

# Lymphocyte interactions with the extracellular matrix of malignant cells *in vitro*: A morphological and immunocytochemical study

H. Logothetou-Rella

Department of Experimental Physiology, Medical School, University of Athens, Athens, Greece

**Summary.** The interactions of lymphocytes with the glycosaminoglycans-protease-membrane extracellular matrix, produced by mixed cell cultures of normal with malignant cell clones, were examined. Pre-activated and activated heterologous peripheral lymphocytes were used. Co-cultures of activated lymphocytes with all cell types used, formed identical cell nodules. Histology of cell nodules showed that activated lymphocytes were cytolytic to pure normal or malignant cell clones. On the contrary, lymphocytes in nodules with mixed cell clones (normal with malignant cell clones) or embryonic cells, underwent degeneration changing the fusiform type tumor nodule into the adenoid type. The adenoid type cell nodule consisted of cells with high nuclear to cytoplasm ratio and mitotic activity. In addition, leukocyte common antigen was deposited in the extracellular matrix and on the cell membrane of target tumor cells. Pre-activated lymphocytes, in mixed cell cultures, failed to lyse the target tumor cells and underwent abnormal cell divisions, producing subsets similar to nuclear vlimata, which remained attached to the extracellular matrix. The morphological and immunocytochemical observations of lymphocytes were discussed and attributed to the presence of the specific extracellular matrix of glycosaminoglycans-protease-membranes.

**Key words:** Lymphocytes, Extracellular matrix, Malignant cells, Normal cells, Embryonic cells, Nuclear vlimata, Glycosaminoglycans-protease-membranes

## Introduction

The use of human primary malignant cell cultures has revealed that glycosaminoglycans (GSG) are produced

by malignant cells, stored intracellularly in membranous sacs and when exocytosed, participate in an enhanced tumor nodule formation (Logothetou-Rella et al., 1988a,b). Furthermore, GSG were associated with protease which cross-reacted with the antiserum against  $\alpha_1$ -chymotrypsin (Logothetou-Rella et al., 1992a,b). In addition, GSG-protease-membranes were the binding extracellular matrix (ECM) of tumor nodules and were correlated with production of nuclear vlimata (Logothetou-Rella, 1993). This GSG-protease membrane ECM has been found abundant in mixed cell cultures of normal and malignant cell clones (Logothetou-Rella et al., 1992b), embryonic cells (Logothetou-Rella et al., 1989) and absent in normal cell cultures (Logothetou-Rella et al., 1988c).

It is well known that the ECM not only serves a mechanical role in supporting and maintaining tissue structure, but also modulates a multitude of cell functions such as development, migration and proliferation. Also, lymphocytes have been reported to interact with a variety of components found in the ECM (Shimizu and Shaw, 1991). Hence, ECM may play a role in the ability of tumor target cells to adopt a multitude of ways to escape from immune attack.

This study examines the interaction of heterologous lymphocytes with the ECM of GSG-protease-membranes, focusing mainly on the morphology and immunocytochemistry of lymphocytes and tumor target cells.

## Materials and methods

### *Cell culture establishment*

Stationary normal and malignant cell cultures were established from human tissue specimens by enzymatic digestion (Logothetou-Rella et al., 1988b) and were used as target cells.

Two normal cell strains were used: normal fallopian tube F-cells, isolated from tissue specimen from a

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*Offprint requests to:* Dr. Helen Logothetou-Rella, Department of Experimental Physiology, Medical School, University of Athens, P.O. Box 60114, GR-153 10 Agia Paraskevi-Athens, Greece

patient who underwent total hysterectomy; and the bladder N-cells, previously characterized (Logothetou-Rella et al., 1988c).

Three malignant cell strains were used: malignant urothelial Pa-cells, established from tumor tissue specimen from a patient with invasive bladder carcinoma (Logothetou-Rella, 1993); and the lung cell lines, M and P-cells, consisting of mixed normal and malignant cells, recently characterized (Logothetou-Rella et al., 1992b).

Normal amniotic embryonic cells from two pregnant women cultivated for prenatal diagnosis were also used as target cells.

Heterologous lymphocytes were isolated from human blood of healthy donors, by density gradient centrifugation (Boyum, 1968).

All cell cultures were grown in complete medium RPMI-1640 (Gibco) supplemented with 10% foetal bovine serum (Gibco), penicillin (100 U/ml, Gibco) and streptomycin (100 µg/ml, Gibco) and incubated at 37 °C in a CO<sub>2</sub>-humidified incubator. Stock cells are stored frozen in liquid nitrogen.

#### *Interaction of lymphocytes with normal and malignant cells*

Normal, malignant and embryonic cells were grown in glass petri-dishes (10 cm in diameter) to post-confluency. Isolated peripheral lymphocytes were then added to post-confluent cell cultures, at  $7 \times 10^6$  lymphocytes per petri-dish. To the co-culture four drops of Phytohaemagglutinin (PHA) were added every three days (activated lymphocytes). Co-cultures were allowed to interact while incubating for ten days.

In another set up of experiments lymphocytes were first activated with PHA for 24 hours (pre-activated), washed in complete medium by centrifugation at 100 g for 10 min and then added to post-confluent target cell cultures and allowed to co-cultivate in incubation for 15 days, in the absence of PHA.

Normal or malignant cell cultures in the continuous presence of PHA and in the absence of lymphocytes were used as control cultures.

#### *Cytology, histology and immunohistochemistry*

Cell nodules formed by the co-culture of lymphocytes with target cells, in the continuous presence of PHA,

were centrifuged at 400 g for 5 min. The cell nodule pellets were fixed in formalin, embedded in paraffin and tissue sections were stained with haematoxylin-eosin (HE), PAS and PAS-diacetate.

Paraffin nodule sections were also used for immunohistochemistry (Hsu et al., 1981) using antiserum against lymphocyte common antigen (1:150, LCA, DAKO, M 701), B-cell antigen (1:500, L26, Dako, M 755) and T-cell antigen (1:400, UCALT, Dako, M 742).

Co-cultures containing pre-activated lymphocytes were fixed in cold acetone (-20 °C) for 10 min and were immunocytologically examined, using antiserum against LCA (1:10), B-cell antigen (1:50, L26) and T-cell antigen (1:40, UCALT).

All cell cultures as well as mechanically dispersed nodule cells were fixed in 50% ethanol and stained by the Papanicolaou method.

Cell nodules and nodule dispersed cells were stained with 4% trypan blue for viability assessment.

## Results

### *Interaction of pre-activated lymphocytes with lung cells (malignant + normal cell clones)*

Pre-activated or non-activated lymphocytes stayed attached on top of the GSG ECM which covered the target M and P stationary cell cultures (Figs. 1, 2). Non-activated lymphocytes retained the round form while pre-activated ones attained the nuclear vlima morphology. No cytolytic effect was detected and the target cells kept growing intact.

After 15 days of co-culture, most of the lymphocytes took the morphology of a spermatozoon, consisting of a round or elongated nuclear head and tail. The cytoplasm covered the front part of the nucleus and resembled the acrosome of a spermatozoon. The end of the tail formed a plate which was found attached to the ECM of the cell culture (Fig. 3). There were forms with two tails (Fig. 4) or two nuclear heads with very little cytoplasm (Fig. 4, inset).

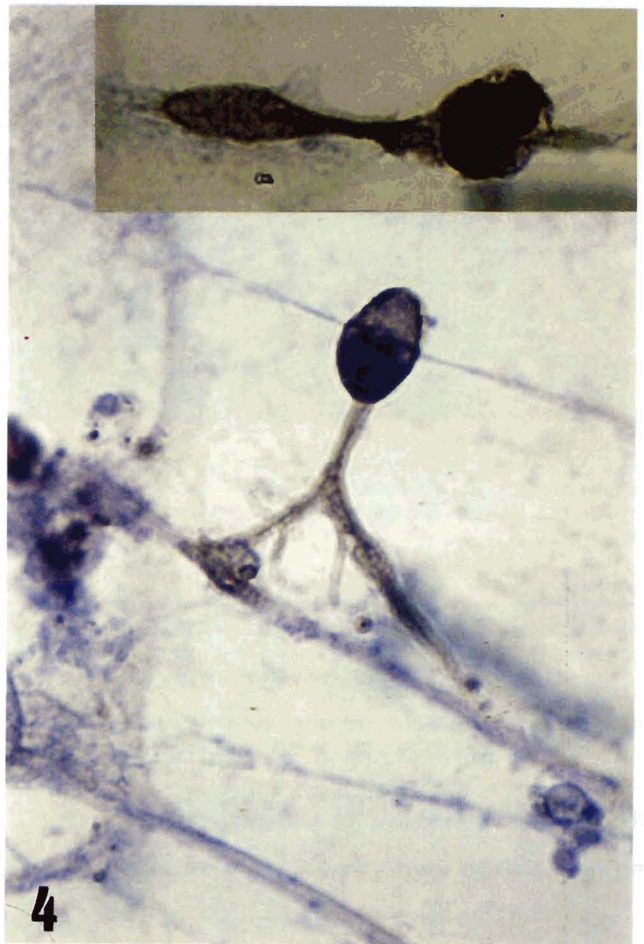
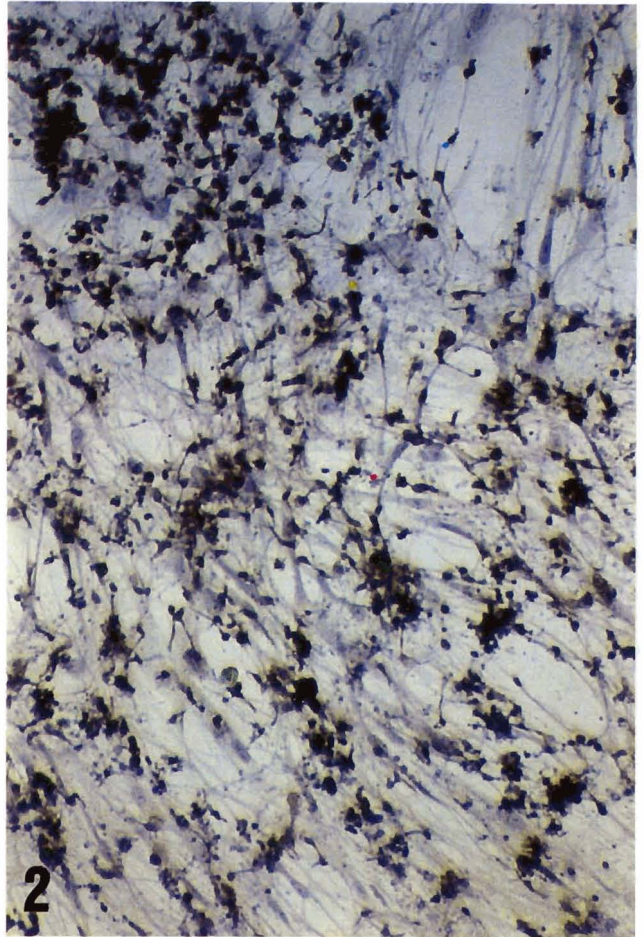
These lymphocytic forms resulted from abnormal lymphocyte division. Figure 5 shows a lymphocyte with a tail plate which contains a small nucleus. During their division the mother lymphocyte is connected to the daughter by a thin cytoplasmic bridge which is then found broken apart. Some lymphocytic mother cells raised their nucleus away from their

**Fig. 1.** Co-culture of non-activated heterologous lymphocytes with post-confluent P-cells for 7 days. Mainly round-shaped lymphocytes lie attached to the green, fibrillar, translucent, GSG-protease-membrane ECM. Target cells are intact. Papanicolaou. x 200

**Fig. 2.** Co-culture of pre-activated heterologous lymphocytes with M-cells for 15 days. Most lymphocytes show the nuclear vlima morphology, on top of the target cells which remain intact. LCA. x 100

**Fig. 3.** Co-culture of pre-activated lymphocytes with M-cells for 15 days. One lymphocyte exhibits the morphology of a nuclear vlima or spermatozoon. LCA. x 1,000

**Fig. 4.** Co-culture of pre-activated lymphocytes with M-cells for 15 days. This lymphocyte exhibits branched tail, attached to the ECM and the cytoplasm of the head resembles the acrosome of a spermatozoon. LCA. x 1,000. Inset: A lymphocyte showing binucleated head. LCA. x 1,000



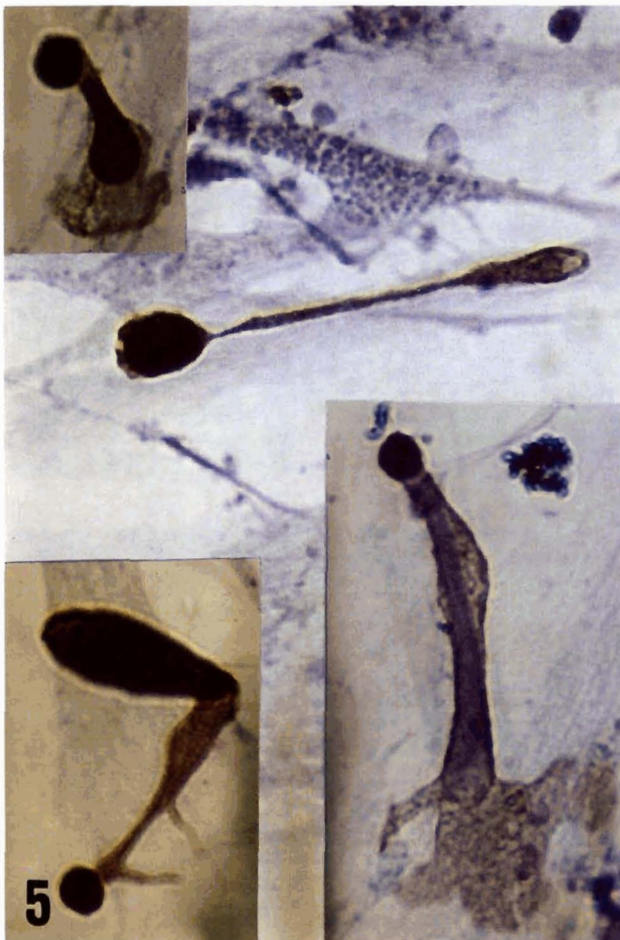
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cytoplasm and gave round daughter cells of different size, almost naked of cytoplasm and negative to antiserum against LCA (Fig. 5, insets). The lymphocytic subsets produced were of small size and inert in the sense that they failed to penetrate the ECM and target cells.

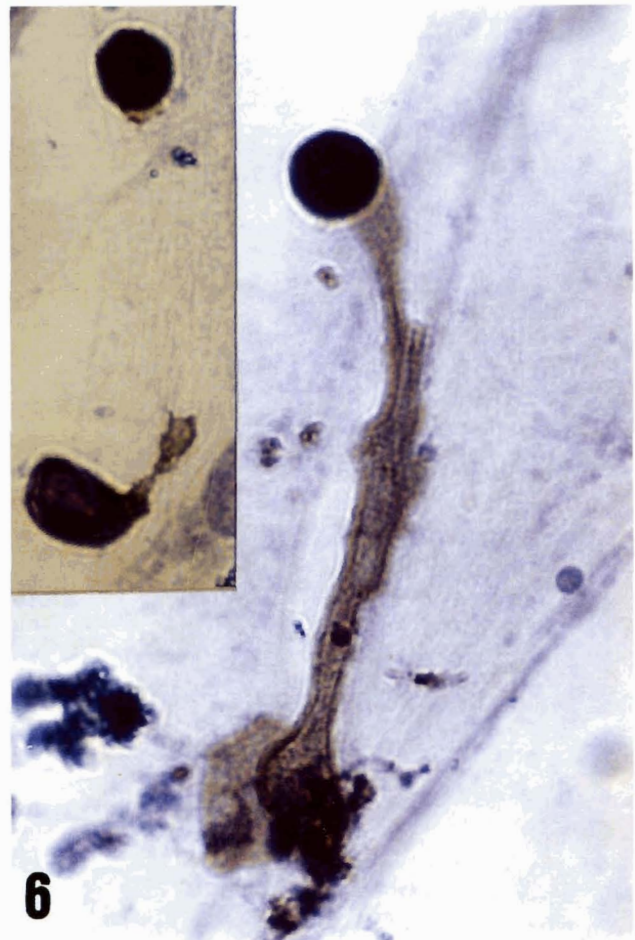
There were also lymphocytes with their tails attached to the ECM and their nucleus set free, naked of

cytoplasm and LCA (Fig. 6). Round daughter cells, naked or with little cytoplasm and LCA, formed clumps which were inert in the sense that they did not lyse the target stationary heterologous cells, lying on top of the target cell culture (Fig. 7).

Lymphocytes of these co-cultures showed positive immunoreactivity to LCA and T-cell antigen and negative to B-cell antigen.



**Fig. 5.** Co-culture of pre-activated lymphocytes with M-cells for 15 days. Various morphologies of lymphocytes during division and production of smaller inert subsets. Division is identical to that of nuclear vlimata. LCA. x 1,000



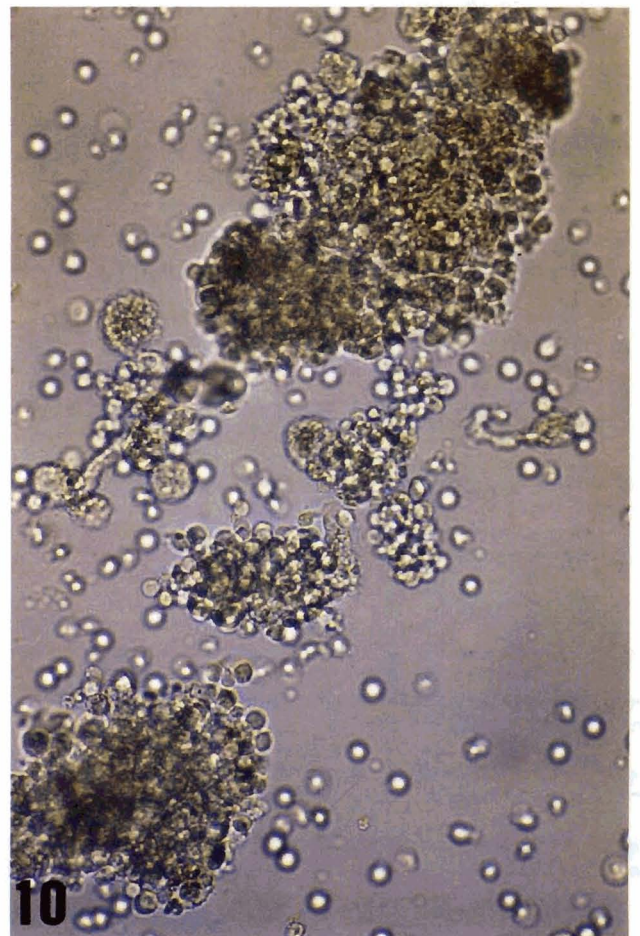
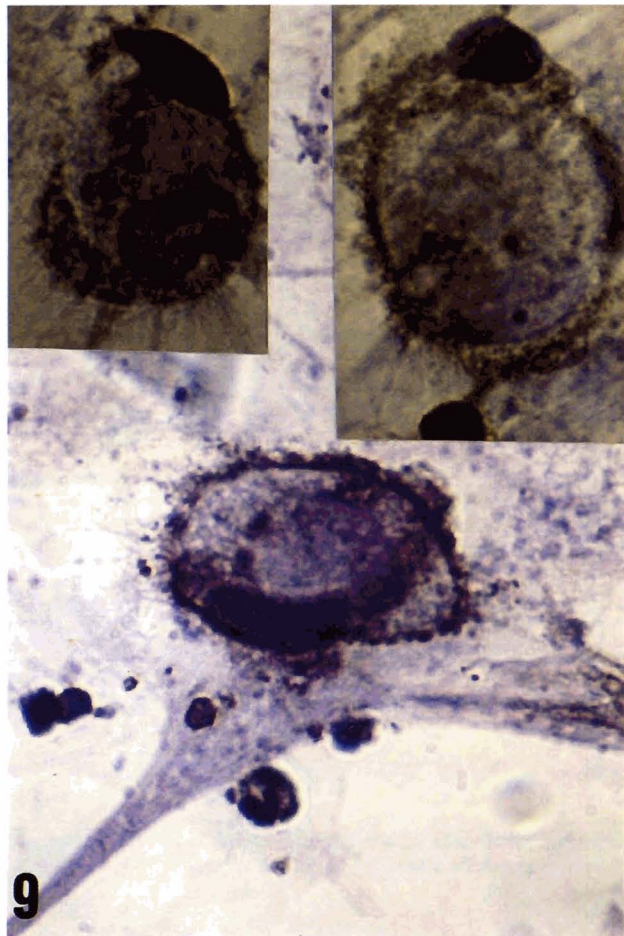
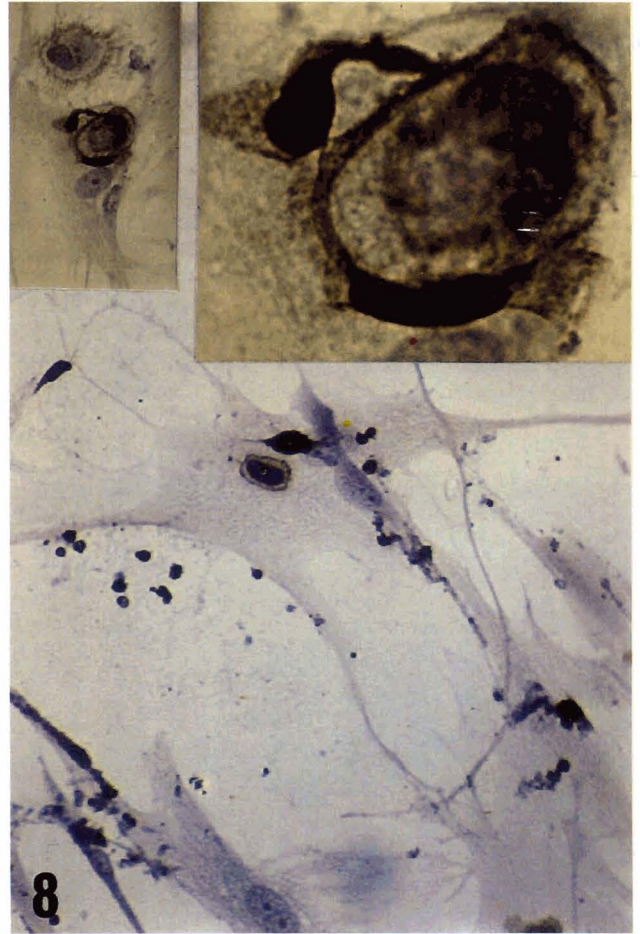
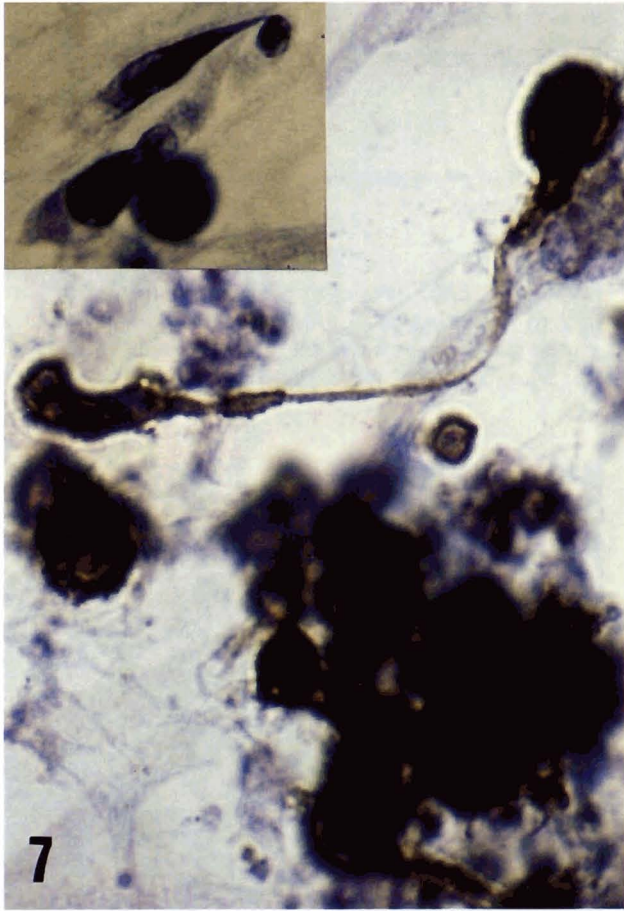
**Fig. 6.** Co-culture of pre-activated lymphocytes with M-cells for 15 days. A lymphocyte, attached to the ECM by its tail, releases its nucleus, devoid of cytoplasm. LCA. x 1,000. Inset: Released naked nucleus (right) free of LCA, on top of target cells. LCA. x 1,000

**Fig. 7.** Co-culture of pre-activated lymphocytes with M-cells for 15 days. A lymphocyte during its assymetrical division. Mother and daughter cells are connected by a cytoplasmic bridge. Also, a clump of variously-sized lymphocytic inert subsets are visible. LCA. x 1,000. Inset: Production of lymphocytic subset, daughter cell is very small and round with very little cytoplasm. Papanicolaou. x 1,000

**Fig. 8.** Co-culture of pre-activated lymphocytes with M-cells for 15 days. LCA is deposited at the cytoplasm around the target cell nucleus at a non-confluent culture area. LCA. x 200. Insets: Lymphocytes have wrapped the nucleus of a target cell by their tails. Bottom, LCA. x 1,000. Top, LCA. x 2,000

**Fig. 9.** The same subjects as in Fig. 8, different fields. LCA. x 1,000

**Fig. 10.** Co-culture of activated lymphocytes with P-cells for 10 days. Large and small nodules in suspension. Trypan blue. x 200

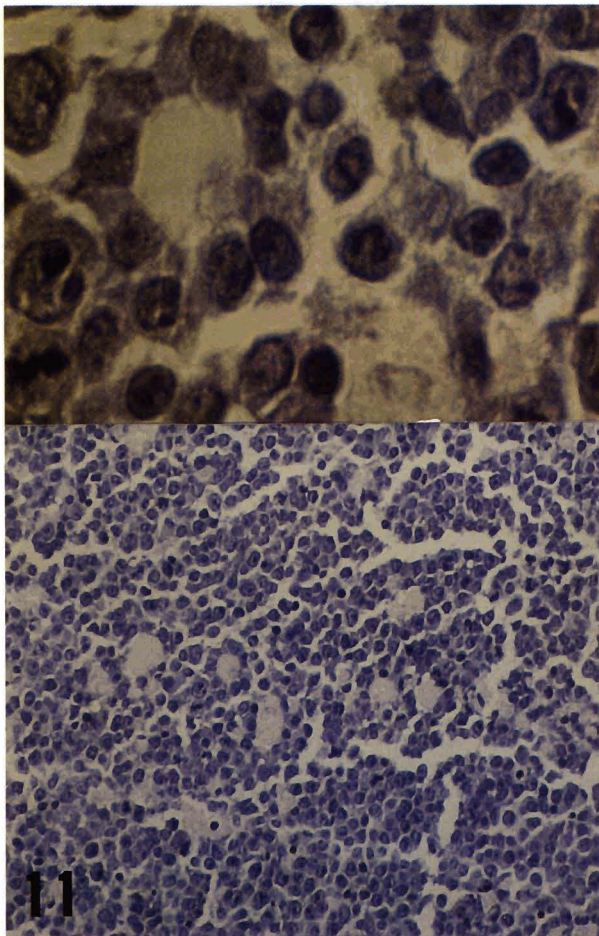


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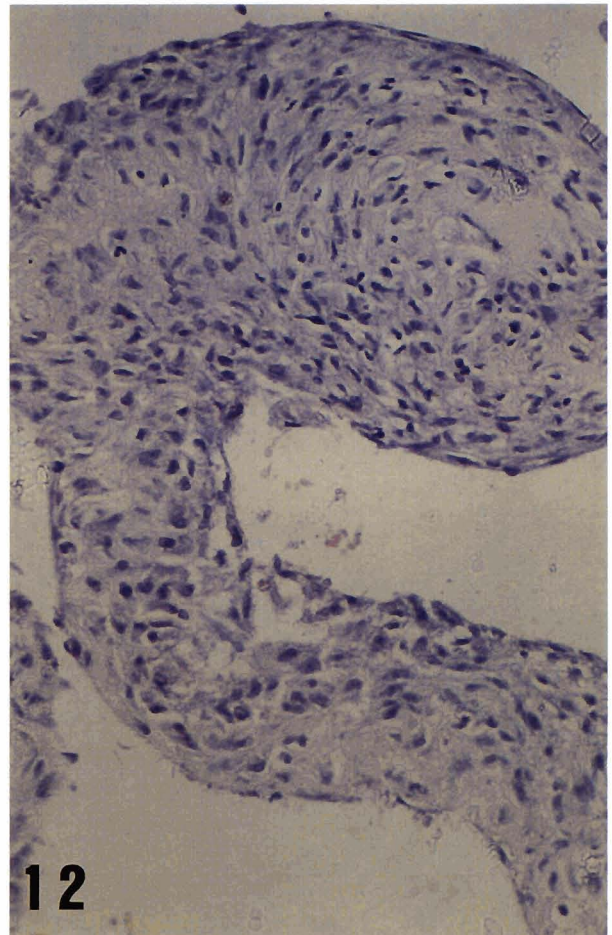
The cytoplasm around the nucleus of some stationary lung target cells was positive to antiserum against LCA (Fig. 8). It was realized, from different fields, that lymphocytes wrapped the nucleus of the target cells with their tails. The wrapped nuclei with very little cytoplasm, positive to antiserum against LCA were observed detached from the rest of their abundant cytoplasm (Figs. 8, 9).

*Interaction of pre-activated lymphocytes with normal and embryonic cells*

There was no cytolytic effect detected in normal F or N-cells after 15 days of co-cultivation with pre-activated lymphocytes. Lymphocytes failed to attach or enter the target cells; they were found intact, in the round form. Most of them were rinsed away during fixation,



**Fig. 11.** Histological picture of cell nodules from the co-culture of activated lymphocytes with P-cells. The nodule appears adenoid. The ECM has been aggregated into a mass around which the cells, in their round form are attached. HE. x 200. Inset. HE. x 1,000



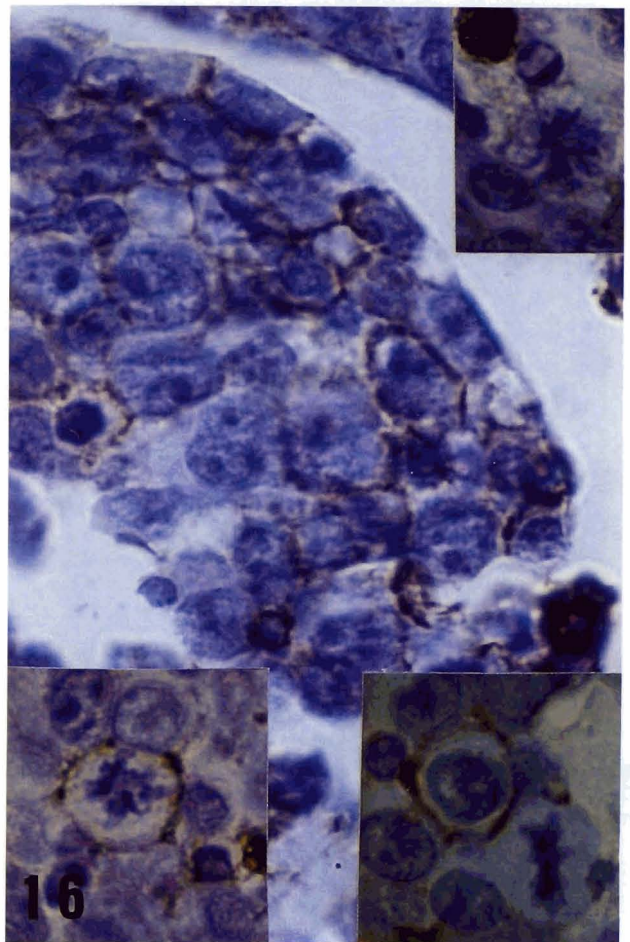
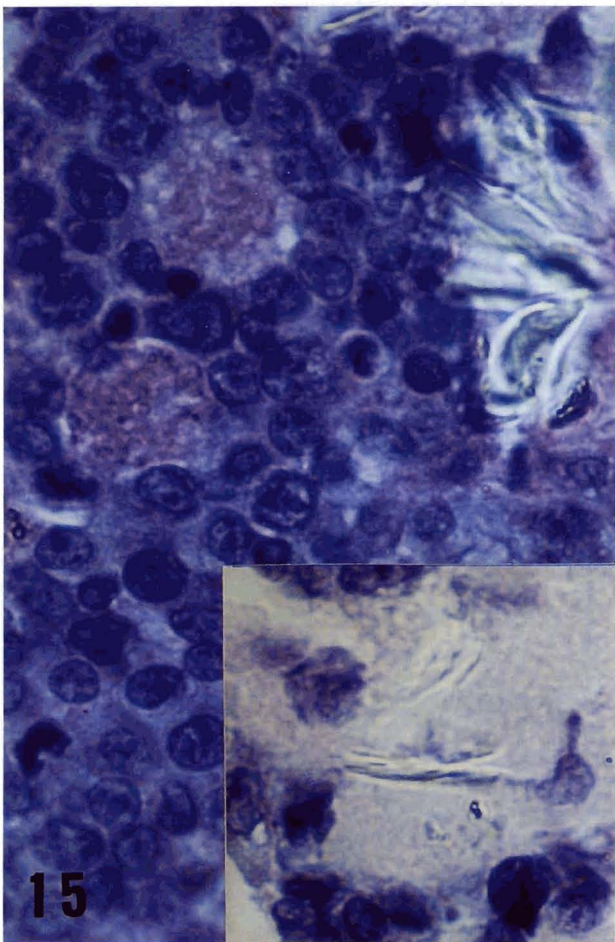
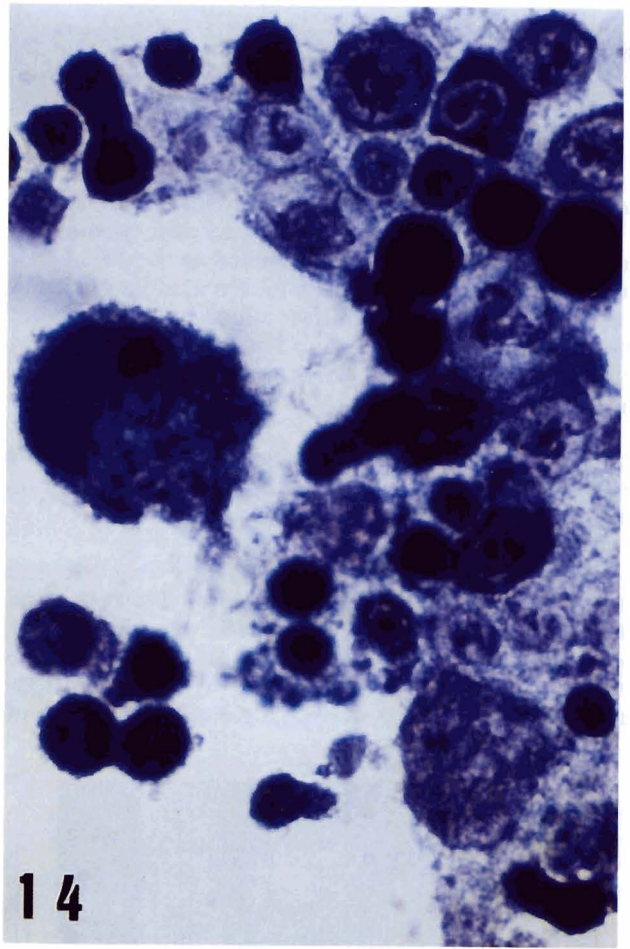
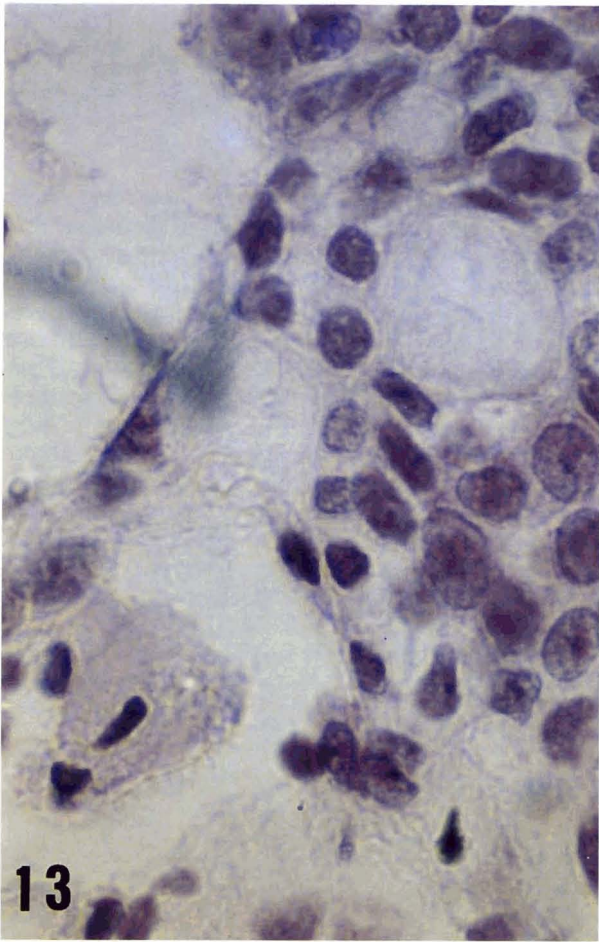
**Fig. 12.** Histological picture of tumor nodule formed by P-cells in culture after 30 days of cultivation, in the absence of lymphocytes. This nodule consists of fusiform cells with small nucleus, abundant cytoplasm and indistinct cell boundaries. HE. x 200

**Fig. 13.** Histological picture of the patient's tumor where P-cells were derived. The picture is identical to that of Fig. 11. HE. x 1,000

**Fig. 14.** co-culture of activated lymphocytes with P-cells. Remaining stationary cells after cell nodule formation. Target cells show high mitotic activity and N/C. Papanicolaou. x 1,000

**Fig. 15.** Histological picture of cell nodules from the co-culture of activated lymphocytes with P-cells. The round aggregated, fibrillar ECM is constituted of GSG. Crystal formation is visible PAS-diastrase. x 1,000

**Fig. 16.** Histological picture of cell nodules from the co-culture of activated lymphocytes with P-cells. LCA is deposited at the cell membrane of resting and dividing epithelial target cells. LCA. x 1,000. Inset: Top left, a degenerating lymphocyte side by side with a target cell in mitosis. LCA. x 1,000



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stained and retained immunoreactivity for LCA.

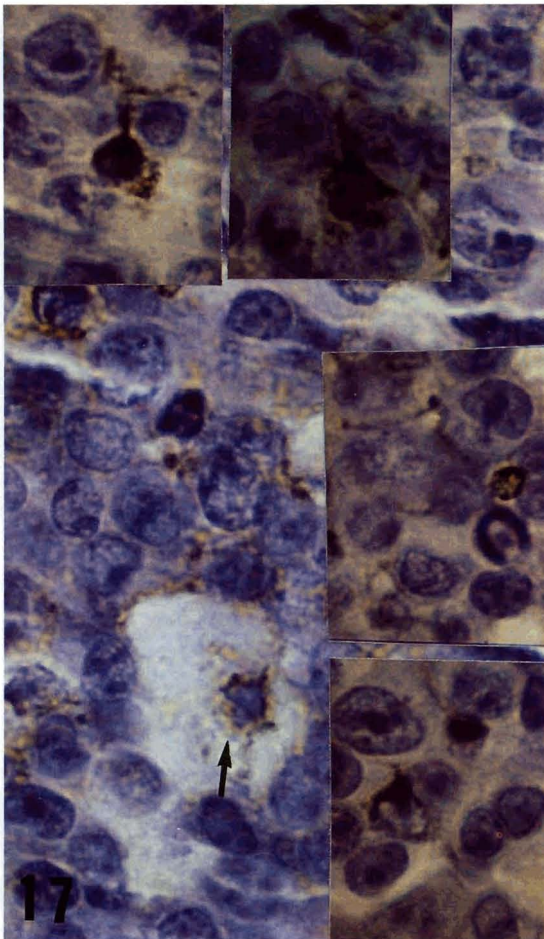
On the contrary, lymphocytes in embryonic cell cultures were denatured and attached to the ECM. All embryonic target cells were positive against the LCA antiserum, at the cytoplasmic region around the nucleus. The overall interaction was identical to that of pre-activated lymphocytes with the lung (malignant + normal clones) cells.

*Interaction of activated lymphocytes with lung cells (malignant + normal cell clones) in the continuous presence of PHA*

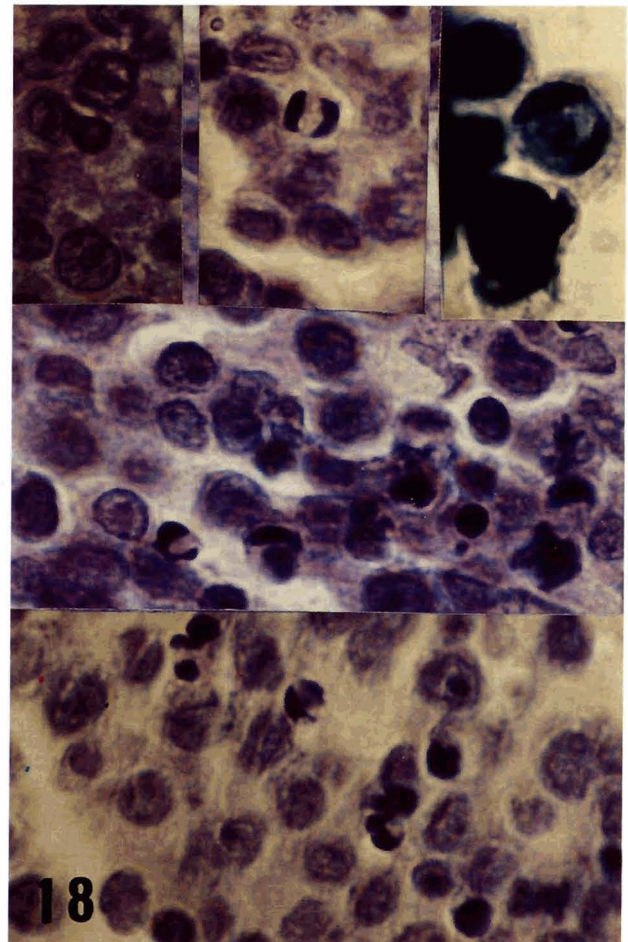
Lymphocytes interacted with stationary lung cells and formed large cell nodules, consisting of live cells even after 10 days of co-culture (Fig. 10). Stationary cells were all detached and formed nodules with the lymphocytes in the first 24 hours of co-cultivation. Paraffin nodule sections showed organoid rearrangement

of epithelial cells to adenoid type (Fig. 11), different from the fusiform cell arrangement of the tumor nodules formed by lung cell cultures in the absence of lymphocytes (Fig. 12), but identical to the histological picture of the patient's tumor (Fig. 13). Moreover, the adenoid form consisted of epithelial cells of high nuclear to cytoplasm ratio (N/C) and high mitotic activity (Fig. 14). The diffuse GSG-protease-membrane, an ECM in rivulets, observed in the tumor nodules formed by lung cell cultures (Logothetou-Rella et al., 1992b) was aggregated into a round, eosinophilic, PAS and PAS diastase positive ball, with formation of large crystals in the presence of lymphocytes (Fig. 15). Target cells derived from mechanically dispersed cell nodules, although viable, failed to attach to the culture vessel surface.

Immunohistochemistry of the nodule tissue sections of lung cells-lymphocytes showed positive immunoreactivity for LCA at the cell membrane of some



**Fig. 17.** The same subject as Figure 16. Lymphocyte is trapped in the ECM (arrow) and its cell membrane is broken. Ellipsoid lymphocytic forms are free of LCA. LCA. x 1,000



**Fig. 18.** Histological picture of cell nodules from the co-culture of activated lymphocytes with P-cells. The various degenerating forms of lymphocytes are shown. HE. x 1,000. Inset: Top left, ellipsoid lymphocyte in the remaining stationary co-culture. Papanicolaou. x 1,000



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epithelial cells (Fig. 16). Dividing epithelial cells, with cell membrane positive to antiserum against LCA were observed.

On the other hand, lymphocytes were in a degenerative stage, near the dividing epithelial cells, and lacked immunoreactivity to antiserum against LCA (Fig. 16, insets). Lymphocytes were trapped in the round ECM and underwent de-generation (Fig. 17). Degeneration started with dissolution of their cell membrane which was then attached to epithelial cells (Fig. 17, right insets). Degenerative stages of lymphocytes negative to immunoreactivity against LCA, B and T-cell antigen were also observed (Fig. 17, bottom left insets). In this stage they were recognizable by their pyknotic, hyperchromatic degenerating nucleus. Some degenerative forms of lymphocytes were of consistent morphology of a hyperchromatic, pyknotic ring or ellipse-shaped nucleus separated into two halves

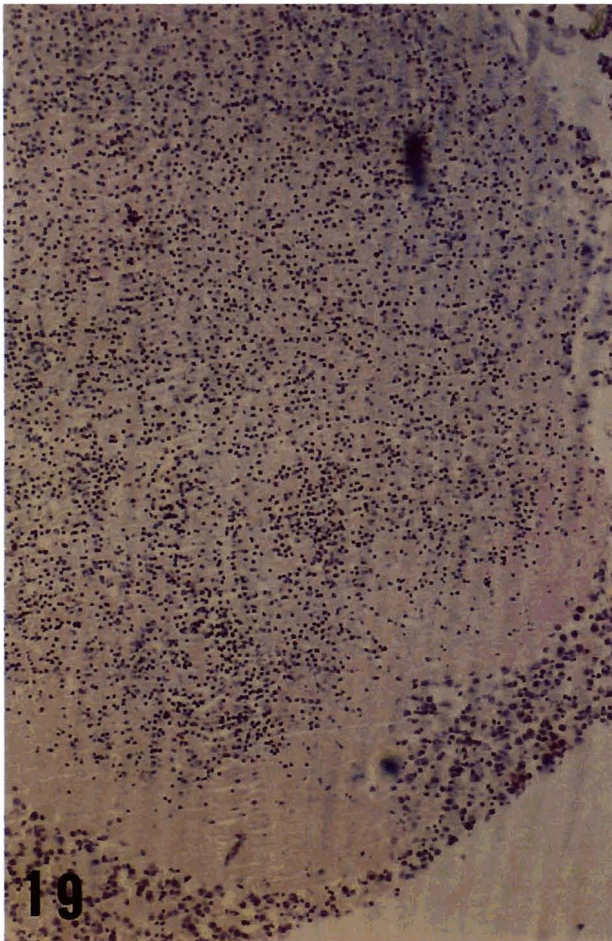
(Fig. 18). The few intact lymphocytes remaining in the nodule tissue sections showed positive immunoreactivity to LCA and T-cell antigen and negative to B-cell antigen. Positive immunoreactivity to LCA was also observed around the round ECM.

Histochemical, cytological and immunohistochemical interaction of the amniotic embryonic cells with lymphocytes was identical to that described for lung cells.

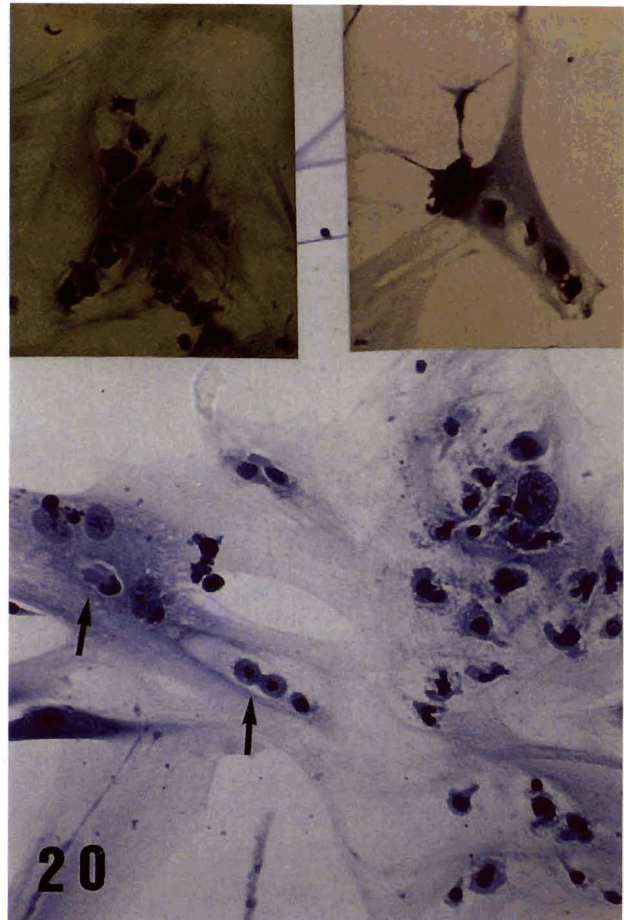
*Interaction of activated lymphocytes with normal cells in the continuous presence of PHA*

The co-cultures of lymphocytes with normal F or N-cells also formed large cell nodules. Tissue sections of these nodules showed large necrotic areas with a high number of lymphocytes (Fig. 19) which showed positive immunoreactivity to LCA, B and T-cell antigen.

Cytology of these co-cultures, before the formation of



**Fig. 19.** Histological picture of cell nodule from the co-culture of activated lymphocytes with normal cells. Large necrotic area and high number of lymphocytes are obvious. HE. x 100



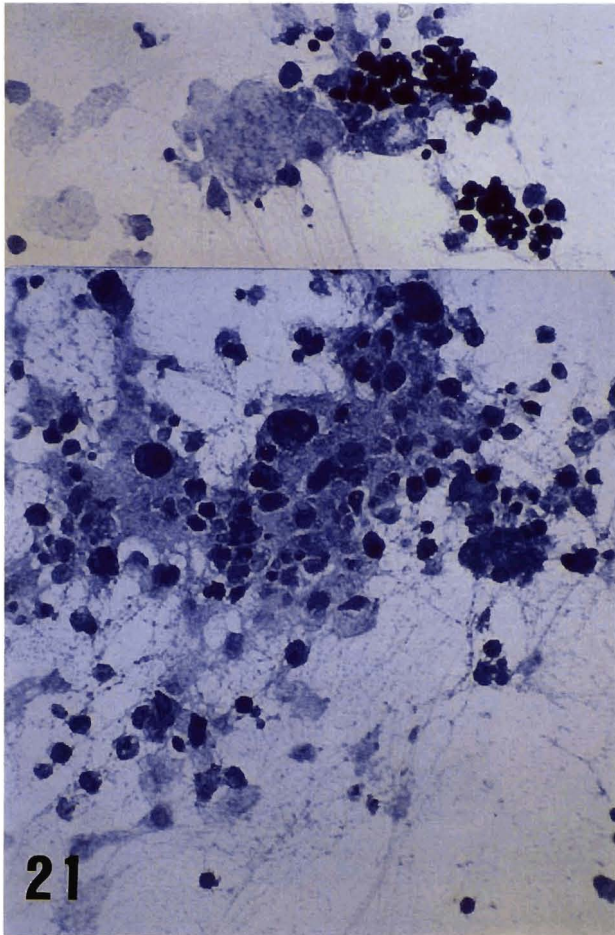
**Fig. 20.** Remaining stationary cells, after cell nodule detachment, from the co-culture of activated lymphocytes with normal cells. A high number of lymphocytes (12/cell) are located inside the target cells. The halo around the lymphocytes characterizes their intracellular location. An intracellular symmetrical division of a lymphocyte is apparent (arrow). Papanicolaou. x 200

nodules (24 hours of co-cultivation), showed lymphocytes not only bound to but also located inside the normal target cells. Lymphocytes were observed intact, in the round form, undergoing normal symmetrical division in the cytoplasm of the target cells. Intracellular location of the lymphocytes was recognized by the characteristic halo around them (Fig. 20). Target cell nuclei were devoid of their cytoplasm and the cells were destroyed by the invasion of many lymphocytes per target cell (Fig. 20, bottom inset).

#### *Interaction of activated lymphocytes with pure malignant cells in the continuous presence of PHA*

The co-culture of lymphocytes with pure malignant Pa-cells formed a few small cell nodules which could not be embedded in paraffin as they were dissolved during processing, due to cell necrosis.

Cytology showed necrotized target cells in a fibrillar basophilic ECM, and remnants of cytoskeletons



**Fig. 21.** Remaining stationary cells, after cell nodule detachment, from the co-culture of activated lymphocytes with pure malignant cells (Pa-cells). Necrotized target cells, remnants of cytoskeletons and abundant lymphocytes are obvious. Papanicolaou. x 200

(Fig. 21). Round-shaped lymphocytes were observed in clumps, in between the malignant target cells (Fig. 21, inset). Viability assessment showed all target cells dead and alive lymphocytes.

Control normal or malignant cell cultures in the continuous presence of PHA, and in the absence of lymphocytes did not form cell nodules.

The overall interaction of lymphocytes with target cells is shown in Fig. 22.

#### **Discussion**

Lymphocytes possess a diversity of cell surface receptors that mediate their adhesion to other cells. These adhesion molecules have been shown to be critical not only to lymphocyte recognition of foreign antigen, but also to the movement or migration of lymphocytes from one anatomic site to another. However, besides interacting with other cells, lymphocytes *in vivo* interact with the extracellular matrix, as they move within various microenvironments. The most extensively studied interactions for lymphocytes and ECM have been glycoproteins, fibronectin, collagen and laminin. Lymphocytes also interact with hyaluronic acid, one of several major GSG of the ECM (Shimizu and Shaw, 1991).

In this study, the interaction of lymphocytes with the GSG-protease-membrane ECM has been examined. ECM has been found to be abundant in mixed cultures of normal and malignant cells, forming a layer which covers the cell cultures (Logothetou-Rella et al., 1992b) and it is considered identical to the *in vivo* ECM, since tumors *in vivo* and these cell cultures show heterogeneity of cell clones. This ECM is distinguished in Papanicolaou-stained cell cultures by its green translucent fibrillar texture.

The results indicate that this ECM acts as a barrier between lymphocytes and target tumor cells. Since GSG is also present in normal cells and tissues, the observed behaviour of lymphocytes may be attributed to the protease associated with GSG. Under the influence of such ECM, lymphocytes were dividing identically to nuclear vlimata but failed to penetrate the ECM and lyse the target cells, mainly because of their attachment and immobilization on the ECM.

This study clearly indicates that activated lymphocytes are cytolytic to pure normal or pure malignant cell clones, as expected; this is attributed to the absence of the GSG-protease-membrane ECM. On the other hand, lymphocytes are degenerated by the GSG-protease-membrane ECM produced by the mixed cell cultures (malignant with normal cell clones) and embryonic cells.

Pre-activation of lymphocytes did not cause cell nodule formation of any of the co-culture examined. The continuous activation of lymphocytes caused cell nodule formation of normal, embryonic, malignant and mixed cell cultures. All these cell nodules, although appearing identical under phase microscopical examination,

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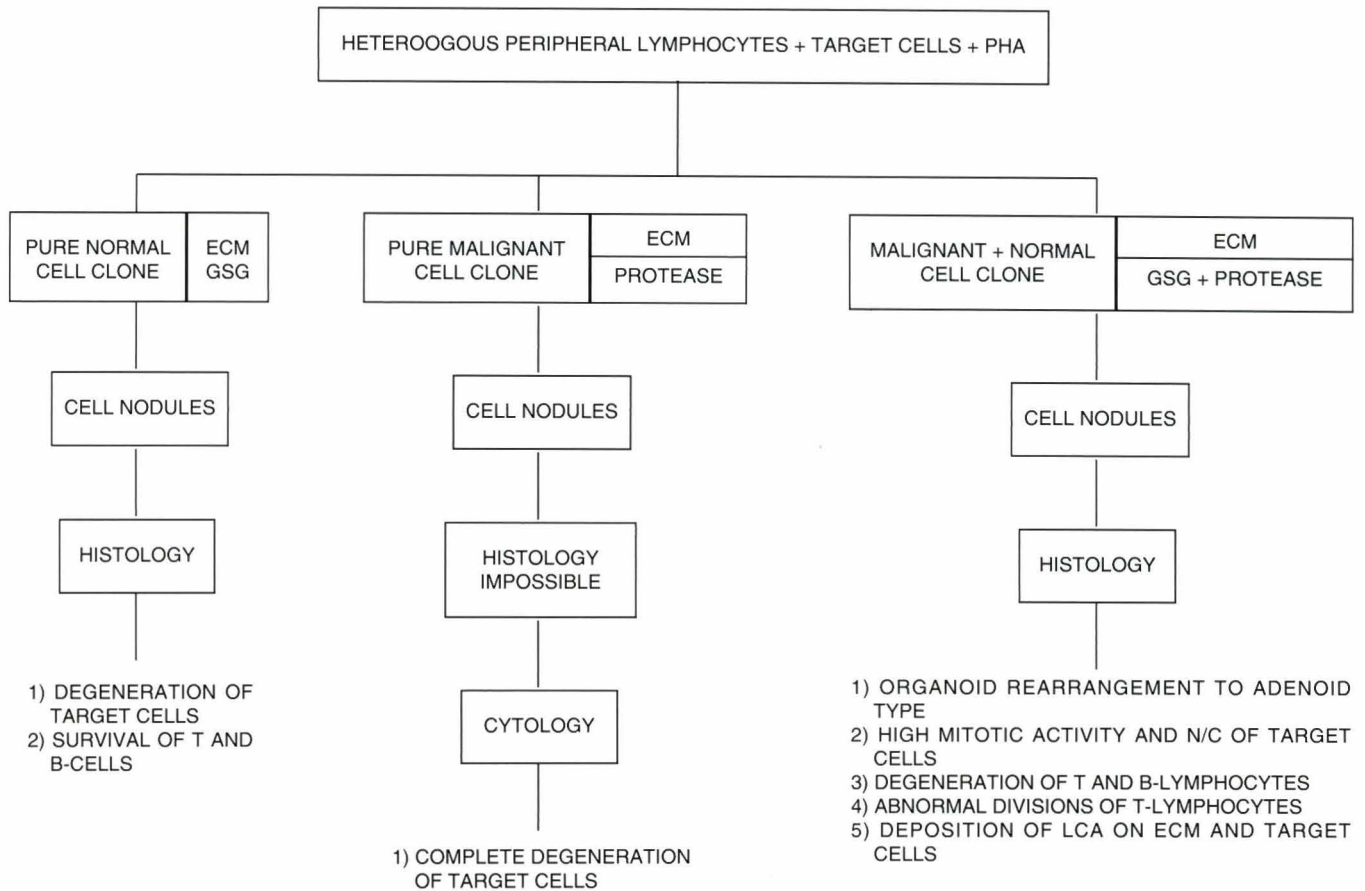


Fig. 22. Overall interaction of lymphocytes with target cells.

showed a different histological picture. Immunohistology of cell nodules of normal cell-lymphocyte co-cultures showed survival of both B and T-cells while those of mixed, pure malignant, or embryonic cells showed only T-cells.

Pre-activated lymphocytes, although heterologous, did not attach to and affect normal target cells. On the contrary, pre-activated lymphocytes divided abnormally, attached to the ECM and degenerated in the mixed cell cultures.

M and P-cells have been reported to produce abundant GSG-protease-membrane ECM and form tumor nodules of fusiform type, consisting of cells with abundant cytoplasm and low mitotic activity (Logothetou-Rella et al., 1992b). Addition of activated lymphocytes caused rearrangement of the fusiform type tumor nodule to adenoid, consisting of cells with high N/C ratio and high mitotic activity, identical to the tumor of cell origin.

It is therefore obvious that adenoid type nodules are formed *in vitro* by the interaction of normal, malignant cells, ECM and activated lymphocytes, by an as yet unknown mechanism.

Under the influence of this ECM, all T-lymphocytes obtain the morphology of a nuclear vlima produce inert

subsets and undergo lysis, depositing the LCA on the ECM and the target tumor cells. Deposition of LCA in the cytoplasm around the nucleus of target cells (in areas where ECM was not visible) indicates some movement of lymphocytes and LCA receptor involvement.

Although this behaviour and morphology of lymphocytes has been detected *in vitro*, ellipsoid forms of lymphocytes can also be identified in the histological picture of tumors *in vivo*. Such ellipsoid lymphocytic forms have been mostly ignored.

In conclusion, the ECM produced by the interaction of malignant with normal cells, may be responsible for the ability of tumor target cells to adopt a multitude of strategies to escape from immune attack *in vitro* and may constitute a major obstacle to the success of immunotherapy *in vivo*.

Further study is in progress to elucidate the aforementioned interaction of lymphocytes with the ECM.

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*Lymphocyte-extracellular matrix interactions***References**

- Boyum A. (1968). Isolation of leucocytes from human blood. Further observations. *Scand. J. Clin. Lab. Invest.* 21 (Suppl. 97), 31-50.
- Hsu S.M., Raine L. and Fanger H. (1981). A comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. *Am. J. Clin. Pathol.* 75, 734-738.
- Logothetou-Rella H. (1993). Spermatozoa-like cell invaders (nuclear vlimata) in human neoplasia. *Histol. Histopath.* 8, 407-423.
- Logothetou-Rella H., Nesland J.M., Vamvassakis E., Karayiannis A., Hadjiminis J. and Dimpoulos C. (1988a). Common characteristics of primary cell cultures originated from invasive urothelial carcinomas. *Eur. Urol.* 14, 61-64.
- Logothetou-Rella H., Vamvassakis E., Nesland J.M., Hadjiminis J. and Dimpoulos C. (1988b). Morphological and immunohistochemical characteristics of a cell line originated from non-invasive human bladder transitional carcinoma. *Eur. Urol.* 14, 65-71.
- Logothetou-Rella H., Nesland J.M., Karayiannis A. and Dimpoulos C. (1988c). Growth characteristics of cultured human normal bladder epithelial cells: A comparison with urothelial carcinoma cell cultures. *Eur. Urol.* 15, 259-263.
- Logothetou-Rella H., Kotoulas I.-G., Nesland J.M., Kipiotos D. and Abazis D. (1989). Early human trophoblast cell cultures: A morphological and immunocytochemical study. *Histol. Histopath.* 4, 367-374.
- Logothetou-Rella H., Karayiannis A., Vamvassakis E., Livaditou A., Pikramenos D., Nesland J.M. and Dimpoulos C. (1992a). A cytologic, ultrastructural and immunocytochemical comparison of tumor cells and cell cultures originating in invasive bladder carcinoma. *Eur. Urol.* 21, 146-150.
- Logothetou-Rella H., Stamboulieh-Abazis D., Tentas K., Nesland J.M., Kipiotos D., Dosios T. and Stavridis I. (1992b). Common characteristics of primary cell cultures originated from metastatic lung carcinomas. *J. Exp. Clin. Cancer Res.* 11, 285-301.
- Shimizu Y. and Shaw S. (1991). Lymphocyte interactions with extracellular matrix. *FASEB J.* 5, 2292-2299.

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