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Volume 4

Metastatic Tumor Growth

Edited by
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Metastatic Tumor Growth

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Foreword

It is not the primary tumor but the metastatic spread that determines the fatal outcome of cancer in more than 90%, as every physician knows. Looking over the current scientific publications in cancer research we shall find that the majority is devoted to problems of carcinogenesis, including aspects of molecular biology and immunology. Problems of tumor growth, of interaction between tumor cells and neighboring normal cells and basement membranes, and of primary and secondary growth in general are studied by only a few groups. Clinicians interpreting the development of metastases as a bad omen, will often revert to a kind of therapeutic nihilism. On the other hand some authors have recently tried to put the blame for metastatic spread on certain diagnostic procedures such as palpation, surgical and punch biopsy, offering even mathematical proof for their ideas. If anything, these studies may help us to remember that our factual knowledge about metastatic tumor spread is still very limited.

To meet the challenge we attempted to present the current status of cancer research on such topics as the origin of metastasis, the relationship between cell spreading and invasion, the various host defense mechanisms, and also in the vast field of organ specific problems in metastatic tumor growth.

This volume will deal with morphological and biochemical interactions between tumor cell surface, microenvironment, and adjacent structures. Recent studies have emphasized the coexistence of several different cell populations within a malignant neoplasm and their relevance for the understanding of metastasation as such. Immunospecific host-defense mechanisms can be distinguished from nonspecific reactions, their role in metastatic tumor spread being investigated by morphological and biochemical studies.

Clinically, hemostasis might be an important factor in metastatic growth. Another point is made in biostatistical research about the distribution of metastases over the whole body trying to determine the causative factors of these patterns. New radiodiagnostic methods have improved the basis for therapeutic measures in radiology, surgery and possibly immunology.

Far from covering the entire spectrum of problems connected with metastatic tumor growth we just wanted to collect some of the safely established facts and promising developments in order to clear the path towards prevention and therapy.

E. Grundmann

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Interaction of Locomotive and Lytic Activities of Tumor Cells in Invasion[1])

PETER STRÄULI and GISELA HAEMMERLI

Local spread of cancer is primarily accomplished by invasive growth. In contrast to the expansive growth of benign tumors, invasive growth is characterized by an irregular distribution of cell multiplication. We can assume that the sites at which cell proliferation takes place are conjointly determined by topical kinetic conditions of the tumor and by local physicochemical and metabolic properties of the host.

Cell multiplication as basic mechanism of invasion can be supplemented by other activities of cancer cells, mainly locomotion and lytic action. It is reasonable to assume, but difficult to prove, that these two cellular activities are functionally interconnected. Some evidence in favor of such an interaction is presented in the following report.

Locomotion is displayed by single cancer cells as they occur in leukemias or when they are, under certain conditions, detached from the penetration front of solid tumors. Locomotive cells possess the machinery that generates the driving force for locomotion and translates this force, by combining it with an adequate pattern of adhesions and dehesions, into propulsion (WOHLFAHRTH-BOTTERMANN, 1980). The crucial question in the present context is whether the pressure of propulsion alone, its momentum, allows the passage of moving cells across tissue constituents, particularly the extracellular matrix, or whether lytic effects exerted on host structures are necessary.

As concerns lytic effects of cancer cells, we suggest to distinguish between dissociative and destructive effects. Dissociative action results in the loosening of tissue organization, but not in tissue destruction. At present, this distinction is exclusively based on morphologic evidence. On the other hand, the agents responsible for both effects may well be the same. All we know is that the most likely candidates, lysosomal hydrolases, are synthesized by many invading tumors. But the significance of these enzymes at the level of the single cell and its microenvironment is open to debate (STRÄULI et al., 1980).

In the following some observations of our group in Zürich will be presented. They relate to the transplantable undifferentiated leukemia L 5222 of the BD IX rat. The cells of this leukemia are highly locomotive (HAEMMERLI et al., 1976). During locomotion, the cells have a characteristic polarized shape *(Figs. 1 a and b)* which became evident during microcinematography and was substantiated by concurrent scanning electron microscopy (FELIX et al., 1978).

If these leukemia cells are confronted with spread normal cells, e.g. fibroblasts, or sheets of mesonephros cells, they squeeze under the spread cells where they continue to locomote in the familiar shape (HAEMMERLI et al., 1977). Proof for the position of the leukemia cells between the spread cells and their substrate is provided by transmission electron micrographs *(Figs. 2 a–c)*.

Reflection microscopy applied to the fibroblast-leukemia cell model reveals patterns of substrate adhesions of the involved cells. Fibroblasts show a complicated mixture of dark streaks and medium-grey and light areas. The dark streaks, termed focal contacts (IZZARD

[1]) These studies were supported by an institutional grant from the Zürich Cancer League.

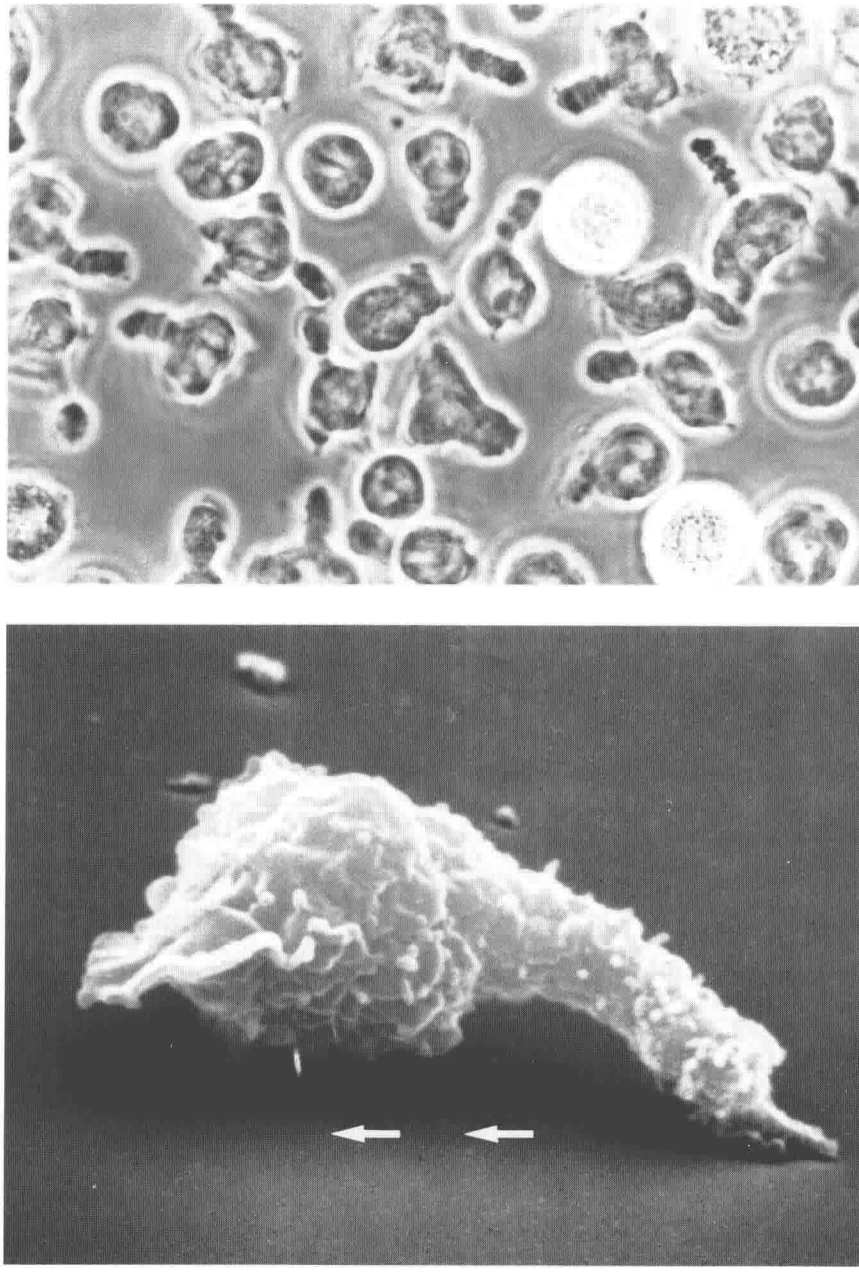


Fig. 1: Cells of the transplantable leukemia L 5222 of the BD IX rat

a) Leukemia cells in culture chamber, most of them in the polarized configuration displayed during locomotion. Phase contrast (from film sequence) × 800

b) Scanning electron micrograph of single leukemia cell fixed during locomotion. Direction of migration indicated by arrows. Note veil-like extension at front end. × 8.500

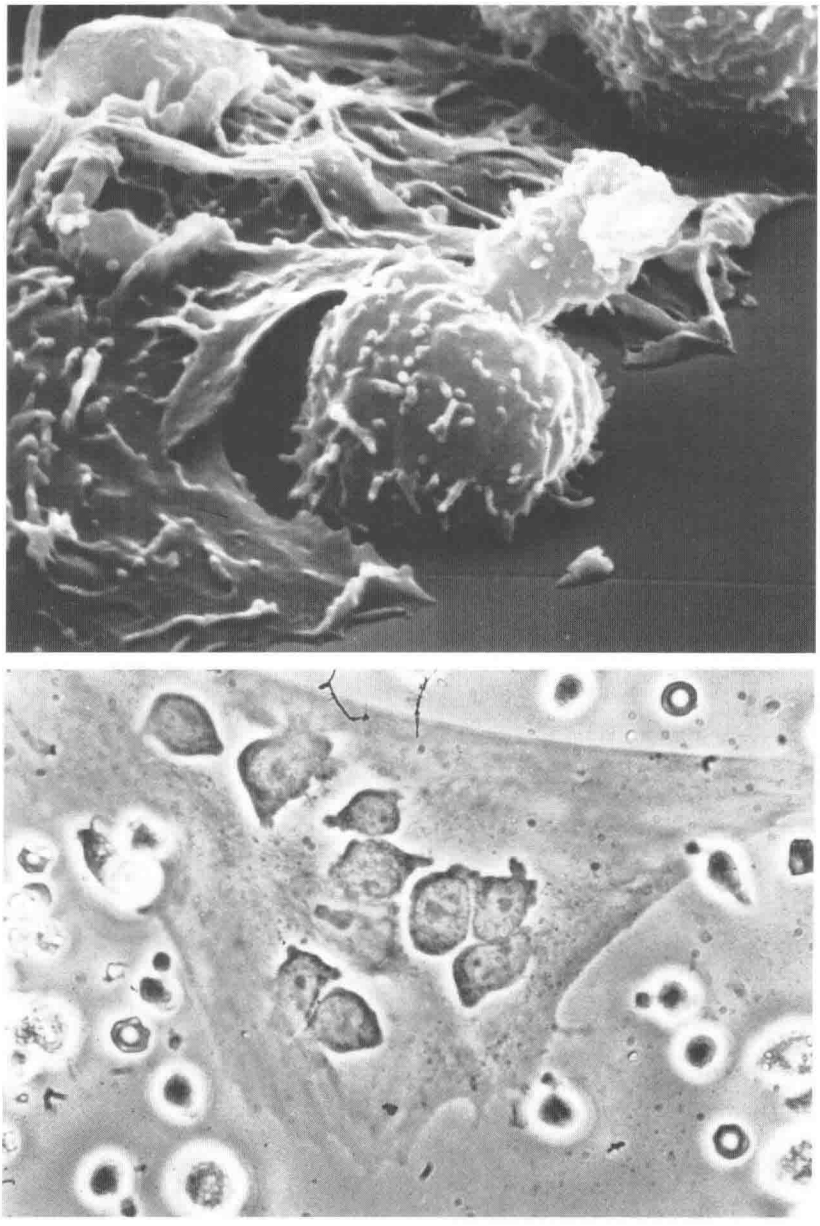


Fig. 2: Confrontation of L 5222 leukemia cells and spread normal cells (chick embryo mesonephros cells) in vitro

a) Scanning electron micrograph of leukemia cell facing lifted part of spread normal cell. Configuration and posture of leukemia cell indicate that it was fixed while attempting to squeeze under normal cell. × 7.700

b) Leukemia cells locomoting under spread normal cells. Other leukemia cells, not engaged in this type of activity, are out of focus. Phase contrast (from film sequence). × 600

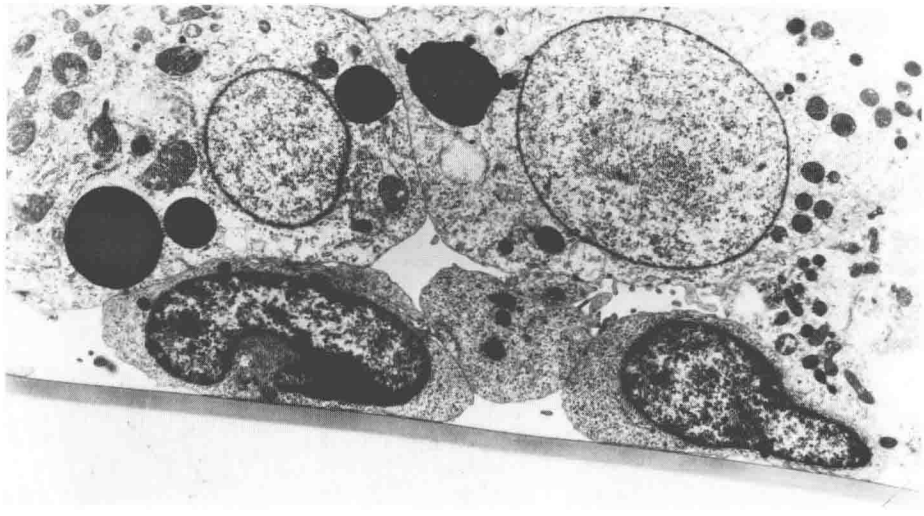


Fig. 2: c) Transmission electron micrograph of leukemia cells localized between normal cells and substrate. × 5.400

and LOCHNER, 1976), are thought to be the sites of strongest attachment, while the light areas represent zones without contact. Leukemia cells have no focal contacts, but are completely outlined in a shade of grey suggestive of an intermediate contact *(Figs. 3 a and b)*. Reflection microscopy also shows that the leukemia cells are surrounded by a zone from which all attachment sites of fibroblasts have disappeared. This indicates that in these areas the fibroblasts are completely lifted off the substrate. As revealed by microcinematography, the clear spaces accompany the locomotive tracks of the leukemia cells (HAEMMERLI and PLOEM, 1979).

At the moment, our interpretation is that fibroblast adhesions obstructing the advance of migrating leukemia cells can yield to the momentum of locomoting cells. But lytic factors cannot be excluded.

Progressing from in vitro- to in vivo-conditions, we have studied the invasion of rat leukemia cells into the mesentery after intraperitoneal implantation of the L 5222 leukemia. The first morphologically recognizable event is contraction and rounding-up of the mesothelial cells. Whether this requires a direct contact between leukemia and host cells, or whether it can also be produced by intraperitoneal injection of a cell-free leukemia filtrate is under investigation. At any rate, the contraction of mesothelial cells could be due to lytic effects exerted on intercellular contacts. This dissociative process exposes areas of the submesothelial basal lamina. It is to the denuded zones that the leukemia cells preferentially adhere. Some of them display the polarized shape and the posture indicative of locomotion *(Figs. 4 a and b)*. As a next step, destructive lytic effects are recognizable, resulting in the disappearance of the basal lamina and the formation of holes partly filled with leukemia cells *(Figs. 4 c and d)*.

The presence of leukemia cells within the mesentery, indicating a further step of invasion, is visualized by semithin sections *(Fig. 5 a)*. Within the mesentery, the leukemia cells continue their locomotive activity, as was shown by microcinematography *(Fig. 5 b)* (HAEMMERLI and STRÄULI, 1978). L 5222 leukemia cells locomote between the main architectural elements of the mesentery, collagen bundles and elastic fibers. This requires continous adaptation of the cell configuration to the texture of the momentary environment. Although the interfibrillar

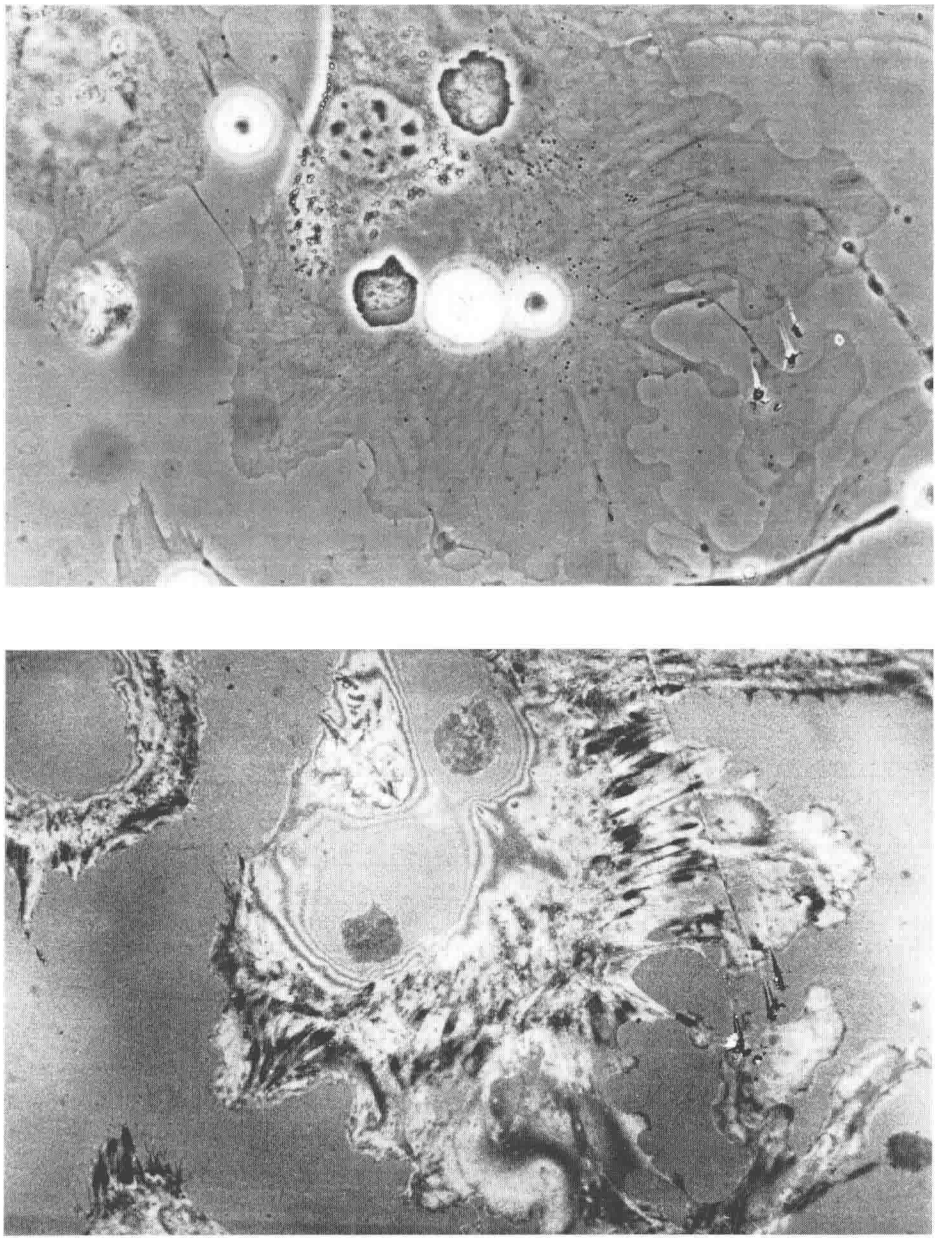


Fig. 3: L 5222 leukemia cells locomoting under a spread normal cell: comparison of phase contrast and reflection contrast

a) Phase contrast. × 600

b) The same situation in reflection contrast. Note the dark streaks representing focal contacts of the normal cell. The two leukemia cells are surrounded by a zone from which all substrate contacts of the normal cell have disappeared. × 600

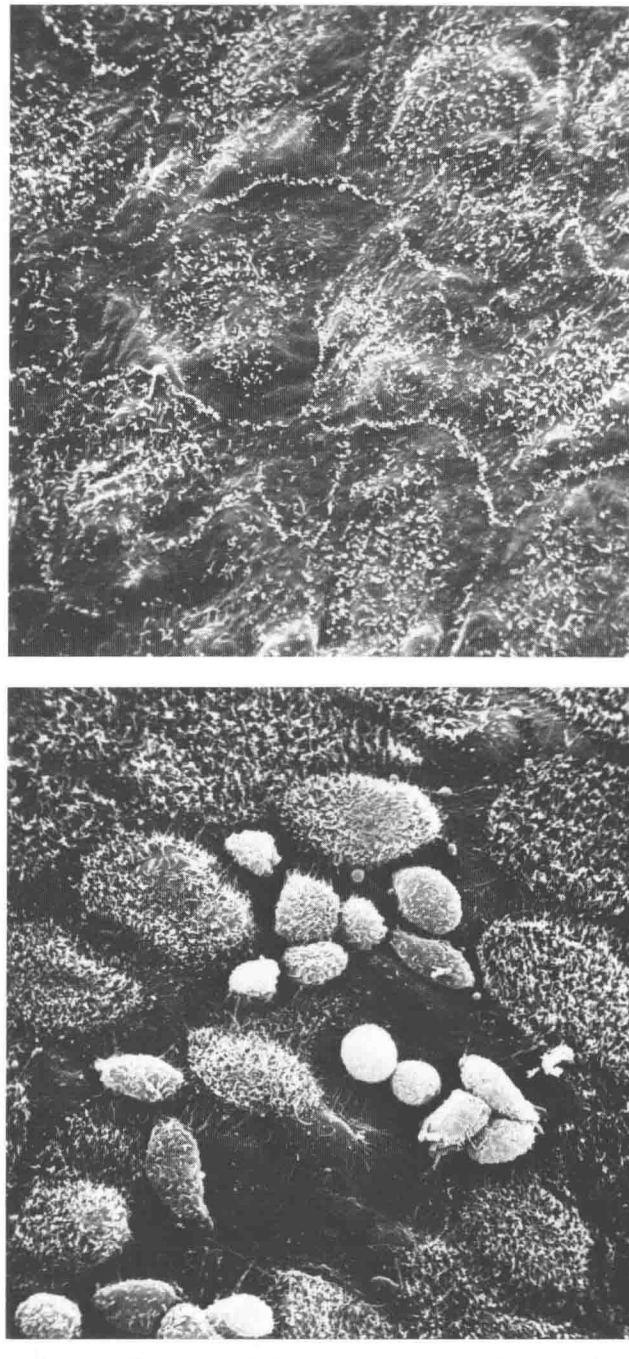
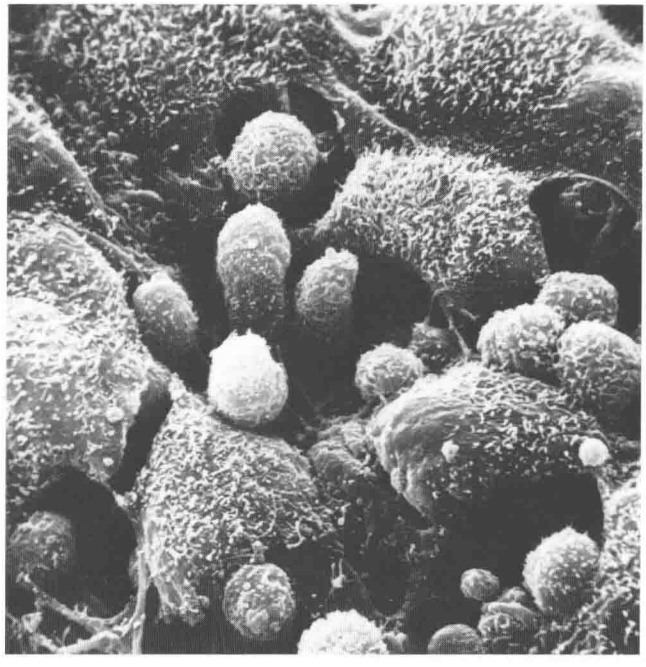
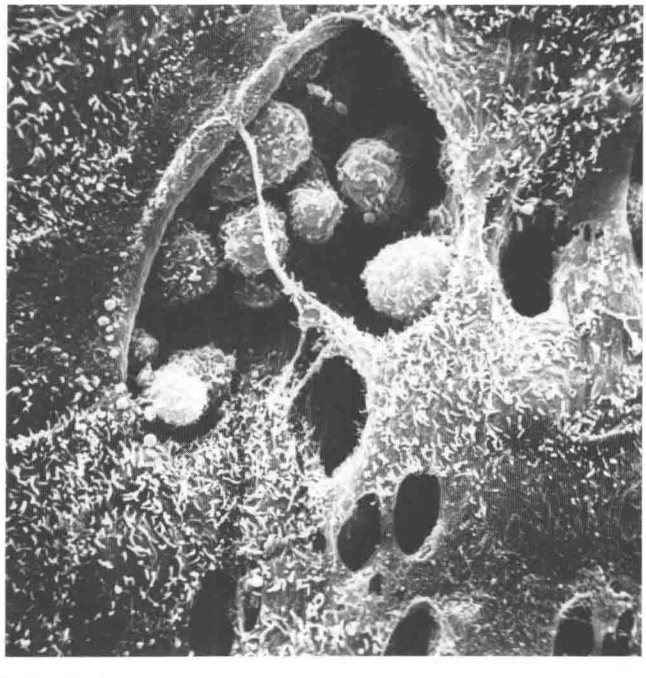


Fig. 4: Infiltration of L 5222 leukemia cells into the mesentery after i.p. transplantation, visualized by scanning electron microscopy

a) Normal mesothelium of mesentery. × 900

b) Contracted mesothelial cells and exposed areas of submesothelial layer. Leukemia cells in spherical and polarized shape are attached to mesothelium – free zone. × 900





c) Leukemia cells fixed while penetrating into the mesentery between contracted mesothelial cells. × 1800

d) Leukemia cells within holes of mesentery. The mesothelium is partly perforated, partly undermined. × 1800

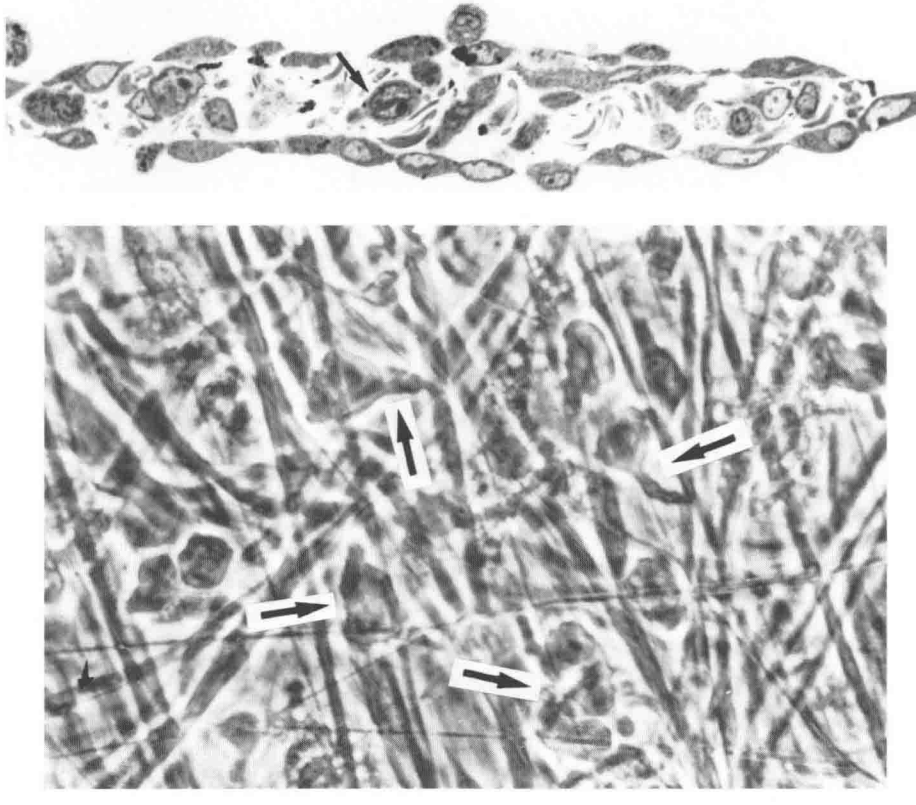


Fig. 5: L 5222 leukemia cells within the mesentery

a) Semi-thin section. Loosely distributed leukemia cells, one in polarized configuration (arrow). Composite photograph. × 700

b) Leukemia cells (arrows) locomoting between collagen bundles and elastic fibers of mesentery. Phase contrast (from film sequence). × 500

space appears blank in phase contrast and transmission electron microscopy, we cannot assume it to be empty or merely filled with fluid. It must be expected to contain a highly organized molecular network of proteoglycans. It is within this network that the leukemia cells migrate. Do they push it aside, do they lyse it, or do they combine pressure and lysis? This question cannot be answered at present.

So we can say that in the L 5222 leukemia-mesentery model, infiltration seems to operate by a combination of dissociative and destructive lytic effects and of locomotion, both superimposed upon proliferation.

However, other combinations are possible. For instance, another transplantable rat leukemia, the BNML, is composed of non-locomotive cells. After intraperitoneal implantation, the cells of this leukemia adhere to intact mesothelial cells, begin to multiply and to form aggregates. At a later date, such foci are also found within the mesentery, but no emigration of leukemia cells from these aggregates could be recorded by microcinematography (HAEM-

MERLI and FELIX, 1977). It thus appears that in this leukemia invasion is accomplished by proliferation possibly supported by lytic action.

Conclusion

We conclude that locomotion and lytic action are accessory factors of the main mechanism of local spread of cancer, uncontrolled growth. It appears that the two activities can operate either singly or in combination. In those instances in which they proceed conjointly, it is likely that their interaction is effective at the macromolecular rather than at the cellular level. The central problem, so far largely unexplored, is the penetrability of the extracellular matrix under normal conditions and in the vicinity of invading cancer.

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