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THE LICHEN PLANUS LIKE AND SCLEROTIC PHASES OF THE GRAFT VERSUS HOST DISEASE IN MAN: AN ULTRASTRUCTURAL STUDY OF SIX CASES

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Abstract. Skin biopsies from 6 patients with chronic graft-versus-host disease (GVHD) were studied ultrastructurally. The 6 patients experienced an early lichenoid phase 65–135 days after the graft and 3 of them progressed to a late sclerotic phase 200–340 days after the grafting. Damage to the basal membrane and to the keratinocytes of the basal layer and low spinous layers, and presence of epidermal regenerative cells were features common to the lichenoid phase of chronic GVHD and idiopathic lichen planus. The late sclerotic phase of GVHD with persistence of basal cell injury, normal periodicity and structure of the collagen fibres and numerous active fibroblasts in the upper third of the dermis were findings that distinguished GVHD from scleroderma. Satellite cell necrosis, i.e. lymphocyte satellites of necrotic keratinocyte, was observed in the two phases of chronic GVHD. Thus at the ultrastructural level the early phase of chronic GVHD mimics lichen planus, but the late sclerotic phase is distinct from scleroderma.

Key words: Chronic graft-versus-host disease; Lichenoid phase; Sclerotic phase; Idiopathic lichen planus; Lupus erythematosus; Scleroderma

Chronic graft-versus-host disease (GVHD) mimics some cutaneous diseases: the early phase was described as lichen-planus-like eruption (LPLE) (30, 31, 32), and the late, sclerotic phase may remind us of scleroderma (30, 34, 37). It was the purpose of this work to compare the electron-microscopic features of LPLE with lichen planus (LP) and lupus erythematosus (LE), and of the sclerotic phase of chronic GVHD with scleroderma.

MATERIAL AND METHODS

Six patients, 19 to 25 years old, underwent allogenic bone marrow graft for idiopathic bone marrow aplasia. Some 65 to 135 days after the graft, they experienced an oral and cutaneous lichen planus like eruption, i.e. the early phase of chronic GVHD, and were biopsied some 200 to 340 days after grafting. Three of these patients went through a late phase of chronic GVHD, with atrophic and sclerotic cutaneous changes, which were biopsied. One patient had on one foot an LPLE lesion which, 4 months later, resembled morphea. Biopsies were performed on this plaque in the two different phases of chronic GVHD.

After removal under local anesthesia, the skin specimens, which included whole dermis and part of the hypodermis, were cut in two halves. Half of the biopsy specimen was fixed in formalin and further processed for optical microscopy. The other half was immediately fixed in glutaraldehyde 1% buffered cacodylate for one hour at +4°C, washed in cacodylate buffer three times for 10 minutes, postfixed in osmium tetroxide for one hour at +4°C, dehydrated in ethanol and propylene oxide series and embedded in T.A.A.B. medium. Sections were cut with an OM U_2 Reichert microtome. Semi-thin sections were examined after toluidine blue staining to check whether they had been cut strictly perpendicular to the epidermis, whether epidermal appendages and hypodermis were present, and to observe the mast cells.

Ultrathin sections were contrasted with uranylacetate solution and citrate lead stain. Ultrastructural study was performed with an Hitachi $HU_{12}A$ electron microscope at 75 kV. The analysis of skin lesions throughout chronic GVHD was concentrated on three aspects: injury of basal layer, low spinous layers, and dermo-epidermal junction; (ii), inflammatory infiltrate: its composition and topography; (iii), dermal collagen and fibroblasts.

RESULTS

LPLE Stage

Uniform changes were seen in all 6 patients studied.

Epidermal changes

Injury was most prevalent in keratinocytes of the basal layer. The most severe change was complete necrosis of the keratinocytes, with pyknotic nu-

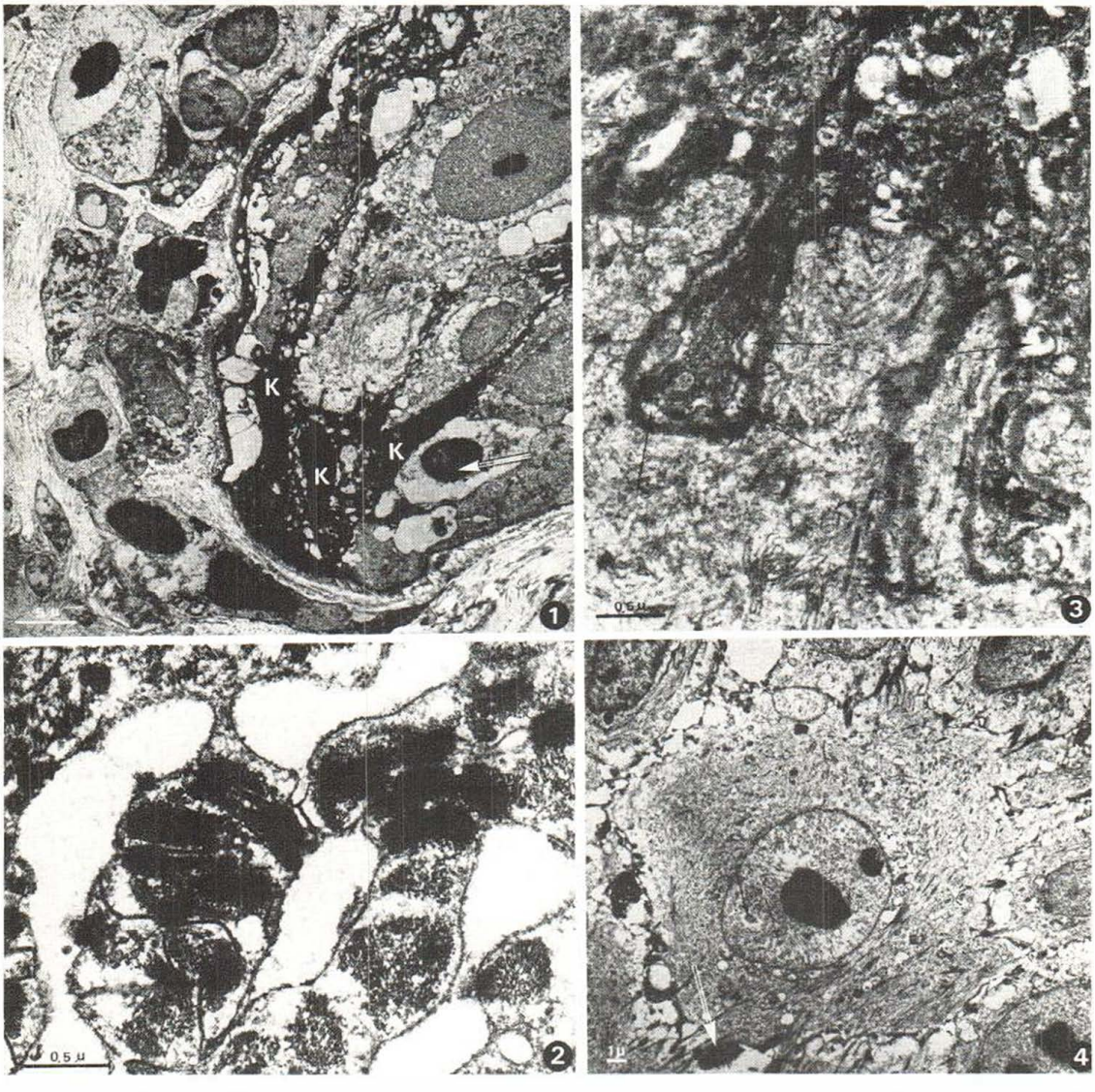

Fig. 1 (lichenoid phase). Three necrotic keratinocytes (K) with a satellite lymphocyte (twin arrow) in the basal layer. Cytoplasmic vacuolization of the keratinocyte and widening of intercellular space in the lower spinous layer. In the papillary dermis, the infiltrate is composed of lymphocytes and macrophages.

Fig. 2 (lichenoid phase). Cluster of desmosomes in a widened intercellular space in low spinous layers. These desmosomes have a normal ultrastructural appearence and segments of cytoplasmic membrane are constantly attached to them.

Fig. 3 (lichenoid phase). Dermo-epidermal junction with irregular widening of the lamina lucida (arrows), absence of electron-dense deposits and very sparse anchoring filaments.

Fig. 4 (lichenoid phase). Large clear cell with voluminous nucleolus and nuclear microbodies (black arrows). A lymphocyte satellite of this cell is indicated by the white arrow.

cleus and condensation of cytoplasm (Fig. 1), often accompanied by fractures and duplication of basal membrane. Less severe were the following changes: cytoplasmic vacuolization of the keratinocyte, tonofilament bundles irregularly aggregated, basal cell axis parallel to the dermo-epidermal junction, and broadening of intercellular spaces which contained numerous clustered desmosomes. Seg-

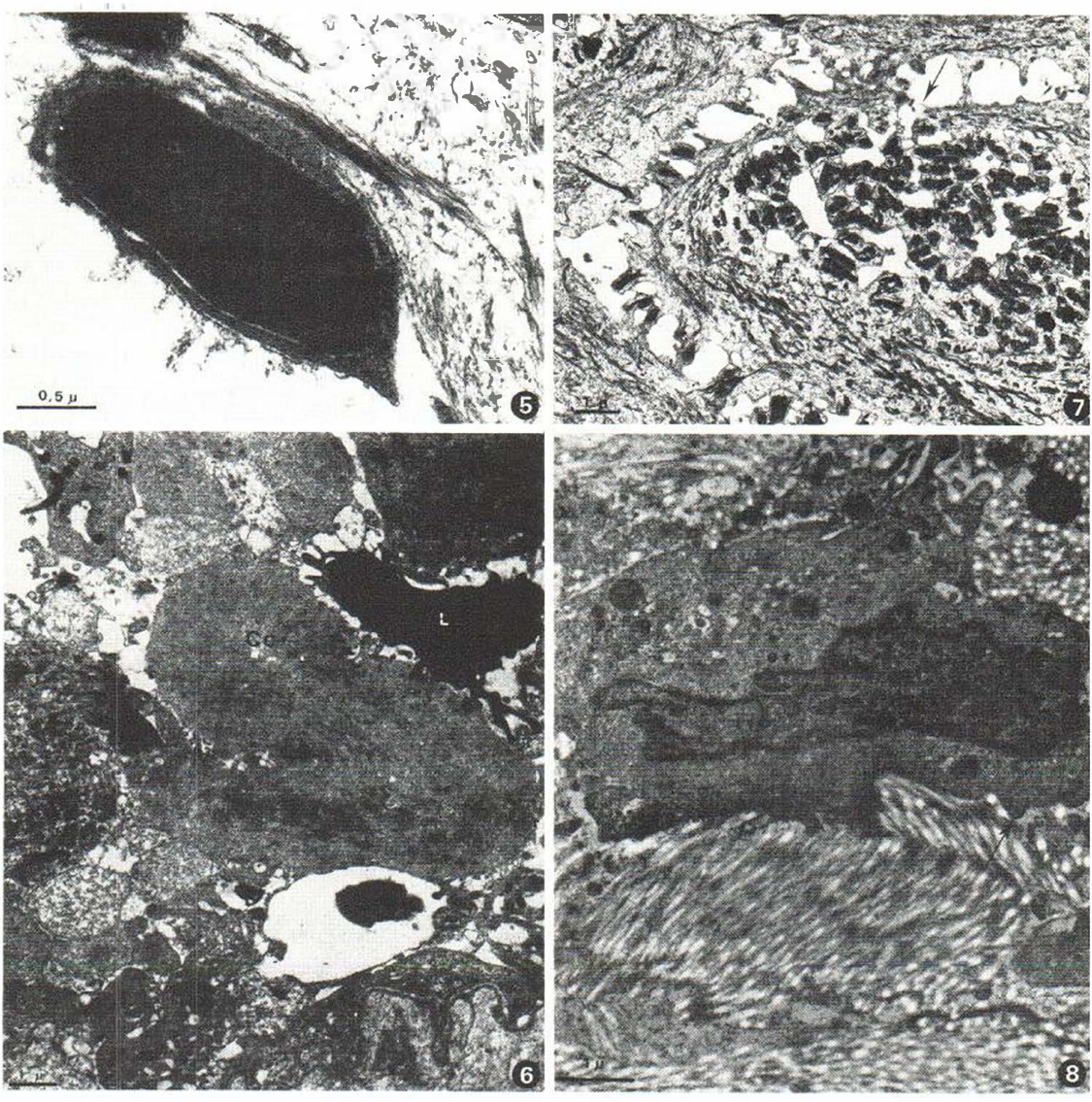

Fig. 5 (lichenoid phase). Broad contact between the cytoplasm of the satellite lymphocyte and the cytoplasm of the keratinocyte.

Fig. 6 (lichenoid phase). Point-contacts between a colloid body (*Cc*) and elongated cytoplasmic processes of a satellite lymphocyte (*L*) in the basal layer.

Fig. 7 (sclerotic phase). This cluster of ultrastructurally normal desmosomes is not situated in the cytoplasm of the keratinocyte, but in the intercellular space (arrow indicates the channel between the intercellular space and the area where the desmosomes are located).

Fig. 8 (sclerotic phase). Active fibroblast in upper third of dermis, with prominent Golgi (*g*), abundant ergastoplasm (*er*) and partial thickening of cytoplasmic membrane (arrows).

ments of cell membrane were always seen with these desmosomes. The dense plaques remained parallel and desmosomal fine structure was intact (Fig. 2). The hemidesmosomal fine structure was also preserved.

Careful study of the dermo-epidermal junction in early lesions of LPLE showed irregular widening of lamina lucida (Fig. 3), with paucity of anchoring filaments, and fibrin deposits on the dermal side in two cases.

Analysis of the different types of epidermal cells failed to reveal any transformation in melanocyte or

Langerhans' cell structure, but two kinds of change were observed in keratinocytes of the basal or the low spinous layers. Besides damaged cells, some keratinocytes in the low spinous layers showed intense synthetic activity: large and clear cytoplasm with abundant granular endoplasmic reticulum, numerous mitochondria and free ribosomes, and nucleus with voluminous nucleolus and spherical microbodies (Fig. 4). Such large cells could have broad cytoplasmic contacts with elongated "satellite" lymphocytes of the inflammatory infiltrate (Fig. 5). These active cells were not found in the basal layer.

Colloid bodies were numerous in low epidermal layers (Fig. 6) and papillary dermis, where they lay under severely damaged epidermal zones, often with broken basal lamina. Most of these dermal colloid bodies were phagocytosed by macrophages.

Infiltrate

The infiltrate was most prominent in the papillary dermis, while in the lower layers of the epidermis it hugged the epidermis, and had irregular and spotty borders. It was denser at the sites of major damage to epidermal zones. A hundred cells were counted on contiguous areas at different levels: 67 were lymphocytes, 18 were macrophages, 10 mast cells with intact granules, 2 polymorphonuclear leukocytes, 2 fibroblasts, and 1 plasma cell. Langerhans' cells were not seen in the dermis. Mast cells, macrophages and lymphocytes were seen in the papillary dermis. In the epidermis, we observed both small and long, stretched lymphocytes adjacent to damaged keratinocytes or to colloid bodies.

Two kinds of morphological change could be interpreted as constituting contact between "satellite" lymphocytes and epidermal cells: point contact with long, slender cytoplasmic processes between lymphocytes and keratinocytes (Fig. 1) or colloid bodies (Fig. 6), and broad contact via cytoplasmic membranes of lymphocytes and keratinocytes joined side by side (Fig. 5).

The dermis at this LPLE stage had normal collagen bundles and elastic fibres; there were very few fibroblasts. Capillaries and venules had endothelial pinocytic vesicules but no deposits or breaks in their basal membrane.

Sclerotic Stage

Two patients were biopsied 200 to 250 days after the graft and the third one was biopsied 340 days after grafting. The ultrastructural features were rather similar.

1) The epidermis was atrophic, with an almost straight dermo-epidermal junction. It preserved changes from the lichenoid phase: mild injury of the basal cells, orientation of these cells parallel to the dermo-epidermal junction, "satellite" lymphocytes, and few colloid bodies in the upper dermis. Many desmosomes were still clustered in narrowed intercellular spaces (Fig. 7) but no phagocytosed desmosomes could be found.

2) The infiltrate was sparser than in the LPLE phase and was located mostly in the papillary dermis, around dermal vessels. Of 100 cells counted, 58 were lymphocytes, 19 macrophages (with fragments of colloid bodies and basal lamina in their lysosomes), 12 fibroblasts, 9 mast cells, and 2 plasma cells. Mast cells had cytoplasmic microvilli and granules with irregular shape and inhomogeneous content.

3) Dermal involvement in this phase was prevalent on papillary dermis. The collagen bundles were broad, densely packed and interbundle spaces were reduced. Studied in longitudinal sections, collagen fibres were of normal axial periodicity and cross-band pattern. The fibroblasts were numerous and showed signs of intense synthetic activity (Fig. 8) with prominent Golgi, numerous granular endoplasmic reticula, partial thickening of cytoplasmic membrane, and protocollagen fibrils on the periphery of their cytoplasmic membrane. In the perifollicular and reticular dermis, collagen bundles were dense but neither lymphocytic infiltrates nor active fibroblasts could be found. The eccrine sweat glands that we observed were located in the lower dermis and had a normal ultrastructural aspect. Dermal capillaries were often dilated, but no endothelial proliferation or deposits could be seen along the basal membrane.

DISCUSSION

Graft-versus-host disease resembles several skin diseases such as lichen planus (LP), lupus erythematosus (LE) and scleroderma (30, 34, 37). We concentrated our study on an analysis of the ultrastructural features of chronic GVHD in comparison with those of LP, LE, and scleroderma.

1) The early chronic phase is clinically lichenoid (31, 32) and histologically very similar to LP.

At the ultrastructural level, LPLE is distinct from

LE regarding the following points: damage was prevalent in the basal and low spinous layers; there was absence of electron-dense deposits, or alteration of elastic fibres.

In LPLE, keratinocytes of the basal layer, as well as keratinocytes of the low spinous layers were damaged, in contrast to the necrosis and the disintegration of the dermo-epidermal zone only, in LE (36).

No electron-dense deposits could be found in LPLE, either near the dermo-epidermal junction (16) or within the blood vessel walls (16, 19).

Elastic fibres in LPLE were not, as in LE, coated with dense granular material (24). Their fibrillar components did not show the ultimate formation of fibrillar bodies as in LE (33).

LPLE is similar to idiopathic LP in that epidermal damage is prevalent in the basal layer and low spinous layers, and in its similar basal cell orientation. Furthermore the two disorders, having the same topography of their colloid bodies and presence of large active cells, may also have similar epidermal kinetics.

Johnson (21), Brody (4), Sarkany (29) and Medenica (27) described in LP damage of the basal layer and low spinous layers: cytoplasmic vacuolization, widening of intercellular spaces and ruptures of the dermo-epidermal junction, where the epidermal damage was the most severe and the lymphocytic infiltrate the densest. Brody (4) reported a change in basal cell axis, becoming parallel to dermo-epidermal junction. All these features were seen in LPLE, but in LP, desmosomes from damaged epidermal zones were sparse and gradually disintegrated. In our cases, desmosomes were clustered in widened intercellular spaces, but their fine structure was intact and segments of cytoplasmic membrane remained attached to their extremities. In LPLE, clusters of ultrastructurally normal desmosomes may result from ruptures in cytoplasmic membranes of injured keratinocytes. Their multiplicity may also suggest an associated process of neoformation.

Colloid bodies—except for a few located in the upper epidermis (transepidermal elimination? (10)—were situated as in LP (9) in the lower epidermal layers and in the upper dermis where they underwent phagocytosis. This apoptosis (18, 38) is an epidermal kinetic common to LP and LPLE.

Half-and-half cells as described in LP (8) could not be found in LPLE. The large active cells in low spinous layers may be similar to the large pera-shaped cells observed in LP papules only 24 hours after injury (7). Furthermore, an autoradiographic study showed increased cellular proliferation in LP (26). LPLE and LP may thus combine to exert a continuous process of basal layer destruction and epidermal repair.

Several observations were made in LPLE which are not classical features of LP: (i) the widening of the lamina lucida; (ii) the presence of numerous mastocytes in the dermal infiltrate; and (iii) the picture of lymphocyte satellites of necrotic keratinocytes. Such a picture of satellite cell necrosis (17) or dyskeratosis (6) is a characteristic feature of GVHD during the acute phase; it has also been reported during the chronic phase (5, 15). It is regarded as a sign of lymphocytotoxicity, but we have observed a broad contact between a lymphocyte and a large active keratinocyte (Fig. 4). Finally, at the ultrastructural level the LPLE of GVHD more closely mimics LP and LE.

2) The sclerotic phase of GVHD retains to a lesser degree the epidermal morphologic characteristics of LPLE. But at this stage, dermal lesions prevail and may be reminiscent of scleroderma.

They differ from scleroderma in the following respects: topography of the infiltrate, localization of active fibroblasts and of densely packed collagen bundles in the upper third of the dermis.

The infiltrate in the papillary dermis is different from the infiltrate of the LPLE phase: it is sparser and is principally located around capillaries. Neither inflammatory cells between collagen bundles of the lower two-thirds of reticular dermis, as described in scleroderma (11), nor panniculitis with lymphocytes, fibroblasts and histiocytes in intercellular spaces of subcutaneous tissue (13) could be found in our three cases. But mast cells, which looked degranulated in histologic sections with toluidine blue stain, were seen by electron microscopy to have cytoplasmic microvilli and irregular granules as in scleroderma (23).

Elastic fibres in scleroderma, unlike in our cases, are altered in the deeper part of the dermis (13, 14) and their pathological fragments are phagocytosed by macrophages (23).

Collagen bundles in scleroderma have morphological characteristics: thin fibrils (100–200 A) within fibre bundles, considered by Braun Falco (3) to be a morphological sign of increased fibrilloneogenesis, and "beaded filaments" (20) typical

of embryonic collagen. Biochemical changes (subhydroxylated collagen) (1, 2) are associated with these morphological characteristics. Fleischmajer (12) introduced a topographic concept, assuming that the main alteration in scleroderma took place in subcutaneous tissue. This was consistent with experimental data (28). In the sclerotic stage of chronic GVHD, no thin fibrils or beaded filaments were observed. Dense collagen bundles, lymphocytic infiltrates and fibroblasts with morphological signs of active synthesis were seen, but only in the papillary dermis.

If skin induration in scleroderma corresponds to an active synthesis of abnormal collagen in subcutaneous tissue, skin induration in the sclerotic stage of chronic GVHD may be due to active synthesis of morphologically normal collagen in the upper third of the dermis. A "descending process" of the sclerosis in chronic GVHD could be opposed to an "ascending process" in true scleroderma.

Experimental works have demonstrated that, in certain circumstances, lymphocytes could stimulate collagen synthesis (22). This may be relevant to the pathogenesis of scleroderma (25–35). In chronic GVHD a similar process might be operating. The lymphocyte stimulation of collagen synthesis may explain the progression from the LPLE, where the lymphocytic infiltrate prevails to the sclerotic phase, where the collagen accumulation prevails. The localization in the papillary dermis of both lymphocytes and active fibroblasts favours the "descending process" theory of dermal sclerosis in GVHD, as opposed to the ascending process in scleroderma.

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