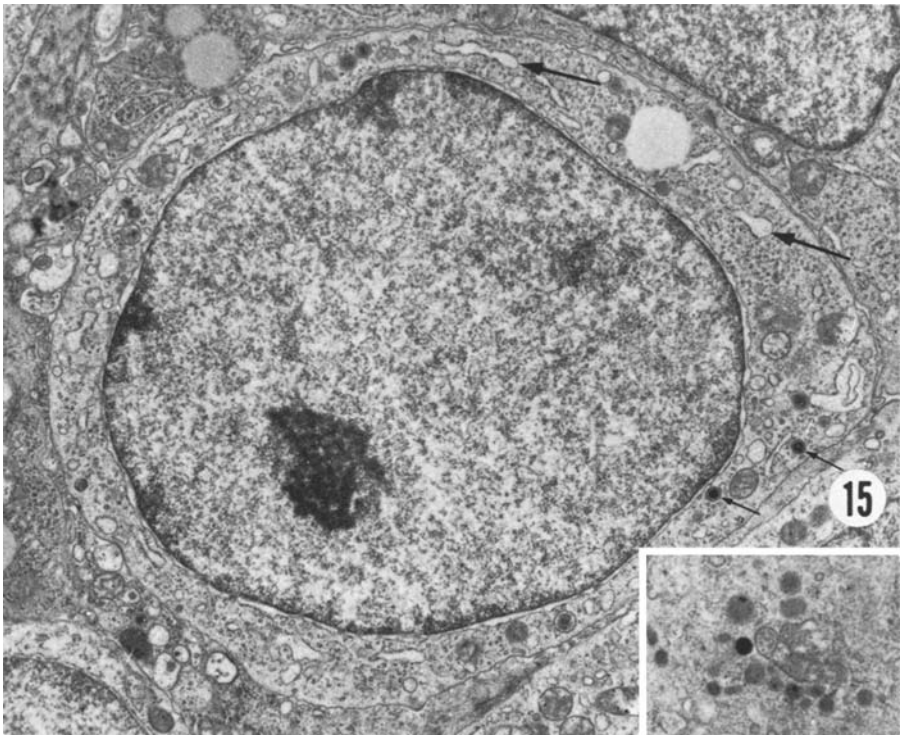

Fig. 15. Representative KG-1a cell from sc myelosarcoma shows small dense core granules (*small arrows*) as well as dilated ER (*large arrows*) (×10,000). *Inset*: Cytoplasmic granules show positive MPO reaction (×17,000)

from the host's sc tissues. The fibroblasts, fibrils and neocapillaries formed the biological framework that supported the neoplastic cells arrangement, nutrition, and evolution of the tumor (Fig. 14).

Pulmonary nodules were formed by closely-packed, intravascular and extravascular leukemia cells. As in the sc myelosarcomas, the neoplastic cells induced fibrovascular reaction from the pulmonary tissue, forming actual tumor nodules.

Chromosomal and antigenic analyses. The karyotypic examination of HL-60, KG-1 and KG-1a cells proliferating in culture and derived from sc and pulmonary tumors from the first inoculation and 3rd and 4th passages did not show differences in the chromosomal pattern. Similarly, cells from KG-1, KG-1a and HL-60 myelosarcomas in nude mice preserved the surface antigenic determinant and cross-reacted with an antibody to K-562 cells, as demonstrated by the ADCMC technique, and by positive immunoperoxidase reaction in tumor tissue sections.

Hematologic findings. Atypical cells, occasionally found in smears of the peripheral blood of the mice after sc transplantation of KG-1, KG-1a and

HL-60 cells, were an expression of the release and dissemination of neoplastic cells from the tumors developed in the sc and visceral tissues. However, the total and percent numbers of normal mouse blood cells remained unchanged. The cellular composition of bone marrow imprints from tumor-bearing mice was also normal. Histologic examinations of bone marrow, spleen, liver, and lymph nodes of nude mice with myelosarcomas confirmed that these tissues were free of neoplastic cell proliferation.

Discussion

The results reported here demonstrate that HL-60, KG-1 and KG-1a human myeloid leukemia cell lines, transplanted into immunodeficient mice, develop discrete local and metastatic tumors and do not involve the hematopoietic tissues of the host.

Sequential histopathologic examinations demonstrated that the leukemia cells induced capillaries and fibroblastic proliferation from the tissue of the host. After a characteristic lag period, the steady proliferation of cells led to the formation of myelosarcomas with a fibrovascular stroma which supported the neoplastic cell arrangement and provided the required circulatory influx.

The neoplasias composed of leukemia cells had a host's age-associated pattern of growth identical to that observed after sc transplantation of cells derived from epithelial solid tumors (Sordat 1982; Lozzio and Machado 1982). Thus, leukemia cells yielded localized tumors at the site of inoculation in *adult* mice while, in contrast, the transplantation of the cells into *newborn* nude mice developed a disseminated neoplastic growth involving lungs and kidneys.

The biological factors that determine the changing behavior of neoplastic cells, according to the age of the host at the time of transplantation, are a matter of debate. Presumably, the lack of natural killer (NK) lymphocytes during the first three weeks of life of nude mice could facilitate the dissemination of transplanted tumor cells (Herberman et al. 1975) while NK cells already active in adult mice would restrict the tumor growth to the site of inoculation. Thus, the NK cells are either cytotoxic for circulating neoplastic cells or may impair the arrest and extravasation of these cells in distant organs although incapable of inhibiting a localized tumor growth. Obviously, non-immunologic (metabolic or tissular) factors (DeWys 1972) could have an important role in determining the modality of neoplastic growth of heterologous transplanted cells in mice. Work in progress in our laboratories suggest that the content of acid mucopolysaccharides of sc tissues in 45 h and 2-week-old mice is higher than in 4–8-week old nude mice. Although we lack sufficient data to establish a correlation between this observation and the pattern of neoplastic growth, it is possible that the different structural and chemical conditions of the sc tissues of the *newborn* mice, among other factors, may facilitate intravasation.

It could be argued that, due to their small size, the production of disseminated neoplastic growth in *newborn* nude mice may be the result of an

accidental intravascular inoculation of the cells during the sc injection. However, it has been demonstrated that nude mice do not support the proliferation of iv-injected heterologous tumor cells (Fidler et al. 1976). In previous experiments, we found that the human myeloid cell line K-562, which consistently yields tumors after sc transplantation, does not proliferate following iv-inoculation into nude mice (Machado et al. 1982). Besides, disseminated tumor growth occurs several weeks after sc transplantation of the cells following a random time pattern in different mice. Thus, it appears that the myeloid neoplastic cells enter the circulation of the mouse from the sc site of inoculation at varying intervals; how this process is regulated by the individual host remains to be determined. It is possible, however, that the heterologous cells, during their stay in the sc tissues of the mice, undergo changes that permit them to circulate, extravasate, and develop metastases in distant organs.

The random occurrence of a transient "dormant stage" in HL-60 myelosarcomas suggests that these quiescent neoplasms preserve the basic cellular features. This finding is important in regard to the apparent low tumorigenic ability of HL-60 cells. As demonstrated, mouse-to-mouse passages of quiescent transplants can lead to a recovery of the "normal" pattern of growth of HL-60 sarcomas. Thus, it is not justified to qualify the "dormant" sc HL-60 tumors as an expression of the lack of tumorigenic properties in this cell line. This finding again stresses the need for adequate histopathological examinations and not relying strictly on just a gross evaluation of results in studies on the transplantation of solid tumors or suspended neoplastic cells.

As shown in these experiments, the HL-60 and KG-1 myelosarcomas initially developed in *newborn* mice were maintained as established tumors by serial passages through *adult* mice. In contrast, KG-1a myelosarcomas, although formed by highly undifferentiated cells, could only be successfully transplanted into *newborn* hosts. It would appear that these latter cells may be extremely sensitive to immunologic and non-immunologic factors present in *adult* nude mice. On the contrary, the absence of those hypothetical factors in *newborn* hosts should enhance the proliferation of KG-1a cells. It must be noted that KG-1a cells are an undifferentiated variant subpopulation of the KG-1 line with a peculiar in vitro behavior in that they do not respond to treatment with retinoids which, in cultures of the parent cell line, induce a macrophage-like differentiation (Lehrer et al. 1973).

From a general standpoint, the changing behavior of KG-1a cells in nude mice of different ages is one more example of the interaction between a particular type of neoplastic cell and the host's environment which influences the characteristics of tumor growth.

The three cell lines tested consistently attained varying degrees of differentiation along the myeloid series in vitro. These cellular features were maintained during proliferation in the mice except for the KG-1a cells that developed cytoplasmic MPO-positive granules not seen in cultured cells. The presence of readily identifiable markers in the neoplastic cells makes these models particularly useful for studying the influence of a biological environ-

ment on cell differentiation and neoplastic growth. Thus, these systems can be ideally suited for defining the in vivo influence and therapeutic potentials of compounds that stimulate cell differentiation in vitro or for the screening of other anti-neoplastic agents. A similar model, the K-562 myelosarcomas in nude mice developed in our laboratories, has proven to be extremely useful in investigations of the effectiveness of specific (antibody) and non-specific (chemical) compounds on neoplastic growth (Lozzio and Machado 1982; Machado et al. 1983).

Finally, it must be emphasized that the results of this and previous studies demonstrate beyond doubt that the characteristic tumorigenicity of human atypical cells in nude mice does not depend on the epithelial or mesenchymal origin but, rather is associated with the "malignant" qualities of the transplanted cells. Thus, human leukemia cells that do not involve the hematopoietic tissues of the host are as suitable as cells from solid tumors for experimental studies on local and metastatic neoplastic growth.

References

Barka T, Anderson PJ (1962) Histochemical methods for acid phosphatase using hexazonium pararosanilin as coupler. J Histochem Cytochem 10:741–753

Bessis M (1973) Living Blood Cells and Their Ultrastructure. New York, Springer-Verlag

Cawley JC, Hayhoe FGJ (1973) Ultrastructure of Haemic Cells. A Cytological Atlas of Normal and Leukaemic Blood and Bone Marrow. Philadelphia, WB Saunders, Co, Ltd, p 55

Collins SJ, Gallo RC, Gallagher RE (1977) Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. Nature 270:347–349

Fidler IJ, Caines S, Dolan Z (1976) Survival of hematogenously disseminated allogeneic tumor cells in athymic nude mice. Transplantation 22:208–212

Fukuda M, Koeffler HP, Minowada J (1981) Membrane differentiation in human myeloid cells: Expression of unique profiles of cell surface glycoproteins in myeloid leukemic cell lines blocked at different stages of differentiation and maturation. Proc Natl Acad Sci USA 78:6299–6303

Gallagher R, Collins S, Trujillo J, McCredie K, Ahearn M, Tsai S, Megzgar R, Aulakh G, Ting R, Ruscetti F, Gallo R (1979) Characterization of the continuous, differentiating myeloid cell line (HL-60) from a patient with acute promyelocytic leukemia. Blood 54:713–733

Huberman E, Callaham MF (1979) Induction of terminal differentiation in human promyelocytic leukemia cells by tumor-promoting agents. Proc Natl Acad Sci USA 76:1293–1297

Koeffler HP, Golde DW (1978) Acute myelogenous leukemia: A human cell line responsive to colony-stimulating activity. Science 200:1153–1154

Koeffler HP, Billing R, Lusis AJ, Sparkes R, Golde DW (1980) An undifferentiated variant derived from the human acute myelogenous leukemia cell line (KG-1). Blood 56:265–273

Latif ZA, Lozzio BB, Wust CJ, Krauss S, Aggio MC, Lozzio CB (1980) Evaluation of drug-antibody conjugates in the treatment of human myelosarcomas transplanted in nude mice. Cancer 45:1326–1333

Lehrer RI, Cohen LE, Koeffler HP (1983) Specific binding of [^{3}H]phorbol dibutyrate to phorbol diester-responsive and -resistant clones of a human myeloid leukemia (KG-1) line 1. Cancer Res 43:3563–3566

Lozzio BB, Lozzio CB, Machado E (1977) Human myelogenous (Ph^1+) leukemia cell line: Transplantation into athymic mice. J Natl Cancer Inst 56:627–630

Lozzio BB, Machado EA, Lair SV, Lozzio CB (1977) Suppression of human myelosarcoma growth in athymic mice by a primate antiserum. Cancer Treat Rep 61:1679–1684

Lozzio BB, Machado EA (1982) Transplantation and dissemination of hematopoietic malignancies in the nude and lasat mice. In: Fogh H, Giovanella B (eds) The nude mouse in experimental and clinical research, vol II. Academic Press, New York, pp 521–567

Lozzio BB, Machado EA, Mitchell J, Lozzio CB, Wust CJ, Golde DW (1983) Proliferation of human malignant hematopoietic cells in immunodeficient mice. Suppression by antibody to pluripotent K-562 leukemia cells involves direct cytolysis and effector cells. Blood 61:1045–1053

Lozzio CB, Lozzio BB (1975) Human chronic myelogenous leukemia cell line with positive Philadelphia chromosome. Blood 45:321–334

Machado EA, Lozzio BB, Lair SV (1976) Characterization of hereditarily athymic-asplenic mice. In: Battisto JR, Streilein JW (eds) Immuno-Aspects of the Spleen. Amsterdam, Elsevier/North-Holland Biomedical Press, pp 215–226

Machado EA, Lozzio BB, Lozzio CB, Lair SV, Aggio MC (1977) Development of myelosarcomas from human myelogenous leukemia cells transplanted in athymic mice. Cancer Res 37:3995–4002

Machado EA, Lozzio BB, Lozzio CB, Lair SV, Maxwell PA (1982a) Study of metastases of human malignant cells in nude mice. In: Reed ND (ed) Proceedings of the Third International Workshop on Nude mice. New York, Fischer-Verlag, pp 391–402

Machado EA, Gerard DA, Mitchell JR, Lozzio BB, Lozzio CB (1982b) Arrest and extravasation of neoplastic cells. An electron microscopy study of serial sections at sequential stages. Virchows Arch [Pathol Anat] 396:73–89

Machado EA, Lozzio BB, Lozzio CB, Gerard DA, Mitchell JR, Wust CJ, Bamberger EG (1984) Antibody therapy of metastatic proliferation of human neoplastic cells heterotransplanted into immunodeficient mice. In: Torisu M (ed) Proceedings of the International Symposium on Basic Mechanisms and Clinical Treatments of Tumor Metastasis, Fukuoka, Japan, December, 1982. New York, Academic Press (in press)

Metcalf D (1982) Sources and biology of regulatory factors active on mouse myeloid leukemic cells. J Cell Physiol Suppl 1:175–183

Novikoff AB, Goldfischer S (1968) Visualization of microbodies for light and electron microscopy (abstract). J Histochem Cytochem 16:507

Olsson I, Olofsson T (1981) Induction of differentiation in a human promyelocytic leukemia cell line (HL-60). Exp Cell Res 131:225–230

Rovera G, Olashaw N, Meo P (1980) Terminal differentiation in human promyelocytic leukaemic cells in the absence of DNA synthesis. Nature 284:69–70

Sumner AT, Evans HJ, Buckland RA (1971) New technique for distinguishing between human chromosomes. Nature [New Biol] 232:31–32

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