

LICHEN PLANUS: AN ULTRASTRUCTURAL STUDY

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Abstract. Ultrastructural features of skin lesions are described in 11 patients with lichen planus. Keratinocytes and melanocytes in the basal layer show loss of nuclear membrane, disappearance of nucleoli, homogenization of nuclear material, and aggregation of tonofilaments around the nucleus to resemble cells in the late prophase of the mitotic cycle except that there is no clumping of chromosomes. These cells undergo fibrillar transformation to form densely packed fibrillar bodies (colloid bodies) which are the size and shape of normal cells and frequently have cytoplasmic organelles. Ten per cent of the mainly lympho-histiocytic cells in the dermal infiltrate show changes such as multiple cell membrane discontinuities, disintegration of cytoplasm, and breaks in the nuclear membrane with spilling of nuclear substance either into the cytoplasm or the extracellular space. The shape, size and occasional fibrillar changes in the nuclei and cytoplasm of these cells resemble colloid bodies. Contact sites frequently occurred between cells in the dermal infiltrate, especially between lymphocytes and macrophages. It appears that primary injury to the basal layer cells occurs during the early phase of the mitotic cycle and antigen from this primary site may evoke a cell-mediated type of hypersensitivity reaction. Colloid bodies evolve from cells which have injured nuclei and undergo fibrillar transformation. Dermal as well as epidermal cells may contribute to colloid body formation.

Key words: Lichen planus; Ultrastructure of lichen planus; Lichenoid reaction; Colloid bodies; Basal layer injury; Prophase arrest

The clinical features of lichen planus were described for the first time in 1869 by Erasmus Wilson (20) and the classical histopathological picture was described by Darier (5) in 1909. Samman (19) and Black (4) have recently reviewed lichen planus. Despite distinctive clinical and histopathological features the etiology of this skin disease remains unknown.

In 1972 Sarkany & Gaylarde (17) offered some preliminary evidence in favor of an autoimmune pathogenetic mechanism in lichen planus. They cultured peripheral blood lymphocytes from 6 patients with lichen planus in the presence of a homogenate made from a lesion taken from each patient's own skin. Lymphocyte transformation in response to skin homogenate was obtained in 2 out of the 6 cases.

Electron microscopic observations in lichen planus have previously been recorded by Johnson & Fry (13), Sarkany & Gaylarde (18), Ebner & Gebhart (9), El Laban & Kramer (10), Ebner (8) and Flaxman et al. (11). In these studies (13, 18, 9) epidermal changes were noted to precede dermal changes and initially involved liquefaction of basal layer cells and disruption of the basal lamina. Colloid bodies (9) were found to have fibrillar structures and often contained cell organelles, suggesting that they originate via fibrillar transformation of degenerating basal cells. El Laban & Kramer (10) in an electron microscopic study of oral lichen planus further suggested that such bodies probably arise via degenerative changes in epidermal cells in various stages of mitosis.

There are very few electron microscopic observations (8, 11) regarding the dermal infiltrate in lichen planus. Recently Ebner (8) examined the dermal infiltrate in 3 cases of lichen planus by electron microscopy and found a predominance of lymphocytes as well as remarkable numbers of monocytes and macrophages and transitional forms between these latter two cell types. He also found some mast cells, neutrophils, eosinophils and Lutzner cells. He concluded that the infiltrate resembles

that seen in delayed hypersensitivity reactions and assumed that this infiltrate in lichen planus represents immunologic reaction to an epidermal allergen. Flaxman et al. (11) also found Lutzner cells in the dermal infiltrate of lichen planus.

The present investigation was undertaken on 11 patients with widespread classical lichen planus. Five of these had acute lichen planus. Ultrastructural changes associated with this dermatosis were examined, with particular attention to changes in the epidermal basement zone, the lower epidermis and the dermal infiltrate, in the hope of finding further information which might be helpful in elucidating the etiology of this skin disease.

MATERIALS AND METHODS

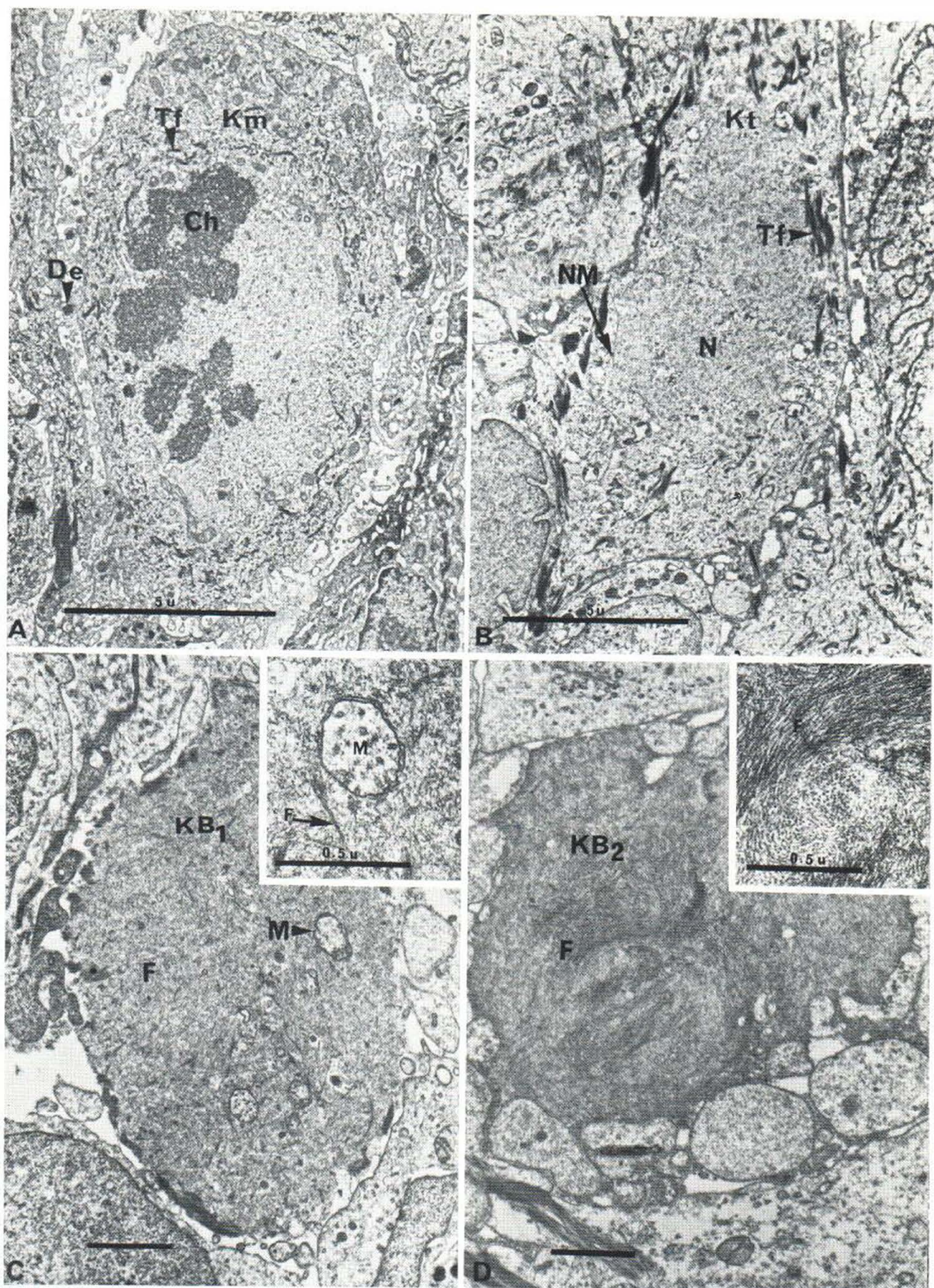
Biopsy specimens were obtained from skin lesions by 4 mm punch and bisected. One half was fixed in formalin, embedded in paraffin and routinely sectioned and stained with H&E for light microscopy. The other half was cut into four pieces. Two were fixed in 3% glutaraldehyde with Millonig's buffer kept at pH 7.4 and then post-fixed for 1½ hours with collidine-buffered osmic acid. The other two were fixed in collidine-buffered osmic acid for 1½ hours. All four pieces were then processed in the same way. They were dehydrated in ethanol, embedded in Epon or Araldite and sectioned on a Porter-Blum ultramicrotome. Sections 600–800 Å thick were stained with uranyl acetate and lead citrate and examined with an RCA Emu-3 electron microscope. Additionally, sections 1–2 µm thick were stained with a mixture of 1% pyronine and 1% toluidine blue in ratio of 1:4 and viewed with a light microscope.

RESULTS

Light microscopic examination of the specimens from all 11 patients showed typical changes of lichen planus, namely hyperkeratosis, hypergranulosis, saw-toothed acanthosis, liquefaction degeneration of the basal cell layer and a band-like infiltrate of mononuclear cells in the upper dermis. Colloid bodies were also seen frequently in the lower epidermis and papillary dermis. Electron microscopic examination showed the following changes. The stratum corneum had increased numbers of cell layers and more densely packed filaments. The cells in the malpighian layer showed increased filamentous material, and the number of keratinosomes was also increased. Keratohyaline granules were somewhat smaller and had round or oval instead of stellate shapes. The number of tonofilaments associated with granules was smaller. The

most striking changes were found in the lower epidermis. There was spongiosis with overstretching of cellular connections so that they ruptured at the bases of desmosomes. The remaining desmosomal protrusions tend to disappear at later stages and cell surfaces then often acquire a smooth appearance. Tonofilaments retract from the injured desmosomes and accumulate around the nucleus or clump in disorderly fashion throughout the cytoplasm. Some cells showed severe degenerative changes with degeneration of organelles, disappearance of the nucleus, and clumping of tonofilaments. These non-specific changes are most likely secondary to injury associated with the inflammatory infiltrate. In the areas where the epidermis did not show these severe injury changes, some individual cells were notably enlarged. The nuclei of these cells were also large and showed partial or complete loss of nuclear membrane and disappearance of the nucleolus. At each end of the long axis of these cells there was an increase in the perinuclear endoplasmic reticulum and the presence of ribosome clusters. These areas were also free of tonofilaments which were displaced peripherally. These individually altered enlarged cells resemble cells in late prophase (15) of the mitotic cycle except for the lack of chromosome aggregation. These cells also had decreased numbers of desmosomes even in the absence of intercellular edema. Cells with more advanced changes showed gradual fibrillar transformation of the cytoplasm. Frequently, non-membrane bound fibrillar bodies were seen which contained larger or smaller numbers of cytoplasmic organelles (Fig. 1C) such as mitochondria or melanin granules. As a final stage there remained fibrillar bodies (Fig. 1D) consisting simply of densely packed fibrils 80–100 Å wide. These bodies (Fig. 1C, 1D) either singly or in

Fig. 1 (A) Keratinocyte in mitosis (Km). There is loss of the nucleolus and nuclear membrane. Chromosomes (Ch) are aggregated in the center of the cell. Tonofilaments are accumulated closer to the periphery of the cell. A desmosome is labelled (De). (B) Basal keratinocyte in lichen planus which resembles the cell in Fig. 1A except chromosomes are absent. The nucleus (N) shows remnants of nuclear membrane (NM). Tonofilaments (Tf) are accumulated peripherally. (C) Colloid body (Kb₁) is a non-membrane limited body similar in size and shape to a keratinocyte. It is finely filamentous (see insert) and contains cell organelles such as mitochondria (M). Filaments are labelled (F). (D) Colloid body (Kb₂) is a pure fibrillar body devoid of cell organelles. (See insert).



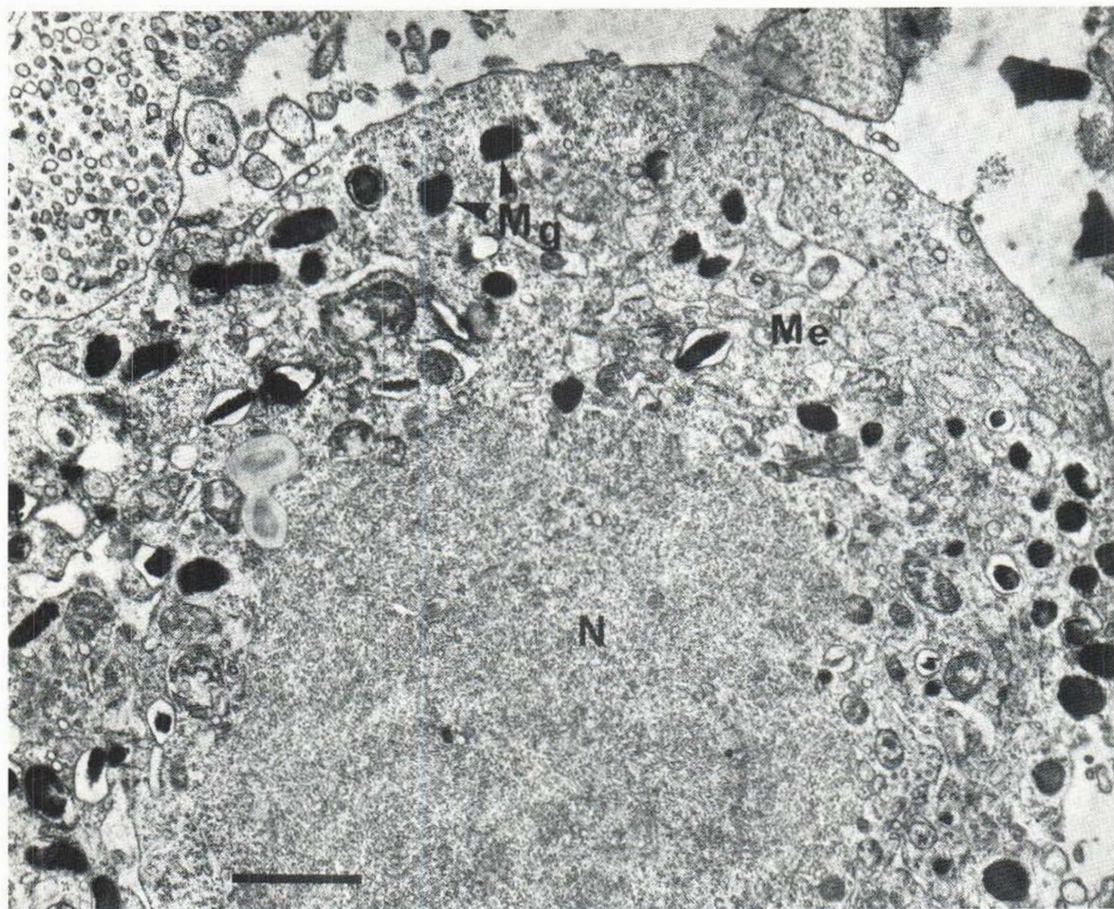


Fig. 2. Melanocyte (*Me*) which shows the same changes as the keratinocyte in Fig. 1 B. The nucleus (*N*) shows loss of

nucleolus and nuclear membrane. A melanin granule is labelled (*Mg*).

groups were the size of normal cells and correspond to the colloid bodies described by light microscopists. Occasionally they were seen to protrude from the epidermis into the dermis. Occasionally melanocytes (Fig. 2) also showed loss of the nuclear membrane, disappearance of nucleoli and homogenization of nuclear material with aggregation of melanosomes and other cytoplasmic organelles at the periphery of the cell. The basal lamina showed multiple discontinuities and occasional new formation of basal laminar material. Areas of increased separation between the basal cell membrane and the basal lamina appeared along with disappearance of anchoring filaments and fewer anchoring fibrils. Where basal cells had disintegrated, only cellular debris was seen between the basal lamina and the epidermis. In the upper dermis there was an infiltrate consisting mainly of lymphocytes and mac-

rophages. Lymphocytes including small, large and transformed lymphocytes were also seen. The remainder of the infiltrate included a few monocytes, polymorphonuclear leukocytes, plasma cells, mast cells and fibroblasts. Approximately 10% of the cells of the dermal infiltrate, mainly lymphocytes, presented the following kinds of abnormalities. The cell membrane may show multiple small discontinuities. The nuclei may be large and show large breaks in the nuclear membrane with spilling of

Fig. 3 (A) Several mononuclear cells are seen. The cell in the center is damaged (*DC*₁) with focal disruption of nuclear and cell membranes. There is spilling of nuclear substance into the extracellular space, somewhat fibrillar (arrow head). (B) Damaged cell (*DC*₂) shows folding of its nucleus (*N*) with entrapped cytoplasm (*Cy*). Remnants of cytoplasm (arrow head) and the nuclear membrane (*NM*) are seen at the periphery of the nucleus.

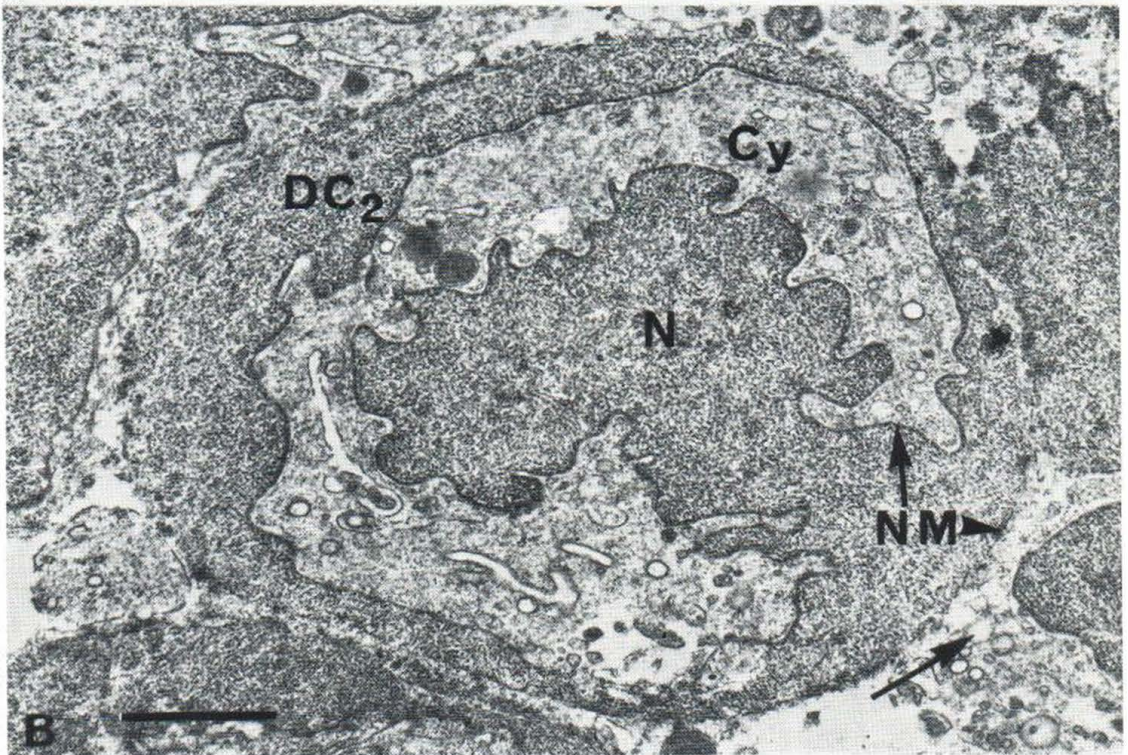
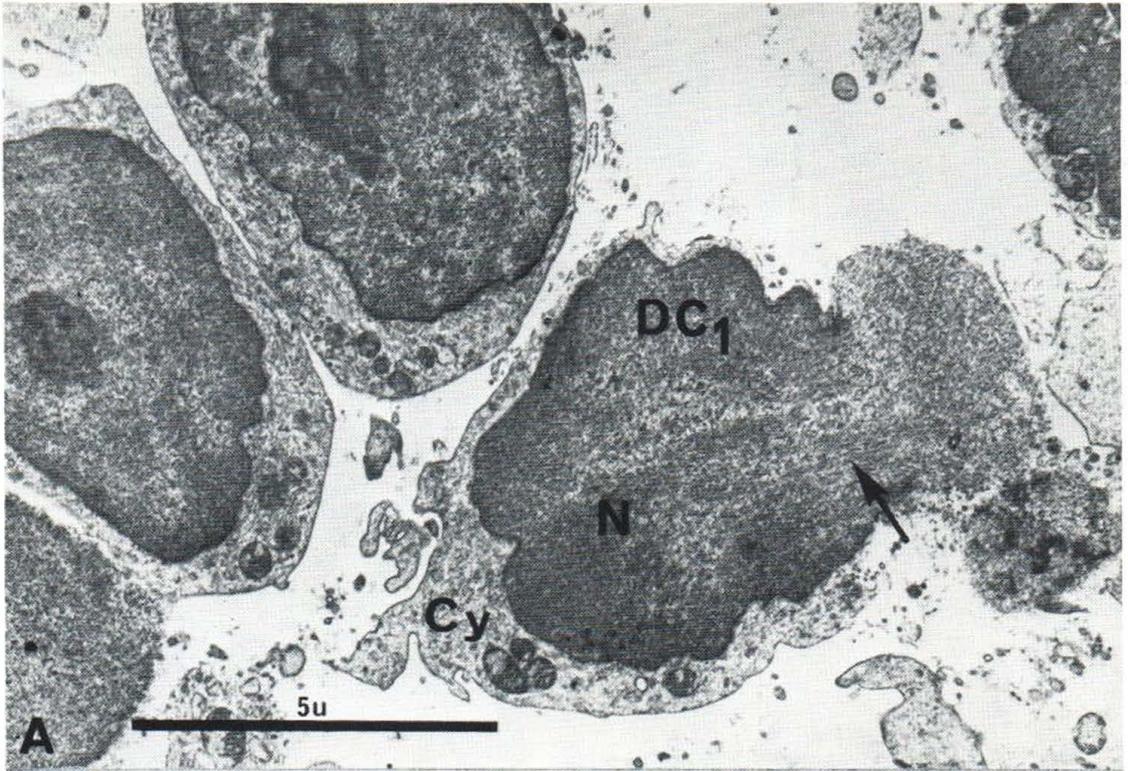




Fig. 4. Lymphocyte (Ly) and histiocyte (Hi) form a contact site (arrow head). Fine fibrils are seen between the two cells at the contact site.

nuclear substance (Fig. 3 A) either into the cytoplasm which is frequently scanty or into the extracellular space. The nuclear substance may show fibrillar areas and some cells show extranuclear accumulation of fibrillar material. A few cells showed only loss of the nuclear membrane and homogenization of nuclear material. These changes resembled the changes already described in the epidermal cells. Occasionally cells with extremely folded nuclei were seen. In some cells the cytoplasm had disintegrated leaving only remnants attached to the nuclear membrane. Where such a denuded nucleus was folded, some cytoplasm could be trapped within the folds to give the appearance of a cell with its nucleus situated peripherally while the cyto-

plasm appeared centrally located (Fig. 3 B). Contact sites (Fig. 4) were frequently observed between cells in the dermal infiltrate and especially between lymphocytes and macrophages. These contact sites were focal and consisted of close approximation of the two cell membranes. Both membranes showed increased density at these points and often this density was associated with numerous fine filaments crossing between the two membranes. A large number of lymphocytes contained centrioles. A few large macrophages were present with numerous secondary lysosomes containing granular, amorphous and fibrillar material. Fibrillar bodies singly or in groups were frequently encircled with macrophages. Fibrin was also observed in the upper der-

mis and at the peripheries of fibrillar bodies. The capillaries were dilated and endothelial cells showed numerous filaments and increased numbers of pedunculated processes.

COMMENT

The most striking ultrastructural changes in lichen planus are in the basal layer, at the epidermo-dermal junction and in the upper dermis. Intercellular edema, a decrease in desmosomes, intracytoplasmic and intranuclear fibrillar changes, and colloid bodies have been described previously, as discussed earlier. The keratinocytes and melanocytes that show increase in nuclear size, disappearance of nucleolus and partial or complete loss of nuclear membrane (Fig. 1 B) resemble cells in the late prophase of the mitotic cycle except that there is no clumping of chromosomes (Fig. 1 A). The most conspicuous changes in prophase of the epidermal mitotic cycle are increased in size of the cell and its nucleus, perinuclear increase of endoplasmic reticulum, ribosome clusters at each end of the long axis of the cell with peripheral displacement of tonofilaments in the same area, gradual disappearance of the nucleolus and nuclear membrane and aggregation of chromosomes into the center of the nuclear region. It appears that in affected cells in lichen planus the mitotic cycle may have been initiated and proceeded toward breakdown of the nuclear region but then just prior to point of chromosome aggregation the cycle became blocked and the cells deviated to degenerative fibrillar transformation of the nucleus and cytoplasm (Fig. 1 A, B, C and D). Occasional cells in the dermal infiltrate show similar changes, loss of nuclear membrane and homogenization of the nuclear material. Cells with disintegrating cytoplasm and showing various other forms of cell injury (Fig. 3 A, B) are seen in the dermis, as described earlier. These cells resemble colloid bodies as regards shape, size and occasionally observed fibrillar changes in the nuclei. It appears that not only degenerating epidermal cells but also possibly dermal cells may contribute to the formation of colloid bodies. Multiple discontinuities of the basal lamina (13, 18) in lichen planus have been observed previously and associated with the presence of the inflammatory infiltrate. The numerous intraluminal pedunculated processes observed in dermal capillaries are possibly related to the passage of many lymphocytes through the capillaries. Fibrin deposits noted around capillaries and in the

upper dermis also have been described (1) previously and were interpreted as resulting from enhanced capillary permeability. Some of the lymphocytes in the dermal infiltrate have hyperconvoluted nuclei and appear indistinguishable from Sezary cells, as have been previously noted (8, 11). The nature of the infiltrate, which consists of macrophages, small and large lymphocytes and the frequent presence of attachment sites between lymphocytes and macrophages (Fig. 4) is similar to that previously noted in allergic contact dermatitis (14). Similar attachment sites have been described between fetal fibroblasts, odontoblasts and osteoblast (16) and it has been postulated that the attachment sites contribute to maintenance of structural integrity of the connective tissue. Close contacts between the reticulohistiocytic cells and erythroblastic, lymphocytic or plasmacytic cells have also been described (3). It was speculated that reticulohistiocytic cells may provide molecules carrying information necessary to the function of the various cell types which they contact in this manner. Lymphocytes (2) may bind antigen directly and it might be speculated that possibly surface bound antigen-antibody complexes on lymphocytes may be transferred via the contact sites to monocyte-macrophage type cells which then phagocytize and finally digest them. In the infiltrates of lichen planus and allergic contact dermatitis the presence of attachment sites between lymphocytes and macrophages suggests active operation of the cell-mediated immune mechanism. One may speculate that lichen planus is associated with a cell-mediated type of hypersensitivity reaction similar to that in allergic contact dermatitis but directed against some basal cell nuclear antigen.

Basophils were not observed in the infiltrate and only a few monocytes were seen despite both of these cell types being numerous in the early first 24 hour phase of allergic contact dermatitis (6, 7, 12, 14). The infiltrate in lichen planus rather resembles that in the post-72-hour stages of allergic contact dermatitis in which basophils are rare and macrophages—rather than monocytes—predominate.

CONCLUSION

The site of primary injury in lichen planus appears to be in the nuclei of basal epidermal cells during the early phase of the mitotic cycle. Antigen from this primary site may evoke a cell-mediated delayed type of hypersensitivity reaction. The colloid

bodies in this disease originate from cells which have injured nuclei and undergo fibrillar transformation. Not only epidermal but also possibly dermal cells may contribute to the formation of colloid bodies. A moderate number of cells in the dermal infiltrate show nuclear membrane damage with spilling of nuclear material into the cytoplasm of the cell or extracellularly. Attachment sites between lymphocytes and macrophages in the infiltrate are frequently present.

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