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Cells in Peripheral Leg Lymph of Normal Men

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Summary

The cells of human peripheral lymph collected from leg lymphatic of healthy volunteers have been studied by light- and electron microscopy. More than 80% of the cells were lymphocytes. The rest of the cells were neutrophils, monocytes, phagocytes, intermediate forms between lymphocytes and monocytes, erythrocytes and large cells. Ultrastructurally these large cells had many features in common with the Langerhans cell sof normal human epidermis and may represent this cell type or a closely related one.

There is a large scale migration of cells from the blood vascular system into lymph. Lymphocytes continually leave the circulation through post-capillary venules in the lymph nodes and enter the efferent lymph (1). However, some lymphocytes also migrate through the blood vessel into non-lymphoid tissue (for instance sub-cutis) and hence into the lymphatics and back to the lymph nodes (2, 3). Experiments in animals have revealed that the peripheral lymph contains few cells compared with the intermediate and central lymph which, after passage through lymph nodes, has been enriched by numerous lymphocytes. The kinetics of circulation of granulocytes, monocytes and erythrocytes is not known to the same extent although these cells also migrate from the blood into the tissue and can be found in peripheral lymph. In animals a significant difference has been found between the number of macrophages in peripheral and central lymph. Macrophages present in peripheral lymph are only rarely seen in lymph after it has passed through a lymph node and are lacking in central lymph (3).

The cellularity of peripheral lymph in normal men has been studied in this laboratory for many years. As in animals we have found a low number of cells which are mostly small lymphocytes with some few monocytes and erythrocytes. Granulocytes are extremely rare. We have observed, however, a great variation in cell output depending on the position of the body and physical activity. In all groups studied we have found that there is an increased output of cells after rest in horizontal position (for details see 4 and 5). For instance in the early morning samples after night rest the mean output of lymphocytes is about 80 times that of the night. Throughout the day output drops to about 10 times that of the night. The high output of cells in the morning is probably caused by a wash-out of cells which have been accumulated in the tissue and lymph capillaries during night rest when the lymph flow is close to zero. The output of monocytes and erythrocytes follows the same pattern as the output of lymphocytes. This was also the case when the volunteers took part in experiments increasing capillary filtration like ergometer cycling, leg venous hypertension and warming of the foot. Following some of these experiments a marked increase in cell output was observed.

Our studies have shown that the variation in cell output in peripheral leg lymph is caused mainly by variation in lymph and blood flow in the tissue and not by variation in the capillary wall's permeability for cells (4, 5).

Peripheral lymph contains also a variable number of very large cells probably belonging to the macrophage group. In a group of healthy volunteers these cells were studied by light and electron microscopy.

Material and methods

The study was performed on 3 healthy volunteers aged 19 to 22 in which peripheral lymph

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was collected from cannulated superficial leg lymphatic according to the techniques previously described (6).

Leg lymph was sampled for 3 hours in the middle of the day during normal everyday activity at day 2 after cannulation. Heparin was used as anticoagulant. Smears for differential counts were made on concentrations prepared with a Shandon Cytocentrifuge operated at 800 r.p.m. for 8 minutes. After fixation the smears were stained with May Grünwald-Giemsa and tested for acid esterase activity (7). Differential counts were performed by counting 1000 cells for each volunteer. Damaged cells which could not be identified were excluded.

For electron microscopy the lymph was centrifuged and the pellet fixed in 2% phosphate buffered glutaraldehyde, postfixed in osmium tetroxide, dehydrated through graded ethanols and embedded in an Epon-Araldite mixture (8). Semithin sections were made with an LKB Pyramitome equipped with glass knives and stained with toluidine blue for light microscopic evaluation and comparison with the smears. Ultrathin sections were cut with diamond knives mounted on an LKB Ultratome III or IV, collected on naked copper grids, contrasted with uranyl acetate and lead citrate and examined in a Philips EM 201 electron microscope.

Normal human skin was processed for electron microscopy in a similar way and used as a control for Langerhans cells and Birbeck granules.

Results and discussion

About 15% of the cells in peripheral lymph were erythrocytes. Differential counts of the nucleated cells revealed that 83% of the cells were small and medium sized lymphocytes. Most of the lymphocytes had an invaginated or kidney shaped nucleus and a high nucleus cytoplasma ratio and a typical chromatin pattern.

It was however difficult to distinguish all of them from small monocytes and it might be that some of the cells interpreted as lymphocytes belong to the monocyte group. About 3% of the cells were morphologically intermediate forms between lymphocytes and monocytes and could not be placed in any of these groups. Neither did the testing for acid esterase give any sharp distinction between these two cell types because in the cells morphologically classified as lymphocytes there was a variable esterase activity, from intense (localized or diffuse) to low or no activity. More than 50% of typical lymphocytes contained large esterase positive dots which might indicate that they are T cells (7).
The 5.5% typical monocytes and 0.5% large lymphocytes were similar to these seen in blood smears in man.

About 6% of the cells were classified as phagocytes. They had abundant, vacuolized cytoplasm and different amounts of phagocytized material. Most of them were esterase positive with a diffuse staining of the cytoplasm. Some of them had numerous cytoplasmic extensions.

Neutrophils constituted 0.9% and eosinophils 0.2% of the cells.

Some of the mononuclear cells showed radial nuclear segmentation. Such segmentation appears in blood cells left at room temperature but the nature of this phenomenon is unknown (9). The fact that the lymph in our study was collected into a vial fastened to the leg at room temperature may explain the radial segmentation in some of the cells.

Our interest was focused on the presence of 1.5% large mononuclear cells 20–30 μm in diameter with abundant pale cytoplasm often with small vacuoles. A very large Golgi zone occupied most of the cytoplasm. The cell border was unsharp with many cytoplasmic extensions. The nucleus was round or kidney shaped and excentrically located. There was a fine chromatin pattern similar to that seen in reticulum cells. Some of the cells had a visible nucleolus. The cells were esterase negative or slightly positive.

By electron microscopy their nuclei were seen to have marginated chromatin and a folded nuclear membrane, in particular where this faced the centre of the cell (Fig. 1). The Golgi apparatus was composed of vesicles, saccules and dense bodies interpreted as lysosomes. In addition, numerous rod-, racket- or circularly-

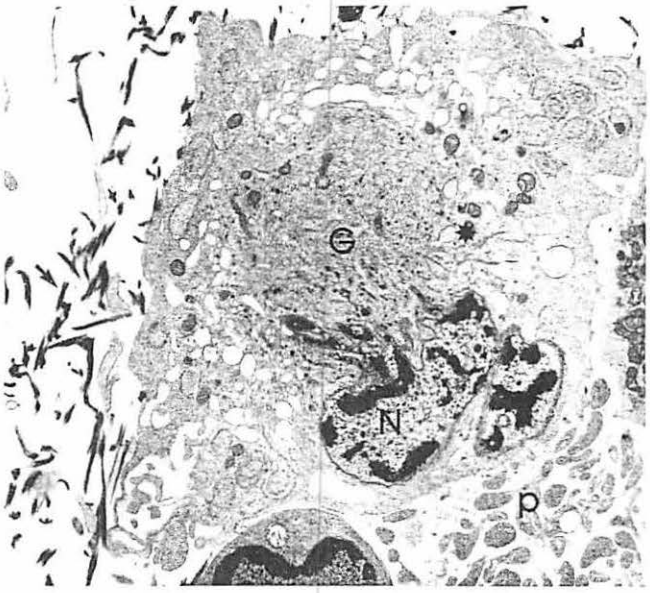

Fig. 1 "Large cell" with an excentric indented nucleus (N), an extensive Golgi apparatus (G) and numerous cytoplasmic projections. The asterisk marks the area shown at higher magnification in Fig. 2. Strands of fibrin are seen outside the cell (arrow). Electron micrograph. Uranyl acetate and lead citrate stain. Magn. 5 500x.

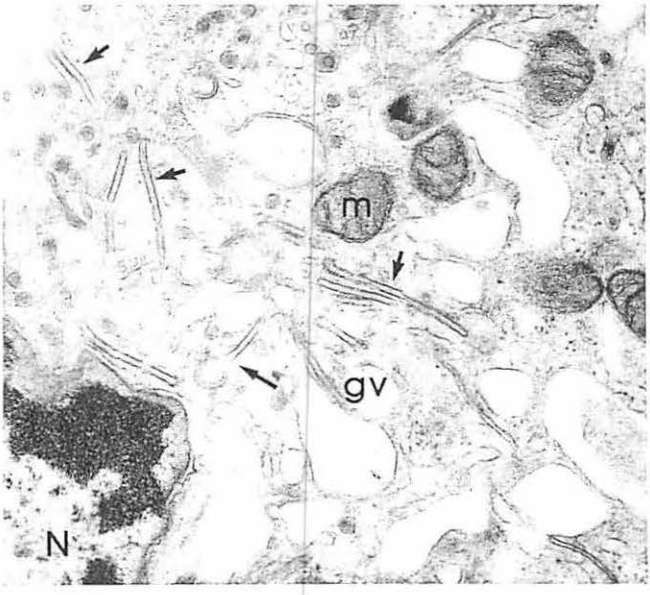

Fig. 2 Larger magnification of the Golgi area showing vesicles (gv) rod shaped (short arrows) and racket shaped (long arrow) granules. Mitochondrias are also seen (m) and a part of the nucleus is shown at lower left (N).
Electron micrograph. Uranyl acetate and lead citrate stain. Magn. 24750x.

shaped bodies were seen in the Golgi region (Figs. 2–4). The rod shaped granules were 400 Å wide and up to 3000 Å long. They seemed to be composed of three lamellas where the one in the middle was barely visible. In areas a faint cross-striated periodicity was observed. The bleb-like dilatation of the racket-shaped bodies bore a strong resemblance to the Golgi vesicles.

The cytoplasm furthermore contained a few and usually collapsed cisternas of rough endoplasmic reticulum, scattered ribosomes and polyribosomes, and normal mitochondria. The majority of the cells showed numerous and

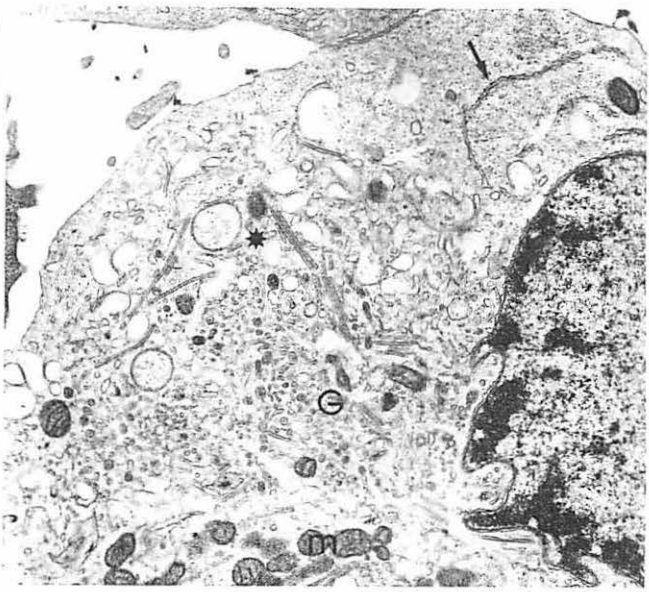

Fig. 3 The extensive Golgi region (G) of another "large cell" containing numerous, and mostly rod shaped granules. The asterisk marks the area shown at higher magnification in Fig. 4. Inconspicuous cisterna of rough endoplasmic reticulum (arrow) are also seen as well as several mitochondrias (m).
Electron micrograph. Uranyl acetate and lead citrate stain. Mag. 11000x.

finger-like cytoplasmic extensions. At the ultrastructural level these cells resembled the so-called Langerhans cells. These were described over 100 years ago in the epidermis and although they have been subject to numerous studies since, their origin and function is still somewhat obscure (10, 11, 12).

Ultrastructurally these cells are characterized by a markedly indented nucleus and an abundant cytoplasm with an extensive Golgi apparatus and characteristic rod-shaped, tennis-racket-shaped granules sometimes referred to as Birbeck granules. (Fig. 5–6). The rod-shaped structures or the racket handles show a median striated line which represents a paracrystalline net or lattice with a 90 Å periodicity.

While some workers believe that the granules represent a unique variety of endocytotic vesicles others regard them as "secretory" granules arising from the Golgi apparatus and discharging their contents to the cell surface. Although this question remains unsettled the Birbeck granules remain the most important characterizing hallmark of Langerhans cells. These specific granules have now been found in various squamous epithelia of man and experimental animals, in histiocytosis X, in reticulum cell sarcoma, in macrophages of

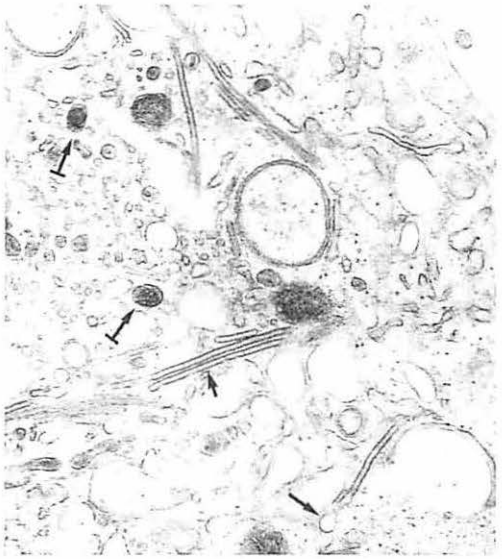

Fig. 4 The area of the Golgi region shown at higher magnification and containing rod shaped granules (short arrow) incipient racket shaped granules (long arrow) as well as dense bodies (labelled arrows). Electron micrograph. Uranyl acetate and lead citrate stain. Magn. 22 000x.

pityriasis rosea, in human lymph nodes of dermatopathic lymphadenopathy as well as in normal lymph nodes of rabbit and hyperplastic

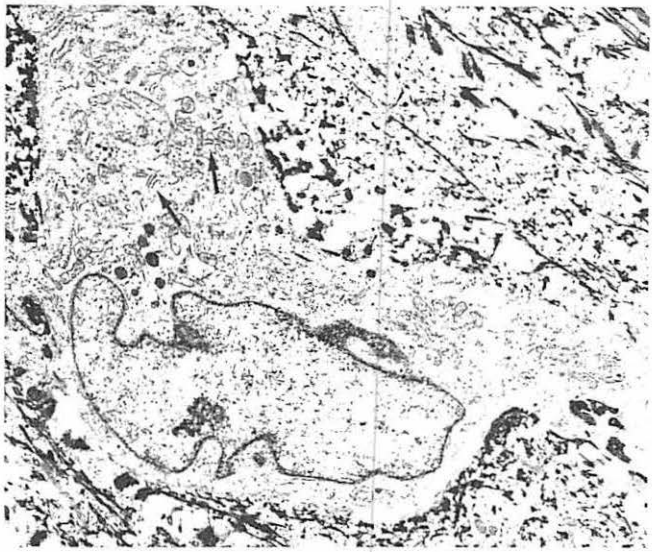

Fig. 5 Langerhans cell in the normal human epidermis surrounded by keratinocytes. At this magnification the Birbeck granules are barely visible (arrows).
Electron micrograph. Uranyl acetate and lead citrate stain. Mag. 5500x.

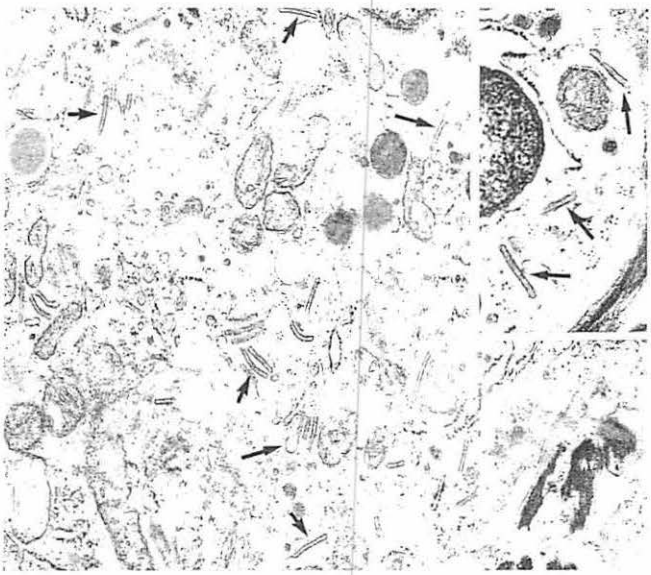

Fig. 6 Epidermal Langerhans cell at higher magnification revealing rod shaped (short arrows) and racket shaped (long arrows) Birbeck granules. Note their faint median line. Inset shows rod shaped granules at higher magnification (arrows). The median line is barely visible.
Electron micrographs. Uranyl acetate and lead citrate stain.
Magn. 16 500x and 24 750x (inset).

lymph nodes of man (for reference see review in 13).

The Langerhans cells are now regarded as histiocytes with phagocytic properties. They have been seen dividing and also crossing basement membranes (13).

The large cells found in human peripheral lymph in the present study share many properties with the Langerhans cell. The observed granules resemble the Birbeck granules, although the median striated line is somewhat less well demarcated and furthermore lacks a distinct cross striation.

The Langerhans cells found in the control specimen also contained granules with a rather faint median line (Fig. 5–6). We therefore interpret the large cells found in peripheral lymph as Langerhans cells or relatives of such

cells, although the possibility exists that the observed granules are more closely related to those found in some paracortical interdigitate cells where the axial cores and the cross striations are missing.

Our results are only representative for the part of peripheral lymph which drains skin, subcutaneous tissue and perimuscular tissue of the foot, and the cell content only representative for "everyday activities".

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