The Lymphocyte and Human Lung Cancers

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SUMMARY

With an autologous tissue culture system, a quantitative determination has been made of random and special lymphocyte interactions with neoplastic and nonneoplastic cells derived from 68 surgically resected human lung cancers. Random activities consist of lymphocyte movements among neoplastic and nonneoplastic cells, whereas special activities (clustering, congregation, emperipolesis) involve attachments of three or more lymphocytes to other cells or penetration into a cell. The frequencies of the interactions and the ratios of special-to-random interactions were determined for nonneoplastic epithelial, spindle, and histiocytic cells and for neoplastic cells. Cancer cells showed the lowest levels of overall lymphocyte interactions and were characterized by high ratios of special-to-random interactions, with epidermoid cancer as an exception in the latter respect. High ratios were also found for nonneoplastic cells derived from cancer-bearing explants, whereas the ratios for cancer-free counterparts were consistently low. The nonneoplastic epithelial cell was the outstanding example of the reversal in ratios. These findings support the concept that a defective lymphocyte-cancer cell interaction occurs in neoplasia and also indicate that cancer tissue influences the in vitro response of lymphocytes to nonneoplastic cells. These new measurements of autochthonous and distinctive lymphocyte-cancer cell interactions with neoplastic and nonneoplastic cells are of potential value for the evaluation of host response and cancer tissue uniqueness in a given patient.

INTRODUCTION

Lymphocytes unquestionably play an important role in host resistance to cancer (3–6, 19), but there is remarkably little information about lymphocyte-cancer cell interactions, especially with respect to what kinds of interactions occur, the frequency of each type of interaction, their functional significance, and how they may be evaluated. This is particularly true for human lymphocyte-cancer cell interactions, and in this respect a new approach has recently been proposed (23, 27) that appears to offer an opportunity to extend our knowledge about such interactions substantially beyond that afforded by other presently used methods. In essence, the method uses primary explants of human cancers in autologous and short-term tissue culture systems and permits several useful measurements of distinctive types of lymphocyte activities. In particular, the presence or absence of different types of lymphocyte activities in "colonies" (to be defined) of neoplastic and nonneoplastic cells appears to be not only a valid means of establishing in part the degree of lymphocyte response but also seems to reflect special functional capabilities of the lymphocyte. Moreover, both colony counts and ratio determinations, i.e., frequencies of each type of lymphocyte interaction in a given cell population, the number of lymphocytes participating in each interaction, and the types of target cells involved, can be achieved with biopsy samples of cancer tissues. Thus, this new approach has the potential to bypass much of the sampling and evaluation problems of the histological preparation.

Although there are a number of recent publications on lymphocyte interactions with neoplastic and nonneoplastic cells in vitro (10, 11, 15, 21, 22, 25, 28, 32, 33, 41–43), there are few reports wherein lymphocyte interactions with human cancer cells have been studied in an autologous tissue culture system (2, 7, 8, 16). Most pertinently, there have been no studies except for our own preliminary reports (24, 26, 27) specifically designed to evaluate intrinsic autologous lymphocyte-cancer cell interactions, i.e., where lymphocytes are already a part of the cancer mass and are not secondarily introduced. Further, the evaluation includes 1 random and 3 special types of lymphocyte activities [clustering (31), congregation (24), and emperipolesis (16)] occurring among human neoplastic and nonneoplastic cells in primary tissue cultures.

The emigrating lymphocytes in primary tissue cultures of lung are classified as migratory and ameboid lymphocytes because of their differences in appearance and behavior (24). The migratory lymphocytes are rounded or pear-shaped cells, while the ameboid lymphocytes are cells with remarkably "spread" cytoplasm and nucleus, the spreading indicating an extraordinary increase in surface area contact with other cells. The lymphocyte interactions with neoplastic and nonneoplastic lung cells are divided into "random" and 3 "special" types of interactions, determined through phase-contrast observations, time-lapse film reviews, and examination of stained preparations. Both migratory and ameboid lymphocytes participate in random interactions with neoplastic and nonneoplastic cells; on occasion, 1 or 2 lymphocytes may localize briefly on a cell of the monolayer, but penetration of the cells by the lymphocyte does not occur.

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Phase-contrast microscopy was used for daily observation of the Rose chamber cultures.

The cells emigrating from the cancerous and “normal” lung explants were identified by a correlative methodology that included whole-lung thin sections (14), routine histological preparations, phase-contrast microscopy, May-Grünwald-Giemsa-stained monolayers, and time-lapse filming. Criteria for the special type of lymphocyte activities and the identification of cancer cells have been previously described (24, 37).

In brief, identification of cancer cells is based on a recently recognized tissue or organoid form of in vitro cancer (37). In essence, identification begins with in vitro tissue culture, which is the equivalent of an unstained, but living, histological preparation. Architectural integrity is preserved, e.g., the cell-to-cell relationship of epidermoid cancer is presented as a “whorl” with a typical epithelial “pearl” (36, 37). The emigration and spreading of cancer cells from the organoid form of the cancer leads to monolayer formation (Fig. 5). Identification of the cancer monolayers is achieved through the finding of areas transitional between the monolayer and tissue forms and through continuous time-lapse filming of the emigration. Also, a number of nuclear and cytoplasmic characteristics of the in vitro cancer tissue persist in the monolayer.

Nonneoplastic epithelial cells originating from ciliated epithelium, a common component of cultures derived from normal lung parenchyma, were readily recognized by the persistence of cilia during the early period of monolayer outgrowth. The cytostructure, staining properties, and growth patterns of these cells readily distinguished them from the other 2 nonneoplastic cell categories, i.e., spindle cells and macrophages. Other nonneoplastic epithelial cells, presumably derived from the mucosal and alveolar linings, shared a number of properties with those of ciliated cell origin and conformed to the pattern of growth commonly observed in tissue culture preparations of CF lung parenchyma. Thus, the characteristics of the parenchymal epithelial cells and the positive identification of the cancer cells as described served to distinguish nonneoplastic from neoplastic epithelial cells.

All fusiform cells with the characteristics of connective tissue monolayers were classified as spindle cells, and cells placed in the macrophage category were characterized by individual cell movements, distinctive membrane patterns, phagocytic activity, and staining properties. In regard to lymphocytes, no distinction was made as to size or organ of origin. All of the nonneoplastic cells were derived from 2 major sources, normal lung parenchyma from a CB lung and lung parenchyma within the cancer tissue itself, i.e., the bronchioloalveolar and other structures that persist despite cancerous invasion and destruction.

In this study, a cell colony was defined as either an outgrowth or an isolated group of 100 or more cells of a single type. Three neoplastic and 3 nonneoplastic cell types were studied; namely, epidermoid cancer, adenocarcinoma, and undifferentiated carcinoma on one hand and nonneoplastic epithelial cells, spindle cells, and macrophages on the other. The colonies were observed for the presence or absence of special and random lymphocyte interactions, and they were

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3 The abbreviations used are: SI, special interaction; CB, cancer bearing; CF, cancer free; TCI, total combined interactions; RI, random interaction.
scattered as positive when the frequency of either interaction with respect to the number of cells in the colony was at or above the level of 1%.

RESULTS

Of 68 lung cancers explanted in vitro, 48 (71%) yielded cancer cell outgrowth in Leighton tube preparations. However, cancer tissue was demonstrated in all histological preparations of the explants following sacrifice after varying periods of in vitro life. In respect to lymphocyte emigration from the explant, an 82% yield was obtained when the findings of both Leighton tubes and Rose chambers were combined. The Rose chamber was advantageous for this purpose since the cellophane strip in the chamber retains lymphocytes within the immediate vicinity of the explant, a feature particularly helpful when only a small number of lymphocytes emerge and when the medium is changed. The 56 lung cancers with preparations positive for emigrating lymphocytes included 18 adenocarcinomas (50% of total adenocarcinomas), 20 undifferentiated cancers (69% of total undifferentiated cancers), and 21 epidermoid carcinomas (90% of total epidermoid carcinomas). In respect to the number of lymphocytes emerging from the various explants, there was a range of a few per low-power field to a densely populated band 2.0- to 3.0-mm thick, with the maximal number of lymphocytes found during the 2nd day of in vitro life. Most of the lymphocytes emerging during the 1st few days of in vitro life were of the “migratory” type, i.e., pleomorphic, freely moving, and refractile (phase-contrast microscopy) cells. As other cells emerged from the explants, some of the migratory lymphocytes spread on their surfaces to become ameboid lymphocytes (24), thus not only appearing to be much larger than the migratory form but exhibiting clearly visible nuclei, delicate cytoplasmic membranes, and well-defined cytoplasmic organelles.

In respect to the interactions of lymphocytes in colonies of neoplastic and nonneoplastic cells, there were several outstanding findings (Chart 1). (a) In reference to the TCI, i.e., a percentile tabulation that combines SI and RI of lymphocytes, the cancer cell colonies show a much lower level of response than any of the nonneoplastic cell colonies. (b) The TCI’s were also consistently lower for the 3 types of nonneoplastic cell colonies derived from CB as opposed to CF explants. (c) When the TCI’s are separated into SI and RI categories, the lowest frequencies for the various cells in each of these 2 categories are again shown by the cancer cells with 1 exception, i.e., the SI of epithelial cells (Chart 1). (d) Cancer cell colonies show approximately twice as many SI as RI. (e) There were 2 outstanding findings with respect to nonneoplastic cells. (i) RI levels are related to the presence or absence of cancer in the explant, i.e., the percentage of colonies positive for RI was higher for all nonneoplastic types having a CF as opposed to a CB explant derivation (Chart 1). The outstanding example is the epithelial cell, where a 3-fold difference is found between the CF and CB categories, 48 and 16%, respectively. (ii) SI levels are also related to the presence or absence of cancer in the explant, but the relationship is essentially the reverse of that found for RI (Chart 1). The best example is again the epithelial cell where a 4-fold difference is found between the CF and CB categories, the respective figures being 8 and 32%. (f) The type of cancer cell present in the explant was found to have a bearing on the levels of RI and SI occurring with both neoplastic and nonneoplastic cells. As shown in Table 1, epithelial cells from CB explants containing adenocarcinoma or undifferentiated cancer have RI levels that are 34.8 and 13.8%, respectively, but the corresponding figure for epidermoid cancer is less than 1%. Conversely, the CF epithelial cell counterpart (Table 2) for epidermoid cancer shows a very high RI. An increase in RI was also noted for undifferentiated cancer, but the level for adenocarcinoma fell slightly. A trend towards a relatively high RI, with a concomitant fall in SI, was generally true for all cells in the CF as opposed to the CB category.

In respect to the cancer cells themselves (Table 1), RI’s were relatively high for epidermoid cancers (15.1%), being 3 times greater than those for adenocarcinomas (5.7%) and 5 times the levels for undifferentiated cancers (3.2%). On the other hand, SI’s were notably low for epidermoid cancers but high for the other 2 cancers, the respective average levels being 7.5, 21.1, and 24.1%. (g) A detailed listing of SI:RI ratios (Table 3) shows high ratios for nonneoplastic cells (and cancer cells themselves) in the CB categories and low ratios for the CF categories with few exceptions. (h) In respect to the subdivisions of SI, emperipolesis was by far the dominant response to cancer cells (Chart 2). Prominent levels of both emperipolesis and congregation were also found in the CB categories of both epithelial and spindle cells. Clustering was primarily a phenomenon involving macrophages.

When the special lymphocyte interactions are plotted
Table 1

Percentage of cell colonies derived from CB explants that are positive for lymphocyte interactions

The colonies are grouped according to cell types and then subdivided according to the type of cancer present in the explant.

<table>
<thead>
<tr>
<th>Interactions</th>
<th>Cancer cell colonies</th>
<th>Epithelial cell colonies</th>
<th>Spindle cell colonies</th>
<th>Macrophage colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U^a</td>
<td>A</td>
<td>E</td>
<td>U</td>
</tr>
<tr>
<td>Total</td>
<td>27.3</td>
<td>26.8</td>
<td>22.6</td>
<td>44.0</td>
</tr>
<tr>
<td>Random</td>
<td>3.2</td>
<td>5.7</td>
<td>15.1</td>
<td>13.8</td>
</tr>
<tr>
<td>Special</td>
<td>24.1</td>
<td>21.1</td>
<td>7.5</td>
<td>30.2</td>
</tr>
<tr>
<td>Emperiploïsis</td>
<td>14.8</td>
<td>21.1</td>
<td>7.5</td>
<td>21.0</td>
</tr>
<tr>
<td>Congregation</td>
<td>7.0</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>6.9</td>
</tr>
<tr>
<td>Clustering</td>
<td>2.3</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

^a U, explants containing undifferentiated carcinoma; A, explants containing adenocarcinoma; E, explants containing epidermoid carcinoma.

Table 2

Percentage of cell colonies derived from CF explants that are positive for lymphocyte interactions

The colonies are grouped according to cell types and then subdivided according to the type of cancer present in the lung. The explant does not contain cancer.

<table>
<thead>
<tr>
<th>Interactions</th>
<th>Epithelial cell colonies</th>
<th>Spindle cell colonies</th>
<th>Macrophage colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U^a</td>
<td>A</td>
<td>E</td>
</tr>
<tr>
<td>Total</td>
<td>55.6</td>
<td>43.8</td>
<td>80.0</td>
</tr>
<tr>
<td>Random</td>
<td>44.5</td>
<td>25.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Special</td>
<td>11.1</td>
<td>18.8</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Emperiploïsis</td>
<td>&lt;1</td>
<td>12.5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Congregation</td>
<td>11.1</td>
<td>6.3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Clustering</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

^a U, nonneoplastic tissue from lung containing an undifferentiated carcinoma; A, nonneoplastic tissue from lung containing adenocarcinoma; E, nonneoplastic tissue from lung containing epidermoid carcinoma.

Table 3

The ratios of SI to RI

<table>
<thead>
<tr>
<th>Type of colony</th>
<th>Type of explant</th>
<th>Undifferentiated carcinoma (SI:RI)</th>
<th>Adenocarcinoma (SI:RI)</th>
<th>Epidermoid carcinoma (SI:RI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer cells</td>
<td>CB</td>
<td>7.5</td>
<td>3.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Epithelial cells</td>
<td>CB</td>
<td>2.1</td>
<td>1.0</td>
<td>33.3+</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>0.2</td>
<td>0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Spindle cells</td>
<td>CB</td>
<td>4.0</td>
<td>2.0</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>1.5</td>
<td>0.8</td>
<td>25.0+</td>
</tr>
<tr>
<td>Macrophages</td>
<td>CB</td>
<td>1.0</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>CF</td>
<td>0.3</td>
<td>0.5</td>
<td>0.2</td>
</tr>
</tbody>
</table>

against time of in vitro life (Charts 3 to 6), there is a trend for most interactions to peak at 7 to 14 days. By the 28th day of in vitro life, there were few instances of SI or RI, but isolated examples of each could be found in cultures as old as 100 days.

The ultimate fate of the cells involved in SI was observed in relatively few instances, but it was evident that in some cases 1 or both of the interacting cells, the lymphocytes or the target cell, underwent degenerative change.

**DISCUSSION**

It appears that short-term cultures of human cancers can provide data on lymphocyte-cancer cell interactions, which are in some way a reflection of "host resistance." Although clinical correlation of the data obtained in this study must await long-term follow-up, some of the findings tend to parallel and supplement the histological indicators of prognosis (3, 38). Of more immediate importance, the methodology has a number of attributes that strongly support its future usefulness. (a) The exclusively autologous cell populations studied and the short-term observation periods (beginning immediately after explantation and rarely extending beyond 6 weeks) assure preparations that are more representative of the in vivo state than the more commonly used tissue culture methods, i.e., cell lines, cell strains, artificially constructed mixed cell populations, and heterologous systems. (b) The appearances and interactions of cell populations derived from
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some other factors involved in the interaction. The nonneoplastic epithelial cells, which are in a sense benign counterparts of the cancer cells, show a response parallel to the cancer cells in the situation where they are derived from CB explants. The fact that this is not the case for those derived from CF explants tends to imply that a defective lymphocyte interaction exists with cancer-associated epithelial cells as well and is a reflection of the cancerous process, i.e., epithelial cells in the vicinity of the cancer are in some way either influenced by or are a part of the neoplastic process, the latter idea being in line with the concept of a “field effect” (39).

A very intriguing aspect of the data is the fact that cancer cells and nonneoplastic cells in the vicinity of cancer tissue are dominated by special, not random, types of lymphocyte interactions. Some of these SI have already been linked in part to lymphocyte-cancer cell responses. Lymphocyte clustering, for example, can be produced experimentally by sensitizing lymphocytes to a specific cancer (1) or, for that matter, to a number of nonneoplastic cells. Emperipolesis has been shown in this study to be the predominant, almost exclusive,

CB as opposed to CF tissue of the same lung can be compared under essentially identical environmental conditions. (c) A number of lymphocyte properties can be detected and measured as part of a host defense evaluation, including viability, longevity, special structural forms and transitions, special forms of behavior, and responses to selected challenges or special environmental conditions.

Insofar as prognostic considerations are concerned, it is noteworthy that, of all the lymphocyte interactions, i.e., with epithelial cells, spindle cells, macrophages, and cancer cells, those involving cancer cells were the least frequent (Chart 1). This is consistent with our earlier observations (34) and the hypotheses of others (9) that a defective lymphocyte-cancer cell interrelationship exists. However, there is little insight at the present time as to whether the defect is mainly of the lymphocyte, the cancer cell, a combination of the 2, or of

some other factors involved in the interaction. The nonneoplastic epithelial cells, which are in a sense benign counterparts of the cancer cells, show a response parallel to the cancer cells in the situation where they are derived from CB explants. The fact that this is not the case for those derived from CF explants tends to imply that a defective lymphocyte interaction exists with cancer-associated epithelial cells as well and is a reflection of the cancerous process, i.e., epithelial cells in the vicinity of the cancer are in some way either influenced by or are a part of the neoplastic process, the latter idea being in line with the concept of a “field effect” (39).

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interaction of lymphocytes with cancer cells. This is in accord with the study of Humble et al. (16), who concluded that “the lymphocyte has a remarkable affinity for malignant cells.” However, as our report points out, the affinity of lymphocytes for lung cancer cells is essentially restricted to this 1 interaction, whereas the overall interactions are clearly diminished. With respect to emperipolesis in nonneoplastic cells, a possible link to atypism and perhaps precancerous change is also suggested by the findings. Particularly significant is the fact that nonneoplastic epithelial cells derived from CB explants were found to exhibit an almost 4-fold greater frequency of emperipolesis than do the corresponding cells from CF explants (Chart 2). Far less significant differences in this respect were noted for spindle cells and macrophages. Emperipolesis was much less frequent for epidermoid cancer than for undifferentiated carcinoma and adenocarcinoma (Table 1), a finding that may have “host-status” meaning, considering the generally more favorable prognosis for epidermoid cancer of the lung. At the same time, it should be noted that random interactions for epidermoid carcinoma are twice as frequent as emperipolesis, whereas for the other 2 cancers there is an outstanding reversal. The latter findings emphasize the value of subdividing lymphocyte interactions, inasmuch as the overall total interactions showed relatively little difference.

The specific mechanisms involved in the various lymphocyte interactions and the functional meaning of the interactions are to date essentially unexplained. Emperipolesis is a remarkable phenomenon, but little can be said about its meaning other than that it may be an extraordinary means of internal surveillance of the cell. There is a speculative opinion that the ameboid form of the lymphocyte may represent a “conditioning” response (24), but neither this activity nor the related phenomenon of congregation have been linked in any way to cancer, other than the implications of this study. Congregation has also been reported for macrophages, and the phenomenon has been related to certain in vitro cells derived from the lungs of guinea pigs exposed to nitrogen dioxide (35).

An obvious aspect of all of the SI is the intimate contact of the lymphocyte to the target cell, either in the form of “foot appendages” (18) (migratory lymphocyte) or extended cell surface area [ameboid lymphocyte (24)]. Therefore, it is likely that alterations at the cell surfaces, especially electrostatic disturbances (9, 13), or the presence of an antibody coat (15) may be responsible for prevention of intimate cell contacts by the lymphocyte. Thus, it is possible for a large number of lymphocytes to be near cancer cells and yet not be effective. The seemingly paradoxical situation where emperipolesis is increased under these circumstances may possibly be explained on the basis of lymphocyte-host cell antagonism, the lymphocytes penetrating into the cell but becoming trapped inside it. That trapping is an actual event is supported by the fact that lymphocytes within lung cancer cells often die, producing inclusion bodies (Fig. 9), and that “near entrapment” of lymphocytes in nonneoplastic cells has been observed via time-lapse films. It is also possible that the lymphocytes are functionally defective although able to penetrate cancer cells, an aspect supported by the fact that delayed or otherwise impaired homograft rejection phenomena are often found in cancer patients (40). Further, lymphocyte populations are heterogeneous, and it may be, as Mikulska et al. (20) have suggested, that cancer states are associated with decreased numbers of “antitumor” lymphocytes.

There is a great need for information about the relationship of each patient’s clinical course to lymphocyte-cancer cell interactions, and in this respect this study represents a beginning. The primary short-term cultures, the documented cancer cells in vitro, the quantitative approach to evaluation of lymphocyte-cancer cell interactions, and the attention to unique lymphocyte activities can provide, with refinement and amplification, a means of obtaining needed insight into the problem of human host resistance to cancer.

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Figs. 1 to 4. Lymphocyte interactions. Phase-contrast photomicrographs.

Fig. 1. Random lymphocyte interactions. Ameboid lymphocytes (solid arrows) and migratory lymphocytes (open arrows) are actively moving on top of a monolayer derived from bronchial epithelium. x 160.

Fig. 2. Congregation of lymphocytes. The activity of numerous ameboid lymphocytes (arrow) is essentially confined to a single large spindle cell. x 160.

Fig. 3. Emperipolesis. A single lymphocyte (arrow) is actively moving within an intracytoplasmic vacuole of a binucleated cell. x 1000.

Fig. 4. Clustering. Numerous migratory lymphocytes (arrow) have become attached to a single cell. x 160.

Figs. 5 to 9. Appearance of neoplastic and nonneoplastic cells and their lymphocyte interactions in May-Grünwald-Giemsa-stained preparations.

Fig. 5. Adenocarcinoma of the lung. The tissue culture preparations were characterized by the presence of numerous gland-like tumor masses composed of thin and thick cords (solid arrows). The organoid cords often showed transitional areas in continuity with monolayers (open arrow). x 250.

Fig. 6. Clustering of migratory lymphocytes around macrophages. A number of macrophages (indicated by arrows) show several migratory lymphocytes attached to their surfaces. The "tail processes" of the lymphocytes are the means of attachment, but they cannot as a rule be seen in stained preparations. In this instance, the spindle cells are free of lymphocyte clustering. x 400.

Fig. 7. Congregation of ameboid lymphocytes on a spindle cell. Seven lymphocytes localized to 1 area are spread on the surface of a spindle cell (arrow), effecting very intimate and extensive surface-to-surface contacts. Note the finer detail of the nucleus and cytoplasm of these lymphocytes as compared to those engaged in clustering (Figs. 4 and 6). x 400.

Fig. 8. Emperipolesis in a spindle cell. Four lymphocytes are present in a large juxtanuclear vacuole (arrow), the latter indenting the nucleus. A short distance away, a 5th intravacuolar lymphocyte can be seen in association with 2 intravacuolar inclusions interpreted as degenerated lymphocytes. A 6th instance of emperipolesis is at the opposite cytoplasmic pole of the spindle cell. x 400.

Fig. 9. Monolayer of lung cancer cells exhibiting intracellular lymphocytes (arrows) in various stages of degeneration, including inclusion body formation. x 400.
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