

LYMPHOCYTE-EPIDERMIS INTERACTIONS IN MALIGNANT EPIDERMOTROPIC LYMPHOMAS: I. ULTRASTRUCTURAL ASPECTS

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Abstract. The interactions between lymphocytes, Langerhans cells and keratinocytes, are described by electron microscopy in cutaneous lesions of malignant epidermotropic lymphomas (mycosis fungoides and Sézary's syndrome). Various contacts are observed between the different cells. During the degenerating process of keratinocytes, Langerhans cells disappear and histiocytic cells are bound to necrosing epidermal cells. The immunological process of Pautrier microabscess formation is discussed by comparison with ultrastructural aspects of cutaneous lesions of G.V.H. reaction in man.

Key words: Mycosis fungoides; Sézary's syndrome; Epidermis; Lymphocytes.—Langerhans cells —Keratinocytes interactions; Electron microscopy

Among the malignant cutaneous lymphomas, the epidermotropic lymphomas (mycosis fungoides, Sézary's syndrome and Worringer-Kolopp disease) are characterized by dense dermal infiltrates and epidermal exocytosis of proliferating cells in skin lesions. Tumoral cells infiltrate the epidermis and give rise to Pautrier microabscesses by lysis of keratinocytes. A preferential replication of proliferating cells appears in the epidermis (19). The attraction and exocytosis of the tumoral cells into the epidermis seems to be the morphological aspect of an immunological process. In this way, the epidermis is considered as the target and the origin of antigens which are implicated in the immunological process.

The origin of the exocytosis is unknown and various hypotheses are proposed to explain this phenomenon: either an antigenic modification of the epidermis or a modification of recognition capacities of the proliferating lymphoid cells (13) against the epidermis.

To elucidate the nature of exocytosis stimulation and the formation of epidermal lesions, we have

performed an ultrastructural, immunological and experimental study of the various cells present in cutaneous lesions.

We report in this paper the ultrastructural aspects of the lymphocyte-keratinocyte interactions in lesions of mycosis fungoides and Sézary's syndrome.

MATERIAL AND METHODS

Patients

Twelve patients, typical cases of mycosis fungoides or Sézary's syndrome, were considered. Clinical data of the patients are summarized in Table I. Specimens of skin were obtained by means of punch biopsy under local anaesthesia. All specimens were obtained from infiltrative lesions before treatment and especially before oral photochemotherapy (11). Each specimen was examined by standard light microscopy after paraffin embedding, by light microscopy after embedding in epoxy medium, and by electron microscopy.

E.M. studies

For electron microscopy, the biopsy specimens were fixed in 2% glutaraldehyde for 2 hours, post-fixed with osmium tetroxide for 2 hours, dehydrated in alcohol and embedded in epoxy medium. After polymerization, semi-thin sections were obtained on Reichert ultramicrotome and stained with Azur II methylene blue. Ultrathin sections were stained with uranyl acetate and lead citrate. The observation was performed with Hitachi HU 12 A and Philips EM 300 electron microscopes.

RESULTS

(A) Histological findings

In all the cases studied, the standard routine histopathology of skin lesions revealed the presence of proliferating lymphoid cells which infiltrate both the upper dermis and the epidermis.

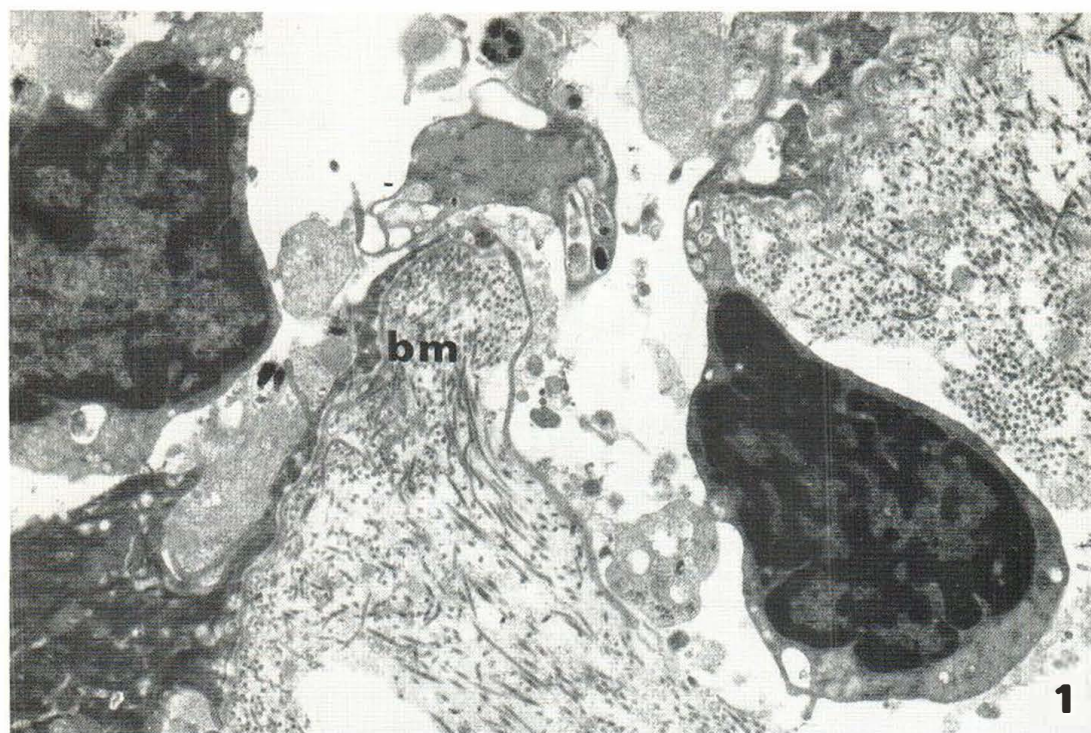


Fig. 1. Disruption of the basement membrane zone (bm) during the migration of proliferating lymphoid cells. $\times 11\,400$.

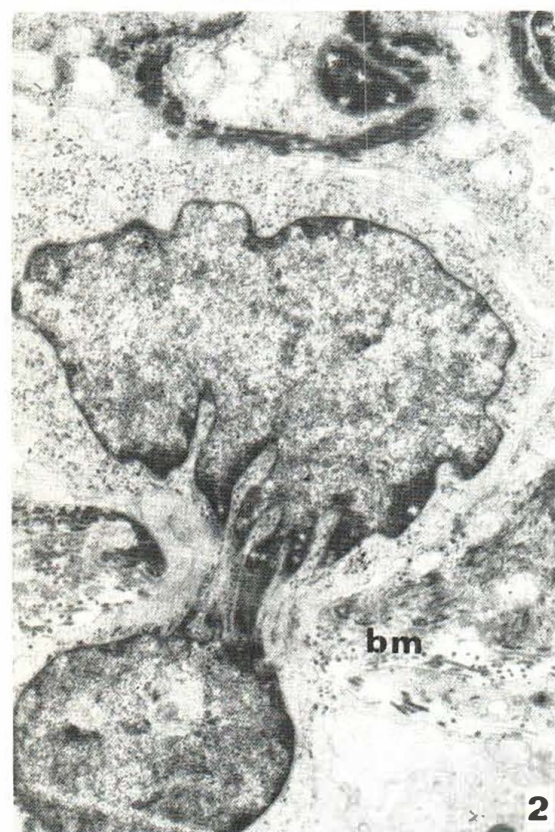


Fig. 2. Detail of the nuclear aspects of the mononuclear cell during migration through the basement membrane zone (bm). $\times 9\,000$.

The epoxy sections observed by light microscopy show the presence of Pautrier microabscesses, intra-epidermal aggregations and isolated lymphoid cells. In Pautrier microabscesses, Sézary cells with dense and lobulated nuclei can be seen.

(B) Ultrastructural findings

If we disregard the different and consecutive steps of the process we can describe three different basic situations in the epidermis lesions:

(i) the passing of the proliferating cells through the basement membrane zone (BMZ) and the presence of individual lymphoid cells in the lower part of the epidermis.

(ii) the association or binding of lymphocytes (L), Langerhans cells (LC) and keratinocytes within the epidermis.

(iii) the binding of lymphocytes, histiocytic cells

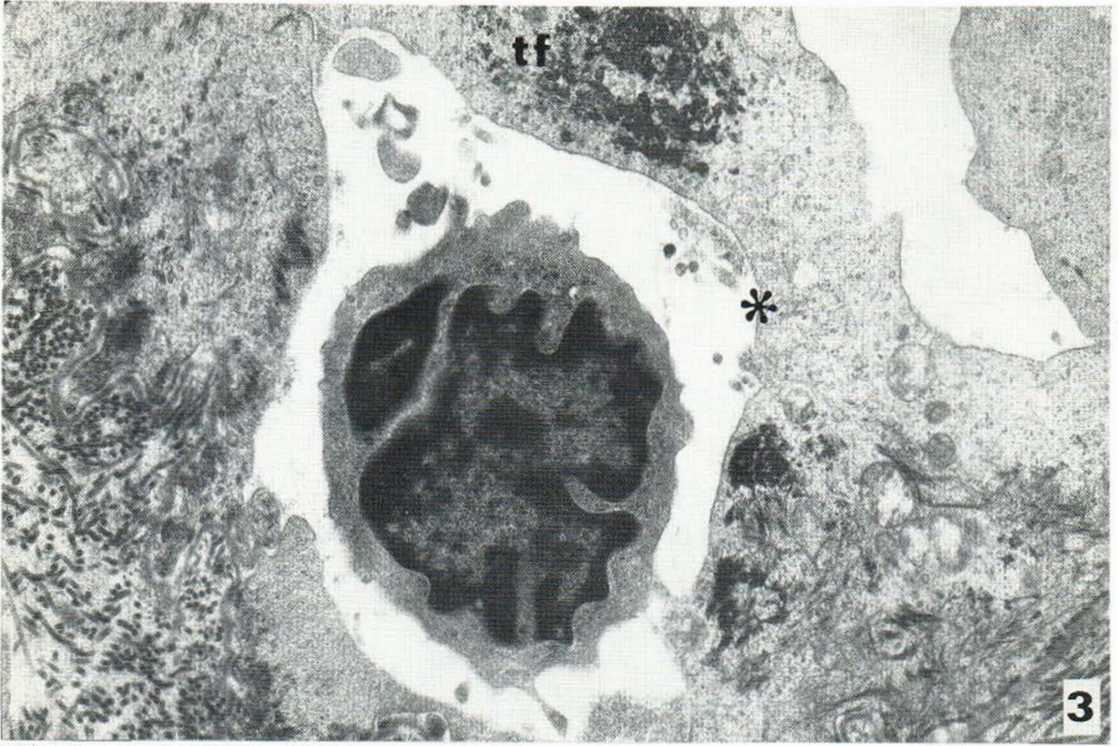


Fig. 3. Isolated lymphocyte in the lower layer of the epidermis. Disappearance of desmosomes on keratinocyte membrane (*) and formation of tonofilament aggregates. $\times 13\,300$.



Fig. 4. Formation of Pautrier microabscesses with lymphoid cells and Langerhans cells. $\times 7\,500$.

and keratinocytes, and the presence of Langerhans cells in the upper part of the epidermis.

(a) *Dermal-epidermal junction and proliferating cells.* The proliferating cells pass through the BMZ by disruption of the basement membrane lamina and disruption of the desmosomes of the basal keratinocytes (Fig. 1). The lymphoid cells passing through the BMZ display an elongated nucleus (Fig. 2).

(b) *Individual lymphoid cells in the epidermis.* Frequently, individual lymphoid cells were seen penetrating the lower layer of the epidermis. These cells are of various sizes and have electron-dense nuclei which are more or less indented. Isolated epidermal lymphocytes do not show any particular contacts with the adjoining keratinocyte cytoplasmic membranes. These keratinocytes often lack desmosomes and display an aggregation of keratin tonofilaments (Fig. 3).

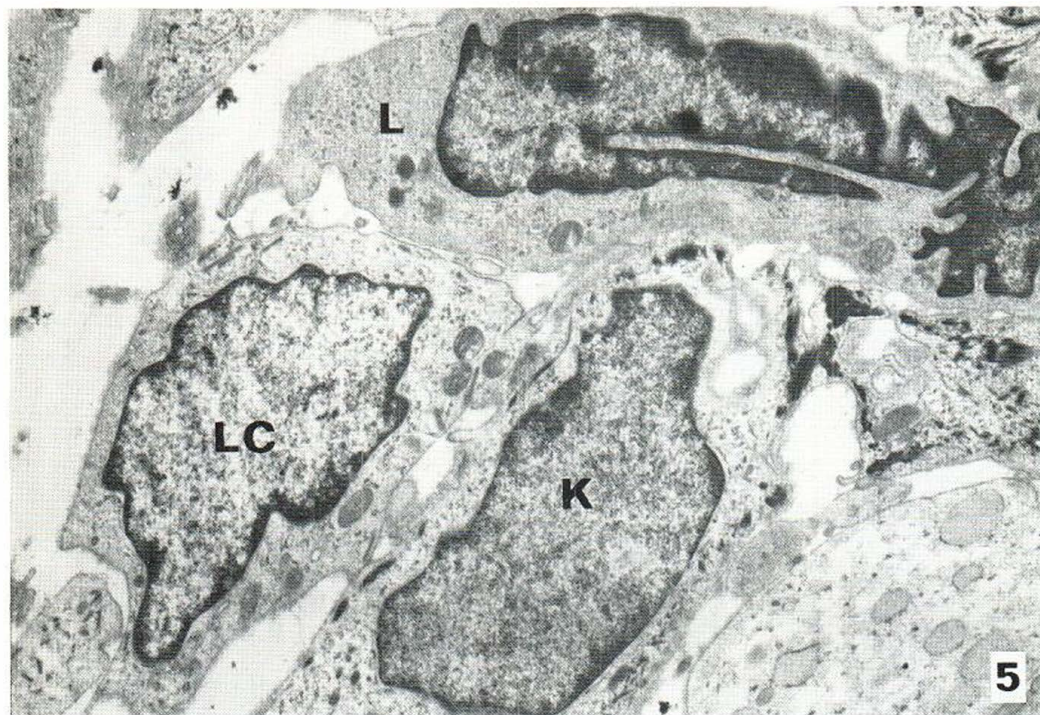


Fig. 5. Contacts between Langerhans cell (LC), lymphocyte (L) and keratinocyte (K) in a Pautrier microabscess. $\times 11\,700$.

(c) *Association between lymphocytes, Langerhans cells and keratinocytes.* In Pautrier microabscesses, various mononuclear cells can be observed (Fig. 4). Lymphocytes are sometimes associated with Langerhans cells characterized by

presence of Langerhans granules. Numerous contacts can be observed between lymphocytes, Langerhans cells and keratinocytes (Fig. 5). The formation of Langerhans granules, which become contiguous with the cytoplasmic membrane, takes

Table 1. *Clinical features of patients studied*

MF=mycosis fungoides; SS=Sézary's syndrome; M=male; F=female; ND=not done

Patient	Age (year)	Sex	Diagnosis	Biopsy site	Sézary cells in lymph nodes	Sézary cells in peripheral blood
1	34	M	SS	Leg	+	+
2	71	M	MF	Trunk	+	+
3	62	F	MF	Abdomen	ND	ND
4	34	M	MF	Trunk	0	ND
5	57	M	MF	Trunk	ND	ND
6	69	F	MF	Neck	0	0
7	72	F	SS	Trunk	ND	+
8	30	M	MF	Trunk	+	+
9	67	F	SS	Leg	ND	ND
10	49	M	MF	Trunk	0	0
11	58	M	MF	Trunk	+	+
12	76	M	MF	Trunk	+	+

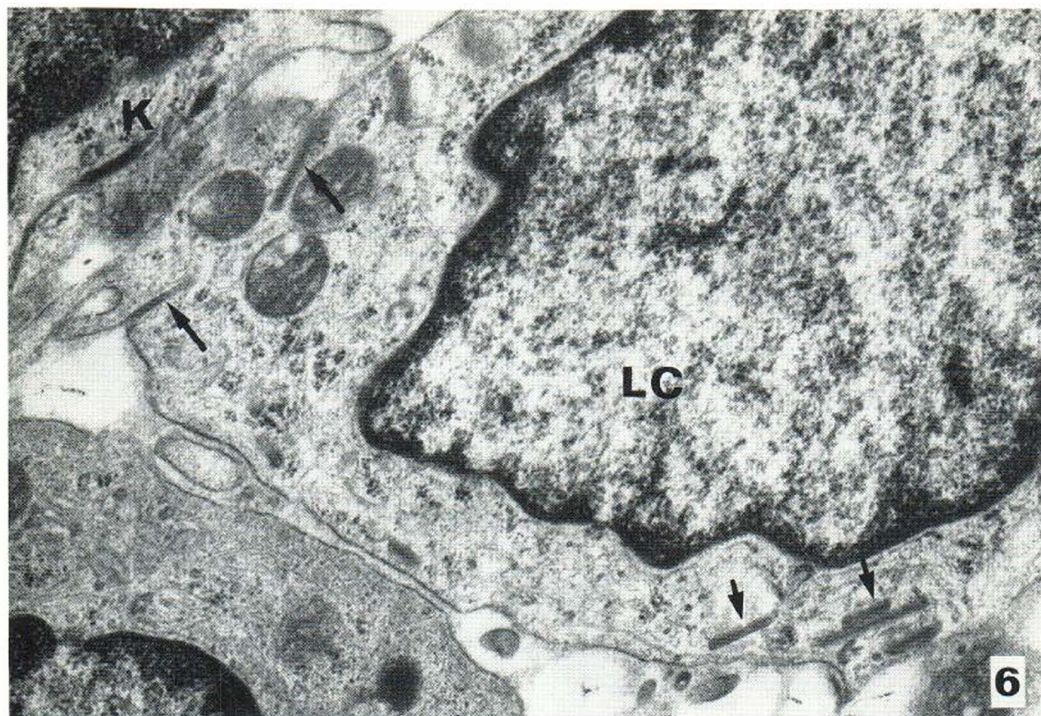


Fig. 6. Detail of the contact between Langerhans cells (LC) and keratinocytes (K) with Langerhans granules

being extended by cytoplasmic membrane of LC (arrows). $\times 22\,500$.

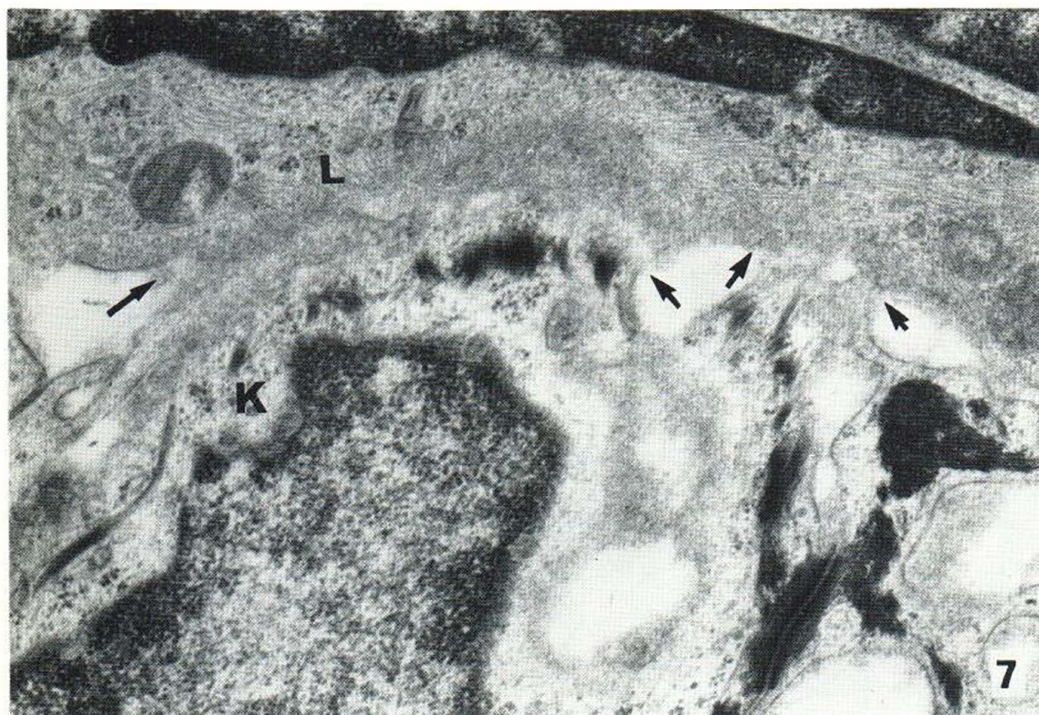


Fig. 7. Detail of contact between lymphocyte (L) and keratinocyte (K) in a Pautrier microabscess. $\times 27\,000$.

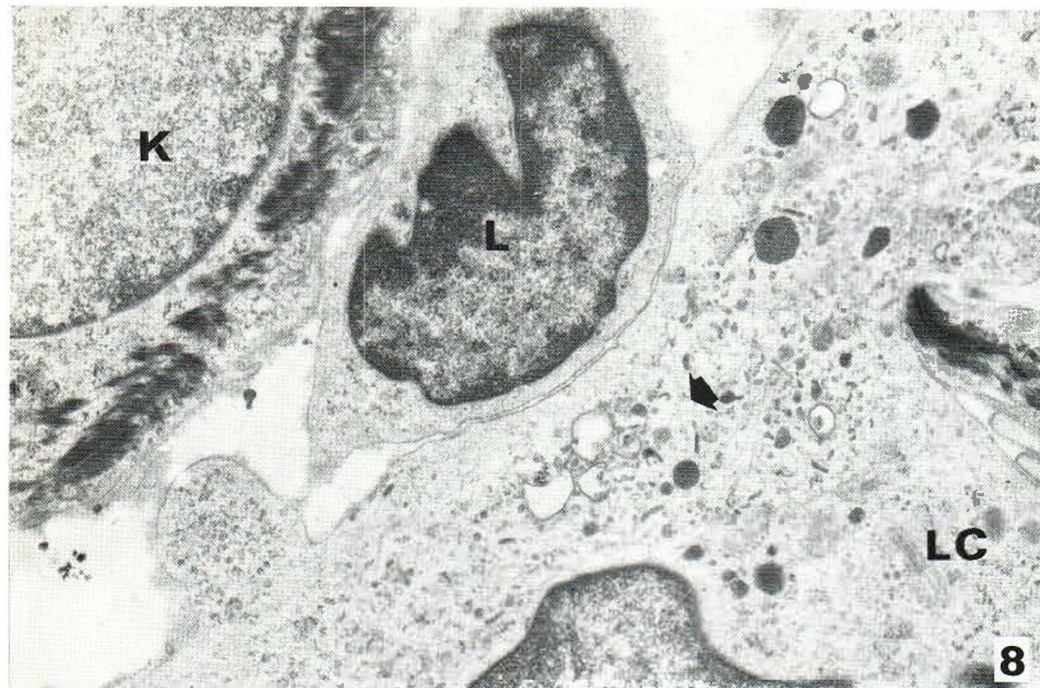


Fig. 8. Detail of the cytoplasm (arrow) of a Langerhans cell (LC) during interaction with keratinocyte (K) and lymphocyte (L). $\times 13\,500$.

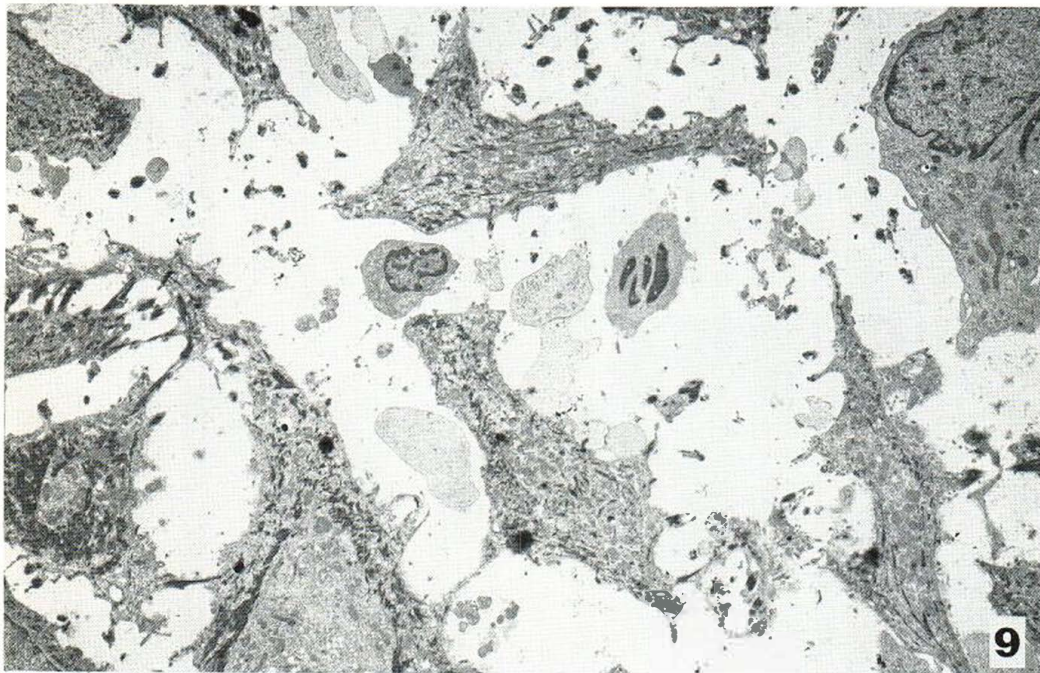


Fig. 9. Disruption and disappearance of desmosomes and isolation of keratinocytes in a Pautrier microabscess $\times 3\,420$.

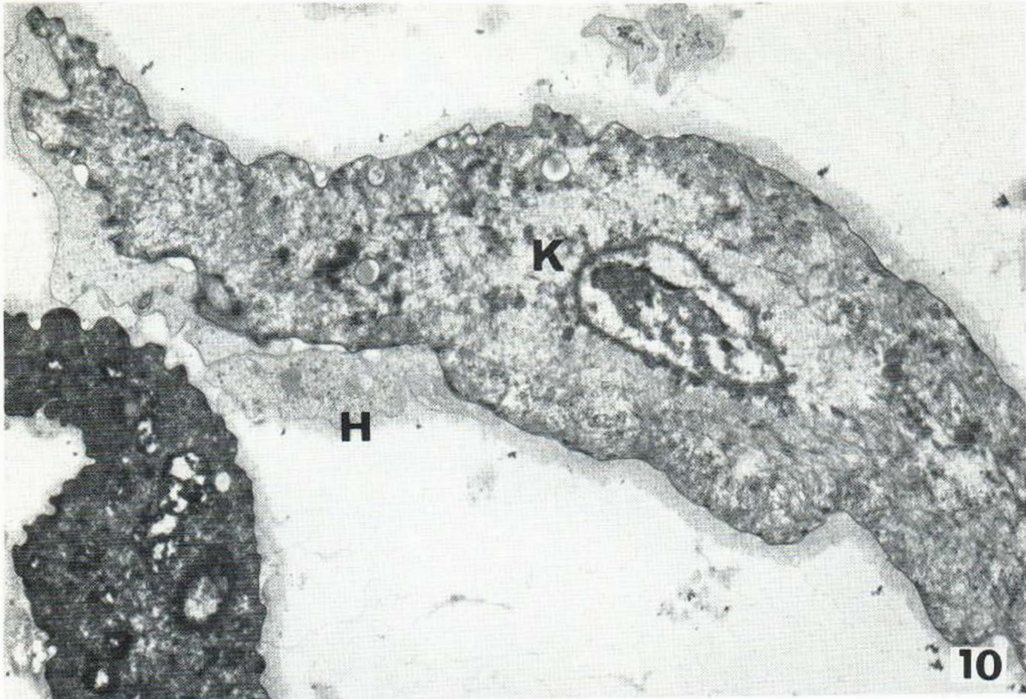


Fig. 10. Degenerating keratinocyte (K) with disappearance of cytoplasmic organelles, lipid droplets and electronic densification. H: histiocytes. $\times 7\,200$.

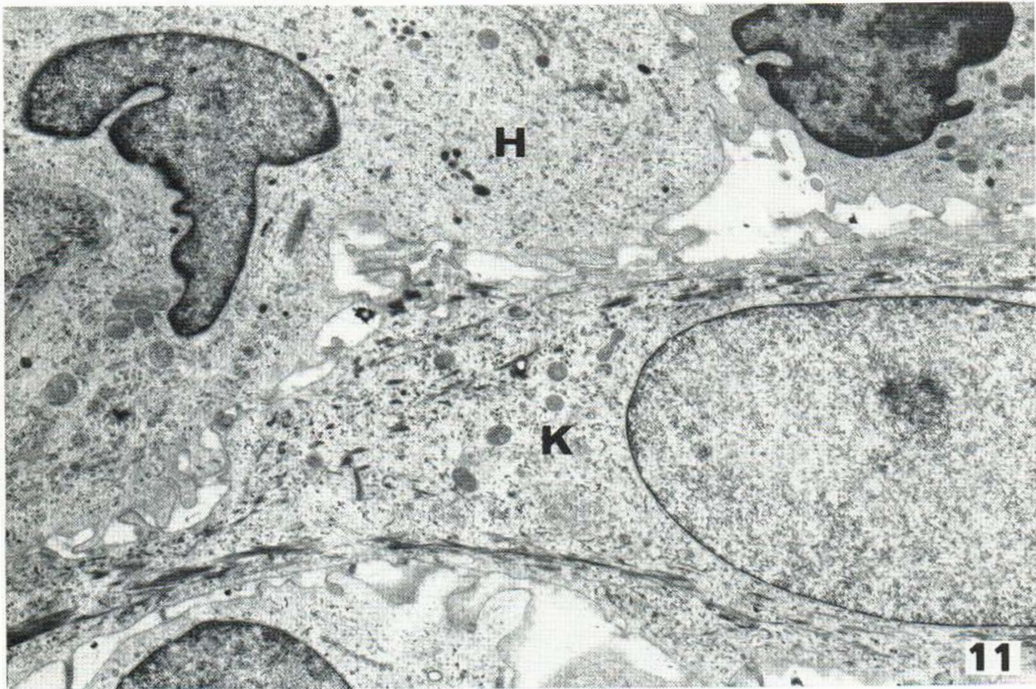


Fig. 11. Aspects of histiocytes (H) during the interactions between lymphocyte and keratinocyte (K). $\times 6\,300$.

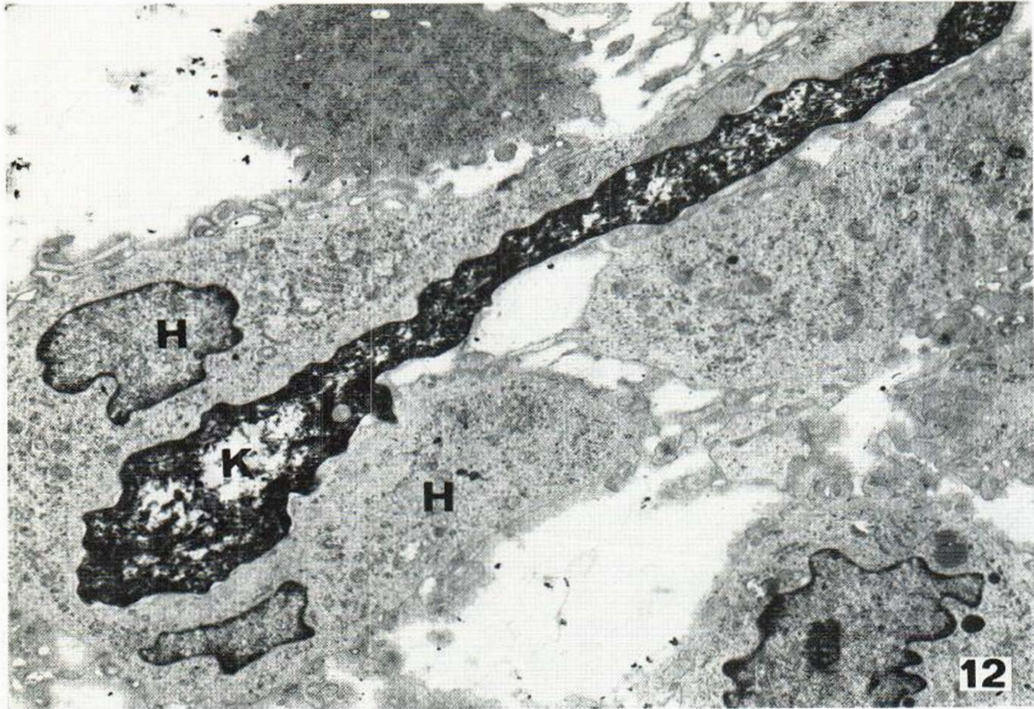


Fig. 12. Degenerating keratinocyte surrounded by histiocytes (H). $\times 4500$.

place at the contact point between keratinocytes and Langerhans cells (Fig. 6). Each cytoplasmic membrane loses its electron density in the area of contact between lymphocytes and keratinocytes (Fig. 7).

The cytoplasm of Langerhans cells often contains Langerhans granules, vacuoles, dense granules, microfilaments and microtubules (Fig. 8). In this area, keratinocytes often lack desmosomes and show a decrease in tonofilament aggregates. Disruption of the desmosomes allows an isolation of the keratinocytes (Fig. 9). These degenerating cells lack cytoplasmic organelles and display localized disruption of the nuclear membrane and dense clumps of tonofilaments (Fig. 10). This suggests an irreversible process.

(d) *Association between lymphocytes, histiocytic cells, and keratinocytes.* In Pautrier microabscesses, degenerating keratinocytes are sometimes bound to lymphocytes and histiocytic cells (Fig. 11). These histiocytic cells possess the same ultrastructural aspects of Langerhans cells though without identifiable specific Langerhans granules.

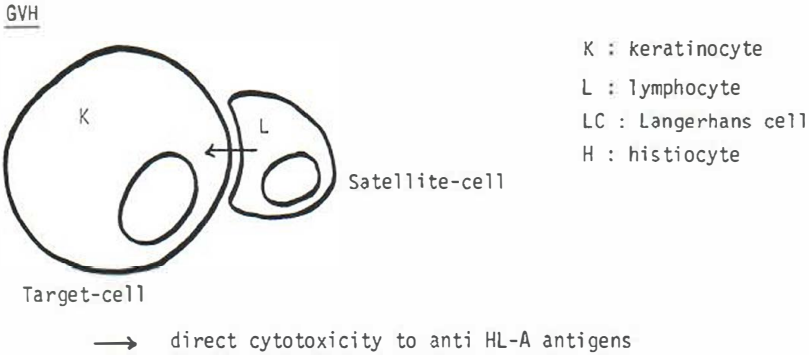
These cells show various contacts with degenerating keratinocytes which occasionally appear to be completely surrounded by histiocytic cells (Fig. 12).

When keratinocytes are largely involved in the degenerating process, the bound mononuclear cells are always histiocytic. In this circumstance, Langerhans cells are restricted to the upper layer of the epidermis above the lesional area and below the granular layer.

DISCUSSION

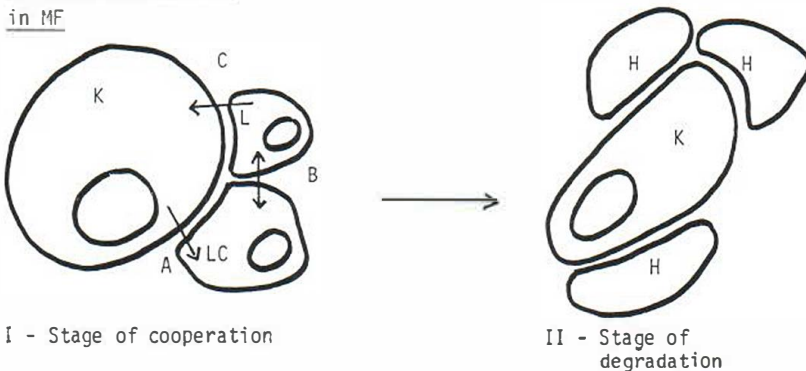
Epidermotropic malignant lymphomas have been the subject of several ultrastructural studies (1, 2, 7, 10, 15) which have often dealt with the ultrastructural characteristics of the dermal infiltrating tumoral cells. Recently, Rowden et al. (18) reported on an ultrastructural study of cutaneous lesions with special regard to epidermal infiltrating cells and the presence of Ia antigens (18). This antigen is shared by mononuclear phagocytes and especially by Langerhans cells (9, 16, 25).

The thymodependent origin of tumoral cells of



PAUTRIER MICROABCESS

in MF



- A- Recognition of Ag X ?
B- T lymphocyte-macrophage-like cell cooperation
C- Cytotoxicity

Fig. 13. Diagram illustrating the involvement of mononuclear cells in Pautrier microabscesses of epidermotropic lymphomas and their possible relationship.

mycosis fungoides and Sézary's syndrome was demonstrated by immunoelectronmicroscopy on freed cells after extraction from skin infiltrates (3, 20, 26). This origin was confirmed in situ by immunohistochemistry on frozen tissue sections in light microscopy in both dermis and epidermis (5, 21, 22, 27).

Pautrier microabscesses are the product of interactions between T lymphoid cells, Langerhans cells and keratinocytes. This association was observed in various lesions, such as those of contact dermatitis (23, 24). In this disease, Silberberg et al. (24) have described by means of electron microscopy the formation of Langerhans granules in the contact zone between Langerhans cells and other cells. Our results show the same aspect between keratinocytes and Langerhans cells in epidermotropic T cell lymphomas. The interaction cells and Langerhans cells is considered to represent confrontation between the antigens and the

immunocompetent cells (14, 25). In mycoses fungoides, the same pattern is suggested by Rowden & Lewis (17). The various contacts between keratinocytes and Langerhans cells before the degenerating process becomes established suggest the existence of a recognition of antigens present on the keratinocyte cytoplasmic membrane.

The disappearance of Langerhans cells, when keratinocytes are involved in the degenerating process, and the appearance of histiocytic cells, suggest a possible replacement of Langerhans cells by histiocytic cells. Recently Rowden et al. (18) observed ultrastructural degeneration of Langerhans cells in Pautrier microabscesses and the presence of cell debris containing Langerhans granules. We have not observed these aspects. Rowden et al. consider Langerhans cells to be target cells. Our results cannot support this hypothesis. If we consider the different steps of keratinocyte degeneration as chronological markers, we can suggest a

possible functional change of Langerhans cell into histiocytes with the disappearance of Langerhans granules.

The degenerating process of keratinocytes is mediated by the interactions between Langerhans cells, which are considered to be the macrophages of the immune reaction, and proliferating cells with cytotoxic properties (12). Degeneration of keratinocytes and cytotoxic epidermotropic lymphocytes was also observed in cutaneous lesions of Graft-versus-host reaction (GVH) after bone marrow transplantation (8). The ultrastructural aspects of GVH cutaneous lesions (4, 6) show a lysis of keratinocytes with disappearance of cytoplasmic organelles and desmosomes, aggregates of tonofilaments and vacuolization. The most characteristic event is the association between the degenerating keratinocytes and isolated satellite lymphoid cells (4, 8) and the absence of Langerhans cells. In this case, the lysis of keratinocytes occurs without the participation of Langerhans cells. In the GVH lesion, lymphoid cells are considered as killer cells and keratinocytes as target cells. The same process in mycosis fungoides needs the presence of Langerhans cells. These observations suggest different immunological mechanisms in the degenerating process of keratinocytes.

Further immunological and experimental studies will enable us to establish precisely the different functional aspects of the epidermotropism of cutaneous lymphomas.

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