Lymphocyte macrophage interactions: peripolesis of human alveolar macrophages

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ABSTRACT: Peripolesis is a phenomenon in which a lymphocyte attaches itself to another cell, usually a macrophage or veiled cell, and proceeds to circle around it. In emperipolesis, a related phenomenon, the lymphocyte invaginates the target cell so deeply that it appears to be intracytoplasmic.

Lung cells in bronchoalveolar lavage fluids from 20 patients were observed in the living state and filmed. Peripolesis of the alveolar macrophages was recorded in six cases. These patients included one case each of carcinoma of the bronchus, tuberculosis, sarcoidosis and asthma, while two patients had no detectable lung disease. Five out of the six positive cases were females. In every instance there was a high number of lymphocytes in the washing. The peripolesed macrophages were not injured, but temporary alteration of the cell membrane was noted in a minority of cell sequences. The peripolesing cells were also examined by transmission and scanning electron microscopy. The lymphocyte was found to be closely attached to the surface of the macrophage, with no invagination and its ultrastructure was that of a small lymphocyte.


The technique of bronchoalveolar lavage (BAL), now widely used in the diagnosis of pulmonary disease [1, 2] has made it possible to recover immunologically active cells in the washing fluid, and to observe them in the living state. Lung macrophages are developmentally separated from gut and other tissue macrophage systems [3] and include several types of cells varying in size, enzyme content, motility and membrane markers [4]. Some lavage fluids contain veiled or dendritic antigen-presenting cells (APCs), which resemble macrophages but are not phagocytic or adherent and are also highly mobile [5]. The two types of APC have different functions. The macrophages ingest particulate antigens, or large molecules, and after lysosomal degradation the fragments are returned to the cell membrane, whereas dendritic cells deal with small antigens and digestion, if required, probably takes place in the cell membrane. There are also variable numbers of large and small lymphocytes, neutrophil leucocytes and ciliated bronchial epithelia. All these cells were observed microscopically and their interactions recorded by time-lapse cinematography. We also studied the same cells by transmission and scanning electron microscopy (TEM and SEM). In this paper we report that the cell interactions known as peripolesis [6-10] can be observed in a proportion of lung washings. The circling cells were lymphocytes and the peripolesed cells alveolar macrophages.

We did not observe peripolesis of dendritic cells, although animal experiments have shown that it does occur, apparently in connection with antigen presentation [11]. In the lung the role of alveolar macrophages is mainly down-regulation of the immune response, but they may acquire the ability to present antigen in conditions such as sarcoidosis [12].

This is the first proof of peripolesis in lung washings. In the literature there are ‘still’ photographs showing lung macrophages with attached lymphocytes [4]. However, contact between these cells is not always followed by peripolesis, which can only be established by cinematography or direct viewing of the living cells at body temperature.

Materials and Methods

Patients

We studied an unselected group of 20 patients undergoing diagnostic bronchoscopy, who gave permission for an additional washing by the usual
Table 1. - Perlpolesis in bronchial lavage fluids: clinical correlations in six positive cases

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Sm.</th>
<th>Diagnosis</th>
<th>Peripolesis of macrophages (cinematography)</th>
<th>%Lympho</th>
<th>%Macro</th>
<th>%Neutro</th>
<th>%Eosino</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>30</td>
<td>+</td>
<td>Retronasal bleeding</td>
<td>42</td>
<td>53</td>
<td>3</td>
<td>2</td>
<td>&lt;1</td>
<td>Bronchoscopy and chest X-ray normal</td>
</tr>
<tr>
<td>F</td>
<td>40</td>
<td>+</td>
<td>Chest infection</td>
<td>22</td>
<td>74</td>
<td>4</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>Thalassaemia; infection with haemoptysis che X-ray normal complete recovery</td>
</tr>
<tr>
<td>F</td>
<td>56</td>
<td>+</td>
<td>Pulmonary tuberculosis</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Asymptomatic Mycobacteria isolated</td>
</tr>
<tr>
<td>F</td>
<td>30</td>
<td>+</td>
<td>Sarcoidosis</td>
<td>25</td>
<td>72</td>
<td>3</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>Active disease; erythema nodosum Hilar adenopathy</td>
</tr>
<tr>
<td>F</td>
<td>38</td>
<td>+</td>
<td>Asthma</td>
<td>45</td>
<td>52</td>
<td>3</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>Past and family history of asthma, urticaria and eczema</td>
</tr>
<tr>
<td>F</td>
<td>74</td>
<td>+</td>
<td>Squamous carcinoma of bronchus</td>
<td>55</td>
<td>43</td>
<td>2</td>
<td>&lt;1</td>
<td>Died</td>
<td></td>
</tr>
</tbody>
</table>

Mean age positive patients = 45 yrs. *: preparation fixed for scanning electron microscopy on day of lavage. BAL: bronchoalveolar lavage; Sm.: smoking; ND: not done; Lympho: Lymphocytes; Macro: Macrophages; Neutro: Neutrophil leucocytes; Eosino: Eosinophil leucocytes.

Table 2. - Clinical details in 14 patients showing no perlpolesis

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Sm.</th>
<th>Diagnosis</th>
<th>%Lympho</th>
<th>%Macro</th>
<th>%Neutro</th>
<th>%Eosino</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>61</td>
<td>+</td>
<td>Squamous carcinoma of bronchus</td>
<td>10</td>
<td>80</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>M</td>
<td>63</td>
<td>+</td>
<td>Oat cell carcinoma of bronchus</td>
<td>12</td>
<td>76</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>F</td>
<td>57</td>
<td>+</td>
<td>Squamous carcinoma of bronchus</td>
<td>8</td>
<td>91</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>F</td>
<td>65</td>
<td>+</td>
<td>Squamous carcinoma of bronchus</td>
<td>23</td>
<td>69</td>
<td>8</td>
<td>&lt;1</td>
</tr>
<tr>
<td>F</td>
<td>45</td>
<td>-</td>
<td>Pulmonary tuberculosis</td>
<td>7</td>
<td>66</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>M</td>
<td>53</td>
<td>+</td>
<td>Pulmonary tuberculosis</td>
<td>16</td>
<td>77</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>M</td>
<td>77</td>
<td>+</td>
<td>Squamous carcinoma of bronchus</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M</td>
<td>47</td>
<td>-</td>
<td>Oat cell carcinoma of bronchus</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M</td>
<td>66</td>
<td>+</td>
<td>Pulmonary embolus</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M</td>
<td>70</td>
<td>+</td>
<td>Metaplasia and haemoptysis</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M</td>
<td>29</td>
<td>-</td>
<td>Carcinoid</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>F</td>
<td>74</td>
<td>-</td>
<td>Pulmonary embolus with pleural effusion</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M</td>
<td>31</td>
<td>+</td>
<td>Haemoptysis, nil found</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>M</td>
<td>73</td>
<td>-</td>
<td>Haemoptysis, nil found</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Mean age negative patients = 57 yrs. Sm.: smoking; ND: not done; Lympho: Lymphocytes; Macro: Macrophages; Neutro: Neutrophil leucocytes; Eosino: Eosinophils leucocytes.

There were 11 males and 9 females, whose clinical details are given in tables 1 and 2; 60% were smokers.

Bronchoalveolar lavage, tissue culture and filming

One hundred and twenty millilitres of prewarmed phosphate buffered saline (PBS) was infused under lignocaine anaesthesia and immediately recovered onto ice. In cases of suspected lung tumour the extra lavage was performed on the opposite side. After filtration the cells were washed twice in PBS and resuspended at a concentration of 5x10^5·ml^-1 in culture medium (RPMI 1640) with 10% blood group AB serum or 10% fetal calf serum (FCS) with added penicillin and streptomycin. Fluids recovered from the lung are often contaminated with yeasts and fungi. We attempted to control this by adding mycostatic drugs including
amphotericin B, flucytosine, miconazole, mycostatin with gentamicin and econazole at recommended concentrations. These drugs were toxic for macrophages and lymphocytes and none had the desired effect of inhibiting the growth of contaminants.

At this time smears and cytospins were prepared from the cell concentrates and stained with May-Grunwald-Giemsa. Five hundred cells were counted in each preparation and the percentage of lymphocytes, alveolar macrophages, neutrophil leucocytes and eosinophil leucocytes calculated. Ciliated epithelial cells and squamous cells were not included in the total counts. The cells were transferred to a Petri dish with a glass inset which was mounted on an inverted phase contrast microscope, provided with a CO₂ gas supply and enclosed in a hot box at 37°C (fig. 1) [14]. This system allows observation and recording of cellular movement by time-lapse filming for up to 48 h. The lignocaine administered to the patients inhibited the movement of the lavage cells and filming was postponed until they had regained their motility, usually 3–4 h. Filming was carried out at a speed of one frame·3 s⁻¹, i.e. speeded up 72 times, using 16 mm Eastman colour negative. If electron microscopy (EM) studies were envisaged, the culture medium was removed at the end of the film and replaced by 3% glutaraldehyde.

Results

Peripolesis

Peripolesis was first observed 3.5 h after setting up cultures of lung washings and was also seen at 18–24 h. It occurred in 6 of the 20 preparations examined. Peripolesis was usually seen on the first and second days but in one case it was observed after 18 h, but not on the first day, possibly due to a prolonged effect of the anaesthetic. Lymphocytes moved randomly, but in positive cultures when one came within reach of a macrophage it turned sharply towards the macrophage, rapidly attached itself and began the characteristic circling movement, with extrusion of a lamellipodium in the direction of motion. Many complete circumnavigations were seen (fig. 2) lasting 3–8 min. Occasionally the lymphocyte changed course from clockwise to anticlockwise, or vice versa,

Electron Microscopy

In order to identify peripolesing cells in fixed preparations a map of the cells was made and reference points marked on the end of the dish.

For TEM the cells were embedded in a gel consisting of 10% bovine serum albumen (BSA) in PBS cross-linked with 3% glutaraldehyde. Although alveolar macrophages are strongly adherent they were easily incorporated into the gel. The position of the peripolesing group was marked on the surface, and the gel was then cut up and processed in the usual way. Thin sections were examined in a Jeol (JEM 1200 EX) electron microscope.

For SEM the glass inset with attached macrophages was cut down to approximately 6x2 mm so that it could be inserted into the stem unit of the electron microscope. A narrow copper strip was attached to the undersurface of the coverslip in line with the reference points and the glass was cut along the edge of the strip, after which the cells were processed for SEM, i.e. critical point drying and gold coating.
Fig. 2. - Frames from a time-lapse film of cells in a lung washing from a female patient with carcinoma of the bronchus. The sequence shows the phenomenon of peripolesis. a) A mobile lymphocyte (arrow) with its lamellipodium extended in the direction of movement was approaching an alveolar macrophage. b) The lymphocyte made contact with the macrophage. c, d, e and f) Clockwise peripolesis was in progress. In this case one complete cycle took 8 min (x600).

cultures, lymphocytes were seen to make short contacts with macrophages. Such cells extended themselves repeatedly away from the macrophage, but did not produce a lamellipodium and finally moved off. At no time did we observe emperipolesis with deep invagination of the target cell [6, 15–18].

Fig. 3. - Frames from a time-lapse film of cells from the same washing as figure 2. The alveolar macrophage developed long spiky processes and appeared to be injured while being subjected to peripolesis by a lymphocyte (arrow). The time interval between a and b was 3 min. c) 1 hour later, the lymphocyte detached itself. d) 25 min later, the lymphocyte moved away and the macrophage regained its normal appearance (x600).
**Alveolar macrophages**

The macrophage population was heterogeneous and included large, medium and small cells [1, 2]. Thirty percent had a distinct yellowish tinge. About 10% of the macrophages extended and retracted a fimbriated or club-shaped process (fig. 4). This structure could have been mistaken for an attached lymphocyte, but cine-observations showed that it came out in several bursts, of variable length and conformation, whereas attached lymphocytes extended and retracted themselves in a more repetitive manner. Sometimes a long thin process made contact with another macrophage and was then withdrawn.

**Other antigen-presenting cells**

Dendritic or veiled cells were seen in 3 of the 12 cultures recorded on film. These cells were easily identified in the living state. They were smaller than the macrophages, they moved at greater speed and continually extended long veils. Two of the washings were from patients with carcinoma of the bronchus and contained 25 and 27% dendritic cells; no peripolesis was seen in these cultures. The third washing from a patient with tuberculosis contained 10% dendritic cells. In this culture the macrophages were peripolesed, but the dendritic cells were not.

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**Fig. 4.** - a) Frames from a film of cells in a lung washing from a male patient with carcinoma of the bronchus. Alveolar macrophage with a fimbriated process (arrow). b) The same cell with process withdrawn (x600). c) Scanning electron micrograph of the macrophage illustrated in a and b. The cell body lies above and behind a circular protrusion covered with ruffles located in the area from which a process was extended and later withdrawn. Only a small proportion of macrophages possessed this parachute-like arrangement. The retracted process looks superficially like a lymphocyte, but we have proof from cine-viewing that no lymphocyte was present, also the deeply ruffled surface is quite different from the ridged surface of the lymphocyte illustrated in figure 5B. Bar=2 μ
Lymphocytes

The number of lymphocytes in the washings varied widely. In stained preparations of the cell concentrates, percentages ranged from 7-55%. In normal subjects there are on average 10% using cytocentrifuge preparations but up to 20% using an improved method [19].

A

Fig. 5. – A) Transmission electronmicrograph (EM) of lymphocyte peripolesing alveolar macrophage in clockwise direction. Washing was from female patient with non-recurrent haemoptysis. Lamellipodium at leading end and mitochondria behind nucleus. Area of contact between the cells is almost flat with small indentations. Free surface of macrophage has processes, except in the area immediately behind peripolesing lymphocyte. Bar=2 µ.

B

TEM and SEM

Figure 5A shows a lymphocyte in the act of peripolesing a macrophage. The cells were filmed and the clockwise direction of motion was correlated with the EM appearance. The lamellipodium at the advancing pole of the lymphocyte can be seen interdigitating with the ruffled surface of the macrophage. Behind the lamellipodium the under-surface of the lymphocyte is flat and closely applied to the macrophage, which is here devoid of processes. There are a few very short interdigitations between the cells in this area, which suggests that the lymphocyte actively flattens the macrophage membrane as it travels round. As usual, during movement the lymphocyte organelles are gathered behind the nucleus. No large granules, or parallel tubular arrays are present, therefore the peripolesing cell was probably not a natural killer (NK) cell [20].

Figure 5B shows a scanning EM of another peripolesing cell. In this case the lymphocyte was moving anti-clockwise. The surface of the macrophage is covered with fairly large ruffles, but the lymphocyte appears smooth, except in the region of the lamellipodium.

Clinical correlations

In the group of six patients whose cells exhibited peripolesis there was one case of carcinoma of the bronchus, one of sarcoidosis, one of pulmonary tuberculosis and one of asthma; the remaining two patients, including the only male, had slight bleeding and no lung disease was found (table 1). These six patients had a mean age of 45 yrs compared with 57 yrs for the negative group, and 5 out of 6 were females. About half were smokers in each group. Peripolesis occurred in washings containing an increased proportion of lymphocytes. In stained preparations the percentage of lymphocytes ranged from 21.8-58.2 mean 35.6% (95% confidence interval 21.8-58.2). In six smears from negative cases it was significantly less, mean 11.6% (95% confidence interval 7.2-18.5); two sample t-test (p=0.01). Unfortunately the remaining negative smears were lost.

In 14 out of 20 washings no peripolesis occurred and the range of diagnoses was similar to the group showing the phenomenon (table 2). Eight of the negative cultures were looked at continuously from 3.5-6 h and also at 18-21 h. The other six cultures were negative on the day of lavage, but could not be examined on the following day, owing to contamination. It is likely that the majority would have been negative at 18 h since, only one of the six cases showing peripolesis became positive on the second day.

Discussion

Previous investigations on cells in lung washings [1, 2] concentrated mainly on the morphology and secretory activity of macrophages, the proportion of lymphocytes and the effects of smoking. More recently the phenotypes of the cells have been examined with monoclonal antibodies [21–23].
We set out to film the behaviour of living cells and this led to the observation of peripolesis, which had not previously been described in alveolar cells, although still photographs of lymphocyte and macrophage rosettes have been published [4, 24]. Peripolesis can be studied without film or video equipment by looking at the cells at 37°C for at least one hour. If nothing happens on the first day the cultures can be viewed again the next day after incubation overnight.

Other workers have observed peripolesis of thyroid epithelial cells [6, 25] and accessory cells [7-11]. Cytotoxic injury to the peripolesed cells was noted in gut preparations made from patients with ulcerative colitis [26], but in autoimmune thyroid no cell killing was observed [6]. In the present study of peripolesed alveolar macrophages, a small proportion developed a spiky appearance suggestive of injury, but later recovered. This appearance was also seen in dendritic cells after an attack by a killer cell and was followed by death of the target cell (unpublished observation).

Peripolesis occurred in washings with high lymphocyte counts (22-55%), whereas only one of the negative cases had a borderline raised count of 23%. To see if blood lymphocytes were capable of this behaviour we chose a washing fluid with a low lymphocyte count and negative peripolesis and added up to 50% autologous peripheral blood lymphocytes to the culture. No peripolesis was seen on the first or second days. This preliminary experiment suggests that lung lymphocytes are a special population. The phenotype of the peripolesing lymphocytes has not yet been determined. The cell examined by TEM was a small lymphocyte without the ultrastructural characteristics of NK cells which have been implicated in emperipolesis of malignant cells [16, 17]. Peripolesis was more common in women (p=0.05, Fishers exact test). In view of the close correlation with the lymphocyte count one might expect females to have higher counts. However, previous BAL studies on normal subjects or smokers did not support this suggestion [1]. Possibly in disease states the lung lymphocytes increase more sharply in female patients. Obviously our clinical sample is too small to detect any firm correlation between individual diseases and lung peripolesis.

Dendritic cells were found in three of the washings, in one case the macrophages were peripolesed, but not the dendritic cells, an interesting difference requiring confirmation. In animal experiments veiled cells (precursors of dendritic cells) from the afferent lymph of rabbits immunized with human gammaglobulin (HGG), were pulsed with the antigen and mixed with autologous blood lymphocytes. Peripolesis of the veiled cells was observed in two of the four cultures, but was not seen in numerous preparations from rabbits immunized with an unrelated antigen [11]. This suggested that surface immunoglobulin might be the signal for the phenomenon. Alveolar macrophages have numerous receptors able to capture immunoglobulin [27] and the flattening of the macrophage membrane during lymphocyte movement would expose the Fe-receptor bearing part of the surface, which lies in the clefts between the membrane ruffles [28], so ensuring contact with the attached immunoglobulin. In HGG cultures peripolesis of the veiled cells was sometimes halted by the attachment of two more lymphocytes to the peripolesing cell, producing a configuration typical of antigen presentation by cells of this type, i.e. one lymphocyte attached to the veiled cell and two more attached to this cell [11]. Antigen-presenting macrophages form similar clusters [29, 30], but there are no reports suggesting that peripolesis of the macrophages precedes, or is associated with, this type of activity. We never observed the attachment of additional lymphocytes to the peripolesing cells in our BAL preparations, but it might occur in advanced cases of sarcoidosis.

The main function of alveolar macrophages is down-regulation of the immune response in the lung [31]. Only one third of the macrophages were peripolesed, which probably reflects the heterogeneity of the population with respect to surface markers [32-34] and the secretion of cytokines [31].

Mandatory investigations to clarify the role of peripolesis in BAL fluids are as follows: to enrich the cultures with lung lymphocytes as well as blood lymphocytes in order to increase the incidence of peripolesis. This would greatly facilitate the phenotyping of the peripolesed macrophages which may represent a non-secretory subgroup. The phenotypes of the lymphocytes and their state of activation should also be determined. Of further interest would be to study precisely defined and larger groups of patients with interstitial lung diseases in order to reveal possible clinical correlations with disease state and activity.

Acknowledgements: The authors thank G.L. Asherson for his support, J.L. Mackenzie for preparing still photographs from the films, N. Webb for the diagram of the apparatus and for the layouts and N. Saunders for typing the manuscript.

References


